School of Applied Chemistry

The Occurrence and Origins of Some Alkylphenols in Crude Oils

Marisa Ioppolo-Armanios

This thesis is presented as part of the
requirements for the award of the
Degree of Doctor of Philosophy
of
Curtin University of Technology

1996

All levels of understanding and teaching
are available in your world.
It is where you yourselves
are able to hear that you will feel an affinity.
When true affinity becomes less compelling
then you seek teachings of another form.

Extract from Emmanual's Book.

TABLE OF CONTENTS

ACKNOWLEDGMENTS

First and foremost, I wish to thank my principal supervisor Professor Robert Alexander for his never-ending enthusiasm, guidance and wisdom throughout the duration of this study. Thank you also goes to my associate-supervisor Professor Robert Ian Kagi for his assistance and moral support when needed most.

I would like to gratefully acknowledge the financial support provided in the form of an Australian Postgraduate Research Award (APRA) and a Mineral and Energy Research Institute of Western Australia Scholarship. The technical assistance of Mr Steve Fisher with the tracer GC-FTIR analysis, and Mr Geoff Chidlow with the GC-MS analysis is gratefully acknowledged. I wish to thank Dr Roger Summons for providing several of the crude oil samples used in this study. Also thanks goes to my colleagues for the many invigorating discussions and experiences shared throughout the years.

A very special thank you is extended to two very special people, my Mother and Mother-in-law for their untiring support throughout the duration of this study. I wish to also thank my family members and friends, particularly Mr Colin Scanlan, for all of their encouragement over the years. Finally, and most importantly, my deepest thanks goes to my husband and best-friend, Dr Carim Armanios for being the greatest source of inspiration, strength, direction and support in my life.

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Analytical procedures have been developed for the quantitative analysis of phenols in crude oils and sedimentary rock pyrolysates and extracts. The procedures involve isolation of the phenolic components of the sedimentary organic matter by extraction with alkaline aqueous methanol, followed by the removal of carboxylic acids using a back extraction step. Co-extracted non-polar components are removed from the alkaline extract by liquid chromatography or by extracting it with hexane. The phenol isolates thus obtained were analysed by capillary GC, GC-MS and GC-FTIR. Recoveries of 70-95% were measured for C_0-C_3 alkylphenol compounds using these procedures.

Crude oil samples (45) representing a range of locations, ages, depositional environments, maturities, source types, and biodegradation levels have been analysed for their phenol contents. A range of C_0-C_5 alkylphenols (~40) were identified in crude oils using co-chromatography on up to three different stationary phases and by comparison of their retention times, mass spectral and infrared spectral properties with reference compounds. Isopropylmethylphenols (six) and *sec*-butylmethylphenols (nine) were synthesised for use in the identification of these compounds. A range of C_0-C_4 alkylphenols (24) were quantified using a dimethyl siloxane column (BP1 or DB1) and found to occur at concentrations ranging from $190x10^3$ ng/g down to the limit of detection of 10 ng/g (ppb).

The crude oil samples were classified into six groups based on their C_0-C_5 alkylphenol compositions. Group 1 crude oils have at least one isomer class in which the relative proportions of the alkylphenol isomers reflect their relative thermodynamic stabilities. The vast majority of samples, however, do not contain relative proportions of phenols which reflect their stabilities, and these differences have been used to group the remaining crude oils. Group 2 crude oils contain predominantly alkylphenols derived from natural product precursors. This group

has been further subdivided into Group 2A in which samples have isopropylmethylphenol distributions dominated by carvacrol and thymol; and Group 2B which is comprised of crude oils that contain high relative abundances of methylphenols which can be derived from tocopherols. Group 3 crude oils have C_2-C_5 alkylphenol compositions dominated by *ortho* and *para* substituted phenols which are proposed to be formed from geosynthetic processes. Group 4 crude oils contain alkylphenol compositions in which the relative abundances of *meta* substituted compounds in six isomer classes are much greater than those expected from chemical equilibration. Group 5 comprises of samples which are biodegraded and as a consequence contain alkylphenols below the limit of detection. Group 6 crude oils contain very low concentrations of alkylphenols and comprises samples which are derived from source rocks that pre-date the widespread occurrence of land plants or contain negligible land plant input.

Some alkylphenols in crude oil are structurally related to natural product precursors and therefore appear to be biomarkers. The monoterpenoid natural products carvacrol and thymol, or its rearrangement product 3-isopropyl-5-methylphenol, which occur in high relative abundances in Group 2A samples are such compounds. Because carvacrol and thymol occur widely in extant conifers and angiosperms, their presence in crude oils derived from source rocks deposited when these plant types were widespread suggests they also originate from these plants. A range of trimethylphenols and tetramethylphenols which occur in high relative abundances in Group 2B samples, are also reaction products obtained from heating α-tocopherol with aluminium smectite. This, together with the reported widespread occurrence of tocopherols in plant photosynthetic tissue and in sedimentary rocks, leads to the conclusion that tocopherols are likely precursors to these petroleum methylphenols.

The lignin components of terrestrial plants also appear to be important precursors to petroleum alkylphenols. Strong evidence for this is provided by the

observation that crude oils derived from source rocks which contain negligible higher plant input contain very low concentrations of alkylphenols (Group 6). In order to determine the likelihood of lignins as precursors of petroleum alkylphenols, the phenol contents of coals of lignitic through to bituminous rank were examined. The unbound phenolic components of the lignite samples were analysed by isolating the phenols from their dichloromethane extracts, and the bound phenolic components were analysed by pyrolysis GC-MS. At lignitic rank the unbound methoxyphenols allowed taxonomical classification of the samples and the bound hydroxyphenols bore structural similarities to lignin moieties. The bound C_0-C_4 alkylphenol components of lignitic, subbituminous and bituminous coals in a sedimentary sequence were quantitatively analysed by isolating the phenols from their hydrous pyrolysates. In the coals of subbituminous and bituminous rank, the bound alkylphenol components could not easily be related to lignin precursors because molecular transformations of lignins are very severe at these ranks. The increases in the individual concentrations and relative proportions of alkylphenols with methyl and/or isopropyl substituent(s) in the *ortho* and *para* positions in the subbituminous coal pyrolysate were attributed to electrophilic methylation and isopropylation reactions occurring to lignin structures in coals during coalification. The dominance of *ortho* and *para* substituted methylphenols in coaly Group 3A crude oils which were also observed in the hydrous pyrolysates of coals suggests that the altered lignin structures in coals may be precursors of some of these petroleum methylphenols.

Methylation, isopropylation and *sec*-butylation are proposed as geosynthetic processes to account for the alkylphenol compositions of crude oils with phenol distributions dominated by *ortho* and *para* alkyl-substituted compounds (Group 3). Many crude oils show high relative abundances of *ortho* and *para* substituted C_1-C_5 alkylphenol isomers and some were also enriched in C_3-C_5 alkylphenols compared to kerogen pyrolysates. Because the alkylphenol products obtained from

the laboratory alkylation of cresols have distributions which closely resemble those in these crude oils, it is proposed that similar alkylation processes occur in source rocks. Alkylation ratios reflecting the degree of methylation, isopropylation and *sec*-butylation, which were based on the relative abundance of the dominant alkylation products compared to their likely precursor *ortho* cresol, indicate that high levels of methylation occurred in crude oils over a wide range of maturities. In contrast, high levels of isopropylation and *sec*-butylation were observed only in mature samples. Selective dissolution of phenol isomers in crude oils by water contact was discounted as an explanation for the observed phenol distributions based on the relative distribution coefficients of phenols between isooctane and water.

The alkylphenol compositions of the remaining crude oils appear to be produced from alteration processes occurring in the subsurface; these include oxidation and biodegradation processes. Oxidation of alkylphenols is proposed to account for the alkylphenol compositions of Group 4 crude oils. Because *ortho* and *para* substituted alkylphenols are more susceptible to oxidation than their *meta* substituted counterparts, the selective removal of these compounds via an oxidation process is suggested as an explanation for the high relative abundances of *meta* substituted isomers observed in these crude oils. A natural product origin for these compounds appears unlikely due to the lack of known natural products which could give rise to *meta* substituted alkylphenols with isopropyl substituents, and the diverse range of organisms required to give rise to the *meta* substituted phenols with *n*-alkyl substituents observed in Group 4 samples.

Crude oils from two Australian basins which have undergone various levels of biodegradation were analysed to assess biodegradation effects on petroleum alkylphenol compositions. Alkylphenols could not be detected in the moderately to severely biodegraded crude oils (Group 5) whereas related non-biodegraded samples contained relatively higher levels of alkylphenols. The very low levels of

phenols in the biodegraded samples (<10 ppb) suggests that phenols are depleted via processes that occur during biodegradation. Because water washing often co-occurs with biodegradation, the very low levels of alkylphenols in these samples may also be due in part to the removal of these polar components via water dissolution.

CHAPTER 1

INTRODUCTION

1.1 JUSTIFICATION FOR THIS STUDY

Phenols are abundant in sedimentary organic matter due to their widespread occurrence in natural products (Sections 1.2) and their capacity to resist microbial degradation. They make an important contribution to the formation of both terrestrial and marine derived humus during diagenesis (refer to Section 1.3), and consequently, phenolic moieties are abundant in kerogen and occur in relatively high concentrations in many kerogen pyrolysates such as coal tars, coal-derived liquids and shale oils (Section 1.4.1). The incorporation of phenolic natural products into the kerogen of petroleum source rocks suggests that the crude oils derived from them may contain phenolic biomarkers.

Phenols remain largely unexploited as a source of geochemical information. This is probably due to the low concentrations of alkylphenols in crude oils (ppb) making their analysis difficult. This study involved the development of analytical procedures for the analysis of petroleum phenols, and a detailed investigation of their geochemistry, particularly in terms of their origins. In addition, alkylphenols have physical and chemical properties which may enable them to be used for determining the nature of a variety of processes occurring in the sedimentary environment. For example, their high polarity relative to hydrocarbon components of petroleum makes them more susceptible to mineral adsorption and water dissolution effects (Section 1.7), and their susceptibility towards electrophilic aromatic substitution reactions and oxidation may allow them to be used as indicators of such processes occurring in the subsurface.

1.2 PHENOLIC NATURAL PRODUCTS

1.2.1 Land Plants

Lignins

Lignins are high molecular weight polymeric substances based on three phenylpropane units (Figure 1.1) and occur in virtually all vascular plants (Wardrop, 1971). After cellulose, lignins are the most abundant organic compounds in nature (Sarkanen and Hergert, 1971; Wardrop, 1971). The structures of lignins in plants are determined mainly by the relative abundances of the basic units contributing to them. For example, gymnosperm lignins are mainly derived from guaiacyl units with a minor contribution of the *para*-hydroxyphenylpropane unit; while lignins from angiosperm woods and grasses are

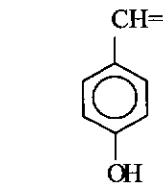
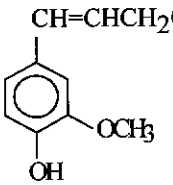
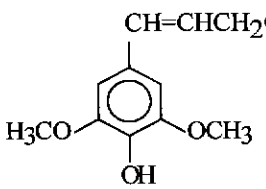
	Gymnosperms (Softwoods)	Angiosperms (Hardwoods)	Grasses
 *p*-hydroxy-phenylpropane	minor component	minor component	minor component
 guaiacyl	major component	significant component	significant component
 syringyl	very seldom observed	significant component	significant component

Figure 1.1 Lignin monomer units and their occurrence in plants.

comprised of these two units as well as the syringyl unit (Figure 1.1) (Sarkanen and Hergert, 1971; Kirk and Farrell, 1987; Stafford, 1988). Some exceptional gymnosperm species from the *Podocarpacaeae* family also contain syringyl units (Sarkanen and Hergert, 1971). Structural models for softwood lignins (guaiacyl lignin) and hardwood lignins (guaiacyl-syringyl lignin) are shown in Figure 1.2 and Figure 1.3 respectively. Lignins occur in some primitive plant groups such as ferns (Sarkanen and Hergert, 1971; Stafford, 1988), however, they are absent from most mosses and plants of lower taxonomic rank (Sarkanen and Hergert, 1971). Lignin-like compounds have been isolated from some fungi which contained *p*-hydroxy-phenylpropane and syringyl units (Towers, 1969; Turner, 1971).

Lignins are formed via random free-radical copolymerisation and therefore are highly complex, heterogenous, cross-linked biopolymers (Isherwood, 1965; Goodwin and Mercer, 1972; Alder, 1977; Kirk and Farrell, 1987; Stafford, 1988). Over ten phenylpropanoid linkages have been identified in lignins. Alkylaryl ether bonds dominate, particularly the β-O-4 type which accounts for 50-60% of the interunit links in most lignins (Figure 1.2, refer to inset for monomer numbering). Other major bonds recognised in lignins include aryl-aryl and alkyl-aryl carbon-carbon bonds.

Lignins units are commonly characterised on the basis of products from oxidative degradation or pyrolysis (Sarkanen and Hergert, 1971; Hedges *et al.*, 1988). Figure 1.4 shows the structures of the most common cupric oxide oxidation products of lignins (Hedges and Parker, 1976; Hedges and Ertel, 1982; Ishiwatari and Uzaki, 1987; Hedges *et al.*, 1988; Meyers and Ishiwatari, 1993). These products are formed from lignin moieties which have been oxidised at the propyl side chain to yield hydroxy and hydroxy-methoxy substituted aldehydes, ketones, carboxylic acids and cinnamyl phenols. The pyrolysis products of lignins are guaiacyl- and syringyl- derived phenols which are formed from the cleavage of

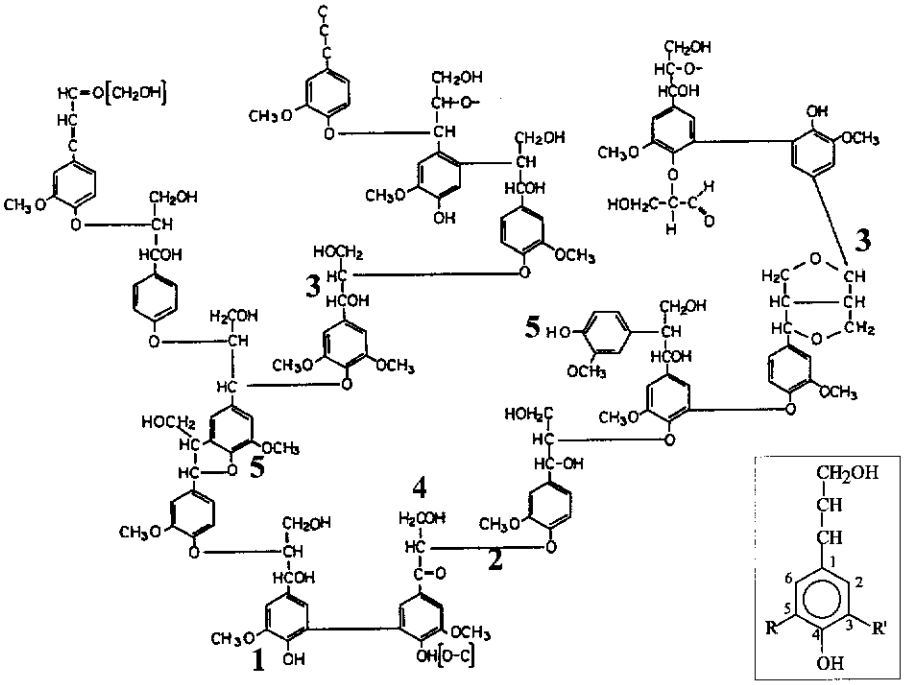


Figure 1.2 Structural model of spruce lignin (Alder, 1977). This guaiacyl lignin is typical of softwoods. Numbers 1-5 show the positions at which molecular transformations occur to give rise to the lignin structures shown in the brown coal (Figure 1.6) and subbituminous coal (Figure 1.7) models.

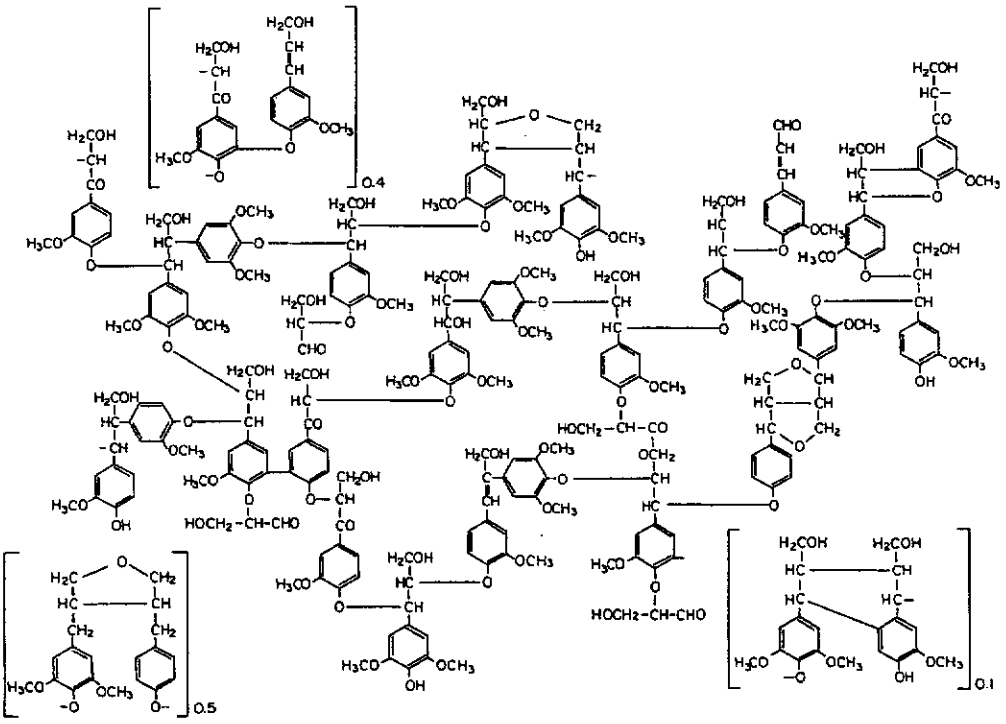


Figure 1.3 Structural model of beech lignin (Nimz, 1974). This guaiacyl-syringyl lignin is typical of hardwoods.

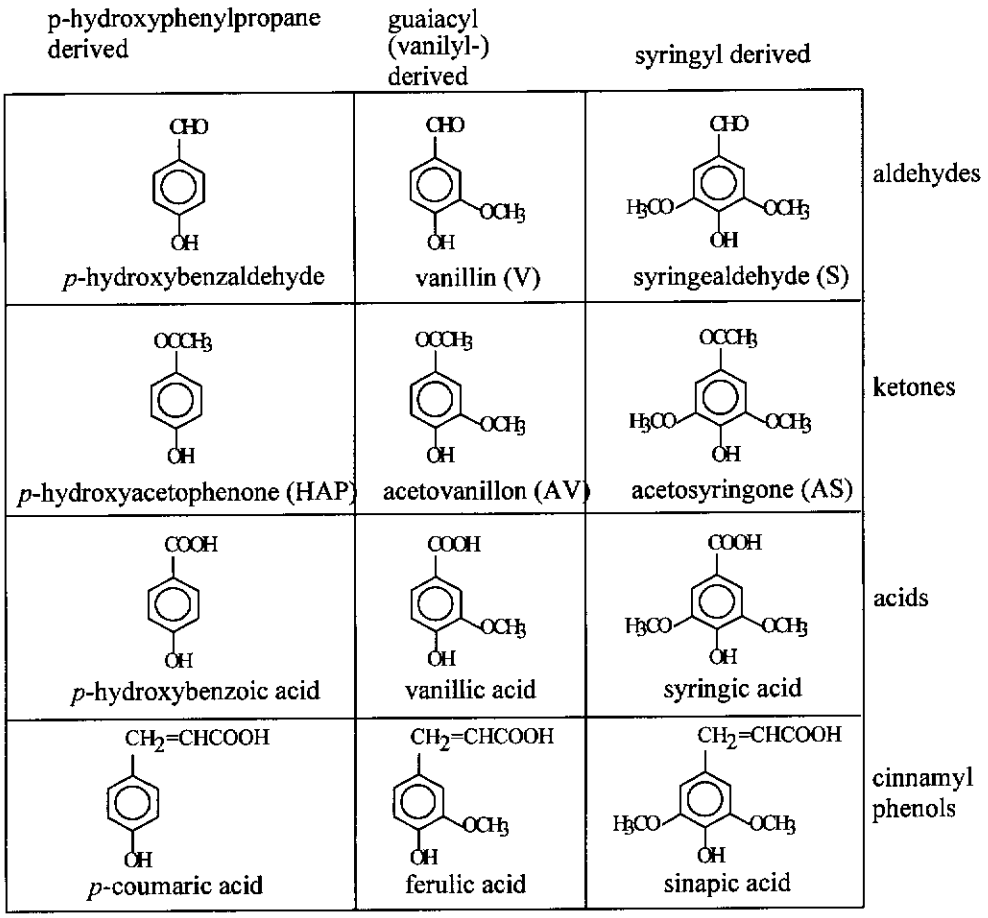


Figure 1.4 Cupric oxide oxidation products of lignins.

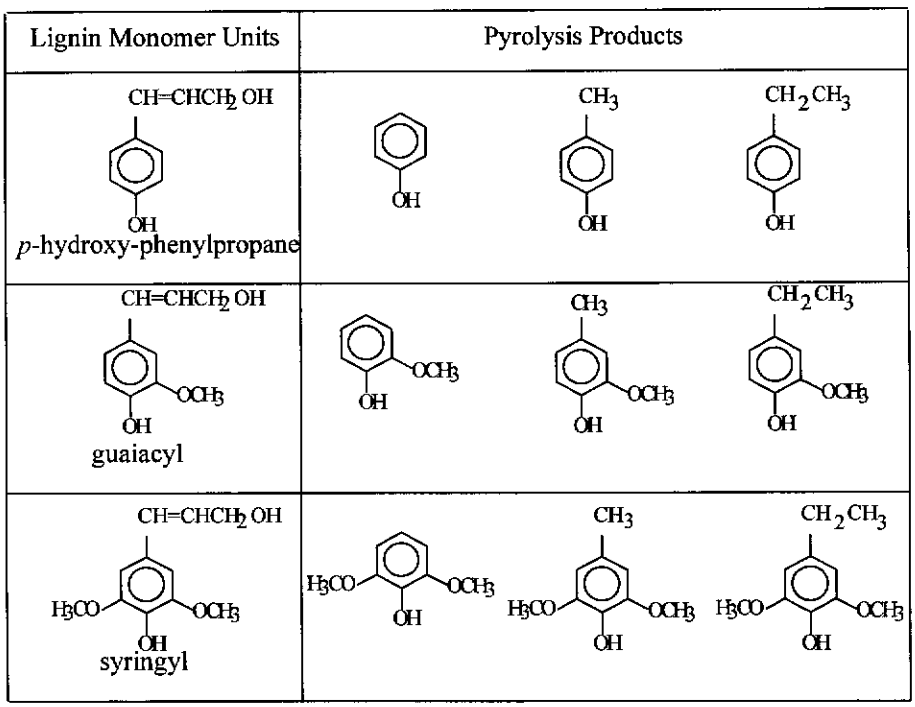


Figure 1.5 Common pyrolysis products of lignins, and their likely lignin precursor moieties.

lignin units at the propyl side chain (Figure 1.5). For example, common pyrolysis products of the guaiacyl unit are reported to be guaiacol (2-methoxyphenol) and 4-alkylguaiacol compounds; and 2,6-dimethoxyphenol, 4-methyl-2,6-dimethoxyphenols and 4-ethyl-2,6-dimethoxyphenols are common pyrolysis products of the syringyl unit (Saiz-Jimenez and de Leeuw, 1986a; Hatcher *et al.*, 1989b). Careful examination of either the oxidative degradation or pyrolysis products of a sample therefore enables the original composition of the lignin to be inferred. Furthermore, because lignins are unique to vascular plants the yield of lignin degradation products from marine sediments has been used to indicate the input of terrestrial-derived organic matter in near shore and deep sea sediments (Gardner and Menzels, 1974; Hedges and Parker, 1976; Hedges and Mann, 1979; Gagosian and Peltzer, 1986; Gough *et al.*, 1993).

Lignins are very resistant to physical and chemical degradation and relatively resistant to microbial degradation. Extensive lignin biodegradation can only occur in aerobic environments (Kirk and Farrell, 1987). White-rot fungi are most efficient in degrading lignins; some can completely degrade the polymer while others can modify the chemical structure of lignins but are unable to cleave the interunit linkages (Alexander, 1977; Kirk and Farrell, 1987). Some aerobic bacteria can also degrade lignin to a small extent (Alexander, 1977; Kirk and Farrell, 1987).

Due to their relatively high resistance to degradation, lignins survive through diagenetic processes (Saiz-Jimenez and de Leeuw, 1986b; Hatcher and Spiker, 1988; de Leeuw and Largeau, 1993). Consequently lignin derivatives have been reported in soils (Ertel and Hedges, 1984), humic substances (Given *et al.*, 1984; Hatcher and Clifford, 1994), Recent sediments (Given *et al.*, 1984; Hedges *et al.*, 1988), buried wood (Sigleo, 1978; Attalla *et al.*, 1988; Bates and Hatcher, 1989; Hatcher *et al.*, 1989c), coals (e.g. Hatcher *et al.*, 1989c) and kerogens (Ishiwatari

and Uzaki, 1987). Furthermore, lignins are considered the major precursor of the vitrinite maceral (Teichmüller and Teichmüller, 1979; Stach *et al.*, 1982). Because lignins are very abundant in nature and appear to be one of the most important sources of sedimentary organic matter, a great deal of work has been carried out on the diagenesis of lignins. The molecular alterations lignins undergo during diagenesis and coalification are particularly important since the molecular structures in mature coals, which may be precursors to petroleum phenols, are very different from those in the source lignins. These changes have been discussed below as they are pertinent to later discussions.

Molecular Alterations of Lignins During Coalification

Molecular transformation of lignin methoxyphenols up to the subbituminous stage of coalification (R_O=0.6) is well established because coalified tissue up to this maturity level can be morphologically related to woody xylem tissue. The transformations which occur during diagenesis of the xylem tissue are summarised below.

1) Demethylation of guaiacyl and syringyl units to form catechol-like structures that are the dominant components of brown coal and lignite (Botto, 1987; Stout *et al.*, 1988; Hatcher *et al.*, 1989a; 1989b).

2) Cleavage of β-O-4-aryl ether bonds to form phenols and reactive carbocations that alkylate the catechol rings (Botto, 1987; Hatcher, 1990).

3) Oxidation of the propyl side chain of lignin monomers giving rise to carbonyl and carboxyl groups (Hatcher, 1990).

4) Dehydroxylation of the propyl side chain (Hatcher, 1990).

5) Dehydration (or dehydroxylation) of the catechol-like structures to form a cross-linked structure dominated by alkylphenols (Hatcher *et al.*, 1989b; 1989c; Hatcher, 1990; Ohta and Venkatesan, 1992).

Based on these transformations, structural models have been developed for lignin from gymnosperm wood of brown coal rank (Figure 1.6, Hatcher, 1990) and subbituminous rank (Figure 1.7, Hatcher *et al.*, 1992). These models were based on spruce lignin (Figure 1.2) and therefore a careful comparison with spruce lignin shows the molecular changes occurring in lignins of brown coal and subbituminous coal. Numbers 1-5, which correspond to the changes listed above, have been included in Figure 1.2 and Figures 1.6-1.7 to facilitate such a comparison.

Molecular transformations of lignin methoxyphenols in high-volatile bituminous and higher rank coals are very severe. In coals of this rank, the coalified tissue can no longer be morphologically related to woody xylem tissue and the lignin methoxyphenols or their demethylated products (dihydroxybenzenes) are not dominant components in the coal pyrolysates. Alkylphenols are the dominant pyrolysis products of bituminous coals and account for up to 40% of the pyrolysate (Senftle *et al.*, 1986; Nip *et al.*, 1988; Hatcher *et al.*, 1992). The chemical changes reported to occur at the early bituminous stage are outlined below (Hatcher *et al.*, 1992):

1) Formation of diaryl ethers, dibenzofurans and dibenzopyrans via the condensation of phenols (cleavage of such structures results in the increased amount of benzene and alkylbenzenes compared to alkylphenols seen in the pyrolysates of higher rank coals).

2) The inclusion of naphthalene by cyclization of a four-carbon side chain with an attached aromatic structure.

3) The most significant change is the rapid decrease in oxygen content with a corresponding increase in carbon content. The total number of oxygen functional groups is reduced.

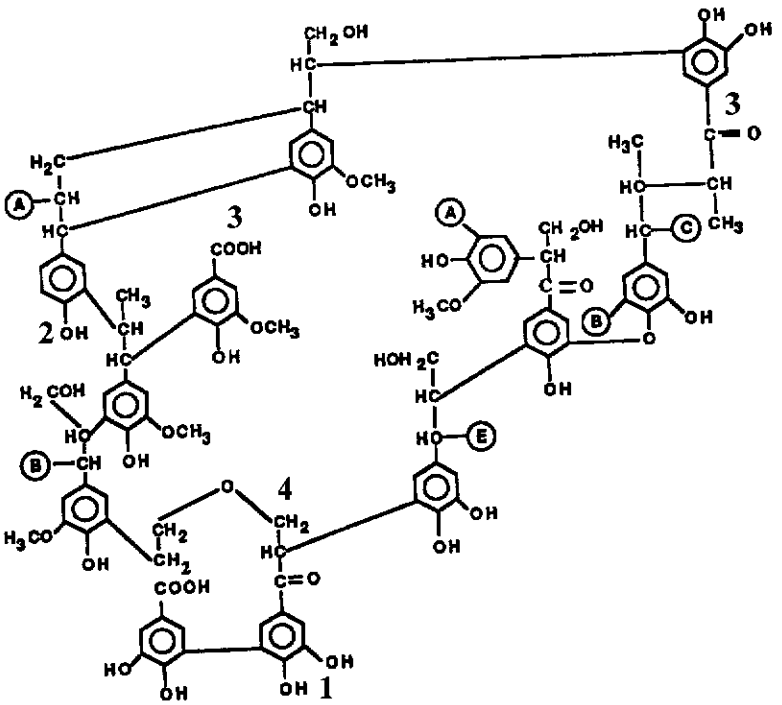


Figure 1.6 Structural model for gymnosperm wood of brown coal rank (*after* Hatcher, 1990). The circled letters indicate cross-link sites to other circled letters. Numbers 1-4 correspond to transformations described in the text.

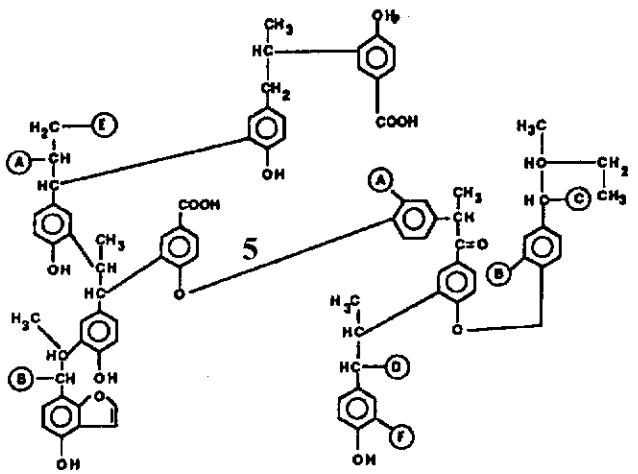


Figure 1.7 Structural model for subbituminous coalified wood (*after* Hatcher *et al.*, 1992). The circled letters indicate cross-link sites to other circled letters. Sites C and D are cross-links to other coal macromolecules. 5 shows the cross-linking formed by the dehydration of catechol-like structures resulting in a polymer dominated by alkylphenols (see text).

Tannins

Tannins are high molecular weight polyphenolic compounds that occur in higher plants and in some algae. Tannins are classified into three groups: 1) proanthocyanidins (previously known as condensed tannins), 2) hydrolysable tannins and 3) phlorotannins. Phlorotannins which are restricted to brown macroalgae will be discussed in Section 1.2.1.

Proanthocyanidins are high molecular weight polymers formed from the condensation of a range of flavan units. The most common flavan monomers in proanthocyanidins are those based on flavan-3-ols (cyanadins) which differ mainly in the hydroxylation pattern of the B-ring (Figure 1.8) (Haslam, 1989). The most widely distributed members of flavan-3-ols in nature are catechin and epicatechin (Figure 1.8) however, many more are known (Haslam, 1989). Some tannins are exclusively based on one flavan monomer but usually there is only a predominance of one unit. These biopolymers are generally made up of flavan units in which C-4 of the heterocyclic ring is linked to C-8 of the adjacent unit (Figure 1.8). A limited number of flavan units (1-4%) are also linked via C-6. The molecular weight of proanthocyanidins can be as high as 20000 (Haslam, 1989). Due to the number of different flavan building units and to the number of different links which may be present, a very large variety of proanthocyanidin structures is observed in nature (Ribereau-Gayon, 1972; Haslam, 1989). Proanthocyanidins are widely distributed in the plant kingdom (Goodwin and Mercer, 1972; Haslam, 1989). They are probably universal in the major groups of gymnosperms and are widespread among woody angiosperms (Foo and Porter, 1980; Shen *et al.*, 1986; Stafford, 1988). They are rare in nonwoody angiosperm families representing both aquatic and herbaceous plants and have not been reported in primitive vascular plants.

Hydrolysable tannins are polyesters of sugars and phenolic acids and are classified into two groups: gallotannins and ellagitannins. The gallotannins contain gallic acid as the phenolic acid, whereas ellagitannins contain hexahydroxydiphenic acid as the major acid constituent (Figure 1.9). D-Glucopyranose is the most common sugar core in hydrolysable tannins (Goodwin and Mercer, 1972; Nishizawa *et al.*, 1985; Tanaka *et al.*, 1985), although other monosaccharides and polyalchohol cores also occur (Ishimaru *et al.*, 1987; Sun *et al.*, 1988). Like proanthocyanidins, hydrolysable tannins are widely distributed in the plant kingdom (Goodwin and Mercer, 1972).

Proanthocyanidins and hydrolysable tannins have different preservation potentials in sediments. Proanthocyanidins demonstrate high chemical stability and exhibit antimicrobial properties (Porter and Woodruffe, 1984; Stafford, 1988), and therefore tend to accumulate in dead or dying cells (Goodwin and Mercer, 1972). Wilson and Hatcher (1988) showed that tannins are selectively preserved in bark during coalification to the brown coal stage. The high preservation potential of proanthocyanidins, and their abundance in higher plants suggests that they may provide a substantial contribution to land-derived sedimentary organic matter (de Leeuw and Largeau, 1993). Stach *et al.* (1982) have suggested that these tannins may also be significant precursors of the vitrinite maceral. Hydrolysable tannins however, are considered to have low preservation potential since they can be enzymatically degraded by a large number of esterases (de Leeuw and Largeau, 1993).

Sporopollenins

The chemically resilient material that comprises the outer cell walls (exines) of spores and pollen grains is known as sporopollenin. Although early reports suggested that sporopollenins were formed from carotenoids and/or

Figure 1.8 Common flavan monomer units in proanthocyanidins. Circles represent macromolecules.

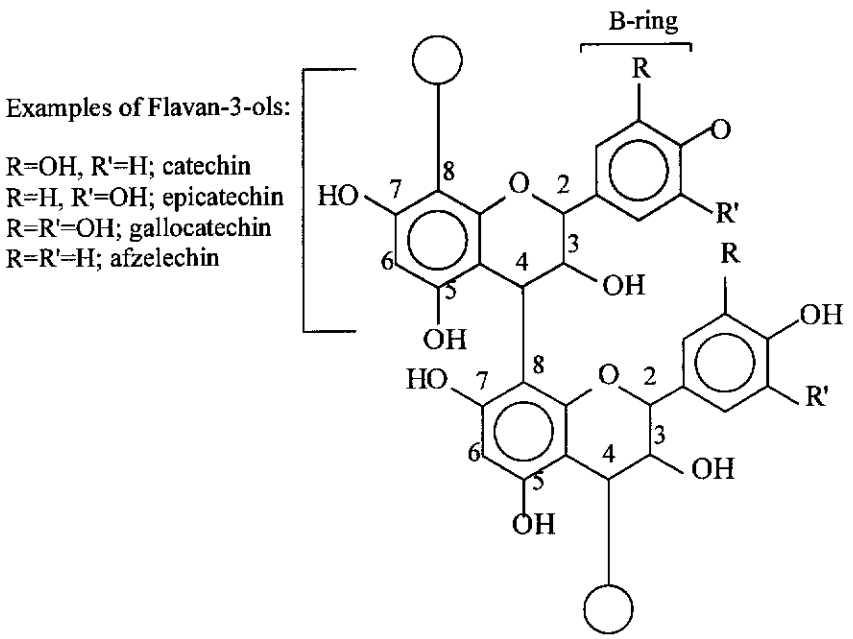
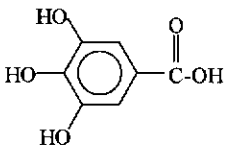
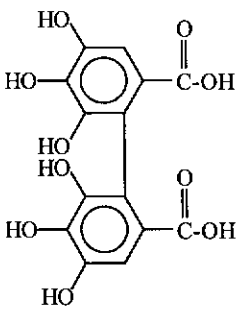


Figure 1.9 Polyhydroxy monomeric units in hydrolyzable tannins.



Gallic acid



Hexahydroxydiphenic acid

carotenoid esters (Brooks and Shaw, 1968; 1978), more recent reports have indicated that carotenoid compounds are not major components of vascular plant sporopollenins (Schenck *et al.*, 1981; Prahl *et al.*, 1985; 1986; Schulze Osthoff and Wiermann, 1987; Guilford *et al.*, 1988). Sporopollenins are thought to be derived mainly from either phenylpropanoid units (Prahl *et al.*, 1986; Schulze Osthoff and Wiermann, 1987) or from long *n*-alkyl chains possibly derived from fatty acids (Guilford *et al.*, 1988).

Sporopollenins are widespread substances since they are present in lower plants (spore exines) and in angiosperms and gymnosperms (pollen exines). Sporopollenin levels generally range from 1.4% to 28% of the total dry weight of the spores and pollens (Brooks, 1971; Brooks and Shaw, 1972), with very high levels of 45% reported to occur in the spores of some ferns (Toia *et al.*, 1985).

Phenolic compounds are produced by oxidative degradation and pyrolysis of sporopollenins. Schultze Osthoff and Wiermann (1987) reported that a large amount of phenols was obtained after nitrobenzene oxidation of *Pinus* pollen, indicating that phenols play an important role as integrated substances in this sporopollenin skeleton. Some of the oxidation degradation products were *p*-coumaric acid, *p*-hydroxybenzoic acid, vanillic acid, vanillin, *p*-hydroxybenzaldehyde and ferulic acid. Phenolic pyrolysis products of sporopollenins, and thermally and chemically altered sporopollenins include phenol, catechol, *p*-vinylphenol and guaiacol (Schenck *et al.*, 1981) and monohydroxy and dihydroxy phenolic acids (Hayatsu *et al.*, 1988).

The rates of degradation of sporopollenin species vary considerably. Under oxic conditions in soils, some spores and pollens entirely degrade within years, while the exines of other species are unaffected by bacterial and fungal attack and chemical oxidation (Havinga, 1971). A similar effect was observed in Recent sediments where some spores and pollens are degraded much more rapidly

than others (Faegri, 1971). This author also found that better preservation of sensitive exines occurred in sediments deposited under acidic conditions. Despite some variability in degradation rates, the highly resistant exines have been reported to occur morphologically intact in many samples (Shaw, 1970; Brooks and Shaw, 1972; Kovach and Dilcher, 1985), and in fact spore and pollen exines are the most widely occurring plant fossils (Brooks, 1971). Due to the high preservation potential of some sporopollenins, they are considered to be the main precursors of the maceral sporonite which often contains fossil exines (Brooks, 1971). Sporonite can account for up to 50% of sapropelic coals (Cooper and Murchison, 1971; Stach *et al.*, 1982) and is also widespread in kerogens with contributions of land-derived organic matter (Johnson, 1985).

Suberins

Suberins represent macromolecular polyesters occurring in vascular plants. Suberins are mainly present as wall components of cork cells and account for up to 30-35% of the dry weight of cork (O'Brian and Carr, 1970; Robards *et al.*, 1973; Espelie *et al.*, 1982). They are thought to play a role in the protection of vascular plants against infection of microorganisms and in preventing desiccation (Espelie *et al.*, 1980).

Although the detailed chemical structure of suberins is still unclear, they are thought to be polymers containing C_{16}-C_{24} fatty acids units (Holloway, 1984). Kolattukudy (1980) suggested that some esterified phenolic acids also occurred in suberins, however Holloway (1982) indicated that the presence of phenolic acids still needed to be established unequivocally. Figure 1.10 shows the working model of suberin tentatively proposed by Kolattukudy (1980).

Suberins appear to have limited preservation potential since they are depolymerised by extracellular enzymes from fungi (Kolattukudy, 1981). Suberins may however be preserved to some extent as some Recent sediments and peats have released series of compounds similar to the building blocks of suberins when hydrolysed with base (Cardoso *et al.*, 1977; Cardoso and Eglinton, 1983; ten Haven *et al.*, 1987a).

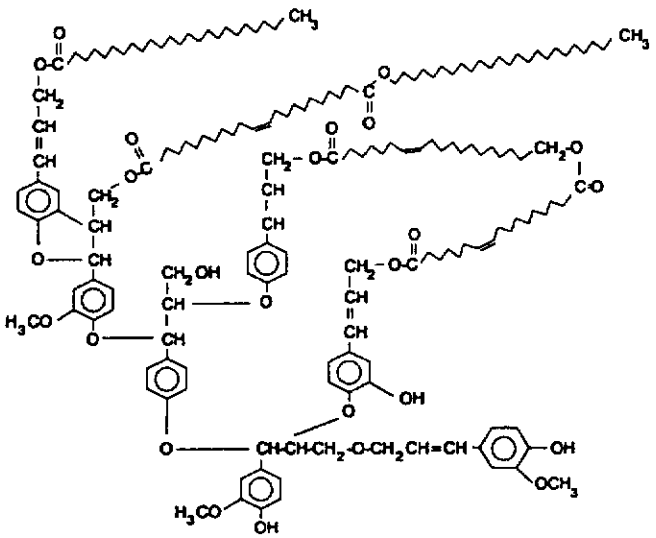


Figure 1.10 Proposed model of the intramolecular structure of suberins (Kolattukudy, 1980).

Tocopherols and Related Compounds

A range of compounds which are based on the 6-hydroxychroman skeleton and have long alkyl moieties are commonly occurring natural products (Figure 1.11). The most ubiquitous of these, the tocopherols (I), are naturally occurring antioxidants found in a large range of plant species which contain photosynthetic tissue such as land plants and algae (Threlfall and Whistance, 1971; Janiszowska and Pennock, 1976; Janiszowska and Rygier, 1985). Although tocopherols have been reported to occur in non-photosynthetic plants such as yeasts and fungi (Diplock *et al.*, 1961; Kubin and Fink, 1961), other authors such as Skinner and

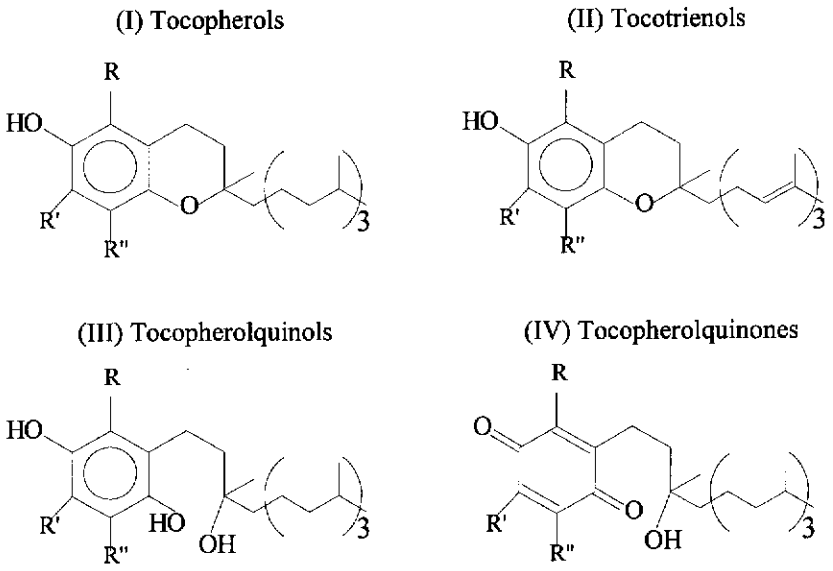


Figure 1.11 Tocopherols and structurally related natural products. R, R', R" = CH_3 and/or H.

Sturm (1968) and Janiszowska and Pennock (1976) were unable to detect tocopherols in a variety of yeasts and fungi, respectively. A structurally related group of unsaturated compounds, tocotrienols (II) have been reported to occur in higher plants and algae (Green, 1963; Threlfall and Whistance, 1971; Janiszowska and Pennock, 1976). Tocopherolquinols (III) and tocopherolquinones (IV), which can be obtained from the oxidation of tocopherols, occur in a range of yeasts and bacteria (Hughes and Tove, 1982) and higher plants and algae (Threlfall and Whistance, 1971; Janiszowska and Rygier, 1985, Newton *et al.*, 1977).

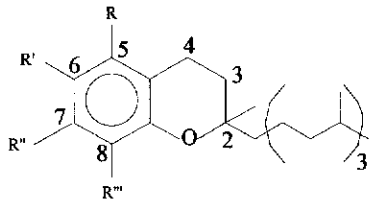
Despite tocopherols high susceptibility to oxidation, they appear to have some preservation potential as indicated by their occurrence in a range of Cretaceous and younger sediments (Brassell *et al.*, 1983; Brassell and Eglinton,

1986). α- and γ-Tocopherols occurred in highest relative abundance in all of the samples, and δ-tocopherol occurred in low relative abundance in a diatomaceous ooze from Walvis Ridge (Brassell and Eglinton, 1986). The abundance of tocopherols in natural products and their occurrence in a wide variety of sediment extracts suggests that tocopherols may be important constituents of kerogens.

Tocopherols have been reported to be precursors of sedimentary pristane. Goossens *et al.* (1984) reported that pristene was produced from the flash pyrolysis and thermal degradation of α-tocopherol. These results, together with the observation that tocopherols occur in many sediments, led these authors to suggest that tocopheryl moieties can be important sources of the pristane found in sedimentary rocks and crude oils.

Methyltrimethyltridecylchromans (MTTCs)

A range of mono-, di- and tri- methyl-2-methyl-2-(4,8,12-trimethyltridecyl)chromans (I) has been reported in a number of sediment extracts and crude oils (Sinninghe Damsté *et al.*, 1987; 1993; ten Haven *et al.*, 1990). Although structurally related to tocopherols, a methyl substituent at position six of some methyl-MTTCs indicates that their direct derivation from tocopherols is unlikely and therefore a direct biosynthetic origin was proposed (Sinninghe Damsté *et al.*, 1987). Recently, Li *et al.* (1995) suggested that MTTCs may be formed during diagenesis from condensation reactions between chlorophyll and alkylphenols. The distribution of MTTCs in sediments and crude oils appears to be dependent on the original environment of deposition and might be used to assess the occurrence of palaeohypersalinity (Sinninghe Damsté *et al.*, 1987).



R, R', R", R'" = CH_3 or H

(I)

Monoterpenoid Phenols in Essential Oils

The monoterpenoids are a large class of compounds which are important components of the essential oils obtained from conifers and angiosperms (Dev, 1989). Thymol (I) and carvacrol (II) (Figure 1.12) are monoterpenoid phenols which are common components of plant-derived essential oils (Albers, 1942; Guenther, 1966). They are the main constituents of several oils derived from species of the *Labiate* family (angiosperm) and occur in various other oils (e.g. from species of *Ocimum*) as significant components (Guenther, 1966). Several wood oils of angiosperms and conifers also contain carvacrol (e.g. oil of *Callitris quadrivalis*), thymol (e.g. Colombo root oil), several derivatives of thymol (e.g. plants from the *Compositae* family) and as much as 96% carvacrol methyl ether (e.g. *Cupressus sempervirens*) (Dev, 1989). Bohlmann *et al.* (1979) and Dinge *et al.* (1981) reported the isolation and structural elucidation of a third monoterpenoid phenol, 2-isopropyl-4-methylphenol (isothymol) from the plant species *Neurolaena oaxacane*.

In addition to thymol and carvacrol, there are several structurally related non-aromatic monoterpenoid ketones which commonly occur in the essential oils of plants (de Mayo, 1959; Pinder, 1960). Of these menthone (III), pulegone (IV) and piperitone (V) have a substitution pattern similar to that of thymol, while carvone (VI) and carvomenthone (VII) are related to carvacrol (Figure 1.12). These compounds undergo aromatisation readily to give the monoterpenoid phenols; for example, carvone and carvomenthone are easily dehydrogenated to

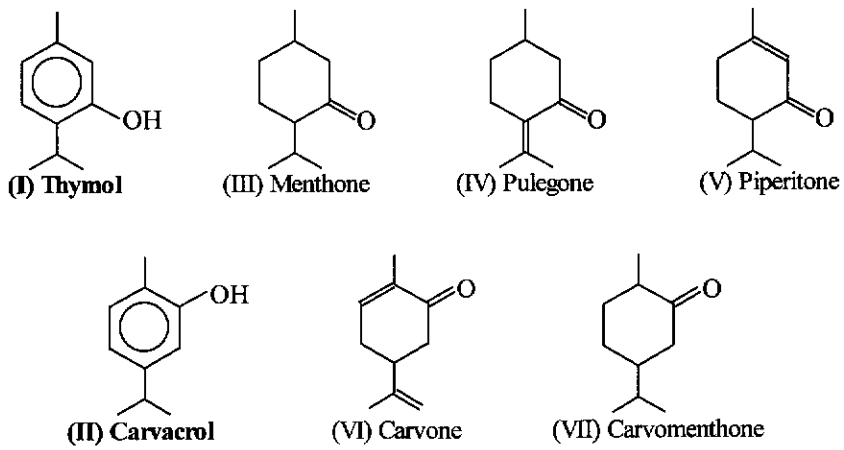


Figure 1.12 Monoterpenoid phenols and related ketones found in the essential oils of land plants.

give carvacrol when treated with acid, and piperitone and menthone can be aromatised to give thymol (Pinder, 1960). As aromatisation is a well known sedimentary process, these non-aromatic oxygenated monoterpenoids may be converted into their respective phenols in sedimentary rocks.

1.2.2 Aquatic Organisms and Microorganisms

Long Chain Alkylphenols Derived from *Botryococcus Braunii.*

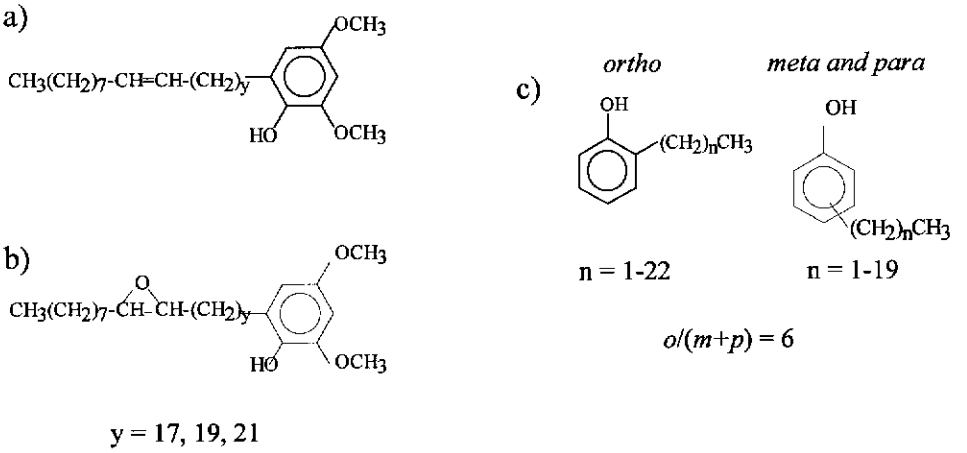
Botryococcus braunii is a ubiquitous, essentially freshwater, green microalgae; however, colonies have also been reported to occur in brackish (Temperley, 1936; Gilson, 1964) and saline (Masters, 1971) environments, and have been tentatively identified in hypersaline lakes (Playford, 1977; Bauld, 1981). Three distinct chemical races (A, B and L) have been recognised, and these are characterised mainly by the differences in their lipid compositions and

differences in the bulk chemical structure of the highly resilient, non-hydrolysable macromolecular material (PRB) which comprises the outer walls of each alga type (Berkaloff *et al.*, 1983; Kadouri *et al.*, 1988; Derenne *et al.*, 1989).

A range of phenolic lipids has been identified in the external lipids (i.e. lipids stored in outer walls) of almost all strains of *B. braunii* Race A studied to date (Metzger and Casadevall, 1989; Gelin *et al.*, 1994). Up to 1.6% of the dry weight of *B. braunii* Race A consists of a series of 6-*n*-alkenyl-2,4-dimethoxyphenols and their epoxide counterparts (Figure 1.13 a) and b) respectively. Phenols have also been generated upon pyrolysis of the PRB A isolated from *B. braunii* cultures (Sabelle *et al.*, 1993). These phenols included a series of *n*-alkylphenols (Figure 1.13 c), together with a much lower relative abundance of *n*-alkylphenols with methyl substituents. The relative abundances of phenolic compounds in the external lipids and pyrolysis products from PRB A of a cultured *B. braunii* colony was found to increase with the salinity of the growth medium (Sabelle *et al.*, 1993).

The phenolic lipids in PRB A appear to have good potential for surviving diagenesis. The PRB of *B. braunii* has been shown to be selectively preserved during diagenesis and thus to play a major role in the formation of lacustrine kerogens derived from *B. braunii* (Largeau *et al.*, 1984; 1986). The phenolic composition in the pyrolysate from a kerogen derived from *B. braunii* (Barigan valley Torbanite, Australia) was shown to be similar to that obtained from similar treatment of a PRB A isolated from *B. braunii* cultures (Sabelle *et al.*, 1993). Therefore the phenol moieties in PRB A also appear to be preserved during diagenesis and were incorporated into the kerogen of the Barigan Torbanite.

Figure 1.13 Phenols in *Botryococcus braunii* (Race A).

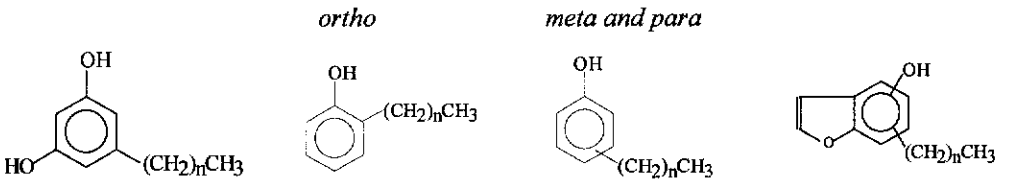


Long Chain Alkylphenols Derived from *Gloeocapsomorpha prisca*.

Gloeocapsomorpha prisca is an extinct marine microorganism whose fossil remains almost entirely constitute many Ordovician kerogens. The affinity of *G. prisca* with extant microorganisms has been suggested by many authors, and *G. prisca* has been considered a cyanobacterium (Klesment and Nappa, 1980; Foster *et al.*, 1990), an extinct green alga (Fowler and Douglas, 1984; Douglas *et al.*, 1991), a *Botryococcus*-type alga (Traverse, 1955; Monin *et al.*, 1980; Glikson *et al.*, 1989), and a planktonic and photosynthetic organism, possibly an eukaryotic lipid-rich alga (Hoffmann *et al.*, 1987). Recently Derenne *et al.* (1990; 1992) suggested that *G. prisca* is very likely to be related to *B. braunii* and was probably a single species of marine planktonic green microalga which had resistant thick walls and adapted to salinity variations which, in turn, controlled its polymorphism. These conclusions were mostly based on parallel morphological and chemical studies carried out on various *G. prisca* rich kerogens and cultured *B. braunii*.

Two distinct types of *G. prisca* colonies have been recognised, only one of which yields very high level of alkylphenols when pyrolysed. For example, the lack of alkylphenols in the pyrolysate of a Guttenberg Oil rock (USA) which almost entirely consists of *G. prisca* colonies, suggested that the alkylphenol content of kerogen pyrolysates can be used to characterise *G. prisca* deposits (Derenne *et al.*, 1992). These authors also noted that the morphology of the *G. prisca* colonies was very distinctive; therefore, based on their morphological and chemical features, two distinct "morpho/chemical" types of *G. prisca* were defined as a "closed/phenolic-rich" type and an "open/phenolic-poor" type. Kukersite, an extensive Estonian oil shale, is the most widely reported "closed/phenolic" rich kerogen derived from *G. prisca* and contains very high levels of alkylphenols (Lindenbeim, 1921; Klesment, 1974; Klesment and Nappa, 1980; Monin *et al.*, 1980). Phenolic compounds accounted for *ca.* 60% of the pyrolysate of the Estonian Kurkersite (Derenne *et al.*, 1990; 1992). Phenol, cresols, dimethylphenols, ethylphenols, naphthols and C_1-C_{10} *n*-alkylbenzenediols were some of the phenols tentatively identified in the base extracts of the Kukersite pyrolysates, however, the bulk of this fraction was not unequivocally identified (Klesment, 1974; Klesment and Nappa, 1980). More recent reports show the occurrence of a series of very long chain phenolic compounds in the pyrolysates of various Ordovician *G. prisca* deposits from Northern Europe and North America (Derenne *et al.*, 1990; 1992). These compounds were 5-*n*-alkyl-1,3-benzenediols, *n*-alkylphenols and *n*-alkylhydroxybenzofurans (Figure 1.14) together with lower relative abundances of methyl substituted derivatives and derivatives with unsaturation in the long alkyl chain. The series of 5-*n*-alkyl-1,3-benzenediols is the most abundant of all the phenolic compounds identified in "closed/phenolic-rich" *G. prisca* derived kerogen pyrolysates.

Figure 1.14 Phenols in the pyrolysate of kerogens rich in*Gloeocapsomorpha prisca*.



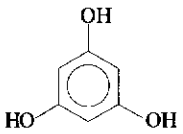
Phlorotannins

Phlorotannins are polymeric substances which consist of phloroglucinol units (Figure 1.15) linked via diaryl ether bridges (Ragan and Glombitza, 1986). They are formed by the dehydropolymerisation of the phloroglucinol monomers and occasionally some of the phloroglucinol units are linked directly via diaryl C-C bonds. Monohalogenated phlorotannins and polymers containing additional hydroxyl substitutes on the aromatic rings have also been identified (Koch and Gregson, 1984; Ragan and Glombitza, 1986). The number of units ranges from three to 13 in most identified phlorotannins, but higher molecular weight polymers have been reported (Grosse-Damhues and Glombitza, 1984; Koch and Gregson, 1984; Ragan and Glombitza, 1986). The occurrence of phlorotannins appears to be restricted to brown macroalgae in which they constitute 4-20% of the dry weight (Glombitza, 1977; Grosse-Damhues and Glombitza, 1984; Koch and Gregson, 1984; Ragan and Glombitza, 1986).

Phlorotannins might play a significant role in kerogen formation in aquatic depositional environments which have a high brown algae input (de Leeuw and Largeau, 1993). The large amounts of yellow-coloured products present in

inshore seawater are considered as possibly derived from tannins of brown algae (Hellebust, 1974). Although no study appears to have been carried out on their biodegradability, their chemical structure suggests that they are chemically stable and therefore they may be preserved during diagenesis (de Leeuw and Largeau, 1993).

Figure 1.15 Phloroglucinol - the major monomer unit in phlorotannins.



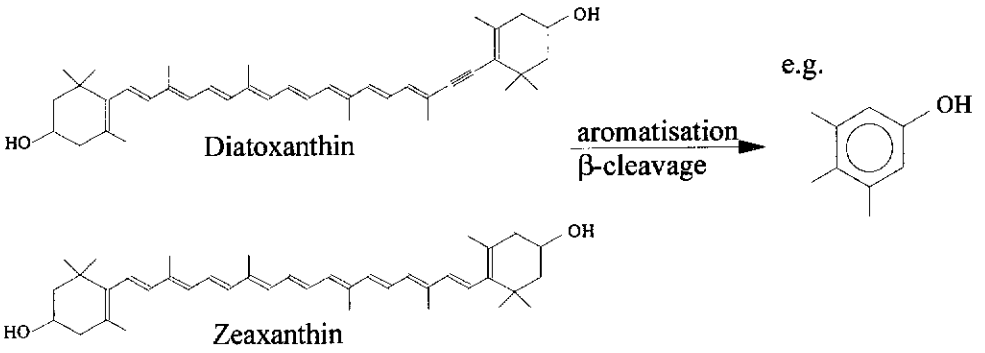
Tocopherols and Related Compounds

Tocopherols, tocotrienols, tocopherolquinols and tocopherolquinones (Figure 1.11) are reported to occur in algae, yeasts and bacteria. Because they also occur in many land plants a detailed discussion of their occurrence in natural products and sediments has already been provided in Section 1.2.1.

Hydroxy Carotenoids (Xanthophylls) as Precursors of Phenols

Carotenoids are yellow to red isoprenoid polyene pigments formally derived from lycopene (Liaaen-Jensen, 1978). They are present in all algae and in the marine environment carotenoids are present in bacteria, yeasts and fungi (Liaaen-Jensen, 1978). It has been estimated that up to 0.1% of the annual production of phytoplankton in the oceans (dry weight) occurs as carotenoids (Fox, 1974) and therefore carotenoids are abundant contributors to sedimentary organic matter in an aquatic environment.

Figure 1.16 Proposed formation of methylphenols from hydroxy carotenoids (xanthophylls) in diatoms and bacteria.



Hydroxy carotenoids are highly specific markers for algal contribution to sediments. For example, diatoxanthin (Figure 1.16) is a highly specific marker for diatoms (Brassell *et al.*, 1980; 1987; Brassell and Eglinton, 1986), and zeaxanthin (Figure 1.16) has been reported as a marker for cyanobacteria (Brassell *et al.*, 1983). Although they are highly specific markers, hydroxy carotenoids are labile compounds and therefore are of limited use as markers of the sources of organic matter in most sedimentary environments (Brassell and Eglinton, 1986; Brassell *et al.*, 1987). In post-glacial lacustrine sediments, the carotenoid record is often well preserved (Züllig, 1982), but in deep sea sediments the number and variety of the carotenoids identified is markedly reduced (Brassell *et al.*, 1983). Unaltered carotenoids have rarely been found in pre-Pliocene sediments (Brassell *et al.*, 1983). As a consequence the presence of their reported degradation products, loliolides and dihydroactinidiols (Klok *et al.*, 1984; Repeta, 1989), in sediments has been used to infer an origin from diatoms (Ten Haven *et al.*, 1987c).

Alkylphenols may also be diagenetic products of these labile hydroxy carotenoids. Aromatisation and β-cleavage, two well known sedimentary processes, of hydroxy carotenoids may yield 3,4,5-trimethylphenol and other methylphenols (Figure 1.16). A high relative abundance of these methylphenols

in crude oils may therefore be useful as markers for hydroxy carotenoid input into the source rocks.

Other Phenolic Natural Products

Other types of phenolic compounds have also been identified in natural products, however, many of these have been reported only in a limited number of organisms. Although not widespread these natural products may still be important contributors to sedimentary phenols, perhaps in certain depositional settings. A brief list of these compounds is given below.

Marine sponges contain a series of linear polyprenyl benzoquinols containing two, and four to eight isoprenic units (Figure 1.17) (Cimino *et al.*, 1972a; 1972b; 1975). The tetraprenyl-1,4-benzoquinol compound has been reported in high concentration (5% dry weight) in the marine sponge *Ircinia muscarum* (Cimino *et al.*, 1972b).

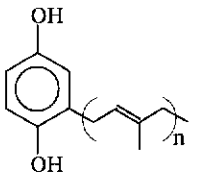
High levels of 5-*n*-alkylresorcinols with C_{19}, C_{21} and C_{23} alkyl-chains (Figure 1.18) have been reported in the aerobic soil bacteria *Azotobacter vinelandii* (Sadoff, 1975; Reusch and Sadoff, 1979) and *Azotobacter chroococcum* (Batrakov *et al.*, 1977).

n-Alkylphenols with the alkyl substituent containing an odd number of carbons ranging from 11 to 17 (Figure 1.19), and 3-n-undecyl-catechol were isolated from a liverwort (Asakawa *et al.*, 1987).

Several alkenyl resorcinols and a phloroglucinol have been reported to occur in the brown alga *Cystophora torulosa* (Moore, 1978).

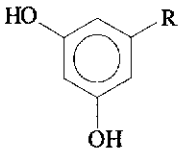
Phenolic compounds were observed in eight cyanobacteria (0.03-1% of the extract) and in the prokaryotic alga *Prochloron* (1.8%-7.1%), however their structures were not elucidated (Barclay *et al.*, 1987).

Figure 1.17 Phenols in marine sponges.



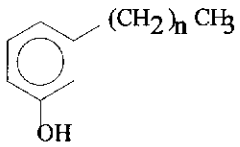
n = 2, 4-8

Figure 1.18 Phenols in aerobic soil bacteria.



R = $C_{19}H_{39}$
$C_{21}H_{43}$
$C_{23}H_{47}$

Figure 1.19 Phenols in liverwort.



n = 11, 13, 15, 17

1.3 HUMIFICATION OF PHENOLIC NATURAL PRODUCTS

The following brief account of humification has been provided to highlight the role of phenolic compounds in the formation of humus which is a precursor of kerogen.

The organic material of plants and animals deposited in sediments undergoes a variety of transformations broadly known as humification. Through a series of enzymic reactions mediated by a multitude of microorganisms or through chemical oxidation, the complex polymers of the biomass such as carbohydrates, proteins, lipids, lignin, tannins and polyphenols are transformed into proto-humus (Rashid, 1985; Stevenson, 1985). During the early aerobic parts of degradation the easily-utilized constituents such as proteins and simple sugars are rapidly consumed by microorganisms (Hollerbach and Dehmer, 1994). Below the surface layer degradation rapidly decreases owing to the lack of oxygen and in coexistence with the much slower degradation, the humification process begins. This involves the conversion of the remaining organic matter and the metabolites

of microorganisms into very complex polymers known as humus. The types of reactions involved include cleavage reactions, aromatisation, polymerisation and condensation. When deposition of organic matter derived from plants is massive compared to inorganic mineral contribution, peats, the precursors to coals, are formed and this process is usually referred to as peatification.

The transformations of phenolic natural products are important steps in the evolution of humus. Enzymatically mediated or chemically initiated oxidative coupling reactions of phenolic compounds allows for the formation of polymeric material which is a very important process occurring during humification (Hollerbach and Dehmer, 1994). Other reactions which phenols undergo are demethylation, decarboxylation, hydroxylation and dehydration (Rashid, 1985). Phenols and polyphenols are also converted into radical quinones which are highly reactive species and capable of reacting with a range of other compounds. Many microorganisms produce polyphenols and hydroxy acids (Kononova, 1966; Haider and Martin, 1967; Martin *et al.*, 1967; 1972; Grishina and Korotkov, 1978) and therefore the microbial biomass is also an important source of phenolic compounds incorporated in humus. Microbially-derived phenols may be particularly important in a marine depositional environment when phenolic natural products are less abundant (Rashid, 1985).

A detailed account of the complex reactions which phenolic compounds undergo during humification is beyond the scope of this study. What is to be stressed is that phenols are important components of humus since they are intimately involved in the formation of these substances and consequently they can be important contributors to the kerogens in petroleum source rocks.

1.4 PHENOLS IN SEDIMENTARY ORGANIC MATTER

1.4.1 Phenols in Kerogen Pyrolysates

Kerogens derived from both terrestrial and marine depositional environments have been reported to yield alkylphenols when pyrolysed. Alkylphenols are most abundant in terrestrially derived sedimentary rocks (especially coals) and are often the most prominent pyrolysis products (e.g. Senftle *et al.*, 1986). They are particularly abundant in vitrinite concentrates and can account for up to 50% of the total pyrolysate (Senftle *et al.*, 1986; Hartgers *et al.*, 1994). The major components in coal pyrolysates are C_0-C_2 alkylphenols (Senftle *et al.*, 1986; Hartgers *et al.*, 1994), with lower relative abundances of C_3 alkylphenols (Nip *et al.*, 1988; Hatcher *et al.*, 1992; Nip *et al.*, 1992) and C_4 alkylphenols (Hatcher *et al.*, 1992). Phenols occur in lower relative abundances in marine (mainly algal) kerogens than in terrestrial and mixed marine/terrestrial kerogen pyrolysates (Larter and Douglas, 1980; van de Meent *et al.*, 1980). Ordovician kerogens which consist almost entirely of accumulations of *G. prisca* yield high levels of long-chain-alkylphenols in their pyrolysates (refer to Section 1.2.2).

The relatively high concentrations of alkylphenols in synthetic fuels also demonstrates that phenols are important components of sedimentary organic matter. Coal-derived liquids (Parees and Zamzelski, 1982; McClennen *et al.*, 1983; Wood *et al.*, 1985; Green *et al.*, 1986) and shale oils (Hertz *et al.*, 1980; Bett *et al.*, 1983; Green *et al.*, 1986; Ekstrom *et al.*, 1987) obtained from the pyrolytic treatment of brown coal and oil shale respectively have been reported to contain high concentrations of hydroxyaromatic compounds. Individual phenolic components are present at 200-400 μg/g in shale oil and up to 30000 μg/g in coal-derived liquids (Hertz *et al.*, 1980; Guenther *et al.*, 1981). Alkylphenols,

alkylindanols, alkylnaphthols, alkyldihydronaphthols, alkyltetrahydronaphthols, alkylhydroxybenzenes and phenanthrols are amongst the phenolic classes reported to occur in synthetic fuels. Specific alkylphenols include the cresols, the six dimethylphenol isomers, 2,3,5-trimethylphenol, 2,3,6-trimethylphenol and 2,4,5-trimethylphenol (Guenther *et al.*, 1981).

A range of alkylphenols, alkylphenylphenols and alkylnaphthols have also been reported in automotive distillate fuel and light cycle oil, products derived from crude oil (Hazlett and Power, 1989). Because these phenols were observed in refined products there is the possibility that the phenols were reaction products of the refining process.

1.4.2 Crude Oils

Few reports appear in the literature on the identification and quantification of phenols in crude oils. Solely on the basis of mass spectral evidence Green *et al.* (1986) reported the occurrence of alkylphenols, indanols, tetralinols, naphthols and hydroxybiphenyls in a Wilmington, Californian petroleum. These authors also carried out a comparative study on the total concentrations of phenols in different types of fuels and concluded that not only does crude oil contain the lowest amount of total acids (<2.5% whole fuel) compared with coal liquids and shale oil, but hydroxyaromatics make up the least amount of the total acids (<0.1% whole fuel). MacCrehan and Brown-Thomas (1987) estimated the concentration of phenol and 2-methylphenol in a crude oil sample to be in the submicrogram/gram range. Recently, a range of C_0-C_3 alkylphenols have been identified in Californian crude oils using a modification of the isolation method developed for the present study (Macleod *et al.*, 1993; Taylor *et al.*, 1993). These authors showed that individual phenolic components occur in concentrations of up to 25000 ng/g in some Californian crude oils.

1.5 ANALYSIS OF PHENOLS IN HYDROCARBON MIXTURES

1.5.1 Enrichment Methods

Methods reported for the separation of phenol-rich fractions from petroleum and related hydrocarbon mixtures include anion-exchange chromatography, liquid chromatography and alkaline extraction.

Anion exchange techniques involve isolating the acid components by passing the hydrocarbon mixture through a resin bed, and removing the bonded compound classes according to their acidity (Jewell *et al.*, 1972; Green *et al.*, 1986). With a careful choice of eluents, this procedure offers good selectivity for the separation of phenols. In some cases, however, the phenolic fraction requires further enrichment using HPLC before GC analysis can be carried out (Green *et al.*, 1986). Ion exchange chromatography is reported to give good reproducibility and phenol recovery, however the analysis is time consuming (Jewell *et al.*, 1972).

