

School of Applied Chemistry

Sedimentary Processes Involving Aromatic Hydrocarbons

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*The real voyage of discovery consists not
in seeking new landscapes but in having new eyes.*

-Marcel Proust-

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Summary

Sedimentary organic matter contains many compounds that have no obvious biogenic precursors, so their formation and occurrence are of geochemical interest. The first part of this thesis (chapters 2-5) discusses the results obtained from studying hydrocarbon racemates. Some of the compounds identified are also suggested as intermediates in the formation of alkylnaphthalenes identified in chapters 6-7. The second part of this thesis (chapters 6-11) covers the identification of a range of alkylnaphthalenes and alkyphenanthrenes in sedimentary organic matter. Possible pathways for the formation of these hydrocarbons are outlined and their application as probes into the processes of petroleum formation are described.

In chapter 2 the use of permethylated cyclodextrin capillary gas chromatography columns to separate hydrocarbon racemates are reported. Chapter 3 reports the synthesis of 1,2,2,5-tetramethyltetralin and 1,2,2,5,6-pentamethyltetralin and identifies them as racemates in crude oil. They are proposed as intermediates in the formation of sedimentary alkylnaphthalenes (identified in chapters 6 and 7).

The identification of isodihydro-*ar*-curcumene in sedimentary organic matter is described in chapters 4 and 5. It co-occurs in crude oil with dihydro-*ar*-curcumene and is suggested to originate from this compound via a sedimentary rearrangement process. Chiral GC-MS techniques have been used to show the presence of both enantiomers of these compounds in crude oils. The elution order of the enantiomers has been established using reference compounds of known configuration. The effects of maturity and biodegradation on dihydro-*ar*-curcumene and isodihydro-*ar*-curcumene enantiomers is reported. Optically pure dihydro-*ar*-curcumene from natural products undergoes rapid racemisation in the subsurface, yielding a racemic mixture before the onset of significant oil formation. 1,2-Alkyl shifts on the aromatic ring also begin at an early stage to yield isodihydro-*ar*-curcumene and these processes continue with increasing maturity. Laboratory

experiments using proton and clay catalysts (Lewis acid catalyst) show that the alkyl shift reaction is catalysed by both proton and Lewis acids, and racemisation is only catalysed by Lewis acids. A moderately biodegraded crude oil has been shown to be depleted in the *R* enantiomer of dihydro-*ar*-curcumene and an extensively degraded oil has dihydro-*ar*-curcumene depleted relative to isodihydro-*ar*-curcumene.

The identification of a number alkylnaphthalenes and their possible origins in sedimentary organic matter is described in chapters 6 and 7. In chapter 6 a previously unreported tetramethylnaphthalene (TeMN) was identified in petroleum. This compound is structurally similar to bicyclic compounds of microbial origin and these are suggested as a likely source, via a tetralin intermediate identified in chapter 3. In chapter 7 isomeric pentamethylnaphthalenes previously unreported in sedimentary organic matter are reported. These isomeric pentamethylnaphthalenes (PMNs) were identified in a number of crude oils and sediments, ranging in age from Proterozoic to Tertiary. 1,2,3,5,6-PMN is suggested to form predominantly from the aromatisation of drimanoid precursors *via* 1,2,2,5,6-pentamethyltetralin identified in chapter 3. In laboratory experiments, the other pentamethylnaphthalenes were generated from 1,2,3,5,6-PMN in proportions that reflect the relative stability of the isomers. By analogy, the other PMNs in sediments are suggested to arise *via* acid catalysed isomerisation or transalkylation processes. A maturity parameter was developed based on laboratory experiments in conjunction with observed distributions of pentamethylnaphthalenes.

The formation of alkylnaphthalenes and alkylphenanthrenes through a methylation process is discussed in chapters 8-10. Several crude oils and shales which contain anomalously high concentrations of 1,6-dimethylnaphthalene, 1,2,5-trimethylnaphthalene, 1,2,7-trimethylnaphthalene, 1,2,3,5-tetramethylnaphthalene, 1,2,3,5,6-pentamethylnaphthalene, 2-methyl-6-isopropyl-1(4-methylpentyl)naphthalene, phenanthrene, 1-methylphenanthrene, 1,7-dimethylphenanthrene and retene have been shown to contain relatively high

concentrations of their corresponding methylated counterparts. In laboratory experiments carried out under mild conditions, each of the alkylnaphthalenes and alkylphenanthrenes have been shown to be methylated in specific positions when heated with a methyl donor in the presence of a clay catalyst. These observations have been interpreted as evidence for a sedimentary methylation process.

The effect of biodegradation on alkylnaphthalenes and alkylphenanthrenes formed from sedimentary methylation is described in chapter 11. Land-plant-derived aromatic hydrocarbons with a range of susceptibilities to reservoir biodegradation have been identified in crude oils. These compounds are the result of reactions of natural products involving aromatisation, rearrangement and methylation in the sediments (chapters 9 and 10). They are therefore suggested as markers for land-plants in severely biodegraded oils in which most of the other biologically derived compounds cannot be recognised. The order of biodegradability of these compounds has been assessed relative to their non-methylated counterparts namely 6-isopropyl-2-methyl-1-(4-methylpentyl)naphthalene and retene. The order of degradation of the four compounds is : retene > 9-methylretene ~ 6-isopropyl-2-methyl-1-(4-methylpentyl)naphthalene > 6-isopropyl-2,4-dimethyl-1-(4-methylpentyl)naphthalene. These results have been used to assess that a crude oil is a mixture of severely biodegraded and less biodegraded crude oil.

CHAPTER 1

1.0 Introduction

Aromatic compounds are major constituents of sedimentary organic matter (Radke, 1987). Examples include the alkylbenzenes, alkyltetralins, alkylnaphthalenes and alkylphenanthrenes, few of which have obvious natural product precursors. Therefore, the formation, occurrence and fate of these compounds in sedimentary organic matter is of great interest. This chapter provides an overview of the geochemistry of these compounds.

1.1 Sedimentary alkylbenzenes

Alkylbenzenes are usually the major mono aromatic components of petroleum (Ostroukhov *et al.*, 1983, Petrov, 1984; Radke 1987). They have been reported in a range of sedimentary organic matter (Martin *et al.*, 1963; Mair and Barnewall, 1964; Smith, 1967; Solli *et al.*, 1979; Kolesnikova, 1972; Petrov, 1984; Sinninghe Damste *et al.*, 1988, 1989; Hartgers *et al.*, 1992, 1994a,b; Dong *et al.*, 1993; Hoefs *et al.*, 1995; Saiz-Jimenez, 1995; Clegg *et al.*, 1997). Natural products such as carotenoids, terpenoids and alkaloids have been proposed as possible precursors of sedimentary alkylbenzenes (Erdman, 1961; Hartgers *et al.*, 1992, 1994a,b; Hoefs *et al.*, 1995; Clegg *et al.*, 1997).

Homologous series of n-alkylbenzenes, n-alkyltoluenes and n-alkylxylenes have been reported in crude oils (Ostroukhov *et al.*, 1983; Albaiges *et al.*,

1986; Williams *et al.*, 1988; Sinninghe Damsté *et al.*, 1988, 1991; Ellis *et al.*, 1992, 1995c), and in sediments (Dong *et al.*, 1993; Sinninghe Damsté *et al.*, 1993b; Dong *et al.*, 1993). Similarly, n-alkyl ethylbenzenes (Ostroukhov *et al.*, 1983) and 1,2 di-n-alkylbenzenes (Sinninghe Damsté *et al.*, 1991) have been reported in crude oils. Suggested origins of these compounds include cyclisation and aromatisation of linear functionalised precursors such as fatty acids and fatty alcohols (Williams *et al.*, 1988; Sinninghe Damsté *et al.*, 1991; Douglas *et al.*, 1991; Dong *et al.*, 1993). Recently, it has been suggested that alkyltoluenes and alkylxylenes may originate through methylation of alkylbenzenes (Ellis *et al.*, 1995b).

Other homologous series of alkylbenzenes identified in crude oils include 1-alkyl-2,3,6-trimethylbenzenes which have been suggested as biological markers for green sulphur bacteria (Ostroukhov *et al.*, 1982; Summons and Powell, 1987). Long chain isoprenoid alkylbenzenes have been identified in sediments and crude oils by Sinninghe Damsté *et al.* (1988). Series of phenylalkanes have been identified in crude oils and sediments and their origin was suggested to be related to *Thermoplasma* bacteria and fossil algae (Ellis *et al.*, 1996a). A series of isohexylalkylbenzenes were also identified and these compounds were suggested to form *via* a rearrangement process during aromatisation (Ellis *et al.*, 1996b).

Recently, dihydro-*ar*-curcumene was identified in crude oils and sediments, and has been proposed as a higher plant biomarker (Ellis *et al.*, 1995a). The structure and numbering system of dihydro-*ar*-curcumene is shown in Figure 1.1.