Solid-liquid chromatography has been used to isolate phenols from synthetic fuels (Schweighardt *et al.*, 1976; Artz and Schweighardt, 1980; Later *et al.*, 1981; Shoup and Mayer, 1982; Marko-Varga and Barceló, 1992). These procedures usually involve the use of a thin plate or column of silica gel or alumina to separate components according to their polarity. The polar material is then re-chromatographed to obtain phenol rich fractions. With careful selection of eluting solvents this method allowed the phenolic components of synthetic fuels to be enriched to a level where they could be analysed directly by GC. A major disadvantage of this method is the difficulty in separating acidic components from certain other polar compounds such as basic nitrogen-containing compounds. Often these two compound classes elute very closely, and this causes interference during GC analysis. To overcome this problem HPLC is included as a further enrichment step for subfractionating the acids from other components, as HPLC

offers higher chromatographic resolution (Green *et al.*, 1986). In fact, a sequence of initial enrichment followed by HPLC subfractionation has been used with both ion-exchange chromatography and alkaline extraction as the initial enrichment step (MacCrehan and Brown-Thomas, 1987; Pauls *et al.*, 1990). This dual enrichment procedure was suggested to be especially suited to fuels which contain low levels of phenols (Green *et al.*, 1986). The major disadvantage of dual-enrichment procedures is the long time periods required to complete the separation.

Alkaline extraction methods allow rapid and efficient separation of phenols from hydrocarbon mixtures (MacCrehan and Brown-Thomas, 1987; Hazlett and Power, 1989; Pauls *et al.*, 1990). In these procedures the total acids are extracted into an alkaline aqueous methanol solution and then recovered by acidification and extraction with an organic solvent. The major disadvantage with this method is its lack of specificity as all acidic classes are extracted into basic solution. This can be overcome by further fractionating the acid isolate using HPLC as discussed above (e.g. MacCrehan and Brown-Thomas, 1987; Pauls *et al.*, 1990). In the present study the separation of phenols from the most abundant acidic class in crude oil, carboxylic acids, was effected by utilising the large differences in the acid dissociation constants (pKa) of phenols and carboxylic acids. Separation of these acid classes was achieved by buffering the aqueous extract to a pH at which the alkylphenols could be isolated by organic extraction whereas the acids remained in aqueous solution as their carboxylate salts (refer to Chapter 4).

1.5.2 Analysis of Phenol Rich Fractions Obtained From Crude Oil and Synthetic Fuel

The analysis of phenol rich fractions isolated from crude oils and synthetic fuels has been performed using gas (GC and GC-MS) and liquid chromatography (HPLC). Liquid chromatography coupled with various detectors, most commonly oxidative electrochemical detection, has been used for the analysis of phenol rich fractions (e.g. Kung-Jou Chao and Suatoni, 1982; MacCrehan and Brown-Thomas, 1987). Electrochemical detection is used to achieve the sensitivity required for the analysis of trace levels of phenols in crude oil (MacCrehan and Brown-Thomas, 1987). Separation of isomeric alkylphenols such as *m*- and *p*-cresols and the dimethylphenols could not be achieved using HPLC even when many combinations of solvents and columns were tried (Kung-Jou Chao and Suatoni, 1982; MacCrehan and Brown-Thomas, 1987). A major advantage, however, of liquid chromatography is that it is less sensitive to sample purity than GC.

Capillary GC is commonly used for the analysis of phenol isolates because it offers excellent separation of isomeric compounds such as *m*- and *p*-cresols and, 2,4-dimethylphenol and 2,5-dimethylphenol (e.g. Later *et al.*, 1981; Green *et al.*, 1986; Hazlett and Powers, 1989; Pauls *et al.*, 1990; This work). The major disadvantage of GC analysis is that it is sensitive to sample purity and therefore an isolate free of interfering components is required for GC analysis. This can easily be overcome for isolates containing low levels of interfering components by using specific ion monitoring in GC-MS analysis. The superior separation efficiency of capillary gas chromatography has led to its use in this study because separation of isomeric petroleum phenols was desired.

1.6 HYDROCARBON-WATER-ROCK INTERACTION EFFECTS ON ALKYLPHENOL COMPOSITIONS

Alkylphenols are an attractive compound class for study of hydrocarbon-water-rock interactions because of their high polarity and their ability to hydrogen bond. The presence of the hydroxyl group renders these organic compounds relatively water soluble and enables them to be adsorbed onto polar mineral surfaces and solid sedimentary organic matter such as kerogen and coal.

The nature of the effects of hydrocarbon-water partitioning on alkylphenol distributions has been determined for systems including heptane/water (Southworth *et al.*, 1983), octanol/water (Meylan and Howard, 1992) and crude oil/saline water (Taylor, 1994). Although the absolute values of the hydrocarbon/water distribution coefficients (K_D) varied with experimental conditions (e.g. temperature, pH, water salinity), it was noted that K_D increased with increasing molecular weight and steric hindrance of the hydroxyl group. These effects are demonstrated using Figure 1.20 which shows the K_D of alkylphenols between a North Sea crude oil and brine at 80°C (*after* Taylor, 1994). It can be seen that the more highly alkylated phenols have an increased affinity for the hydrocarbon phase. For example, the trimethylphenols have greater K_D values than the methylphenols. Figure 1.20 also demonstrates that the positions of the alkyl substituents affects the K_D values of compounds. In particular, *ortho* substituted alkylphenols (*) where the hydroxyl is hindered by adjacent alkyl groups show greater affinity for the hydrocarbon phase than their *meta* and *para* substituted counterparts. For example, 2,6-dimethylphenol (K_D = 25) compared with 3,5-dimethylphenol (K_D = 10). Similar trends in K_D values were determined in this study for C_0-C_4 alkylphenols in an isooctane/water system at 80°C (refer to Chapter 7; Section 7.6).

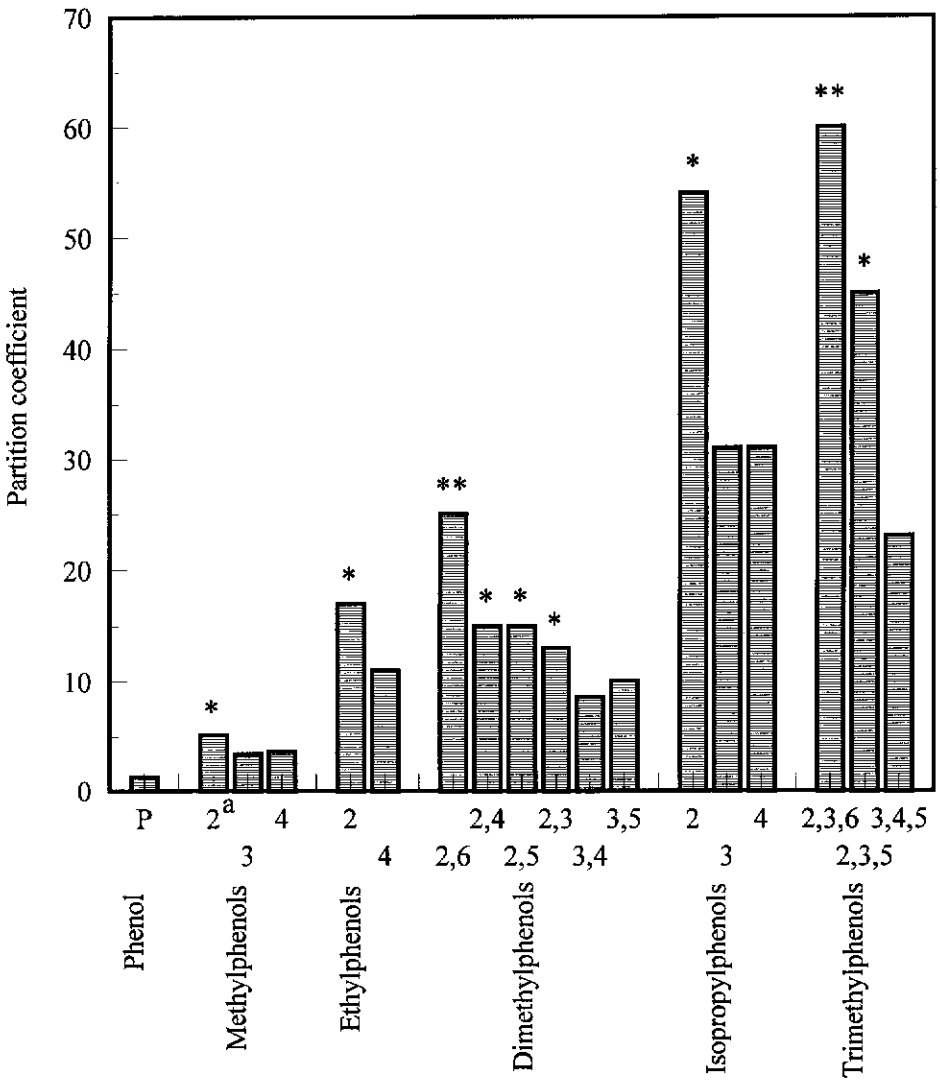


Figure 1.20 A histogram showing oil/water partition coefficients for North Sea oil A29/brine at 80°C and pH 7.0 (Taylor, 1994). * Denotes one *ortho* substituent. a Denotes position of alkyl substituent.

Adsorption of alkylphenols onto both inorganic mineral surfaces and solid sedimentary organic matter may occur from both a hydrocarbon or water phase. The nature of the adsorption effects from a hydrocarbon phase onto polar inorganic minerals can be approximated by the retention order of alkylphenols obtained during TLC (silica gel/benzene; Callmer *et al.*, 1977) and normal phase HPLC with various hydrocarbon mobile phases (Callmer *et al.*, 1977; Husain *et al.*, 1977). These authors showed that the extent of phenolic adsorption on the polar phases was controlled by the steric hindrance of the hydroxyl group. That is, *ortho* substituted phenols were less efficiently adsorbed than their non-hindered counterparts. This suggests that hindered alkylphenols would be relatively enriched in a hydrocarbon phase which is in contact with inorganic minerals. Interestingly the literature data showed that molecular weight did not have an effect on the degree of adsorption. Adsorption of alkylphenols onto minerals from a water phase in a water/mineral system (e.g. water/montmorillonite) appears to be negligible as bound water must be displaced in order for compounds to interact with the mineral surface (Zhang *et al.*, 1990; Taylor, 1994).

The organic matter content of a rock has a major effect upon the adsorption of alkylphenols from both water and hydrocarbon phases (Pepper, 1991; Sandvik *et al.*, 1992; Larter *et al.*, 1994; Taylor, 1994). Zhang *et al.* (1990) determined the partition coefficients for the adsorption of phenol from water onto montmorillonite to be 0.2 L/kg whereas the value is much higher, 148 L/kg, for phenol adsorption onto organic matter (Meylan and Howard, 1992). Larter *et al.* (1994) determined the distribution of 2,5-dimethylphenol between minerals and organic matter as a function of total organic carbon (Figure 1.21). This work showed that 2,5-dimethylphenol is preferentially adsorbed onto organic matter with the majority of it being adsorbed at TOC values above 2% (0.02). Taylor (1994) measured the adsorption of C_0-C_3 alkylphenols on brown coal from a

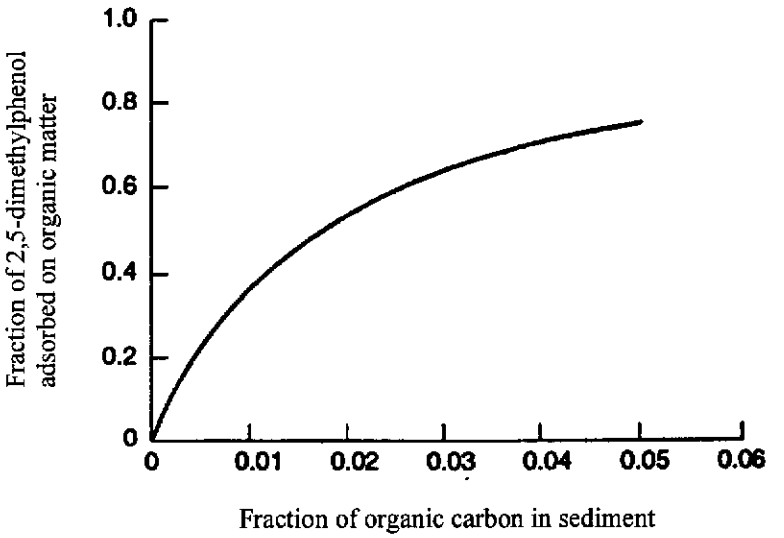


Figure 1.21 A graph showing fraction of 2,5-dimethylphenol adsorbed on organic matter as a function of sediment carbon content (*after* Larter *et al.*, 1994).

hexane/toluene phase and showed that non-hindered alkylphenols are selectively adsorbed. He suggested, however, that phenol and *meta* and *para*-cresols were more likely to be adsorbed by organic matter in the sedimentary environment where alkylphenols must compete for sorption sites with other polar species.

Since alkylphenols are readily partitioned into water and adsorbed by mineral surfaces and solid sedimentary organic matter, the potential exists for petroleum alkylphenol distributions to be significantly modified via these mechanisms during migration. Strong evidence for this has been presented by Macleod *et al.* (1993) who showed that phenol and the cresols dominated the phenol distributions in oil field waters whereas associated oil phases contained higher relative abundances of dimethylphenols and trimethylphenols. Selective adsorption of petroleum components by organic matter in a sedimentary environment has also been suggested to be a major cause of the differences observed between migrated crude oils and bitumens extracted from mature source rocks (Sandvik *et al.*, 1992).

1.7 SCOPE OF THIS STUDY

The scope of this work is to analyse the trace levels of alkylphenols in crude oils in order to determine their geochemical significance. Previously, only a very limited range of alkylphenols has been analysed in a limited number of crude oils. In this study, procedures have been developed enabling phenol isolates to be obtained routinely and quantitatively from crude oils and rock pyrolysates in a form which gives interference-free capillary gas chromatograms. Crude oils (45) and sedimentary rock pyrolysates and extracts (six) from Australia, Indonesia and Canada have been analysed for their phenol compositions using these procedures. The crude oil samples represent a range of locations, ages, depositional environments, maturities, source rock types and levels of biodegradation.

The focus of this study are C_0-C_5 alkylphenol components which have been unequivocally identified and the majority quantitatively analysed. Although higher molecular weight alkylphenols and bicyclic phenols were also tentatively identified in some crude oils, the geochemistry of these compounds was not assessed due to low recoveries in the isolation step and/or lack of reference compounds for unequivocal identifications.

The geochemical significance of C_0-C_5 alkylphenols in crude oils is assessed, with an emphasis on determining the sources of petroleum alkylphenols particularly in terms of natural product precursors and geosynthetic origins. The effects of sedimentary processes such as equilibration, biodegradation/water washing and oxidation on petroleum alkylphenol compositions are also described.

CHAPTER 2

EXPERIMENTAL

2.1 MATERIALS AND REAGENTS

Solvents and Reagents

All solvents were purified by procedures outlined in Perrin *et al.* (1980). All common reagents used were AR grade. The purity of the solvents were checked by evaporating 100 mL of a solvent to a volume of 50-100 μL and analysing the concentrated sample for contaminants using GC (Section 2.3).

C-18 Sep-Pak Cartridge

Sep-Pak cartridges were obtained from Millipore Waters Associates.

Glassware

All glassware was heated at 600°C for 12 h before use to eliminate contamination from glassware.

Silica Gel

Silica gel (Merck, 70-230 mesh) was used for column chromatography. It was activated at 120°C for at least 24 h before use.

Aluminium Smectite

The smectite from a Wyoming bentonite was isolated by dispersing the clay in water and collecting the fraction with a particle size of less than 2 μm. The Wyoming bentonite was purchased from the Standard Chemical Company, Melbourne, Australia. The surface area of the bentonite was found to be 33.0 m^2g^{-1} by the B.E.T. method and its cation exchange capacity, determined titrimetrically, was found to be 67.7 meq/100 g (Fripiat and Van Olphen, 1979).

Aluminium smectite was prepared by treating the isolated smectite with aluminium chloride solution (1 N). The clay was washed free of excess ions by repeatedly centrifuging with deionised water, then dried and crushed and the particles less than 340 μm were collected using a sieve . Organic impurities were removed from the clay by extraction with dichloromethane/methanol (9:1) using an ultrasonic bath. The aluminium smectite was activated at 120°C for 48 h before use.

Molecular Sieves

ZSM-5 molecular sieves (CBV 2802, The PQ Corporation) were used for the separation of the branched and cyclic compounds from crude oil saturate fractions. The molecular sieves were activated at 120°C for at least 24 h before use.

Copper

Elemental copper powder (Ajax) was used to remove elemental sulphur from hydrocarbon mixtures. Before use the copper was activated by washing it with 2 M hydrochloric acid, and then sequentially rinsing it with deionised water, ethanol, acetone and *n*-pentane.

Phenol Reference Compounds

Phenols which were used as reference compounds were obtained from several commercial sources: Aldrich Chemical Company, Inc., Fluka Chemie (Switzerland), Sigma Chemicals and Tokyo Kasei Organic Chemicals. These standards have purities in excess of 90%.

2.2 SYNTHESIS OF ALKYLMETHYLPHENOLS

2-Isopropyl-6-methylphenol

2-Isopropyl-6-methylaniline was converted into 2-isopropyl-6-methylphenol via the diazonium salt. The IR-spectrum and proton NMR (PMR) spectrum were similar to those reported in the literature (Lamartine and Perrin, 1969; Childs and Hor, 1977).

2-Isopropyl-4-methylphenol

2-Isopropyl-4-methylphenol was prepared using the general procedure reported by Kornblum and Lurie (1959) in which phenoxide ions are C-alkylated in heterogeneous suspension. Briefly, a mixture of *p*-methylphenoxide (13.0 g, 0.10 mol) and isopropylbromide (13.5 g, 0.11 mol) in dry toluene (100 mL) was stirred at 70-75°C for 60 h under nitrogen. The product on extraction with aqueous sodium hydroxide (10%) followed by acidification, yielded a dark oil (7 g) which contained *p*-cresol and 2-isopropyl-4-methylphenol (3:2). 2-Isopropyl-4-methylphenol (mp 35-36°C) (1 g, 0.007 mol) was isolated on a silica gel column, eluting with 5-10% diethylether in petroleum spirit. The PMR spectrum was similar to that reported in the literature (Bassus *et al.*, 1974).

4-Isopropyl-2-methylphenol

4-Isopropyl-2-methylphenol was synthesised by formylating *p*-isopropylphenol (6.8 g, 0.05 mol) using stannic chloride, tributylamine and paraformaldehyde (Furniss *et al.*, 1989). The 2-hydroxy-5-isopropylbenzaldehyde (1.6 g, 0.01 mol) thus prepared was isolated on a silica gel column, eluting with 10% diethylether in petroleum spirit. After Clemmensen reduction in the absence of an organic solvent (Adams *et al.*, 1960) 4-isopropyl-2-methylphenol (1 g, 0.007 mol) was recovered

by ether extraction. The IR spectrum and PMR spectrum were similar to those reported in the literature (Lamartine and Perrin, 1969; Childs and Hor, 1977).

3-Isopropyl-4-methylphenol

3-Isopropyl-4-methylphenol was synthesised using the method reported by Bassus *et al.* (1974). Briefly, *p*-cresol (10 g, 0.09 mol) and isopropylalcohol (5.4 g, 0.09 mol) were condensed in the presence of aluminium chloride (18.7 g, 0.14 mol) to yield a mixture of isopropylmethylphenols and di-isopropylmethylphenols. 3-Isopropyl-4-methylphenol (mp 39-40°C) was isolated by radial chromatography on a silica gel plate PF_{254} using hexane-diethylether mixtures as eluents. The PMR spectrum was similar to that reported by Bassus *et al.* (1974).

2-Isopropyl-3-methylphenol

2-Isopropyl-3-methylphenol was synthesised using the procedure reported by Nasipuri and De Dalal (1975). Briefly, 2-isopropyl-3-methylcyclohex-2-eneone was obtained by alkylating Hagemann's ester with isopropyliodide and hydrolysing the product with alkali (Nasipuri *et al.*, 1966). 2-Isopropyl-3-methylcyclohex-2-eneone was then heated with 10% palladium-charcoal in *p*-cymene for 5 h under nitrogen. The product on extraction with aqueous sodium hydroxide (10%) followed by acidification, yielded 2-isopropyl-3-methylphenol (mp 70-71°C). The PMR spectrum was similar to that reported by Nasipuri and De Dalal (1975).

3-Isopropyl-2-methylphenol

3-Isopropyl-2-methylphenol was synthesised by converting 2-amino-6-chlorotoluene to 3-chloro-2-methylphenol via a diazonium salt intermediate and then protecting the phenol by reaction with benzylchloride in the presence of

potassium carbonate (Tietze and Eicher, 1989). 3-Chloro-2-methylphenylbenzylether (1 g, 0.0043 mol) in dry tetrahydrofuran (THF, 3 mL) was then added dropwise to magnesium turnings (0.11 g, 0.0045 mol) in THF (10 mL) and the reaction initiated by refluxing for 16 h as suggested by Ramsden *et al.* (1957). Acetone (0.253 g, 0.0043 mol) was then added and the mixture refluxed for a further 4 h and allowed to stand overnight. After quenching the reaction with saturated ammonium chloride, the THF was evaporated to yield the tertiary alcohol intermediate and unreacted 3-chloro-2-methylphenylbenzylether. The intermediate (0.52 g, 0.002 mol) was isolated on a silica gel column, eluting with 10% diethylether in petroleum spirit followed by diethylether and, then hydrogenolysed (2 atms) in ethanol (10 mL) using 10% palladium-charcoal (0.06 g) for 48 h to yield 3-isopropyl-2-methylphenol (0.26 g, 0.0017 mol) (mp 82-83°C). The IR spectrum and PMR spectrum were similar to those reported by Lamartine and Perrin (1969).

Sec-butyl-2-methylphenols

A mixture of *sec*-butyl-2-methylphenols was prepared by adding 2-bromobutane dropwise to 2-methylphenol and aluminium chloride (0.025 mol: 0.025 mol: 0.038mol respectively) in 1,2-dichloroethane (50 mL). The reaction mixture was maintained at 3-5°C for 2.5 h and then poured onto ice water and extracted with dichloromethane (4 x 20 mL). The reaction products were analysed using GC-MS and GC-FTIR.

Sec-butyl-3-methylphenols

A mixture of *sec*-butyl-3-methylphenols was prepared by adding 2-bromobutane dropwise to 3-methylphenol and aluminium chloride (0.025 mol: 0.025 mol: 0.05 mol respectively) in 1,2-dichloroethane (50 mL). The reaction

mixture was refluxed for 5 h and then poured onto ice water and extracted with dichloromethane (4 x 20 mL). The reaction products were analysed using GC-MS and GC-FTIR.

***Sec*-butyl-4-methylphenols**

A mixture of *sec*-butyl-4-methylphenols were prepared by adding 2-bromobutane dropwise to 4-methylphenol and aluminium chloride (0.025 mol: 0.025 mol: 0.038 mol respectively) in 1,2-dichloroethane (50 mL). The reaction mixture was maintained at 3-5°C for 2.5 h and then poured onto ice water and extracted with dichloromethane (4 x 20 mL). The reaction products were analysed using GC-MS and GC-FTIR.

2.3 INSTRUMENTATION

Gas Chromatography - Flame Ionisation Detection (GC)

GC analysis was performed with a Varian 3500 gas chromatograph with flame ionisation detection. The gas chromatograph was fitted with either a 60 m x 0.22 mm i.d. BP1 fused silica dimethyl siloxane column (SGE Australia), BP5 fused silica 5% diphenyldimethyl siloxane column (SGE) or DB1701 fused silica 14% cyanopropylphenyl siloxane column (J & W). Hydrogen was used as the carrier gas, with a linear flow rate of 30 cm s^{-1}. The oven was programmed from 45 (1 min) to 300°C (15 min) at 4°C min^{-1} (for BP1 and BP5) and 45 (1 min) to 280°C (15 min) at 4°C min^{-1} (for DB1701). Samples of 0.2-0.4 μL were introduced onto the column using a Varian 1097/1098 on-column injector. The injector was programmed from 45 to 280°C at 150°C min^{-1}. A length of 20-30 cm was removed from the front of the column at regular intervals to rectify column activity.

Gas Chromatography-Mass Spectrometry (GC-MS)

Two GC-MS systems were used during the course of this study:

I. Phenol extracts were analysed using a Hewlett Packard 5987 GC-MS system equipped with an RTE/A data system. The GC-MS was fitted with a 60 m x 0.25 mm i.d. column containing either a dimethyl siloxane (DB1, J & W), diphenyldimethyl siloxane (DB5, J & W) or cyanopropylphenyl siloxane (DB1701, J & W) phase. Hydrogen was used as the carrier, with a flow rate of 40 cm s^{-1}. The GC oven was programmed from 40 (1 min) to 280°C (15 min) at 4°C min^{-1}. Samples were introduced onto the column using an OCI-3 on-column injector (SGE) and analysed using the full data acquisition (SCAN) and selected ion monitoring (SIM) modes. Typical mass spectrometer operating conditions were: electron multiplier voltage 2000 V; emission current 220 μA; electron energy 70 eV; source temperature 250°C.

II. Phenol extracts, and the branched and cyclic and aromatic fractions isolated from crude oil were analysed using a 5890 Series II gas chromatograph interfaced to a 5971A mass selective detector. B/C fractions and phenol extracts were analysed using a 60 m x 0.25 mm i.d. column containing a DB1 phase (J & W, 0.25 μm phase thickness) and the GC oven was programmed from 50 to 270°C at 8°C min^{-1} and then to 285°C (20 min) at 1°C min^{-1}. Aromatic fractions and phenol extracts were analysed using a 40 m x 0.18 mm i.d. column containing a DB5 phase (J & W, 0.4 μm phase thickness) and the GC oven was programmed from 70 to 290°C (20 min) at 3°C min^{-1}. Helium was used as the carrier, with a flow rate of 22 cm s^{-1}. Samples were introduced on-column using a Hewlett Packard 7673 automatic sampler and analysed using the SCAN and SIM modes. Typical mass spectrometer operating conditions were: electron multiplier voltage 2000 V; emission current 220 μA; electron energy 70 eV; source temperature 180°C.

Pyrolysis Gas Chromatography-Mass Spectrometry (Py GC-MS)

Pyrolysis gas chromatography of ground, solvent-extracted (dichloromethane) sedimentary rocks was performed with a Pyrojector microfurnace (SGE Scientific) interfaced via the capillary inlet system to a Hewlett-Packard 5890 gas chromatograph fitted with a 25 m x 0.2 mm i.d. WCOT fused-silica cross-linked methylsilicone phase column (HP-1, film thickness 0.33 μm, Hewlett-Packard). Samples (*ca.* 3 mg) were prepared as solid pellets and introduced directly into the pyrolyser, which was maintained at 600°C. Helium was used as both pyrolysis and carrier gas (linear velocity 30 cm s^{-1}), and injections were splitless (1 min hold). The oven was programmed from -20°C (2 min) to 280°C (18 min) at 4°C min^{-1}. Eluting compounds were monitored using a Hewlett-Packard 5970 mass selective detector in the full data acquisition mode, scanning from 40 to 540 dalton in 1.3 s cycles. Typical mass spectrometer operating conditions were: electron multiplier voltage 1750 V; emission current 220 μA; electron energy 70 eV; source temperature 220°C.

Tracer Gas Chromatography-Fourier Transform Infrared (GC-FTIR)

Solid state infrared spectra were obtained using a Bio-Rad FTS-60A FTIR spectrometer equipped with a GC Tracer interface. The phenol samples were separated using a Hewlett-Packard 5890 gas chromatograph fitted with a 50 m x 0.2 mm i.d. column containing a diphenyl dimethyl siloxane (BP5) phase. Hydrogen was used as the carrier gas, with a linear flow velocity of 30 cm s^{-1}. The GC oven was programmed from 50 to 280°C at 4°C min^{-1}. Samples were introduced on-column using an OCI-3 on-column injector (SGE). The column effluent passed through a silica transfer line heated at 290°C and eluted compounds were deposited on a liquid nitrogen cooled zinc-selenide plate (-150°C). A total response chromatogram was obtained operating the spectrometer at a resolution of

2 cm^{-1} with a scan rate of 0.81 sec $scan^{-1}$ and the plate speed was at a nominal setting of 2.

Nuclear Magnetic Resonance Spectroscopy (NMR)

A Bruker nuclear magnetic resonance spectrometer operating at 300 MHz was used to obtain ^1H spectra for the isopropylmethylphenols synthesised. The spectra (chemical shifts in ppm) were recorded in deuterochloroform solution and TMS was used as internal standard.

2.4 GEOCHEMICAL TECHNIQUES

Extraction of Soluble Organic Matter (SOM) from Sedimentary Rocks

Crushed sedimentary rocks (5-10 g) were extracted with dichloromethane (200 mL) using a continuous soxhlet extraction apparatus for 8 h. The majority of solvent was removed from the filtrate by fractional distillation (1-2 mL remaining) and hexane was added. The solvent was sequentially distilled and more hexane added several times to remove any traces of dichloromethane from the SOM extract. The phenols were extracted from the SOM using analytical procedure I (Section 2.7).

Hydrous Pyrolysis of Sedimentary Rock Samples

Hydrous pyrolysis experiments were carried out in a closed 100 mL stainless steel vessel that was pre-tested for leaks using water under pyrolysis conditions. Solvent-extracted sedimentary rocks (0.5-1.5 g) were placed in the pyrolysis chamber along with deionised water (6 mL). The reaction vessel was purged with nitrogen for 3-5 min then sealed and placed in an oven at 330°C for 72 h (MoS_2 was used to coat the vessel thread to prevent seizing at high temperatures). After

cooling to room temperature, the vessel was further cooled in liquid nitrogen, opened and both the pyrolysed sedimentary rock and aqueous supernatant were extracted with dichloromethane (100 mL; 4 x 4 mL respectively). The extracts were combined and dried with anhydrous magnesium sulphate (0.25 g) and then the majority of solvent was distilled from the extract (1-2 mL remaining) and hexane (2 mL) was added. The solvent was sequentially distilled and hexane added (1 mL) two more times to remove trace levels of dichloromethane. The phenols in the hydrous pyrolysate were then analysed using analytical procedure II (Section 2.7).

Fractionation of Crude Oil by Column Chromatography

Crude oils (50-100 mg) were separated into aliphatic, aromatic and polar fractions by liquid chromatography using silica gel. Activated stationary phase (6 g) was introduced into a glass column (40 cm x 1.2 cm i.d.) as a slurry in *n*-pentane (20 mL). The saturated hydrocarbons were eluted with *n*-pentane (20 mL), the aromatic hydrocarbons with 10% dichloromethane in *n*-pentane (40 mL), and the polar compounds with 1:1 mixture of dichloromethane/methanol (30 mL). The saturate fraction was routinely passed through a small column of activated copper (100 mg) to remove elemental sulphur.

Isolation of Branched and Cyclic Alkane Fractions (B/C)

Activated ZSM-5 molecular sieve was dry packed into a Pasteur pipette plugged at the base with cotton wool that had been extracted with dichloromethane. The saturate fraction (30 mg) was introduced onto the top of the column in a minimum of *n*-pentane (200 μL). The column was then eluted with 4 bed volumes of *n*-pentane to yield a branched and cyclic alkane fraction.

2.5 ALUMINIUM SMECTITE CATALYSED HEATING REACTIONS

Methylation of Methylphenols Using a Methyl-Donor

A solution of 2-methylphenol, 3-methylphenol and 4-methylphenol (0.5 mg, 0.25 mg, 0.25 mg respectively), tetradecane (0.5 mg as internal standard) and 1,4,5,8-tetramethylnaphthalene (1.7 mg as methyl donor) in dichloromethane (30 μL) was transferred into a glass ampoule containing aluminium smectite (20 mg). The reaction mixture was thoroughly stirred with a fine glass rod and then the dichloromethane evaporated, this was repeated a second time after the side of the glass ampoule had been washed down with dichloromethane (10-20 μL). The residue was sealed in an evacuated glass ampoule and the reaction mixture was heated at 160°C for 12 h. The ampoule was opened after cooling in liquid nitrogen and the clay was extracted with dichloromethane. Eicosane (0.5 mg) was added as a normalisation standard and the mixture was analysed by GC and GC-MS.

Isopropylation of Methylphenols Using an Isopropyl-Donor

A solution of 2-methylphenol, 3-methylphenol and 4-methylphenol (0.5 mg, 0.25 mg, 0.25 mg respectively), tetradecane (0.5 mg as internal standard) and cadalene (1.8 mg as isopropyl donor) in dichloromethane (30 μL) was transferred into a glass ampoule containing aluminium smectite (20 mg). The reaction mixture was thoroughly stirred with a fine glass rod and then the dichloromethane evaporated, this was repeated a second time after the side of the glass ampoule had been washed down with dichloromethane (10-20 μL). The residue was sealed in an evacuated glass ampoule and the reaction mixture was heated at 150°C for

12 h. The ampoule was opened after cooling in liquid nitrogen and the clay was extracted with dichloromethane. Eicosane (0.5 mg) was added as a normalisation standard and the mixture was analysed by GC and GC-MS.

Heating Reactions of Thymol and α-Tocopherol on Aluminium Smectite

A solution of thymol (1 mg) and dodecane (0.5 mg as internal standard) in dichloromethane (20 μL) was transferred into a glass ampoule containing aluminium smectite (20 mg). The reaction mixture was thoroughly stirred with a fine glass rod and then the dichloromethane evaporated and the residue was sealed in an evacuated glass ampoule. Several ampoules containing the reaction mixture were heated at temperatures ranging from 140-200°C for 12 h and the ampoules were opened after cooling in liquid nitrogen. The clay in each ampoule was extracted with dichloromethane and eicosane (0.5 mg) was added as a normalisation standard. The mixture was analysed by GC and GC-MS.

Blank Experiments for Aluminium Smectite Heating Reactions

A solution of tetradecane (0.5 mg) and 1,4,5,8-tetramethylnaphthalene (1.7 mg) or cadalene (1.8 mg) in dichloromethane (30 μL) was transferred into a glass ampoule containing aluminium smectite (20 mg). The dichloromethane was evaporated and the residue was sealed in an evacuated glass ampoule and the reaction mixture was heated at 160°C for 12 h. The ampoule was opened after cooling in liquid nitrogen and the clay was extracted with dichloromethane. Eicosane (0.5 mg) was added as a normalisation standard and the mixture was analysed by GC and GC-MS. The phenolic reaction products obtained from the aluminium smectite reactions outlined above were not present in the blank reaction products.

2.6 DETERMINATION OF PHENOL PARTITION COEFFICIENTS

To overcome analytical difficulties due to coelution, two mixtures of standard alkylphenols in isooctane (0.5 mg mL^{-1}) were prepared with compositions shown in Table 7.5 (Section 7.6). Samples of the standard mixtures (1 mL) were placed in flasks containing isooctane (15 mL) and distilled water (50 mL), and the two phases were vigorously mixed at 80 ± 1°C in a nitrogen atmosphere. In each case, the system was allowed to equilibrate and after 36 h the two phases were separated while still hot. The aqueous phase was extracted with dichloromethane (4 x 20 mL), the dichloromethane was dried with magnesium sulphate and then distilled on a sand bath to a volume of approximately 1.5 mL. The isooctane phase was extracted with aqueous sodium hydroxide (4 x 4 mL, 1 M) and the combined aqueous extracts acidified with glacial acetic acid and then extracted with dichloromethane (4 x 4 mL). The dichloromethane extract was dried with magnesium sulphate and the solvent distilled on a sand bath to approximately 1.5 mL. The phenol concentrations in the two dichloromethane concentrates were determined by GC analysis on a BP5 phase. The distribution coefficients for each compound in Table 7.5 were determined using the relationship:

K_D (80°C) = [compound] in isooctane phase / [compound] in water phase

2.7 ANALYSIS OF PHENOLS IN SEDIMENTARY ORGANIC MATTER

Extraction of Phenols from Crude Oils (Analytical Procedures I and II)

Figure 4.1 (Section 4.1) is a flow diagram of the analytical procedures developed in this study. Approximately 2-5 g of crude oil was accurately weighed into a test tube, and 2-nitrophenol (1, 2, 10 or 20 μg) was added as an internal

standard. The oil was diluted with hexane (approx. 6 mL) and extracted three times with sodium hydroxide in 5% methanol/95% water (v/v) (0.1 M, 3 mL). Mixing was carried out using a test tube mixer (Super-Mixer; Lab-line Instruments, Inc.). The phases were allowed to separate and emulsions were broken using centrifugation (Sorvall Superspeed Centrifuge) when necessary (5-30 min). The lower alkaline aqueous phase was transferred by Pasteur pipette to a 20 mL glass syringe to which a C-18 Sep-Pak cartridge (pre-washed with 2 mL methanol followed by 5 mL water) was attached (analytical procedure I), or to a test-tube (analytical procedure II).

In procedure I, the non-polar impurities were removed by passing the basic aqueous solution through the Sep-Pak cartridge at a slow rate (approximately one drop every 5 s) and collecting the eluent in a test-tube. The Sep-Pak was washed twice with sodium hydroxide in 5% methanol/95% water (v/v) (0.1 M, 2 mL) and the washings combined with the eluate. The washings were allowed to pass through the Sep-Pak cartridge at a similar rate to the basic aqueous solution. In procedure II, the non-polar impurities were removed by extracting the combined alkaline extracts three times with hexane (4 mL).

The aqueous methanolic sodium hydroxide solution obtained from procedures I or II was then buffered to a pH of 6.8 using glacial acetic acid (approximately 100 μL) and sodium bicarbonate solution (1 mL, 1 M). The buffered solution was extracted four times with dichloromethane (4 mL) using the test tube mixer. The combined dichloromethane extracts were dried with a small amount of anhydrous magnesium sulphate (0.2 g), and filtered through a Pasteur pipette containing a cotton wool plug pre-washed with dichloromethane. The extract was slowly distilled using a sand bath to approximately 1 mL and quantitatively transferred to a small vial. 1-Chloro-octadecane (2 or 10 μg) was added as a normalisation standard for calculation of phenol recoveries, and the extract was further

evaporated to a volume of 50-200 μL. The phenol concentrate was analysed by GC, GC-MS and GC-FTIR.

Extraction of Phenols from Sedimentary Rock Extracts and Pyrolysates

A hexane solution (approximately 8 mL) of the sedimentary rock extract (SOM) or hydrous pyrolysate (refer to Section 2.4) was placed in a test tube, and 2-nitrophenol (1, 2, 10 or 20 μg) was added as an internal standard. Phenols were isolated from the hexane solution of the SOM or hydrous pyrolysate using analytical procedures I and II respectively.

Analytical Procedure Blanks

Blanks for the analytical procedures were measured regularly by subjecting the internal standard (2-nitrophenol, 1 μg) in hexane to the analytical procedures. The chromatograms obtained showed only minor peaks, which were not phenols, in comparison to that of the internal standard indicating that sample contamination had not occurred during analysis.

CHAPTER 3

GEOLOGICAL AND GEOCHEMICAL DESCRIPTION

OF SAMPLES

3.1 SAMPLES

A range of samples from Australia, Indonesia and Canada comprising eight sedimentary rocks and 45 crude oils were used in this study. The geological and geochemical data pertaining to these samples are listed in Tables 3.1 to 3.6. Figure 3.1 shows the geographic location of the Australian and Indonesian sedimentary basins from which these samples were obtained.

3.2 GEOCHEMICAL PARAMETERS

A range of geochemical parameters was measured on the samples used in this study. Table 3.1 shows the definitions of the parameters used and the analytical technique with which they were measured. Table 3.5 and Table 3.6 show the values of the geochemical parameters obtained for the crude oils.

Table 3.2 Geological data pertaining to Australian sedimentary rock samples.

Name	Depth (m)	Basin / Formation	Age	Lithology	Ro (%)
Loy Yang	67-68	Gippsland / Latrobe	Miocene	Brown coal	0.2
Heartbreak	24.3-24.8	Bremer / Werillup	Eocene	Brown coal	0.3
Weena-1	1491	Cooper / Patchawarra	Permian	Siltstone	0.63
Tinga Tingana	1586	Cooper / Patchawarra	Permian	Siltstone	0.68
Sturt East-3	1886	Cooper / Patchawarra	Permian	Siltstone	0.67

Table 3.3 Geological data for GK coal samples from South Sumatra Basin (SSB), Indonesia.

Depth (m)	Formation	Age	Lithology	Ro (%)	Coal rank
380-390	Air Benakat	Mid Miocene	Brown coal	0.3	Lignite
1740-1744	Talang Akar	Lower Miocene	Brown coal	0.6	Subbituminous
2258-2260	Talang Akar	Lower Miocene	Brown coal	0.9	High vol. bituminous

Table 3.1 Definition of geochemical parameters used in Table 3.2-Table 3.6.

Geochemical parameter	Definition	Measurement technique*
Pr/Ph	Pristane/Phytane	GC
C_{29}/C_{27} Sterane	(*20S*+*R*)-14α,17α(H)-Cholestane/ (*20S*+*R*)-14α,17α(H)-Ethylcholestane	m/z 217 (GC-MS)
Diahopane/Hopane	17α-Diahopane/17α,21β(H)-Hopane	m/z 191 (GC-MS)
Diasterane/Sterane	(*20S*+*R*)-13β,17α(H)-Ethyldiacholestane/ (*20S*+*R*)-14α,17α(H)-Ethylcholestane	m/z 217 (GC-MS)
CPI	$(C_{23}+C_{25}+C_{27}+C_{25}+C_{27}+C_{29})/ 2\ (C_{24}+C_{26}+C_{28})$	GC
20S/(20S+20R)	(*20S*)-14α,17α(H)-Ethylcholestane/ (*20S*+*R*)-14α,17α(H)-Ethylcholestane	m/z 217 (GC-MS)
22S/22R	(*22S*)-17α,21β(H)-Homohopane/ (*22R*)-17α,21β(H)-Homohopane	m/z 191 (GC-MS)
Moretane/Hopane	17β,21α(H)-Hopane/17α,21β(H)-Hopane	m/z 191 (GC-MS)
Ts/(Ts+Tm)	18α-22,29,30-Trisnorneohopane/ (18α-22,29,30-Trisnorneohopane + 17α-22,29,30-Trisnorhopane)	m/z 191(GC-MS)
DNR1	(2,6-DMN + 2,7-DMN)/1,5-DMN	m/z 156 (GC-MS)
TNR1	2,3,6-TMN/(1,4,6-TMN+1,3,5-TMN)	m/z 170 (GC-MS)
Rc	$0.6\ \frac{[1,5\ (2\text{-MP}+3\text{-MP})]}{\text{P}+1\text{-MP}+9\text{-MP}} + 0.4$	m/z 178 and m/z 192 (GC-MS)
Ro (%)	Percentage incident light (546 nm) reflected from vitrinite phytoclasts	

DMN = Dimethylnaphthalene, TMN = Trimethylnaphthalene, P = Phenanthrene, MP = methylphenanthrene

* Peak areas were used for the determination of values in Tables 3.5-3.6

Table 3.4 Geological data pertaining to crude oil samples.

	Crude oil	Location Basin / Country	Age of putative source rock	Depositional environment
C1	Nilam	Kutei / Indonesia	Tertiary	Non-marine
C2	A	Kutei / Indonesia	Tertiary	Non-marine
C3	B	Kutei / Indonesia	Tertiary	Non-marine
C4	C	Kutei / Indonesia	Tertiary	Non-marine
C5	D	Kutei / Indonesia	Tertiary	Non-marine
C6	E	Kutei / Indonesia	Tertiary	Non-marine
C7	Iron Duke	- / Indonesia	Tertiary	Non-marine
C8	Basker-1 3091m	Gippsland / Aust.	Cretaceous (Cret)	Non-marine
C9	Tuna-4 1400.5 m	Gippsland / Aust.	Cretaceous	Non-marine
C10	Tuna-4 2820 m	Gippsland / Aust.	Cretaceous	Non-marine
C11	West Seahorse-1	Gippsland / Aust.	Cretaceous	Non-marine
C12	Lakes Entrance	Gippsland / Aust.	Cretaceous	Non-marine
C13	Caroline-1	Otway / Aust.	Cretaceous	Non-marine
C14	Lambert-1 DST-1A	Dampier Sub-basin / Aust.	Jurassic / Cret	Marine
C15	F	Dampier Sub-basin / Aust.	Jurassic / Cret	Marine
C16	G	Dampier Sub-basin / Aust.	Jurassic / Cret	Marine
C17	H	Dampier Sub-basin / Aust.	Jurassic / Cret	Marine
C18	I	Dampier Sub-basin / Aust.	Jurassic / Cret	Marine
C19	J	Dampier Sub-basin / Aust.	Jurassic / Cret	Marine
C20	K	Dampier Sub-basin / Aust.	Jurassic / Cret	Marine
C21	Barrow Deep	Barrow Sub-basin / Aust.	Jurassic	Marine
C22	Barrow UJ 6700 ft	Barrow Sub-basin / Aust.	Jurassic	Marine
C23	L	Barrow Sub-basin / Aust.	Jurassic	Marine
C24	Rough Range-1	Barrow Sub-basin / Aust.	Jurassic	Marine
C25	M	Barrow Sub-basin / Aust.	Jurassic	Marine
C26	N	Barrow Sub-basin / Aust.	Jurassic	Marine
C27	O	Barrow Sub-basin / Aust.	Jurassic	Marine
C28	P	Barrow Sub-basin / Aust.	Jurassic	Marine
C29	Windalia	Barrow Sub-basin / Aust.	Jurassic	Marine
C30	Q	Barrow Sub-basin / Aust.	Jurassic	Marine
C31	Bodalla Sth Basal Jurassic	Eromanga / Aust.	Jurassic	Non-marine
C32	Kenmore-1 DST-1 1275-1288 m	Eromanga / Aust.	Jurassic	Non-marine
C33	Moorari-4	Eromanga / Aust.	Jurassic	Non-marine
C34	Byrock-1 DST-1 1967-1984 m	Cooper / Aust.	Permian	Non-marine
C35	Earlstown-1 DST-2 2340-2355 m	Cooper / Aust.	Permian	Non-marine
C36	Lycium-1 DST-1 6544 ft	Cooper / Aust.	Permian	Non-marine
C37	Malgoona-1 DST-2 7420 ft	Cooper / Aust.	Permian	Non-marine
C38	Sturt-6 DST-3 6180 ft	Cooper / Aust.	Permian	Non-marine
C39	West Terrace	Canning / Aust.	Carboniferous	Marine
C40	Blina-1	Canning / Aust.	Devonian	Non-marine
C41	Husky Mobil Rainbow	Alberta / Canada	Mid Devonian	Marine
C42	Mirbelia	Canning / Aust.	Devonian	Marine
C43	Dodonea	Canning / Aust.	Ordovician	Marine
C44	R	Canning / Aust.	Ordovician	Marine
C45	Jamison	McArthur / Aust.	Mesoproterozoic	Marine

Table 3.5 Source type and depositional environment biomarker data for crude oil samples.

	Crude oil	Pr/Ph	C_{29}/C_{27} Steranes	Diahopane /Hopane	Diasterane /Sterane	CPI
C1	Nilam	6.3	6.2	0.055	1.6	1.08
C2	A	8.6	11	0.032	0.91	1.13
C3	B	8.3	4.8	0.053	1.2	1.07
C4	C	2.3	5.0	0.051	0.90	1.05
C5	D	10	2.4	0.084	2.2	1.10
C6	E	6.8	5.6	0.053	1.1	0.95
C7	Iron Duke	3.4	1.7	<0.01	0.40	1.01
C8	Basker-1	5.6	>50	0.19	1.2	1.04
C9	Tuna-4 1400.5 m	3.0	7.7	0.12	1.1	Bio
C10	Tuna-4 2820 m	5.8	10	0.18	2.8	1.10
C11	West Seahorse-1	5.4	11	0.20	0.92	1.10
C12	Lakes Entrance	Bio	4.5	0.19	2.8	Bio
C13	Caroline-1	5.6	0.91	0.96	2.8	1.05
C14	Lambert-1	2.5	0.91	0.61	2.2	1.02
C15	F	2.4	1.1	0.40	2.2	1.03
C16	G	3.2	1.3	0.22	2.9	1.03
C17	H	5.2	*	*	*	*
C18	I	2.9	1.4	0.092	0.81	1.03
C19	J	nd	1.1	0.19	1.7	nd
C20	K	2.6	0.91	0.42	2.5	1.03
C21	Barrow Deep	2.6	1.5	2.1	1.4	1.05
C22	Barrow UJ	1.8	1.1	0.17	1.3	1.01
C23	L	nd	1.9	0.16	1.2	nd
C24	Rough Range-1	3.7	1.6	0.25	2.9	1.02
C25	M	3.4	0.77	0.34	3.7	1.02
C26	N	2.9	1.1	0.50	2.8	1.05
C27	O	2.6	1.2	0.16	1.2	1.10
C28	P	3.0	1.0	0.41	1.6	1.05
C29	Windalia	2.8	1.5	0.14	0.92	Bio
C30	Q	Bio	1.4	1.3	0.81	Bio
C31	Bodalla Sth BJ	4.9	2.0	0.14	1.9	1.06
C32	Kenmore-1 DST-1	4.4	3.1	0.11	1.9	1.09
C33	Moorari-4	9.1	>50	<0.01	2.3	1.28
C34	Byrock-1 DST-1	5.5	2.1	0.20	1.7	1.10
C35	Earlstown-1 DST-2	5.1	5.0	0.58	1.7	1.01
C36	Lycium-1 DST-1	5.4	3.8	0.06	2.9	1.11
C37	Malgoona-1 DST-2	6.2	3.0	0.30	2.8	1.09
C38	Sturt-6 DST-3	6.4	4.3	0.10	1.6	1.11
C39	West Terrace	1.5	0.59	0.23	2.2	0.97
C40	Blina-1	0.60	0.91	0.30	1.2	1.04
C41	Husky Mobil Rainbow	4.0	7.7	0.05	0.72	*
C42	Mirbelia	1.0	0.91	0.13	1.0	1.04
C43	Dodonea	2.2	1.0	0.13	1.3	1.08
C44	R	1.4	*	<0.01	*	1.07
C45	Jamison	1.1	1.9	0.061	0.82	1.06

Bio = biodegraded, * Denotes concentration too low for meaningful measurement, nd = no data available

Table 3.6 Hydrocarbon maturity data for crude oil samples.

	Crude oil	Hydrocarbons				Aromatics		
		20S/ (20S+20R)	22S/22R	Moretane /Hopane	Ts/ (Ts+Tm)	DNR1	TNR1	Rc
C1	Nilam	0.41	1.8	0.26	0.48	9.3	1.0	0.95
C2	A	0.51	1.5	0.21	0.60	7.5	0.76	0.75
C3	B	0.43	1.5	0.22	0.71	8.9	0.84	0.77
C4	C	0.51	1.5	0.21	0.57	11	0.85	0.90
C5	D	0.46	1.6	0.15	0.60	9.7	0.79	0.82
C6	E	0.42	1.4	0.23	0.71	6.9	0.73	0.70
C7	Iron Duke	0.35	1.4	0.20	0.45	7.2	0.71	0.96
C8	Basker-1	0.48	1.2	0.12	0.41	5.5	0.66	0.88
C9	Tuna-4 1400.5 m	0.44	0.82	0.17	0.27	3.8	0.52	0.73
C10	Tuna-4 2820 m	0.74	1.5	0.12	0.40	6.7	0.75	0.86
C11	West Seahorse-1	0.47	0.94	0.15	0.36	8.6	0.82	0.94
C12	Lakes Entrance	0.76	1.1	<0.1	0.41	Bio	Bio	Bio
C13	Caroline-1	0.42	1.5	0.14	0.25	3.6	0.80	0.64
C14	Lambert-1	0.52	1.7	<0.1	0.80	7.1	0.95	0.80
C15	F	0.66	1.1	<0.1	0.77	5.8	0.85	0.81
C16	G	0.51	1.5	0.090	0.59	8.3	0.87	0.88
C17	H	*	*	*	*	6.8	0.87	0.98
C18	I	0.35	1.4	0.28	0.26	5.7	1.2	0.75
C19	J	0.46	1.4	0.39	0.25	5.0	0.90	0.66
C20	K	0.49	1.6	<0.1	0.74	5.5	0.77	0.78
C21	Barrow Deep	0.76	*	<0.1	1.0	12	1.1	0.92
C22	Barrow UJ	0.54	1.3	0.11	0.73	7.4	0.87	0.81
C23	L	0.28	0.83	0.23	0.46	6.6	0.88	0.76
C24	Rough Range-1	0.43	1.3	0.11	0.30	3.5	0.46	0.63
C25	M	0.56	1.7	<0.1	0.69	6.6	1.0	0.82
C26	N	0.47	1.3	<0.1	0.62	8.3	0.98	0.85
C27	O	0.49	1.3	0.24	0.65	8.4	0.86	0.74
C28	P	0.50	1.7	0.22	0.60	8.4	0.91	0.75
C29	Windalia	0.44	1.4	0.10	0.59	5.9	0.83	0.80
C30	Q	0.37	1.4	1.2	0.61	4.1	0.87	0.72
C31	Bodalla Sth (BJ)	0.48	1.6	<0.1	0.38	2.7	0.44	0.72
C32	Kenmore-1 DST-1	0.48	1.4	0.14	0.29	2.2	0.43	0.50
C33	Moorari-4	0.41	1.2	0.31	0.09	1.4	0.35	0.51
C34	Byrock-1 DST-1	0.54	1.2	<0.1	0.40	4.3	0.66	0.88
C35	Earlstown-1 DST-2	0.56	0.75	<0.1	0.68	7.2	0.92	0.94
C36	Lycium-1 DST-1	0.49	1.5	0.25	0.11	3.4	0.68	0.73
C37	Malgoona-1 DST-2	0.50	1.6	<0.1	0.43	5.4	0.82	0.89
C38	Sturt-6 DST-3	0.47	1.3	0.11	0.25	4.6	0.82	0.85
C39	West Terrace	0.48	1.0	<0.1	0.69	*	*	*
C40	Blina-1	0.50	1.2	<0.1	0.50	6.7	1.1	0.98
C41	Husky Mobil Rainbow	0.50	1.1	0.32	0.63	4.9	0.82	1.09
C42	Mirbelia	0.45	1.2	<0.1	0.54	3.7	0.67	0.70
C43	Dodonea	0.30	1.9	0.11	0.39	2.9	0.90	0.63
C44	R	*	1.3	<0.1	0.10	9.0	1.2	0.90
C45	Jamison	0.49	1.3	0.12	0.45	7.1	0.92	1.0

Bio = biodegraded, * Denotes concentration too low for meaningful measurement

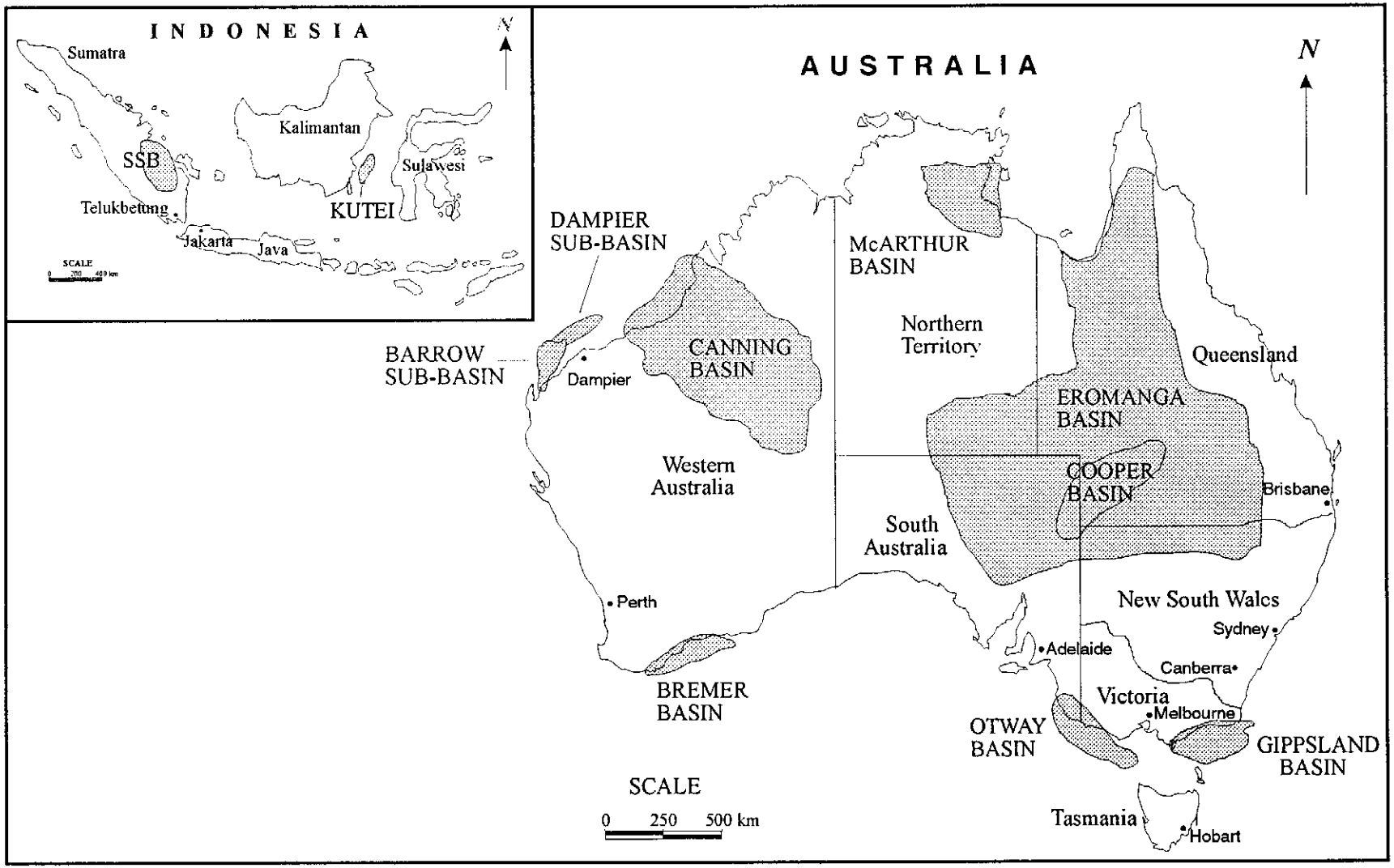


Figure 3.1 Maps showing the locations of some Australian and Indonesian sedimentary basins.

Duplicate measurements were carried out on approximately 5% of the crude oils and based on the reproducibility of the values obtained for these representative samples it was estimated that the values shown in Table 3.5 and Table 3.6 have a measurement error of ± 5%. The values in the tables are therefore reported to two significant figures with the exception of CPI values which are reported to three significant figures. The geochemical parameters have been selected to provide information on the source type of the organic matter and the environment in which the organic matter was deposited (Table 3.5), and the extent to which the organic matter had been matured during burial in the subsurface (Table 3.6). A brief description of the application of and interpretation of each parameter is provided in Sections 3.2.1 and 3.2.2.

3.2.1 Geochemical Parameters Indicating Source Type and Source Depositional Environment (Table 3.5)

Pristane/Phytane (Pr/Ph)

Pristane/phytane ratios of crude oils have been used to indicate the oxicity or redox potential of source rocks (Didyk *et al.*, 1978; Powell, 1988). The values used to describe different depositional conditions are shown below.

Pr/Ph Ratio	Interpretation (Peters and Moldowan, 1993)
>3	Terrestrial organic matter deposited under oxic conditions
<0.6	Anoxic depositional environment (commonly hypersaline)
0.6-3	Inconclusive, other geochemical parameters required

Interferences by source input and thermal maturity of a sample must be considered when interpreting Pr/Ph values. Although pristane and phytane are primarily derived from the side chain of chlorophyll in phototropic organisms, other sources of these acyclic isoprenoids have been recognised. These include

archaebacterial lipids from methogens or halophiles (Volkman and Maxwell, 1986) and tocopherols (Goossens *et al.*, 1984; Brassell and Eglinton, 1986). Thus differences in populations of organisms contributing to sedimentary rocks can affect Pr/Ph ratios. The thermal maturity of samples should also be considered when using the Pr/Ph ratio to describe paleoenvironment. Typically the Pr/Ph ratio increases with thermal maturity of petroleum (Connan, 1974; ten Haven *et al.*, 1987b) however, other reports indicate that although Pr/Ph ratio initially increases with maturity, it decreases at higher levels of maturity (Albrecht *et al.*, 1976; Radke *et al.*, 1980; Connan, 1984).

Cholestane/Ethylcholestane (C_{29}/C_{27} Steranes)

The relative proportions of C_{27}, C_{28} and C_{29} steranes has been used as an indicator of source type. Huang and Meinschein (1979) first suggested that a dominance of C_{29} steranes indicated a strong terrestrial contribution, whereas a dominance of the C_{27} compounds indicated a substantial contribution from marine phytoplankton. When C_{28} steranes occur in similar proportion to the C_{27} and C_{29} steranes it may indicate a contribution by lacustrine algae (Volkman *et al.*, 1983a). The ratio used in this study (C_{29}/C_{27} steranes) was reported by Volkman *et al.* (1983a). The values used to indicate different source type are shown below.

C_{29}/C_{27} Steranes	Interpretation of Source type (Peters and Moldowan, 1993)
>2	Terrestrial
0.7-2	Mixed marine / Terrestrial
<0.7	Marine

Volkman (1986; 1988) suggests that there are also significant marine sources of C_{29} steranes as some marine sediments, including those deposited in pelagic environments far from terrestrial influence, show a predominance of these

steranes. Furthermore, lower Paleozoic and Precambrian sediments predating the widespread occurrence of land plants often contain substantial amounts of C_{29} steranes (Grantham, 1986b; Grantham and Wakefield, 1988). Caution must therefore be employed when interpreting these ratios.

Diahopane/Hopane

A high relative abundance of diahopanes in crude oils has been suggested to indicate that the samples are derived from source rocks with high acidic clay contents which were deposited under sub-oxic conditions or that they have undergone biodegradation. Moldowan *et al.* (1991) suggested that diahopanes may be derived from bacterial hopanoid precursors that have undergone oxidation in the D-ring and rearrangement by clay-mediated acidic catalysis. These authors suggested that such a mechanism could lead to the higher concentrations of 17α-diahopanes observed in petroleum samples derived from clay containing source rocks deposited in oxic to sub-oxic depositional environments. In biodegraded samples high values (>0.5) of this ratio are more likely to indicate that selective biodegradation of hopane has taken place (Armanios *et al.*, 1992).

Diasterane/Sterane

Diasterenes are suggested to be formed during early diagenesis from sterenes via acid catalysed rearrangement reactions on clays (Rubinstein *et al.*, 1975; Sieskind *et al.*, 1979). The diasterenes are subsequently reduced to diasteranes (rearranged steranes). High diasterane/sterane ratios are therefore likely to be observed in petroleum derived from source rocks containing acidic clays. Diasterane/sterane ratios are commonly used to distinguish petroleum from carbonate versus clastic source rocks (e.g. Mello *et al.*, 1988). Low values

generally indicate clay-poor, carbonate source rocks, whereas high ratios indicate clay-rich, clastic source rocks.

High oxicity (Eh) of sediments during deposition also appears to account for high diasterane/sterane ratios in some samples. Moldowan *et al.* (1986; 1992) found a correlation between low pH, high Eh and high diasterane/sterane ratios. Similarly, high values have also been reported in clay-poor limestone from Florida (Palacas *et al.*, 1984) and carbonates (Clark and Philp, 1989).

Other factors which appear to give rise to high diasterane/sterane values in petroleum samples are high thermal maturity (Seifert and Moldowan, 1978) and the selective destruction of steranes during biodegradation (Peters and Moldowan, 1993).

Carbon Preference Index (CPI)

The relative proportions of odd versus even *n*-alkanes in the C_{23} to C_{31} carbon number region has been used to infer thermal maturity and source type of crude oils and source rock extracts (Bray and Evans, 1961; Scanlan and Smith, 1970; Peters and Moldowan, 1993). Recently, Marzi *et al.* (1993) proposed a revised CPI formula which deals with inconsistency in previous CPI calculations. The relationship between CPI values and maturity and source type are shown below.

CPI value	Interpretation	(Peters and Moldowan, 1993)
	Maturity	Source
>1 odd preference	Immature	Terrestrial (higher plant waxes)
<1 even preference	Immature	
1.0	Mature	

3.2.2 Geochemical Parameters Indicating Thermal Maturity (Table 3.6)

Regular Steranes [20S/(20S+20R)]

Thermal maturity of geochemical samples is commonly measured by the relative abundances of 20S and 20R 14α,17α(H)-ethylcholestanes. The biologically produced 20R diastereomer is depleted relative to the more stable 20S diastereomer with increasing maturity (Mackenzie *et al.*, 1980; Marzi and Rullkötter, 1992). Recent reports suggest that interconversion of 20R and 20S is unlikely and the observed effect is the net result of a complex set of processes involving different rates of formation of 20R and 20S from kerogen and different rates of destruction of the free isomers in the bitumen (Abbott *et al.*, 1990). The 20S/(20S+20R) ratio increases from zero to a steady state value of about 0.5 (Seifert and Moldowan, 1986). Oil generation commences at a value of approximately 0.29, however it is recommended that the ratio be calibrated for each basin and source rock by comparison with other maturity and generation parameters before it can be used as an accurate indicator of the onset of petroleum generation (Peters and Moldowan, 1993). A guide to sterane ratio and inferred maturity is given below.

20S/(20S+20R)	Maturity description (Peters and Moldowan, 1993)
0.24-0.33	Immature
0.33-0.42	Onset of petroleum generation
0.42-0.52	Mature
0.52-0.55	Equilibrated

Factors other than thermal maturity can affect the sterane ratio. These include facies (Moldowan *et al.*, 1986; Hwang *et al.*, 1989), biodegradation (Rullkötter and Wendisch, 1982; McKirdy *et al.*, 1983; Seifert *et al.*, 1984; Clayton and King, 1987) and, selective formation of the 20R diastereomer at high maturity

(Peters *et al.*, 1990; Marzi and Rullkötter, 1992). Furthermore, care should be used when applying the sterane maturity ratio for samples from hypersaline sources, as immature bitumens from such sources can have sterane ratios which make them appear mature (ten Haven *et al.*, 1986).

Homohopanes [22S/22R]

With increasing maturity the biologically produced 22R isomers of C_{31} to C_{35} 17α,21β(H)-hopanes isomerise to produce some of the geological isomer 22S (Ensminger *et al.*, 1977; Seifert and Moldowan, 1980). The C_{31} homologue is most often used in this maturity parameter, however, in principle, any of the homologues can be used. The homohopane 22S/22R ratio increases from zero to approximately 1.5 during maturation with equilibration occurring at values of 1.3-1.6 (Seifert and Moldowan, 1986). Samples with 22S/22R in the range 1.0-1.2 are immature with respect to oil generation, while ratios in the range 1.3 to 1.6 indicate that the main phase of oil generation has been reached. The values used to indicate sedimentary rock and crude oil maturity are shown below.

22S/22R	Maturity description (Peters and Moldowan, 1993)
1.0-1.2	Immature
1.2-1.3	Low maturity
1.3-1.6	Mature
1.5-1.6	Equilibrated

Immature rocks have been reported to contain an equilibrated mixture of homohopane diastereomers indicating that factors other than maturity can affect 22S/22R isomerisation. For example, anomalous behaviour in the rate at which free 17α-homohopane isomerises has been suggested to account for the fully isomerised homohopanes found in very immature carbonate rocks from the Adriatic Basin (Moldowan *et al.*, 1992). The unusual diagenetic pathway for hopanes in

hypersaline environments is suggested to be responsible for the mature hopane patterns observed in many bitumens from immature rocks deposited under hypersaline conditions (ten Haven *et al.*, 1986).

Moretane/Hopane

The biological 17β,21β(H)-configuration (ββ) of sedimentary hopanoids is very unstable and readily converts to βα-(moretane) and αβ-hopane configurations (Seifert and Moldowan, 1980). At higher temperatures, the conversion of moretanes back to αβ-hopanes becomes possible through a ββ-hopane intermediate. However due to the high energy barriers involved, little or no conversion of αβ-hopanes back to ββ-hopanes occurs, resulting in an equilibrium mixture favouring αβ-hopanes over βα-moretanes by approximately 20:1 (Peters and Moldowan, 1993). The ratio of C_{30} 17β,21α(H)-hopane (moretane) to the corresponding 17α,21β(H)-hopane decreases with increasing thermal maturity from about 0.8 to a value <0.15 in mature source rocks and crude oils (Mackenzie *et al.*, 1980; Seifert and Moldowan, 1980). Crude oils generated from Tertiary source rocks have higher moretane to hopane ratios (0.1-0.3) than those from older source rocks (<0.1) (Grantham, 1986a). Other factors affecting moretane/hopane ratios are variations in source input or depositional environment (Rullkötter and Marzi, 1988). The values used to indicate different maturity levels are shown below.

Moretane/Hopane	Maturity description (Peters and Moldowan, 1993)
0.8-0.15	Immature to low maturity
<0.15	Mature

Ts/(Ts+Tm)

The 18α-22,29,30-trisnorneohopane (Ts) shows higher relative stability than 17α-22,29,30-trisnorhopane (Tm) during catagenesis (Seifert and Moldowan, 1978). It is unknown whether conversion of Tm to Ts also occurs. The Ts/(Ts+Tm) ratio is both maturity and source dependent and therefore is only a reliable maturity indicator when comparing oils from a common source of consistent organic facies (Peters and Moldowan, 1993). The ratio also appears to be sensitive to lithology or oxicity of the depositional environment (McKirdy *et al.*, 1983; Rullkötter *et al.*, 1985; Moldowan *et al.*, 1986; Price *et al.*, 1987). Thus this ratio is of limited value and must be used with caution as a maturity indicator. The values used to indicate different maturity levels are shown below.

Ts/(Ts+Tm)	Maturity description (Peters and Moldowan, 1993)
<0.5	Immature to low maturity
>0.5	Mature

Maturity Parameters Based on Methylnaphthalenes (DNR1 and TNR1)

The ratio of 2,6-dimethylnaphthalene and 2,7-dimethylnaphthalene (2,6-DMN+2,7-DMN) relative to 1,5-DMN (DNR1) increases with increase in maturity (Radke *et al.*, 1982b). The relative proportions of the less stable α,α substituted isomer 1,5-DMN declines and those of the more stable β,β-substituted isomers 2,6-DMN and 2,7-DMN increase with increasing thermal maturity (Alexander *et al.*, 1985). Similarly the ratio of 2,3,6-trimethylnaphthalene (2,3,6-TMN) relative to 1,4,6-TMN+1,3,5-TMN (TNR1) increases with increasing thermal maturity (Alexander *et al.*, 1985). The proportions of the more stable β,β,β-substituted isomer (2,3,6-TMN) increases relative to the α,α,β-substituted isomers (1,4,6-TMN+1,3,5-TMN) with increasing thermal maturity. Both the DNR1 and TNR1 parameters are based on the fact that α-substituted isomers are subject to greater

steric strain and are therefore less stable than β-substituted isomers which are less strained (Alexander *et al.*, 1985). The aromatic maturity parameters provide information for very high rock maturities as they continue to change throughout the oil window and into the condensate and wet gas zones. Although DNR1 and TNR1 aromatic maturity indicators are the most reliable and useful parameters, limitations do exist and no single parameter should be used in isolation. For example, occasionally samples have anomalous values probably due to an unusually large input into the source rock of one of the methylnaphthalenes used in the parameters (Alexander *et al.*, 1985).

Maturity Parameters Based on Methylphenanthrenes (Rc)

Maturity parameters have been developed based on phenanthrene and methylphenanthrenes (e.g. MPI1, MPI2) which exhibit a correlation with vitrinite reflectance values (Radke *et al.*, 1982b; Radke and Welte, 1983). These parameters were subsequently calibrated against vitrinite reflectance (up to 1.9) using Australian coals (Boreham *et al.*, 1988). Ratios based on methylphenanthrenes were found useful for assessing the maturity of crude oils and condensates (Garrigues *et al.*, 1988). The changes in the relative abundances of phenanthrene and the methylphenanthrenes with maturity has been explained in terms of the relative reactivity of the isomers. In mature samples methylation at the 1- and 9-positions of phenanthrene leads to the dominance of these isomers (Radke *et al.*, 1983; Alexander *et al.*, 1995). With increasing maturity the relative abundance of the more thermodynamically stable isomers 2- and 3-methylphenanthrenes increases. However at higher maturities demethylation reactions become important leading to a lowering of MPI values. The major limitations of methylphenanthrene-based parameters are that they are not useful for low maturity sedimentary rocks and for rocks containing more hydrogen-rich organic matter

(Boreham *et al.*, 1988). In this study a calculated reflectance parameter (Rc) has been used (Table 3.1).

Vitrinite Reflectance (Ro %)

Vitrinite reflectance (Ro) is used for assessing the thermal maturity of the kerogen in sedimentary rocks (Bostick, 1979). Vitrinite reflectance increases throughout the oil window and approximate Ro values have been assigned to the beginning and end of oil generation as shown below.