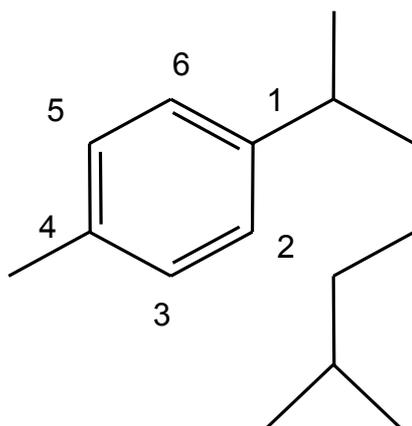


Figure 1.1. The structure and numbering system of dihydro-*ar*-curcumene (1-(1,5-dimethylhexyl)-4-methylbenzene).

1.2 Sedimentary alkyltetralins

Alkyltetralins (alkyl-1,2,3,4-tetrahydronaphthalenes) have received little attention in organic geochemistry. Although the presence of tetralin and its mono-, di- and trimethyl homologues in crude oils have been noted by Gallegos (1971) and Sokolov *et al.* (1972). The first tentative identifications of alkyltetralins in sedimentary organic matter were of 5,6-dimethyltetralin, 1,1,6-trimethyltetralin and 1,1,5,6-tetramethyltetralin in Green River shale (Anders *et al.*, 1973). 1,1,6-Trimethyltetralin has been reported in the pyrolysis products of sporopollenins, a spore coal and Green River shale by Achari *et al.* (1979) and again in the Green River shale by Koopmans *et al.* (1997). The structure and numbering system of 1,1,6-trimethyltetralin (1,1,6-trimethyl-1,2,3,4-tetrahydronaphthalene) is shown in Figure 1.2.

The pentacyclic triterpenoid β -amyrin has been suggested as a possible source of 1,2,5-trimethyltetralin (Bendoraitis, 1974). However, 1,2,5-trimethyltetralin has not been reported previously in sedimentary organic matter. 2,2,7,8-Tetramethyltetralin has also been suggested as a degradation product of β -amyrin and was tentatively identified in a coal by Püttmann and Villar (1987). This tetramethyltetralin was later synthesised and identified in Cretaceous sediments and crude oils, and was proposed as

an angiosperm marker for sedimentary organic matter of Cretaceous and younger ages (Forster *et al.*, 1989a).

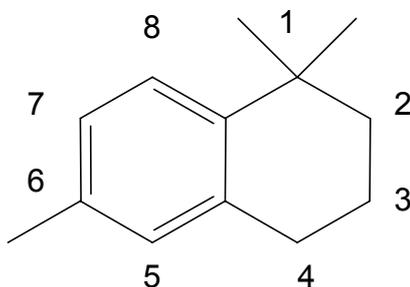


Figure 1.2. The structure and numbering system of 1,1,6-trimethyltetralin (1,1,6-trimethyl-1,2,3,4-tetrahydronaphthalene).

1,1,5,6-Tetramethyltetralin has also been tentatively identified in Green River shale (Anders *et al.*, 1973) and in coals (Püttmann and Villar, 1987; Heppenheimer *et al.*, 1992) and shales (Radke *et al.*, 1986; Püttmann and Villar, 1987; Borrego *et al.*, 1997). This tetramethyltetralin has been suggested to originate from higher plant resinous precursors (Radke *et al.*, 1986; Püttmann and Villar, 1987; Heppenheimer *et al.*, 1992) and microbial triterpenoids (Püttmann and Villar, 1987).

Calamenene (1,2,3,4-tetrahydrocadalene) and 5,6,7,8-tetrahydrocadalene have been identified in the Haselbach Clays (Simoneit *et al.*, 1986), in recent sediments (Hostettler *et al.*, 1989; Otto *et al.*, 1997) and in pyrolysates of dammar resins (van Aarssen *et al.*, 1990, 1991). Compounds with this carbon skeleton are especially abundant in plant resins and essential oils of land plants (Grantham *et al.*, 1983; van Aarssen *et al.*, 1990), and occur in bryophytes and fungi (Bordoloi *et al.*, 1989). van Aarssen *et al.* (1991) tentatively identified two structurally related alkyltetralins in the pyrolysates of dammar resin as 1,6-dimethyltetralin and 2,5-dimethyltetralin .