Ro (%)	Maturation level (Peters and Moldowan, 1993)
~0.6	Beginning oil window
~0.9	Peak oil window
~1.4	End oil window

Terrestrial higher plant debris is the main precursor to vitrinite and therefore the major limitation of this measurement is that it cannot be carried out on very oil-prone source rocks or on source rocks that pre-date the widespread distribution of land plants, both of which often contain little or no vitrinite (Ayres *et al.*, 1982). Also large amounts of oil prone macerals (Price and Barker, 1985) or bitumen (Hutton *et al.*, 1980) retard the normal progression of Ro with maturity. Vitrinite reflectance is most useful within and beyond the zone of petroleum generation (Ro>0.6%) when biomarker parameters are becoming less useful (Peters and Moldowan, 1993).

CHAPTER 4

ANALYSIS OF PHENOLS IN SEDIMENTARY ORGANIC MATTER

4.1 ANALYTICAL PROCEDURES

The analytical procedures used for extracting phenols from crude oil and sedimentary rocks are shown in Figure 4.1. The procedures were based on a method reported by MacCrehan and Brown-Thomas (1987) which involved isolation of the phenolic components of a crude oil using aqueous methanolic sodium hydroxide. An isolate obtained in this way contained both non-polar hydrocarbons and carboxylic acids which needed to be removed in order to obtain a phenol extract which gave interference-free capillary gas chromatograms. Several modifications of the reported procedure were explored and their outcomes assessed in terms of recovery of individual phenols and chromatogram quality. These investigations led to the development of procedure II which is the preferred method for the analysis of C_0-C_5 alkylphenols in crude oil.

Modification A - Separation of Phenols and Carboxylic Acids

The procedure reported by MacCrehan and Brown-Thomas (1987) was found unsuitable for analysis of the trace level of phenols present in crude oil by GC techniques due to the presence of carboxylic acids in the acid isolate. The reported procedure involved extraction of the acid components from crude oil with 0.1 M sodium hydroxide in 60% methanol/water (v/v) and removal of non-polar components and particulate matter by passing the extract through a C-18 Sep-Pak cartridge. After neutralisation the total acid extract was fractionated using HPLC and the phenols analysed using oxidative electrochemical detection. In the present study a large number of individual alkylphenols was to be determined by capillary GC, it was therefore necessary to remove the carboxylic acids, the most abundant acidic components in crude oil (Green *et al.*, 1986), so as to obtain a phenol concentrate.

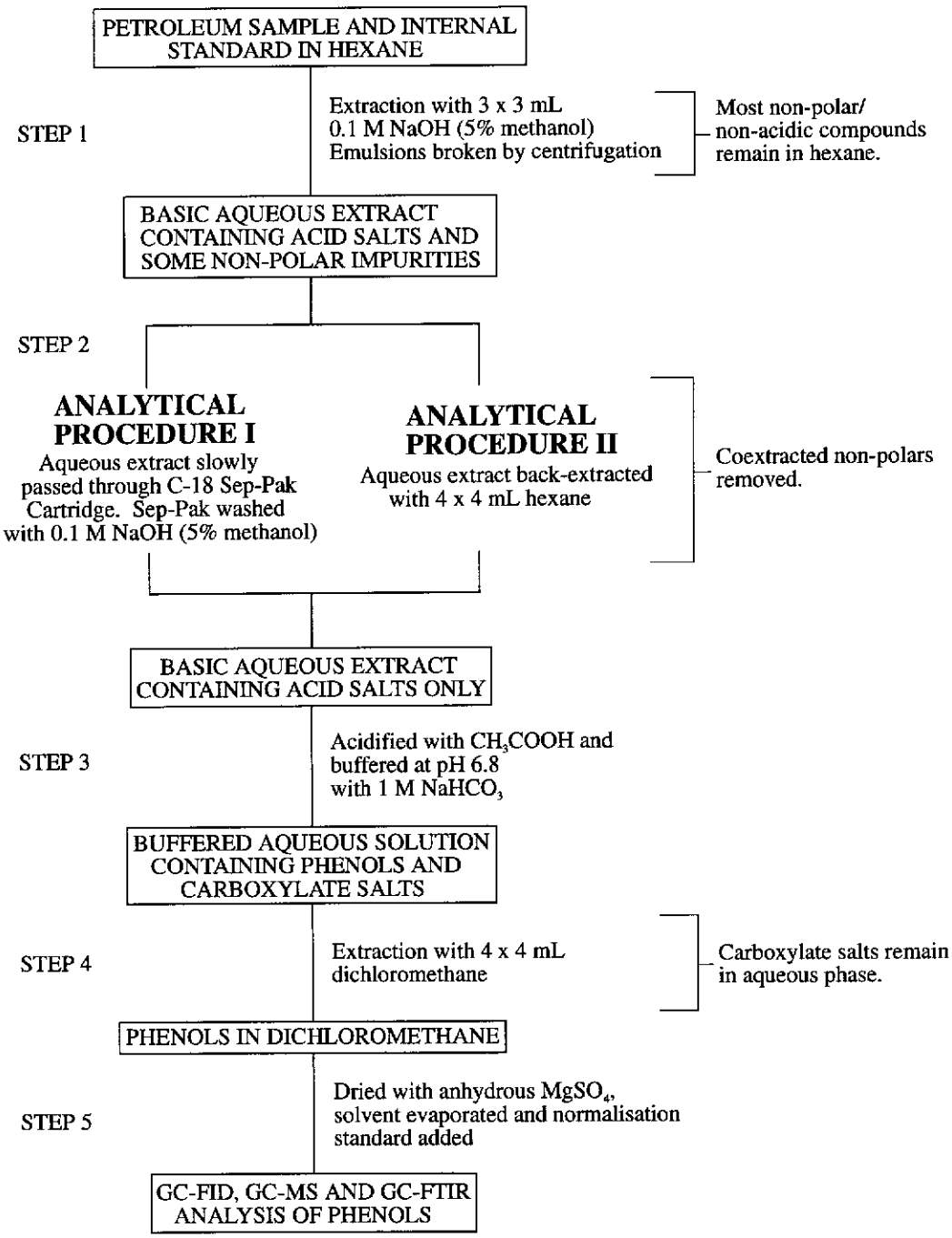


Figure 4.1 Flow diagram of the analytical procedures for extracting phenols from crude oils and sedimentary rock extracts and pyrolysates.

Separation of phenols and carboxylic acids was effected by exploiting the differences in their acid dissociation constants (pK_a). Table 4.1 shows the pK_a values and the percentage dissociated at pH 6.8 for several phenols and carboxylic acids. The values in Table 4.1 indicate that at pH 6.8 all of the carboxylic acids would be dissociated to greater than 98% whereas the phenols would be dissociated to less than 0.35%. The phenols were therefore recovered by dichloromethane extraction from an aqueous solution buffered at pH 6.8 leaving the carboxylate salts in solution (Steps 3 and 4, Figure 4.1). The phenol concentrates thus obtained were free of carboxylic acids but still contained a lot of non-phenolic compounds, indicating that further modifications were required.

Table 4.1 Acid dissociation constants (pK_a) and percentage dissociation at pH 6.8 for phenols and carboxylic acids in aqueous solution at 25°C (unless otherwise stated).

Compound	pK_a	% Dissociated at pH=6.8[c]
4-Methylphenol	10.26[a]	0.035
2,5-Dimethylphenol	10.22[a]	0.038
2-Isopropylphenol	10.49[b]	0.020
2-Isopropyl-5-methylphenol	10.62[b] (20°C)	0.015
1-Naphthol	9.30[a] (20°C)	0.32
4-Methoxy-2,6-dimethylphenol	10.84[b]	0.009
4-Methyl-1,3-benzenediol	9.46[b] (pK_1)	0.22
Octanoic acid	4.89[a]	98.8
Benzoic acid	4.20[a]	99.7
1-Naphthoic acid	3.70[a]	99.9
Adipic acid	4.42[a] (pK_1)	99.6

a Dean, 1992, b Serjeant and Dempsey, 1985,
c Calculated using $pH = pK_a + \log [PhO^-]/[PhOH]$.

Modification B - Increasing the Polarity of the Sodium Hydroxide Solution

MacCrehan and Brown-Thomas (1987) reported that a high concentration of methanol (60%) in Step 1 (Figure 4.1) was critical in order to prevent emulsions. This was found to be correct for some samples; however the high

methanol content also significantly decreased the polarity of the aqueous methanolic sodium hydroxide solution, resulting in the coextraction of significant amounts of non-acidic compounds from the crude oil. By reducing the methanol concentration to 5%, substantially less non-acidic material was coextracted resulting in an extract clean enough to allow trace levels of alkylphenols to be analysed by GC. When emulsions occurred it was essential that a clear aqueous methanolic sodium hydroxide extract was obtained by breaking the emulsion using centrifugation (5-30 min) before proceeding to Step 2. Analytical procedure I (Figure 4.1) shows an outline of the modified procedure.

Table 4.2 shows the recoveries obtained for 31 C_0-C_4 alkylphenols in hexane solution using procedure I. These values were obtained by carrying out extractions on two standard mixtures of phenols in hexane in order to avoid co-elution problems in the GC analysis step. The percentage recoveries obtained for C_0-C_3 alkylphenols and 2-nitrophenol (used as an internal standard) were in the range 75-95%. The values for the C_4 alkylphenols, however, are considerably lower as they are recovered at less than 30%. The low recoveries for the phenols with C_4 alkyl substituents were due to the retention of their phenolate salts on the C-18 phase of the Sep-Pak cartridge. Such an effect is presumably due to the greater lipophilic nature of the more alkylated phenols. The recoveries of these phenols were greatly increased by washing the Sep-Pak cartridge with a solution containing a higher proportion of methanol (>15% methanol); however, the higher methanol content caused other interfering components to be eluted from the cartridge, resulting in an extract that gave a complex pattern of peaks when analysed by GC. Such a modification was therefore not useful for rectifying the problem of low recoveries of the C_4 alkylphenols.

Modification C - Replacing the C-18 Sep-Pak Cartridge with a Solvent Back-Extraction Step

Attempts to increase the recoveries of C_4 alkylphenols were made by back-extracting the aqueous methanolic alkaline extract with an organic solvent, thereby eliminating the need for the use of the C-18 Sep-Pak column (Figure 4.1, analytical procedure II, Step 2). The very good solvent properties of dichloromethane initially made it an attractive candidate for use as the back-extracting solvent, however this feature was also found to be responsible for the unacceptably low recoveries of some *ortho* substituted alkylphenols. Table 4.2 shows the phenol recoveries obtained using a dichloromethane back-extraction step (procedure II, dichlor B/E). Although many alkylphenols were efficiently recovered and the recoveries of some C_4 alkylphenols were increased up to 65%, the recoveries of hindered phenols were greatly reduced. For example 2,6-dimethylphenol was recovered at 60%, 2,3,6-trimethylphenol and 2,4,6-trimethylphenol at 35% and 50% respectively, 2-isopropylphenol at 70% and 2-isopropyl-6-methylphenol at only 3%. The percentages of phenols in the dichloromethane phase were also determined and are shown in parentheses in Table 4.2. These results show that all the phenolate salts of the compounds in the standard mixture were extracted to some degree by the dichloromethane extract, however the hindered salts were lost to the greatest extent. For example, 25% of 2,6-dimethylphenol, 55% and 40% of 2,3,6-trimethylphenol and 2,4,6-trimethylphenol respectively, 20% of 2-isopropylphenol and 90% of 2-isopropyl-6-methylphenol were recovered from the dichloromethane back-extract.

The extraction of organic acids such as acetic acid and oxalic acid into organic solvents such as chloroform, heptane and *o*-xylene using alkylamines has been reported (Marcus and Kertes, 1969). They reported that the mechanism by which this occurs is the extraction of ion-associates or ion-pairs, for example

Table 4.2 Measured recoveries of reference C_0-C_4 alkylphenols and 2-nitrophenol in hexane using three analytical procedures.

Compound	% Recovery		
	Procedure I[a] (Mod A, B)	Procedure II dichlor B/E[b] (Mod A, B, C)	Procedure II hexane B/E[a] (Mod A, B, C)
Phenol	95	85 (0.5)[c]	95
2-Methylphenol	95	85 (3)	95
3-Methylphenol	95	90 (0.5)	95
4-Methylphenol	95	90 (0.4)	95
2-Ethylphenol	90	80 (8)	90
3-Ethylphenol	90	90 (4)	90
4-Ethylphenol	85	90 (4)	90
2,3-Dimethylphenol	85	80 (10)	85
2,4-Dimethylphenol	90	80 (13)	90
2,5-Dimethylphenol	90	80 (7)	90
2,6-Dimethylphenol	90	60 (25)	90
3,4-Dimethylphenol	85	90 (4)	90
3,5-Dimethylphenol	95	90 (3)	90
2,3,6-Trimethylphenol	75	35 (55)	80
2,3,5-Trimethylphenol	80	65 (33)	80
2,4,6-Trimethylphenol	90	50 (40)	90
3,4,5-Trimethylphenol	90	80 (16)	90
2-Isopropylphenol	80	70 (20)	85
3-Isopropylphenol	80	85 (7)	85
4-Isopropylphenol	80	80 (9)	85
2-Isopropyl-6-methylphenol	15	3 (90)	65
2-Isopropyl-4-methylphenol	25	20 (80)	80
2-Isopropyl-3-methylphenol	15	20 (80)	65
2-Isopropyl-5-methylphenol	30	30 (70)	80
4-Isopropyl-2-methylphenol	30	40 (60)	80
5-Isopropyl-2-methylphenol	20	45 (55)	80
3-Isopropyl-5-methylphenol	30	60 (40)	85
3-Isopropyl-2-methylphenol	25	40 (60)	80
4-Isopropyl-3-methylphenol	30	60 (40)	80
3-Isopropyl-4-methylphenol	30	65 (35)	80
2-Nitrophenol	95	99 (<0.1)	95

a Outlined in Figure 4.1,
b Similar to procedure II (Figure 4.1) however using a dichloromethane back extraction in Step 2,
c Values in parentheses are the percentages of phenols recovered from the dichloromethane back extraction phase.

di-butylammonium acetate, into the organic solvent. A similar mechanism is suggested to account for the extraction of sodium alkylphenoxide ion-pairs into dichloromethane when using procedure II-dichlor B/E. Sodium phenoxide is soluble in organic solvents such as ethanol and acetone illustrating that such species do have an affinity for relatively polar solvents (Weast, 1974). As dichloromethane is a solvent used for polar organic compounds (Kirk-Othmer, 1985), it would probably also be a good solvent for phenolate salts, particularly at the low concentrations (ppm) these phenols were present. The increased extraction of phenols with alkyl groups in the *ortho* positions is suggested to be due to steric hindrance where the ionic part of the phenolate salts is shielded by the lipophilic alkyl groups thereby rendering these species less polar and thus increasing their solubility in the organic phase.

The suitability of a less polar solvent for the back extraction step in place of dichloromethane was assessed. Analytical procedure II (Figure 4.1) shows an outline of the procedure which includes a hexane back extraction step. Table 4.2 (procedure II) shows the recoveries obtained for 31 alkylphenols using this procedure. The recoveries for C_0-C_3 alkylphenols are all greater than 80% and more importantly, the *ortho* substituted phenols are no longer discriminated against with the lowest recovered at 65% (2-isopropyl-6-methylphenol). The recoveries for the C_4 alkylphenols were also greatly increased, with all but two having recoveries of greater than 80%. This procedure therefore resulted in the efficient extraction of C_4 alkylphenols without the selective discrimination of hindered alkylphenols.

Analytical procedure II was also employed to measure the recoveries of some representative higher molecular weight phenols. C_5 Alkylphenols, indanol, tetralinol, cyclohexylphenol, phenylphenol, phenanthrol and methoxyphenols were all recovered at greater than 85% (Table 4.3), and therefore analytical procedure II appears efficient for the analysis of these phenol groups. The percentage

recoveries shown in Table 4.3 however, indicate that this procedure was not efficient for analysing 2,4-di-*tert*-butyl-5-methylphenol and 4-nonylphenol; 95% and 80% respectively of which were recovered from the standard hexane solution indicating that virtually none of their phenolate salts were extracted from hexane. The steric hindrance caused by the large *ortho tert*-butyl substituents in 2,4-di-*tert*-butyl-5-methylphenol and the presence of the long lipophilic alkyl chain in 4-nonylphenol are likely to be responsible for the increased solubility of these species in hexane.

Table 4.3 Measured recoveries of standard C_5-C_9 alkylphenols, bicyclicphenols and methoxyphenols from hexane using procedure II (Figure 4.1).

Compound	% Recovery
4-*Sec*-butyl-2-methylphenol	85
5-*Sec*-butyl-2-methylphenol	85
3-*Sec*-butyl-2-methylphenol	85
2,4-Di-*tert*-butyl-5-methylphenol	<0.1
4-Nonylphenol	15
5-Indanol	90
1-Tetralinol	90
2-Naphthol	90
4-Cyclohexylphenol	90
3-Phenylphenol	90
9-Phenanthrol	80
2-Methoxy-4-methylphenol	85
4-Methoxyphenol	95

Reproducibility of and Errors Associated with Analytical Procedure II

The reproducibility with which alkylphenols were extracted using procedure II was estimated by carrying out six separate analyses of standard C_0-C_4 alkylphenols in hexane solution (Table 4.2 shows the alkylphenols measured). Percentage errors were calculated as 1.65 times the standard deviation divided by the average recovery for the six recovery measurements. The values calculated in this manner statistically correspond to a 90% confidence limit. The percentage

errors were determined to be approximately 10% for all of the alkylphenols measured. Although these errors are based on the recovery of alkylphenols from standard phenol mixtures in hexane it is suggested that they are a good estimation of the errors involved in crude oil quantification since high recoveries of the internal standard (>85%), which were comparable to those obtained for the standard mixtures, were measured using 1-chloro-octadecane as a normalisation standard in the analysis of most crude oils (refer to Chapter 2, Section 2.7).

Sample Types Analysed Using Procedures I and II

Whenever possible analytical procedure II was used to isolate phenols from sedimentary rock pyrolysates and crude oil samples as it could be used to efficiently extract a wide range of phenols (Table 4.2 and Table 4.3). Even though analytical procedure I resulted in relatively low recoveries of C_4 alkylphenols it was found necessary to use the LC column (C-18 Sep-Pak) when analysing phenols in low rank coal extracts and some crude oils, particularly biodegraded oils. In these samples, severe emulsions were often formed which resulted in the need for the Sep-Pak cartridge in order to obtain phenol isolates suitable for GC analysis.

4.2 GC ANALYSIS OF DERIVATISED AND UNDERIVATISED ALKYLPHENOLS

Chemical derivatisation has been reported to enhance the GC resolution of complex phenolic mixtures (Green *et al.*, 1986) and to decrease peak tailing in the chromatograms of naturally occurring phenols (Casteele *et al.*, 1976; Vanhaelen and Vanhaelan-Fastre, 1980). Green *et al.* (1986) carried out a comparative study of the GC resolution obtained for a complex mixture of petroleum phenols when chromatographed in the free, silylated and acylated form. These authors reported

that only two C_2 alkylphenols and four C_3 alkylphenols were resolved in GC-MS analysis of an underivatised phenol fraction, whereas six C_2 phenols and nine C_3 phenols were resolved after derivatisation of the fraction. This improved resolution was even more evident in the analysis of higher molecular weight hydroxyaromatics due to the increased number of isomers. The major drawback with derivatisation techniques however, is that they can result in low and variable derivatisation efficiencies and the presence of reaction byproducts such as trifluoroacetic acid which need to be removed before analysis (Casteele *et al.*, 1976; Green *et al.*, 1986). Green *et al.* (1986) measured the derivatisation efficiencies of a range of phenols when silylated, using a mixture of N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) and trimethylchlorosilane, and acylated using trifluoroacetyl chloride (TFACl) and showed that hindered phenols, such as 2,4-dimethyl-6-*tert*-butylphenol, were derivatised to less than 50%. Due to problems such as these which are often associated with derivatisation, the potential for direct GC analysis of underivatised phenols was investigated in this study.

The chromatographic behaviour and elution order of underivatised alkylphenols on BP1, BP5 and DB1701 capillary columns were determined. Figure 4.2 shows that alkylphenols could be chromatographed without derivatisation on the three column phases with minimal tailing. Refer to Table 4.4 for peak identifications. The resolution and elution order of phenols on the BP1 and BP5 columns are similar; however, the elution order of 2-isopropylphenol (peak 14) and 2,4,6-trimethylphenol (peak 15) are readily reversed on the BP5 column with a slight increase in carrier gas flow rate. 3,5-Dimethylphenol and 3-ethylphenol (peaks 10 and 11) were the only compounds in the standard mixture that co-eluted on the BP1 and BP5 phases. Therefore, in contrast to the previous report (Green *et al.*, 1986), good resolution can be obtained for underivatised C_2 and C_3 alkylphenols. The DB1701 column was less effective than the less polar phases and resulted in co-elution of several compounds. For example,

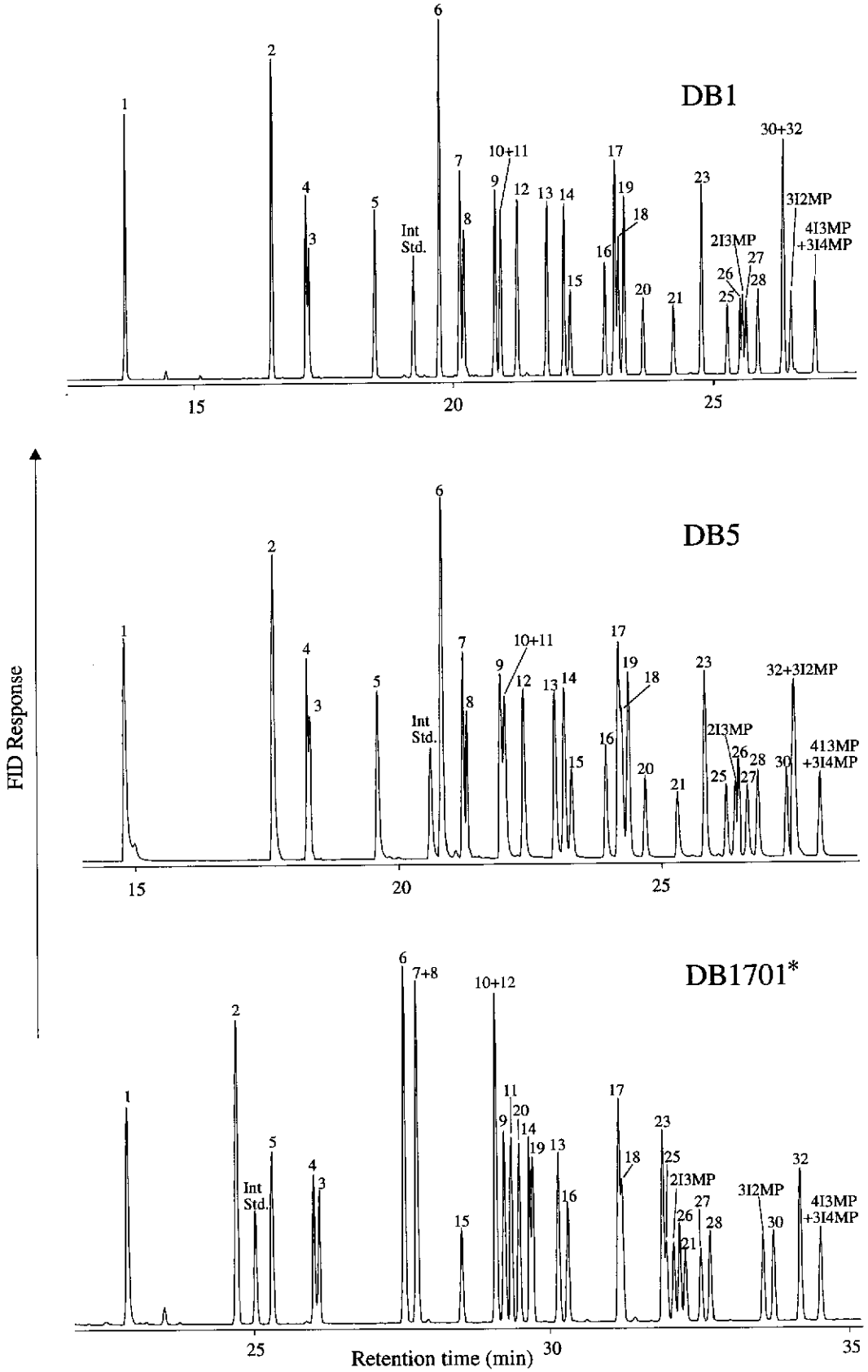


Figure 4.2 Gas chromatograms of a standard mixture of alkylphenols obtained on BP1, BP5 and DB1701 capillary columns. Refer to Table 4.4 for peak identifications. Int Std. = internal standard, xIyMP = x-isopropyl-y-methylphenol.
* Concentration of 3-ethylphenol was greater is the mixture analysed using DB1701 column.

2,4-dimethylphenol and 2,5-dimethylphenol (peaks 7 and 8) and 3,5-dimethylphenol and 2,3-dimethylphenol (peaks 10 and 12) co-eluted, making it unsuitable for the analysis of these methylphenols. The DB1701 column did however give improved resolution of 3-methylphenol and 4-methylphenol (peaks 3 and 4) and completely resolved 3-ethylphenol (peak 11) from 3,5-dimethylphenol (peak 10) . The retention times of the hindered alkylphenols (*ortho* substituted) were decreased relative to the other phenols on the polar DB1701 column; for example, the retention times of 2,6-dimethylphenol (peak 5), 2,4,6-trimethylphenol (peak 15) and 2,3,6-trimethylphenol (peak 19) were decreased. The major drawback in the GC analysis of underivatised phenols was found to be the frequency with which the capillary column became active. This resulted in deterioration in chromatography (peak tailing) which was rectified by removing the front end of the column.

The good resolution achieved for standard phenols in hexane was also evident in the chromatograms of the phenol isolates obtained from crude oils. Figure 4.3 is an example of a gas chromatogram obtained from the phenol extract of crude oil. Clearly the petroleum phenols were chromatographed with minimal tailing and good resolution similar to that observed in the GC analysis of the standard mixture. Further examples of the good chromatography achieved for petroleum alkylphenols can be seen throughout the rest of this thesis.

Table 4.4 Phenols identified in crude oils and sedimentary rock pyrolysates. Compounds 31, 33-50 and b-h were identified only in crude oils. Peaks listed in this table correspond to peaks labelled in all of the proceeding chromatograms unless otherwise stated. * Denoted compounds not previously identified in crude oil, a MacCrehan and Brown-Thomas 1987, b Green *et al.*, 1986.

Peak label	Compound	Method of Identification.	Ref.
1	Phenol	CC (1-3), MS1	a
2	2-Methylphenol	CC (1-3), MS1	a
3	3-Methylphenol	CC (1-3), MS1	a
4	4-Methylphenol	CC (1-3), MS1	a
5*	2,6-Dimethylphenol	CC (1-3), MS1	
6*	2-Ethylphenol	CC (1-3), MS1	
7*	2,4-Dimethylphenol	CC (1-3), MS1	
8*	2,5-Dimethylphenol	CC (1-3), MS1	
9*	4-Ethylphenol	CC (1-3), MS1	
10*	3,5-Dimethylphenol	CC (1-3), MS1	
11*	3-Ethylphenol	CC (1-3), MS1	
12*	2,3-Dimethylphenol	CC (1-3), MS1	
13*	3,4-Dimethylphenol	CC (1-3), MS1	
14*	2-Isopropylphenol	CC (1-3), MS1	
15*	2,4,6-Trimethylphenol	CC (1-3), MS1	
16*	2-*n*-Propylphenol	CC (1-3), MS1	
17*	4-Isopropylphenol	CC (1-3), MS1	
18*	3-Isopropylphenol	CC (1-3), MS1	
19*	2,3,6-Trimethylphenol	CC (1-3), MS1	
20*	2-Isopropyl-6-methylphenol	CC (1-3), MS1, IR	
21*	4-*n*-Propylphenol	CC (1-3), MS1	
22*	2,4,5-Trimethylphenol or 2,3,4-trimethylphenol	MS2	
23*	2,3,5-Trimethylphenol	CC (1-3), MS1	
24*	2-*Sec*-butylphenol	CC (2), MS1	
25*	2-Isopropyl-4-methylphenol	CC (1-3), MS1, IR	
26*	2-Isopropyl-5-methylphenol (thymol)	CC (1-3), MS1, IR	
27*	4-Isopropyl-2-methylphenol	CC (1-3), MS1, IR	
28*	5-Isopropyl-2-methylphenol (carvacrol)	CC (1-3), MS1, IR	
29*	4-*Sec*-butylphenol	CC (2), MS1	
30*	3-Isopropyl-5-methylphenol	CC (1-3), MS1, IR	
31*	2-*Sec*-butyl-6-methylphenol	CC (1-2), MS1	
32*	3,4,5-Trimethylphenol	CC (1-3), MS1	
33*	5-Indanol	CC (2), MS1	
34*	4-Isopropyl-2,6-dimethylphenol	MS2	

Table 4.4 continued.

Peak label	Compound	Method of Identification	Ref.
35*	2-*Sec*-butyl-4-methylphenol	CC (1-2), MS1	
36*	3-Isopropyl-2,6-dimethylphenol	MS2	
37*	2-*Sec*-butyl-5-methylphenol	CC (1-2), MS1	
38*	4-*Sec*-butyl-2-methylphenol	CC (1-2), MS1, IR	
39*	5-*Sec*-butyl-2-methylphenol	CC (1-2), MS1	
40*	3-*Sec*-butyl-5-methylphenol	CC (1-2), MS1	
41*	1-Tetralinol	CC (2), MS1	
42*	2-Tetralinol	CC (2), MS1	
43	1-Naphthol	CC (2), MS1	a
44*	2-Phenylphenol	CC (2), MS1	
45	2-Naphthol	CC (2), MS1	a
46*	2-Cyclohexylphenol	CC (2), MS1	
47*	4-Cyclohexylphenol	CC (2), MS1	
48*	3-Phenylphenol	CC (2), MS1	
49*	4-Phenylphenol	CC (2), MS1	
50*	9-phenanthrol	CC (2), MS1	
C_x	Alkylphenols	MS2	
a*	Ethylmethylphenols	MS2	
b_x	Alkylphenylphenol	MS2	b
c_x	Indanol	MS2	b
d_x	Alkylindanol	MS2	b
e_x	Alkyltetralinol	MS2	b
f_x	Alkylnaphthol	MS2	b
g_x	Fluorenol	MS2	b
h_x	Phenanthrol / Anthracol	MS2	b

CC: Co-chromatography with reference compounds by capillary GC (1=BP1, 2=BP5, 3=DB1701)
MS1: Comparison of mass spectrum with that of a reference compound
MS2: Comparison of mass spectrum with literature spectrum
IR: Comparison of infrared spectrum with that of a reference compound (crude oils only)
x: Number of alkyl substituents.

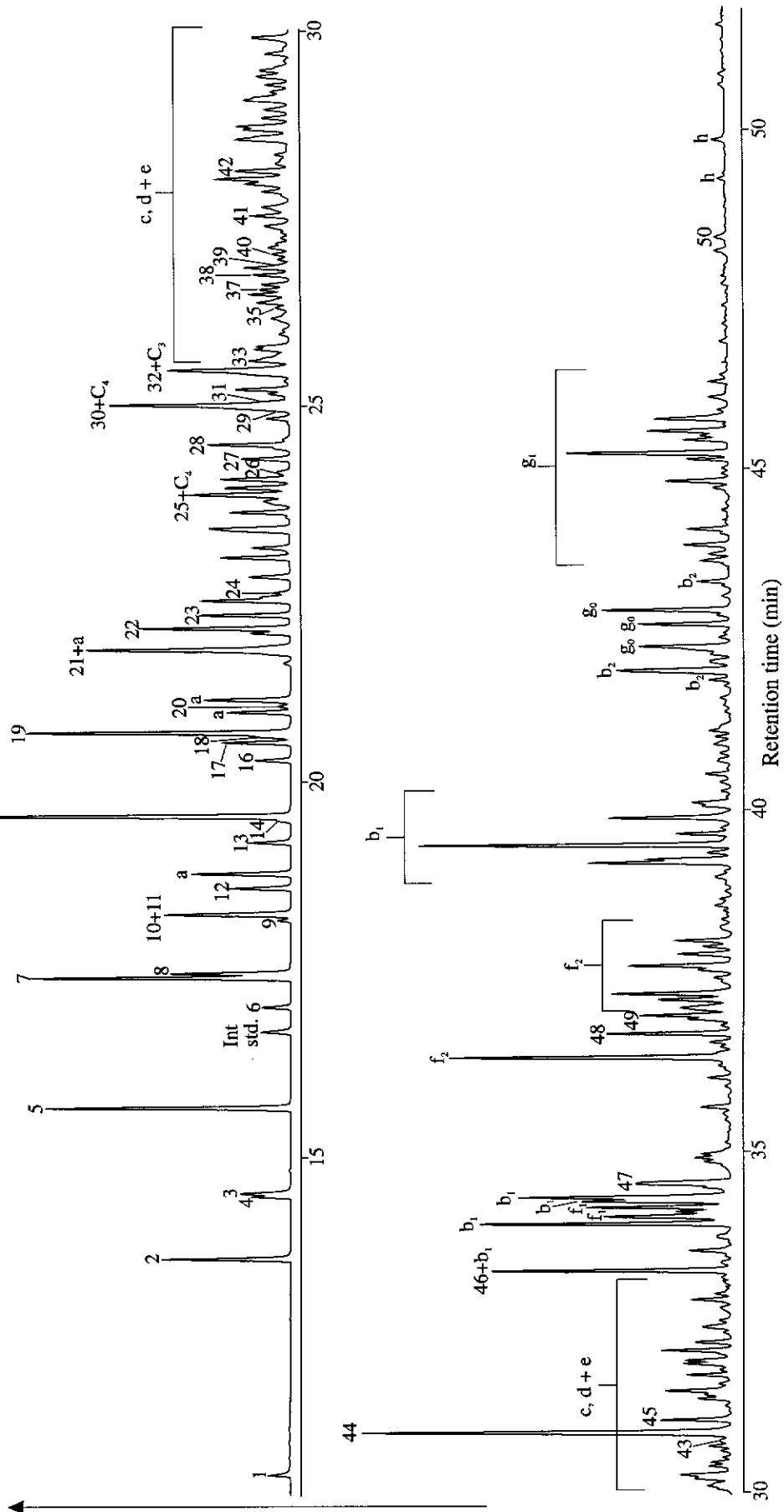


Figure 4.3 Gas chromatogram of the phenol extract of Caroline-1 crude oil obtained using a BP5 capillary column. Refer to Table 4.4 for peak identifications.

4.3 IDENTIFICATION OF PHENOLS IN SEDIMENTARY ORGANIC MATTER

4.3.1 Reference Compounds

Reference compounds used for the identification of phenols in sedimentary organic matter were obtained from either commercial sources or synthesised. Table 4.5 shows a list of the phenol reference compounds obtained from commercial sources.

Table 4.5 Phenol reference compounds obtained from commercial sources.

Compound	Compound
Phenol	2-*tert*-Butylphenol
2-Methylphenol	3-*tert*-Butylphenol
3-Methylphenol	4-*tert*-Butylphenol
4-Methylphenol	2-Isopropyl-5-methylphenol (thymol)
2,3-Dimethylphenol	3-Isopropyl-5-methylphenol
2,4-Dimethylphenol	4-Isopropyl-3-methylphenol
2,5-Dimethylphenol	5-Isopropyl-2-methylphenol (carvacrol)
2,6-Dimethylphenol	2,4-di-*tert*-butyl-5-methylphenol
3,4-Dimethylphenol	5-indanol
3,5-Dimethylphenol	1-tetralinol
2-Ethylphenol	2-tetralinol
3-Ethylphenol	1-naphthol
4-Ethylphenol	2-naphthol
2,3,5-Trimethylphenol	2-Cyclohexylphenol
2,3,6-Trimethylphenol	4-Cyclohexylphenol
2,4,6-Trimethylphenol	2-Phenylphenol
3,4,5-Trimethylphenol	3-Phenylphenol
2-*n*-Propylphenol	4-Phenylphenol
4-*n*-Propylphenol	9-phenanthrol
2-Isopropylphenol	3-methoxyphenol
3-Isopropylphenol	4-methoxyphenol
4-Isopropylphenol	2-methoxy-4-methylphenol
2-*sec*-Butylphenol	
4-*sec*-Butylphenol	

Six isopropylmethylphenols were prepared using the schemes outlined in Figure 4.4. Reaction schemes (I), (II), (III) and (IV) were adaptations of various literature procedures to the synthesis of these isopropylmethylphenols. Reaction schemes (V) and (VI) were previously reported syntheses of the two remaining isomers. The structure of each reaction product was confirmed using NMR and FTIR techniques and comparing melting points with literature data. Figure 4.5 a) shows the partial total ion chromatogram of an all-isomer mixture of reference isopropylmethylphenols.

Sec-butylmethylphenols were prepared as mixtures via Friedel-Crafts alkylation reactions similar to those reported for the preparation of isopropylmethylphenols (Carpenter and Easter, 1955). *Sec*-butylation of the three cresols resulted in the mixtures of *sec*-butylmethylphenols shown in Figure 4.6. It is apparent from the reaction products that alkylation of *ortho*-cresol occurred at all free ring positions (scheme I), *para*-cresol was also alkylated at all free ring positions (scheme II), and *meta*-cresol was alkylated at three of the four free ring positions (scheme III). By analogy with the results obtained by Carpenter and Easter (1955), where 2-isopropyl-3-methylphenol was not produced from the isopropylation of *m*-cresol, it was inferred that 2-*sec*-butyl-3-methylphenol was the isomer not produced in the *sec*-butylation of *m*-cresol. The very low yield of this isomer is presumably due to the steric hindrance at the two position of *meta*-cresol.

In order to determine the GC elution order of the *sec*-butylmethylphenols, peaks in the gas chromatograms of each reaction mixture were assigned by comparison with the retention behaviour of the isopropylmethylphenols (Figure 4.5 a and b). Figure 4.5 a) shows that earlier eluting isopropylmethylphenols have substituents in *ortho*-positions; that is, the phenols with the larger alkyl group (isopropyl) in the *ortho*-position have the shortest retention times, the phenols with an *ortho*-methyl substituent were the next to elute, and phenols with no *ortho*-substituents have the longest retention times. On this basis Figure 4.5 b) shows

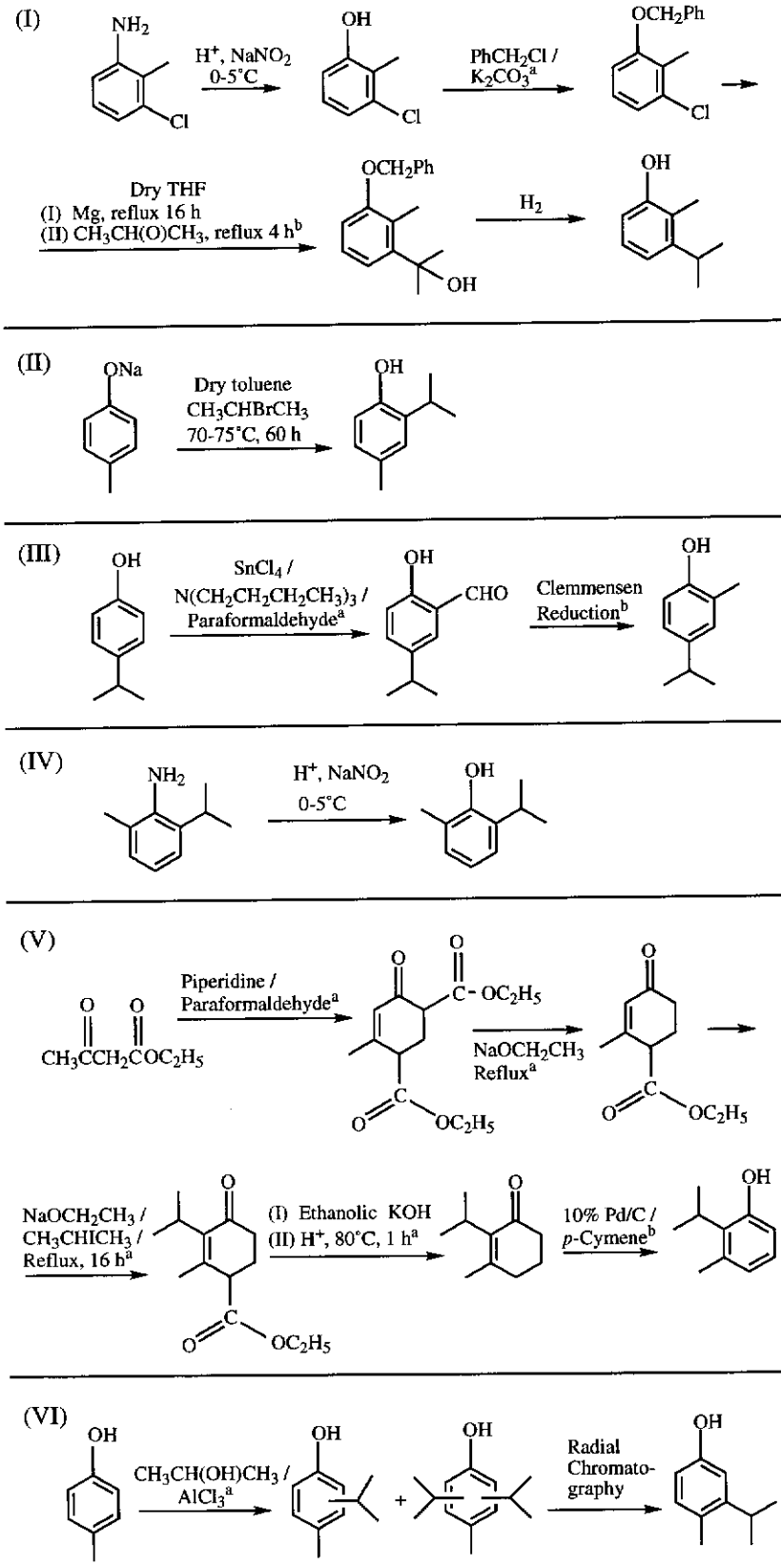


Figure 4.4 Synthetic schemes used to synthesise isopropylmethylphenols.

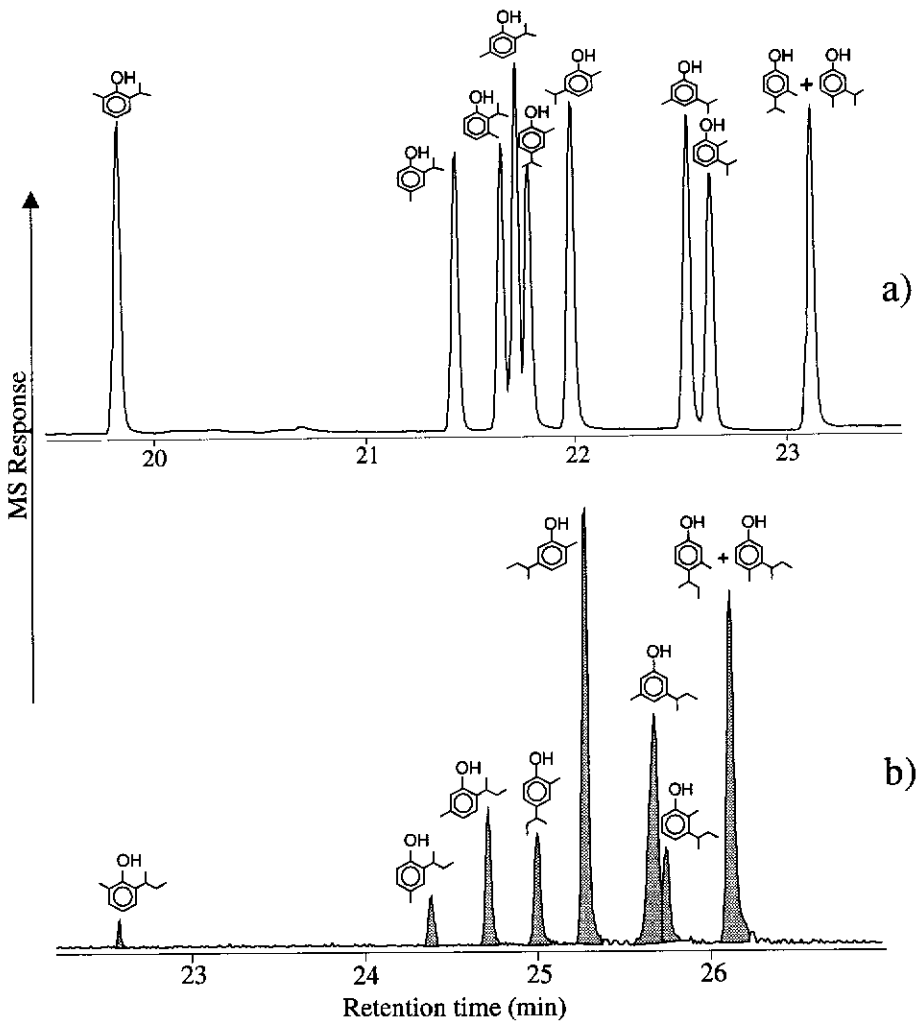


Figure 4.5 Partial total ion chromatograms of a) an all-isomer mixture of reference isopropylmethylphenols and b) a mixture of *sec*-butylmethylphenols obtained using a BP1 capillary column.

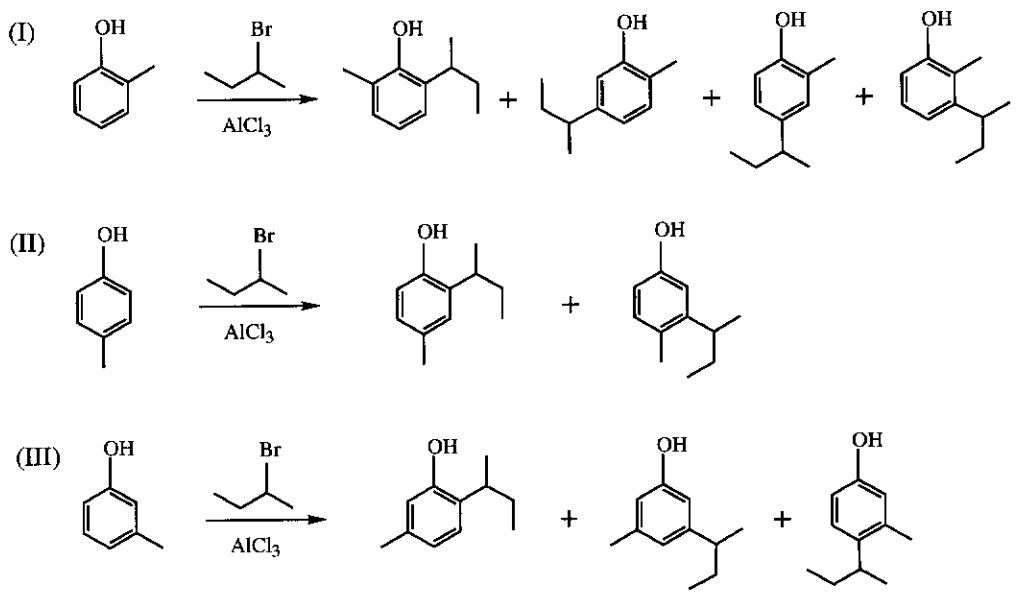


Figure 4.6 Reaction schemes for synthesis of *sec*-butylmethylphenols using Friedel-Crafts alkylation.

that the first eluting *sec*-butylmethylphenol was assigned as that which contains two *ortho*-substituents (2-*sec*-butyl-6-methylphenol) followed by for example 2-*sec*-butyl-4-methylphenol and then 4-*sec*-butyl-2-methylphenol, and 3-*sec*-butyl-4-methylphenol was the last to elute. The remaining assignments were based on retention order, by analogy with the chromatographic behaviour of the corresponding isopropylmethylphenols.

The structural assignments for the *sec*-butylmethylphenols were confirmed by comparison of the FTIR spectra of isopropylmethylphenols and those of similarly substituted *sec*-butyl-methylphenols. The substitution patterns of aromatic compounds are often determined using the highly characteristic C-H out-of-plane bending absorbances at 800-900 cm^{-1} (Shrewsbury, 1960; Nakanishi and Solomon, 1977). The position of the out-of-plane bending absorbances of alkylphenols are sensitive to the size of the substituents, therefore the two bulky groups (isopropyl and *sec*-butyl) are required to be at similar ring positions for meaningful comparison (Shrewsbury, 1960). For example, 2-*sec*-butyl-5-

methylphenol has similar out-of-plane bending absorbances to 2-isopropyl-5-methylphenol, but not 5-isopropyl-2-methylphenol even though both isopropylmethylphenols have a 2,4-dialkyl substitution pattern (Shrewsbury, 1960). Figure 4.7 and Figure 4.8 (a and c) show partial solid-state FTIR spectra of three reference isopropylmethylphenol isomers and the correspondingly substituted *sec*-butylmethylphenols. Dotted lines have been included for each set of spectra to highlight similar bands in the 600-1000 cm^{-1} region. Similarities in the FTIR spectra of each *sec*-butylmethylphenol with the corresponding isopropylmethylphenol therefore confirmed the earlier assignments made on the basis of chromatographic behaviour.

4.3.2 Phenols Identified in Crude Oils

In this study a large number of phenols has been identified in crude oils using several methods of identification. Table 4.4 is a comprehensive list of the phenols found in crude oils and the methods with which they were identified. The range of compounds identified in crude oils is illustrated in Figure 4.3 which shows the gas chromatogram of the phenols isolated from Caroline crude oil. This crude oil was selected because it contains most of the compounds listed in Table 4.4. The most tentative assignments were based on the comparison of the mass spectra of the petroleum phenols with those of literature spectra (Table 4.4; MS2). Such assignments were made when reference compounds were not available. The compounds assigned this way include some C_3-C_5 alkylphenols, alkyl-phenylphenols, alkylindanols and alkyltetralinols. Compound 22 was assigned as either 2,4,5-trimethylphenol or 2,3,4-trimethylphenol based on mass spectral features indicating that it was a trimethylphenol (high molecular ion; m/z 136 compared with M-15; m/z 121), and the fact that it was not one of the remaining isomers for which reference compounds were available. A second group of 48 phenols (compounds 1-21 and 23-50), 42 of which have not been previously

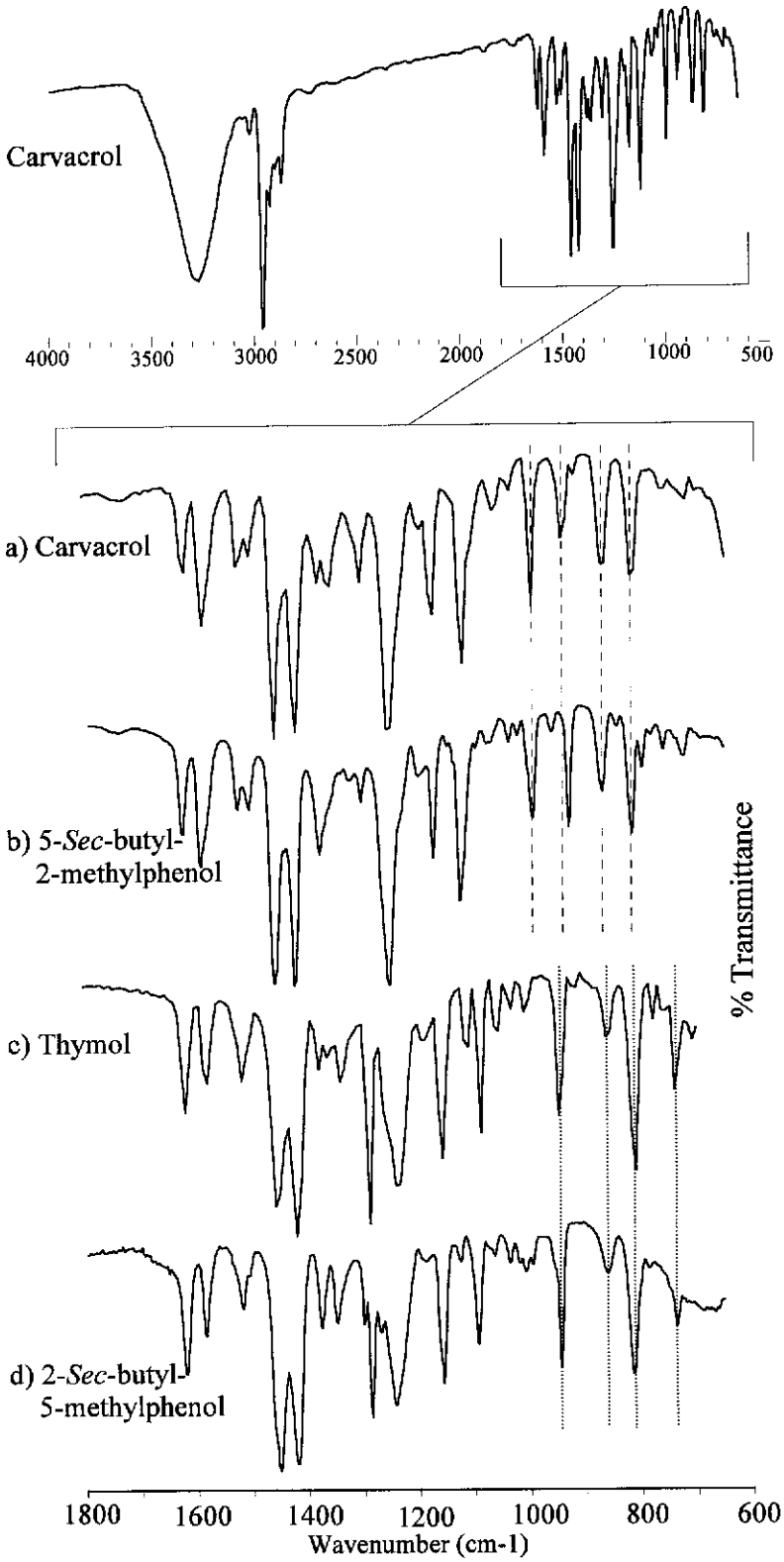


Figure 4.7 Partial infrared spectra of reference isopropylmethylphenols (a, c) and the similarly substituted *sec*-butylmethylphenols (b, d) obtained using direct deposition GC-FTIR.

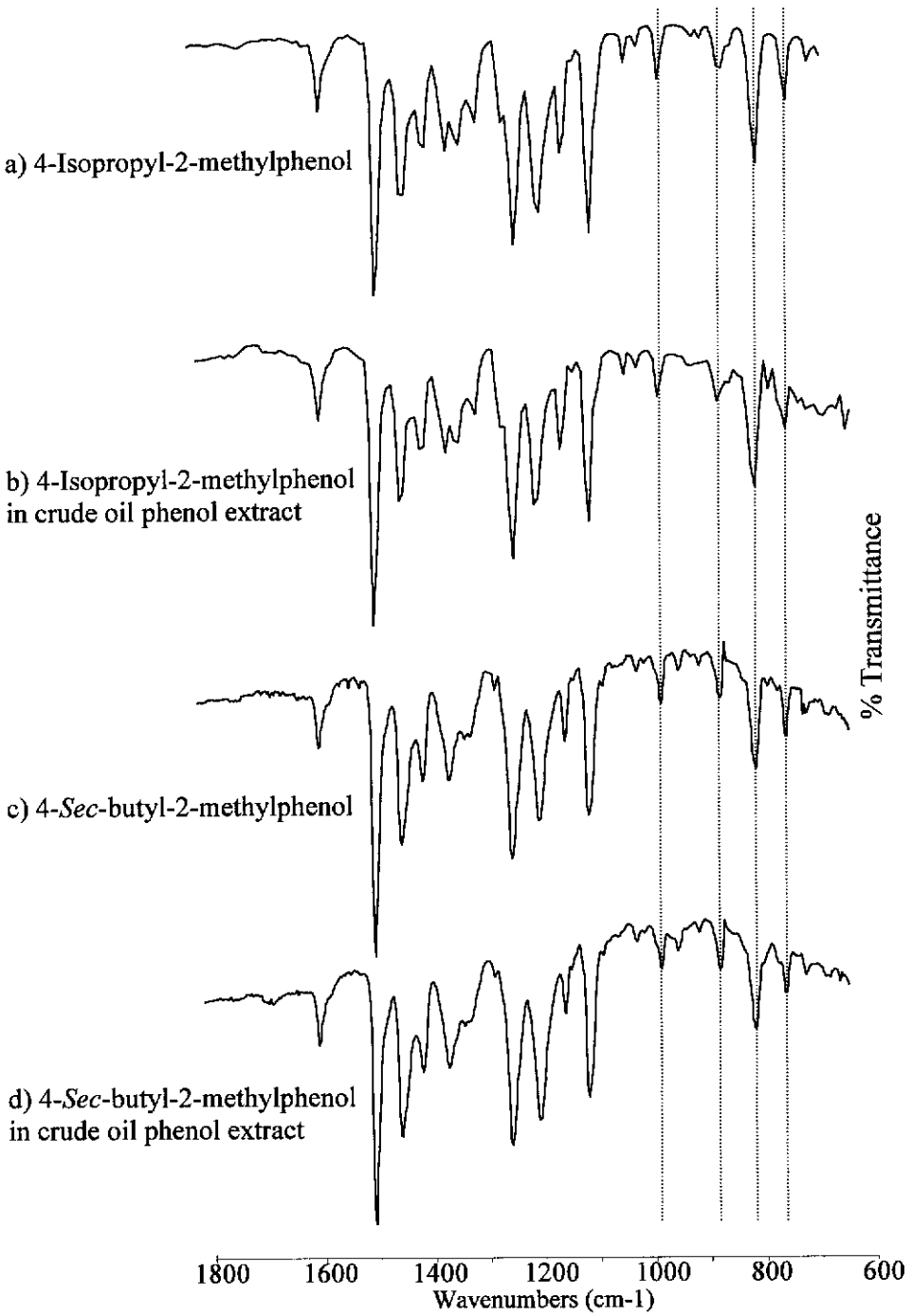


Figure 4.8 Partial infrared spectra of reference compounds a) 4-isopropyl-2-methylphenol and c) 4-*sec*-butyl-2-methylphenol and compounds in the phenol extract of crude oil (b, d) obtained using direct deposition GC-FTIR.

reported in crude oils, were identified by co-chromatography with reference compounds on a BP1, BP5 and/or DB1701 capillary column (Table 4.4; CC 1, 2 and 3). The alkylphenols which are the focus of this study, phenols 1-40 were co-chromatographed with reference compounds (excluding 22) on at least two capillary column phases. Comparison of the mass spectra of these petroleum phenols with those obtained from reference compounds further supported their identification (Table 4.4; MS1). The structures of a third group of phenols were confirmed using direct deposition GC-FTIR techniques (Table 4.4; IR). The FTIR spectra of these petroleum alkylphenols were compared to those of reference compounds. The following two sections present in more detail the identification of this third group of compounds, isopropylmethylphenols and *sec*-butylmethylphenols in crude oils.

Identification of Isopropylmethylphenols in Crude Oils

Six of the 10 isopropylmethylphenol isomers were identified in crude oils by co-chromatography with reference compounds on three stationary phases, and comparison of their mass spectra and GC-FTIR spectra with those of reference compounds. Figure 4.9 shows the partial gas chromatograms of the phenol extract from Basker crude oil and a mixture of reference isopropylmethylphenols. Although the compounds corresponding to peaks labelled (x) in Figure 4.9 b) have similar retention times to those of reference compounds, comparison of their mass spectra showed they were not isopropylmethylphenols. These isomers whose structures are shown in Figure 4.9 a) are characterised by having the alkyl groups on adjacent ring positions. Nesterova *et al.* (1989) reported that compounds with two or more alkyl substituents at the neighbouring carbon atoms of an aromatic ring, including phenols, have low thermodynamic stabilities. Their low stabilities may therefore be responsible for the absence of the four isopropylmethylphenols with adjacent alkyl groups in the crude oils analysed in this study.

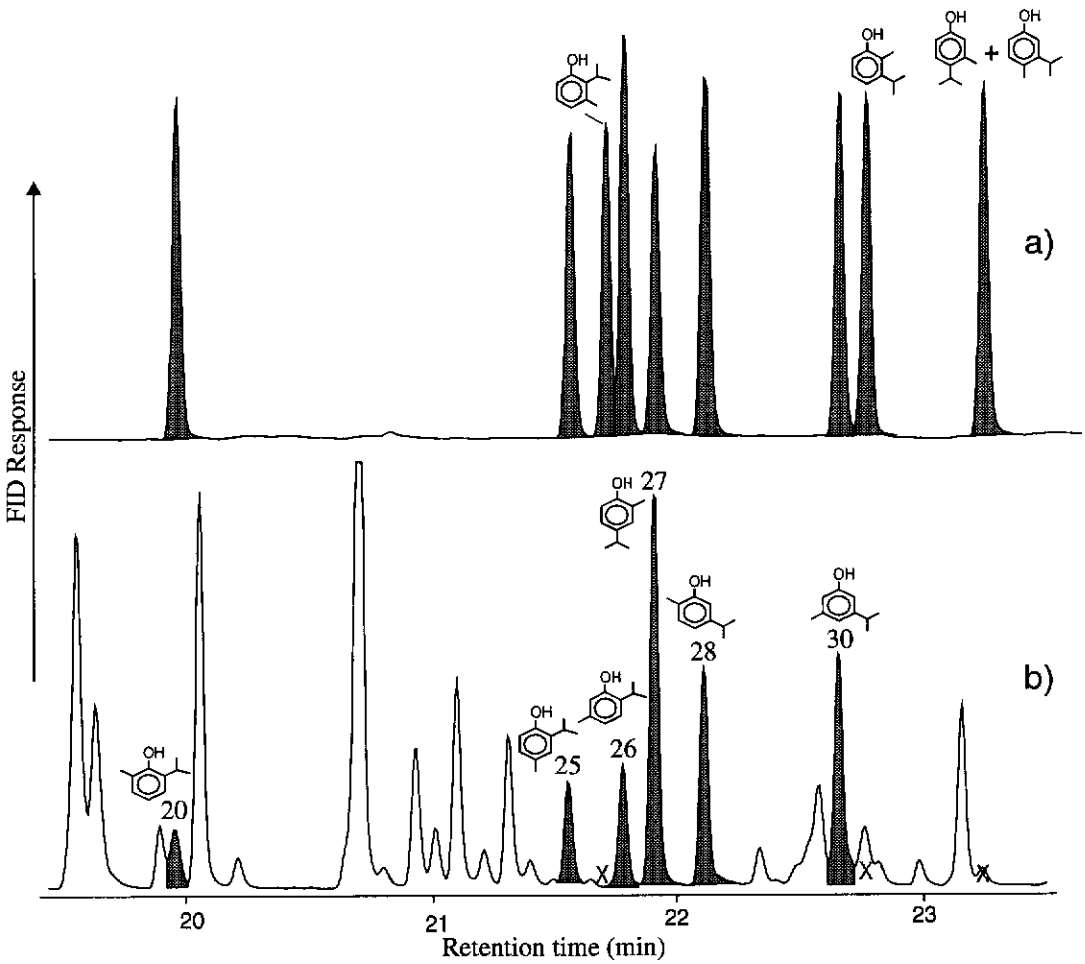


Figure 4.9 Partial gas chromatograms of a) a mixture of reference isopropylmethylphenols and b) the phenol extract from a crude oil, obtained using a BP5 capillary column. Refer to text for discussion of X.

A comparison of GC-MS and GC-FTIR techniques for the identification of the isopropylmethylphenols in phenol extracts was carried out. Figure 4.10 shows the mass spectrum of carvacrol, which is typical of that of all the other isopropylmethylphenols, and that of an unknown C4 alkylphenol present in crude oil. It is clear that the two mass spectra are very similar, both having a base peak at m/z 135 (M-15) and a strong molecular ion at m/z 150. Using mass spectrometry one therefore cannot differentiate between isopropylmethylphenols and some other C4 alkylphenols in crude oils, nor is it possible to unambiguously identify individual isopropylmethylphenol isomers. Figure 4.8 (a, b) and Figure 4.11 show the partial FTIR-spectra (650-1700 cm^{-1}) of some of the geochemically significant isopropylmethylphenols obtained during direct deposition GC-FTIR analysis of reference compounds and a crude oil phenol extract. In contrast to the mass spectra of the isopropylmethylphenols, their infrared spectra are complex and each isopropylmethylphenol has a characteristic infrared spectrum with several diagnostic bands, examples of which are indicated by the dotted lines on the spectra. Comparison of the spectra of the reference compounds and the petroleum isopropylmethylphenols clearly provides an unambiguous method for identifying these compounds in crude oils.

Identification of *Sec*-butylmethylphenols in Crude Oil

Six of the 10 *sec*-butylmethylphenol isomers were identified by co-chromatography on two column phases and by the comparison of their mass spectra and FTIR spectra (4-*sec*-butyl-2-methylphenol only) with those of reference compounds. Selected ion chromatograms of a reference mixture and a phenol extract from a crude oil are shown in Figure 4.12. As was the case with the isopropylmethylphenols, the isomers not identified in the crude oils studied were

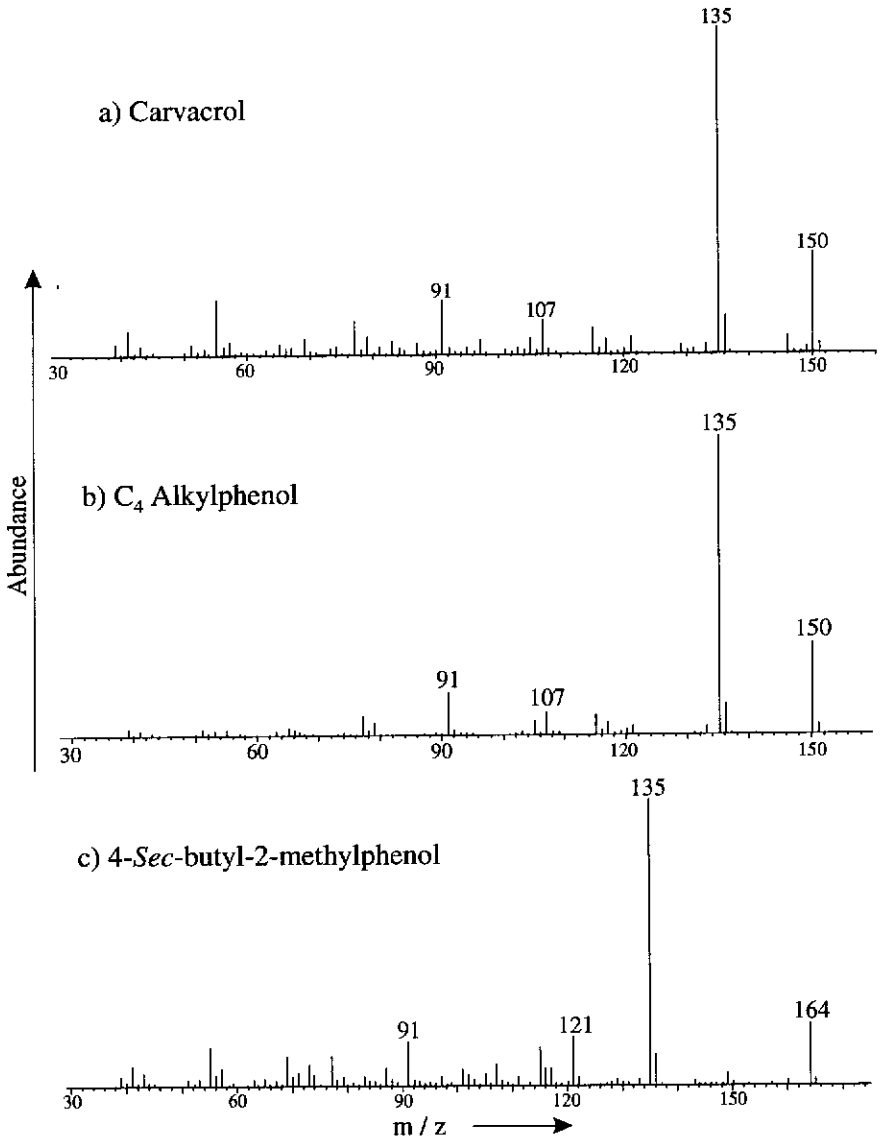


Figure 4.10 The mass spectra of C_4 and C_5 alkylphenols.

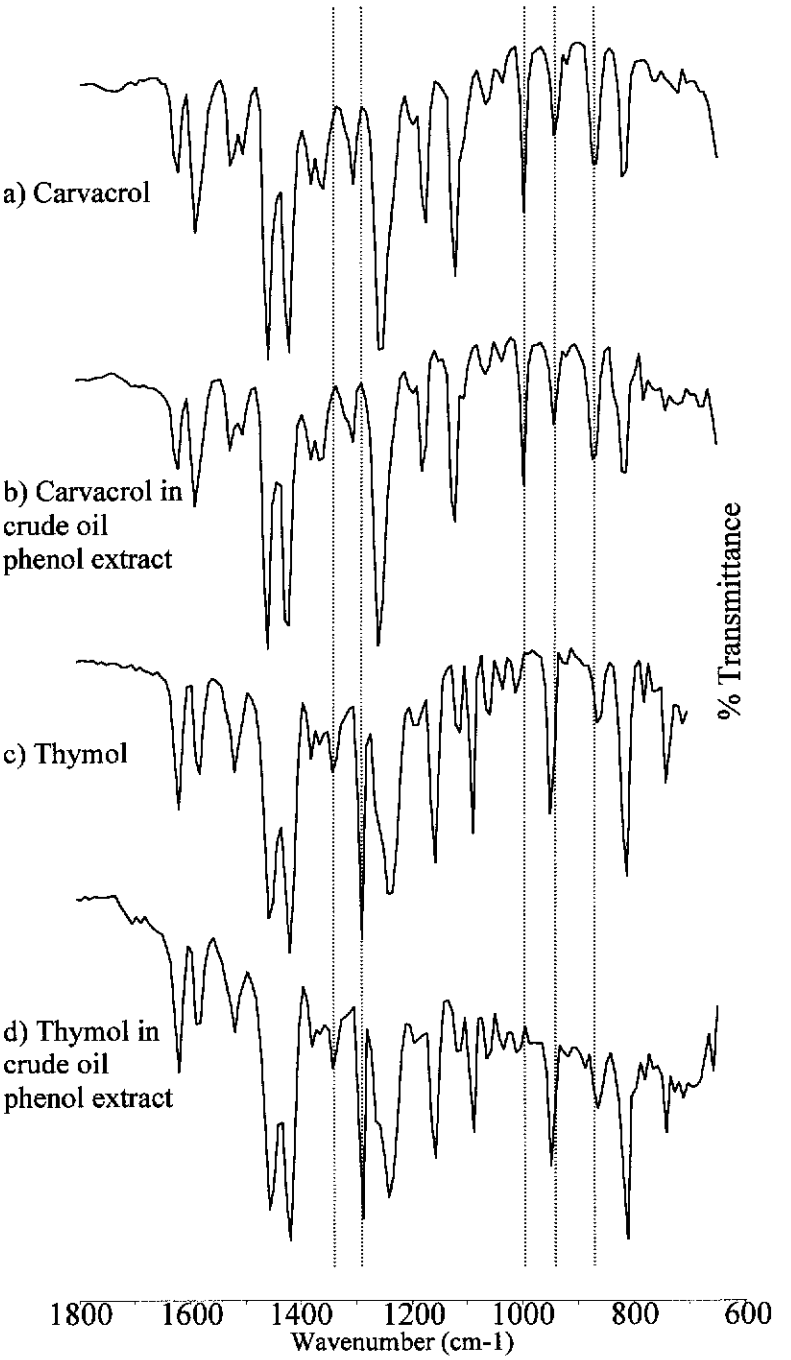


Figure 4.11 Infrared spectra of reference compounds a) carvacrol and c) thymol and compounds in the phenol extract of a crude oil (b, d) obtained using direct deposition GC-FTIR.

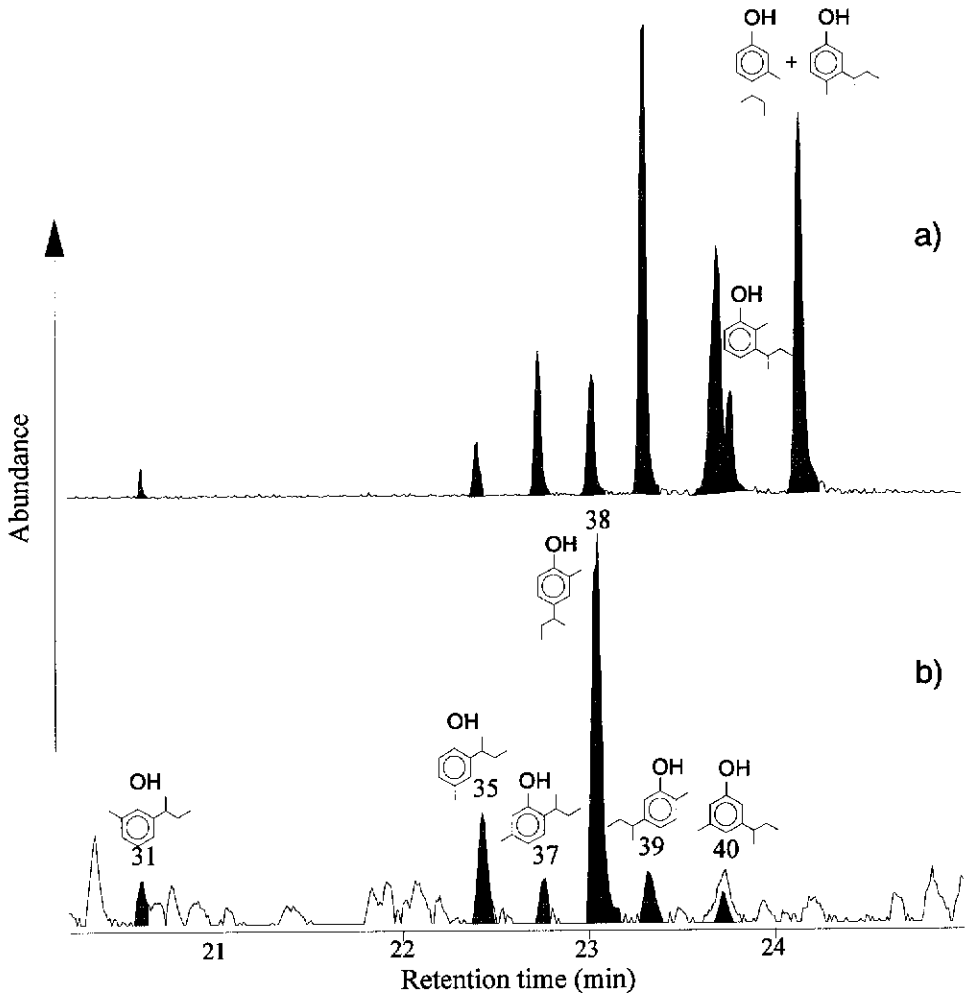


Figure 4.12 Partial m/z 135 mass chromatograms of a) a mixture of reference *sec*-butylmethylphenols and b) the phenol extract of a crude oil, obtained using a BP1 capillary column.

those with adjacent alkyl groups (Figure 4.12 a) which are suggested to have low thermodynamic stabilities (Nesterova *et al.*, 1989). Peak number 40 represents co-eluting compounds, and examination of the mass spectrum revealed that approximately 50% of the components represented by this peak was due to 3-*sec*-butyl-5-methylphenol (co-eluted with C_4 alkylphenol; % determined using parent ion m/z 164).

In contrast to mass spectrometry GC-FTIR techniques provided unambiguous identification of 4-*sec*-butyl-2-methylphenol in crude oils. Figure 4.10 shows the mass spectrum of 4-*sec*-butyl-2-methylphenol. The presence of a base peak at m/z 135 (M-29) and a molecular ion at m/z 164 in the mass spectrum is typical of all other *sec*-butylmethylphenol isomers and therefore isomeric *sec*-butylmethylphenols cannot be differentiated using mass spectrometry. In contrast to their mass spectra, their infrared spectra are complex and characteristic and provide an unambiguous method of identification for these compounds. Therefore because of its high relative abundance in crude oils (Figure 4.12; peak 38) additional evidence as to the identity of 4-*sec*-butyl-2-methylphenol was obtained from its solid state FTIR spectrum. The infrared spectra of 4-*sec*-butyl-2-methylphenol and the compound present in a crude oil are shown in Figure 4.8 (c, d). Clearly the two spectra are very similar and therefore confirm the presence of 4-*sec*-butyl-2-methylphenol in the crude oil extract. FTIR spectra free of interferences could not be obtained for other *sec*-butylmethylphenol isomers in crude oils due to their lower relative abundances and the presence of co-eluting compounds.

4.3.3 Phenols Identified in Sedimentary Rock Pyrolysates and Extracts

The bound phenolic components of sedimentary rocks were investigated using pyrolysis-GC-MS and hydrous pyrolysis. Flash pyrolysis GC-MS enabled the rapid identification of alkylphenols in the pyrolysate by a comparison of their mass spectra and retention times with those of reference compounds; however, due to the very low relative abundances of C_3-C_4 alkylphenols in the flash pyrolysates the identification of such compounds was found to be ambiguous using this technique. In order to study the C_3-C_4 alkylphenols in more detail preparative scale hydrous pyrolysis of rocks was carried out and the alkylphenols isolated using analytical procedure II (Figure 4.1). Figure 4.13 shows the partial total ion chromatogram of a phenol extract thus obtained. Twenty six alkylphenols were identified in the coal pyrolysates by co-chromatography with authentic standards (Table 4.4; CC). Comparison of the mass spectra of the phenols in the extracts with those obtained from authentic standards further supported their identification (Table 4.4; MS1). Although phenol and the cresols are well known components of sedimentary rocks (e.g. Allan and Larter, 1981), the xylenols and 4-ethylphenol have only recently been reported in sedimentary rock pyrolysates (Hatcher *et al.* 1992; Hartgers *et al.* 1994; Veld *et al.* 1994). The remaining 16 compounds have not been previously reported in sedimentary rock pyrolysates.

Phenols were isolated from the dichloromethane extract of low rank coals (Loy Yang and Heartbreak coals) using analytical procedure I and analysed by GC-MS. The major components in the phenol extracts were assigned as hydroxybenzaldehydes and hydroxymethoxybenzaldehydes based on mass spectral data; phenol and the cresols were also present in some extracts, but in lower relative abundances. A detailed discussion on the phenols in these coal extracts is provided in Chapter 6 (Section 6.1).

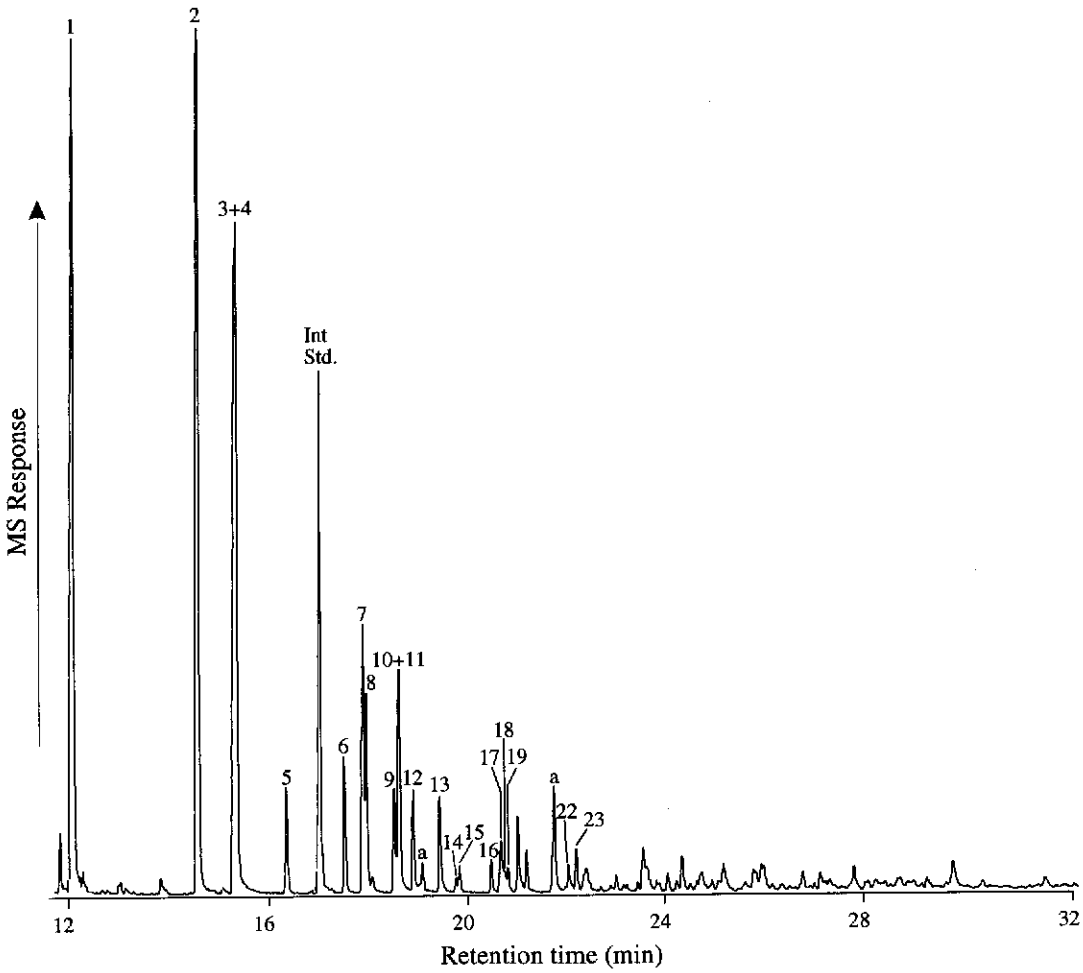


Figure 4.13 Partial total ion chromatogram of the phenol extract from the hydrous pyrolysate of GK coal (2258-2262m). Refer to Table 4.4 for peak identifications.

4.4 QUANTIFICATION OF C_0-C_4 ALKYLPHENOLS IN CRUDE OILS

The quantification of phenols in crude oil was performed using an internal standard method and phenol peak areas from the gas chromatograms of crude oil phenol extracts. Standards containing accurately known amounts of internal standard and phenol analytes in hexane were subjected to the analytical procedures to determine correction factors. The correction factors obtained therefore reflect not only differences in FID and MS responses but also chemical and physical differences of phenols which are responsible for different extraction efficiencies. The phenols in the majority of crude oils were quantified using GC analysis on a BP1/DB1 phase capillary column due to the superior resolution of alkylphenols on this phase (refer to Section 4.2). Peak areas from the addition of the m/z 109 and 139 mass chromatograms (*o*-nitrophenol, internal standard), and the parent peak and base peak of alkylphenols were used for the analysis of crude oils quantified using GC-MS.

Alkylphenol concentrations were calculated using Equation I:

$$[P]_{oil} = \frac{P_{oil}}{Int_{oil}} \times \frac{[P]_{std}}{P_{extd\ std} / Int_{extd\ std}} \times \frac{1}{Mass\ oil} \quad \text{..... (I)}$$

$[P]_{oil}$ = concentration of phenol P in the oil (ng/g)
P_{oil} = peak area of phenol P in the crude oil extract
Int_{oil} = peak area of the internal standard in the crude oil extract
$[P]_{std}$ = concentration of phenol P in the standard solution (ng/g)
$P_{extd\ std}$ = peak area of phenol P in the extracted standard solution
$Int_{extd\ std}$ = peak area of the internal standard in the extracted standard solution
Mass oil = mass of crude oil extracted (g)
$[P]_{std} / (P_{extd\ std} / Int_{extd\ std})$ = correction factor for phenol P

The concentrations of C_0-C_4 alkylphenols in a broad range of crude oils have been measured in this study (Table 4.6). The percentage errors associated with the values in Table 4.6 are estimated to be approximately 10% (the calculation of these percentage errors is discussed in Section 4.1-Reproducibility of and Errors Associated with Analytical Procedure II). Table 4.6 shows that alkylphenols were analysed down to a limit of detection of 10 ng/g (using GC-MS analysis). Individual compounds occur in crude oils in the range 10 - 190×10^3 ng/g and the total concentration of C_0-C_4 alkylphenols in crude oils range from 427 - 1091×10^3 ng/g. The values in parentheses in Table 4.6 are the relative amounts (%w/w) of alkylphenols among similarly substituted isomers. The concentrations of 3-ethylphenol and 3,5-dimethylphenol were calculated for Group 4 crude oils (Chapter 5; Section 5.5) by analysis on a DB1701 column phase on which they were resolved. Some alkylphenols were below the limit of detection (nd), and in some crude oils which contained very low levels of alkylphenols, the concentrations of some compounds could not be obtained due to insufficient resolution and/or ambiguous identifications (Table 4.6; IR, e.g. Rough Range).

4.5 CRUDE OIL SAMPLE CONTAMINATION

In view of the low concentrations of alkylphenols in crude oils it is necessary to address the possibility of sample contamination. Sample contamination could occur during recovery of crude oils from the reservoir, during storage, or during analysis in the laboratory. In order to assess contamination during analysis, blanks were measured by subjecting the internal standard (2-nitrophenol, 1 μg) in hexane to the analytical procedures. The chromatograms obtained showed only minor peaks in comparison to that of the internal standard and it was estimated that the maximum non-phenolic contribution to the values

Table 4.6 Concentrations of C_0-C_4 alkylphenols in crude oils (nanogram/gram)[a].

Compound	C1 Nilam	C2 Crude oil A	C3 Crude oil B	C4 Crude oil C	C5 Crude oil D
Phenol	5600	3800	7500	4300	24000
2-Methylphenol	17000 (63)[b]	180 (14)	830 (16)	35000 (69)	160000 (65)
3+4-Methylphenol	10000 (37)	1100 (86)	4400 (84)	16000 (31)	85000 (35)
2-Ethylphenol	1800 (66)	52 (13)	180 (12)	8000 (65)	13000 (60)
4-Ethylphenol	940 (34)	51 (13)	280 (20)	4400 (35)	8600 (40)
3-Ethylphenol		290 (74)	1000 (68)		
3,5-Dimethylphenol	12000 (20)[c]	1900 (98)	4800 (91)	24000 (25)	58000 (12)
2.6-Dimethylphenol	13000 (22)	nd	nd	10000 (11)	97000 (21)
2,4-Dimethylphenol	17000 (28)	nd	82 (1)	30000 (32)	160000 (34)
2,5-Dimethylphenol	13000 (21)	nd	240 (5)	20000 (21)	100000 (21)
2,3-Dimethylphenol	3000 (5)	nd	43 (1)	6100 (6)	30000 (6)
3,4-Dimethylphenol	2300 (4)	41 (2)	120 (2)	4300 (5)	27000 (6)
2,3,6-Trimethylphenol	4200 (23)	nd	400 (100)	3500 (25)	20000 (33)
2,3,5-Trimethylphenol	7200 (39)	nd	nd	10000 (71)	24000 (39)
2,4,6-Trimethylphenol	7100 (38)	nd	nd	570 (4)	17000 (28)
2-Isopropylphenol	4000 (27)	32 (1)	450 (5)	7300 (26)	12000 (25)
3-Isopropylphenol	5600 (39)	2200 (93)	6700 (70)	8500 (31)	15000 (31)
4-Isopropylphenol	4900 (34)	150 (6)	2400 (25)	12000 (43)	21000 (44)
2-Isopropyl-6-methylphenol	6000 (12)	nd	nd	6300 (6)	23000 (10)
2-Isopropyl-4-methylphenol	4600 (9)	140 (3)	180 (2)	9400 (9)	23000 (10)
2-Isopropyl-5-methylphenol	2700 (5)	170 (3)	360 (3)	4800 (5)	10000(5)
4-Isopropyl-2-methylphenol	18000 (35)	380 (8)	1100 (10)	43000 (43)	98000 (45)
5-Isopropyl-2-methylphenol	10000 (20)	160 (3)	370 (4)	15000 (15)	39000 (18)
3-Isopropyl-5-methylphenol	10000 (19)	4100 (83)	8600 (81)	22000 (22)	26000 (12)
Total (x 10^{-3})	180	14.8	40.0	304	1091

a) Alkylphenols were below detection limit in Tuna-4 (1400.5 m), West Seahorse, Lakes Entrance, Windalia, Crude oil Q, Husky Mobil Rainbow, Mirbelia, Dodonea and Jamison crude oils. Data not available for isopropylmethylphenols in Crude oil L, West Terrace and Crude oil R, b) Values in parentheses are relative amounts (%w/w) among similar substituted isomers, c) Sum of 3-ethylphenol and 3,5-dimethylphenol, nd = not detected, IR = insufficiently resolved to obtain meaningful measurement.

Table 4.6 continued.

Compound	C6 Crude oil E	C7 Iron Duke	C8 Basker-1	C10 Tuna 2820 m	C13 Caroline-1
Phenol	27000	230	3500	4000	14000
2-Methylphenol	13000 (36)	210 (17)[b]	5100 (58)	9800 (59)	47000 (54)
3+4-Methylphenol	23000 (64)	1000 (83)	3700 (42)	6900 (41)	40000 (46)
2-Ethylphenol	2800 (21)	130 (46)	380 (58)	750 (51)	12000 (62)
4-Ethylphenol	2400 (18)	150 (54)	270 (42)	710 (49)	7300 (38)
3-Ethylphenol	8200 (61)				
3,5-Dimethylphenol	12800 (76)	570(73)	2900 (27)	4300 (14)	60000 (17)
2.6-Dimethylphenol	nd	nd	1100 (10)	6300 (21)	88000 (24)
2,4-Dimethylphenol	410 (2)	25 (3)	2600 (25)	10000 (33)	110000 (30)
2,5-Dimethylphenol	1600 (9)	67 (9)	2700 (26)	6000 (20)	49000 (13)
2,3-Dimethylphenol	820 (5)	46 (6)	740 (7)	1900 (6)	33000 (9)
3,4-Dimethylphenol	1300 (8)	73 (9)	530 (5)	1800 (6)	25000 (7)
2,3,6-Trimethylphenol	nd	39 (14)	260 (18)	2200 (23)	140000 (37)
2,3,5-Trimethylphenol	310 (100)	240 (86)	1100 (75)	2500 (26)	48000 (13)
2,4,6-Trimethylphenol	nd	nd	110 (7)	5000 (51)	190000 (50)
2-Isopropylphenol	7400 (21)	110 (10)	760 (31)	510 (17)	4600 (9)
3-Isopropylphenol	13000 (37)	680 (61)	580 (24)	690 (23)	16000 (30)
4-Isopropylphenol	15000 (42)	320 (29)	1100 (45)	1800 (60)	33000 (61)
2-Isopropyl-6-methylphenol	nd	650 (6)	340 (6)	150 (4)	14000 (11)
2-Isopropyl-4-methylphenol	550 (2)	800 (7)	440 (8)	300 (7)	39000 (30)
2-Isopropyl-5-methylphenol	2600 (8)	1500 (13)	450 (9)	900 (22)	11000(8)
4-Isopropyl-2-methylphenol	4100 (13)	860 (8)	2000 (37)	300 (7)	38000 (30)
5-Isopropyl-2-methylphenol	3900 (13)	3700 (32)	920 (17)	1400 (35)	17000 (13)
3-Isopropyl-5-methylphenol	20000 (64)	3900 (34)	1200 (23)	1000 (25)	10000 (8)
Total (x 10^{-3})	160	15.3	32.8	69.2	1046

Table 4.6 continued.

Compound	C14 Lambert-1	C15 Crude oil F	C16 Crude oil G	C17 Crude oil H	C18 Crude oil I
Phenol	780	29	190	1100	1700
2-Methylphenol	690 (46)	16 (19)	530 (61)[b]	1400 (50)	2200 (52)
3+4-Methylphenol	800 (54)	70 (81)	340 (39)	1400 (50)	2000 (48)
2-Ethylphenol	100 (70)	nd	160 (76)	320 (36)	440 (57)
4-Ethylphenol	43 (30)	nd	50 (24)	240 (27)	330 (43)
3-Ethylphenol		10 (100)		330 (37)	
3,5-Dimethylphenol	570 (44)	130 (90)	470 (24)	1270 (55)	1000 (13)
2.6-Dimethylphenol	93 (7)	nd	300 (16)	nd	1400 (19)
2,4-Dimethylphenol	170 (13)	nd	370 (19)	30 (1)	2500 (33)
2,5-Dimethylphenol	240 (18)	14 (10)	470 (24)	420 (18)	1400 (19)
2,3-Dimethylphenol	150 (12)	nd	210 (11)	200 (9)	650 (9)
3,4-Dimethylphenol	82 (6)	nd	120 (6)	390 (17)	560 (7)
2,3,6-Trimethylphenol	35 (38)	nd	170 (28)	58 (39)	1600 (38)
2,3,5-Trimethylphenol	48 (51)	29 (100)	390 (65)	91 (61)	630 (15)
2,4,6-Trimethylphenol	10 (11)	nd	40 (7)	nd	2000 (47)
2-Isopropylphenol	80 (17)	nd	150 (25)	200 (26)	86 (8)
3-Isopropylphenol	220 (48)	130 (83)	160 (27)	210 (28)	450 (40)
4-Isopropylphenol	160 (35)	26 (17)	280 (48)	350 (46)	590 (52)
2-Isopropyl-6-methylphenol	70 (5)	nd	240 (10)	46 (9)	280 (9)
2-Isopropyl-4-methylphenol	110 (8)	nd	410 (17)	37 (8)	460 (15)
2-Isopropyl-5-methylphenol	130 (10)	nd	170 (7)	54 (11)	150 (5)
4-Isopropyl-2-methylphenol	270 (20)	21 (9)	590 (25)	72 (14)	1100 (36)
5-Isopropyl-2-methylphenol	230 (17)	17 (8)	560 (23)	120 (24)	370 (12)
3-Isopropyl-5-methylphenol	540 (40)	190 (83)	420 (18)	170 (34)	700 (23)
Total (x 10^{-3})	5.62	0.682	6.79	8.51	22.6

Table 4.6 continued.

Compound	C19 Crude oil J	C20 Crude oil K	C21 BarrowDeep	C22 Barrow UJ	C23 Crude oil L
Phenol	7500	2400	4800	470	55
2-Methylphenol	12000 (67)	3400 (54)	6900 (58)	3500 (57)[b]	430 (82)
3+4-Methylphenol	5800 (33)	2900 (46)	5000 (42)	2600 (43)	96 (18)
2-Ethylphenol	2200 (52)	310 (63)	990 (61)	850 (57)	130 (90)
4-Ethylphenol	2000 (48)	180 (37)	630 (43)	630 (43)	14 (10)
3-Ethylphenol					
3,5-Dimethylphenol	5000 (32)	1900 (28)	2700 (28)	1000 (4)	210 (18)
2.6-Dimethylphenol	2900 (18)	980 (15)	1400 (14)	6000 (23)	72 (6)
2,4-Dimethylphenol	3700 (23)	1300 (20)	2200 (23)	13000 (49)	130 (11)
2,5-Dimethylphenol	2400 (15)	1300 (20)	2000 (21)	3600 (14)	420 (35)
2,3-Dimethylphenol	910 (6)	760 (11)	670 (7)	1400 (5)	270 (23)
3,4-Dimethylphenol	890 (6)	420 (6)	700 (7)	1400 (5)	92 (8)
2,3,6-Trimethylphenol	nd	360 (30)	350 (31)	4100 (11)	120 (31)
2,3,5-Trimethylphenol	1200 (100)	520 (44)	640 (58)	2700 (8)	250 (66)
2,4,6-Trimethylphenol	nd	310 (26)	120 (11)	29000 (81)	10 (3)
2-Isopropylphenol	640 (26)	330 (21)	140 (12)	260 (11)	58 (27)
3-Isopropylphenol	1000 (40)	540 (35)	500 (42)	nd	79 (37)
4-Isopropylphenol	830 (34)	680 (44)	540 (46)	2200 (89)	75 (36)
2-Isopropyl-6-methylphenol	150 (2)	830 (19)	95(4)	540 (4)	
2-Isopropyl-4-methylphenol	430 (6)	340 (8)	210(9)	2800 (24)	
2-Isopropyl-5-methylphenol	90 (1)	470 (11)	250 (11)	500 (4)	
4-Isopropyl-2-methylphenol	1300 (20)	1400 (32)	420(18)	5300 (44)	
5-Isopropyl-2-methylphenol	1000 (15)	260 (6)	420(18)	1800 (15)	
3-Isopropyl-5-methylphenol	3800 (55)	1000 (24)	930(40)	1100 (9)	
Total (x 10^{-3})	55.7	22.9	32.6	84.8	2.51

Table 4.6 continued.

Compound	C24 Rough Range	C25 Crude oil M	C26 Crude oil N	C27 Crude oil O	C28 Crude oil P
Phenol	90	2000	2300	51	20
2-Methylphenol	57 (61)	250 (28)	8500 (55)	590 (72)	550 (92)[b]
3+4-Methylphenol	37 (39)	630 (72)	6900 (45)	230 (28)	49 (8)
2-Ethylphenol	19 (63)	70 (21)	1800 (51)	130 (65)	140 (82)
4-Ethylphenol	11 (37)	57 (17)	1700 (49)	70 (35)	31 (18)
3-Ethylphenol		210 (62)			
3,5-Dimethylphenol	38 (27)	990 (68)	4000 (32)	240 (9)	180 (7)
2.6-Dimethylphenol	23 (17)	67 (5)	340 (3)	720 (27)	800 (29)
2,4-Dimethylphenol	15 (11)	120 (8)	1300 (10)	910 (34)	930 (34)
2,5-Dimethylphenol	30 (22)	120 (8)	3400 (27)	460 (17)	480 (17)
2,3-Dimethylphenol	13 (9)	55 (4)	1500 (12)	270 (10)	280 (10)
3,4-Dimethylphenol	20 (14)	110 (7)	2100 (16)	96 (3)	96 (3)
2,3,6-Trimethylphenol	IR	55 (21)	1100 (48)	550 (29)	700 (30)
2,3,5-Trimethylphenol	IR	59 (22)	1100 (48)	240 (13)	290 (13)
2,4,6-Trimethylphenol	IR	150 (57)	100 (4)	1100 (58)	1300 (57)
2-Isopropylphenol	IR	250 (11)	820 (17)	84 (22)	58 (16)
3-Isopropylphenol	IR	1600 (69)	2200 (46)	140 (36)	69 (19)
4-Isopropylphenol	IR	460 (20)	1800 (37)	160 (42)	240 (65)
2-Isopropyl-6-methylphenol	nd	44 (3)	880 (11)	72 (11)	90 (13)
2-Isopropyl-4-methylphenol	nd	130 (9)	720 (9)	160 (25)	180 (26)
2-Isopropyl-5-methylphenol	10(13)	96 (7)	520 (7)	130 (20)	150 (21)
4-Isopropyl-2-methylphenol	nd	160 (11)	2600 (34)	170 (27)	160 (23)
5-Isopropyl-2-methylphenol	54(73)	33 (2)	1800 (23)	54 (8)	60 (9)
3-Isopropyl-5-methylphenol	10(14)	960 (67)	1300 (17)	50 (8)	60 (8)
Total (x 10^{-3})	0.427	8.68	48.8	6.68	6.91

Table 4.6 continued.

Compound	C31 Bodalla Sth BJ	C32 Kenmore-1	C33 Moorari-4	C34 Byrock-1	C35 Earlstown-1
Phenol	85	10	40	150	29
2-Methylphenol	180 (55)	48 (58)	2200 (67)	1500 (70)	36 (29)
3+4-Methylphenol	150 (45)	35 (42)	1100 (33)	630 (30)	89 (71)
2-Ethylphenol	22 (55)	11 (50)	270 (59)	130 (69)	10 (50)
4-Ethylphenol	18 (45)	11 (50)	190 (41)	58 (31)	10 (50)
3-Ethylphenol					
3,5-Dimethylphenol	110 (15)	26 (11)	2100 (20)	520 (13)	18 (10)
2.6-Dimethylphenol	160 (22)	45 (19)	1900 (18)	910 (24)	28 (16)
2,4-Dimethylphenol	190 (26)	78 (32)	3100 (29)	1300 (34)	34 (20)
2,5-Dimethylphenol	140 (19)	46 (19)	1800 (17)	760 (20)	70 (41)
2,3-Dimethylphenol	82 (11)	28 (12)	1000 (9)	200 (5)	10 (6)
3,4-Dimethylphenol	55 (7)	18 (7)	800 (7)	170 (4)	12 (7)
2,3,6-Trimethylphenol	120 (43)	IR	1500 (33)	150 (27)	220 (55)
2,3,5-Trimethylphenol	71 (25)	49 (47)	2200 (48)	310 (56)	50 (12)
2,4,6-Trimethylphenol	89 (32)	56 (53)	900 (19)	91 (17)	130 (33)
2-Isopropylphenol	10 (15)	nd	260 (12)	51 (16)	10 (13)
3-Isopropylphenol	26 (39)	IR	1900 (85)	100 (30)	nd
4-Isopropylphenol	31 (46)	IR	60 (3)	180 (54)	68 (87)
2-Isopropyl-6-methylphenol	11 (10)	30 (13)	460 (7)	100 (12)	380 (19)
2-Isopropyl-4-methylphenol	36 (33)	35 (15)	260 (4)	97 (12)	170 (8)
2-Isopropyl-5-methylphenol	5 (4.5)	22 (10)	130 (2)	57 (7)	40 (2)
4-Isopropyl-2-methylphenol	10 (9)	29 (13)	320 (5)	330 (40)	680 (34)
5-Isopropyl-2-methylphenol	42 (39)	94 (42)	3300 (53)	180 (22)	520? (26)
3-Isopropyl-5-methylphenol	5 (4.5)	15 (7)	1800 (29)	61 (7)	230 (11)
Total (x 10^{-3})	1.65	0.686	27.6	8.04	2.84

Table 4.6 continued.

Compound	C36 Lycium-1	C37 Malgoona-1	C38 Sturt-6	C39 West Terrace	C40 Blina-1	C44 Crude oil R
Phenol	12	160	180	270	21	110
2-Methylphenol	790 (69)b	1500 (64)	1300 (66)	250 (46)	55 (56)	140 (54)
3+4-Methylphenol	350 (31)	840 (36)	670 (34)	290 (54)	44 (44)	120 (46)
2-Ethylphenol	54 (64)	710 (63)	220 (61)	190 (100)	47 (53)	46 (44)
4-Ethylphenol	30 (36)	420 (37)	140 (39)	nd	41 (47)	58 (56)
3-Ethylphenol						
3,5-Dimethylphenol	200 (9)	440 (3)	440 (7)	110 (100)	100 (12)	77 (22)
2.6-Dimethylphenol	520 (24)	4000 (29)	1800 (28)	nd	180 (22)	nd
2,4-Dimethylphenol	840 (39)	6200 (45)	2500 (38)	nd	220 (27)	74 (22)
2,5-Dimethylphenol	370 (17)	1900 (14)	1100 (17)	nd	150 (18)	99 (29)
2,3-Dimethylphenol	120 (6)	550 (4)	400 (6)	nd	110 (13)	44 (13)
3,4-Dimethylphenol	110 (5)	690 (5)	300 (4)	nd	68 (8)	48 (14)
2,3,6-Trimethylphenol	140	4300	990	nd	520 (39)	160 (49)
2,3,5-Trimethylphenol	180	2400	750	nd	340 (25)	150 (45)
2,4,6-Trimethylphenol	480	15000	2900	nd	490 (36)	20 (6)
2-Isopropylphenol	nd	550 (8)	330 (25)	nd	59 (29)	79 (30)
3-Isopropylphenol	37 (31)	1500 (22)	110 (8)	nd	23 (11)	180 (70)
4-Isopropylphenol	81 (69)	4900 (70)	880 (67)	nd	121 (60)	nd
2-Isopropyl-6-methylphenol	80 (29)	2800 (11)	1300 (18)		110 (8)	
2-Isopropyl-4-methylphenol	20 (7)	3000 (13)	930 (12)		310 (23)	
2-Isopropyl-5-methylphenol	10 (4)	2500 (10)	440 (6)		90 (7)	
4-Isopropyl-2-methylphenol	70 (25)	13000 (53)	4200 (56)		320 (23)	
5-Isopropyl-2-methylphenol	60 (21)	2400 (10)	430 (6)		90 (7)	
3-Isopropyl-5-methylphenol	40 (14)	790 (3)	190 (2)		430 (32)	
Total (x 10^{-3})	4.59	70.6	22.5	1.11	3.94	1.40

reported for petroleum phenols was 10% (calculated by comparison with Blina-1), however in most cases it was lower than 1%.

Assessing contamination of crude oil during recovery from reservoirs is much more difficult due to the wide range of chemicals used in the drilling and maintenance of wells, and in crude oil production. An attempt has been made to summarise the current chemicals used in such operations in order to assess the likelihood of phenol contamination. Table 4.7 is a list of the common chemicals reported to be used in the oil industry. A careful examination of Table 4.7 shows that no free phenols are commonly used, even amongst classes such as the biocides (as phenols are well known for their antibacterial properties). There are however two polymeric products; ethoxylated phenol resin and lignins which could theoretically degrade to produce phenol monomers. The naturally occurring polymer lignin is primarily comprised of three phenol building units which contain methoxy- and propyl- type substituents. Ethoxylated phenolic resins are prepared from ethylene oxide and phenols with up to three hydroxymethyl ($-CH_2OH$) substituents. Therefore, if the lignins or the ethoxylated phenol resins were to degraded, the dominant contaminants that would be observed in crude oil are likely to be phenols containing methoxy- and hydroxymethyl-substituents. Such compounds have not been identified in any of the crude oils studied to date and therefore contamination from such sources is not evident. Although it is possible that other phenols may be components of patented formulations and have therefore not been reported in the literature, Table 4.7 provides encouragement that the phenolic products known to be used in the oil industry are unlikely to be responsible for the alkylphenols present in crude oil.

Furthermore, crude oils recovered at different times over intervals of many years, from the same reservoir, have been analysed and shown to contain similar distributions and concentrations of alkylphenols. Figure 4.14 shows the gas chromatograms of the phenols isolated from Blina crude oil samples recovered in

Class	Subclass	Common chemicals used	References
Water-based drilling muds	Weighting agents (density modifiers)	Barytes, iron oxides, calcium carbonate	Callaghan (1991) Lundie (1988)
	Viscosifiers	Bentonite, Xanthan gum, Guar gum, acrylic/acrylamide co-polymers	
	Flocculants/ Clay stabilisers	Polymers such as hydrolysed polyacrylamides and cellulose ethers	
	Dispersants	Ferrochrome lignosulphonates, **lignosulphonates and lignins**, sodium polyacrylates and phosphates	
	Fluid loss additives	Carboxymethyl cellulose, polyacrylates, pre-gelatinised starch	
Oil-based drilling muds		Diesel oil emulsions has been superseded by low aromatic mineral oil emulsions. Additives; emulsifiers, wetting agents, detergents	
Cementing		Portland cement. Additives; for better fluidity and setting characteristics	Callaghan (1991)
Completion fluids		Filtered brine. Additives; polymers such as hydroxy-ethylcellulose or polysaccharides	Callaghan (1991)
Demulsifiers		Ethylene oxide (EO) condensates, propylene oxides (PO) products, oxyalkylenes of alkylformaldehyde resins, sulphonates, cationic fatty acids, **ethoxylated phenolic resin**	Callaghan (1991); Muijs (1991); Graham *et al.* (1983)
De-oilers	Surface tension reducers	Polyamine or polyamine quaternary compounds	Kelley (1983); Muijs (1991)
	Coagulants/flocculants	Similar substances used as demulsifiers	
Defoamers		Modified silicon-based EO/PO adducts	Callaghan (1991)
Corrosion inhibitors		Amides/imidazoles, salts of nitrogenous molecules with carboxylic acids, nitrogen quaternaries, polyoxy alkylated amines, amide + imidazolines, nitrogen heterocyclics, phosphates + phosphonates	Kelley (1983); Muijs (1991)
Scale inhibitors		Polyacrylamide, polyacrylate, phosphate esters, polycarboxylates, organic phosphonates	Johnson (1983); Pennington (1988)
Wax inhibitors	Dispersants	Polyolesters or amine ethoxylates	Callaghan (1991); Gilby (1983)
	Pour-point depressants	Polyalkyl acrylates and methacrylates, polyethylene waxes, ethylene-vinyl acetate copolymers	
Gas drying + hydrate suppression		Glycol, methanol	Callaghan (1991)
Asphaltene strippers		Aromatic solvents; eg. xylenes, toluene and alkylbenzenes	Benson *et al.*, (1991)
Biocides		Aldehydes, quaternary ammonium compounds and chlorine release agents	Pennington (1988)
Secondary oil recovery		Water treated with filtration aids, oxygen scavengers, corrosion inhibitors, scale inhibitors, biocides	Callaghan (1991)

Table 4.7 Summary of the chemicals reported to be used in the oil industry during drilling, maintenance of wells and crude oil production.

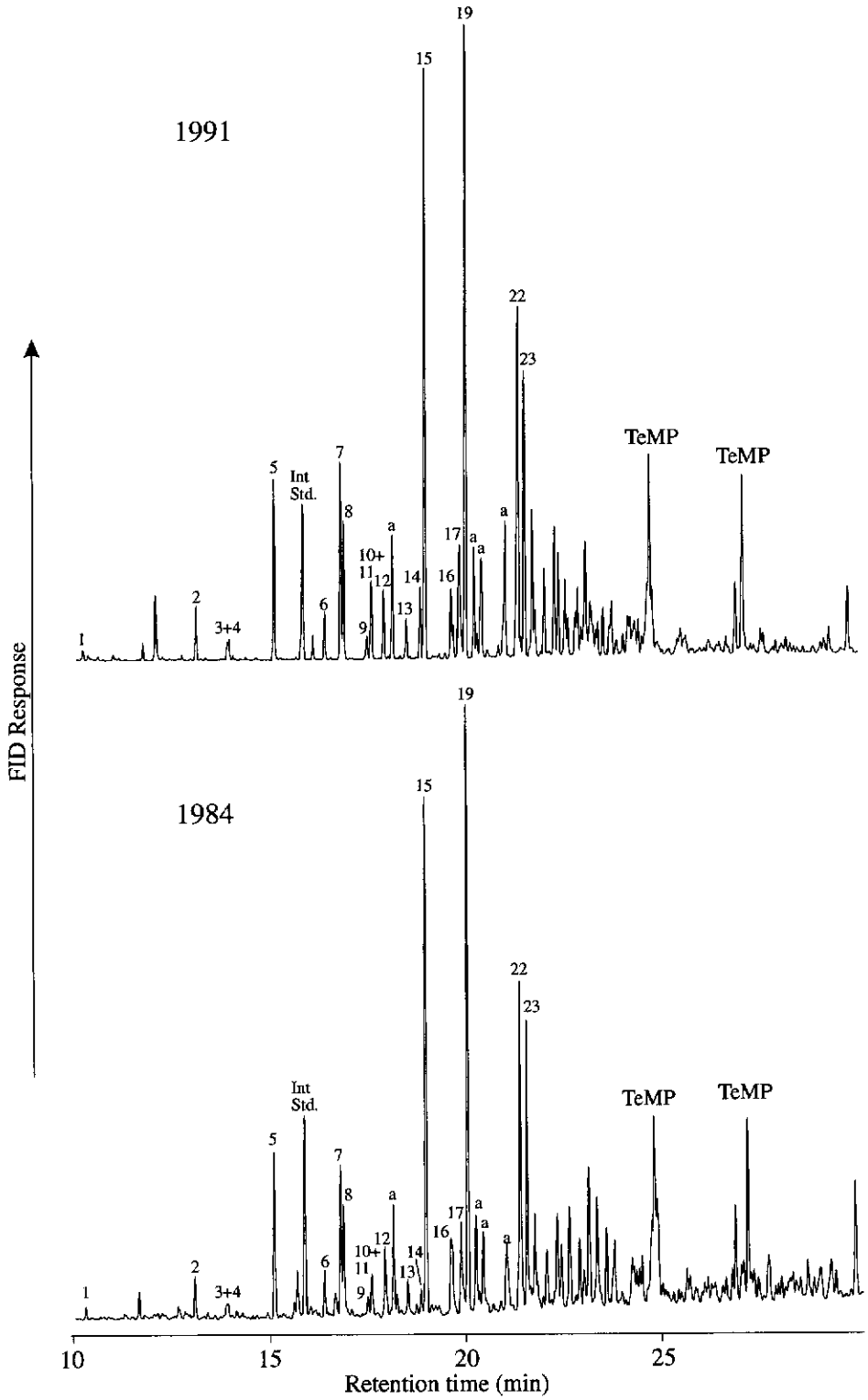


Figure 4.14 Partial gas chromatograms of Blina-1 crude oil recovered in 1991 and 1984. Refer to Table 4.4 for peak identifications.

1984 and 1991. Clearly the phenol distributions and concentrations (relative to internal standard; Int Std.) are similar in the two samples. Such results are unlikely to be obtained if the phenols were contaminants used in drilling or production operations.

4.6 CONCLUSIONS

1) Analytical procedures were developed which enabled the trace level analysis of phenols in a broad range of crude oils and several hydrous pyrolysates and brown coal extracts. The procedures involved extraction of acid components from crude oil using alkaline 5% aqueous methanol solution followed by the removal of non-phenolic coextracted material by back-extracting with hexane (analytical procedure II). In samples which formed severe emulsions, the coextracted material was removed by passing the alkaline solution through a C-18 Sep-Pak cartridge (analytical procedure I) . Phenols were then separated from carboxylic acids by buffering the alkaline solution to pH 6.8 and recovering the phenols with dichloromethane extraction thereby leaving carboxylate salts in aqueous solution.

2) Recoveries of 65-95% were measured for a range of individual phenols from hexane solution using analytical procedure II. The phenols included C_0-C_5 alkylphenols, indanol, tetralinol, naphthol, cyclohexylphenol, phenanthrol and methoxyphenols. The procedure was not efficient in extracting phenols which were very lipophilic in nature (e.g. 2,4-di-*tert*-butylphenol and 4-nonylphenol).

3) A large range of compounds were identified in crude oils (60+) and sedimentary rocks (35+) by comparison of their chromatographic behaviour and infrared and mass spectral properties with commercial and synthesised reference compounds. Forty two and sixteen alkylphenols were identified for the first time in crude oils and rock pyrolysates respectively.

5) The concentrations of C_0-C_4 alkylphenols in the crude oils were determined with errors of approximately 10%. Individual compounds occurred in crude oils in the range 10 - 190x10^3 ng/g and the total concentration of C_0-C_4 alkylphenols in crude oils ranged from 427 - 1091x10^3 ng/g. The low levels of phenols measured in crude oils are unlikely to be due to crude oil contamination.

CHAPTER 5

CLASSIFICATION OF CRUDE OILS BASED ON ALKYLPHENOL COMPOSITIONS

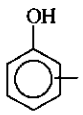
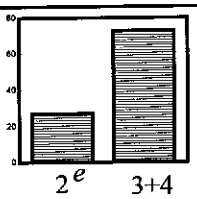
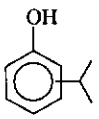
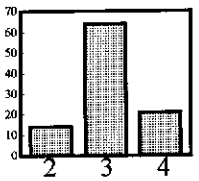
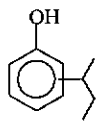
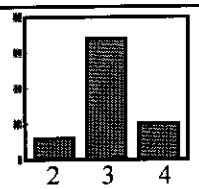
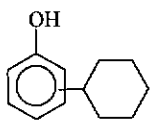
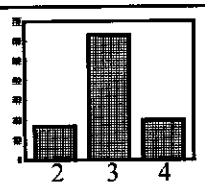
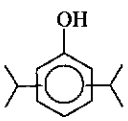
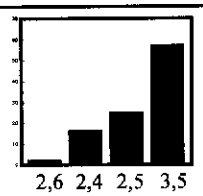
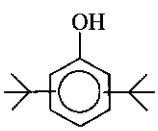
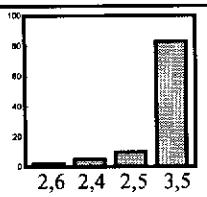
5.1 METHOD OF CLASSIFICATION

The crude oil samples analysed in this study have been classified into six groups based on their C_0-C_5 alkylphenol compositions. Some crude oils have isomer classes in which the relative proportions of the alkylphenols reflects their relative thermodynamic stabilities. In most cases, however, the relative proportions of phenols within an isomer class do not reflect thermodynamic stabilities, and these differences have been used to group crude oils. Some samples were placed in more than one group since the phenol distributions of different isomer classes deviate from reported equilibrium distributions in different ways. The characteristic features of the phenol compositions of the six groups of crude oils and the processes which are likely to give rise to the observed compositions are the topics of this chapter.

5.1.1 Reported Equilibrium Distributions

The relative abundances of alkylphenol isomers in equilibrated mixtures were obtained from reported laboratory equilibration experiments or calculated using thermodynamic data. Figure 5.1 shows the relative abundances of isomers in the equilibrated mixtures of six classes of alkylphenols. Examination of the bar graphs shows that in all classes the *meta* substituted isomers are the most abundant (most stable) and the *ortho* substituted isomers are the least abundant (least stable). For example, in mixture number 5, 3,5-diisopropylphenol occurs in high relative abundance (57%) whereas the di-*ortho* substituted compound, 2,6-diisopropylphenol, occurs in very low relative abundance (2%); compounds with only one *ortho* substituent, ie. 2,4-diisopropylphenol (16%) and 2,5-diisopropylphenol (25%) occur in proportions in between these end values. The relative abundances of isomers with two substituents at neighbouring carbon atoms

Figure 5.1 Bar graphs of the relative percentages of alkylphenols in equilibrated mixtures reported in the chemical literature.

Mixture number	Isomer class	Temp (°C) [phase]	Relative % in equilibrated mixture
1		170[a] [gas]	
2		170[b] [liquid][f]	
3		170[c] [liquid][f]	
4		300[c] [liquid][f]	
5		170[b,c] [liquid][f]	
6		200[d]	

a Equilibrium distribution calculated from $\Delta Gf°$, $\Delta Hf°$, S° reported by Dean, 1992,
b Nesterova *et al.*, 1983, c Nesterova *et al.*, 1989, d Bolton *et al.*, 1968,
e Denotes positions of alkyl substituents, f No solvent.

of an aromatic nucleus, ie. 2,3-diisopropylphenol and 3,4-diisopropylphenol, were not reported due to their low thermodynamic stabilities (Nesterova *et al.*, 1989).

Comparison of the equilibrium distribution of di-*tert*-butylphenols (Figure 5.1; mixture number 6) to that of the diisopropylphenols (mixture number 5) showed that isomers with substituents at similar ring positions occurred in similar relative abundances indicating that the position of the alkyl substituent predominantly governs isomer stability in alkylphenol mixtures with different alkyl substituents. The equilibrium distributions reported for diisopropylphenols was therefore selected as an approximation of the equilibrium distribution of dialkylphenols such as the dimethylphenols and isopropylmethylphenols, for which experimental values were not available.

5.2 GROUP 1 - CRUDE OILS CONTAINING ALKYLPHENOL ISOMERS THAT REFLECT THEIR RELATIVE STABILITIES

The crude oils in Group 1 contain alkylphenols in proportions which reflect their relative stabilities in at least one isomer class. Figure 5.2 shows bar graphs of the equilibrium distributions of alkylphenols reported in the chemical literature and the alkylphenol distributions in Group 1 crude oils.

The relative abundances of C_2-C_4 alkylphenol isomers in Lambert crude oil are similar to those observed in the reported laboratory equilibrated mixtures. The all-*meta* isomers occur in highest relative abundances (e.g. 3-isopropylphenol, 3,5-dimethylphenol and 3-isopropyl-5-methylphenol), there are lesser amounts of the compounds with one *ortho* substituent (e.g. 2-isopropylphenol, 2,4-dimethylphenol and 2-isopropyl-5-methylphenol) and finally the isomers with two *ortho* substituents are in much lower relative abundances (e.g. 2,6-dimethylphenol and 2-isopropyl-6-methylphenol). In the case of the isopropylmethylphenols the isomers

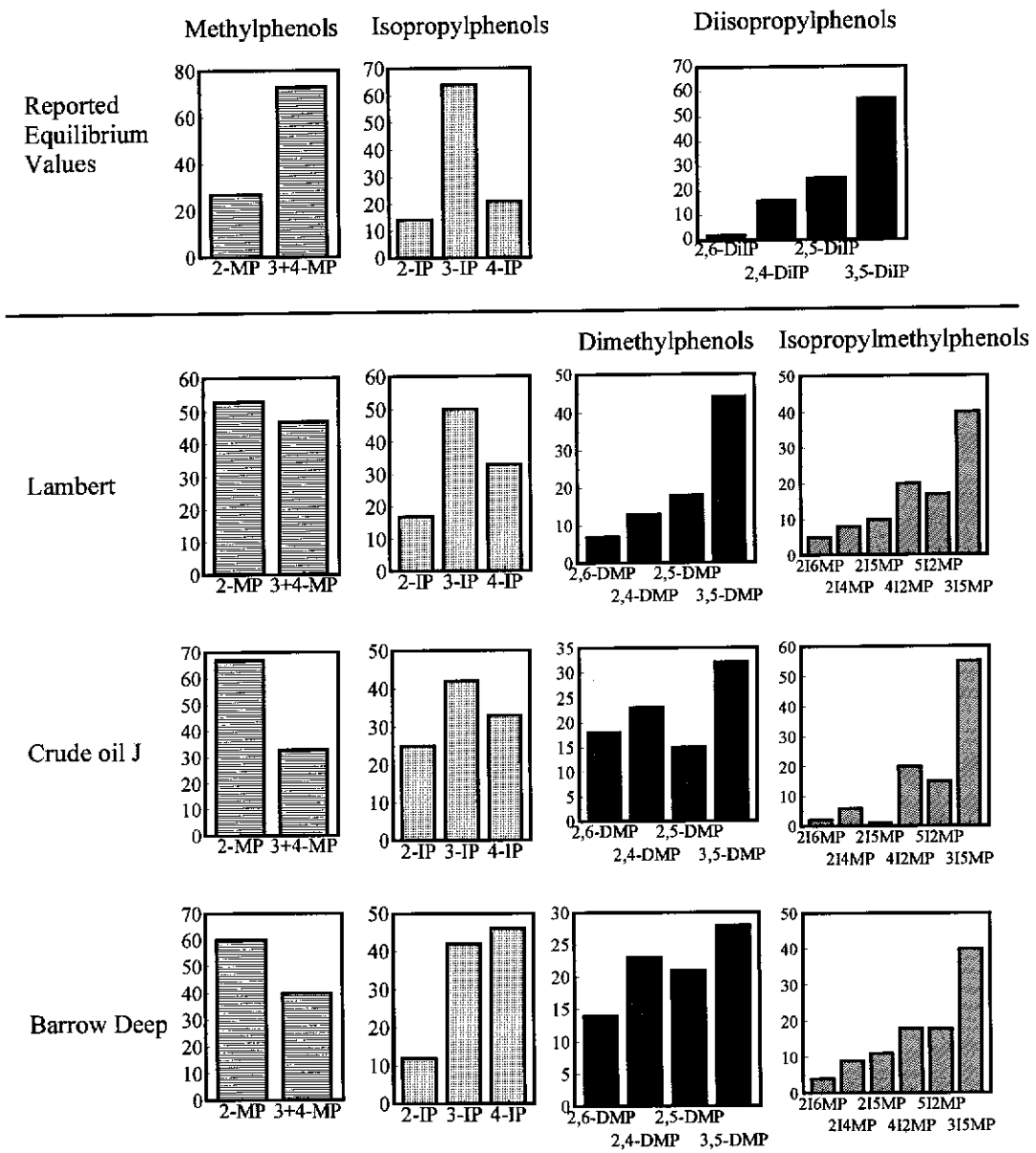


Figure 5.2 Bar graphs showing the alkylphenol compositions in Group 1 crude oils and those in reported equilibrated mixtures. MP = methylphenols, IP = isopropylphenols, DMP = dimethylphenols, xIyMP = x-isopropyl-y-methylphenol.

with one *ortho* isopropyl group (e.g. 2-isopropyl-5-methylphenol) occur in lower relative abundance than those with one *ortho* methyl group (e.g. 5-isopropyl-2-methylphenol) due to the bulkier isopropyl group in the *ortho* position making the former compounds less stable. The similarities in the relative abundance of C_2-C_4 alkylphenol isomers in Lambert crude oil and the reported equilibrated mixtures suggests that these alkylphenols in Lambert crude oil may be at or approaching equilibrium proportions. Comparison of the proportions of methylphenols in Lambert to those in the equilibrated mixture, however, shows that, unlike the C_2-C_4 alkylphenols, the relative proportions of the C_1 alkylphenols do not reflect their relative stabilities.

The rate at which petroleum alkylphenols attain equilibrium may be dependent on the number and type of alkyl substituent(s). Examination of the phenol distributions in Crude oil J and Barrow Deep crude oils (Figure 5.2) shows that the isopropylmethylphenol and isopropylphenol (Crude oil J) isomers are approaching their equilibrium proportions whereas the methylphenols and dimethylphenols are not. The observation that alkylphenols with branched substituents (isopropyl) occur in proportions which reflect their relative stabilities but those with only methyl substituents have largely not attained equilibrium proportions may be explained in terms of the isomerisation mechanism. Presumably isomerisation of alkylphenols occurs via an intramolecular 1,2-shift where the transition state is suggested to be a protonated cyclopropane species formed by the migrating group and two adjacent aromatic carbons (March, 1992). Since more stable transition states are formed when substituents with a secondary carbon migrate, rearrangement of alkylphenols with such substituents would isomerise more readily than those with methyl substituents. Based on this isomerisation mechanism and data obtained from samples in Group 1, the rate of

attainment of equilibrium for alkylphenols in crude oils appears to be isopropylmethylphenols > isopropylphenols > dimethylphenols > methylphenols.

There is no simple relationship between the observation of equilibrium proportions of alkylphenols in crude oils and the maturity of the crude oil as measured by well established maturity parameters. Figure 5.3 shows plots of the relative percentages of the most stable dimethylphenol and ethylphenol isomers (% 3,5-dimethylphenol+3-ethylphenol) and isopropylmethylphenol isomer (% 3-isopropyl-5-methylphenol) in the crude oils analysed in this study versus aromatic maturity parameter DNR1 and sterane maturity parameter 20S/20S+20R. Crude oils from the Dampier, Barrow and Kutai basins have been labelled for easy comparison of samples from the same basin. The lines on the plots represent the estimated equilibrium values for the alkylphenol ratios. From the data it is apparent that the majority of crude oils have percentages of the most stable isomers below the equilibrium values. The crude oils with values above that of equilibrium comprise Group 4 crude oils and are discussed in Section 5.5. Figure 5.3 shows that the most mature crude oils (highest DNR1 and 20S/20S+20R values) do not necessarily contain alkylphenol ratios close to equilibrium values, even among crude oils from the same basin. It therefore appears that there is no simple relationship between the relative amount of the most stable all-*meta* isomers and the thermal maturity of the crude oils in which they occur. Since these results indicate that thermal maturity does not play a dominant role in the equilibration of alkylphenols in crude oils, perhaps other factors, such as the acid catalytic nature of the mineral or maceral matrix of a source rock, may be more significant.

In order to assess the relationship between the acid catalytic nature of source rocks and the abundances of the most stable alkylphenols in an isomer class, the relative percentages of 3,5-dimethylphenol+3-ethylphenol and 3-isopropyl-5-methylphenol have been plotted versus diasterane/sterane ratios (Figure 5.4). High

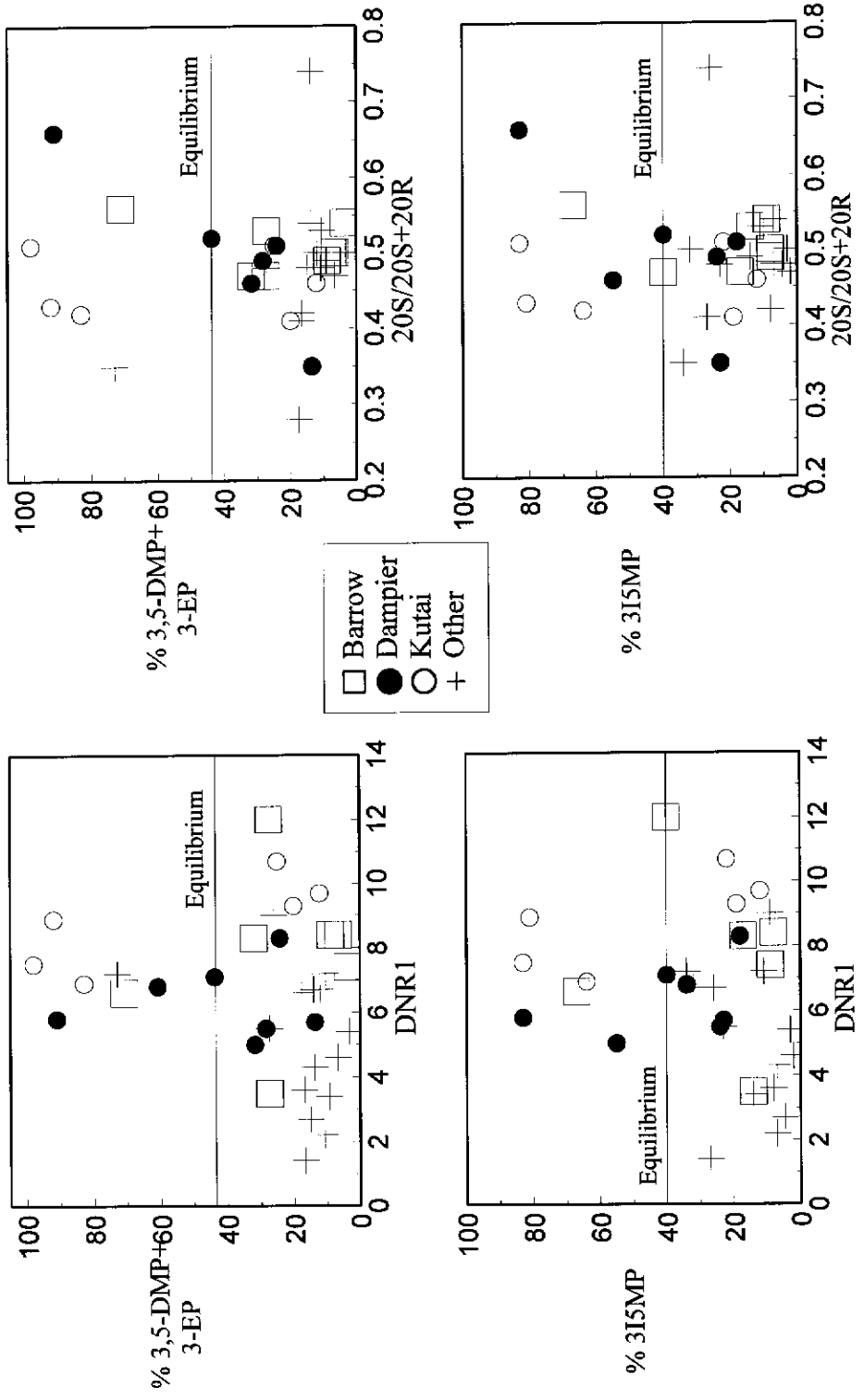


Figure 5.3 Plots of relative % 3,5-dimethylphenol+3-ethylphenol [(3,5-DMP+3-EP)/Σ Dimethylphenols x 100] and relative % 3-isopropyl-5-methylphenol (3I5MP/Σ Isopropylmethylphenols x 100) versus maturity parameters DNR1 and 20S/20S+20R for crude oils. DNR1 = (2,6-DMN + 2,7-DMN)/1,5-DMN; 20S /20S+20R = C29 (20S) regular sterane/C29 (20S+R) regular sterane.

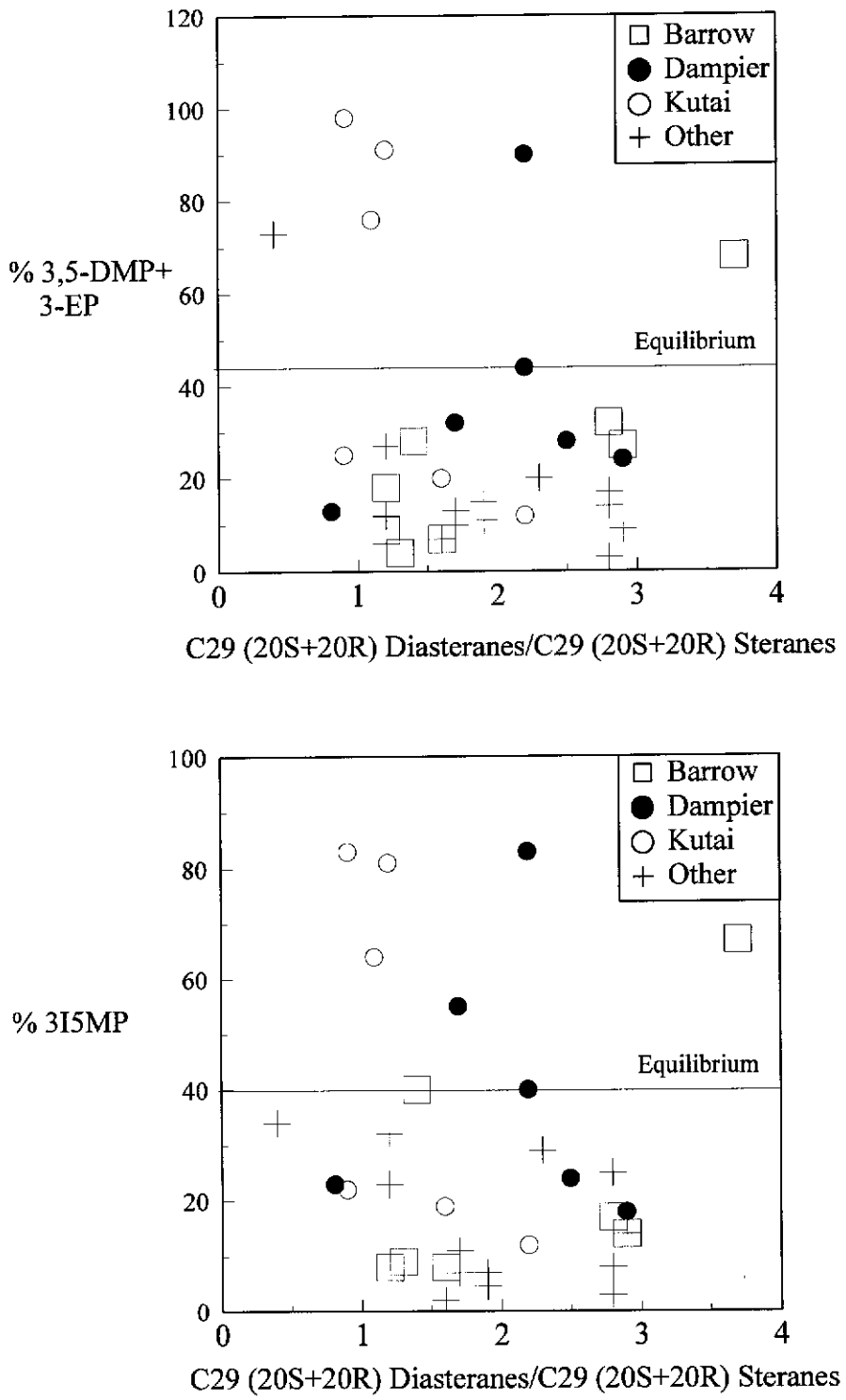


Figure 5.4 Plots of relative % 3,5-dimethylphenol+3-ethylphenol [(3,5-DMP+3-EP)/Σ Dimethylphenols x 100)] and relative % 3-isopropyl-5-methylphenol (3I5MP/Σ Isopropylmethylphenols x 100) versus C29 (20S+20R) diasteranes/C29 (20S+20R) steranes for crude oils.

diasterane/sterane ratios are suggested to be observed in petroleum derived from rocks containing acidic clays (refer to Chapter 3; Section 3.2.1). Such samples would be expected to have alkylphenols approaching equilibrium proportions if acid clay catalysed rearrangement reactions play a dominant role in alkylphenol equilibration. Figure 5.4 shows that high values for the percentages of the most stable all-*meta* isomers do not correlate with high diasterane/sterane ratios. Even the most reactive compound class, isopropylmethylphenols, do not show a simple relationship with the ratio. From the data it is apparent that there is not a simple relationship between diasterane/sterane ratios and extent of alkylphenol equilibration.

It is important to note that the majority of crude oils (excluding Group 1) do not contain alkylphenols in proportions which reflect their relative stabilities. In fact, only the crude oils shown above; eight percent of all samples analysed contain at least one alkylphenol isomer class which may be approaching equilibrium proportions. Therefore thermodynamic stability is not a major factor in controlling the alkylphenol compositions of crude oils. Attempting to understand the processes giving rise to the phenol compositions in the majority of crude oils has therefore been a natural outcome of this study.

5.2.1 Conclusions

1) Some crude oils contain at least one alkylphenol isomer class in which the relative proportions of isomers reflect their relative stabilities, however the majority of crude oils do not contain phenols in equilibrium proportions.

2) Alkylphenols with branched substituents (e.g. isopropylmethylphenols) appear to attain equilibrium proportions in crude oils at a faster rate than those with methyl substituents (e.g. methylphenols).

3) There is no simple relationship between the relative amounts of the most stable all-*meta* alkylphenol isomers in crude oils and crude oil thermal maturity or the acid catalytic nature of their source rocks as measured by diasterane/sterane ratios.

5.3 GROUP 2 - CRUDE OILS CONTAINING PREDOMINANTLY ALKYLPHENOLS DERIVED FROM NATURAL PRODUCTS

5.3.1 Group 2A - Crude Oils with Isopropylmethylphenols Dominated by Monoterpenoid Phenols (Carvacrol and Thymol)

The crude oils in Group 2A contain isopropylmethylphenol distributions which are dominated by carvacrol, thymol and/or 3-isopropyl-5-methylphenol. Figure 5.5 shows the isopropylmethylphenol compositions in the crude oils in this group, along with that in a Group 1 crude oil (Lambert) and an equilibrated mixture of diisopropylphenols. Comparison of the isopropylmethylphenol distributions in Group 2A crude oils with that of an equilibrated mixture shows that all of these samples contain carvacrol (C) in elevated levels and, Rough Range, Kenmore and Tuna-4 also contain high levels of thymol (T) relative to 3-isopropyl-5-methylphenol (3,5). Group 2A crude oils may also have a high relative abundance of 3-isopropyl-5-methylphenol which may be formed from the rearrangement of thymol.

Evidence that thymol readily undergoes reaction on acid clay surfaces to form 3-isopropyl-5-methylphenol was obtained by heating thymol with aluminium smectite at 180°C. After 12 hours the reaction products were analysed and shown to contain predominantly 3-isopropyl-5-methylphenol (50%), presumably formed by the isomerisation of thymol to yield the more stable all-*meta* isomer, and small amounts of other isopropylmethylphenols (Table 5.1). Therefore a high relative

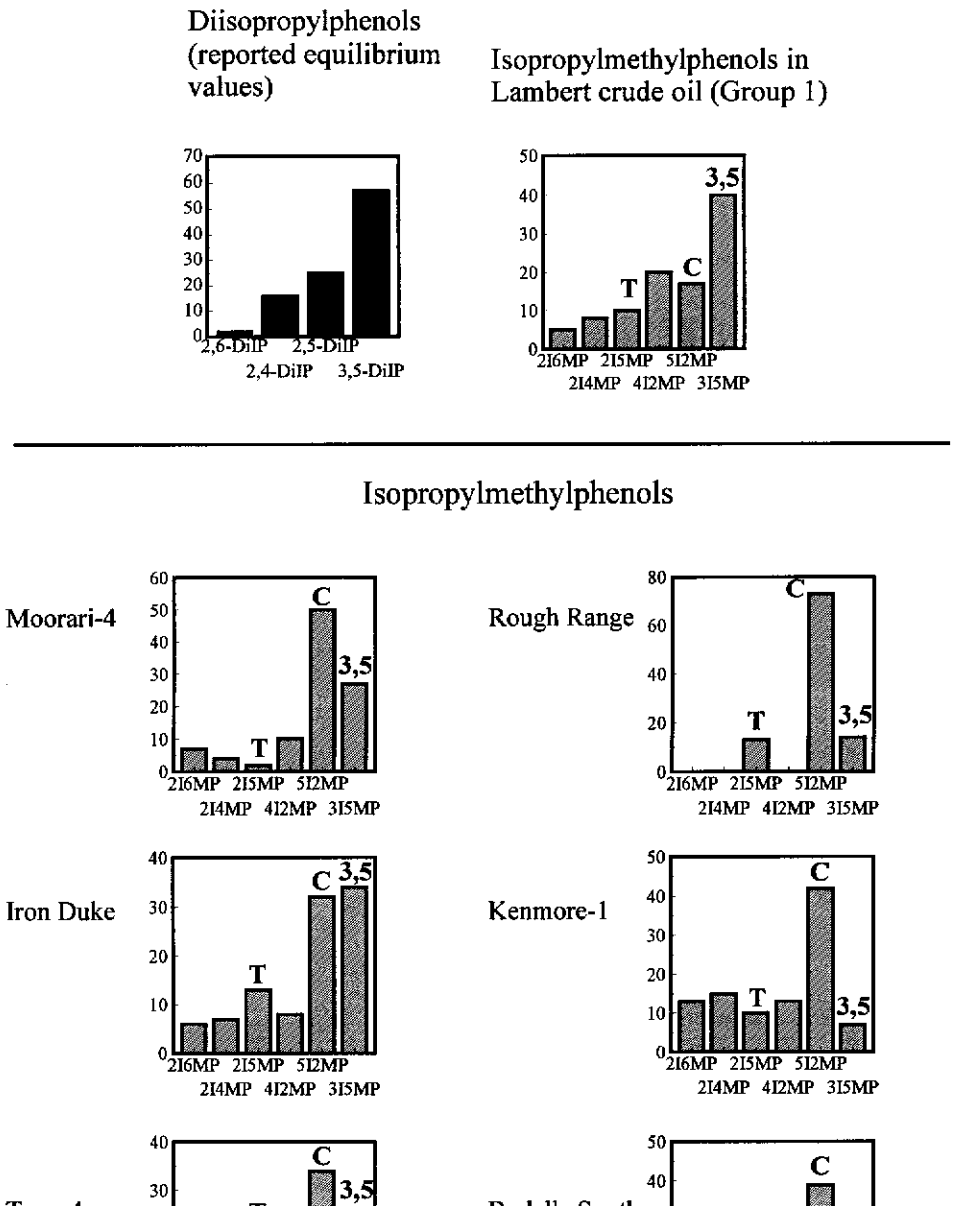


Figure 5.5 Bar graphs showing the isopropylmethylphenol distributions in Group 2A crude oils and Lambert crude oil. **T** = thymol; **C** = carvacrol; **3,5** = 3-isopropyl-5-methylphenol; xIyMP = x-isopropyl-y-methylphenol.

abundance of 3-isopropyl-5-methylphenol, when associated with elevated levels of carvacrol, is likely to be formed from the rearrangement of thymol.

The elevated levels of carvacrol, thymol and 3-isopropyl-5-methylphenol in crude oils is indicative of an input of land-plant-derived natural products. A detailed discussion of these phenols as biomarkers is provided in Section 6.3 (Chapter 6).

Table 5.1 Relative percentages of isopropylmethylphenol reaction products obtained from heating thymol with aluminium smectite at 180°C for 12 hours.

Compound	Relative %
2-Isopropyl-6-methylphenol	<0.1
2-Isopropyl-4-methylphenol	0.4
2-Isopropyl-3-methylphenol	<0.1
2-Isopropyl-5-methylphenol (thymol; reactant)	40.1
4-Isopropyl-2-methylphenol	0.8
5-Isopropyl-2-methylphenol (carvacrol)	0.4
3-Isopropyl-5-methylphenol (dominant product)	50.2
3-Isopropyl-2-methylphenol	<0.1
3-Isopropyl-4-methylphenol	<0.1
4-Isopropyl-3-methylphenol	8.1

5.3.2 Group 2B - Crude Oils Containing Predominantly Alkylphenols Derived From Tocopherols

The crude oils in Group 2B contain mixtures of phenols dominated by methylphenols which appear to be derived from the alteration of tocopherol or structurally-related natural products. Laboratory heating reactions of α-tocopherol on acid clay surfaces produce a range of trimethylphenol and tetramethylphenol products (refer to Chapter 6; Section 6.3), many of which are also present in Group 2B crude oils. For example, the gas chromatogram of the phenol extract of a Group 2B crude oil (Blina) is shown in Figure 4.14 (Chapter 4). This crude oil contains mainly methylphenols which are also clay catalysed reaction products of α-tocopherol such as 2,3,6-trimethylphenol (peak 19), 2,4,5-trimethylphenol (peak 22), 2,3,5-trimethylphenol (peak 23) and tetramethylphenols (peaks TeMP). The

occurrence of high relative abundances of tocopherol derived methylphenols in Group 2B crude oils suggests that the incorporation of tocopherol natural products in source rocks may give rise to these petroleum methylphenols. A detailed discussion of the methylphenols produced from tocopherols and their occurrence in group 2B crude oils is given in (Chapter 6; Section 6.3).