1.3 Sedimentary alkylnaphthalenes

Polymethylnaphthalenes are common constituents of sedimentary organic matter (Tissot and Welte, 1984). Methylnaphthalenes (MNs), dimethylnaphthalenes (DMNs) and trimethylnaphthalenes (TMNs) have been reported in shales (e.g. Radke *et al.*, 1982a; Alexander *et al.*, 1985, 1986; Radke *et al.*, 1986; Strachan *et al.*, 1988; Budzinski *et al.*, 1993b), coals (e.g. Radke *et al.*, 1982b, 1984a, 1990b) and in crude oils (e.g. Gavât and Irimescu, 1942; Carruthers and Douglas, 1955; Rossini, 1960; Mair and Mayer, 1964; Yew and Mair, 1966; Alexander *et al.*, 1983b; Alexander *et al.*, 1984c; Rowland *et al.*, 1984; Alexander *et al.*, 1985; Radke *et al.*, 1990a). More recently, tetramethylnaphthalenes (TeMNs) have been reported in sedimentary rocks and petroleum (e.g. Püttmann and Villar, 1987; Forster *et al.*, 1989b; Heppenheimer *et al.*, 1992; Yawanarajah and Kruege, 1994; Borrego *et al.*, 1997).

The first identifications of alkylnaphthalenes in sedimentary matter were made through isolation and characterisation from crude oils. This includes 2,3,6-TMN (Gavât and Irimescu, 1942), 1,2,5-TMN, 1,2,7-TMN and 2,3,6-TMN (Carruthers and Douglas, 1955), 2,3,6,7-TeMN (Mair and Martinez-Pico, 1962), and in a major study the MNs, the ethylnaphthalenes (ENs), nine of the ten DMN isomers and eight of the 14 TMN isomers (Mair and Mayer, 1964; Yew and Mair, 1966). Later studies carried out using synthetic standards and high resolution gas chromatography revealed distributions of MNs (Radke *et al.*, 1982b; Alexander *et al.*, 1983b), DMNs (Alexander *et al.*, 1983b), TMNs (Rowland *et al.*, 1984) and TeMNs in crude oils and sediments (Püttmann and Villar, 1987; Forster *et al.*, 1989b).

Some polymethylnaphthalenes have been reported in high relative abundances in sediments and crude oils of low maturity. Examples include 1,6-DMN (van Aarssen *et al.*, 1992), 1,2,5-TMN (Püttmann and Villar, 1987; Strachan *et al.*, 1988; Killops *et al.*, 1991; Heppenheimer *et al.*, 1992), 1,2,7-TMN (Püttmann and Villar, 1987; Strachan *et al.*, 1988; Forster *et al.*, 1989a) and 1,2,5,6-TeMN (Püttmann and Villar, 1987; Killops *et al.*, 1991;

Heppenheimer *et al.*, 1992). The presence of these alkylnaphthalenes in sediments and crude oils of low maturity has led to the suggestion that they originate from triterpenoids derived from microbial and land-plant sources (Püttmann and Villar, 1987; Strachan *et al.*, 1988; Forster *et al.*, 1989a). Other methyl isomers that increase with maturity are suggested to originate from isomerisation and transalkylation processes (Strachan *et al.*, 1988).

Ethylmethylnaphthalenes (EMNs) have been reported in coal tars (Chang and Karr, 1962) and crude oils (Mair and Mayer, 1964; Rowland *et al.*, 1986). Audino *et al.* (1996) synthesised a number of these isomers and identified 13 of the EMN isomers, using GC-MS techniques, in crude oils. A microbial source has been suggested as a possible origin for these compounds due to their ubiquitous occurrence throughout the geological record (Audino *et al.*, 1996).

Cadalene (4-isopropyl-1,6-dimethylnaphthalene) is a well known alkylnaphthalene and occurs widely in recent and Ancient sediments (Bendoraitis, 1974; Baset *et al.*, 1980; Radke *et al.*, 1984a; Simoneit, 1986; Wang and Simoneit, 1990; Noble *et al.*, 1991; Heppenheimer *et al.*, 1992; Alexander *et al.*, 1994; Singh *et al.*, 1994). Compounds with this carbon skeleton are especially abundant in plant resins and essential oils of land plants (Grantham *et al.*, 1983; van Aarssen *et al.*, 1990), and in bryophytes and fungi (Bordoloi *et al.*, 1989).

Long chain naphthalenes have been identified in rock extracts (Connan *et al.*, 1986; Gontcharov, 1993; Ellis *et al.*, 1995c) and crude oils (Gontcharov, 1993; Ellis *et al.*, 1995c). Phytanylnaphthalene has also been identified in crude oils and rock extracts (Gontcharov, 1993). A mechanism for the formation of these compounds via ring closure and aromatisation of alkylstyrenes has been proposed by Ellis *et al.*, (1995c).