5.4 GROUP 3 - CRUDE OILS CONTAINING PREDOMINANTLY *ORTHO* AND *PARA* SUBSTITUTED ALKYLPHENOLS (GEOSYNTHEIS OF ALKYLPHENOLS)

The crude oils in Group 3 contain mixtures of alkylphenols with isomer distributions dominated by *ortho* and *para* substituted compounds. These samples contain low relative abundances of *meta* substituted compounds compared to the equilibrated distributions indicating they are not close to equilibrium. Group 3 has been subdivided into two additional groups, the first contains crude oils dominated by *ortho* and *para* methylphenols (Group 3A). The second, Group 3B, contains isopropylmethylphenols and *sec*-butylmethylphenol distributions dominated by 4-isopropyl-2-methylphenols and 4-*sec*-butyl-2-methylphenol respectively. Figure 5.6 shows the proportions of some C_1-C_4 alkylphenols in four representative Group 3 crude oils, together with those reported for equilibrated mixtures of related alkylphenol classes and a Group 1 crude oil (Lambert). The data show that the alkylphenol distributions of Group 3 crude oils are dominated by *ortho* and *para* substituted alkylphenols such as 2-methylphenol, 2-isopropylphenol, 2,6-dimethylphenol, 2,4-dimethylphenol and 4-isopropyl-2-methylphenol. Although not shown in Figure 5.6, these samples are also characterised by a high relative abundance of 2,4,6-trimethylphenol (refer to Chapter 7). Since *ortho* and *para* substituted phenols are kinetic products of electrophilic aromatic substitution

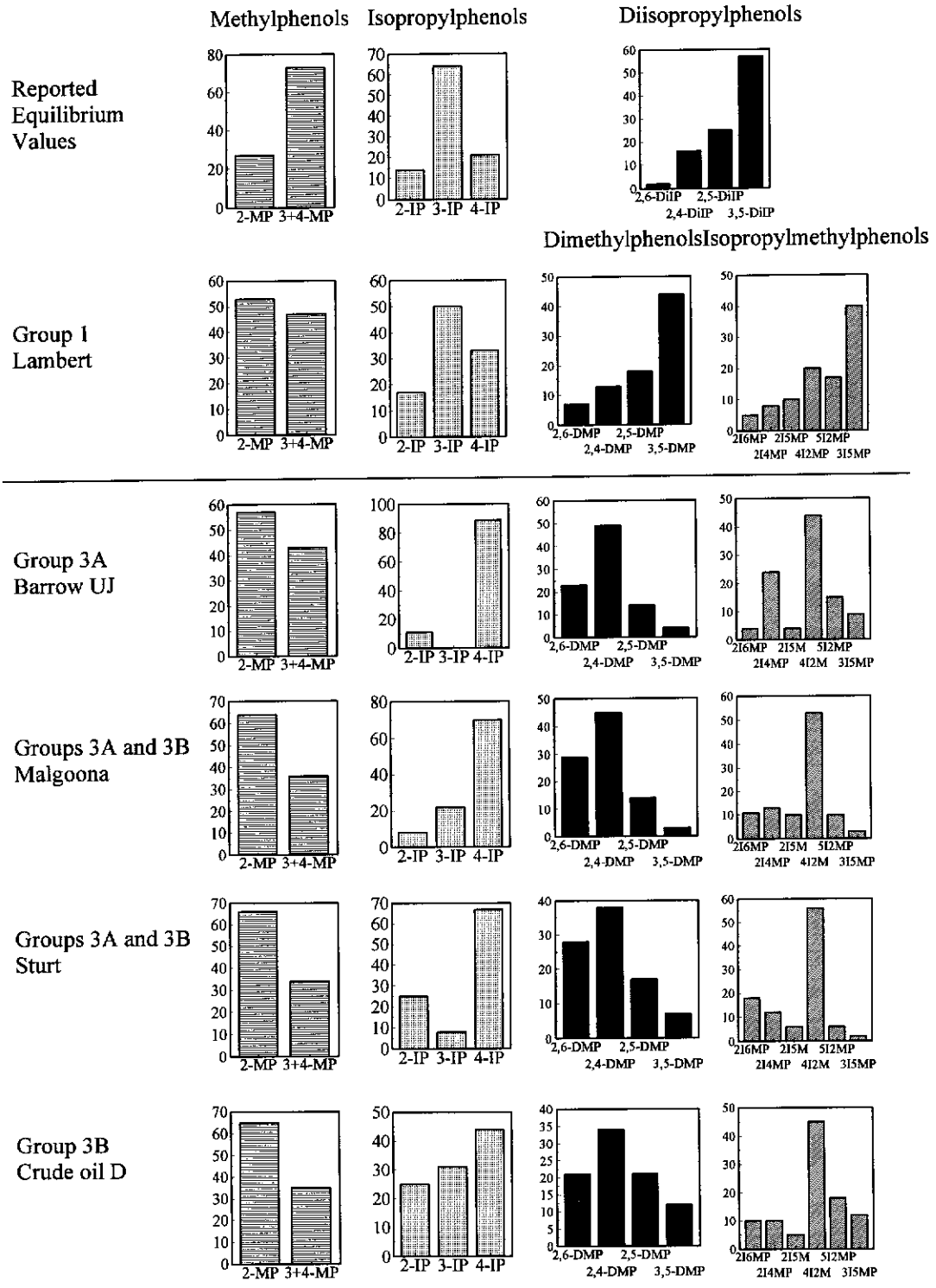


Figure 5.6 Bar graphs showing some alkylphenol distributions in representative Group 3A, Group 3B and Group 1 crude oils and those in reported equilibrated mixtures.
MP = methylphenol, IP = isopropylphenol, DMP = dimethylphenol,
xIyMP = x-isopropyl-y-methylphenol.

reactions it is proposed that *ortho* and *para* substituted petroleum alkylphenols are formed by geosynthetic processes involving alkylation of simpler phenols in source rocks. A detailed discussion of the geosynthesis of petroleum alkylphenols comprises Chapter 7.

5.5 GROUP 4 - CRUDE OILS CONTAINING PREDOMINANTLY *META* SUBSTITUTED ALKYLPHENOLS

The crude oils in Group 4 contain mixtures of alkylphenols in which the *meta* substituted isomers dominate in six isomer classes. Figure 5.7 shows the proportions of some alkylphenol isomers in Group 4 crude oils, and the distributions of some alkylphenol isomers in equilibrated mixtures reported in the literature and a Group 1 sample (Lambert). Comparison of the alkylphenol distributions in the equilibrated mixtures to those in Group 4 crude oils clearly shows that the *meta* substituted isomers among the methylphenols, isopropylphenols, dimethylphenols and isopropylmethylphenols occur in Group 4 crude oils in amounts greater than their equilibrium proportions. In addition to these isomer classes, the gas chromatogram of the phenol extract of a Group 4 crude oil (Figure 5.8) shows that 3-ethylphenol (peak 11) and 3-ethyl-5-methylphenol (peak 3E5MP) are also the major compounds in their respective isomer classes. Because the *meta* substituted isomers occur in greater than their equilibrium proportions, simple equilibration processes cannot give rise to the very high relative abundances of these isomers in Group 4 crude oils.

The possibility that a natural product origin could account for the high relative abundance of *meta* substituted isomers in these crude oils was investigated. There are several long-chain phenolic natural products that could undergo cleavage in the sedimentary environment to yield predominantly *meta* substituted shorter

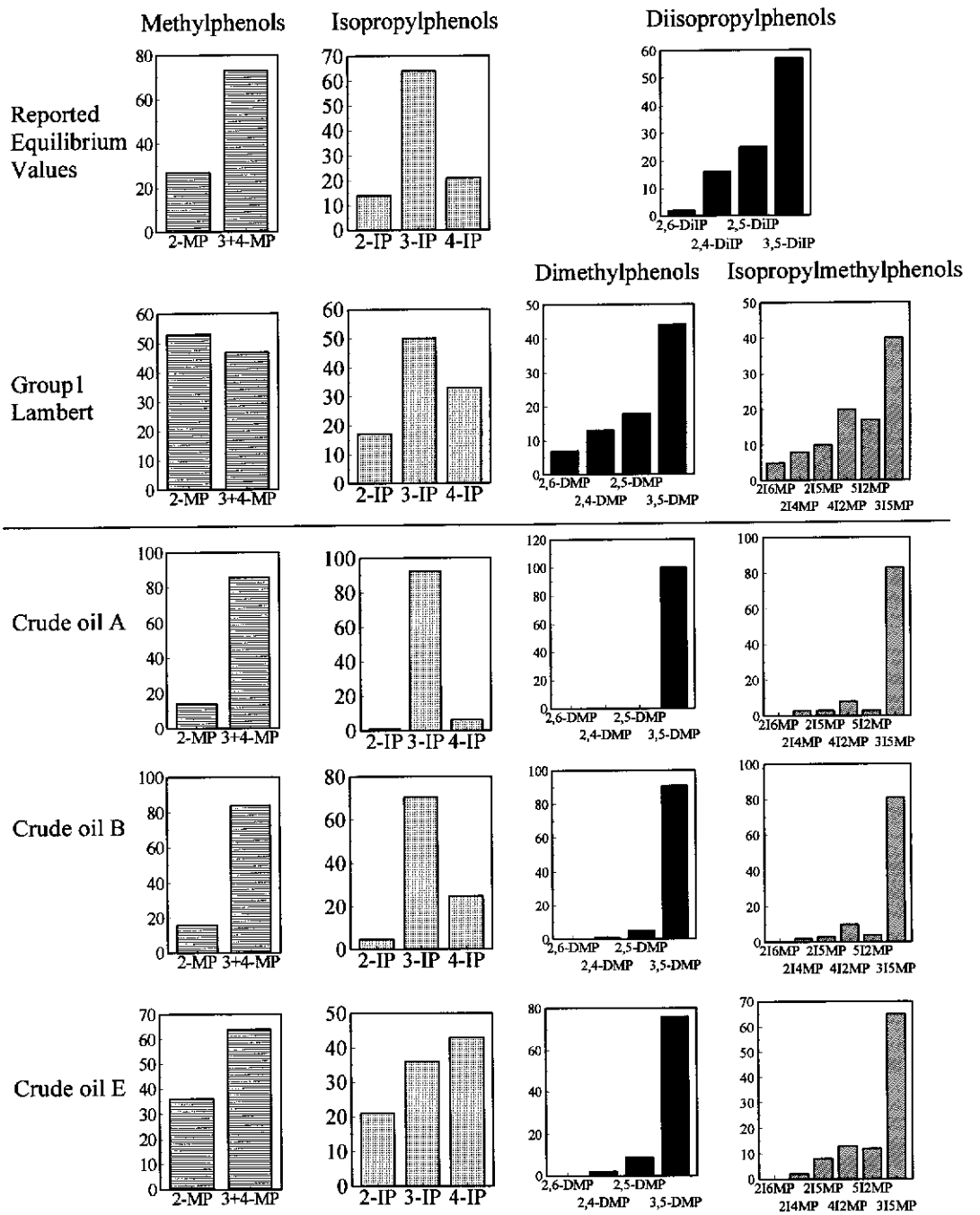
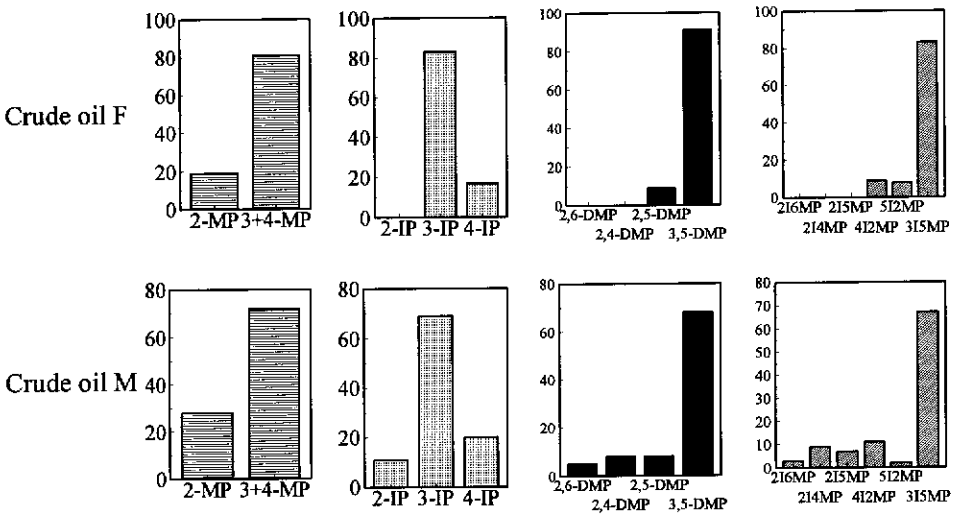


Figure 5.7 Bar graphs showing some alkylphenol distributions in Group 4 crude oils, Lambert and reported equlibrated mixtures. MP = methylphenol, IP = isopropylphenol, DMP = dimethylphenol, xIyMP = x-isopropyl-y-methylphenol.

Figure 5.7 continued.



n-alkyl chain compounds. Natural products which may give rise to *meta* mono-substituted *n*-alkylphenols include 5-*n*-alkyl-1,3-benzenediols in kerogens derived from *G. prisca* (Derenne *et al.*, 1990; 1992) and *meta* substituted long chain alkylphenols in aerobic soil bacteria (Sadoff, 1975) and liverwort (Asakawa *et al.*, 1987). Natural products which have two *meta*-substituents and could therefore yield *meta* di-*n*-alkylphenols include the hydroxycarotenoids (refer to Section 1.2.2). A natural product origin for *meta*

alkylphenols with branched substituents, however, appears less likely. Although it was previously shown that the commonly occurring natural product thymol yielded the all-*meta* isomer (3-isopropyl-5-methylphenol) when heated with acidic clay (Section 5.3.1), this conversion did not proceed beyond the equilibrium proportions even when high temperatures and reaction times were employed. Furthermore, the crude oils which have been classified as containing a high relative abundances of natural products (carvacrol and thymol) always contained 3-isopropyl-5-methylphenol below its equilibrium proportion. Therefore a natural product source

for very high levels of 3-isopropyl-5-methylphenol is unlikely. The lack of known natural product precursors for *meta* alkylphenols with branched chains, ie. 3-isopropylphenol and 3-isopropyl-5-methylphenol and the diverse range of natural products required to give rise to mono and di-substituted *n*-alkylphenols, ie. 3-methylphenols, 3-ethylphenols, 3,5-dimethylphenols and 3-methyl-5-ethylphenol, suggests that a natural product source for these compounds is unlikely.

The possibility that the high relative abundance of *meta* substituted compounds in Group 4 crude oils is the result of the physical properties of phenols was investigated. *Meta* and *para* substituted isomers are selectively depleted from

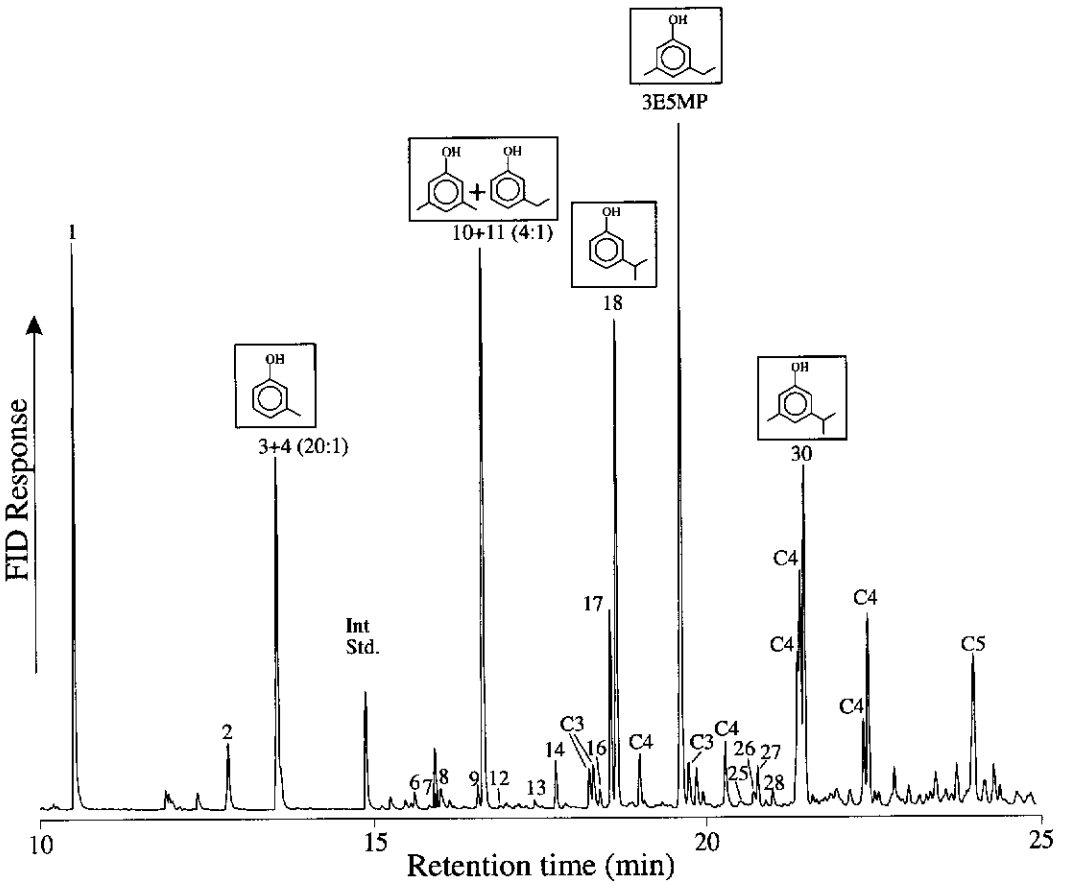


Figure 5.8 Gas chromatogram of a Group 4 crude oil phenol extract (Crude oil B). Compound in boxes are all *meta* substituted alkylphenols. For peak identification s refer to Table 4.4.

hydrocarbon mixtures via both water dissolution and adsorption effects compared to their *ortho* substituted (hindered) counterparts (refer to Section 1.6). Therefore, both water contact and mineral/solid organic matter contact would deplete, rather than enrich, crude oils of the *meta* substituted isomers. Perhaps more importantly, because *meta* and *para* substituted isomers have similar distribution coefficients between a hydrocarbon phase and water (Section 1.6 and Table 7.5), and similar absorption affinities for solid surfaces, these processes cannot selectively enrich/deplete one of the isomers over the other. Since water dissolution or adsorption effects cannot differentiate between *meta* and *para* substituted phenols, the selective enrichment of *meta* isomers may be occurring via a chemical process.

The possibility that the high relative abundances of *meta* substituted compounds in Group 4 crude oils is the result of the selective depletion of *ortho* and *para* substituted isomers via chemical oxidation processes was investigated. Chemical evidence indicates that phenoxy-radical formation is the initial step in the majority of phenol oxidations (Musso, 1963). The radical species then reacts further to produce a variety of phenol oxidation products of which the most common are quinones, coupling products and quinone methides (Whiting, 1978). The stability of the phenoxy radical intermediate is dependent on the presence of *ortho* and *para* substituents which give increased steric protection and/or resonance stabilisation. Steric protection is provided by large *ortho* groups such as *tert*-butyl which "shield" the oxy-radical (I, Figure 5.9). This effect is demonstrated by the widespread use of hindered (di-*ortho*) phenols as anti-oxidants (Johnson, 1975; Kikugawa *et al.*, 1990). Resonance stabilisation is provided by both *ortho* and *para* alkyl substituents which provide electron delocalisation and hence stabilise the resonance structures II-IV (Figure 5.9). The stabilising effect of *para* substituents is demonstrated by the lower stability of 2,6-di-*tert*-butyl-4-methyl-phenoxy radical compared to radicals in which the *para* position contains larger

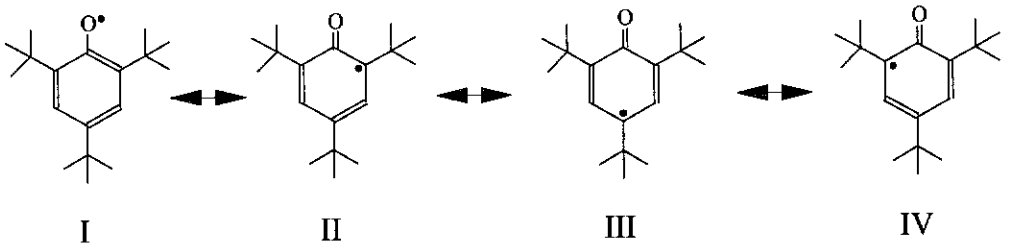


Figure 5.9 Resonance structures of 2,4,6-tri-*tert*-butylphenoxide. *Ortho* substituents provide steric stability (I) and, *ortho* and *para* substituents provide resonance stability (II)-(IV).

alkyl groups such as isopropyl ie. 2,6-di-*tert*-butyl-4-isopropyl-phenoxy radical (Altwicker, 1967). This effect is further demonstrated using the measured rate constants for the oxidation of unhindered phenols using 2,4,6-tri-*tert*-butylphenoxide as oxidant. The rate constants for phenols with an electron releasing group in the *para* position were higher than those with the same substituent in the *meta* position (e.g. 4-*tert*-butylphenol k = 92.5 ± 6.5 $l.mol^{-1}sec^{-1}$ *cf.* 3-*tert*-butylphenol k= 20.8 ± 1.9 $l.mol^{-1}sec^{-1}$) (Altwicker, 1967). In addition to their higher rate of reaction and stability, *ortho* and *para* substituted alkylphenoxy radicals in which the alkyl substituent(s) bears an α-hydrogen disproportionate readily to the starting phenol and a quinone methide (Figure 5.10). Generally *para*-quinone methides are more stable than *ortho*-quinone methides and therefore form more readily (Turner, 1964). This latter oxidation pathway is particularly attractive for the *ortho* and *para* substituted petroleum phenols since their substituents all contain at least one α-hydrogen. Because *ortho* and *para* substituted alkylphenols are more readily oxidised than their *meta* substituted counterparts, the selective loss (via oxidation) of the former more reactive phenols may account for the high relative abundance of *meta*-substituted isomers observed in Group 4 crude oils.

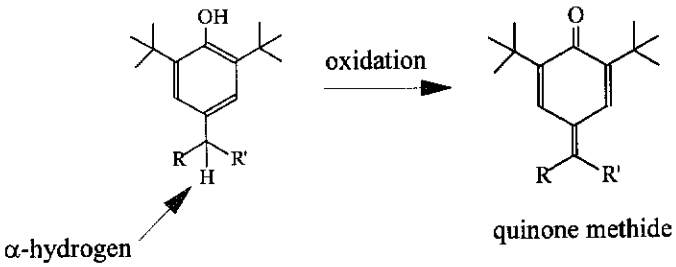


Figure 5.10 Formation of quinone methides from the oxidation of alkylphenols with *ortho* and *para* substituents bearing an α-hydrogen.

There are various oxidants in a petroleum environment which may oxidise alkylphenols. Phenoxy radicals are readily produced in the laboratory using a variety of oxidising agents, the most common of which are inorganic oxidants such as iron and copper salts. These reactions usually proceed in a heterogeneous system involving a solution of the phenol in an inert organic solvent coming in contact with a suspension or aqueous phase of the oxidising agent (Altwicker, 1967). It is therefore likely that petroleum phenols may be oxidised by solid mineral phases or dissolved inorganic oxidants in water or crude oil in the subsurface. Although the exact nature of the oxidising agent is speculative some potential oxidants in a petroleum environment include complexed species of transition metals (e.g. Fe^{3+} complexes), and oxidised sulphur species such as disulphides and $(S_xO_y)_n$ which can readily be reduced to species such as thiols, hydrogen sulphide or even elemental sulphur (Oae, 1991). Inorganic ions (e.g. Cu^{2+}) may also act as catalysts for oxidation via oxygen, followed by the regeneration of the metal ion.

5.5.1 Conclusions

1) Some crude oils contain alkylphenol mixtures in which the *meta* substituted compounds in six different isomer classes of C_1-C_4 alkylphenols occur well above their equilibrium proportions.

2) A natural product origin or the selective enrichment via water dissolution or adsorption processes are unlikely causes of the high relative abundance of *meta* substituted isomers in these crude oils.

3) Because *ortho* and *para* substituted phenols are more susceptible to oxidation compared to *meta* substituted isomers, the selective removal of these compounds via an oxidation process was suggested as an explanation for the high relative abundances of *meta* substituted isomers observed in Group 4 crude oils.

5.6 GROUP 5 - CRUDE OILS THAT ARE BIODEGRADED

Suites of crude oils from two Australian basins which have undergone various degrees of biodegradation have been analysed for their phenol contents. Table 5.2 shows geological and geochemical data pertaining to each of the crude oils. The sample suites from the two basins contain a range of non-degraded to severely biodegraded crude oils; each sample suite having a suggested common source so that compositional differences in the crude oils should largely reflect alterations occurring during biodegradation.

The alkane compositions of the five Gippsland basin crude oils were examined to assess their state of biodegradation. Figure 5.11 shows the gas chromatograms of the alkanes separated from Tuna-4 (2820 m), Tuna-4 (1440 m) and Lakes Entrance crude oils. The alkane fractions of these three crude oils were used to represent the three different levels of biodegradation observed in the suite. The gas chromatogram of Tuna-4 (2820 m) shows a saturated hydrocarbon distribution which is dominated by the *n*-alkane homologous series. The presence of these readily biodegradable alkanes (Volkman *et al.*, 1983b) in this crude oil indicates that it contains material which has not been subjected to bacterial degradation. The gas chromatogram of Tuna-4 (1400 m), on the other hand,

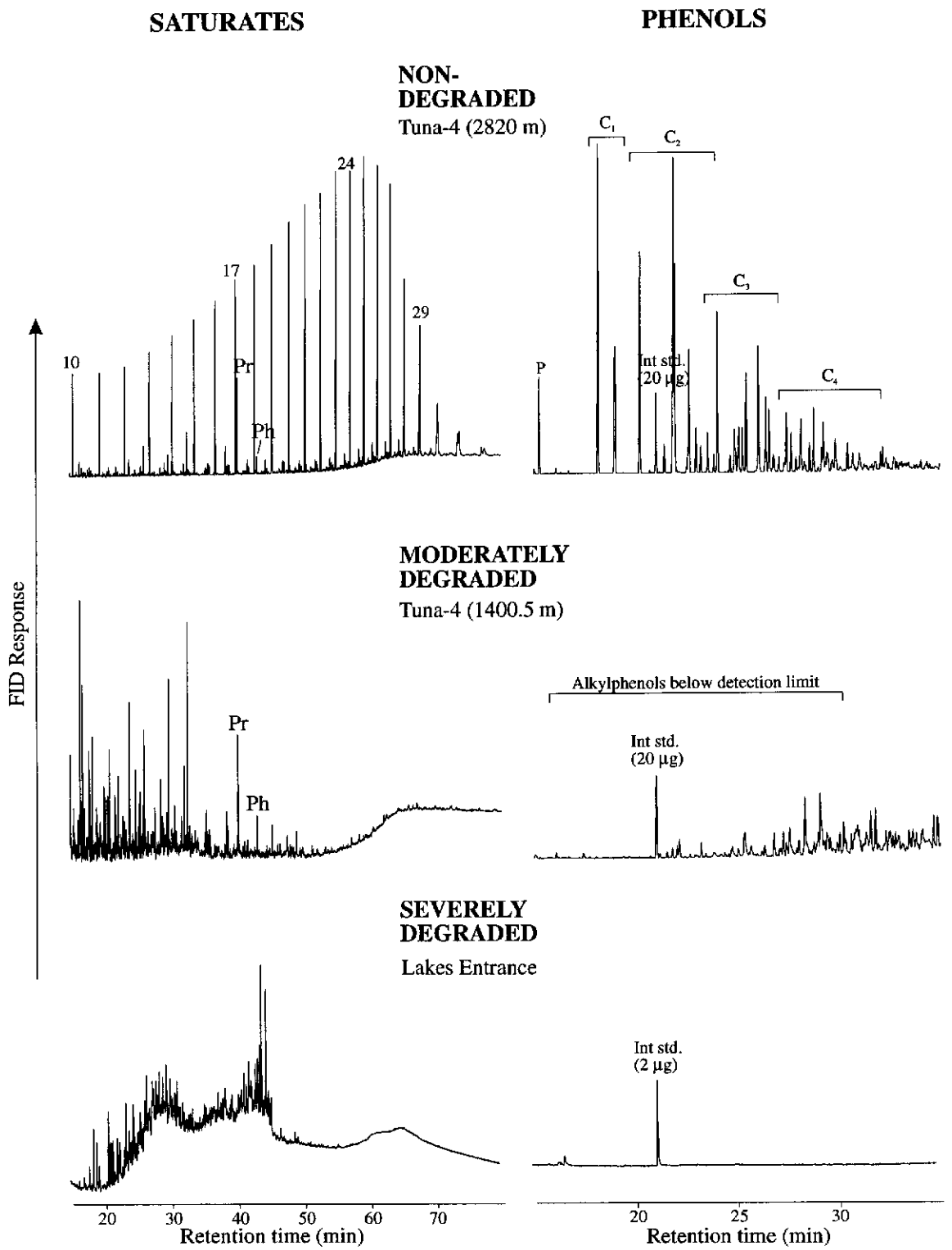


Figure 5.11 Gas chromatograms of the saturated hydrocarbon isolates and the phenol extracts of crude oils from the Gippsland Basin, Australia. P = phenol; C_1-C_4 = C_1-C_4 alkylphenols.

shows no evidence of *n*-alkanes and is comprised dominantly by acyclic isoprenoids and drimanes, indicating that this crude oil contains material which has been subjected to a moderate level of biodegradation (Volkman *et al.*, 1983b). Finally the gas chromatogram of Lakes Entrance is similar to that previously published (Alexander *et al.*, 1983) and is comprised of an unresolved complex mixture of branched and cyclic alkanes consistent with crude oils having been severely biodegraded.

Table 5.2 Biodegradation level and phenol concentration for crude oils from the Gippsland and Barrow basins, Australia.

Basin	Crude oil	Proposed source	Biodegradation level[4]	Phenol conc.(ng/g)[5]
Gippsland	Basker	Latrobe Group[1]	Non-degraded	32780
	Tuna-4 (2820m)		Non-degraded	69300
	Tuna-4 (1400m)		Moderate	nd
	West Seahorse		Moderate	nd
	Lakes Entrance		Severe	nd
Barrow	Barrow UJ	Dingo Claystone[2,3]	Non-degraded	84580
	Windalia		Moderate	nd
	Crude oil Q		Moderate	nd

1 Stainforth,1984; 2 Volkman *et al.*, 1983a; 3 McLerie *et al.*, 1991, 4 Volkman *et al.*, 1983b, 5 Total concentration of C_0-C_4 alkylphenols; nd = phenols below detection limit

The phenol compositions of the crude oils from the Gippsland basin were examined to assess the effect of biodegradation on petroleum alkylphenols. Table 5.2 shows the total concentrations of C_0-C_4 alkylphenols in the five crude oils. Also shown in Figure 5.11 are the gas chromatograms of the phenol extracts of three crude oils. The non-degraded crude oil, Tuna-4 (2820 m) was found to contain a relatively high concentration of alkylphenols (32780 ng/g). The moderately and severely degraded crude oils, Tuna-4 (1400 m) and Lakes Entrance respectively, however do not contain detectable levels of alkylphenols.

Crude oils from the Barrow sub-basin which ranged from non-degraded to moderately degraded samples were also analysed for their phenol contents. Table 5.2 shows the level of biodegradation and the total C_0-C_4 alkylphenol

concentrations in the three Barrow sub-basin samples. Analogous to the observations made for the Gippsland basin, the non-degraded crude oil was found to contain a high concentration of phenols (84580 ng/g) whereas the two moderately degraded samples contained alkylphenols below the limit of detection. The very low levels of phenols in biodegraded crude oils from the Gippsland and Barrow Basins indicates that alkylphenols are depleted in biodegraded crude oils.

The removal of phenols via water dissolution is an attractive alternative explanation for their very low levels in the biodegraded crude oils. Although water washing and biodegradation might occur independently, they occur in most cases parallel to each other (Tissot and Welte, 1978). Because simple alkylphenols are very water soluble components in crude oil they are likely to be removed from crude oil during water washing processes that occur in conjunction with biodegradation processes.

The observation that biodegraded/water washed crude oils contain very low concentrations of phenols is inconsistent with that reported by Taylor *et al.* (1993) who observed a range of alkylphenols in a moderately degraded Californian crude oil. These authors also observed an increase in the concentrations of C_2 and C_3 alkylphenols and a decrease in C_1 alkylphenols with increasing level of biodegradation as measured by pristane/*n*-C_{17} alkane ratio. The variations in concentrations with biodegradation levels were suggested to be dependent on the relative volumes of water accessing the fields, the change in partition coefficients of the species in the oil-water system as degradation proceeds, and possible generation of new phenolic species during degradation (Taylor *et al.*, 1993). These factors in some combination may therefore be responsible for the discrepancy between the observations made in the Australian crude oils and those reported by Taylor *et al.* (1993).

The contribution of simple alkylphenols to biodegraded crude oils by microorganisms appears unlikely. Although some fungi, bacteria and actinomycete are reported to synthesise a variety of phenolic acids and polyphenols (di- and tri-hydroxy) from non aromatic carbon, e.g. aliphatic precursors (Martin and Haider, 1971; Vaughan and Malcolm, 1985), monohydroxybenzenes (alkylphenols) are not reported to be significant biosynthetic products of microorganisms. Furthermore, biodegradation of aromatic compounds usually results in the formation of di- and tri-hydroxyaromatic compounds rather than monohydroxy metabolites (Vaughan and Malcolm, 1985). Therefore, the contribution of simple alkylphenols to biodegraded oils via biosynthesis or aromatic compound metabolism appears unlikely.

5.6.1 Conclusions

Biodegraded crude oils from the Gippsland and Barrow basins contained very low levels of alkylphenols indicating that these compounds were depleted during crude oil biodegradation. Because water washing and biodegradation often co-occur the very low levels of alkylphenols in the crude oils may also be due in part to the removal of alkylphenols via water dissolution.

5.7 GROUP 6 - CRUDE OILS THAT CONTAIN NEGLIGIBLE LAND PLANT INPUT

Group 6 is comprised of crude oil which are derived from source rocks that pre-date the widespread distribution of land plants or contain negligible higher plant input. Three of the samples in this group have source rocks which pre-date the widespread occurrence of land plants and the two Devonian oils are derived

from marine algal material which contains no indication of higher plant input (Table 5.3).

The phenol composition of these crude oils were examined to assess the importance of land-plant-derived natural products as precursors to petroleum phenols. Figure 5.12 shows the gas chromatograms of the phenol extracts of four crude oils with source rocks of Devonian, Ordovician and Mesoproterozoic ages. All of the crude oils shown in Figure 5.12 contain very low concentrations of C_0-C_5 alkylphenols. Table 5.3 shows that alkylphenols were below the limit of detection in all Group 6 crude oils except Crude oil R.

Table 5.3 Source type, source age and phenol concentration in Group 6 crude oils.

Crude oil	Source type	Age of source rock	References	Phenol conc. (ng/g)
Husky Mobil Rainbow	Marine Bacteria and Algae	Mid Devonian	Isaksen, 1992	nd
Mirbelia	Marine Algae	Devonian	Romine *et al.*, 1994; Summons, 1995	nd
Dodonea	Marine Algae	Ordovician	Hoffman *et al.*, 1987	nd
Crude oil R	Marine Algae	Ordovician	BHP Petroleum, 1985	1403
Jamison	Marine Algae	Mesoproterozoic	Taylor *et al.*, 1994	nd

1 Total concentration of C_0-C_4 alkylphenol, nd = Alkylphenols below detection limit

The occurrence of some alkylphenols in Crude oil R indicates that sources of petroleum phenols other than land-plants did exist. Figure 5.13 shows the gas chromatogram of the phenol extract of Crude oil R. The high relative proportions of methylphenols which can be produced from acid catalysed reactions of tocopherols e.g. 2,5-dimethylphenol (peak 8), 2,3-dimethylphenol (peak 12), 2,3,6-trimethylphenol (peak 19) and 2,3,5-trimethylphenol (peak 23) indicates that the petroleum phenols in Pictor are probably largely derived from tocopherol natural products. The importance of tocopherols as alkylphenol precursors is discussed in Section 6.3 (Chapter 6).

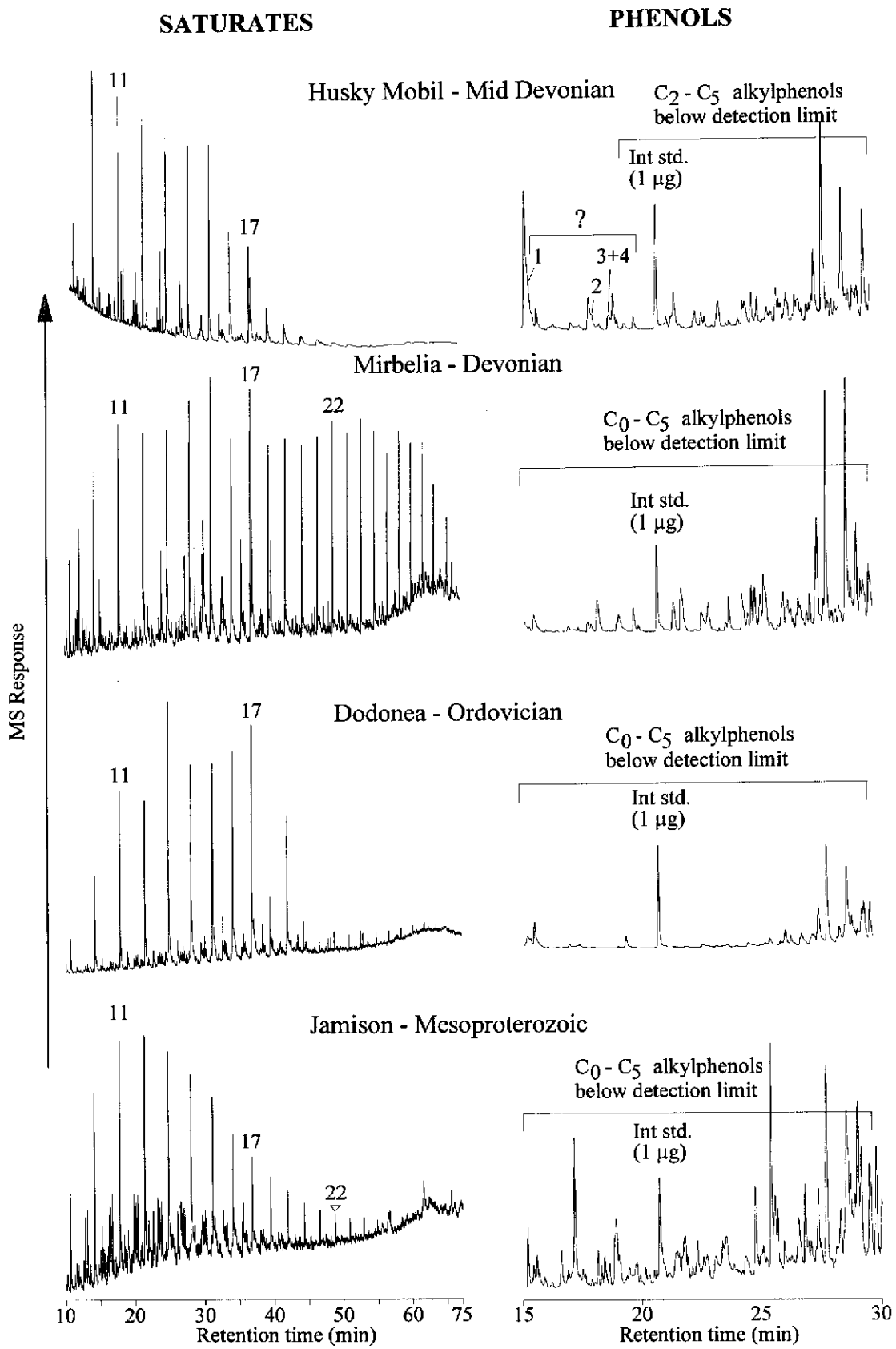


Figure 5.12 Gas chromatograms of the saturated hydrocarbon isolates and the phenol extracts of crude oils with source rocks that pre-date the widespread distribution of land plants or contain negligible higher plant input.

A comparison of the total C_0-C_4 alkylphenol concentrations of Group 6 crude oils with those of other crude oils analysed in this study which postdate the evolution of land plants further indicates that Group 6 samples contain very low levels of phenols (Figure 5.14). Crude oils with source rocks of Tertiary and Cretaceous age in the suite studied contain very high relative concentrations of alkylphenols whereas the average concentration decreases in Jurassic through to Carboniferous sourced crude oils, and finally, the Devonian to Mesoproterozoic sourced crude oils contain the lowest average concentration of alkylphenols. The high concentration of phenols in many crude oils derived from source rocks that

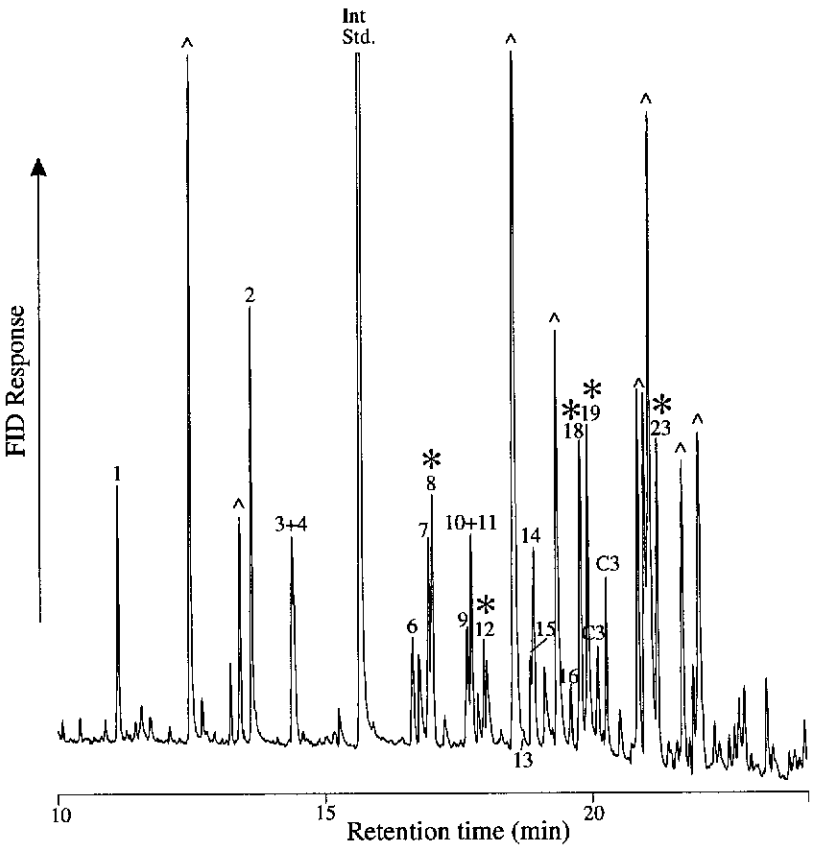


Figure 5.13 Gas chromatogram of the phenol extract of Crude oil R.
* Denotes methylphenols likely to be derived from tocopherols.
^ Peaks do not represent alkylphenols.

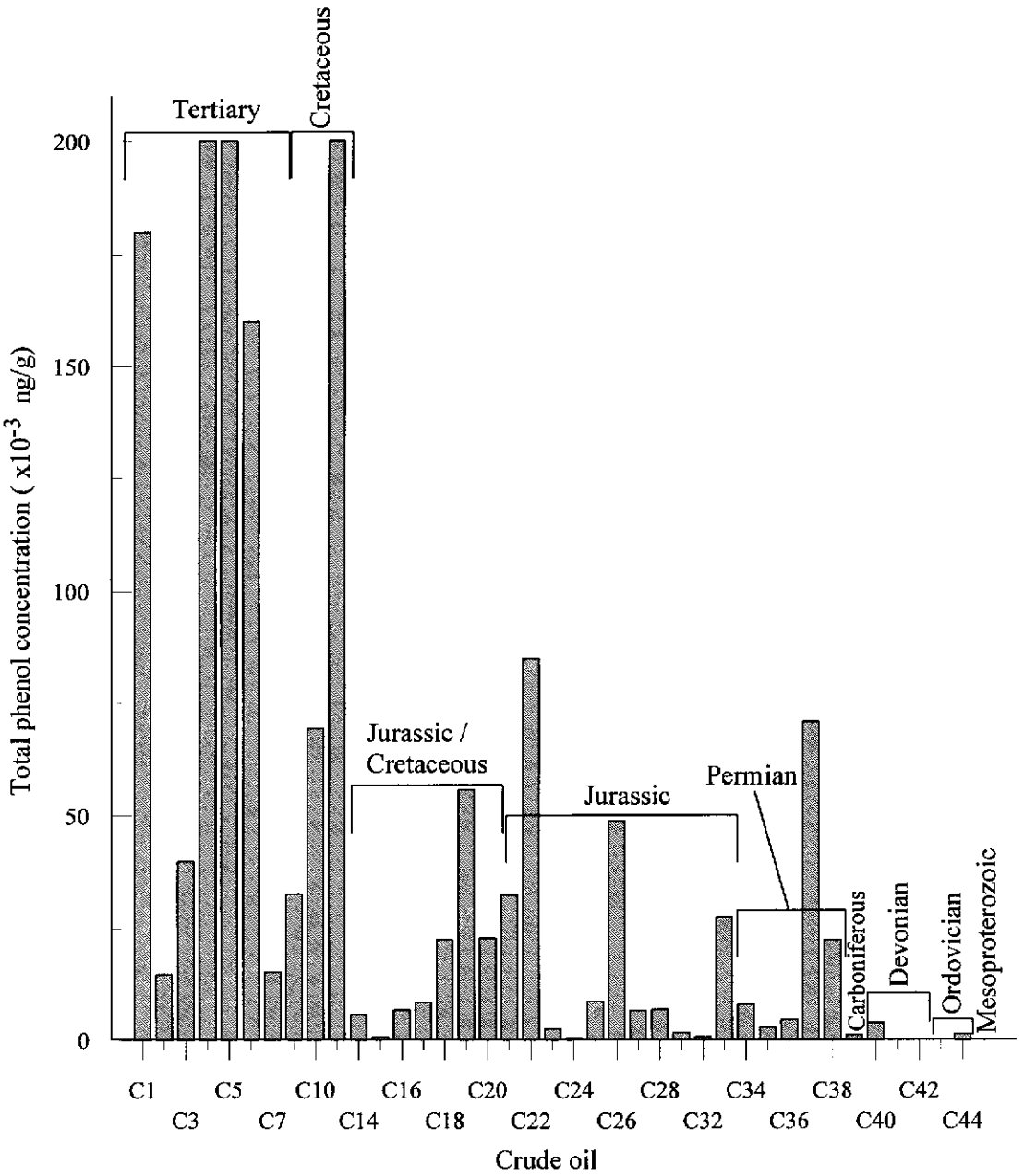


Figure 5.14 Total C-0 to C-4 alkylphenol concentrations in crude oils with source rocks of Tertiary to Mesoproterozoic ages. Biodegraded/water washed crude oils have been omitted (ie. C9, C11, C12, C29 and C30).

postdate the widespread distribution of land plants and the very low concentration in crude oils derived from source rocks which pre-date the evolution of land plants or contain negligible land plant input, suggests that many petroleum alkylphenols originate from land plants or other organisms which become widespread at a similar time in history.

5.8 CONCLUSIONS

The crude oils used in this study were classified into six groups (Table 5.4) on the basis of their C_0-C_5 alkylphenol compositions. Figure 5.15 shows a summary of the distributions of some C_1-C_4 alkylphenols in each group. Samples in Group 1 contained proportions of alkylphenols in at least one isomer group which reflected their relative thermodynamic stabilities. Group 2 samples contained predominantly phenols which could be directly related to natural product precursors such as carvacrol, thymol and tocopherols. Samples in Group 3 predominantly contained alkylphenols which appear to be products of electrophilic aromatic alkylation reactions occurring in source rocks (geosynthetic products). Group 4 and Group 5 contained samples in which the alkylphenol compositions appear to be produced from alteration processes occurring in the subsurface. Such processes included oxidative depletion of *ortho* and *para* substituted phenols and depletion of phenols via biodegradation and/or water washing. Group 6 contained samples which are derived from source rocks which pre-date the widespread distribution of land plants or contained no indication of land plant input. These crude oils contained very low levels of alkylphenols which suggests that land-plant-derived natural products are important precursors to petroleum phenols. The alkylphenol compositions observed in crude oils are therefore dependent on the origins of the phenols (natural products) and the alterations which occurs in the

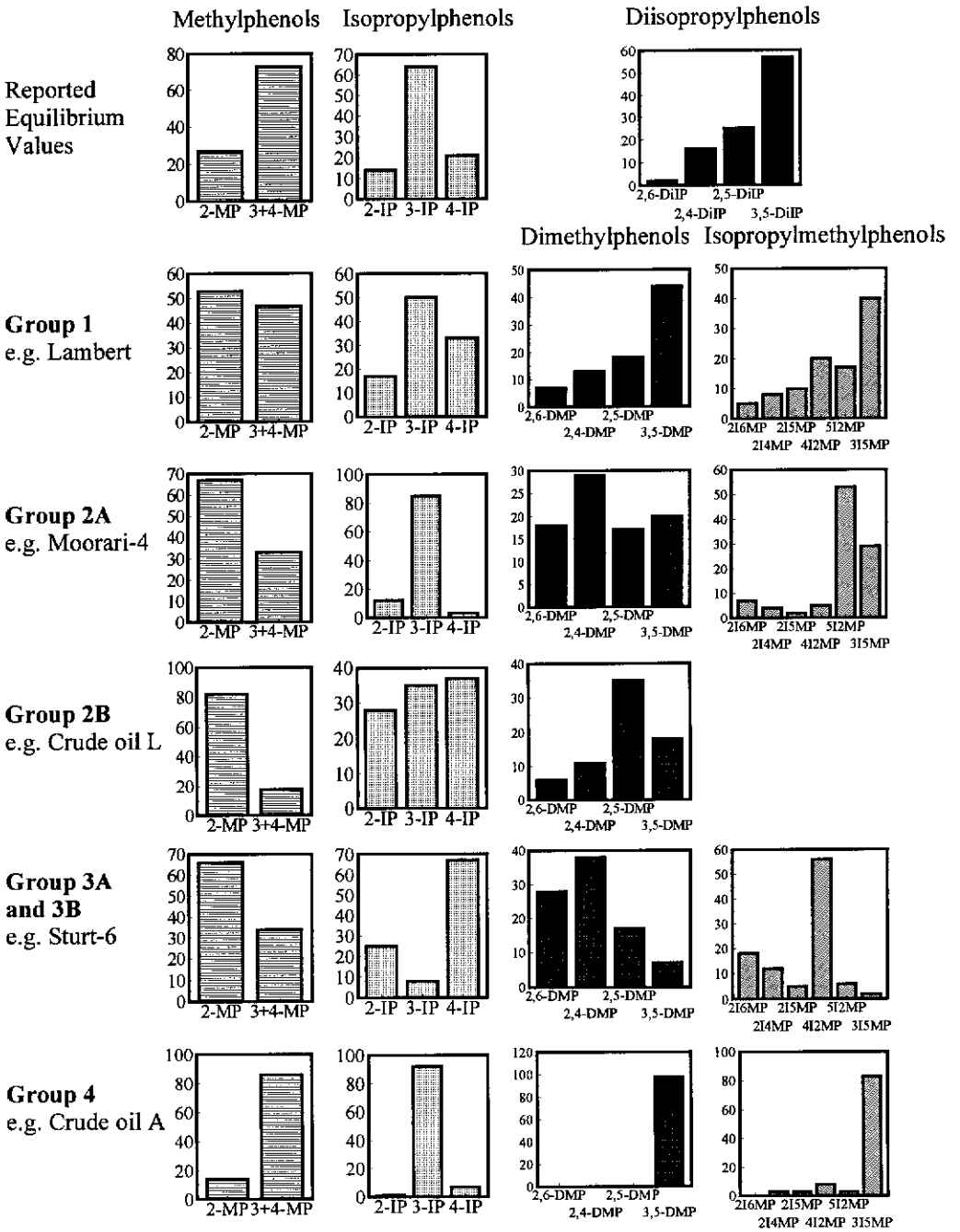


Figure 5.15 Bar graphs showing alkylphenol distributions in equilibrated mixtures and in typical Group1 - Group 6 crude oils. MP = methylphenols, IP = isopropylphenols, DMP = dimethylphenols, xIyMP = x-isopropyl-y-methylphenols.

subsurface (equilibration, geosynthesis, oxidative removal of phenols, biodegradation effects, removal via water washing).

Table 5.4 Classification of crude oils based on alkylphenol composition.

Group 1	**Group 2**		**Group 3**	
Lambert	**2A**	**2B**	**3A**	**3B**
Crude oil J	Iron Duke	Crude oil L	Crude oil D	Nilam
Barrow Deep	Tuna-4 2820 m	Moorari-4	Tuna-4 2820 m	Crude oil C
	Rough Range	Blina	Caroline-1	Crude oil D
	Bodalla South BJ	Crude oil R	Crude oil I	Basker
	Kenmore-1		Barrow UJ	Caroline-1
	Moorari-4		Crude oil O	Earlstown
			Crude oil P	Malgoona-1
			Bodalla South BJ	Sturt
			Kenmore-1	
			Moorari-4	
			Byrock	
			Lycium	
			Malgoona-1	
			Sturt	

Group 4	**Group 5**	**Group 6**	**Ungrouped**
Crude oil A	Tuna-4 1400 m	Husky Mobil Rain.	Crude oil G
Crude oil B	West Seahorse	Mirbelia	Crude oil H
Crude oil E	Lakes Entrance	Dodonea	Crude oil K
Crude oil F	Windalia	Crude oil R	Crude oil N
Crude oil M	Crude oil Q	Jamison	West Terrace

CHAPTER 6

SOME NATURAL PRODUCT PRECURSORS OF PETROLEUM ALKYLPHENOLS

6.1 INTRODUCTION

The abundance and widespread occurrence of phenolic natural products in nature and their relatively high resistance to microbial degradation leads to their incorporation in sedimentary rocks. In principle therefore, some petroleum phenols are likely to be derived from naturally occurring phenols which have contributed to the source rocks. It is important to bear in mind that these natural products may have undergone diagenetically and/or catagenetically induced changes and hence occur in a slightly altered form in petroleum. In this chapter the natural product origins of some petroleum alkylphenols are investigated. Lignins are the focus of the first section, while monoterpenoid phenols and tocopherol natural products make up the remaining part of this chapter. Where appropriate, the alterations which these natural products may have undergone in the sedimentary environment are investigated in order to deduce the petroleum alkylphenols most likely to be derived from them.

6.2 LIGNINS

Lignins are the most abundant and resistant phenolic biopolymers found in nature. They are dominant components of terrestrial plants and therefore are the major contributors to peat and the vitrinite maceral in coals. In order to link lignin natural product precursors to petroleum alkylphenols on a molecular level it is necessary to understand the transformations lignin phenols undergo during coalification. The aim of this section is to examine in detail the molecular changes that take place in a suite of coal samples from lignitic through to bituminous rank.

The unbound phenolic components of two brown coals (Loy Yang and Heartbreak) are determined by isolating the phenols from their dichloromethane extracts and analysing them by GC-MS. The bound components of these immature samples are also determined by flash pyrolysis GC-MS techniques and a comparison made of the alkylphenols with those of the SOM.

In a later section, the bound phenolic components of subbituminous and bituminous coals are quantitatively analysed by isolating the phenols from their hydrous pyrolysates. This enabled minor components (C_3-C_4 alkylphenols) to be examined which are particularly important in the higher rank samples since coals of this rank are more likely to give rise to petroleum alkylphenols.

6.2.1 Samples

Five coal samples from Australia and Indonesia have been used in this study. Geological and geochemical data pertaining to the samples are provided in Table 3.2 and Table 3.3 (Chapter 3). Loy Yang coal is a medium-light lithotype coal (onshore Loy Yang Field, Latrobe Valley, core LY1277) from the extensive Miocene brown coal deposits of the Gippsland Basin, Victoria. The brown coal deposits in the Latrobe valley are endowed with fossil angiosperm and gymnosperm wood (Chaffee *et al.*, 1984; George *et al.*, 1984; Bates and Hatcher, 1989; Hatcher *et al.*, 1989b). Heartbreak coal is a late Eocene lignite from the Werillup Formation of the Bremer Basin, Western Australia. The GK coal samples are from the South Sumatra Basin, Indonesia.

6.2.2 Phenols in Brown Coal Extracts and Flash Pyrolysates

Loy Yang Coal

The phenolic components in the SOM of Loy Yang coal were extracted using analytical procedure I (Figure 4.1, Chapter 4). Figure 6.1 shows the total ion chromatogram of the phenol isolate. Based on mass spectral data it was determined that aldehydes and ketones were the major components, and alkylphenols (peaks 1, 2 and 3) were relatively minor components in the phenol extract. The major components were deduced to be vanillin (peak V), acetovanillon (peak AV) and methylacetovanillon (peak MAV) which are structurally related to the guaiacyl unit of lignins whereas syringealdehyde (peak S), and acetosyringone (peak AS) are structurally related to the syringyl unit (*cf.* Figure 1.1; Chapter 1). The presence of these phenolic aldehydes and ketones in the SOM of Loy Yang coal is consistent with the reported widespread occurrence of angiosperm and gymnosperm lignin in the brown coal deposits of the Latrobe Valley (Chaffee *et al.*, 1984; George *et al.*, 1984; Bates and Hatcher, 1989; Hatcher *et al.*, 1989b). The occurrence of syringyl related phenols in the SOM indicates that angiosperm wood was a significant precursor to this coal sample whereas the guaiacyl related phenols indicate both angiosperm and/or gymnosperm wood precursors. This conclusion relies heavily on the assumption that the SOM is inherent to this coal sample, however it must be recognised that the SOM may be a migrated product and hence not necessarily be indicative of the coal sample from which it was extracted.

The occurrence of oxidised lignin units such as phenolic aldehydes and ketones, and the absence of intact phenylpropane units in the Loy Yang coal extract suggests that this sample has been biodegraded. Although lignins, particularly the phenylpropane units, are resistant to physical and chemical

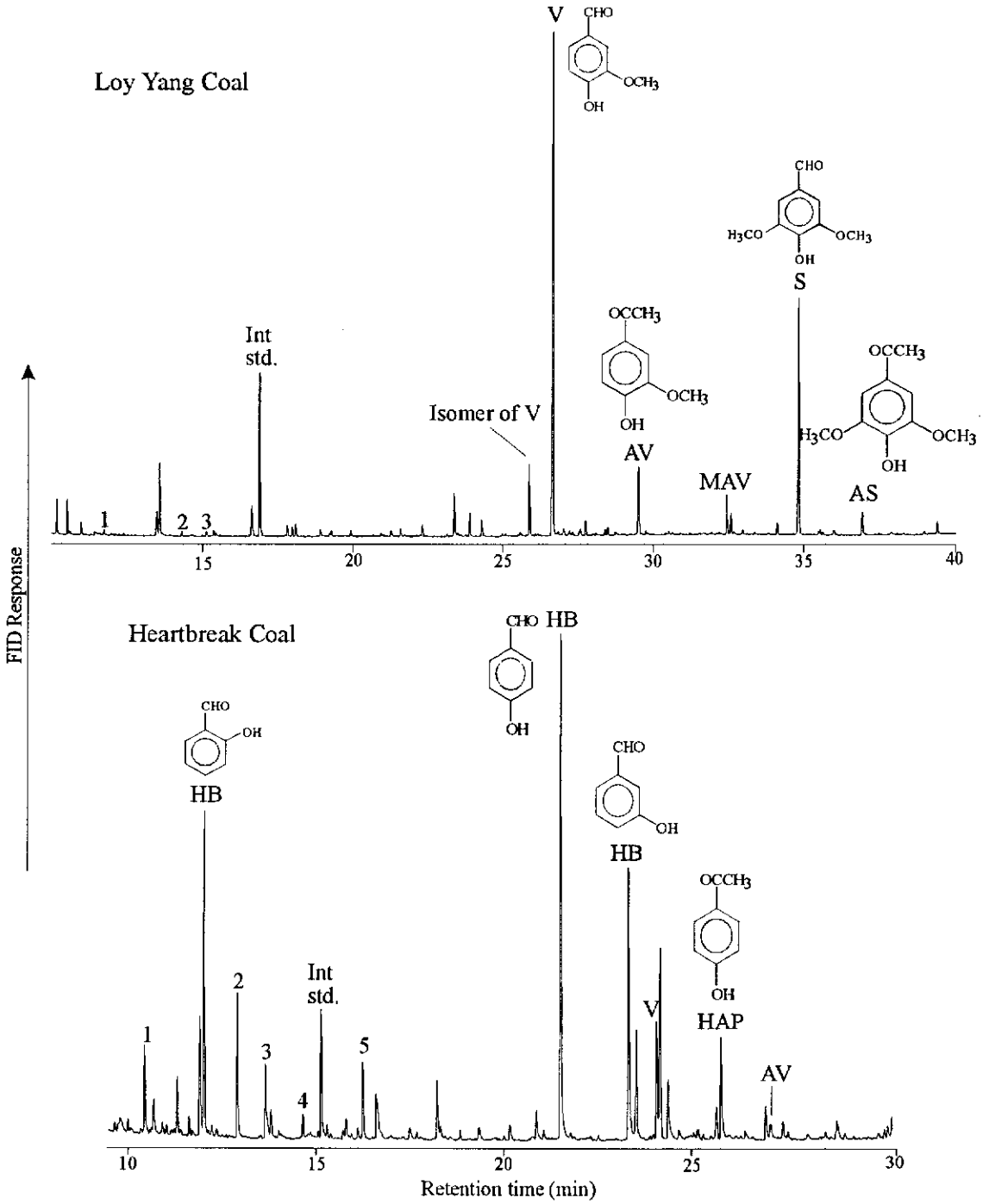


Figure 6.1 Total ion chromatograms of the phenol isolates of Loy Yang Coal and Heartbreak Coal dichloromethane extracts. 1 = phenol, 2 = *o*-cresol, 3 = *m*+*p*-cresol, 4 = 2,6-dimethylphenol, 5 = 2,4-dimethylphenol, V = vanillin, HAP = *p*-hydroxy-acetophenone, AV = acetovanillon, MAV = methylacetovanillon, S = syringealdehyde, AS = acetosyringone, HB = hydroxybenzaldehyde, Int std. = *o*-nitrophenol.

degradation, many microorganisms are able to degrade the polymers to varying extents (refer to Chapter 1; Section 1.2.1). Some of the phenolic compounds reported to be derived from lignin metabolism are vanillin, *p*-hydroxybenzaldehyde, syringealdehyde and vanillic acid (Alexander, 1961; Hatta *et al.*, 1966; Christman and Oglesby, 1971). The high relative abundances of the lignin metabolites vanillin and syringealdehyde in the phenol isolate indicates that Loy Yang coal has been subjected to microbial attack.

The bound phenolic components of Loy Yang coal were analysed using flash pyrolysis GC-MS. Figure 6.2 shows a) the total ion chromatogram of the flash pyrolysis products of dichloromethane-extracted Loy Yang coal, and selected ion chromatograms showing b) alkylphenols and c) hydroxyphenols in the flash pyrolysate. Examination of the mass spectra of major peaks in the total ion chromatogram showed that hydroxyaromatic compounds (alkylphenols and hydroxyphenols) were the major flash pyrolysis products of Loy Yang coal. In contrast to the SOM, the pyrolysis products of Loy Yang coal did not contain detectable levels of methoxyphenols (guaiacyl and syringyl compounds). The pyrolysis products obtained from this sample are very similar to those previously reported for gelified wood from the Latrobe Valley, where alkylphenols and hydroxyphenols were the major products (Chaffee *et al.*, 1984). In contrast, fossil non-gelified gymnosperm and angiosperm woods from this area gave pyrograms dominated by methoxyphenols (Philp *et al.*, 1982; Chaffee *et al.*, 1984). Therefore, the pyrolysis products obtained from the Loy Yang sample used in this study appear to have been derived from diagenetically altered lignins which have undergone demethylation and dehydroxylation consistent with coalification processes (Botto, 1987; Stout *et al.*, 1988; Hatcher *et al.*, 1989a; 1989b). In contrast to the methoxyphenols in the SOM, the phenolic pyrolysis products do

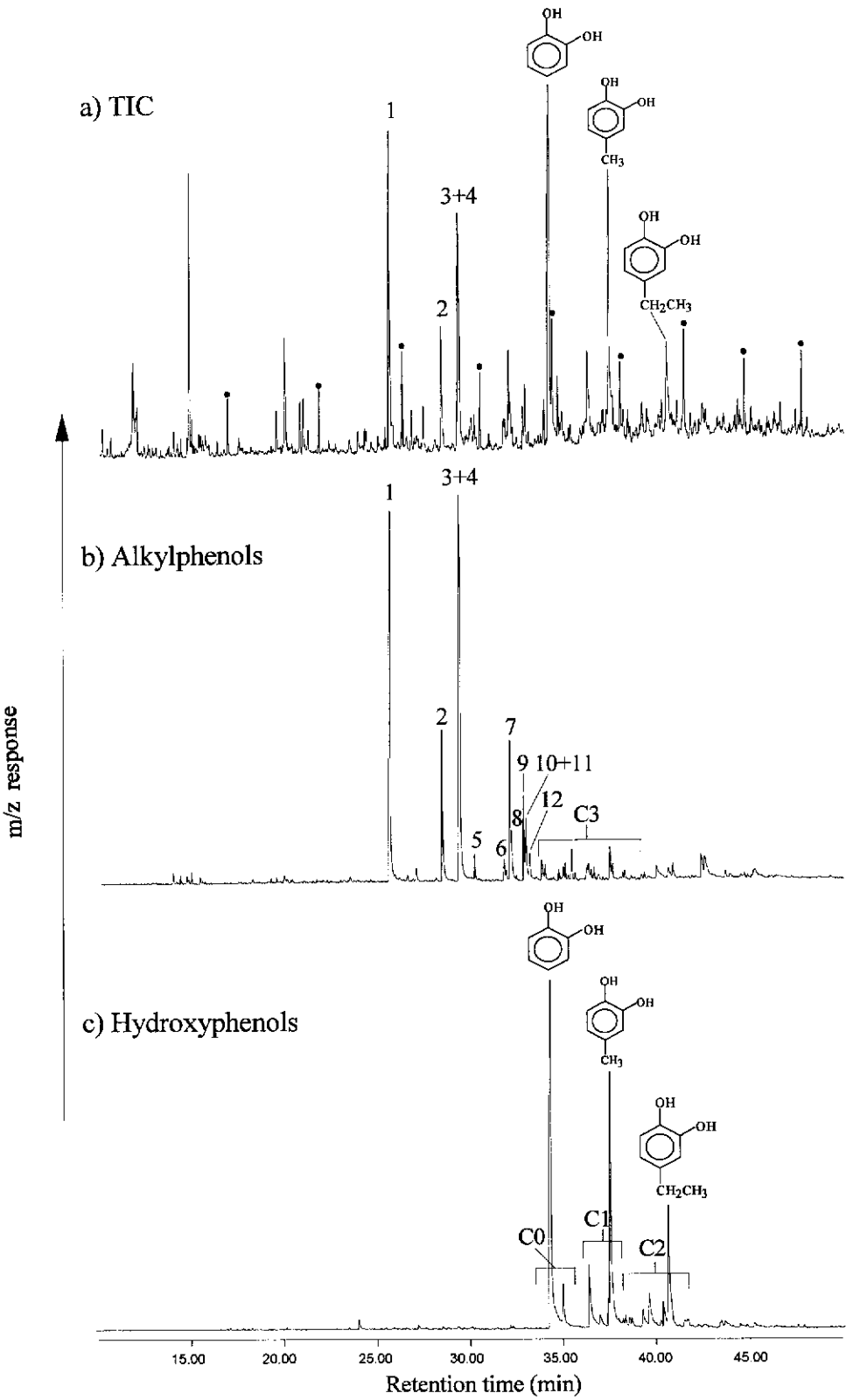


Figure 6.2 a) Total ion chromatorgam, b) selected ion chromatogram (sum m/z 94,107, 108, 121, 122, 136) for C-0 to C-3 alkylphenols and c) selected ion chromatogram (sum m/z 110, 123, 124, 138) for C-0 to C-2 hydroxyalkylphenols of the flash pyrolysis products of Loy Yang coal (600°C).
• Represent *n*-alkanes. See Table 4.4 for peak identifications.

not allow taxonomical classification of this sample, however they do indicate a lignin origin.

The differences in the phenol composition of the SOM and pyrolysate of Loy Yang coal suggests that this sample contains coal structures which have undergone different levels of diagenesis. The suggestion that coalified tissue of lignitic rank is composed of a mixture of structures, some of which have evolved more than others has been made previously (Hatcher, 1990). The methoxyphenols in the SOM of Loy Yang coal appear to have been produced from microbial attack on comparatively unaltered lignins whereas the phenols and hydroxyphenols in the flash pyrolysate are produced from more severely diagenetically altered structures. An estimation of the relative abundances of the lignin structures at different levels of diagenesis can be obtained by comparing phenol yields obtained from them. It was necessary to extract the phenols from the SOM of ten times more coal in order to obtain the equivalent amount of phenols yielded in the flash pyrolysate. This suggests that the absence of methoxyphenols in the pyrolysate of Loy Yang coal is due to the very low abundance of relatively intact lignins compared with more modified structures resulting from demethylation and dehydroxylation.

The high relative abundance of methoxyphenols in the coal extract however suggests that these compounds are selectively concentrated in the SOM. One process by which this could occur is via the selective cleavage of methoxyphenols from lignins by microbes. In this process the compounds from intact lignin structures may be "bio-selectively" cleaved compared to the diagenetically produced alkylphenol and hydroxyphenol based geopolymers. Therefore, the selective biodegradation of the natural biopolymer may be responsible for the dominance of methoxyphenols in the coal extract.

Heartbreak Coal

The phenolic components in the dichloromethane extract of Heartbreak coal were isolated using analytical procedure I (Figure 4.1, Chapter 4). Figure 6.1 shows the total ion chromatogram of the phenol isolate. Based on mass spectral data, the major components in the isolate were assigned as hydroxybenzaldehydes (peaks HB) with lower relative abundances of vanillin and acetovanillon (peaks V and AV) and alkylphenols (peaks 1-5). The absence of methoxyphenols which are structurally related to the syringyl unit of angiosperm lignins suggests that angiosperm wood was not a significant contributor to this coal sample. The hydroxyaromatics, particularly the compounds related to the guaiacyl lignin unit (peak V and AV) in the Heartbreak coal phenol isolate, are therefore most likely derived from gymnosperm lignins. Further, the presence of phenolic aldehydes and ketones, and the absence of intact phenylpropane units in the coal extract suggests that this coal sample has been biodegraded, as these compounds are products of lignin metabolism (Alexander, 1961; Hatta *et al.*, 1966; Christman and Oglesby, 1971).

The bound phenolic components of Heartbreak coal were analysed using flash pyrolysis GC-MS. Figure 6.3 shows a) the total ion chromatogram and selected ion chromatograms showing b) alkylphenols and c) hydroxyphenols of the flash pyrolysis products. Examination of mass spectra of major peaks in the total ion chromatogram showed that hydroxyaromatic compounds were relatively minor pyrolysis products and aliphatic compounds were the major pyrolysis products of Heartbreak coal. Among the hydroxyaromatic pyrolysis products, alkylphenols (Figure 6.3 b) occurred in higher relative abundance than hydroxyphenols (Figure 6.3 c). In contrast to the phenols in the SOM of this sample, the flash pyrolysate did not contain detectable levels of hydroxybenzaldehydes or methoxyphenols. The occurrence of a high relative

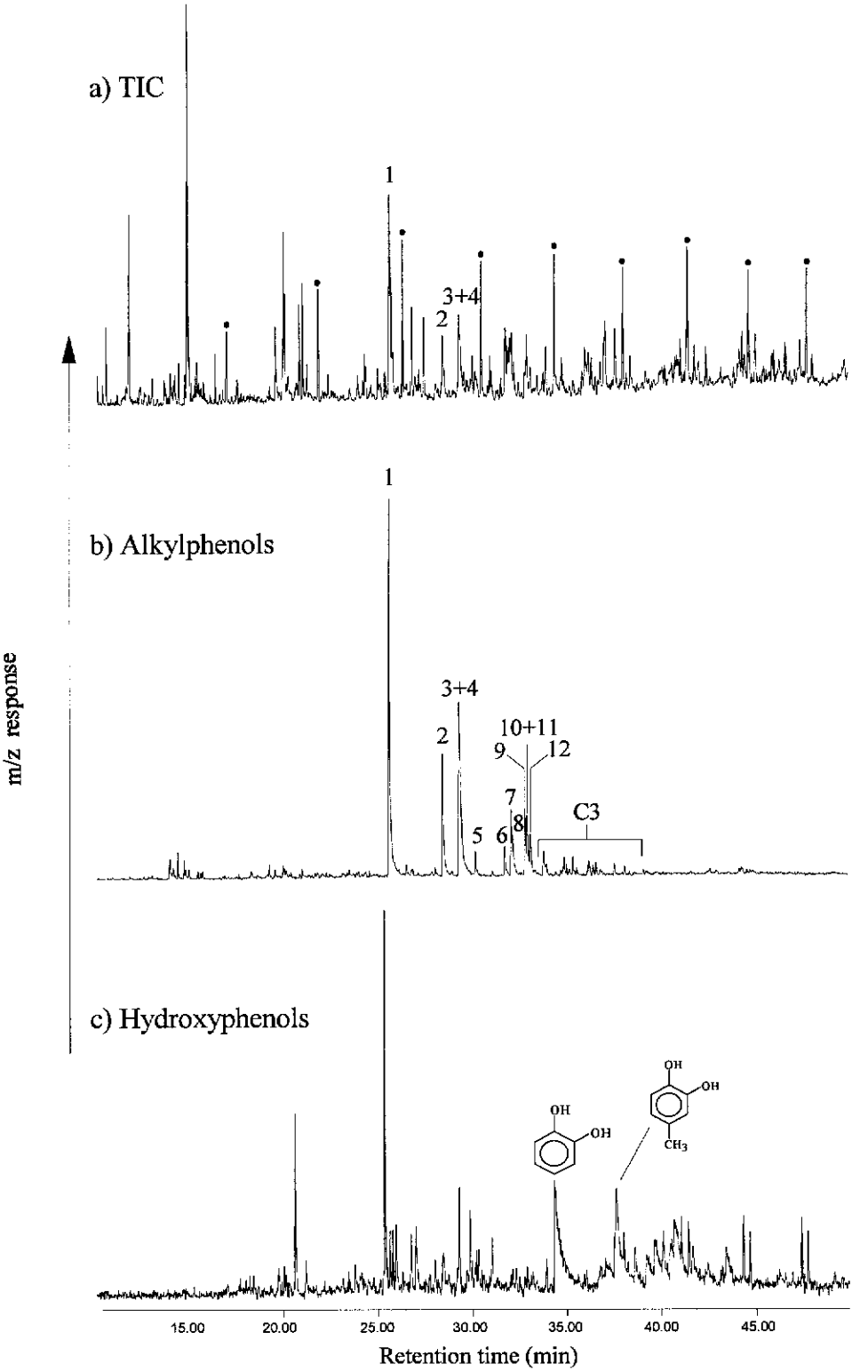


Figure 6.3 a) Total ion chromatorgam, b) selected ion chromatogram (sum m/z 94,107, 108, 121, 122, 136) for C-0 to C-3 alkylphenols and c) selected ion chromatogram (sum m/z 110, 123, 124, 138) for C-0 to C-2 hydroxyalkylphenols of the flash pyrolysis products of Heartbreak coal (600°C).
• Represent *n*-alkanes. See Table 4.4 for peak identifications.

abundance of alkylphenols compared to hydroxyphenols in the pyrolysate indicates that this coal contains lignin structures which have undergone coalification processes such as demethylation and dehydroxylation. Dehydroxylation is an important process leading to the formation of structures in subbituminous coal which yield alkylphenols when pyrolysed (Hatcher, 1990). The Heartbreak sample therefore, appears to contain some lignin derived structures which have undergone a degree of metamorphism consistent with coal approaching subbituminous rank.

6.2.3 Alkylphenols in the Hydrous Pyrolysates of South Sumatra Basin Coals of Subbituminous and Higher Rank

The bound phenolic components of GK coal samples in a sedimentary sequence from South Sumatra Basin have been analysed by isolating the phenols from their hydrous pyrolysates (Chapter 4; analytical procedure II). A lignite sample (Ro = 0.3) which shows no evidence of methoxyphenols in the pyrolysate has been included as the first sample in the sequence. The two remaining samples are coals of subbituminous and bituminous rank (Ro = 0.6 and 0.9 respectively). The total ion chromatograms of the phenol extracts are shown in Figure 6.4. The phenol distributions in these chromatograms are consistent with reported distributions in which phenol and cresols dominate with lesser amounts of C_2 alkylphenols and very low relative abundances of higher substituted phenols (*cf.* Allan and Larter, 1981; Senftle *et al.*, 1986; Nip *et al.*, 1988).

Phenol concentrations in the GK coal hydrous pyrolysates were determined using the internal standard method and the correction factors discussed in Section 4.4 (Chapter 4). Table 6.1 shows the concentrations, expressed in milligram/gram of pyrolysate, for a range of C_0-C_4 alkylphenols. These concentrations are approximate, since the efficiencies with which the alkylphenols

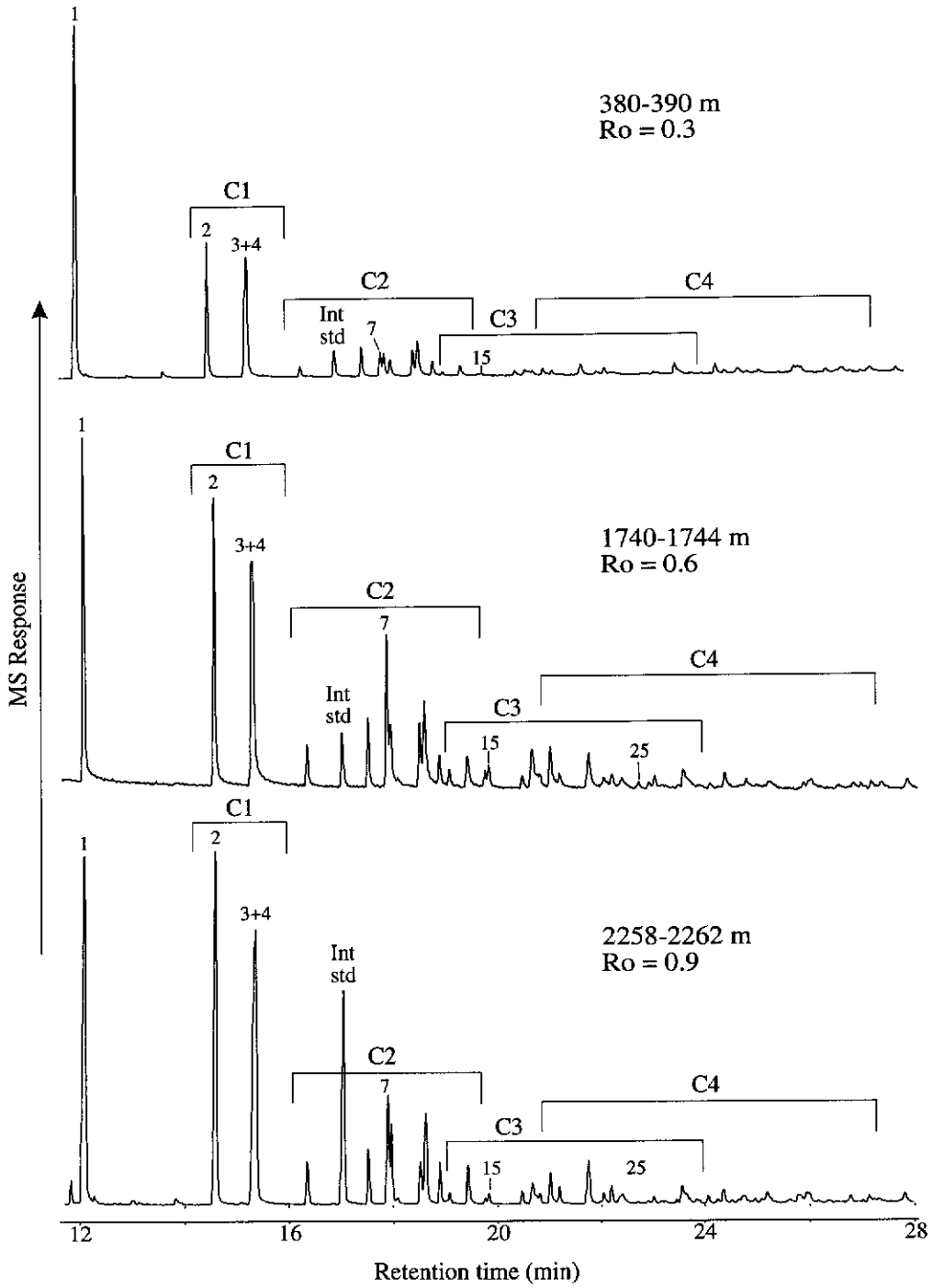


Figure 6.4 Partial total ion chromatograms of the phenol extracts from GK coal hydrous pyrolysates. Refer to Table 4.4 for peak identifications.

Table 6.1 Phenol concentrations (mg/g of pyrolysate) in the hydrous pyrolysates of GK coals.

Peak	Compound	Sample			1740m/ 380m [1]
		380 m Ro = 0.3	1740 m Ro = 0.6	2258 m Ro = 0.9	
1	phenol	92	14	4.6	0.15
2	2-methylphenol	23 (35)[3]	8.8 (35)	3.3 (40)	0.38
3+4	3-+4-methylphenol [2]	42 (65)	16 (65)	5.0 (60)	0.38
6	2-ethylphenol	6.0 (42)	2.4 (38)	0.56 (47)	0.40
9	4-ethylphenol	8.2 (58)	3.9 (62)	0.64 (53)	0.48
10+11	3-ethylphenol + 3,5-dimethylphenol [2]	9.2	4.2	1.26	0.47
5	2,6-dimethylphenol	1.4 (9)	1.0 (10)	0.35 (11)	0.71
7	2,4-dimethylphenol	3.7 (24)	4.2 (40)	0.98 (30)	1.1 *
8	2,5-dimethylphenol	3.5 (23)	2.1 (20)	0.86 (26)	0.60
12	2,3-dimethylphenol	3.3 (22)	1.4 (13)	0.48 (14)	0.42
13	3,4-dimethylphenol	3.3 (22)	1.8 (17)	0.62 (19)	0.55
19	2,3,6-trimethylphenol	1.3 (36)	0.64 (31)	0.15 (29)	0.49
23	2,3,5-trimethylphenol	1.3 (36)	0.60 (30)	0.20 (39)	0.46
15	2,4,6-trimethylphenol	0.52 (15)	0.66 (33)	0.11 (21)	1.3 *
32	3,4,5-trimethylphenol	0.45 (13)	0.13 (6)	0.053 (10)	0.29
14	2-isopropylphenol	0.55 (17)	0.56 (16)	0.031 (7)	1.02 *
18	3-isopropylphenol	1.1 (34)	0.68 (20)	0.18 (42)	0.62
17	4-isopropylphenol	1.6 (49)	2.2 (64)	0.22 (51)	1.4 *
20	2-isopropyl-6-methylphenol	0.077 (6)	0.28 (11)	0.017 (9)	3.6 *
25	2-isopropyl-4-methylphenol	0.10 (8)	0.44 (17)	0.011 (6)	4.4 *
26	thymol	0.40 (33)	0.46 (18)	0.026 (14)	1.1 *
27	4-isopropyl-2-methylphenol	0.15 (12)	0.93 (37)	0.079 (42)	6.2 *
28	carvacrol	0.43 (36)	0.17 (7)	0.019 (10)	0.40
30	3-isopropyl-5-methylphenol	0.055 (5)	0.24 (10)	0.036 (19)	4.4 *
Total		204	67.8	19.8	0.33

1 Ratio of absolute concentration in 1740 m sample to that at 380 m. * Denotes phenols with ratios greater than one. 2 Individual yields could not be calculated due to co-elution.
3 Values in parentheses are the percentages of the isomers among similarly substituted phenols. Relative percentage of 3-ethylphenol and 3,5-dimethylphenol were not included in the ethylphenol and dimethylphenol groups respectively due to co-elution.

were recovered from the pyrolysed coal and aqueous phase were not determined. The relative proportions expressed as percentages of the isomers among groups of similarly substituted phenols (ie. methylphenols, dimethylphenols, trimethylphenols, isopropylphenols and isopropylmethylphenols) are shown in parenthesis in Table 6.1. Ratios of the concentrations of individual compounds obtained for the sample of subbituminous rank (1740 m) to those obtained from the shallowest sample were also calculated (Table 6.1). The subbituminous coal sample was selected for

comparison to the lignite sample because the molecular transformations occurring to lignin phenols during coalification were found to be most interesting at subbituminous rank.

The total phenol concentration in the GK coals hydrous pyrolysates decreased with increasing rank. Table 6.1 shows the total concentration of phenols decreased from 204 mg/g for the most immature sample (380 m, Ro = 0.3) to 19.8 mg/g for the most mature sample (2258 m, Ro = 0.9). These results are consistent with literature reports showing that the alkylphenol concentrations obtained from coal and vitrinite concentrate pyrolysates are strongly and inversely rank related (Senftle *et al.*, 1986; Senftle and Larter, 1987; Øygard *et al.*, 1988). The decrease in the phenol content of coals with increasing rank is in line with the decrease in oxygen shown by type III kerogen and humic substances in the Van Krevelen diagram with increase in maturity (Tissot and Welte, 1978).

Comparison of the relative proportions of alkylphenols isolated from the hydrous pyrolysates of the GK coals showed that the proportions of *ortho* and *para* substituted alkylphenols increased with increasing rank. In fact, the proportions of *ortho* and *para* substituted phenols increased to such a high extent in the subbituminous sample that their individual concentrations increased in this sample despite the decrease observed in the total alkylphenol concentration. The

higher relative proportions and individual concentrations of *ortho* and *para* alkylphenol in the higher rank coal pyrolysate are attributed to the electrophilic aromatic substitution reactions which have been reported to occur in coals (refer to Section 1.2.1; Lignins). A detailed discussion of the changes observed in five alkylphenol isomer classes; methylphenol, dimethylphenol, trimethylphenol, isopropylphenol, isopropylmethylphenol, and the electrophilic alkylation processes likely to give rise to them comprise the remaining part of this section.

The gas chromatograms in Figure 6.4 show the proportions of phenol and methylphenols in the GK coal pyrolysates. A decrease in phenol (peak 1) relative to the cresols (peaks 2, 3 and 4) is observed with increasing rank. The relative proportions of the cresols however do not change significantly with increasing rank, with only a small relative increase of 2-methylphenol observed in the most mature samples (Figure 6.5 a).

Figure 6.5 b) shows a plot of the proportions (measured as percentages) of dimethylphenols in the GK coal hydrous pyrolysates, and Figure 6.6 shows the selected ion chromatograms of the C_2 alkylphenols in the GK coal pyrolysates. These figures illustrate that the most outstanding change in the C_2 alkylphenol composition of the pyrolysates is the increase in the relative proportions of 2,4-dimethylphenol (peak 7) in the sample at 1740 m as compared to the lignite sample. Examination of the ratios in Table 6.1 shows that there was actually an increase in the individual concentration of 2,4-dimethylphenol in the pyrolysate of the sample at depth 1740 m (1740 m/380 m = 1.1), whereas the concentrations of all other C_2 alkylphenols decreased. Therefore, despite the general trend that phenol concentrations in the pyrolysate decrease with increasing rank, the individual concentration of 2,4-dimethylphenol has increased in the subbituminous coal sample.

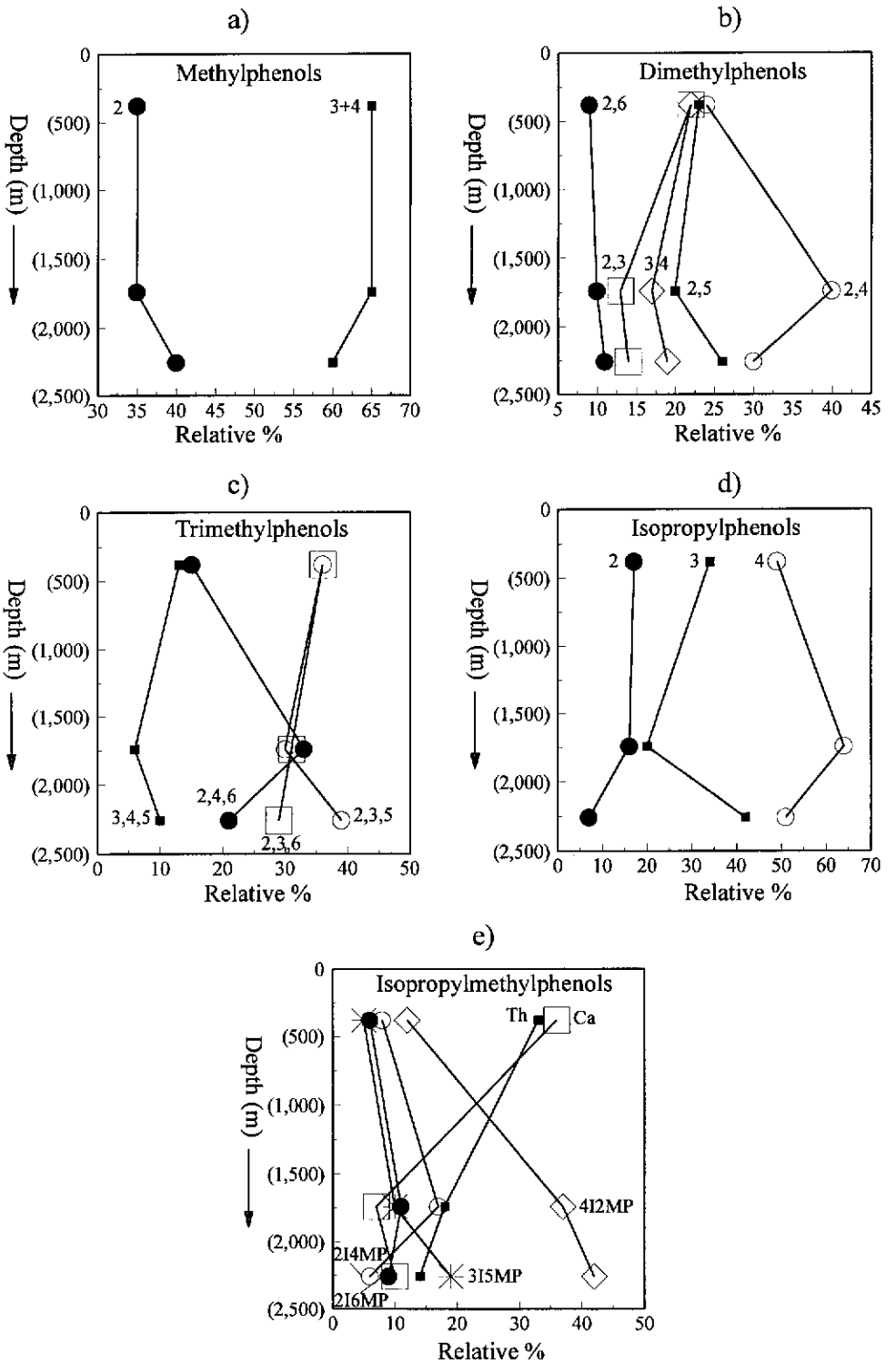


Figure 6.5 Plots showing the percentages of individual alkylphenol isomers within an isomer class in the hydrous pyrolysis products of GK coal samples. Numbers in the plots refer to position(s) of alkyl substituent(s). Th = thymol, Ca = carvacrol, xIyMP = x-isopropyl-y-methylphenol.

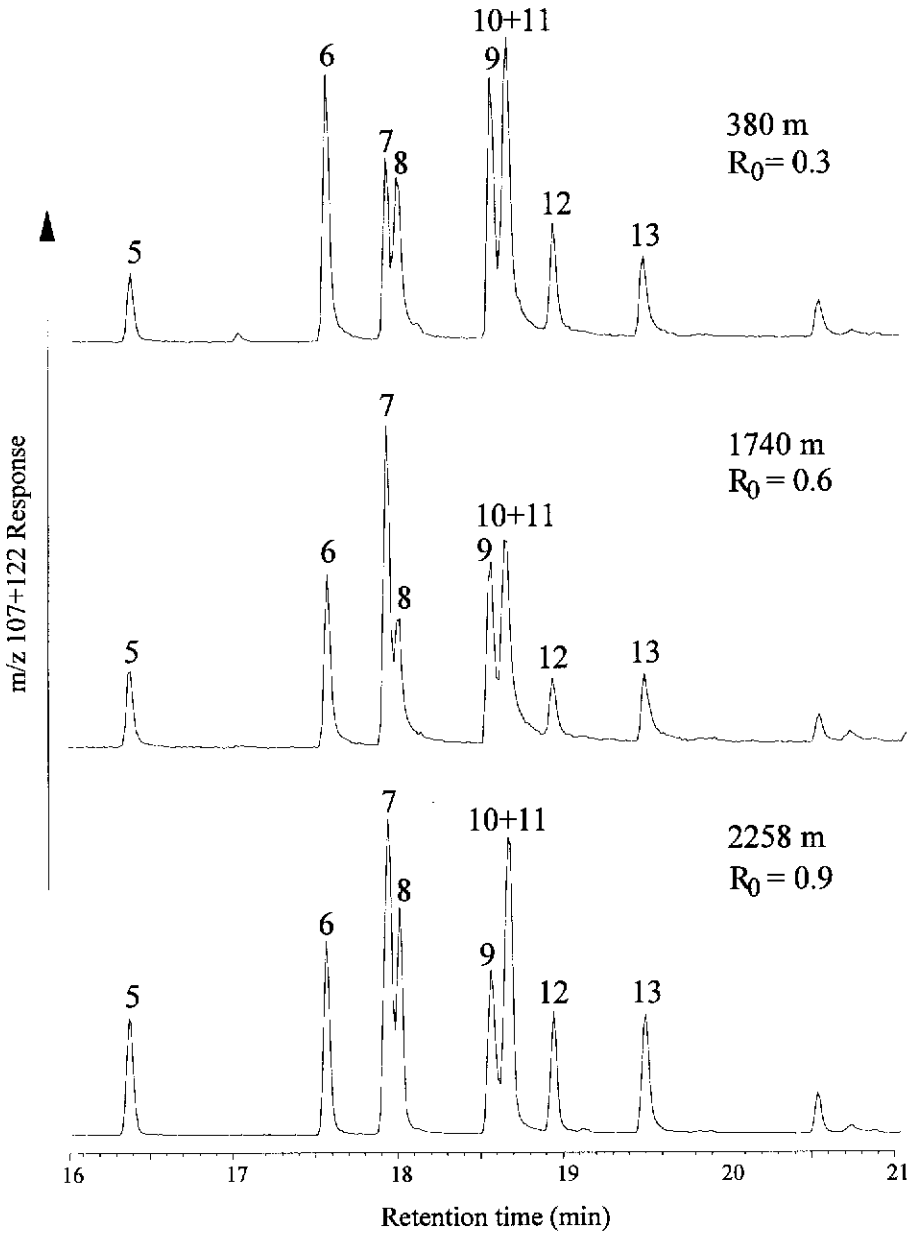


Figure 6.6 Partial selected ion chromatograms (m/z 107+122) of C2 alkylphenols extracted from the hydrous pyrolysates of GK coals. Refer to Table 4.4 for peak identifications.

Figure 6.7 shows the selected ion chromatograms of the C_3 alkylphenols in the GK coal hydrous pyrolysates. The lignite (380 m) sample contains high relative abundances of *n*-propylphenols (peaks 16 and 21) which are likely to be derived from the phenylpropyl units of lignin compared with the more mature samples. An unknown ethylmethylphenol coelutes with 4-propylphenol, and in order to estimate the relative abundances of these phenols contributing to peak 21+a the relative abundances of the M-29 ion (m/z 107-propylphenol) and M-15 ion (m/z 121-ethylmethylphenol) contributing to the peak were determined. Plots of the proportions of trimethylphenols and isopropylmethylphenols in the coal pyrolysates are shown in Figure 6.5 c) and d) respectively. Among the trimethylphenols, the relative percentage of 2,4,6-trimethylphenol was higher in the sample at depth 1740 m compared to the lignite sample (Figure 6.5 c). The relative percentage of 4-isopropylphenol was also higher in the subbituminous sample pyrolysate (Figure 6.5 d). Table 6.1 shows that the concentration of 2,4,6-trimethylphenol was higher in the pyrolysate of the sample at depth 1740 m (1740 m/380 m = 1.3), whereas the concentrations of the remaining trimethylphenols were less. Similarly, the individual concentrations of 2-isopropylphenol (1740 m/380 m = 1.02) and 4-isopropylphenol (1740 m/380 m = 1.4) were higher in the deeper sample.

Figure 6.8 shows the selected ion chromatograms of isopropylmethylphenols in the GK coal pyrolysates. The gas chromatograms reveal some remarkable changes in the relative distribution of isopropylmethylphenols in the lignite sample (380 m) to those in the two deeper samples (1740 m and 2258 m). The isopropylmethylphenol distribution in the lignite sample pyrolysate is dominated by thymol (peak 26) and carvacrol (peak 28), two isoprenoid phenols which occur in extant higher plants (refer to Chapter 1; Section 1.2.1). Their occurrence in the lignite sample is likely to be indicative

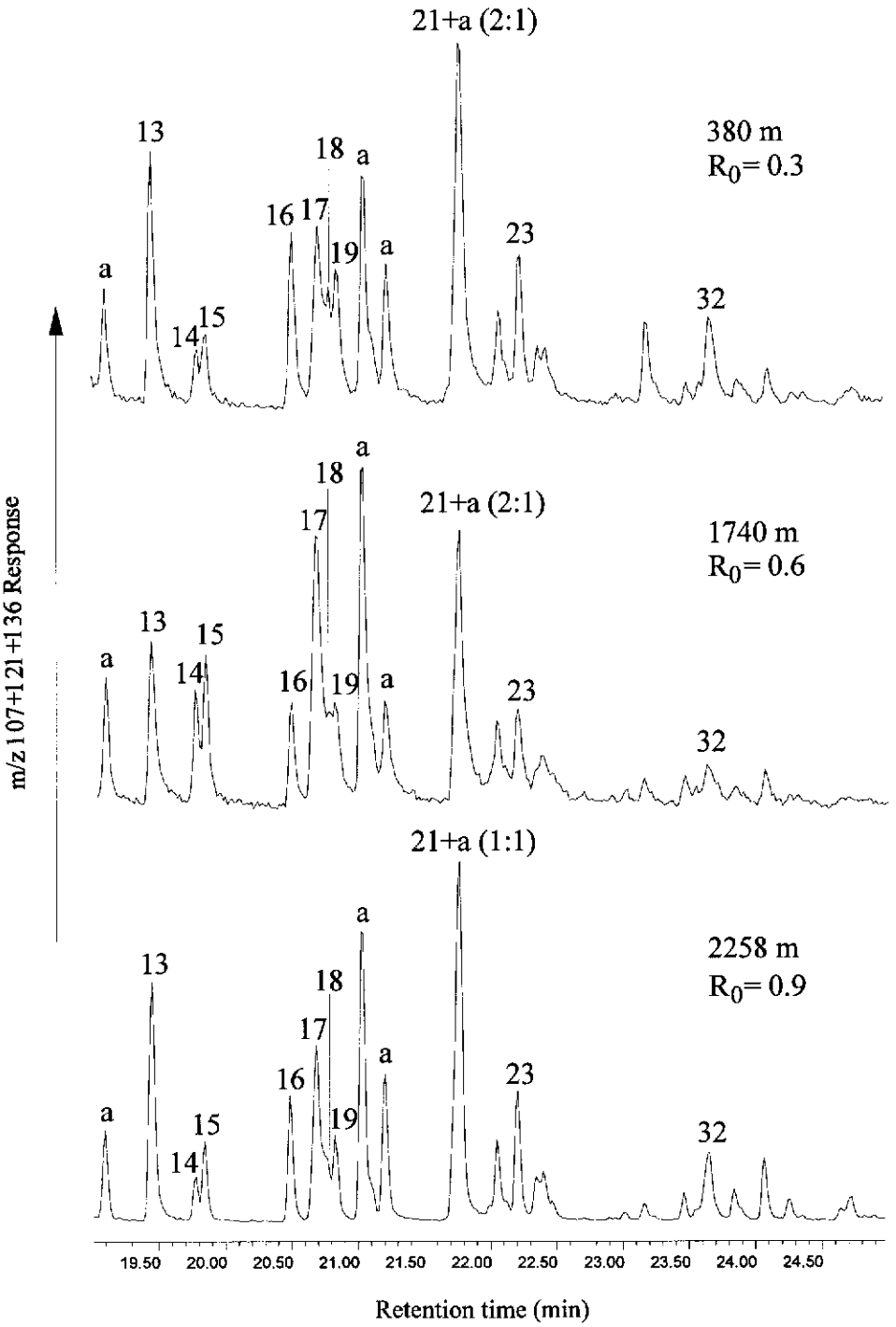


Figure 6.7 Partial selected ion chromatograms (m/z 107+121+136) of C3 alkylphenols extracted from the hydrous pyrolysates of GK coals. Refer to Table 4.4 for peak identifications.

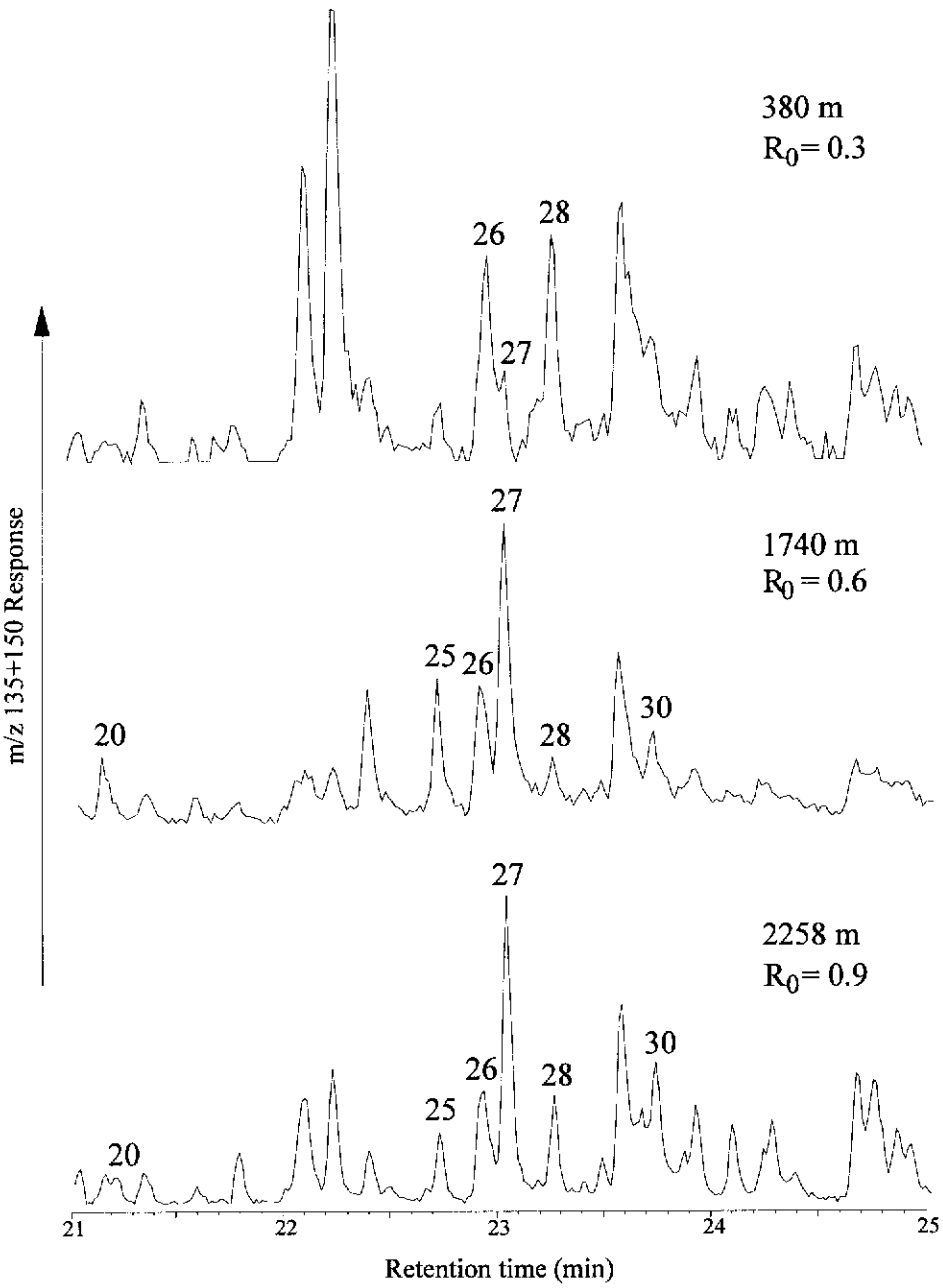


Figure 6.8 Partial selected ion chromatograms (m/z 135+150) of C4 alkylphenols extracted from the hydrous pyrolysates of GK coals. Refer to Table 4.4 for peak identifications.

of an input of appropriate isoprenoid phenol precursors. The isopropylmethylphenol distributions in the deeper samples however, are dominated by 4-isopropyl-2-methylphenol (peak 27). The changes in the relative proportions of isopropylmethylphenols in the GC coal pyrolysates are clearly shown in Figure 6.5 e) where thymol and carvacrol occur in greatest relative abundance in the lignite sample (>30 %) whereas, 4-isopropyl-2-methylphenol is the major isomer in the deeper samples (> 35 %). Furthermore, Table 6.1 shows that the individual concentrations for all of the isopropylmethylphenols except thymol and carvacrol have increased by a factor of greater than 2 in the pyrolysate of the 1740 m sample, with the greatest increase observed for 4-isopropyl-2-methylphenol (1740 m/380 m = 6.2).

The higher relative proportions and individual concentrations of *ortho* and *para* substituted alkylphenols among the dimethylphenols, trimethylphenols, isopropylphenols and isopropylmethylphenols in the subbituminous coal (1740 m) pyrolysate relative to that in the lignite pyrolysate discussed above are likely to be the result of electrophilic aromatic alkylation reactions which occur during coalification (Botto, 1987; Hatcher, 1990; Hatcher *et al.*, 1992). Examination of Table 6.1 and Figure 6.5 shows that eight of the nine alkylphenols for which individual concentrations (denoted by * in Table 6.1) and relative proportions increased contain substituents exclusively in the *ortho* and *para* positions and are therefore suggested to be kinetic products of electrophilic substitution reactions. Bound state methylation giving rise to phenol precursors which are *ortho* and *para* substituted are discussed in detail in Chapter 7 (Section 7.2.2; Methylation Products in Crude Oil). Briefly, during early coalification the methoxy substituents of lignin undergo extensive demethylation resulting in the methylation of the *ortho* positions of hydroxyaromatic structures (Carrado *et al.*, 1990; Hatcher, 1990). Hatcher *et al.* (1992) developed a model for subbituminous

coal consisting of *ortho* and *para* substituted phenol moieties (refer to Figure 7.4). The relative increase of 2,4-dimethylphenol in the pyrolysate of coals of increasing rank has been previously attributed to these phenol moieties (Hatcher *et al.*, 1992; Hartgers *et al.*, 1994). Table 6.1 shows that in addition to 2,4-dimethylphenol, the concentration of 2,4,6-trimethylphenol has also increased in the subbituminous coal pyrolysate indicating that this trimethylphenol is produced from similar phenol structures in subbituminous coal. Although the subbituminous coal model proposed by Hatcher *et al.* (1992) contains phenol units which are predominantly di-substituted it is likely that in some cases both *ortho* positions of the phenol moieties are alkylated. The formation of these 2, 4 and 6-trisubstituted units during coalification is likely to be responsible for the increase in the relative proportion and individual concentration of 2,4,6-trimethylphenol in the subbituminous coal sample. The lower relative abundance of 2,4,6-trimethylphenol (peak 15) compared to 2,4-dimethylphenol (peak 7) in the coal pyrolysates (Figure 6.4) however, is consistent with the lower relative abundance of tri-substituted structures present in the subbituminous coal model.

The remaining compounds which were higher in individual concentrations (* in Table 6.1) and relative proportions (Figure 6.5) in the subbituminous coal pyrolysate contain isopropyl substituents in the *ortho* and *para* positions. Electrophilic isopropylation reactions are proposed to give rise to these compounds with isopropyl substituents. It is unclear as to the source of isopropyl groups in coal, however one likely source is the propyl side chain of lignin methoxyphenols. Botto (1987) reported that the β-carbon of the *n*-propyl side chains of lignin units underwent aromatic substitution in the artificial coalification of ^{13}C-labelled lignin. Figure 6.4 however, shows that isopropylation products occur in much lower abundance than methylation products. For example, 2-isopropyl-4-methylphenol (peak 25) occurs in much lower relative abundance than

2,4-dimethylphenol (peak 7). This suggests that isomerisation of the C_3 side-chain occurs less readily than the methylation processes.

6.2.4 Lignins as Precursors to Petroleum Alkylphenols

The lignin components of terrestrial plants appear to be important precursors to petroleum alkylphenols. The high contribution of lignins to humic materials has led many authors to conclude that lignins are the most feasible precursor to sedimentary alkylphenols (e.g. Hartgers *et al.*, 1994). This conclusion, together with the observation that crude oils derived from source rocks which contain negligible higher plant input contain very low concentrations of alkylphenols (refer to Section 5.7), indicates that lignin components of land plants may be important precursor to petroleum alkylphenols. Further evidence for this is the high relative abundances of *ortho* and *para* substituted methylphenols which are also produced in the hydrous pyrolysates of mature coals and in some crude oils derived from coaly source rocks (e.g. Group 3A; Section 7.2.2). This suggests that lignin-derived structures may be precursors to the *ortho* and *para* methylphenols observed in the coaly Group 3A crude oils.

Perhaps the most contradictory evidence regarding lignins as a source of petroleum alkylphenols is the large discrepancy in the concentrations of alkylphenols obtained from the hydrous pyrolysis of coals and those normally found in crude oils. Table 6.2 shows the percentage of alkylphenols obtained from the hydrous pyrolysate of GK coal samples and two crude oils derived from source rocks which have had significant land plant input (Mahakam Delta and Eromanga Basin). The crude oils were selected on the basis that they are both Group 3A samples and that they span the range of alkylphenol concentrations observed in crude oils. The values in Table 6.2 shows that the pyrolysates contain 10 to 30 000 times more alkylphenols than the crude oils. Therefore, if lignins

were the precursors to petroleum alkylphenols one might expect their concentration to be considerably greater in crude oils.

Table 6.2 Percentage of C_0-C_4 alkylphenols (% w/w) in the hydrous pyrolysates of GK coal samples and in crude oils derived from coaly source rocks.

Sample	% C_0-C_4 Alkylphenols
Hydrous Pyrolysate	
GK 1740 m	7 %
GK 2258 m	2 %
Crude Oil	
Crude oil D	0.1 %
Kenmore	7×10^{-5} %

The apparent discrepancy between petroleum alkylphenol concentrations and those in sedimentary rock pyrolysates may be explained in terms of the physical and chemical properties of phenols. As alkylphenols are relatively polar components of crude oils they are likely to be inefficiently expelled from source rocks. The retention of hydrocarbons generated within petroleum source rocks has been reported to significantly affect the amount and compositions of expelled hydrocarbons (Burnham and Braun, 1990; Ungerer, 1990; Sandvik *et al.*, 1992). The observation that alkylphenols readily adsorb onto polar mineral surfaces and solid organic matter (refer to Chapter 1; Section 1.6) suggests that the alkylphenol concentrations relative to non-polar hydrocarbons in expelled crude oils may be reduced by adsorption processes occurring during primary migration. Furthermore, their polarity makes alkylphenols susceptible to water dissolution and adsorption effects during secondary migration, which would also result in a decrease in the concentration of alkylphenols in migrated crude oils.

The low concentrations of alkylphenols in crude oil might also be due to their chemical reactivity. They may behave as intermediate reactive species like, for example, alkenes. Although alkenes occur in high relative abundance in

sedimentary rock flash pyrolysates they are not observed in crude oil because they are readily saturated. Similarly, alkylphenols which occur in high relative abundance in sedimentary rocks may be reactive intermediates readily forming other products. Coupling reactions which give rise to polymeric phenolic material may be one mechanism by which petroleum is depleted of alkylphenols. Although the exact nature of these processes requires further investigation, the physical and chemical properties of phenols are most likely responsible for the large discrepancy in the absolute concentration of alkylphenols in sedimentary rock pyrolysates and crude oils.

6.2.5 Conclusions

The maturation level of coals has a major effect on the molecular structures of unbound and bound alkylphenol components. At brown coal rank the unbound methoxyphenols allowed taxonomical classification of the samples and the bound hydroxyphenols bore structural similarities to lignin moieties. In coals of subbituminous and bituminous rank however, the bound alkylphenols could not easily be related to lignin precursors as demethylation and dehydroxylation of the lignin units have occurred at these ranks (Hatcher *et al.*, 1992; refer to Section 1.2.1).

The total concentrations of alkylphenols in the hydrous pyrolysates of coal samples in a sedimentary sequence decreased with increasing rank. In contrast, the individual concentrations and relative proportions of eight alkylphenols with methyl and/or isopropyl substituent(s) in the *ortho* and *para* positions were higher in the pyrolysate of the subbituminous sample (1740 m) compared with the less mature lignite sample (380 m). The higher amounts of these *ortho* and *para* substituted alkylphenols in the deeper sample has been attributed to electrophilic

methylation and isopropylation reactions occurring to lignin structures in coals during coalification.

Lignins appear to be important precursors to petroleum alkylphenols since crude oils with source rocks which contain negligible higher plant input (Group 6) contain very low concentrations of alkylphenols. Further evidence that lignin-derived structures may yield petroleum methylphenols is provided by the high relative abundances of *ortho* and *para* substituted methylphenols which were also obtained in subbituminous coal hydrous pyrolysates, in coaly Group 3A crude oils.

6.3 MONOTERPENOID PHENOLS

It was previously shown in Chapter 5, Section 5.3 that Group 2A crude oils contain isopropylmethylphenol compositions dominated by carvacrol, thymol and/or 3-isopropyl-5-methylphenol, which is a rearrangement product of thymol. Figure 6.9 shows the partial gas chromatogram of a typical Group 2A crude oil in which these phenols are major components of the isopropylmethylphenols. Table 6.3 shows that all crude oils contain carvacrol and sometimes thymol, clearly in excess of the equilibrium proportions as shown by the Group 1 crude oil, Lambert. Because carvacrol and thymol are ubiquitous in land-plant-derived essential oils and can also be derived from non-aromatic natural products (refer to Section 1.2.1; Monoterpenoid Phenols), they may be biomarkers for land-plants.

The crude oils containing a high relative abundance of the monoterpenoid phenols also contain biomarkers indicative of higher plants. These crude oils have source rocks of Jurassic age and younger and contain a range of higher plant biomarkers (Table 6.4). Samples from the Barrow, Eromanga and Gippsland basins contain biomarkers characteristic of conifers and the Tertiary crude oil

Table 6.3 Relative percentages (% w/w) of thymol, carvacrol and 3-isopropyl-5-methylphenol in crude oils.

Crude oil	Group	Relative percentage		
		Thymol	Carvacrol	3-Isopropyl-5-methylphenol
Moorari-4	2A	2	50	27
Kenmore-1	2A	10	42	7
Bodalla South	2A	4.5	39	4.5
Rough Range	2A	13	73	14
Tuna-4 2820 m	2A	22	34	26
Iron Duke	2A	13	32	34
Lambert	1	10	17	40

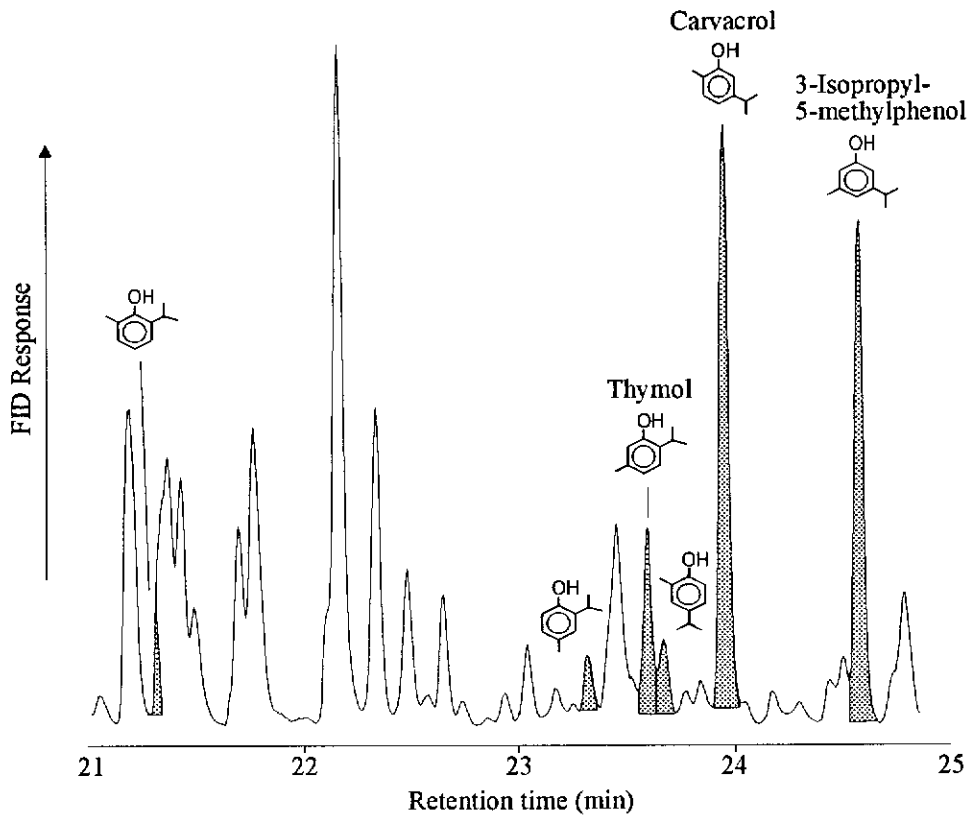


Figure 6.9 Partial gas chromatogram of the phenol extract from Iron Duke crude oil.

contains biomarkers for angiosperms. Because some extant conifers and angiosperms contain carvacrol, thymol and structurally related compounds it seems likely that the carvacrol and thymol (and its rearrangement product 3-isopropyl-5-methylphenol) in Group 2A crude oils are derived from similar sources. Elevated relative proportions of carvacrol and thymol in crude oils are therefore attributed to natural products derived from higher plants incorporated into the source rocks.

Table 6.4 Location, age of putative source rocks and occurrence of higher plant biomarkers in Group 2A crude oils .

Crude oil	Location Basin / Country	Age of putative source rock	Higher plant biomarkers	Refs
Moorari-4	Eromanga / Aust.	Jurassic	isopimarane, labdane	a
Kenmore-1	Eromanga / Aust.	Jurassic	isopimarane, labdane	a
Bodalla south	Eromanga / Aust.	Jurassic	isopimarane, labdane	a
Rough Range	Barrow / Aust.	Jurassic	retene, cadalene	b
Tuna-4 2820 m	Gippsland / Aust.	Cretaceous	phyllocladane, kaurane	c
Iron Duke	- / Indonesia	Tertiary	bicadinanes (WTR), oleanane	b

a Alexander *et al.*, 1992; b This work; c Alexander *et al.*, 1987.

6.4 TOCOPHEROLS AND STRUCTURALLY RELATED COMPOUNDS

Tocopherols and structurally related natural products occur widely in both marine and terrestrial organisms and are preserved during diagenesis as demonstrated by their occurrence in sedimentary rocks (refer to Section 1.21; Tocopherols and Related Compounds). In this section the potential for tocopherols to yield petroleum phenols was investigated by carrying out acid catalysed heating reactions of α-tocopherol on an acidic clay. Similarities in the methylphenols generated in the heating reactions and those identified in crude oils (Group 2B) suggests that tocopherols may be important precursors to petroleum methylphenols. Detailed discussions of the reaction products and the occurrence of methylphenol reaction products in crude oils are given below.

6.4.1 Laboratory Acid-Catalysed Reactions of α-Tocopherol

Heating reactions of α-tocopherol were carried out to investigate the formation of alkylphenols and isoprenoid hydrocarbons from tocopherol precursors. These reactions were carried out by heating α-tocopherol and aluminium smectite at temperatures ranging from 130°C to 180°C (seven hours), and reaction products were observed at temperatures of 150°C-180°C. Figure 6.10 shows the partial gas chromatogram of the reaction products obtained at 170°C. The major isoprenoid hydrocarbons in the reaction products were pristane and phytane (6:1). These compounds were identified by comparison of their retention time and mass spectra with those of reference compounds. The formation of pristene from flash pyrolysis and thermal degradation of α-tocopherol has been previously reported (Goossens *et al.*, 1984). These authors proposed that pristene was formed via a mechanism involving an intramolecular rearrangement leading to β-cleavage at C3 of α-tocopherol (Figure 6.11a). Because pristane was produced from the clay heating reactions in the present study, a similar mechanism to that proposed by Goossens *et al.* (1984) may also be operating on acidic clays. Subsequent saturation of the intermediate, pristene, may then give rise to pristane. The formation of phytane from tocopherols has not been previously reported. The phytane observed in the reaction products of α-tocopherol and aluminium smectite may have been formed via proton-catalysed dealkylation at C4 of α-tocopherol as such a process is likely to occur in the presence of acid clay catalysts.

2,3,5,6-Tetramethyl-1,4-benzoquinone and 2,3,6-trimethyl-1,4-benzoquinone were also tentatively identified in the reaction mixture by comparison of their mass spectra to literature data. The formation of trimethylbenzoquinone from α-tocopherol was previously reported by Goossens *et al.* (1984).

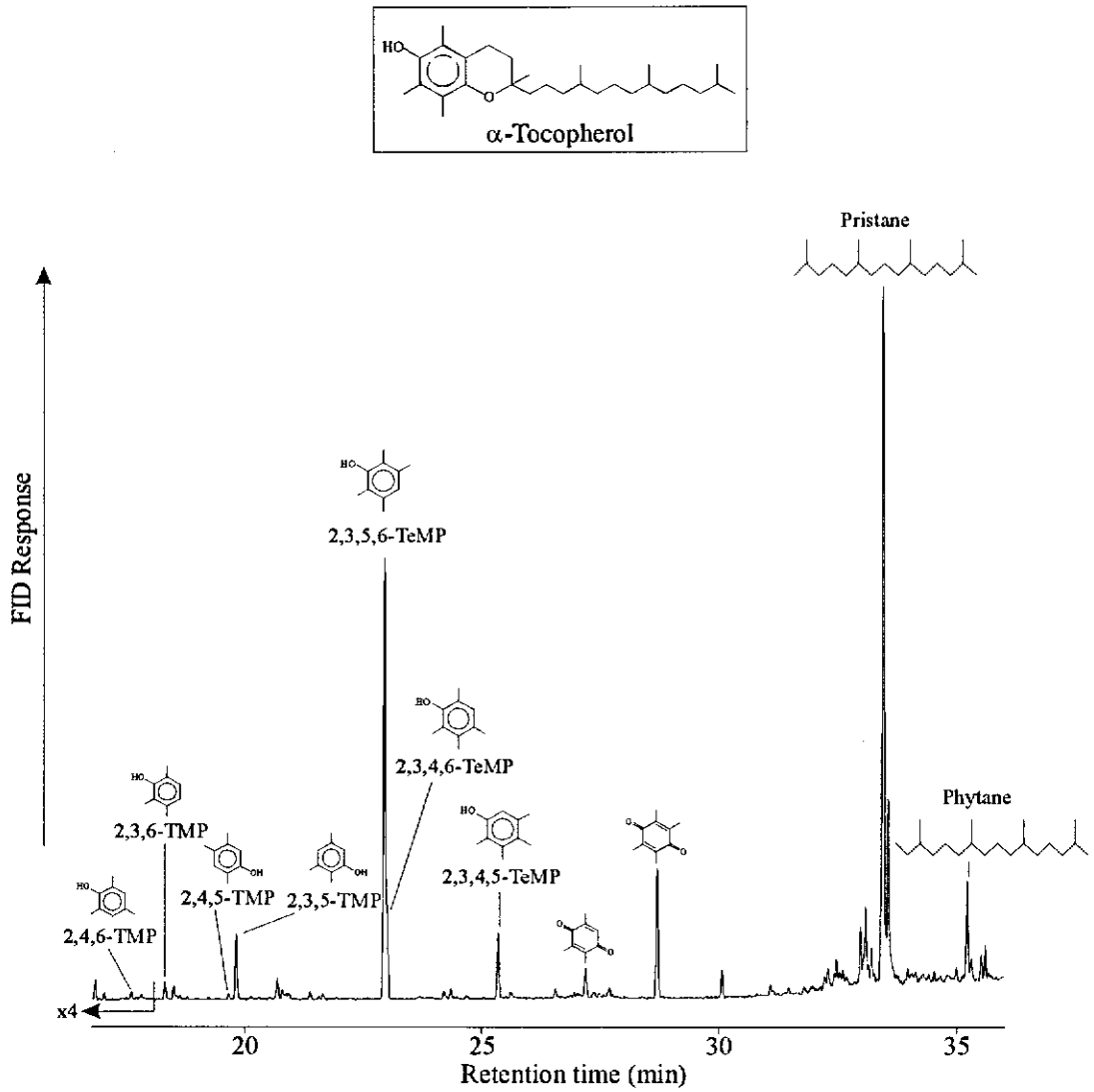
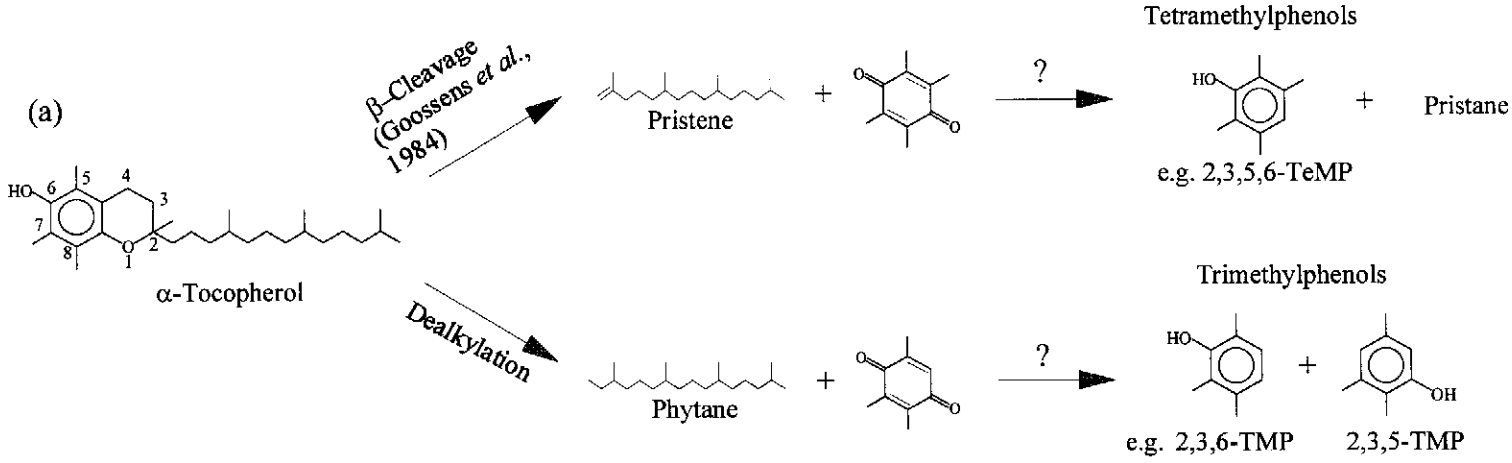


Figure 6.10 Partial gas chromatogram of the reaction products obtained from heating α-tocopherol and aluminium smectite at 170°C/7 h. TMP = trimethylphenol, TeMP = tetramethylphenol.

(a)



(b)

Compound	Structure	β-Cleavage	Dealkylation
I. α-tocopherol	5,7,8-trimethyltocol	2,3,5,6-TeMP	2,3,6-TMP; 2,3,5-TMP
II. β-tocopherol	5,8-dimethyltocol	2,3,5-TMP; 2,3,6-TMP	2,5-DMP; 2,5-DMP
III. γ-tocopherol	7,8-dimethyltocol	2,3,5-TMP; 2,3,6-TMP	2,3-DMP; 2,3-DMP
IV. δ-tocopherol	8-methyltocol	3,5-DMP; 2,6-DMP	3-MP; 2-MP
V. ϵ-tocopherol	5-methyltocol	2,3-DMP; 2,3-DMP	2-MP; 3-MP
VI. ζ-tocopherol	5,7-dimethyltocol	2,3,6-TMP; 2,3,5-TMP	2,6-DMP; 3,5-DMP
VII. η-tocopherol	7-methyltocol	2,5-DMP; 2,5-DMP	2-MP; 3-MP

Figure 6.11 a) Reaction products obtained from heating α-tocopherol and aluminium smectite at 170°C/7 h and b) proposed formation of alkylphenols from other naturally occurring tocopherols. MP = methylphenol; DMP = dimethylphenol; TMP = trimethylphenol; TeMP = tetramethylphenol.

Trimethylphenols and tetramethylphenols were the major alkylphenol reaction products obtained from heating α-tocopherol with aluminium smectite (Figure 6.10). 2,3,6-Trimethylphenol, 2,3,5-trimethylphenol and 2,4,6-trimethylphenol were identified by comparison of their retention times and mass spectra with authentic standards, and 2,4,5-trimethylphenol was tentatively identified on the basis of its mass spectrum. The phenolic products 2,3,6-trimethylphenol and 2,3,5-trimethylphenol are directly structurally related to the aromatic moiety of α-tocopherol and are therefore likely to be formed from the reduction (deoxygenation) of the intermediate trimethylbenzoquinone reaction product (Figure 6.11 a). In turn, *para* substituted trimethylphenol reaction products which cannot be directly related to α-tocopherol may be formed via clay-catalysed isomerisation reactions of the primary trimethylphenols produced. For example, isomerisation of 2,3,6-trimethylphenol and 2,3,5-trimethylphenol may have produced 2,4,6-trimethylphenol and 2,4,5-trimethylphenol, respectively.

The C_4 alkylphenol reaction products were identified on the basis of their mass spectra and their relative retention order on BP5 and DB1701 capillary columns. The high relative abundance of molecular ion (m/z 150) relative to the base peak at m/z 135 (M-15) in their mass spectra (e.g. Figure 6.12 a) indicated that they were tetramethylphenols and not secondary-alkyl substituted phenols which have mass spectra containing a lower relative abundance of the molecular ion (e.g. Figure 6.12 b). The longer retention time (approximately 2 min) of the C_4 alkylphenol reaction products compared to C_4 phenols with branched alkyl substituents such as isopropylmethylphenols, provided further evidence that they were likely to contain unbranched substituents. The major tetramethylphenol reaction product was assigned as 2,3,5,6-tetramethylphenol since this phenol can be directly structurally related to the aromatic moiety of α-tocopherol and is likely to be formed from the reduction of the tetramethylbenzoquinone reaction product

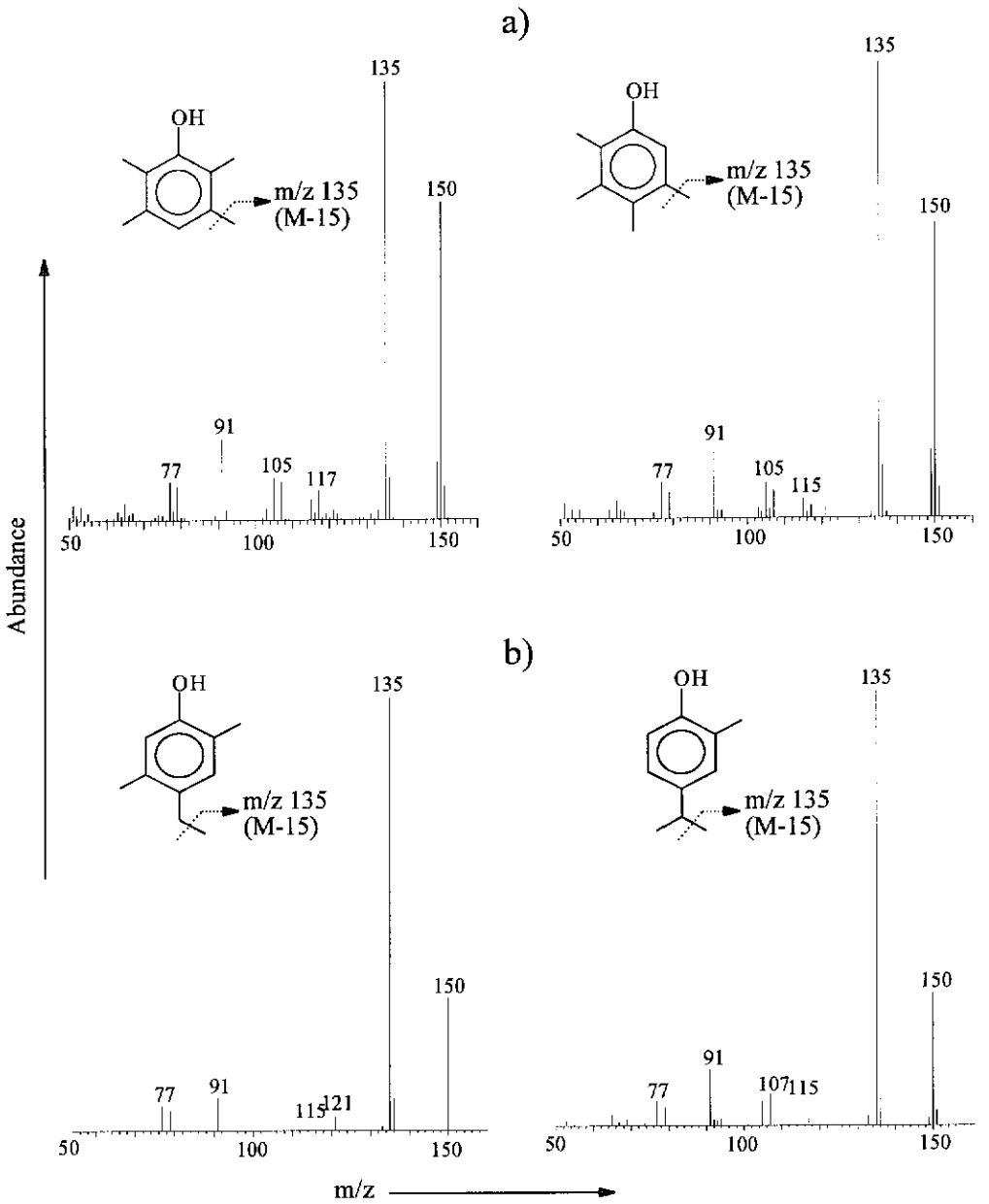


Figure 6.12 Mass spectra of a) the C4 alkylphenols extracted from clay-catalysed α-tocopherol reaction products (170°C/7 h), and b) some secondary-alkyl substituted phenols.

(Figure 6.11 a). The other C_4 alkylphenols are likely to be acid clay catalysed isomerisation products of this major tetramethylphenol product. Since the presence of *ortho* substituents decreases the retention time of alkylphenols on GC capillary columns (refer to Section 4.2), the tetramethylphenol with a similar retention time to 2,3,5,6-tetramethylphenol was assigned as 2,3,4,6-tetramethylphenols as this isomer also contains two *ortho* substituents. The isomer with the longest retention time was assigned as 2,3,4,5-tetramethylphenol based on the facts that no other tetramethylphenol isomers are possible and that it contains only one *ortho* substituent. This is the first report of the formation of alkylphenols in laboratory clay-catalysed heating reactions of α-tocopherol and demonstrates the possibility that methylphenols may be produced from tocopherols in sediments.

Based on the two proposed reaction mechanisms operating when α-tocopherol is heated with an acidic clay (β-cleavage and dealkylation), the major alkylphenol products from six other tocopherols have been inferred (Figure 6.11 b). These products include a range of monomethyl-, dimethyl- and trimethyl-substituted phenols. Examination of the structure of these products showed that 92% and 71% of the phenols produced from β-cleavage and dealkylation respectively contain a *meta* methyl substituent. This is in sharp contrast to the *ortho* and *para* methylphenols produced via an electrophilic alkylation mechanism (*cf.* Section 7.2). *Ortho* methyl substituents are also very common among the methylphenols derived from tocopherols. Therefore the phenolic reaction products derived from tocopherols are predominantly *ortho* and *meta* substituted methylphenols.

6.4.2 Tocopherol-Derived Methylphenols in Crude Oil

Moorari Crude Oil

In order to determine whether tocopherols are potential precursors to petroleum methylphenols, the methylphenols in Moorari crude oil were compared to those obtained from the clay-catalysed reaction products of α-tocopherol. Moorari crude oil was selected because it contains a very high abundance of pristane compared to *n*-C_{17} alkane (1.72) as well as a very high pristane/phytane ratio (9.1) (Jenkins, 1989). The very high abundance of pristane, the major hydrocarbon reaction product of α-tocopherol, in Moorari may indicate that tocopherols are important contributors to this crude oil. Figure 6.13 shows the partial gas chromatograms of a) Moorari crude oil phenol extract and b) the phenol extract of the reaction products obtained from heating α-tocopherol and aluminium smectite at 170°C/7 h. Moorari crude oil contained a high relative abundance of the trimethylphenols and some of the tetramethylphenols observed in the reaction products (peaks denoted by * in Figure 6.13). The relative abundances of compounds in Moorari however are different to those observed in the reaction mixture. The occurrence of a high relative abundance of, for example, 2,4,5-trimethylphenol compared to 2,3,5-trimethylphenol and 2,3,4,6-tetramethylphenol compared to 2,3,5,6-tetramethylphenol may be attributed to a contribution from *in situ* methylation of 2,5-dimethylphenol and 2,3,6-trimethylphenol respectively. The alternative explanation that they are produced from the isomerisation of initially formed 2,3,5-trimethylphenol and 2,3,5,6-tetramethylphenol is less likely because these isomerisations did not proceed to an appreciable extent in the clay catalysed reactions of α-tocopherol.

The relative proportions of trimethylphenols to tetramethylphenols which may be derived from tocopherols are also greater in Moorari crude oil compared

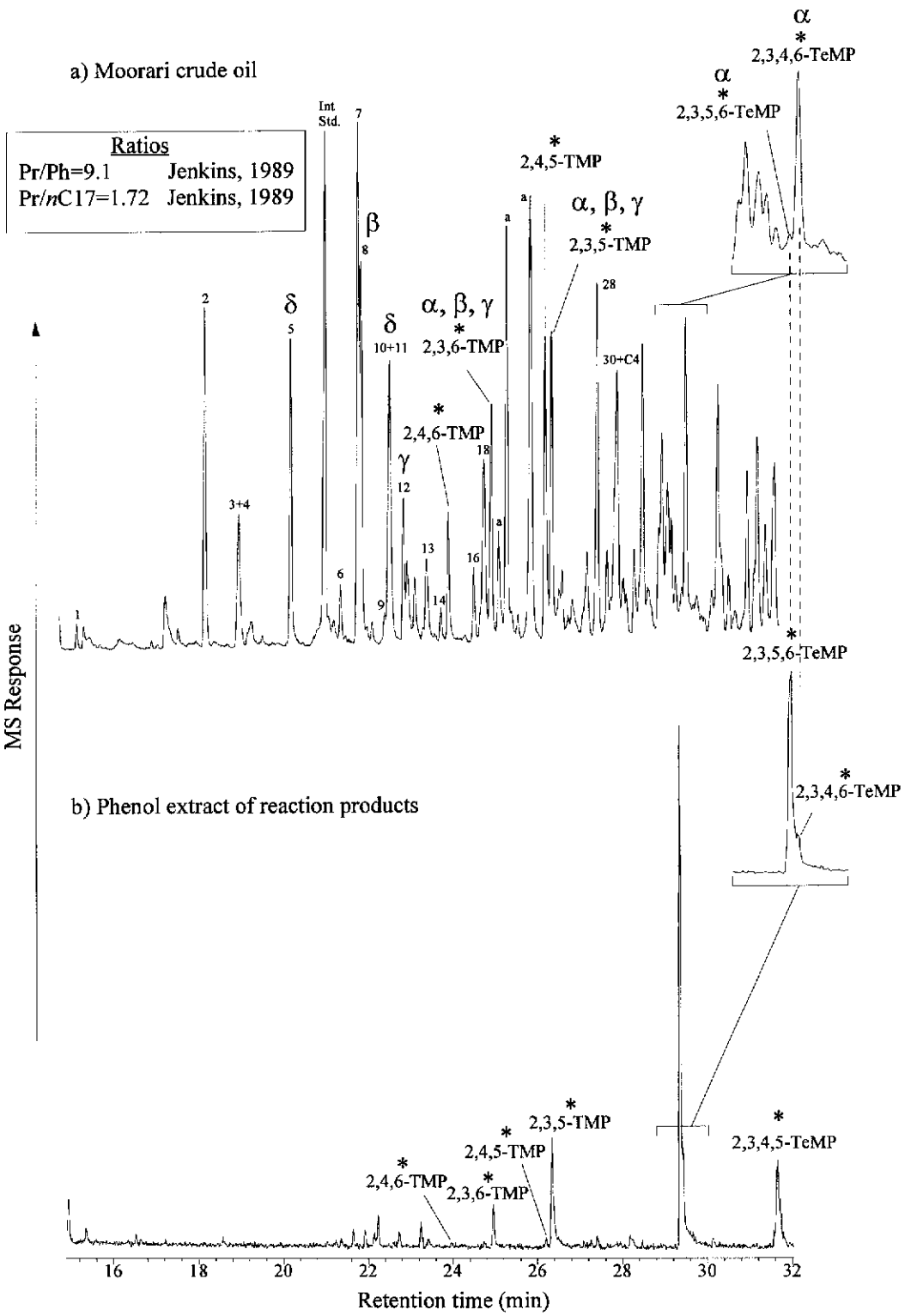


Figure 6.13 Partial total ion chromatograms of the phenol extracts of a) Moorari crude oil and b) the reaction products obtained from heating α-tocopherol and aluminium smectite at 170°C/7 h. * Denotes methylphenols in reaction mixture. α, β, γ and δ = Methylphenols derived from α-, β-, γ- and δ-tocopherols respectively (refer to Figure 6.11 b). TMP = trimethylphenol; TeMP = tetramethylphenol.

to that observed in the reaction products of α-tocopherol. One explanation for this may be that other tocopherols which yield greater proportions of trimethylphenols were present in the source rocks of Moorari. The methylphenols which can be derived directly from α-, β-, γ- or δ-tocopherol have been labelled in Figure 6.13 a). It can be seen that β-tocopherol and γ-tocopherol can also produce trimethylphenols and hence may have occurred in higher relative abundance than α-tocopherol (which yields mainly tetramethylphenols) in the source rocks of Moorari. Other alkylphenols that might be derived from tocopherols other than α-tocopherol sources include 2,6-dimethylphenol (peak 5), 2,5-dimethylphenol (peak 8) and 3,5-dimethylphenol (peak 10) (Figure 6.13 a). A mixed input of tocopherols is likely given the reported co-occurrence of α-, γ- and δ-tocopherol in a Pliocene diatomaceous ooze from Walvis Ridge (Brassell and Eglinton, 1986) and the co-occurrence of α- and γ-tocopherol in a range of sediments (Brassell *et al.*, 1983). However, the contribution of tocopherols to the alkylphenols in the Moorari crude oil remains speculative because of other possible sources for many of these phenols.

Blina Crude Oil

The high relative abundances of tocopherol-derived methylphenols in Blina crude oil may indicate the importance of tocopherols as phenol precursors in crude oils derived from marine algae. Blina crude oil was selected because it contains very high relative abundance of trimethylphenols and tetramethylphenols and several geochemical parameters strongly indicate that it was derived from marine algae (Alexander *et al.*, 1984). A strong odd to even preference in the *n*-C_{13} to *n*-C_{21} range (CPI = 1.16), a high relative abundance of C_{27} steranes (C_{29}/C_{27} regular steranes = 0.9) and an extremely high sterane to hopane ratio (16) all suggest a marine algal/bacterial source for Blina. Unlike Moorari

however, Blina does not contain a high relative abundance of pristane relative to phytane and *n*-C_{17} alkane which was described above as a potential indicator for crude oils derived from source rocks which had a significant input of tocopherols. Figure 6.14 shows the partial gas chromatograms of a) Blina crude oil phenol extract and b) the phenol extract of the reaction products obtained from heating α-tocopherol and aluminium smectite at 170°C/7 h. The phenol extract of Blina crude oil contains a high relative abundance of trimethylphenols and tetramethylphenols which were also reaction products (denoted by * in Figure 6.14). As was observed in Moorari, the relative abundances of tocopherol-derived trimethylphenols and tetramethylphenols are very different to those observed in the reaction products of α-tocopherol. This may therefore, be another example of a mixed tocopherol contribution to the source rocks of Blina. An alternative explanation is that dealkylation processes may be more important in the source rocks of Blina giving predominantly dealkylation products of α-tocopherol which are 2,3,6-trimethylphenol and 2,3,5-trimethylphenol. 2,3,6-Trimethylphenol may then be preferentially methylated *in situ* to give 2,3,4,6-tetramethylphenol. Some support for this latter possibility may be the high relative abundance of phytane, the dealkylation hydrocarbon product of α-tocopherol, in Blina crude oil (pristane/phytane = 0.6).

The large difference in the concentrations of alkylphenols and pristane and phytane in crude oils such as Moorari and Blina (in the order of 1:1000) may be another example of the impact the physical and chemical properties of alkylphenols have on their concentrations in crude oils. If pristane and some of the methylphenols in Moorari crude oil for example, were originating from the same source one might expect their concentrations to be similar, or at least not up to three orders of magnitude different. As was discussed in detail in Section 6.2.4, the polar nature and high reactivity of alkylphenols compared to relatively inert

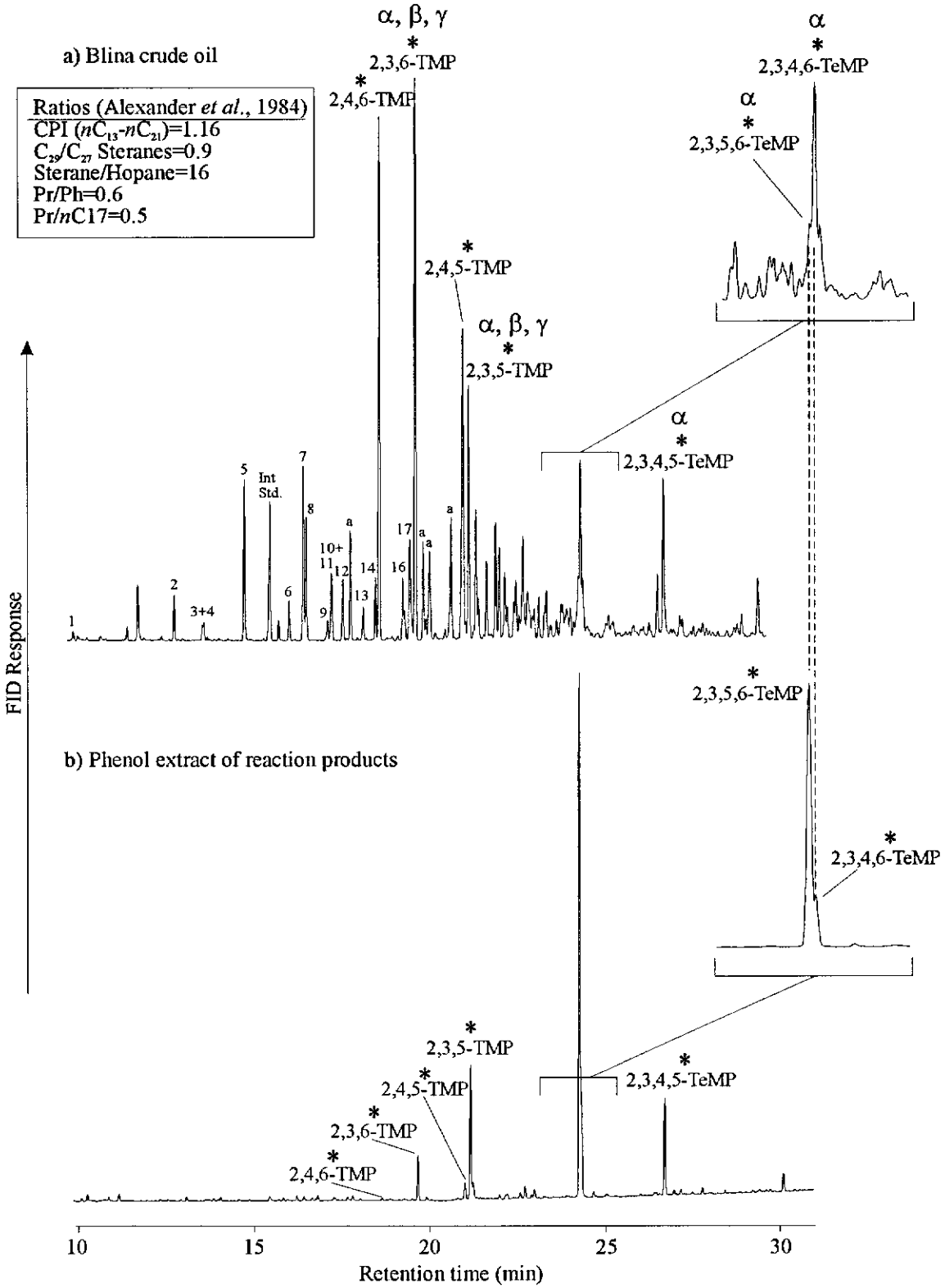


Figure 6.14 Gas chromatograms of the phenol extracts of a) Blina crude oil and b) the reaction products obtained from heating α-tocopherol and aluminium smectite at 170°C/7 h. * Denotes methylphenols in reaction mixture. α, β, γ, δ = Methylphenols derived from α-, β-, γ- and δ-tocopherols respectively (refer to Figure 6.11 b). TMP = trimethylphenol; TeMP = tetramethylphenol.

isoprenoid hydrocarbons are likely causes for the discrepancy between the concentrations of tocopherol-derived methylphenols and isoprenoid hydrocarbons in crude oils. In addition to the possibilities already discussed, the susceptibility of phenols to undergo electrophilic reactions enables the tocopherol-derived methylphenols, particularly sterically crowded tetramethylphenols, to donate methyl groups which may participate in methylation processes occurring in source rocks. This would lead to the depletion of tetramethylphenols in these crude oil, and perhaps to the formation of less substituted, more stable methylphenols.

6.4.3 Conclusions

Because tocopherol natural products are widespread in nature and have been reported to occur in sedimentary rocks, the occurrences in some crude oils (Group 2B) of high relative abundances of methylphenols which can be obtained from heating α-tocopherol and aluminium smectite is suggested to indicate an input of these natural products to the source rocks.

CHAPTER 7

GEOSYNTHESIS OF ALKYLPHENOLS

7.1 INTRODUCTION

Geosynthesis of organic compounds has been suggested to account for the diversity of hydrocarbon structural types found in sedimentary organic matter, many of which have no obvious natural product precursor. For example, Radke *et al.* (1982a; 1983) invoked geosynthesis to explain the high relative abundances of 1- and 9- methylphenanthrenes observed in coals of low maturity, and further suggested that dimethylphenanthrenes and trimethylphenanthrenes might also be derived from geosynthetic processes because they are structurally unrelated to polycyclic isoprenoids. The presence in rocks of dibenzothiophenes with methyl substituents in the ring positions most susceptible to electrophilic substitution (2 and 4) was also taken as support for sedimentary methylation processes.

The capacity of sedimentary organic matter to alkylate appropriate acceptor compounds has been demonstrated in laboratory heating experiments. Derbyshire and Whitehurst (1981) showed that when pyrene was used as a solvent in coal liquefaction experiments, up to 1.7% of the carbon from the coal was transferred to pyrene in the form of methyl substituents. More recently, Smith *et al.* (1994) demonstrated the methylation of phenanthrene, anthracene and pyrene when a mixture of coal and methane was heated at temperatures ranging from 220°C to 400°C.

Phenols are an attractive compound class for study of sedimentary alkylation processes because of their high susceptibility to alkylation. Alkylation reactions occurring via an electrophilic substitution mechanism occur more readily with phenols than with most other aromatic hydrocarbons (Whiting, 1978; March, 1992). The ease of alkylation of phenols in the presence of acidic clay catalysts is well documented (*cf.* Balogh and Laszlo, 1993). For example, this work shows that when alkenes were used as the alkylating reagents phenols were alkylated predominantly in the *ortho* and *para* positions.

The presence in crude oils of alkylphenols that do not bear an obvious structural relationship to natural product precursors suggests that many of the sedimentary phenols, like the alkylaromatic hydrocarbons, are formed by geosynthetic processes involving alkylation of simpler compounds. In this chapter, it is shown that the distributions of dimethylphenols, trimethylphenols, isopropylmethylphenols and *sec*-butylmethylphenols in some crude oils (Group 3) are consistent with geosynthetic processes involving methylation, isopropylation and *sec*-butylation processes. The representative crude oils of Groups 3A and 3B which will be used to discuss the geosynthesis of alkylphenols are shown in Table 7.1.

Table 7.1 Selected aromatic and sterane maturity indicators, and phenol ratios used to indicate methylation (MR), isopropylation (IR) and *sec*-butylation (BR) in crude oil.
MR = (2,4-dimethylphenol + 2,6-dimethylphenol + 2,4,6-trimethylphenol) / 2-methylphenol; IR = 4-isopropyl-2-methylphenol / 2-methylphenol; BR = 4-*sec*-butyl-2-methylphenol / 2-methylphenol.

		Maturity				Phenol alkylation ratio		
Group	Crude oil	20S/20R	DNR1	TNR1	Rc	MR	IR	BR
	Nilam	0.7	9.3	1.05	0.95	2.2	1.1	-
	Barrow UJ	1.0	7.4	0.87	0.81	13.7	1.5	0.40
3A	Crude oil P	1.0	8.4	0.91	0.75	5.6	0.29	0.21
	Moorari	0.7	1.4	0.35	0.51	2.7	0.13	<0.05
	Bodalla Sth	0.8	2.7	0.44	0.72	2.4	0.12	<0.05
	Lycium	0.9	3.4	0.68	0.73	2.3	0.09	0.07
	Byrock	1.0	4.3	0.66	0.88	1.5	0.22	0.18
3B	Sturt	0.9	4.6	0.82	0.85	5.5	3.3	2.8
	Malgoona	1.0	5.4	0.82	0.89	17.2	8.6	6.5
	Earlstown	1.1	7.2	0.92	0.94	5.3	19	26
1	Lambert	1.1	7.1	0.95	0.80	0.40	0.39	0.15

20S/20R = C_{29} 20S Regular sterane / C_{29} 20R Regular sterane
DNR 1 = (2,6 DMN + 2,7-DMN) / 1,5-DMN; TNR1 = 2,3,6-TMN / (1,4,6-TMN + 1,3,5-TMN)
DMN = Dimethylnaphthalene, TMN = Trimethylnaphthalene
Rc = 0.6 [1.5(2-MP+3-MP)/(P+1-MP+9-MP)] + 0.4; P = Phenanthrene, MP = Methylphenanthrene

7.2 GROUP 3A - METHYLATION

7.2.1 Laboratory Acid-Catalysed Methylation Reactions

A series of laboratory methylation reactions of the simple, most abundant sedimentary phenols were carried out to investigate the likelihood of formation of methylphenols via an electrophilic alkylation mechanism. Table 7.2 shows the experimental conditions used in the methylation experiments A-E. A sterically hindered naphthalene (1,4,5,8-tetramethylnaphthalene) was used as the methyl donor as it released methyl groups under relatively mild conditions (160°C) which did not cause appreciable isomerisation of the reaction products. Figure 7.1 shows that the products obtained from the acid (clay) catalysed methylation reaction of phenol and cresols were those from methylation predominantly in the *ortho* and *para* positions. Further, in the cases where the *para* position was free, this position was the more favoured site of alkylation. For example, over three times more *para*-cresol than *ortho*-cresol was formed from the methylation of phenol (experiment A). Also, the reaction products in experiment B showed that *ortho*-cresol was methylated in the *para* position resulting in the formation of 2,4-dimethylphenol (33%), 2,4,6-trimethylphenol (42%) and 2,4,5-trimethylphenol (10%). This is consistent with the preferred *para* alkylation of simple phenols reported in the literature (Brouwer *et al.*, 1970; Olah and Mo, 1972; Whiting, 1978; March, 1992). An exception is seen in the case of the methylation of *meta*-cresol (experiment C), where products resulting from methylation in the *ortho* positions (e.g. 2,5-dimethylphenol) were nearly three times more abundant than those resulting from *para* alkylation (e.g. 3,4-dimethylphenol). This indicates that alkylation adjacent to the methyl substituent was relatively unfavoured, probably due to steric interactions during alkylation.

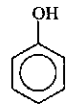
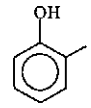
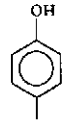
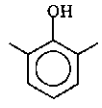
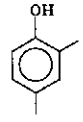
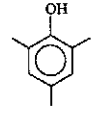
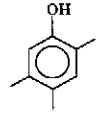
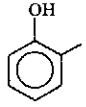
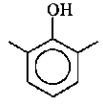
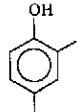
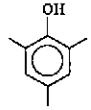
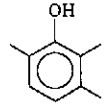
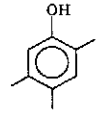
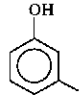
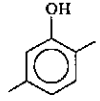
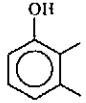
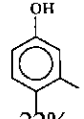
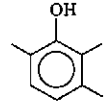
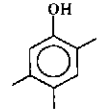
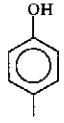
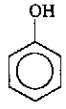
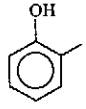
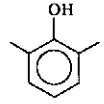
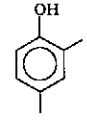
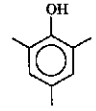
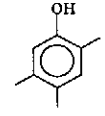
Experiment	Phenolic reactant	Relative percentage of methylphenols in reaction products					
A		 12%	 44%	 3%	 30%	 9%	 2%
B		 10%	 33%	 42%	 5%	 10%	
C		 54%	 4%	 22%	 3%	 17%	
D		 17%	 17%	 5%	 45%	 12%	 4%
E	2-MP, 3-MP + 4-MP (2:1:1)	Alkylphenol products are shown in Figure 7.2.					

Figure 7.1 Methylphenol, dimethylphenol and trimethylphenol products obtained from clay catalysed methylation reactions of phenol and cresols. Values below structures are relative percentages of alkylphenols in the reaction products. Refer to Table 7.2 for reaction conditions pertaining to experiments A-E.
MP = methylphenol.

Table 7.2 Experimental conditions pertaining to heating experiments on aluminium smectite. Experiments A-E were carried out at 160°C/12 h, and F-L were carried out at 150°C/12 h. The reaction products obtained in experiments A-L are shown in Figure 7.1 and Figure 7.5.

Experiment	Phenolic substrate	Alkyl donor	Substrate / alkyl donor ratio
A	phenol:	1,4,5,8-TeMN	1:2
B	2-MP:	1,4,5,8-TeMN	1:2
C	3-MP:	1,4,5,8-TeMN	1:2
D	4-MP:	1,4,5,8-TeMN	1:2
E	2-MP:3-MP:4-MP:	1,4,5,8-TeMN	2:1:1:8
F	2-MP:	Daucalene	1:2
G	3-MP:	Daucalene	1:2
H	4-MP:	Daucalene	1:2
I	2,6-DMP:	Daucalene	1:2
J	2-MP:3-MP:4-MP:	Daucalene	1:1:1:3
K	2-MP:3-MP:4-MP:	Daucalene	2:1:1:4
L	2-MP:2,6-DMP:	Daucalene	1:1:2

MP = methylphenol; DMP = dimethylphenol; TeMN = tetramethylnaphthalene; Daucalene = 4-isopropyl-1,7-dimethyl-naphthalene.

Products obtained from the methylation of an all-isomer mixture of cresols suggests that *ortho*-cresol was the most reactive isomer. Figure 7.2 a) shows the gas chromatogram of the reaction products obtained from experiment E and, the partial gas chromatogram of the cresol reactant mixture (inset). Comparison of the relative abundance of *ortho*-cresol (peak 2) to *meta*- + *para*-cresols (peaks 3+4) in the reactant mixture to that observed after heating shows that *ortho*-cresol reacted to a greater extent than *meta* + *para*-cresols. Therefore alkylation products of *ortho*-cresol would contribute to the reaction products to a greater extent, not only because of its higher concentration in the reactant mixture (o:m:p; 2:1:1), but also because of its higher reactivity in alkylation processes (*cf.* similar effect seen in the isopropylation of *ortho*-cresol - Section 7.3.1).

7.2.2 Methylation Products in Crude Oil

A comparison of the methylphenol compositions of Barrow UJ crude oil (Figure 7.2 b) with that of the reaction products obtained from laboratory methylation of the cresols (Figure 7.2 a) show some remarkable similarities. The phenol distributions revealed in the partial gas chromatograms shown in Figure 7.2 are representative of crude oils which contain methylphenols in proportions which appear to have arisen from electrophilic methylation reactions. These distributions are dominated by *ortho* and *para* substituted methylphenols which is typical of a product mixture from kinetically controlled electrophilic methylation processes such as those outlined in the preceding section. It should be noted that the proportions of methylphenols in the crude oils shown in Figure 7.2 are markedly different from those in crude oils which contain alkylphenols in proportions which reflect their relative stabilities (*cf.* Section 5.2).

The extent to which electrophilic methylation processes have occurred in source rocks may be inferred from the relative abundance of *ortho* and *para* substituted methylphenols present in crude oil. Figure 7.3 shows the gas chromatogram of the phenol extracts of two crude oils from the Cooper basin, Australia in which *ortho* and *para* substituted methylphenols occur in high relative abundances. The gas chromatogram of the Malgoona petroleum phenols (Figure 7.3 a) is very similar to that of Barrow UJ phenols (cf. Figure 7.2 b) as 2,4,6-trimethylphenol (peak 15) is a predominant peak. In contrast to these crude oils, the gas chromatogram of the phenol extract of Lycium (Figure 7.3 b) is dominated by 2,4-dimethylphenol (peak 7) with a lower relative abundance of 2,4,6-trimethylphenol (peak 15). The relative lack of the higher methylated phenol in Lycium indicates that methylation has been less extensive in this case. The group of crude oils which are dominated by methylphenol isomers originating from alkylation processes may therefore be further subdivided in terms of the extent of methylation.

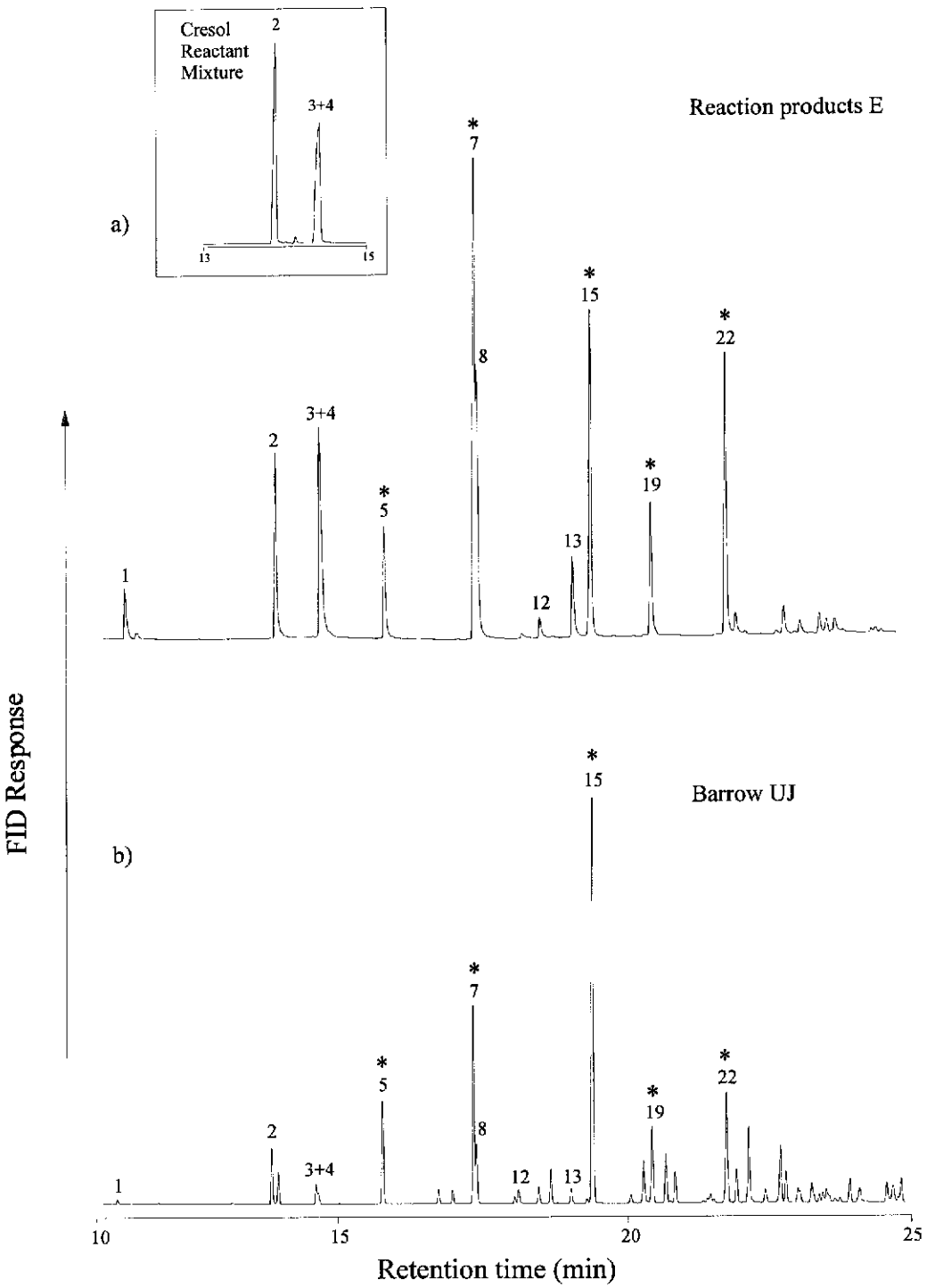


Figure 7.2 Partial gas chromatogram of a) the alkylphenol reaction products from reaction mixture E (refer to Table 7.2), and b) the phenol extract of Barrow UJ crude oil. Peaks labelled * represent *ortho* and *para* substituted methylphenols.

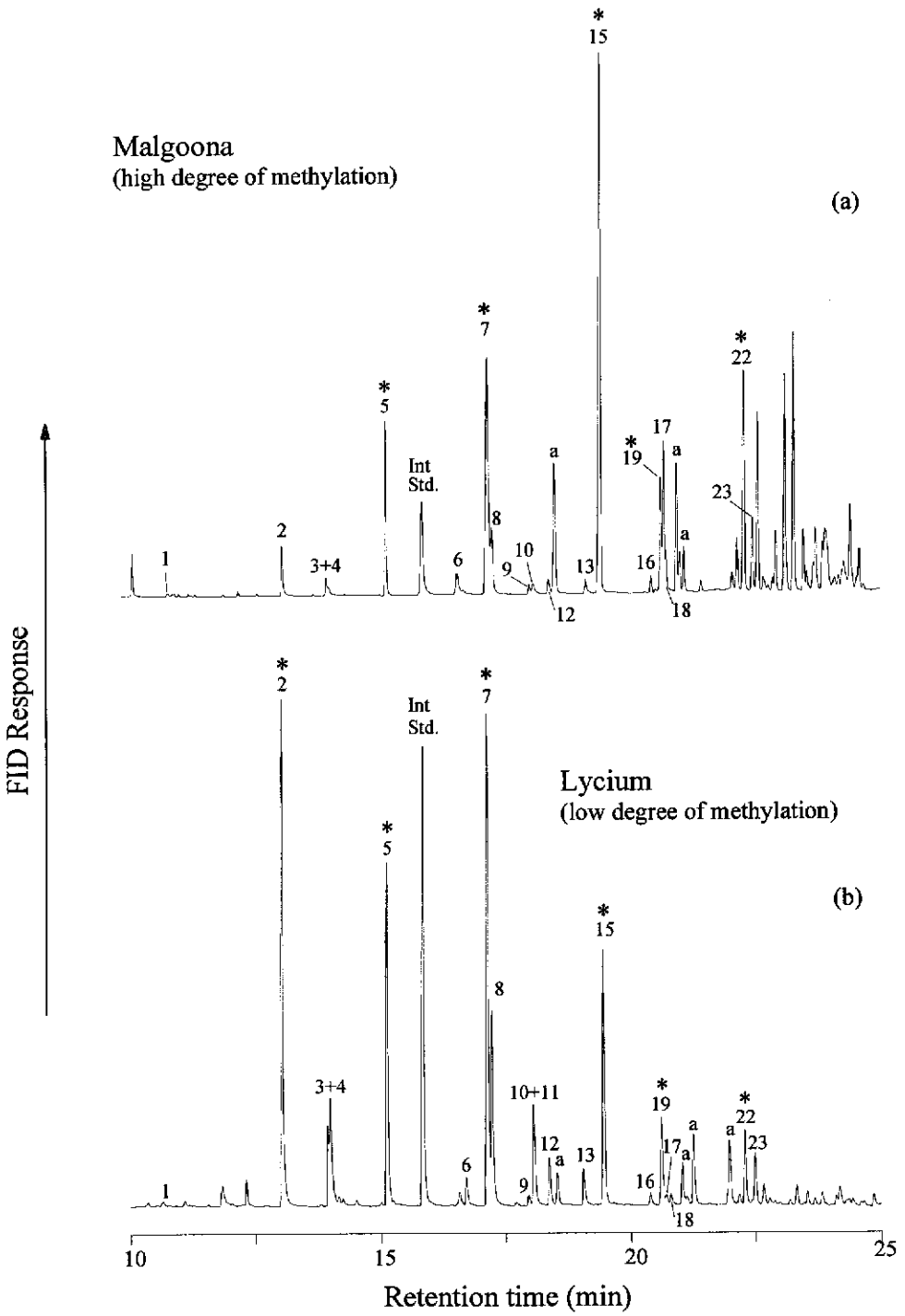


Figure 7.3 Partial gas chromatograms of the phenol extracts of a) Malgoona and b) Lycium crude oils. Peaks labelled * represent *ortho* and *para* substituted methylphenols.

Methylation products present in crude oil should reflect the initial cresol mixture in the source rocks. For example, in Malgoona crude oil (Figure 7.3 a; peak 8) the minor amount of 2,5-dimethylphenol which is the methylation product of *meta*-cresol, may indicate that *meta*-cresol was a minor cresol in the source rock. On the other hand, the high relative abundance of 2,4-dimethylphenol and 2,4,6-trimethylphenol (peaks 7 and 15) may suggest that *ortho* and/or *para*-cresols were the major components in the source rock.

Methylation may occur to either free or bound cresols as there is considerable evidence suggesting that methylation can occur in the bound state in coals. During the early stages of coalification the methoxyl substituents of lignin units undergo extensive demethylation resulting in a biopolymer consisting of phenol and catechol structures (Botto, 1987; Stout *et al.*, 1988; Hatcher *et al.*, 1989b; Corrado *et al.*, 1990; Hatcher, 1990). During this process electrophilic methylation of the phenol rings also occurs, leading to predominantly *ortho* substitution of the phenolic units (Corrado *et al.*, 1990; Hatcher, 1990). Negligible amounts of *para* substitution occurs as this position in lignin units is occupied by a propyl substituent (Sarkanen and Hergert, 1971). Based on these transformations occurring to lignin during coalification, Hatcher *et al.* (1992) developed a model for subbituminous coal which is comprised of phenolic moieties that are largely *ortho* and *para* substituted (Figure 7.4). A variety of *ortho* and *para* substituted methylphenols could be produced from these phenolic moieties via processes such as β-cleavage, and proton dealkylation of the *para* propyl substituent (Figure 7.4). Methylation of phenol precursors in the bound state of coaly material is therefore a potential source of *ortho* and *para* substituted methylphenols. The formation of methyl substituted phenols either directly from unbound phenols or from bound phenols that are subsequently released from the kerogen could account for the formation of methylphenols during early catagenesis.

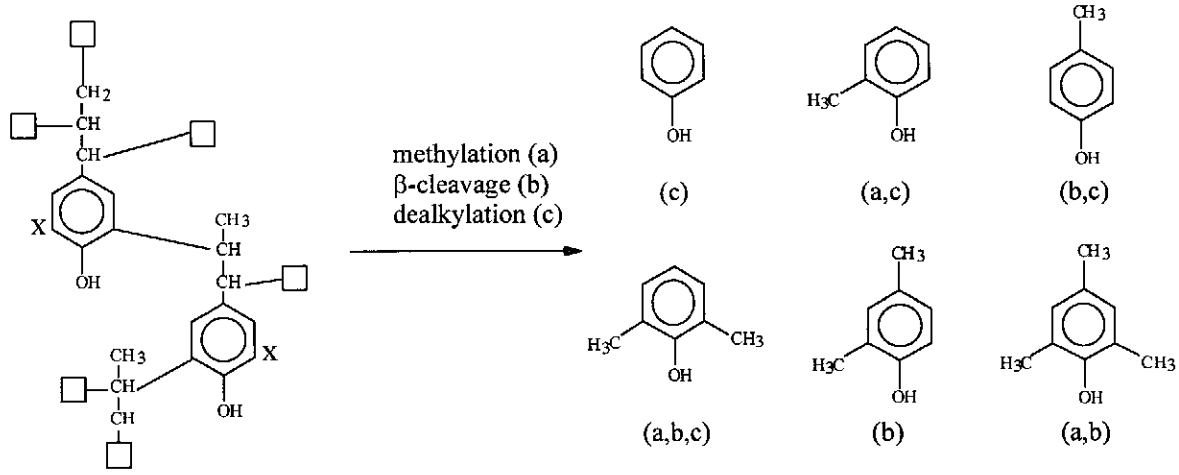


Figure 7.4 Suggested mechanism of formation of *ortho* and *para* substituted methylphenols from subbituminous coal. The coal model was adapted from Hatcher *et al.* (1992); boxes indicate coal macromolecule, x denotes favoured position of alkylation.

7.3 GROUP 3B - ISOPROPYLATION AND *SEC*-BUTYLATION

7.3.1 Laboratory Acid-Catalysed Isopropylation Reactions

A series of laboratory isopropylation reactions of the cresols were carried out to investigate the formation of isopropylmethylphenols via an electrophilic alkylation mechanism. Table 7.2 shows the experimental conditions used for isopropylation experiments F-K. Daucalene was used as the isopropyl donor in these alkylation reactions because this compound released alkyl groups under relatively mild conditions (150°C) giving kinetically controlled products. Figure 7.5 shows that the reaction products obtained from the acid (clay) catalysed isopropylation reactions of cresols were those from isopropylation predominantly in the *ortho* and *para* positions. In the case of *ortho*-cresol, the *para* position was preferentially alkylated giving 4-isopropyl-2-methylphenol as the major reaction product (90%). This is consistent with the preferred *para* alkylation of simple phenols reported in the literature (Brouwer *et al.*, 1970; Olah and Mo, 1972; Whiting; 1978; March, 1992). The reaction products of *meta*-cresol however indicated that *ortho* isopropylation giving 2-isopropyl-5-methylphenol (99%) was greatly favoured compared to the *para* position. Steric hindrance by the methyl group in *meta*-cresol is probably the reason for the lower proportion of *para* substitution. Although a similar effect was noted in the methylation reactions of *meta*-cresol (Section 7.2.1), the decreased *para* isopropylation of *meta*-cresol is much more pronounced. It therefore appears that not only does steric hindrance make alkylation adjacent to a methyl substituent less favourable, bulkier alkylating groups enhance this effect.

The relative reactivity of the cresols to acid (clay) catalysed isopropylation reactions can be inferred from the reaction products obtained from reaction mixture J (Figure 7.5). This equimolar mixture of the three cresols was

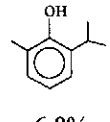
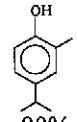
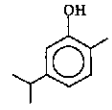
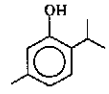
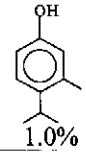
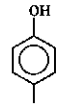
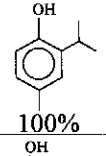
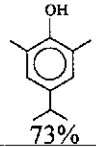
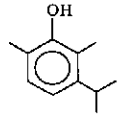
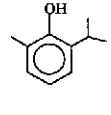
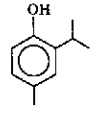
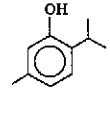
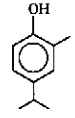
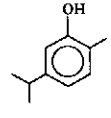
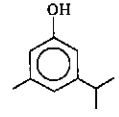
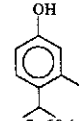
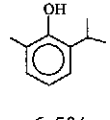
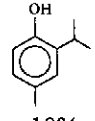
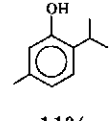
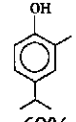
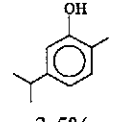
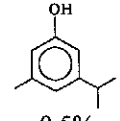
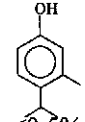
Experiment	Phenolic reactant	Relative percentage of isopropylmethylphenols in reaction products
F		 6.8%  90%  3.9%
G		 99%  1.0%
H		 100%
I		 73%  27%
J	2-MP, 3-MP + 4-MP (1:1:1)	 6.2%  12%  24%  46%  4.8%  1.3%  5.6%
K	2-MP, 3-MP + 4-MP (2:1:1)	 6.5%  10%  11%  69%  3.5%  0.5%  <0.5%

Figure 7.5 Isopropylmethylphenol products obtained from clay catalysed isopropylation reactions of cresols. Values below structures are relative percentages of isopropylmethylphenols in the reaction products. Refer to Table 7.2 for reaction conditions pertaining to experiments F-K. MP = methylphenol.

isopropylated to yield the six isopropylmethylphenol kinetically controlled products (*cf.* mixtures F-H), together with a small amount of the thermodynamically most stable isomer, 3-isopropyl-5-methylphenol. This isomer is likely to be the result of acid catalysed isomerisation reactions, which only become predominant at higher reaction temperatures. The lower abundance of the alkylation products of *meta*-cresol (e.g. 2-isopropyl-5-methylphenol) and *para*-cresol (e.g. 2-isopropyl-4-methylphenol) compared to alkylation products of *ortho*-cresol (e.g. 4-isopropyl-2-methylphenol) demonstrates the lower susceptibility of *meta*-cresol to alkylation, because the vacant activated positions (*ortho* and *para*) are sterically hindered by the presence of an adjacent methyl or hydroxyl substituent. A comparison of the yields of reaction products from each of the cresols shows therefore that the order of reactivity to isopropylation to be *ortho*-cresol > *meta*-cresol > *para*-cresol (ratio of reaction products in mixture J was 4.8:2.5:1 respectively).

Acid catalysed isopropylation reactions of 2,6-dimethylphenol were also carried out to investigate the potential for formation of isopropyldimethylphenols via an electrophilic alkylation mechanism. Figure 7.5 shows that two products were obtained from experiment I and these were tentatively assigned as 4-isopropyl-2,6-dimethylphenol and 3-isopropyl-2,6-dimethylphenol. 4-Isopropyl-2,6-dimethylphenol was assigned as the major product, as this isomer is produced from the alkylation at the activated *para* position of 2,6-dimethylphenol. Isopropylation at the *meta* position occurred to a relatively high extent possibly because this position is considerably activated by the two methyl substituents, which are activating and *ortho* and *para* directing in electrophilic alkylation processes. The relative reactivities of *ortho*-cresol and 2,6-dimethylphenol to acid catalysed isopropylation reactions was inferred from the reaction products obtained from experiment L (Table 7.2). In this reaction mixture the alkylation products of 2,6-dimethylphenol occurred in only slightly higher relative

abundances than those of *ortho*-cresol (0.83:1.0 respectively), indicating that 2,6-dimethylphenol is only slightly more reactive towards isopropylation under these conditions.

7.3.2 Higher Alkylation Products in Crude Oil

The product mixtures obtained from the laboratory isopropylation of cresols shows remarkable similarities to the isopropylmethylphenol distributions in Group 3B crude oils. Figure 7.6 shows the partial gas chromatograms of reaction products obtained from a) experiment K and b) experiment J and, c) the isopropylmethylphenols extracted from Sturt crude oil. The reaction products shown were obtained from two different mixtures of reactants so as to span the range of relative concentrations of cresols observed in kerogen pyrolysates (*cf.* Senftle *et al.*, 1986; Nip *et al.*, 1988; Powell *et al.*, 1991). The major isomer in the reaction products and in the crude oil is that which corresponds to isopropylation of *ortho*-cresol in the *para* position, 4-isopropyl-2-methylphenol (peak 27). There are also minor amounts of isopropylmethylphenols in the crude oil which correspond to alkylation products of *ortho*-cresol (peaks 20 and 28), *meta*-cresol (peak 26) and *para*-cresol (peak 25). The very low relative abundance of the most thermodynamically stable isomer, 3-isopropyl-5-methylphenol, indicates that this distribution is well out of equilibrium (refer to Section 5.2). The high relative abundance of the kinetically controlled alkylation products in the crude oil suggests that a similar isopropylation process is occurring in source rocks and is giving rise to the isopropylmethylphenols found in some crude oils.

The similarities between isomer distributions of isopropylmethylphenol and *sec*-butylmethylphenol in the same crude oil suggests that the *sec*-butylmethylphenols also arise from alkylation (*sec*-butylation) of sedimentary cresols. Table 7.3 shows the relative percentages of *sec*-butylmethylphenols in the

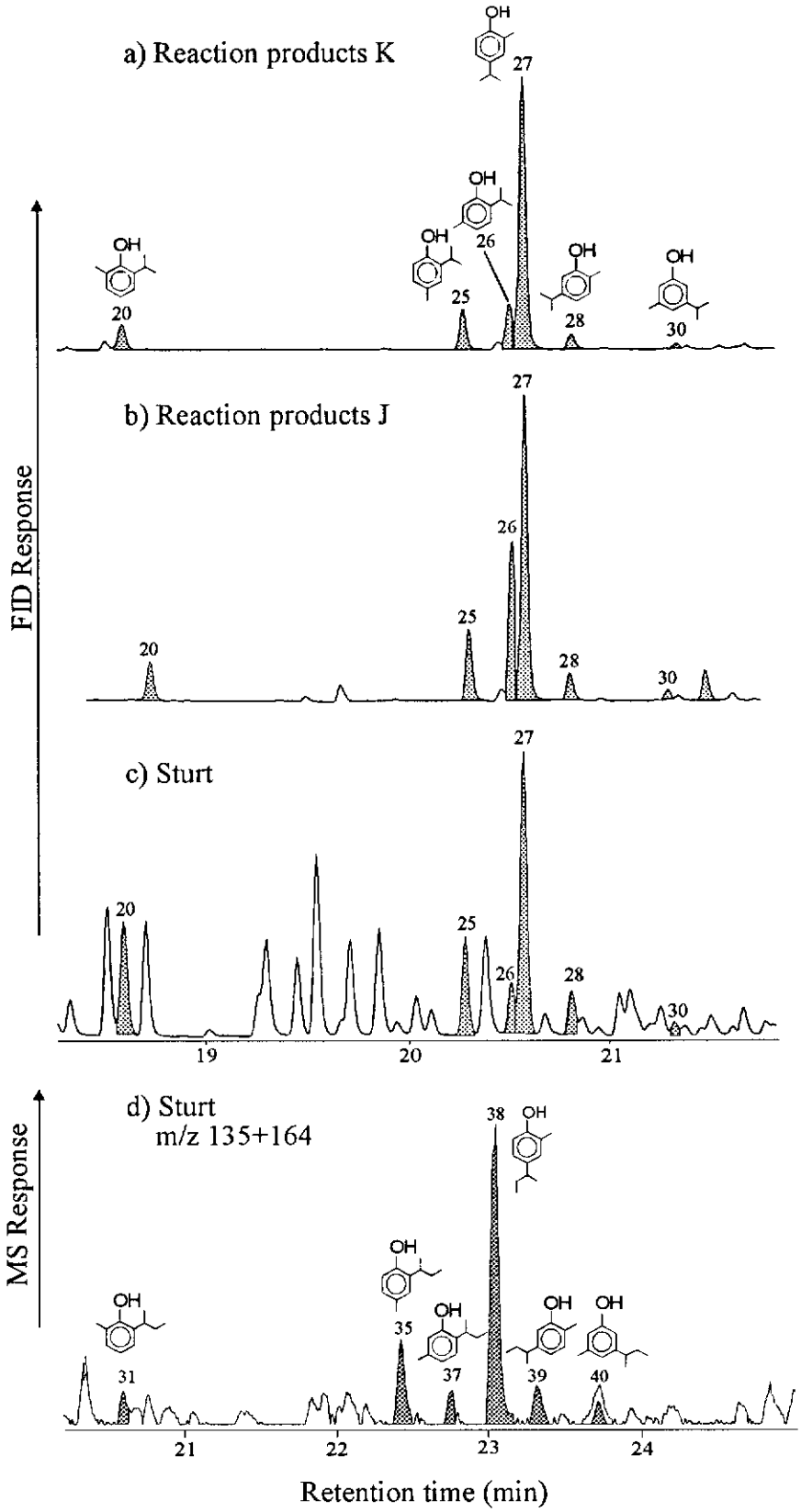


Figure 7.6 Partial gas chromatograms of a) the isopropylmethylphenols obtained from experiment K, b) the isopropylmethylphenols obtained from experiment J (see Table 7.2), c) the phenol extract from Sturt crude oil showing isopropylmethylphenols and d) partial m/z 135+164 mass chromatogram of Sturt crude oil showing *sec*-butylmethylphenols.

Table 7.3 Relative percentage of *sec*-butylmethylphenols in crude oils.

Group	Crude oil[1]	 31	 35	 37	 38	 39	 40
	Barrow UJ	2.2	10	3.8	34	18	32
3A	Crude oil P	7.3	7.7	10	40	17	18
	Lycium	nd	nd	nd	70	18	12
	Byrock	0.9	4.0	4.1	43	19	29
3B	Sturt	1.8	15	5.0	59	8.2	11
	Malgoona	2.1	7.0	4.3	62	18	6.6
	Earlstown	0.6	3.0	3.4	34	25	34
1	Lambert	1.8	6.4	3.8	20	10	58

nd = below limit of detection

1 *sec*-Butylmethylphenols were below detection limit in Moorari and Bodalla South crude oils, no data available for Nilam crude oil.

selected group of crude oils. Figure 7.6 c) and Figure 7.6 d), show the distributions of isomeric isopropylmethylphenols and *sec*-butylmethylphenols in the same crude oil. For each set of isomers the most abundant compound is that with the larger alkyl group at position 4 and the methyl at position 2. In each case the less abundant isomers are those which would result from the alkylation of *meta*-cresol and *para*-cresol, and the most stable all-*meta* isomers (peaks 30 and 40). Such a product set is expected from the alkylation of the three cresols with bulky alkylating agents where steric hindrance of adjacent substituents limits the most reactive cresol (*ortho*-cresol) from being substituted at position 2, and also limits *meta*-cresol and *para*-cresol from being alkylated at positions 2, 4 and 6. The high abundance of the kinetically controlled alkylation products, and the low relative abundance of the most stable, all-*meta* isomer among the *sec*-butylmethylphenols, suggests that these phenols may have also originated from an alkylation process occurring in source rocks.

In addition to isopropylmethylphenols and *sec*-butylmethylphenols, several other alkylphenols which have been tentatively identified in Sturt crude oil

are also likely to be products of sedimentary alkylation. Figure 7.7 shows a) the partial

total ion chromatogram, b) the partial m/z 135 mass chromatogram and c) the partial m/z 149 mass chromatogram of Sturt crude oil. Based on mass spectral data 2-methyl-4-*sec*-pentylphenol has been tentatively identified in the crude oil (Figure 7.7; peak A). The formation of this isomer is consistent with electrophilic attack of a secondary *sec*-pentyl carbocation on *ortho*-cresol in a manner similar to that observed in the isopropylation and *sec*-butylation cases above (*cf.* peaks 27 and 38). The identification of 4-isopropyl-2,6-dimethylphenol (peak 34) and 3-isopropyl-2,6-dimethylphenol (peak 36) in this crude oil (Figure 7.7 c) indicates that electrophilic alkylation reactions are also occurring on dimethylphenols. The isopropyldimethylphenols were identified based on co-chromatography experiments and comparison of mass spectra with the products obtained from the acid catalysed isopropylation of 2,6-dimethylphenol (Figure 7.5 - experiment I). Alkylation products of 2,6-dimethylphenol were the major isopropyldimethylphenols observed as this xylenol has a vacant *para* position with no adjacent substituents and is often a major xylenol in crude oils of this group. Two further phenols corresponding to 1) the isopropylation of 2,4-dimethylphenol (2-isopropyl-4,6-dimethylphenol-peak B), another abundant xylenol in crude oil and, 2) the *sec*-butylation of 2,6-dimethylphenol in the *para* position (4-*sec*-butyl-2,6-dimethylphenol-peak C) have also been tentatively identified in crude oil based on mass spectral data (Figure 7.7 c).

7.4 RELATIONSHIP BETWEEN MATURITY AND ALKYLATION

The effect of maturity on the relative abundances of phenols that could be formed from alkylation of the most reactive sedimentary cresol (*ortho*-cresol) suggests that methylation can occur in less mature source rocks than

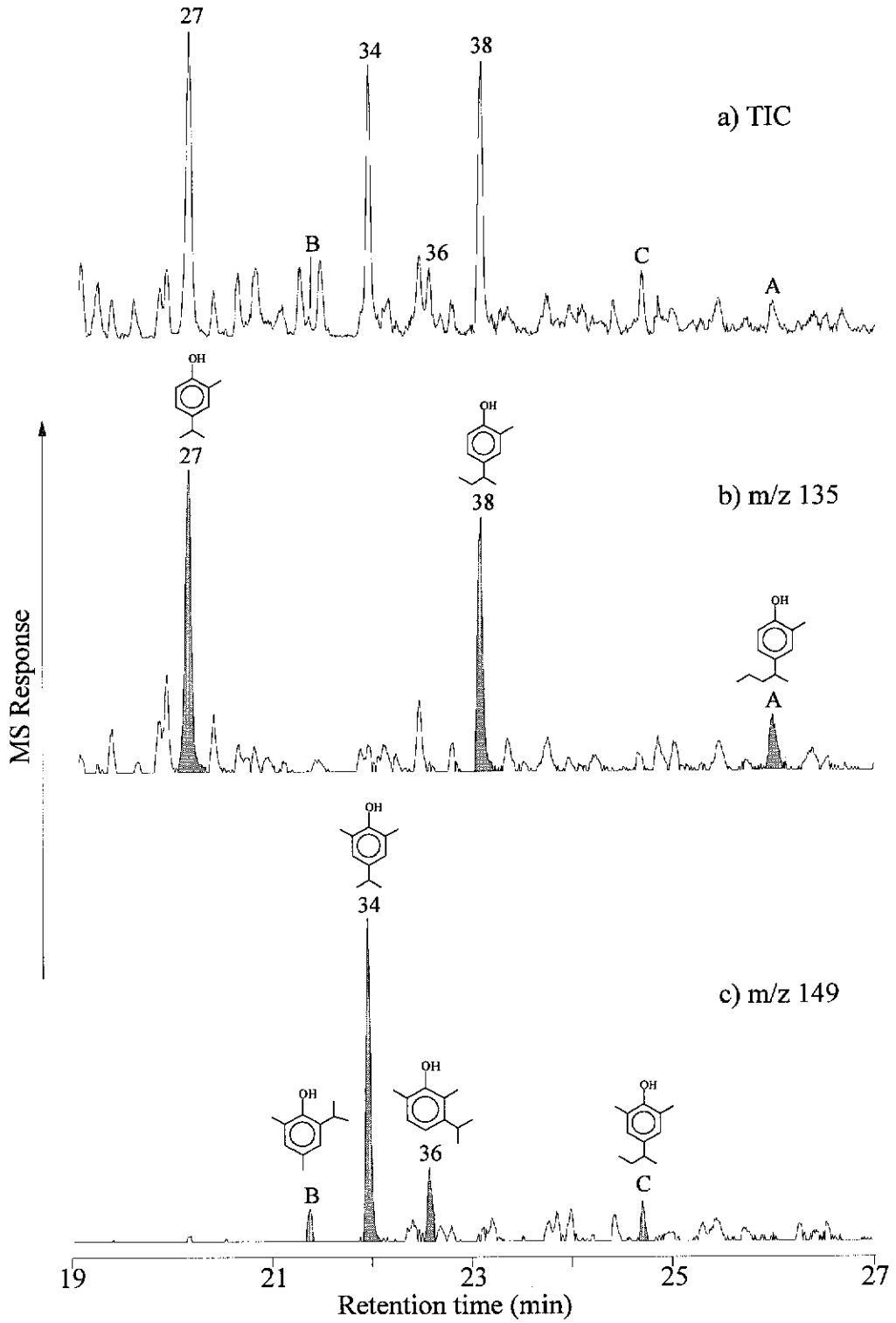


Figure 7.7 a) Partial total ion chromatogram, b) partial m/z 135 mass chromatogram and c) partial m/z 149 mass chromatogram of Sturt crude oil. Compounds A, B and C have been tentatively assigned based on mass spectral data.

isopropylation and *sec*-butylation. Table 7.1 shows aromatic and sterane maturity data and various phenol ratios designed to reflect methylation, isopropylation and *sec*-butylation processes on *ortho*-cresol. Seven of the crude oils (Moorari through to Earlstown) are from the Cooper and Eromanga Basins, Australia, and have been derived from broadly similar coaly, non-marine organic matter (Vincent *et al.*, 1985; Heath *et al.*, 1989; Jenkins, 1989). Based on the four maturity parameters shown in Table 7.1 these seven crude oils are presented in order of increasing maturity. The ratio of the 2-, 4- and 6-methyl substituted phenols to *ortho*-cresol (MR) was used to reflect the extent to which methylation processes had occurred. Similarly the ratio of 4-isopropyl-2-methylphenol to *ortho*-cresol (IR) and 4-*sec*-butyl-2-methylphenol to *ortho*-cresol (BR) should reflect isopropylation and *sec*-butylation of *ortho*-cresol respectively. Data are also shown for Lambert crude oil which has been included as a reference to show typical values of the phenol ratios for a sample previously classified as having a near equilibrated distribution of C_2-C_4 alkylphenols (Section 5.2). Figure 7.8 shows a plot of DNR1 versus the methylation ratio (MR), isopropylation ratio (IR) and sec-butylation ratio (BR) for the Cooper and Eromanga Basin crude oils and Lambert crude oil. It is apparent that the methylation parameter for all the crude oils, even the one with the lowest maturity (Moorari), exceeds the equilibrium value shown by Lambert crude oil. However in the case of the isopropylation ratio only the more mature Cooper basin samples (Sturt, Malgoona and Earlstown) have values exceeding that of the equilibrated sample, and the highest value is shown by the most mature sample (Earlstown). A similar trend to that for IR is seen for the ratio indicating *sec*-butylation (BR) where only the more mature crude oils have a high value. These results suggest that the methylation processes can occur at lower maturity than isopropylation and *sec*-butylation; the latter processes possibly only become significant in the latter part of the oil window.

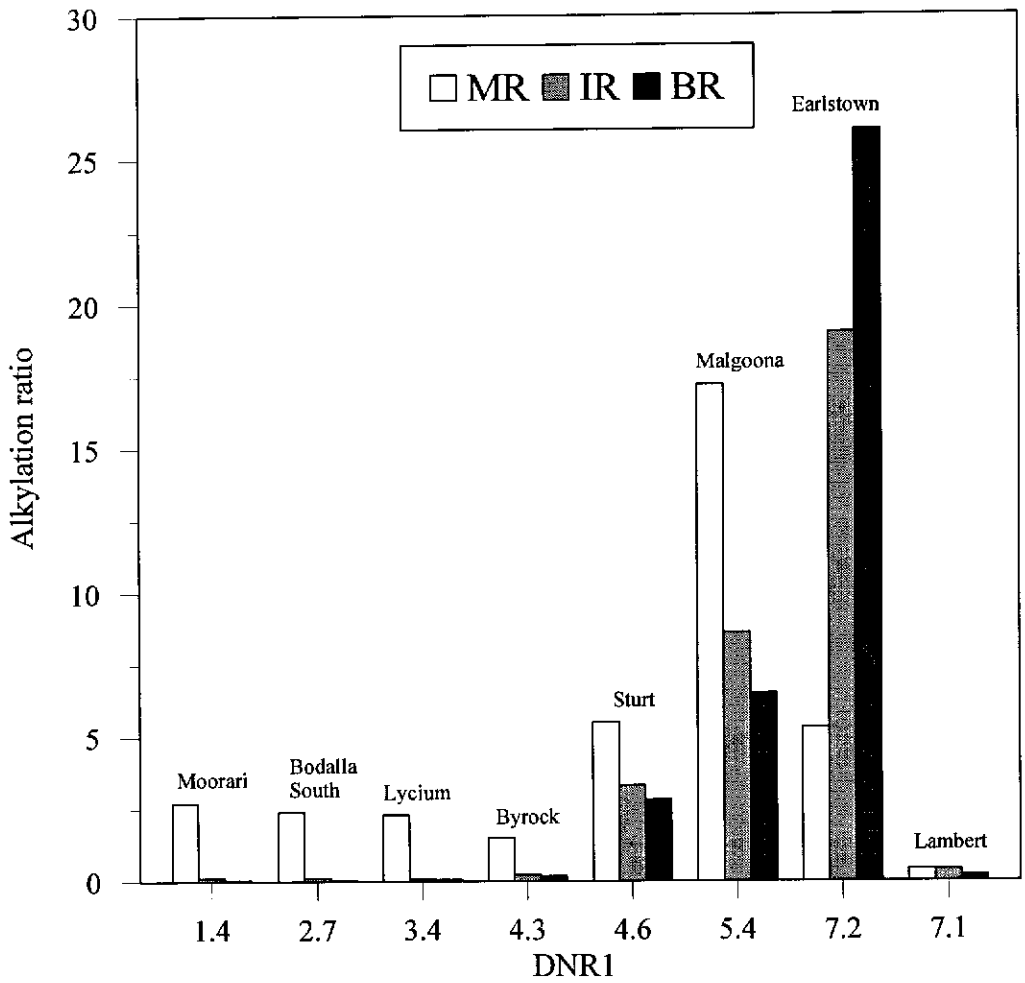


Figure 7.8 Bar graph of aromatic maturity parameter DNR1 versus alkylation ratios: methylation ratio (MR), isopropylation ratio (IR) and *sec*-butylation ratio (BR).

The suggestion that methylation of phenols can occur in rocks that are near the beginning of oil generation is consistent with other reported methylation processes. In the case of phenanthrenes Radke *et al.* (1982a; 1983) attributed the high relative abundance of 9-methylphenanthrene in low maturity rocks to such processes. Methylation has also been recognised as an important process occurring during the early stages of coalification (refer to discussion in Section 5.4.1).

The formation of isopropyl and *sec*-butyl derivatives in the higher maturity zone is consistent with secondary carbocations undergoing electrophilic substitution to the reactive phenol substrate. The carbocations might be formed from the protonation of intermediate alkenes during catagenesis. Alkenes readily undergo such processes under laboratory conditions especially when acid catalysts such as clays are available (Balough and Laszlo, 1993). Isopropylation and *sec*-butylation products however, are not present in all mature crude oils, indicating that there are other factors controlling the extent to which alkylation products occur. Included in Table 7.1 are three mature crude oils (Nilam, Barrow UJ and Crude oil P) which, in contrast to the mature crude oils of the Cooper Basin, do not have very high values of IR and BR. One explanation is that isopropylation and *sec*-butylation has only occurred to a small extent since IR and BR are still above the equilibrium value (*cf.* Lambert) in these crude oils. An alternative explanation is that dealkylation may also be occurring in rocks and hence the more alkylated phenols may undergo dealkylation to give more stable, less substituted compounds such as the cresols. Such a process might be occurring in higher maturity zones when abundant acid catalysts, from dehydration of clays, are present. The nature of the mineral or maceral matrix of a source rock may therefore be a determining factor in preservation of the alkylation products in expelled crude oil.

7.5 C_4 and C_5 ALKYLPHENOL COMPONENTS OF SEDIMENTARY ROCK PYROLYSATES FROM THE COOPER BASIN

An alternative explanation to that given in Section 7.4 for the high relative abundances of *ortho* and *para* substituted alkylphenols in Group 3 crude oils is that they occur in these proportions in the kerogen and are released during catagenesis. The distributions of alkylphenols in Group 3B crude oils (two examples of which are shown in Figures 7.9 b) and 7.9 c) are in marked contrast to those usually found in source rock pyrolysates. Pyrolysates are characterised by their relatively high concentrations of phenol and C_1 alkylphenols with lesser amounts of C_2 and C_3 alkylphenols and a very low relative abundance of higher phenols (*cf.* Allan and Larter, 1981; Senftle *et al.*, 1986; Nip *et al.*, 1988.) In contrast, some of the crude oils from the Cooper basin (e.g. Figure 7.9) contain much higher relative abundances of more highly alkylated phenols.

Therefore the phenolic components of three Permian sedimentary rock samples from the Cooper Basin (Australia) and associated Cooper-reservoired oils were selected for detailed comparison of their C_4 and C_5 alkylphenols. For this purpose three crude oils from reservoirs in the Patchawarra formation were selected for comparison with pyrolysates from three source rocks from the Patchawarra, because these source rocks are most likely genetically related (Kantsler *et al.*, 1983; Yew and Mills, 1989). Isopropylmethylphenols and *sec*-butylmethylphenols were not detected in the flash pyrolysates of the three sedimentary rock samples even when pyrolysis GC-MS was employed and their base (m/z 135 and 149) and parent peaks (m/z 150 and 164) were monitored (Table 7.4). In order to carry out a more detailed study of these C_4 and C_5 alkylphenols, one of the Cooper Basin sedimentary rocks was also subjected to preparative scale hydrous pyrolysis so that the phenols in the pyrolysate could be isolated. GC-MS analysis of the phenols from the pyrolysate revealed that

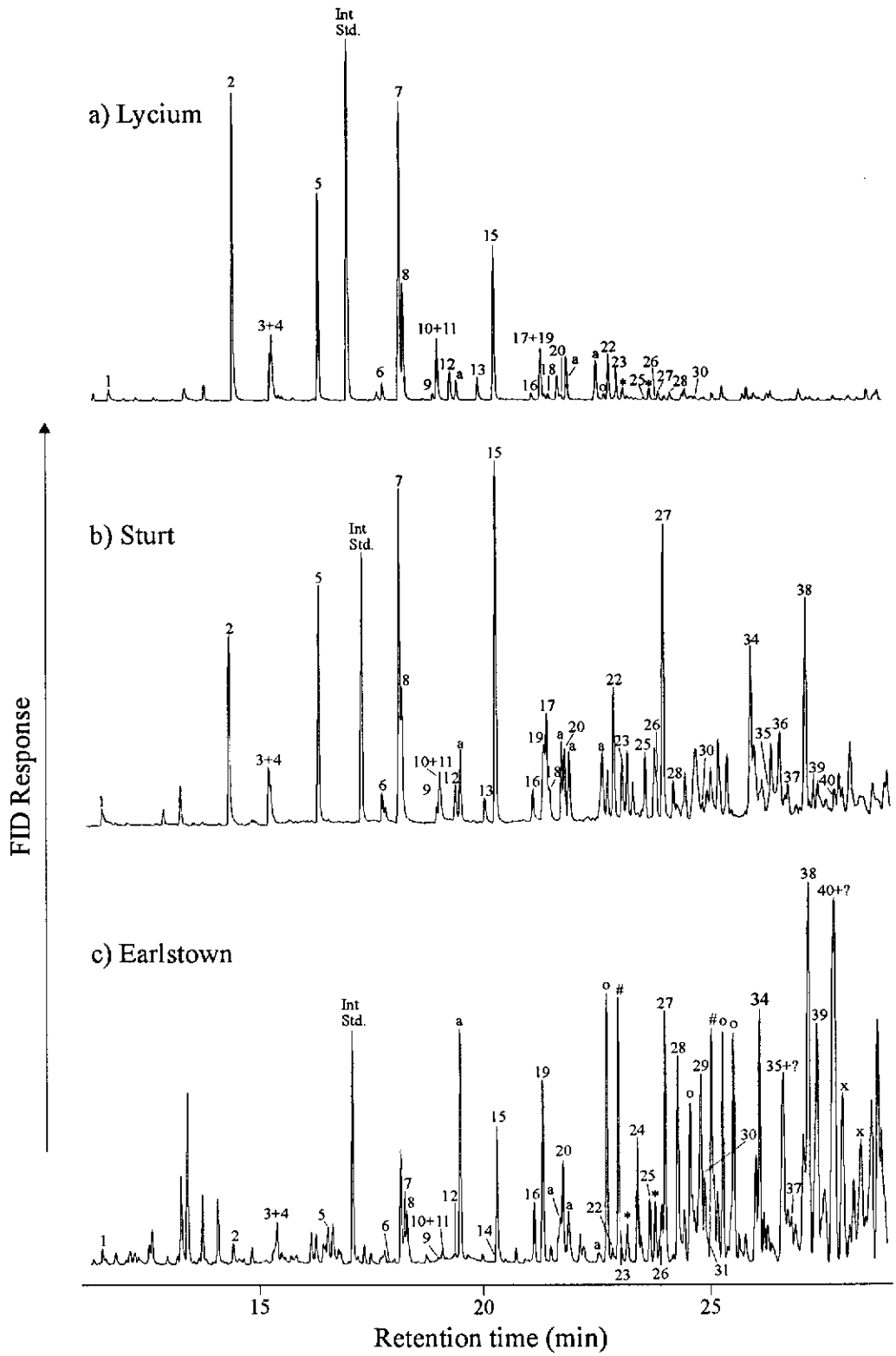


Figure 7.9 Partial gas chromatograms of the phenol extracts of a) Lycium, b) Sturt and c) Earlstown crude oils from the Patchawarra formation, Cooper basin.
Refer to Table 4.4 for peak identifications.

isopropylmethylphenols were present in very low relative abundances (e.g. 4-isopropyl-2-methylphenol / 2-methylphenol = <0.01) compared to the Cooper Basin crude oils (e.g. 4-isopropyl-2-methylphenol / 2-methylphenol = 19, see IR values in Table 7.1) and further, all *sec*-butylmethylphenols were still below the limit of detection. It is therefore concluded that the very high relative abundances of isopropylmethylphenols and *sec*-butylmethylphenols in these crude oils is not due to the cracking of these compounds from kerogen during maturation of sedimentary organic matter, but is most likely the result of the geosynthetic processes occurring in sedimentary rocks where simpler compounds such as phenol and the cresols are alkylated.

Table 7.4 Relative percentage of 4-isopropyl-2-methylphenol and 4-*sec*-butyl-2-methylphenol, and the ratio of 2,4-dimethylphenol and 2,3-dimethylphenol in Patchawarra crude oils and sedimentary rock pyrolysates.

Group	Sample name	Basin	% 4I2MP[1]	% 4sB2MP[2]	2,4-DMP / 2,3-DMP
3A	Lycium		25	70	7.0
3B	Sturt		56	59	6.3
3B	Earlstown		34	34	3.4
Pyrolysates[3]	Tinga Tingana (flash)	Cooper	nd	nd	3.8
	Weena (flash)		nd	nd	2.8
	Sturt East (flash)		nd	nd	2.6
	Tinga Tingana (hydrous)		30	nd	3.0
1	Lambert	Dampier	20	20	1.1

1 % 4I2MP = (4-isopropyl-2-methylphenol / ∑ isopropylmethylphenols) x 100
2 % 4sB2MP = (4-*sec*-butyl-2-methylphenol / ∑ *sec*-butylmethylphenols) x 100
3 Ratios obtained from GC-MS analysis of pyrolysate
DMP = dimethylphenol, nd = isomers were below detection limit

7.6 EFFECTS OF WATER CONTACT ON ALKYLATION PRODUCTS IN CRUDE OILS

An alternative explanation which may account for the observed distributions of alkylphenols in Group 3 crude oils is that they result from

alteration of the phenol distribution in the crude oil by water contact during petroleum expulsion and migration, or in a reservoir. Different distribution coefficients of individual phenols between water and oil could change the composition of an initially formed phenol mixture into that of a migrated product. In order to investigate the nature of these effects, the isooctane/water distribution coefficients of some of the more common C_0 - C_4 substituted petroleum phenols have been measured (Table 7.5). Measurements were carried out using two mixtures of standard phenols in order to avoid co-elution problems in the GC analysis step. An increase in affinity for the hydrocarbon phase was observed for the more highly alkylated phenols, and for the *ortho* substituted phenols compared with their *meta* and *para* substituted counterparts. For example, 2,6-dimethylphenol (K_D = 14) compared with 3,5-dimethylphenol (K_D = 2.9); 2,4,6-trimethylphenol (K_D = 39) compared with 3,4,5-trimethylphenol (K_D = 7.4); 2-isopropyl-6-methylphenol
(K_D = 98) compared with 3-isopropyl-5-methylphenol (K_D = 28). These results are consistent with those observed in the literature (refer to Section 1.6). Therefore, in principle *ortho* substituted phenols might become enriched in crude oil if water partitioning was a major process effecting petroleum phenols during migration or during reservoir water washing. However a better indication as to whether water contact has caused the high abundance of *ortho* compounds observed in many crude oils is provided by the relative abundances of 2,4-dimethylphenol (K_D = 6.0) and 2,3-dimethylphenol (K_D = 5.0). Because they have very similar distribution coefficients their ratio should not be significantly changed by water contact and therefore the ratio of these compounds produced in the source rock should be the same as that in the migrated crude oil. Comparison of the ratios of 2,4-dimethylphenol and 2,3-dimethylphenol for Patchawarra formation (Cooper Basin) crude oils and source rock pyrolysates in Table 7.4 shows that the relative abundance of the 2,4-dimethylphenol is appreciably greater

Table 7.5 Distribution coefficients (K_D) of phenols between isooctane and water at 80°C.

Compound X	K_D[1]
Phenol (1)[2]	0.3
2-Methylphenol (1)	1.6
3-Methylphenol (1)	0.9
4-Methylphenol (2)	0.9
2,3-Dimethylphenol (1)	5.0
2,4-Dimethylphenol (1)	6.0
2,5-Dimethylphenol (2)	5.6
2,6-Dimethylphenol (2)	14
3,5-Dimethylphenol (2)	2.9
3,4-Dimethylphenol (1)	2.6
2-Ethylphenol (1)	5.7
4-Ethylphenol (1)	3.2
2,3,5-Trimethylphenol (1)	17
2,3,6-Trimethylphenol (1)	35
2,4,6-Trimethylphenol (2)	39
3,4,5-Trimethylphenol (1)	7.4
2-Isopropylphenol (2)	18
3-Isopropylphenol (1)	8.3
4-Isopropylphenol (2)	8.5
2-Isopropyl-3-Methylphenol (2)	58
2-Isopropyl-4-Methylphenol (2)	52
2-Isopropyl-5-Methylphenol (1)	58
2-Isopropyl-6-Methylphenol (2)	98
3-Isopropyl-2-Methylphenol (2)	32
3-Isopropyl-4-Methylphenol (1)	21
3-Isopropyl-5-Methylphenol (2)	28
4-Isopropyl-2-Methylphenol (2)	43
4-Isopropyl-3-Methylphenol (2)	21
5-Isopropyl-2-Methylphenol (2)	47

1 K_D = Distribution Coefficient at 80°C (isooctane / water)
1 K_D = [X] in isooctane / [X] in water
2 Number in brackets refer to the standard phenol mixture used in experiments to determine K_D (*cf.* Experimental)

in the oils than in the pyrolysates. Since the ratio is unlikely to change during migration or subsequent water washing it is suggested that these differences are most likely to be the result of processes occurring in the source rock rather than an artefact of migration.

Comparison of the distribution coefficients for the isopropylmethylphenol isomers in crude oil provides further evidence that water contact is not a dominant factor in determining phenol distributions in these crude oils. 4-Isopropyl-2-methylphenol is the most abundant isopropylmethylphenol in Group 3B crude oils (Figure 7.6 and Figure 7.9; peak 27) and has a distribution coefficient of 43 (Table 10). Comparison of this value to the distribution coefficients of the other petroleum isopropylmethylphenol isomers indicates that 4-isopropyl-2-methylphenol should be depleted relative to all isomers, except 3-isopropyl-5-methylphenol, on contact with water. Table 7.4 shows the percentage of 4-isopropyl-2-methylphenol in the crude oils of this type along with Lambert crude oil, which has previously been classified as a crude oil having phenols near their equilibrium distribution (Section 5.2). In most cases 4-isopropyl-2-methylphenol is the dominant isomer, and in all crude oils it is above the predicted equilibrium concentration. Since 4-isopropyl-2-methylphenol is an isomer likely to be depleted rather than enhanced during water contact, it is concluded that such a process does not cause enrichment of this compound above its equilibrium value.

7.7 CONCLUSIONS

1) Crude oils containing high relative amounts of *ortho* and *para* substituted alkylphenols were found to have a much higher relative abundance of C_3-C_5 alkylphenols than those in kerogen pyrolysates.

2) Group 3 crude oils were shown to contain dimethylphenols, trimethylphenols, isopropylmethylphenols and *sec*-butylmethylphenols with

isomer distributions similar to those formed in laboratory alkylation experiments of ubiquitous sedimentary cresols. These results are interpreted as evidence for the geosynthesis of phenols in sedimentary rocks.

3) A high relative abundance of the dimethylphenols and trimethylphenols produced from alkylation processes was observed in a suite of crude oils with a range of maturities (DNR1 = 1.4 to 9.3), whereas a high relative abundance of the products from isopropylation and *sec*-butylation only occurred in the crude oils with DNR1 values greater than 4. These results indicate that methylation can occur at lower maturity than isopropylation and *sec*-butylation.

4) Based on measured distribution coefficients of phenols between isooctane and water, the high relative concentration of 2,4-dimethylphenol and 2-isopropyl-4-methylphenol (phenols which are the dominant isomers produced from the methylation and isopropylation of *ortho*-cresol, respectively) in these crude oils is unlikely to be caused by enrichment of these isomers during crude oil-water contact.

REFERENCES

Abbott G. D., Wang G. Y., Eglinton T. I., Home A. K. and Petch G. S. (1990) The kinetics of sterane biological marker release and degradation process during the hydrous pyrolysis of vitrinite kerogen. *Geochim. Cosmochim. Acta* **54**, 2451-2462.

Adams R., Bachmann W. E., Fieser L. F., Johnson J. R. and Sinyder H. R. (1960) *Organic Reactions*. Vol 1, John Wiley and Sons, Inc, New York, pp.164.

Albers C. C. (1942) The occurrence of thymol and carvacrol in the plant kingdom and their significance in botanical systematics. *Pharm. Arch.* **13**, 39-48.

Albrecht P., Vandenbroucke M. and Mandengue M. (1976) Geochemical studies on the organic matter from the Douala Basin (Cameroon). I. Evolution of the extractable organic matter and the formation of petroleum. *Geochim. Cosmochim. Acta* **40**, 791-799.

Alder E. (1977) Lignin chemistry - past, present and future. *Wood Sci. Technol.* **11**, 169-218.

Alexander M. (1961) *Introduction to Soil Micrology*. John Wiley and Sons, New York, pp. 472.

Alexander M. (1977) *Introduction to Soil Micrology*. John Wiley and Sons, New York, pp. 457.

Alexander R., Kagi R. I., Woodhouse G. W. and Volkman J. K. (1983) The geochemistry of some biodegraded Australian crude oils. *APEA J.* **23**, 53-63.

Alexander R., Cumbers M. and Kagi R. I. (1984) Geochemistry of some Canning basin crude oils. In *The Canning Basin, W.A.: Proceedings of Geol. Soc. Aust./Pet. Expl. Soc. Aust. Symposium, Perth, 1984*. (Edited by Purcell P. G.), pp. 353-358.

Alexander R., Kagi R. I., Rowland S. J., Sheppard P. N. and Chirila T. V. (1985) The effects of thermal maturity on distributions of dimethylnaphthalenes and trimethylnaphthalenes in some ancient sediments and petroleum. *Geochim. Cosmochim. Acta* **49**, 385-395.

Alexander R., Noble R. A. and Kagi R. I. (1987) Fossil resin biomarkers and their application in oil to source-rock correlation, Gippsland Basin, Australia. *APEA J.* **27**, 63-72.

Alexander R., Larcher A. V., Kagi R. I. and Price P. L. (1992) An oil-source correlation study using age-specific plant-derived aromatic biomarkers. In *Biological Markers in Sediments and Petroleum* (Edited by Moldowan J. M., Albrecht P. and Philp R. P.), Prentice-Hall, Inc, pp. 201-221.

Alexander R., Bastow T. P., Fisher S. J. and Kagi R. I. (1995) Geosynthesis of organic compounds. II - Methylation of phenanthrene and alkylphenanthrenes. *Geochim. Cosmochim. Acta,* in press.

Allan J. and Larter S. R. (1981) Aromatic structures in coal maceral extracts and kerogens. In *Advances in Organic Geochemistry 1981* (Edited by Bjorøy M. *et al.*), J. Wiley and Sons, New York, pp. 534-545.

Altwicker E. R. (1967) The chemistry of stable phenoxy radicals. *Chem. Rev.* **67** (5), 475-531.

Armanios C., Alexander R. and Kagi R. I. (1992) High diahopane and neohopane abundances in a biodegraded crude oil from the Barrow Sub-basin of Western Australia. *Org. Geochem.* **18**, 641-645.

Artz R. J. and Schweighardt F. K. (1980) Thin layer chromatographic screening of coal liquids. *J. Liq. Chromatogr.* **3** (12), 1807-1827.

Asakawa Y., Masuya T., Tori M. and Campbell E. O. (1987) Long chain alkyl phenols from the liverwort *Schistochila Apeendiculata. Phytochem.* **26**, 735-738.

Attalla M. I., Serra R. G., Vassallo A. M. and Wilson M. A. (1988) Structure of ancient buried wood from *Phyllocladus trichomanoides. Org. Geochem.* **12**, 235-244.

Ayres M. G., Bilal M., Jones R. W., Slentz L. W., Tartir M. and Wilson A. O. (1982) Hydrocarbon habitat in main producing areas, Saudi Arabia. *Am. Assoc. Pet. Geol. Bull.* **66**, 1-9.

Balogh M. and Laszlo P. (1993) *Organic Chemistry Using Clays*. Springer-Verlag, Berlin, pp. 15-20.

Barclay W. R., Kennish J. M., Goodrich V. M. and Fall R. (1987) High levels of phenolic compounds in *Prochloron* species. *Phytochem.* **26**, 739-743.

Bassus J., Bertholon G., Decoret C. and Perrin R. (1974) Étude physicochimique des phénols. XI. Comparaison entre les réactivitiés théoriques et expérimentales du méthyl-4 phénol lors réactions d'isopropylation. Étude physicochimique des composés obtenus. *Bull. Soc. Chim. Fr.* **12**, 3031-3038.

Bates A. L. and Hatcher P. G. (1989) Solid-state ^{13}C NMR studies of a large fossil gymnosperm from the Yallourn Open Cut, Latrobe Valley, Australia. *Org. Geochem.* **14** (6), 609-617.

Batrakov S. G., Pridachina N. N., Kruglyak E. D. and Novogrudskaya E. D. (1977) Phenolic lipid from *Azotobacter chroococcum. Khim. Prir. Soedin.* **4**, 494-499.

Bauld J. (1981) Occurrence of benthic microbial mats in saline lakes. *Hydrobiologia* **81**, 87-111.

Benson C. W., Simcox R. A. and Huldal I. C. (1991) Tailoring aromatic hydrocarbons for asphaltene removal. In *Chemicals in the Oil Industry: Developments and Applications* (Edited by Ogden P. H.), The Royal Society of Chemistry, Cambridge, pp. 215-233.

Berkaloff C., Casadevall E., Largeau C., Metzger P., Peracca S. and Virlet J. (1983) Hydrocarbon formation in the green alga *Botryococcus braunii.* Part 3. The resistant polymer of the walls of the hydrocarbon-rich alga *B. braunii. Phytochem.* **22**, 389-397.

Bett G., Harvey T. G., Matheson T. W. and Pratt K. C. (1983) Determination of polar compounds in Rundle shale oil. *Fuel* **62**, 1445-1454.

BHP Petroleum Pty. Ltd. (1985) *Pictor-1: Well Completion Report Part II: Interpretation*, EP-175, unpublished.

Bohlmann F., Natu A. A. and Kerr K. (1979) Thymol-derivate aus *Neurolaena-arten. Phytochem.* **18**, 489-490.

Bolton A. P., Lanewala M. A. and Pickert P. E. (1968) The isomerisation of *t*-butylphenols using zeolite catalysts. *J. Org. Chem.* **33** (9), 3415-3418.

Boreham C. J., Crick I. H. and Powell T. G. (1988) Alternative calibration of the methylphenanthrene index against vitrinite reflectance: Application to maturity measurements on oils and sediments. *Org. Geochem.* **12** (3), 289-294.

Bostick N. H. (1979) Microscopic measurement of the level of catagenesis of solid organic matter in sedimentary rocks to aid exploration for petroleum and to determine former burial temperatures-A review. *Society of Economic Paleontologists and Mineralogists Special Publication No. 26*, 17-43.

Botto R. E. (1987) Solid state ^{13}C NMR tracer studies to probe coalification. *Energy and Fuels* **1**, 228-230.

Brassell S. C. and Eglinton G. (1986) Molecular geochemical indicators in sediments. In *Organic Marine Geochemistry*, American Chemical Society Symposium Series 305, USA, pp. 10-32.

Brassell S. C., Comet P. A., Eglinton G., Isaacson P. J., McEvoy J., Maxwell J. R., Thompson I. D., Tibbetts P. J. C. and Volkman J. K. (1980) The origin and fate of lipids in the Japan Trench. In *Advances in Organic Geochemistry 1979* (Edited by Douglas A. G. and Maxwell J. R.), Pergamon Press, Oxford, pp. 375-391.

Brassell S. C, Eglinton G. and Maxwell J. R. (1983) The geochemistry of terpenoids and steroids. *Biochem. Soc. Trans.* **11**, 575-586.

Brassell S. C., Eglinton G. and Howell V. J. (1987) Palaeoenvironmental assessment of marine organic-rich sediments using molecular organic geochemistry. In *Marine Petroleum Source Rocks* (Edited by Brooks J. and Fleet A. J.), Geological Society Special Publication No. 26, Blackwell Scientific Publications, London, pp. 79-98.

Bray E. E. and Evans E. D. (1961) Distributions of *n*-paraffins as a clue to recognition of source beds. *Geochim. Cosmochim. Acta* **22**, 2-15.

Brooks J. (1971) Some chemical and geochemical studies on sporopollenin. In *Sporopollenin* (Edited by Brooks J., Grant P., Muir M. D., Shaw G. and Van Gijzel P.), Academic Press, London, pp. 351-407.

Brooks J. and Shaw G. (1968) The post-tetradontogeny of the pollen wass and the chemical structure of the sporopollenin of *Lilium henryii*. *Grana Palynol.* **8**, 227-234.

Brooks J. and Shaw G. (1972) Geochemistry of sporopollenin. *Chem Geol.* **10**, 69-87.

Brooks J. and Shaw G. (1978) Sporopollenin: A review of its chemistry, palaeobiochemistry and geochemistry. *Grana* **17**, 91-97.

Brouwer D. M., Mackor E. L. and Maclean C. (1970) In *Carbonium Ions* (Edited by Olah G. A. and Schleyer P. V. R.), Wiley-Interscience, New York, pp. 837.

Burnham A. K. and Braun R. L. (1990) Development of a detailed model of petroleum formation, destruction and expulsion from lacustrine and marine source rocks. In *Advances in Organic Geochemistry 1989*, (Edited by Durand B. and Behar F.), Pergamon Press, Oxford, pp. 27-40.

Callaghan D. (1991) Chemicals in oil production: A producers perceived needs. In *Chemicals in the Oil Industry: Developments and Applications* (Edited by Ogden P.H.), The Royal Society of Chemistry, Cambridge, pp. 17-31.

Callmer K., Edholm L. -E. and Smith B. E. F. (1977) Study of retention behaviour of alkylphenols in straight and reversed-phase liquid chromatography and application to the analysis of complex phenolic mixtures in conjunction with gas chromatography. *J. Chromatgr.* **136**, 45-62.

Cardoso J. N. and Eglinton G. (1983) The use of hydroxyacids as geochemical indicators. *Geochim. Cosmochim. Acta* **47**, 723-730.

Cardoso J. N., Eglinton G. and Holloway P. J. (1977) The use of cutin acids in the recognition of higher plant contribution to recent sediments. In *Advances in Organic Geochemistry 1975* (Edited by Campos R. and Goni J.), ENADIMSA, Madrid, pp. 273-287.

Carpenter M. S and Easter W. M (1955) The isopropyl cresols. *J. Org. Chem.* **20**, 401-411.

Carrado K. A., Hayatsu R., Botto R. E. and Winans R. E. (1990) Reactivity of anisole on clay and pillared clay surfaces. *Clays and Clay Minerals* **38**, 250-256.

Casteele K. V., De Footer H. and Van Sumere C. F. (1976) Gas chromatographic separation and analysis of trimethylsilyl derivatives of some naturally occurring non-volatile phenolic compounds and related substances. *J. Chromatogr.* **121**, 49-63.

Chaffee A. L., Johns R. B., Baerken M. J., de Leeuw J. W., Schenck P. A. and Boon J. J. (1984) Chemical effects in gelification processes and lithotype formation in Victorian brown coal. *Org. Geochem.* **6**, 409-416.

Childs R. F. and Hor Y. C. (1977) Photoisomerization of the boron trihalide complexes of eucarvone. *Can. J. Chem.* **55**, 3501-3508.

Christman R. F. and Oglesby R. T. (1971) Microbiological degradation and the formation of Humus. In *Lignins. Occurrence, Formation, Structure and Reactions.* (Edited by Sarkanen K. V. and Ludwig C. H.), Wiley-Interscience, New York, pp. 769-795.

Cimino G., de Stefano S. and Minale L. (1972a) Polyprenyl derivatives from the sponge *Ircinia spinosula*. 2-Polyprenylbenzoquinones, 2-polyprenyl-benzoquinols, prenylated furans, and a C_{31} difuranoterpene. *Tetrahedron* **28**, 1315-1324.

Cimino G., de Stefano S. and Minale L. (1972b) Prenylated quinones in marine sponges. *Ircinia* species. *Experientia* **28**, 1401-1402.

Cimino G., de Luca P., de Stefano S. and Minale L. (1975) Disidein, a pentacyclic sesterterpene condensed with a hydroxyhydroquinone moiety, from the sponge *Disidea pallascens. Tetrahedron* **31**, 271-275.

Clark J. P. and Philp R. P. (1989) Geochemical characterization of evaporite and carbonate depositional environments and correlation of associated crude oils in the Black Creek Basin, Alberta. *Can. Pet. Geol. Bull.* **37**, 401-416.

Clayton J. L. and King J. D. (1987) Effects of weathering on biological marker and aromatic hydrocarbon composition of organic matter in Phosphoria shale outcrop. *Geochim. Cosmochim. Acta* **51**, 2153-2157.

Connan J. (1974) Diagènese naturelle et diagenèse artificielle de la matière organique a élément végétaux prédominants. In *Advances in Organic Geochemistry 1973* (Edited by Tissot B. P. and Bienner F.), Editions Technip, Paris, pp. 73-95.

Connan J. (1984) Biodegradation of crude oils in reservoirs. In *Advances in Petroleum Geochemistry*, Vol. 1 (Edited by Brooks J. and Welte D. H.), Academic Press, London. pp. 299-335.

Cooper B. S. and Murchison D. G. (1971) The petrology and geochemistry of sporinite. In *Sporopollenin* (Edited by Brooks J., Grant P., Muir M. D., Shaw G. and Van Gijzel P.), Academic Press, London, pp. 545-568.

Dean J. A. (1992) *Lange's handbook of chemistry* (14th Ed.), McGraw-Hill, Inc., pp. 8.19-8.72.

Derbyshir F. J. and Whitehurst D. D. (1981) Study of coal conversion in polycondensed aromatic compounds. *Fuel* **60**, 655-662.

Derenne S., Largeau C., Casadevall E. and Berkaloff C. (1989) Occurrence of a resistant biopolymer in the *L* race of *Botryococcus braunii*. *Phytochem.* **28**, 1137-1142.

Derenne S., Largeau C., Casadevall E., Sinninghe Damsté J. S., Tegelaar E. W. and de Leeuw J. W. (1990) Characterization of Estonian Kukersite by spectrocopy and pyrolysis: Evidence for abundant alkyl phenolic moieties in an Ordovician, marine, type II/I kerogen. In *Advances in Organic Geochemistry 1989* (Edited by Durand B. and Behar F.), Pergamon Press, Oxford, pp. 873-888.

Derenne S., Metzer P., Largeau C., Van Bergen P. F., Gatellier J. P., Sinninghe Damsté J. S., de Leeuw J. W. and Berkaloff C. (1992) Similar morphological and chemical variations of *Gloeocapsomorpha prisca* in Ordovician sediments and cultures *Botryococcus braunii* as a response to changes in salinity. In *Advances in Organic Geochemistry 1991* (Edited by Eckardt C. B. *et al.*), Pergamon Press, *Org. Geochem.* **19** (4-6), pp. 299-313.

Dev S. (1989) Terpenoids. In *Natural Products of Woody Plants* II. (Edited by Rowe J.W.), Springer-Verlag, Berlin, pp. 704.

Didyk B. M., Simoneit B. R. T., Brassell S. C. and Eglinton G. (1978) Organic geochemical indicators of palaeoenvironmental conditions of sedimentation. *Nature* **272**, 216-222.

Dinge A. S., Kirtany J. K. and Paknikar S. K. (1981) Isothymol: synthesis and biogenesis. *Ind. J. Chem.* **29B**, 245-246.

Diplock A. T., Green J., Edwin E. E. and Bunyan J. (1961) Tocopherols, ubiquinones and ubichromenols in yeasts and mushrooms. *Nature* **189**, 749-750.

Douglas A. G., Sinninghe Damsté J. S., Fowler M. G., Eglinton T. I. and de Leeuw J. W. (1991) Flash pyrolysis of Ordovician kerogens: unique distributions of hydrocarbons and sulphur compounds released from the fossilized alga *Gloeocapsomorpha prisca. Geochim. Cosmochim. Acta* **55**, 275-291.

Ekstrom A., Fookes C. J. R., Loeh H. J., Randall C. H., Rovere C., Ellis J. and Crisp P. T. (1987) Chemical and pyrolysis characteristics of two types of oil shale from the Condor deposit in Queensland, Australia. *Fuel* **66**, 1133-1138.

Engel M. H. and Macko S. A. (1993) *Organic Geochemistry: Principles and Applications*, Plenum Press, New York.

Ensminger A., Albrecht P., Ourisson G. and Tissot B. (1977) Evolution of polycyclic alkanes under the effect of burial (Early Toarcian shales, Paris Basin). In *Advances in Organic Geochemistry 1975* (Edited by Campos R. and Goni J.), ENADIMSA, Madrid, pp. 45-52.

Ertel J. R. and Hedges J. I. (1984) The lignin components of humic substances: distribution among soil and sedimentary humic, fulvic, and base-insoluble fractions. *Geochim. Cosmochim. Acta* **48**, 2065-2074.

Espelie K. E., Davis R. W. and Kolattukudy P. E. (1980) Composition, ultrastructure and function of the cutin- and suberin- containing layers in the leat, fruit peel, juice-sac and inner seed coat of grapefruit (*Citrus paradisi* Macfed). *Planta* **149**, 498-511.

Espelie K. E., Wattendorf J. and Kolattukudy P. E. (1982) Composition and ultrastructure of the suberized cell wall of isolated crystal idioblasts from *Agave americana L.* leaves. *Planta* **153**, 166-175.

Faegri K. (1971) The preservation of sporopollenin membranes under natural conditions. In *Sporopollenin* (Edited by J. Brooks *et al.*), Academic Press, London, pp. 256-272.

Foo L. Y. and Porter L. J. (1980) The phytochemistry of proanthocyanidin polymers. *Phytochem.* **19**, 1747-1754.

Foster C. B., Wicander R. and Reed J. D. (1990) *Gloecapsomorpha prisca* Zalessky, 1917: a new study. Part II: Origin of Kukersite, a new interpretation. *Geobios.* **23**, 133-140.

Fox D. L. (1974) Biochromes. Occurrence, distribution and comparative biochemistry of prominent natural pigments in the marine world. In *Biochemical and Biophysical Perspectives in Marine Biology* (Edited by Malins D. C. and Sargent J. R.), Academic Press, New York, pp. 169-211.

Fowler M. G. and Douglas A. G. (1984) Distribution and structure of hydrocarbons in four organic-rich Ordovician rocks. In *Advances in Organic Geochemistry 1983* (Edited by Schenck P. A., de Leeuw J. W. and Lijmbach G. W. M.), Pergamon Press, Oxford, pp. 105-114.

Fripiat J. J. and Van Olphen V. (1979) *Data Handbook for Clay Materials and Other Non-metallic Minerals*, Pergamon Press, pp. 195-204.

Furniss B. S., Hannaford A. J., Smith P. W. G. and Tatchell A. R. (1989) *Vogel's Textbook of Practical Organic Chemistry* (5th Ed.), Longman Group UK Ltd., United Kingdom, pp. 993.

Gagosian R. B.and Peltzer E. T. (1986) The importance of atmospheric input of terrestrial organic material to deep sea sediments. In *Advances in Organic Geochemistry 1985* (Edited by Leythaeuser D. and Rullkötter J.), Pergamon Press, Oxford, pp. 661-669.

Gardner W. S. and Menzel D. W. (1974) Phenolic aldehydes as indicators of terrestrially derived organic matter in the sea. *Geochim. Cosmochim. Acta* **38**, 813-822.

Garrigues P., Connan J., Parlanti E., Belloca J. and Ewald M. (1988) Relationship between rank and distribution of methylaromatic hydrocarbons for condensates of different origins. *Org. Geochem.* **13**, 1115-1121.

Gelin F., de Leeuw J. W., Sinninghe Damsté J. S., Derenne S., Largeau C. and Metzger P. (1994) Scope and limitations of flash pyrolysis-gas chromatography/mass spectrometry as revealed by the thermal behaviour of high-molecular-weight lipids derived from the green microalga *Botryococcus braunii*. *J. Anal. Appl. Pyrol.* **28**, 183-204.

George A. M., Attwood D. H., Borsaru R. M., King H. D., MacKay T. N and Stacy W. O. (1984) *Wood in brown coal*. State Electricity Commission of Victoria Report No. SO/84/84.

Gilby G. W. (1983) The use of ethylene-vinyl acetate copolymers as flow improvers and wax deposition inhibitors in waxy crude oil. In *Chemicals in the Oil Industry* (Edited by Ogden P.H), The Royal Society of Chemistry, London, pp. 108-124.

Gilson H. C. (1964) "Lake Titicaca". *Int. Ass. Theor. Appl. Limnol.* **15**, 112-127.

Given P. H., Spackman W., Painter P. C., Rhoads C. A. and Ryan N. J. (1984) The fate of cellulose and lignin in peats: an exploratory study of the input to coalification. *Org. Geochem.* **6**, 399-407.

Glikson M., Lindsay K. and Saxby J. (1989) *Botryococcus* - A planktonic green alga, the source of petroleum through the ages: transmission electron microscopical studies of oil shales and petroleum source rocks. *Org. Geochim.* **14**, 595-608.

Glombitza K. W. (1977) Highly hydroxylated phenols of the Phaeophyceae. In *Marine Natural Products Chemistry* (Edited by Faulkner D.J. and Fenical W.H.), Plenum Press, New York, pp. 191-204.

Goodwin T. W. and Mercer E. I. (1972) *Introduction to Plant biochemistry*, Pergamon Press, Oxford.

Goossens H., de Leeuw J. W., Schenck P. A. and Brassell S. C. (1984) Tocopherols as likely precursors of pristane in ancient sediments and crude oils. *Nature* **312** (29), 440-442.

Gough M. A., Fauzi R., Mantoura C. and Preston M. (1993) Terrestrial plant biopolymers in marine sediments. *Geochim. Cosmochim. Acta* **57**, 945-964.

Graham D. E., Stockwell A. and Thompson D. G. (1983) Chemical demulsification of produced crude oil emulsions. In *Chemicals in the Oil Industry* (Edited by Ogden P.H), The Royal Society of Chemistry, London, pp. 73-91.

Grantham P. J. (1986a) Sterane isomerisation and moretane/hopane ratios in crude oils derived from Tertiary source rocks. *Org. Geochem.* **9**, 293-304.

Grantham P. J. (1986b) The occurrence of unusual C_{27} and C_{29} sterane predominance in two types of Oman crude oil. *Org. Geochem.* **9**, 1-10.

Grantham P. J. and Wakefield L. L. (1988) Variations in the sterane carbon number distribution of marine source rock derived crude oils through geological time. *Org. Geochem.* **12**, 61-73.

Green J. (1963) Tocopherols. In *Enzyme Chemistry of Phenolic Compounds* (Edited by Pridham J . B.), Pergamon Press, New York, pp. 47-56.

Green J. B., Thomas J. S., Treese C. A., Yu S. K-T., Renaudo C. P. and Stierwalt B. X. (1986) *Separation and Analysis of Hydroxyaromatic Species in Liquid Fuel,* National Institute for Petroleum and Energy Research, Bartlesville, O.K., unpublished Topical Report, pp. 1-34.

Grishina L. A. and Korotkov K. O. (1978) Modes of formation of specific organic substances in soil. *Soviet Soil Science* **10**, 23-26.

Grosse-Damhues J. and Glombitza K. W. (1984) Isofuhalols, a type of phlorotannins from the brown alga Chorda filum. *Phytochem.* **23**, 2639-2642.

Guenther E. (1966) *The Essential Oils*, Vol 2, Van Nostrand Company Inc. New York, pp. 499-546.

Guenther F. R., Parris R. M., Chesler S. N. and Hilpert L. R. (1981) Determination of phenolic compounds in alternative fuel matrices. *J. Chromatogr.* **207**, 256-261.

Guilford W. J., Schneider D. M., Labovitz J. and Opella S. J. (1988) High resolution solid state ^{13}C NMR investigation of sporopollenin from different plant taxa. *Plant Physiol.* **86**, 134-136.

Haider K. and Martin J. P. (1967) Synthesis and transformation of phenolic compounds by *Epicoccum nigrum* in relation to humic acid formation. *Soil Science Society of America Proceedings* **31**, 766-771.

Hartgers W. A., Sinninghe Damsté J. S., de Leeuw J. W., Ling Y. and Dyrkacz G. R. (1994) Molecular characterisation of flash pyrolyzates of two Carboniferous coals and their constituting maceral fractions. *Energy Fuels* **8** (5), 1055-1067.

Haslam E. (1989) *Plant Polyphenols*. Cambridge University Press, Great Britain.

Hatcher P. G. (1990) Chemical structural models for coalified wood (vitrinite) in low rank coal. In *Advances in Organic Geochemistry* 1989 (Edited by Durand B. and Behar F.), Pergamon Press, Oxford, pp. 959-968.

Hatcher P. G. and Clifford D. J. (1994) Flash pyrolysis and *in situ* methylation of humic acids from soil. *Org. Geochem.* **21**, 1081-1092.

Hatcher P. G. and Spiker E. C. (1988) Selective degradation of plant biomolecules. In *Humic Substances and Their Role in the Environment* (Edited by Frimmel F. H. and Christman R. F.), John Wiley & Sons, New York, pp. 59-74.

Hatcher P. G., Lerch III H. E. and Verheyen T. V. (1989a) Organic geochemistry studies of the transformation of gymnosperms xylem during peatification and coalification to subbituminous coal. *Int. J. Coal Geol.* **13**, 65-97.

Hatcher P. G., Wilson M. A., Vassallo A. M. and Lerch III H. E. (1989b) Studies of angiospermous wood in Australian brown coal by nuclear magnetic resonance and analytical pyrolysis: new insights into the early coalification process. *Int. J. Coal Geol.* **13**, 99-126.

Hatcher P. G., Lerch III H. E., Bates A. L. and Verheyen T. V. (1989c) Solid-state ^{13}C nuclear magnetic resonance studies of coalified gymnosperm xylem tissue from Australian brown coal. *Org. Geochem.* **14**, 145-155.

Hatcher P. G., Faulon J. L., Wenzel K. A. and Cody G. D. (1992) A structural model for lignin-derived vitrinite from high-volatile bituminous coal (coalified wood). *Energy and Fuels* **6**, 813-820.

Hatta K., Schubert W. J. and Nord F. F. (1966) *Arch. Biochem. Biophys.* **113**, 250-251.

ten Haven H. L., de Leeuw J. W., Peakman T. M. and Maxwell J. R. (1986) Anomalies in steroid and hopanoid maturity indices. *Geochim. Cosmochim. Acta* **50**, 853-855.

ten Haven H. L., Baas M., de Leeuw J. W. and Schenk P. A. (1987a) Late Quaternary Mediterranean sapropels I. On the origin of organic matter in sapropel S_7. *Mar. Geol.* **75**, 137-156.

ten Haven H. L., de Leeuw J. W., Rullkötter J. and Sinninghe Damsté J. S. (1987b) Restricted utility of the pristane/phytane ratio as a palaeoenvironmental indicator. *Nature* **330**, 641-643.

ten Haven H. L., Baas M., Kroot M., de Leeuw J. W., Schenk P. A. and Ebbing J (1987c) Late Quaternary Mediterranean sapropels. III: Assessment of source of input and palaeotemperature as derived from biological markers. *Geochim. Cosmochim Acta* **51**, 803-810.

ten Haven H. L., Rullkötter J., Sinninghe Damsté J. S. and de Leeuw J. W. (1990) Distribution of organic sulphur compounds in Mesozoic and Cenozoic deep sea sediments from the Atlantic and Pacific Oceans and the Gulf of California. In *Geochemistry of Sulfur in Fossil Fuels* (Edited by Orr W. L. and White C. M.), Am. Chem. Soc., Washington, pp. 613-632.

Havinga A. J. (1971) An experimental investigation into the decay of pollen and spores in various soil types. In *Sporopollenin* (Edited by Brooks J., Grant P., Muir M. D., Shaw G. and van Gijzel P.), Academic Press, London, pp. 446-479.

Hayatsu R., Botto R. E., McBeth R. L., Scott R. G. and Winans R. E. (1988) Chemical alteration of a biological polymer "sporopollenin" during coalification: Origin, formation and transformation of the coal maceral sporinite. *Energy and Fuels* **2**, 843-847.

Hazlett R. N. and Power A. J. (1989) Phenolic compounds in Bass Strait distillate fuels: their effect on deposit formation. *Fuel* **68**, 1112-1117.

Heath R., McIntyre S. and Gibbins N. (1989) A Permian origin for Jurassic reservoired oil in the Eromanga Basin. In *The Cooper and Eromanga Basins, Australia* (Edited by O'NEIL B. J), Proceedings of Petroleum Exploration Society of Australia, Society of Petroleum Engineers, Australian Society of Exploration Geophysicists (SA Branches), Adelaide, pp. 405-416.

Hedges J. I. and Parker P. L. (1976) Land-derived organic matter in surface sediments from the gulf of Mexico. *Geochim. Cosmochim. Acta* **40**, 1019-1029.

Hedges J. I. and Mann D. C. (1979) The lignin geochemistry of marine sediments from the southern Washington coast. *Geochim. Cosmochim. Acta* **43**, 1809-1818.

Hedges J. I. and Ertel J. R. (1982) Characterisation of lignin by gas capillary chromatography of cupric oxide oxidation products. *Anal. Chem.* **54**, 174-178.

Hedges J. I., Blanchette R. A., Weliky K. and Devol A. H. (1988) Effects of fungal degradation on the CuO oxidation products of lignin: A controlled laboratory study. *Geochim. Cosmochim. Acta* **52**, 2717-2726.

Hellebust J. A. (1974) Extracellular products. In *Algal Physiology and Biochemistry*, Botanical Monographs Vol. 10 (Edited by Stewart W. D. P.), Blackwell, Oxford, pp. 838-863.

Hertz H. S., Brown J. M., Chesler S. N., Guenther F. R., Hilbert L. R., May W. E., Parris R. M. and Wise S. A. (1980) Determination of individual organic compounds in shale oil. *Anal. Chem.* **52**, 1650-1657.

Hoffmann C. F., Foster C. B., Powell T. G. and Summons R. E (1987) Hydrocarbon biomarkers from Ordovician sediments and fossil alga *Gloeocapsomorpha prisca* Zalessky 1917. *Geochim. Cosmochim. Acta* **52**, 2681-2697.

Hollerbach A. and Dehmer J. (1994) Diagenesis of organic matter. In *Diagenesis, IV* (Edited by Wolf K. H. and Chilingarian G. V. *et al.*), Elsevier, Netherlands, pp. 309-357.

Holloway P.J. (1982) The chemical constitution of plant cutins. In *The Plant Cuticle* (Edited by Cutler D. F. *et al.*), Linnean Society Symposium Series No. 10, Academic Press, London, pp. 45-85.

Holloway P. J. (1984) Cutins and suberins, the polymeric plant lipids. In *CRC Handbook of Chromatography Lipids*, Vol. 1 (Edited by Mangold H. K.), CRC Press, Boca Raton, Florida, pp. 321-346.

Huang W. Y. and Meinschein W. G. (1979) Sterols as ecological indicators. *Geochim. Cosmochim. Acta* **43**, 739-745.

Hughes P. E. and Tove S. B. (1982) Occurrence of α-tocopherolquinone and α-tocopherolquinol in microorganisms. *J. Bacteriol.* **151**, 1397-1402.

Husain S., Kunzelmann P. and Schildknecht H. (1977) Separation of isomeric alkylphenols by high-performance liquid chromatographic and gas-liquid chromatographic techniques. *J. Chromatogr.* **137**, 53-60.

Hutton A. C., Kantsler A. J., Cook A. C. and McKirdy D. M. (1980) Organic matter in oil shales. *APEA J.* **20**, 44-67.

Hwang R. J., Sundararaman P., Teerman S. C. and Schoell M. (1989) Effect of preservation on geochemical properties of organic matter in immature lacustrine sediments. In *Organic Geochemistry - Abstracts from 14th International Meeting on Organic Geochemistry* (Edited by Durand *et al.*), Paris, Abstract No. 351.

Isaksen G. H. (1992) *Exxon Oil-Set Program*, Exxon Production Research Company, unpublished.

Isherwood F. A. (1965) Biosynthesis of lignin. In *Biosynthetic Pathways in Higher Plants* (Edited by Pridham J. B. and Swain T.), Academic Press, London, pp. 133-146.

Ishimaru K., Nonaka G. I. and Nishioka I. (1987) Gallic acid esters of proto-quercitol, quinic acid and (-)shikimic acid from *Quercus mongolica* and *Q. myrsinaefolia*. *Phytochem.* **26**, 1501-1504.

Ishiwatari R. and Uzaki M. (1987) Diagenetic changes of lignin compounds in a more than 0.6 million-year-old lacustrine sediment (Lake Biwa, Japan). *Geochim. Cosmochim. Acta* **51**, 321-328.

Janiszowska W. and Pennock J. F. (1976) The biochemistry of vitamin E in plants. *Vitamins and Hormones Advances in Research and Applications* **34**, 77-105.

Janiszowska W. and Rygier J. (1985) Changes in the levels of prenylquinones and tocopherols in *Calendula officinalis* during vegetation. *Physiol. Plant.* **63**, 425-430.

Jenkins C. C (1989) Geochemical correlation of source rocks and crude oils from the Cooper and Eromanga Basins. In *The Cooper and Eromanga Basins, Australia* (Edited by O'NEIL B. J), Proceedings of Petroleum Exploration Society of Australia, Society of Petroleum Engineers, Australian Society of Exploration Geophysicists (SA Branches), Adelaide, pp. 525-540.

Jewell D. M., Weber J. H., Bunger J. W., Plancher H. and Lantham D. R. (1972) Ion-exchange, coordination and adsorption chromatography separation of heavy-end petroleum distillates. *Anal. Chem.* **44**, 1391-1395.

Johnson J. C. (1975) *Antioxidants synthesis and applications*. Noyes Data Corporation, USA, pp. 3-39.

Johnson K. S. (1983) Water scaling problems in the oil production industry. In *Chemicals in the Oil Industry* (Edited by Ogden P.H), The Royal Society of Chemistry, London, pp.125-149.

Johnson N. G. (1985) Early Silurian palynomorphs from the Tuscarora formation in central Pennsylvania and their paleobotanical and geological significance. *Rev. Palaeobot. Palynol.* **45**, 307-360.

Kadouri A., Derenne S., Largeau C., Casadevall E. and Berkaloff C. (1988) Resistant biopolymer in the outer walls of *Botryococcus braunii, B* race. *Phytochem.* **27**, 551-557.

Kantsler A. J., Prudence T. J. C., Cook A. C. and Zwigulia M. (1983) Hydrocarbon habitat of the Cooper/Eromanga basin, Australia. *APEA J.* **23**, 75-92.

Kelley J. A. (1983) The chemistry of corrosion inhibitors used in oil production. In *Chemicals in the Oil Industry* (Edited by Ogden P.H), The Royal Society of Chemistry, London, pp. 150-158.

Kikugawa K., Kunugi A. and Kurechi T. (1990) Chemistry and implications of degradation of phenolic antioxidants. In *Food Antioxidants* (Edited by Hudson B. J. F.), Elsevier Applied Science, London, pp. 65-98.

Kirk T. K. and Farrell R. L. (1987) Enzymatic "combustion": The microbial degradation of lignin. *Annu. Rev. Microbial.* **41**, 465-505.

Kirk-Othmer (1985) *Concise encyclopedia of chemical technology* (3rd Ed.), John Wiley and Sons, USA, pp. 262.

Klesment I. (1974) Application of chromatographic methods in biogeochemical investigations. Determination of the structures of sapropelites by thermal decomposition. *J. Chromat.* **91**, 705-713.

Klesment I. and Nappa L. (1980) Investigation of the structure of Estonian oil shale kukersite by conversion in aqueous suspension. *Fuel* **59**, 117-122.

Klok J., Baas M., Cox H. C., de Leeuw J. W. and Schenck P. A. (1984) Loliolides and dihydroactinidiolide in a recent marine sediment probably indicate a majoe transformation pathway od carotenoids. *Tet. Letters* **25** (48), 5577-5580.

Koch M. and Gregson R. P. (1984) Brominated phloroethols and nonhalogenated phlorotannins from the brown alga *Crystophora congesta. Phytochem.* **23**, 2633-2637.

Kolattukudy P. E. (1980) Biopolyester membranes of plants: Cutin and suberin. *Science* **208**, 990-1000.

Kolattukudy P. E. (1981) Structure, biosynthesis, and biodegradation of cutin and suberin. *Annu. Rev. Plant Physiol.* **32**, 539-567.

Kononova M. M. (1966) *Soil organic matter*, Oxford Press, Oxford, pp. 544.

Kornblum N. and Lurie A. P. (1959) Hetrogeneity as a factor in the alkylation of ambident anions: phenoxide ions. *J. Am. Chem. Soc.* **81**, 2705-2715.

Kovach W. L. and Dilcher D. L. (1985) Morphology, ultrastructure and paleoecology of *Paxillitriletes vittatus*, new species from the mid-Cretaceous cenomanian of Kansas, USA. *Palynology* **9**, 85-94.

Kubin H. and Fink H. (1961) Detection of the total vitamin E content and of separate tocopherols in vegetable and animal matter containing protein. *Fette, Seifen, Anstrichm.* **63**, 280-283.

Kung-Jou Chao G. and Suatoni J. C. (1982) Determination of phenolic compounds by HPLC. *J. Chromatgr. Sci.* **20**, 436-440.

Lamartine R. and Perrin R. (1969) Étude physicochimique des phénols. VII. Préparation, séparation et purification des méthyl-2 isopropylphénols. Caractérisation spectrale et chromatographique. *Bull. Soc. Chim. Fr.* **2**, 437-442.

Largeau C., Casadevall E., Kadouri A. and Metzger P. (1984) Formation of *Botryococcus*-derived kerogens - comparative study of immature Torbanites and of the extant alga *Botryococcus braunii*. *Org. Geochem.* **6**, 327-332.

Largeau C., Derenne S., Casadevall E., Kadouri A. and Sellier N. (1986) Pyrolysis of immature Torbanite and of the resistant biopolymer (PRB *A*) isolated from extant alga *Botryococcus braunii*. Mechanism of formation and structure of Torbanite. In *Advances in Organic Geochemistry 1985* (Edited by Leythaeser D. and Rullkötter J.), Pergamon Press, Oxford, *Org. Geochem.* **10**, pp. 1023-1032.

Larter S. R. and Douglas A. G. (1980) A pyrolysis-gas chromatographic method for kerogen typing. In *Advances in Organic Geochemistry 1979* (Edited by Douglas A. G. and Maxwell J. R.), Pergamon Press, Oxford, pp. 579-583.

Larter S. R., Aplin A. C., Taylor P. and Mei C. (1994) Reservoir geochemistry: A link between reservoir geology and engineering? In the *Proceedings of the European Petroleum Conference, October 1994*, Society of Petroleum Engineers, London, pp. 441-450.

Later D. W., Lee M. L., Bartle K. D., Kong R. C. and Vassllaros D. L. (1981) Chemical class separation and characterization of organic compounds in synthetic fuels. *Anal. Chem.* **53**, 1612-1620.

de Leeuw J. W. and Largeau C. (1993) A review of macromolecular organic compounds that comprise living organisms and their role in kerogen, coal and petroleum formation. In *Organic Geochemistry: Principles and Applications* (Edited by Engel M. H. and Macko S. A.), Plenum Press, New York, pp. 23-73.

Li M., Larter S. R., Taylor P., Jones D. M., Bowler B. and Bjorøy M. (1995) Biomarkers or not biomarkers? A new hypothesis for the origin of pristane involving derivation from methyltrimethyltridecylchromans (MTTCs) formed during diagenesis from chlorophyll and alkylphenols. *Org. Geochem.* **23** (2), pp. 159-167.

Liaaen-Jensen S. (1978) *Photosynthetic Bacteria* (Edited by Clayton R. K. and Sistrom W. R.), Plenum, New York.

Lindenbeim H. A. K. (1921) La Kukersite, étude d'un dépôt marin phytogène du silurien inférieur d'Estonie. *Arch. Sci. Phys. Nat.* **3**, 379-413.

Lundie P. R. (1988) Drilling fluids: A challenge to the chemist. In *Chemicals in the Oil Industry 3rd International Symposium* (Edited by Ogden P.H), The Royal Society of Chemistry, London, pp. 1-13.

McClennen W. H., Meuzelaar H. L. C., Metcalf S. G. and Hill G. R. (1983) Characterisation of phenols and indanols in coal-derived liquids. *Fuel* **62**, 1422-1429.

MacCrehan W. A. and Brown-Thomas J. M. (1987) Determination of phenols in petroleum crude oils using liquid chromatography with electrochemical detection. *Anal. Chem.* **59**, 477-479.

Mackenzie A. S., Patience R. L., Maxwell J. R., Vandenbroucke M. and Durand B. (1980) Molecular parameters of maturation in the Toarcian shales, Paris Basin, France - I. Changes in the configuration of acyclic isoprenoid alkanes, steranes, and triterpanes. *Geochim. Cosmochim. Acta* **44**, 1709-1721.

McKirdy D. M., Aldridge A. K. and Ypma P. J. M. (1983) A geochemical comparison of some crude oils from Pre-Ordovician carbonate rocks. In *Advances in Organic Geochemistry 1981* (Edited by Bjorøy M. *et al.*), J. Wiley and Sons, New York, pp. 99-107.

Macleod G., Taylor P. N., Larter S. R. and Aplin A. C. (1993) Dissolved organics in formation waters: Insights into water-oil-rock ratios in petroleum systems. In *Geofluids - Contributions to an International Conference on Fluids Evolution, Migration and Interaction in Rocks* (Edited by Parnell T., Ruffell A. H. and Moles N. R.), Geol. Soc., London, pp. 18-24.

McLerie M. K., Tait A. M. and Sayers M. J. (1991) The Yammaderry, Cowle and Roller discoveries in the Barrow Sub-basin, Western Australia. *APEA J.* **31**, 32-41.

March J. (1992) *Advanced Organic Chemistry: Reactions, Mechanism, and Structure* (5th Ed.), John Wiley and Sons, Inc., New York, pp. 447-511.

Marcus Y. and Kertes A. S. (1969) *Ion Exchange and Solvent Extraction of Metal Complexes*, John Wiley and Sons Ltd., Bristol.

Marko-Varga G. and Barcelo D. (1992) Liquid chromatographic retention and separation of phenols and related aromatic compounds on reversed phase columns. *Chromatographia* **34**, 146-154.

Martin J. P. and Haider K. (1971) Microbial activity in relation to soil humus formation. *Soil Sci.* **111**, 54-63.

Martin J. P., Richards S. J. and Haider K. (1967) Properties and decomposition and binding action in soil of "humic acid" synthesized by *Epicoccum nigrum*. *Soil Science Society of America Proceedings* **31**, 657-662.

Martin J. P., Haider K. and Wolf D. (1972) Synthesis of phenols and phenolic polymers by *Hendersonula toruloidea* in relation to humic acid formation. *Soil Science Society of America Proceedings* **36**, 311-315.

Marzi R. and Rullkötter J. (1992) Qualitative and quantitative evolution and kinetics of biological marker transformations-Laboratory experiments and application to the Michigan Basin. In *Biological Markers in Sediments and Petroleum* (Edited by Moldowan J. M. *et al.*), Prentice Hall, Englewood Cliffs, pp. 18-41.

Marzi R., Torkelson B. E. and Olson R. K. (1993) A revised carbon preference index. *Org. Geochem.* **20**, 1303-1306.

Masters M. J. (1971) The occurrence of *Chrytridium marylandicum* on *Botryococcus braunii* in School Bay of the Delta Marsh. *Can. J. Bot.* **49**, 1479-1485.

de Mayo P. (1959) *The Chemistry of Natural Products Volume II. Mono- and Sesquiterpenoids* (Edited by Bentley K.W.), Interscience Publishers, Inc., USA, pp. 58-86.

van de Meent D., Brown S. C., Philp R. P. and Simoneit B. R. T. (1980) Pyrolysis-high resolution gas chromatography and pyrolysis gas chromatography-mass spectrometry of kerogens and kerogen precursors. *Geochim. Cosmochim. Acta* **44**, 999-1013.

Mello M. R., Gaglianone P. C., Brassell S. C. and Maxwell J. R. (1988) Geochemical and biological marker assessment of depositional environments using Brazilian offshore oils. *Mar. Pet. Geol.* **5**, 205-223.

Metzger P. and Casadevall E. (1989) Aldehydes, very long chain alkenylphenols, epoxides and other lipids from an alkadiene-producing strain of *Botryococcus braunii*. *Phytochem.* **28**, 2097-2104.

Meyers P. A. and Ishiwatari R. (1993) Lacustrine organic geochemistry-an overview of indicators of organic matter sources and diagenesis in lake sediments. *Org. Geochem.* **20** (7), 867-900.

Meylan W. and Howard P. (1992) Experimental dataset for LOGOW, octanol - water partition coefficient program. Syracuse Research Corporation Chemical Hazard Assessment Division, Environmental Chemistry Center, Syracuse, New York, U.S.A.

Moldowan J. M., Sandararaman P. and Schoell M. (1986) Sensitivity of biomarker properties to depositional environment and/or source input in the Lower Toarcian of SW-Germany. *Org. Geochem.* **10**, 915-926.

Moldowan J. M., Fago F. J., Carlson R. M. K., Young D. C., van Duyne G., Clardy J., Schoell M., Pillinger C. T. and Watt D. S. (1991) Rearranged hopanes in sediments and petroleum. *Geochim. Cosmochim. Acta* **55**, 3333-3353.

Moldowan J. M., Sandararaman P., Salvatori Y., Alajbeg A., Gjukic B., Lee C. Y. and Demaison G. J. (1992) Source correlation and maturity assessment of select oils and rocks from the Central Adriatic Basin (Italy and Yugoslavia). In *Biological Markers in Sediments and Petroleum* (Edited by Moldowan J. M. *et al.*), Prentice Hall, Englewood Cliffs, pp. 370-401.

Monin J. C., Durand B., Vandenbroucke M. and Huc A. Y. (1980) Experimental simulation of the natural transformation of kerogen. In *Advances in Organic Geochemistry 1979* (Edited by Douglas A. G. and Maxwell J. R.), Pergamon Press, Oxford, pp. 517-529.

Moore R. E. (1978) Algal nonisoprenoids. In *Marine Natural Products* (Edited by Scheuer P. J.). Academic Press, New York, pp. 43-124.

Muijs H. M. (1991) Surfactants in oil production. In *Chemicals in the Oil Industry: Developments and Applications* (Edited by Ogden P.H.), The Royal Society of Chemistry, Cambridge, pp. 277-297.

Musso H. (1963) Oxidation of phenols. *Angew. Chem.* **75** (20), 965-977.

Nakanishi K. and Solomon P. H. (1977) *Infrared Absorption Spectroscopy* (2nd Ed.), Holden-day Inc., Oakland, pp. 19-21.

Nasipuri D. and De Dalal I. (1975) Polycyclic systems. Part XVI. Synthesis of 1-isopropyl-2,8-dimethyl-phenanthrene. *Ind. J. Chem.* JCS Perkins I, 2052-2055.

Nasipuri D., Sarker G., Roy R. and Guha M. (1966) Cycloheneone derivatives. Part IV. Evidence of C_1 alkylation of Hagemann's ester. *J. Ind. Chem. Soc.*, 383-390.

Nesterova T. N., Piltchikov V. A. and Rozhnov A. M. (1983) Isomerisation of isopropylphenols and diisopropylphenols in the liquid state. *Zh. Prikl. Khim.* **6**, 1338-1342.

Nesterova T. N., Pimerzin A. A., Rozhnov A. M. and Karlina T. N. (1989) Equilibria for the isomerisation of (secondary-alkyl)phenols and cyclohexylphenols. *J. Chem. Thermod.* **21**, 385-395

Newton R. P., Walton T. J. and Moyse C. D. (1977) Non α-tocopherols in the unicellular blue-green alga *Gloeocapsa. Biochem. Soc. Trans.* **5**, 1486-1489.

Nimz H. H. (1974) Beech lignin - proposal of a constitutional scheme. *Angew Chem. Int. Ed.* **13**, 313-321.

Nip M., De Leeuw J. W. and Schenck P. A. (1988) The characterisation of eight maceral concentrates by means of Curie point pyrolysis-gas chromatography and Curie point pyrolysis-gas chromatography-mass spectrometry. *Geochim. Cosmochim. Acta* **52**, 637-648.

Nip M., De Leeuw J. W. and Crelling J. C (1992) Chemical structure of bituminous coal and its constituting maceral fractions as revealed by flash pyrolysis. *Energy and Fuels* **6**, 125-136.

Nishizawa M., Yamagishi T., Nonaka G. I., Nishioka I. and Ragan M. A. (1985) Gallotannins of the freshwater green alga *Spirogyra sp. Phytochem.* **24**, 2411-2413.

Oae S. (1991) *Organic Sulphur Chemistry: Structure and Mechanism*, CRC Press, USA, pp. 293-368.

O'Brian T. P. and Carr D. J. (1970) A suberized layer in the cell walls of the bundle sheath of grasses. *Aust. J. Biol. Sci.* **23**, 275-287.

Ohta K. and Venkatesan M. I. (1992) Pyrolysis of wood specimens with and without mineral: Implications for lignin diagenesis. *Energy and Fuels* **6**, 271-277.

Olah G. A. and Mo Y. K. (1972) Stable carbocations. CXXXV. Protination of trihydroxybenzenes and their methyl ethers in superacids. *J. Amer. Chem. Soc.* **94** (15), 5341-5349.

Øygard K., Larter S. and Senftle J. (1988) The control of maturity and kerogen type on quantitative analytical pyrolysis data. In *Advances in Organic Geochemistry 1987* (Edited by Mattavelli L. and Novelli L.), Pergamon Press, *Org. Geochem.* **13** (4-6), pp. 1153-1162.

Palacas J. G., Anders D. E. and King J. D. (1984) South Florida Basin - A prime example of carbonate source rocks in petroleum. In *Petroleum Geochemistry and Source Rock Potential of Carbonate Rocks* (Edited by Palacas J. G.), American Association of Petroleum Geologists, Studies in Geology No. 18, pp. 71-96.

Parees D. M and Zamzelski A. Z. (1982) Characterization of coal-derived liquids using fused silica capillary column GC-MS. *J. Chromatogr. Sci.* **20**, 441-448.

Parkhurst R. M. and Skinner W. A. (1981) Chromanols and tocopherols. In *Chemistry of Heterocyclic Compounds,* Vol. 36, SRI International, California, pp. 59-137.

Pauls R. E., Bambacht M. E., Bradley C., Scheppele S. E. and Cronauer D. C. (1990) Distribution and characterization of phenolics in distillates derived from two-stage coal liquefaction. *Energy Fuels* **4**, 236-242.

Pennington J. (1988) An overview of alternative approaches to the development of monitoring methods for scale inhibitors in oil-field produced waters. In *Chemicals in the Oil Industry 3rd International Symposium* (Edited by Ogden P.H), The Royal Society of Chemistry, London, pp. 108-120.

Pepper A. S. (1991) Estimating the petroleum expulsion behaviour of source rocks: a novel quantitative approach. In *Petroleum Migration* (Edited by England W. A. and Fleet A. J.), Geol. Soc. Spec. Pub. No. 59, pp. 9-31.

Perrin D. D., Armarego L. F. and Perrin D. R. (1980) Purification of Laboratory Chemicals (2nd Ed.), Pergamon Press.

Peters K. E., Moldowan J. M. and Sundararaman P. (1990) Effects of hydrous pyrolysis on biomarker thermal maturity parameters: Monterey Phosphatic and Siliceous Members. *Org. Geochem.* **15**, 249-265.

Peters K. E. and Moldowan J. M. (1993) *The Biomarker Guide*, Prentice Hall, Englewood Cliffs, New Jersey.

Philp R. P., Russell N. J., Gilbert T. D. and Friedrich M. J. (1982) Characterization of Victorian soft brown coal wood by microscopic techniques and curie-point pyrolysis combined with gas chromatography-mass Spectrometry. *J. Anal. and Appl. Pyrol.* **4**, 143-162.

Pinder A. R. (1960) *The Chemistry of the Terpenes*, The Universities Press, Belfast, pp. 56, 61, 65-71.

Playford P. E. (1977) Geology and ground water potential. In *Geology and Hydrology of Rottnest Islands* (Edited by Playfors P. E. and Leach R. E. J.), Geol. Surv. W.A. Rep. 6, pp. 1-53. .

Porter L. J. and Woodruffe J. (1984) Haemanalysis: The relative astringency of proanthocyanidin polymers. *Phytochem.* **23**, 1255-1256.

Powell T. J. (1988) Pristane/phytane ratio as an environmental indicator. *Nature* **333**, 604.

Powell T. J., Boreham C. J., Smyth M., Russell N. and Cook A. C. (1991) Petroleum source rock assessment in non-marine sequences: Pyrolysis and petrographic analysis of Australian coals and carbonaceous shales. *Org. Geochem.* **17**, 75-394.

Prahl A. K., Springstubbe H., Grumbach K. and Wiermann R. (1985) Studies on sporopollenin biosynthesis: The effect of inhibitors of carotenoid biosynthesis on sporopollenin accumulation. *Z. Naturforsch.* **40**, 621-626.

Prahl A. K., Rittscher M. and Wiermann R. (1986) New aspects of sporopollenin biosynthesis. In *Biotechnology and Biology of Pollen* (Edited by Mulcahy D. L., Bergami G. and Ottaviani E.), Springer-Verlag, Berlin, pp. 313-318.

Price P. I. and Barker C. E. (1985) Suppression of vitrinite reflectance in amorphous rich kerogen-A major unrecognised problem. *J. Pet. Geol.* **26**, 151-159.

Price P. I., O'Sullivan T. and Alexander R. (1987) The nature and occurrence of oil in Seram, Indonesia. In *Proceedings of the Indonesian Petroleum Association 16th Annual Convention*, Vol. 1, Indonesian Petroleum Association, Jakarta, pp. 141-173.

Radke M. and Welte D. H. (1983) The methylphenanthrene index (MPI): A maturity parameter base on aromatic hydrocarbons. In *Advances in Organic Geochemistry 1981* (Edited by Bjorøy M. *et al.*), J. Wiley and Sons Ltd., pp. 504-512.

Radke M., Schaefer R. G., Leythaeuser D. and Teichmüller M. (1980) Composition of soluble organic matter in coals: relation to rank and liptinite fluorescence. *Geochim. Cosmochim. Acta* **44**, 1787-1800.

Radke M., Welte D. H. and Willsch H. (1982a) Geochemical study on a well in the Western Canada Basin: relation of the aromatic distribution pattern to maturity of organic matter. *Geochim. Cosmochim Acta* **46**, 1-10.

Radke M., Willsch H., Leythaeuser D. and Teichmuller M. (1982b) Aromatic components of coal: relation of distribution pattern to rank. *Geochim. Cosmochim Acta* **46**, 1831-1848.

Radke M., Budzinski H., Pierard C., Willsch H. and Garrigues P. (1983) Chromatographic retention behaviour of trimethylphenanthrenes and their identification by GC-FTIR in geological samples. *J. Polycyclic Aromatic Compounds* **3**, 467-474.

Ragan M. A. and Glombitza K. W. (1986) Phlorotannins, brown alga polyphenols. In *Progress in Phycological Research* (Edited by Round F. E. and Chapman D. J.), Biopress Ltd., Bristol, pp. 129-253.

Ramsden H. E., Balint A. E., Whitford W. R., Walburn J. J. and Cserr R. (1957) Arylmagnesium chlorides. Preparation and characterisatizations. *J. Org. Chem.* **22**, 1202-1206.

Rashid M. A. (1985) *Geochemistry of Marine Humic Compounds*, Springer-Verlag, New York, pp. 35-65; 66-72.

Repeta D. J. (1989) Carotenoid diagenesis in recent marine sediments: II. Degradation of fucoxanthin to loliolide. *Geochim. Cosmochim Acta* **53**, 699-707.

Reusch R. N. and Sadoff H. L. (1979) 5-*n*-Alkylresorcinols from encysting *Azotobacter vinelandii*: Isolation and Characterisation. *J. Bacter.* **139**, 448-453.

Ribereau-Gayon P. (1972) *Plant Phenolics*, Oliver and Boyd, Great Britian.

Robards A. W., Jackson S. M., Clarkson D. T. and Sanderson J. (1973) The structure of barley roots in relation to the transport into the stele. *Protoplasma* **77**, 291-311.

Romine K. K., Southgate P. N., Kennard J. M. and Jackson M. J. (1994) The Ordovician to Silurian phase of the Canning Basin Western Australia: Structure and sequence evolution. In *The Sedimentary Basins of Western Australia: Proceedings of Petroleum Exploration Society of Australia Symposium, Perth, 1994* (Edited by Purcell P. G. and Purcell R. R.), pp. 677-696.

Rubinstein I., Sieskind O. and Albrecht P. (1975) Rearranged sterenes in a shale: Occurrence and simulated formation. *J. Chem. Soc. Perkin Trans.* I. 1833-1836.

Rullkötter J. and Marzi R. (1988) Natural and artificial maturation of biological markers in a Toarcian shale from Northern Germany. *Org. Geochem.* **13**, 639-645.

Rullkötter J. and Wendisch D. (1982) Microbial alteration of 17α(H)-hopane in Madagascar asphalts: Removal of C-10 methyl group and ring opening. *Geochim. Cosmochim. Acta* **46**, 1543-1553.

Rullkötter J., Spiro B. and Nissenbaum A. (1985) Biological marker characteristics of oils and asphalts from carbonate source rocks in a rapidly subsiding graben, Dead Sea, Israel. *Geochim. Cosmochim. Acta* **49**, 1357-1370.

Sabelle S., Oliver E., Metzger P., Derenne S. and Largeau C. (1993) Variability in phenol moieties in the resistant biomacromolecules of the *A* and *B* races of *Botryococcus braunii*. Geochemical implications. In *Organic Geochemistry-Poster Sessions from the 16th International Meeting on Organic Geochemistry* (Edited by Øygard K.), Falch Hurtigtrykk, Stavanger, pp. 558-562.

Sadoff H. L. (1975) Encystment and germination in *Azotobacter vinelandii*. *Bacteriol. Rev.* **91**, 304-310.

Saiz-Jimenez C. and de Leeuw J. W. (1984a) Pyrolysis-gas chromatography-mass spectrometry of soil polysaccharides, soil fulvic acids and polymaleic acid. *Org. Geochem.* **6**, 287-293.

Saiz-Jimenez C. and de Leeuw J. W. (1986b) Chemical characterization of soil organic matter fractions by analytical pyrolysis-gas chromatography-mass spectrometry. *J. Anal Appl. Pyrolysis* **9**, 99-119.

Sandvik E. I., Young W. A. and Curry D. J. (1992) Expulsion from hydrocarbon sources: the role of organic absorption. In *Advances in Organic Geochemistry 1991* (Edited by Eckardt C. B. *et al.*), *Org. Geochem.* **19** (1-3), Pergamon Press, Oxford, pp. 77-87.

Sarkanen K. V. and Hergert H. L. (1971) Classification and distribution. In *Lignins. Occurrence, Formation, Structure and Reactions* (Edited by Sarkanen K. V. and Ludwig C. H.), Wiley-Interscience, New York, pp. 43-94.

Scanlan R. S. and Smith J. E. (1970) An improved measure of the odd-even predominance in the normal alkanes of sediment extracts and petroleum. *Geochim. Cosmochim. Acta* **34**, 611-620.

Schenck P. A., de Leeuw J. W., van Graas G., Haverkamp J. and Bouman M. (1981) Analysis of recent spores and pollen and of thermally altered sporopollenin by flash pyrolysis-mass spectrometry and flash pyrolysis-gas chromatography-mass spectrometry. In *Organic Maturation Studies and Fossil Fuel Exploration* (Edited by Brooks J.), Academic Press, New York, pp. 225-237.

Schulze Osthoff K. S. and Wiermann R. (1987) Phenols as integrated compounds of sporopollenin from *Pinus* Pollen. *J. Plant Physiol.* **131**, 5-15.

Schweighardt F. K., Bockrath B. C., Friedel R. A. and Retcofsky H. L. (1976) Deuterium magnetic resonance spectrometry as a tracer tool in coal liquefaction processes. *Anal. Chem.* **48** (8), 1254-1255.

Seifert W. K. and Moldowan J. M (1978) Applications of steranes, terpanes, and monoaromatics to maturation, migration, and source of crude oils. *Geochim. Cosmochim. Acta* **42**, 77-95.

Seifert W. K. and Moldowan J. M. (1980) The effects of thermal stress on source rock quality as measured by hopane stereochemistry. *Phys. and Chem. Earth* **12**, 229-237.

Seifert W. K. and Moldowan J. M (1986) Use of biological markers in petroleum exploration. In *Methods in Geochemistry and Geophysics,* Vol. 24 (Edited by Johns R. B.), pp. 261-290.

Seifert W. K., Moldowan J. M. and Demaison G. J. (1984) Source correlation of biodegraded oils. *Org. Geochem.* **6**, 633-643.

Senftle J. T. and Larter S. R. (1987) Comparative geochemistry of vitrinite kerogens from carboniferous and tertiary provinces. *Org. Geochem.* **11** (5), 407-409.

Senftle J. T., Larter S. R., Bromley B. W. and Brown J. H. (1986) Quantitative chemical characterisation of vitrinite concentrates using pyrolysis - gas chromatography. Rank variation of pyrolysis products. *Org. Geochem.* **9** (6), 345-350.

Serjeant E. P. and Dempsey B. (1985) *Ionisation Constants of Organic Acids in Aqueous Solution-IUPAC Chemical Data Series-No.23*, Pergamon Press, Oxford.

Shaw G. (1970) Sporopollenin. In *Phytochemical Phylogeny* (Edited by Harborne J.), Academic Press, London, pp. 31-58.

Shen Z., Haslam E., Falshaw C. P. and Begley M. J. (1986). Procyanidins and polyphenols of *Larix gmelini* bark. *Phytochem.* **25**, 2629-2635.

Shoup R. E. and Mayer G. S. (1982) Determination of environmental phenols by liquid chromatography/electrochemistry. *Anal. Chem.* **54**, 1164-1169.

Shrewsbury D. D. (1960) The infra-red spectra of alkylphenols. *Spectrochim. Acta* **16**, 1294-1311.

Sieskind O., Joly G. and Albrecht P. (1979) Simulation of the geochemical transformation of sterols: Superacid effects of clay minerals. *Geochim. Cosmochim. Acta* **43**, 1675-1679.

Sigleo A. C. (1978) Organic geochemistry of silicified wood, Petrified Forrest National Park, Arizona. *Geochim. Cosmochim. Acta* **42**, 1397-1405.

Sinninghe Damsté J. S., Kock-van Dalen A. C., De Leeuw J. W., Schenck P. A., Guoying S. and Brassell S.C. (1987) The identification of mono-, di-, trimethyl 2-methyl-2-(4,8,12-trimethyltridecyl)chromans and their occurrence in the geoshpere. *Geochim. Cosmochim. Acta* **51**, 2393-2400.

Sinninghe Damsté J. S., Keely B. J., Betts S. E., Baas M. B., Maxwell J. R. and De Leeuw J. W. (1993) Variations in abundances and distributions of isoprenoid chromans and long chain alkylbenzenes in sediments of the Mulhouse Basin: a molecular sedimentary record of paleosalinity. *Org. Geochem.* **20**, 1201-1215.

Skinner W. A. and Sturm P. A. (1968) Investigation of algae and yeast for α-tocopherol and α-tocopherolquinone content. *Phytochem.* **7**, 1893-1896.

Smith J. W., George S. C. and Batts B. D. (1994) Pyrolysis of aromatic compounds as a guide to synthetic reactions in sediments. *APEA J.* **34** (1), 231-240.

Southworth G. R., Herbes S. E. and Allen C. P. (1983) Evaluating a mass transfer model for the dissolution of organics from oil films into water. *Water Res.* **17**, 1647-1651.

Stach E., Mackowsky M. Th., Teichmüller M., Taylor G. H., Chandra D. and Teichmüller R. (1982) *Stach's Textbook of Coal Petrology*, Gebruder Borntraeger, Berlin, pp. 1-138.

Stafford H. A. (1988) Proanthocyanidins and the lignin connection. *Phytochem.* **27**, 1-6.

Stainforth J. G. (1984) Gippsland hydrocarbons-a perspective from the basin edge. *APEA J.* **24**, 91-100.

Stevenson F. J. (1985) Geochemistry of soil humic substances. In *Humic Substances in Soil, Sediments, and Water* (Edited by Aiken G. R. *et al.*), John Wiley and Sons, USA, pp. 13-52.

Stout S. A., Boon J. J. and Spackman W. (1988) Molecular aspects of the peatification and early coalification of angiosperm and gymnosperm woods. *Geochim. Cosmochim. Acta* **52**, 405-414.

Summons R. (1995) *personal communication.*

Sun D., Zhao Z., Wong H. and Foo L. Y. (1988) Tannins and other phenolics from *Myrica esculenta* bark. *Phytochem.* **27**, 579-583.

Tanaka T., Nonaka G. I. and Nishioka I. (1985) Punicafolin and ellagitannin from leaves of *Punica granatum. Phytochem.* **24**, 2075-2078.

Taylor P. N. (1994) *The Petroleum Geochemistry of Phenols*. PhD dissertation, University of Newcastle Upon Tyne.

Taylor P. N., Larter S. R., Jones D. M. and Bromley (1993) The occurrence of phenols in crude oils and their implications for reservoir processes. In *Organic Geochemistry- Poster Sessions from the 16th International Meeting on Organic Geochemistry* (Edited by Øygard K.), Falch Hurtigtrykk, Stavanger, pp. 580-582.

Taylor D., Kontorovich A. E., Larichev A. I. and Glikson M. (1994) Petroleum source rocks in the Roper Group of the McArthur Basin: Source characterisation and maturity determinations using physical and chemical methods. *APEA J.* **34**, pp. 279-296.

Teichmüller M. and Teichmüller R. (1979) Diagenesis of coal (Coalification). In *Diagenesis in Sediments and Sedimentary Rocks* (Edited by Larsen G. and Chilingar G.V.), Elseivier, Amsterdam, pp. 207-246.

Temperley B. N. (1936) The Boghead controversy and the morphology of the Boghead algae. *Trans. R. Soc. Edinburgh* **43**, 855-868.

Threlfall D. R. and Whistance G. R. (1971) Biosynthesis of isoprenoid quinones and chromanols. In *Aspects of Terpenoid Chemistry and Biochemistry* (Edited by Goodwin T.W.), Academic Press, London, pp. 372-376.

Tietze L. F. and Eicher T. H. (1989) *Reactions and Syntheses in the Organic Chemistry Laboratory*, University Science Books, USA, pp. 378.

Tissot B. P. and Welte D. H. (1978) *Petroleum Formation and Occurrence*, Springer-Verlag, Heidelberg.

Toia R. E., Marsh B. H., Perkins S. K., McDonald J. W. and Peters G. A. (1985) Sporopollenin content of the spore apparatus of *Azolla. Am. Fern J.* **75**, 38-43.

Towers G. H. N. (1969) Metabolism of cinnamic acid and its derivatives in Basidiomycetes. In *Perspectives in Phytochemistry* (Edited by Harbourne J. B. and Swain T.), Academic Press, London, pp. 179-191.

Traverse A. (1955) Occurrence of the oil-forming alga *Botryococcus* in lignites and other tertiary sediments. *Micropaleontology* **1**, 343-350.

Turner A. B. (1964) Quinone methides. *Q. Rev.* **18**, 347-360.

Turner W. B. (1971) *Fungal Metabolites*, Academic Press, London.

Ungerer P. (1990) State of the art of research in kinetic modelling of oil formation and expulsion. In *Advances in Organic Geochemistry 1989* (Edited by Durand B. and Behar F.), Pergamon Press, Oxford, pp. 1-25.

Vanhaelen M. and Vanhaelen-Fastre R. (1980) High performance liquid, gas-liquid and thin layer chromatography of naturally occurring flavonoids, phenolics and related compounds. *J. Chromatogr.* **187**, 255-160.

Vaughan D. and Malcolm R. E. (1985) *Soil Organic Matter and Biological Activity,* Martinus Nijhoff/Dr W. Junk Publishers, The Netherlands, pp. 1-216.

Veld H., De Leeuw J. W., Sinninghe Damsté J. S. and Fermont W. J. J (1994) Molecular characterization of vitrinite maturation as revealed by flash pyrolysis methods. In *Reevaluation of Vitrinite as a Maturity Parameter: Petrologic, Kinetic and Geochemical Factors* (Edited by Mukhopadhyay P. K. and Sow W. G.), ACS Symp. Ser.; American Chemical Society: Washington, DC, 1994.

Vincent P. W., Mortimore I. R. and McKirdy D. M. (1985) Hydrocarbon generation, migration and entrapment in the Jackson-Naccowlah area, ATP 259P, Southwestern Queensland. *APEA J.* **25** , 62-84.

Volkman J. K. (1986) A review of sterol markers for marine and terrigenous organic matter. *Org. Geochem.* **9**, 83-99.

Volkman J. K. (1988) Biological marker compounds as indicators of the depositional environments of petroleum source rocks. In *Lacustrine Petroleum Source Rocks* (Edited by Fleet A. J. *et al.*), Geological Society Special Publication No. 40, pp. 103-122.

Volkman J. K. and Maxwell J. R. (1986) Acyclic isoprenoids as biological markers. In *Biological Markers in the Sedimentary Record* (Edited by Johns R. B), Elsevier, New York, pp. 1-42.

Volkman J. K., Alexander R., Kagi R. I., Noble R. A. and Woodhouse G. W. (1983a) A geochemical reconstruction of oil generation in the Barrow Sub-basin of Western Australia. *Geochim. Cosmochim. Acta* **47**, 2091-2105.

Volkman J. K., Alexander R., Kagi R. I. and Woodhouse G. W. (1983b) Demethylated hopanes in crude oils and their applications in petroleum geochemistry. *Cosmochim. Acta* **47**, 785-794.

Wardrop A. B. (1971) Classification and distribution. In *Lignins. Occurrence, Formation, Structure and Reactions* (Edited by Sarkanen K. V. and Ludwig C. H.), Wiley-Interscience, New York, pp. 19-41.

Weast R. C (1974) *CRC Handbook of Chemistry and Physics*, Chemical Rubber Publishing Company, USA.

Whiting D. A. (1978) Phenols. In *Comprehensive Organic Chemistry, the Synthesis and Reactions of Organic Compounds*, Vol. 1 (Edited by Barton D. and Ollis W. D.), Pergamon Press, Oxford, pp. 707-797.

Wilson M. A. and Hatcher P. G. (1988) Detection of tannins in modern and fossil barks and in plant residues by high-resolution solid state ^{13}C nuclear magnetic resonance. *Org. Geochem.* **12** (6), 539-546.

Wood K. V., Albright L. F., Brodbelt J. S. and Cooks R. G. (1985) A tandem mass spectrometry investigation of hydroxyaromatic constituents in coal liquids. *Analytica Chimica Acta* **173**, 117-127.

Yew C. C. and Mills A. A. (1989) The occurrence and search for Permian oil in the Cooper Basin, Australia. In *The Cooper and Eromanga Basins, Australia* (Edited by O'NEIL B. J), Proceedings of Petroleum Exploration Society of Australia, Society of Petroleum Engineers, Australian Society of Exploration Geophysicists (SA Branches), Adelaide, pp. 339-359.

Zhang Z. Z., Low P. E., Cusman J. H. and Roth C. B. (1990) Adsorption and heat of adsorption of organic compounds on montmorillonite from aqueous solutions. *Soil Sci. Soc. Am.* **54**, 59-66.

Züllig H. (1982) Untersuchungen über die Stratigraphie von Carotinoiden im geschichteten Sediment von 10 Schweizer Seen sur Erkundung früherer Phytoplankton-Entfaltungen. *Schweiz Z. Hydrol.* **44**, 1-98.

LIST OF JOURNAL PUBLICATIONS

Ioppolo M., Alexander R. and Kagi R. I. (1992) Identification and analysis of C_0-C_3 phenols in some Australian crude oils. *Organic Geochemistry* **18** (5), 603-609.

Ioppolo-Armanios M., Alexander R. and Kagi R. I. (1994) Identification and origins of isopropylmethylphenols in crude oils. In *Advances in Organic Geochemistry 1993*, Elsevier, *Organic Geochemistry* **22** (3-5), pp. 815-823.

Ioppolo-Armanios M., Alexander R. and Kagi R. I. (1995) Geosynthesis of organic compounds I. Alkylphenols. *Geochimica et Cosmochimica Acta,* **59**, 3017-3027.

Ioppolo-Armanios M., Alexander R. and Kagi R. I. (1995) Tocopherols as precursors to petroleum alkylphenols. *Organic Geochemistry* (in preparation).

LIST OF CONFERENCE PRESENTATIONS

Ioppolo-Armanios M., **Alexander R.** and Kagi R. I. The analysis and geochemical significance of phenols in crude oil. *15th Meeting of the EAOG*, Manchester, England, September 1991, (oral presentation).

Ioppolo M., Alexander R. and Kagi R. I. Identification and analysis of C_0-C_3 phenols in some Australian crude oils. *Australian Organic Geochemistry Conference*, Melbourne, Australia, 1991, (poster presentation).

Ioppolo-Armanios M., **Alexander R.** and Kagi R. I. Identification and analysis of isopropylmethylphenols in crude oil. *16th Meeting of the EAOG*, Stavanger, Norway, September 1993, (oral presentation).

Ioppolo-Armanios M., Alexander R. and **Kagi R. I.** The analysis of trace level phenols in petroleum and petroleum refinery effluents. *RACI 12AC/3EC*, Perth, Australia, September, 1993, (oral presentation).

Ioppolo-Armanios M., Alexander R. and Kagi R. I. The geochemical significance of isopropylmethylphenols in crude oil. *Australian Organic Geochemistry Conference*, Sydney, Australia, March 1994, (oral presentation).

Alexander R., Ioppolo-Armanios M. and Kagi R. I. Identification, analysis and origins of phenols in crude oils. *208th American Chemical Society National Meeting and Exposition*, Washington, DC, August, 1994, (oral presentation).