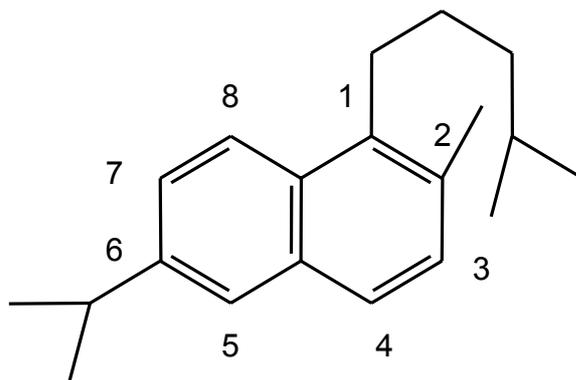


Figure 1.3. The structure and numbering system of 6-isopropyl-2-methyl-1-(4-methylpentyl)naphthalene.

Recently, a series of isohexylalkylnaphthalenes have been identified in crude oils and rock extracts. This homologous series of isohexylalkylnaphthalenes include 2-methyl-1-(4-methylpentyl)naphthalene; 2,6-dimethyl-1-(4-methylpentyl)naphthalene; 6-ethyl-2-methyl-1-(4-methylpentyl)naphthalene and 6-isopropyl-2-methyl-1-(4-methylpentyl)naphthalene (Ellis *et al.*, 1996b), the structure and numbering system of 6-isopropyl-2-methyl-1-(4-methylpentyl)naphthalene is shown in Figure 1.3. These compounds are suggested to form *via* a rearrangement process during aromatisation of terpenoid natural products of higher plant origin (Ellis *et al.*, 1996b). However, one of the isohexylnaphthalenes that is not part of this homologous series, 6-isopropyl-2,4-dimethyl-1-(4-methylpentyl)naphthalene, was tentatively identified as a methylation product of 6-isopropyl-2-methyl-1-(4-methylpentyl)naphthalene (Ellis, 1994; Ellis *et al.*, 1996b).

1.4 Sedimentary alkylphenanthrenes

Polymethylphenanthrenes are common constituents of sedimentary organic matter. Methylphenanthrenes (MPs) and dimethylphenanthrenes (DMPs) have been reported to occur in shales (e.g. Radke *et al.*, 1982a, 1984a, 1986; Garrigues and Ewald, 1983; Garrigues *et al.*, 1987; Budzinski *et al.*, 1992, 1995b; Boreham *et al.*, 1988), coals (e.g. Radke *et al.*, 1982a,b, 1984b, 1990b; Garrigues *et al.*, 1987) and crude oils (e.g. Carruthers and Douglas,

1957; Mair and Martinez-Pico, 1962; Lekveishvili *et al.*, 1980; Melikadze *et al.*, 1985; Garrigues and Ewald, 1983; Boreham *et al.*, 1988; Radke *et al.*, 1990a, 1993; Budzinski *et al.*, 1992, 1995b; Fisher *et al.*, 1996b). More recently, trimethylphenanthrenes have been reported in crude oils and rock extracts (e.g. Killops, 1991; Budzinski *et al.*, 1992, 1993a,b, 1995b; Radke *et al.*, 1993).

One of the first identifications of alkylphenanthrenes in sedimentary matter was through the isolation and characterisation of 3,6-DMP from coal tar (Kruber and Raeithel, 1954). Shortly afterwards, 1,8-DMP and 1,2,8-TMP were isolated from a Kuwait petroleum (Carruthers and Douglas, 1957). Phenanthrene, 1-MP, 2-MP, 3-MP, 9-MP and 1,8-DMP were isolated from crude oil (Mair and Martinez-Pico, 1962). 9-MP, 9-EP, 9-isopropylphenanthrene were also isolated from crude oil (Lekveishvili *et al.*, 1980; Melikadze *et al.*, 1985). Later studies carried out using synthetic standards and high resolution gas chromatography revealed distributions of alkylphenanthrenes such as, phenanthrene, 1-MP, 2-MP, 3-MP, 9-MP (Radke *et al.*, 1982a) and 4-MP in marine sediments (Garrigues and Ewald, 1983). However, studies of ethylphenanthrenes (EPs) and DMP distributions using capillary gas chromatography showed many problems with co-chromatography of many isomers (Radke *et al.*, 1984a,b, 1986). More recent studies have encountered less problems with the co-chromatography of EPs, DMPs and TMPs using capillary gas chromatography with a smectic liquid crystalline phase (Budzinski *et al.*, 1992). These workers reported the retention indices of 25 DMPs and 33 TMPs of which 12 DMPs and 26 TMPs occurred in crude oils and sediments.

1-MP, 1,7-DMP (pimanthrene), 1-methyl-7-ethylphenanthrene and 1-methyl-7-isopropylphenanthrene (retene) are suggested to have specific sources such as resins (Hayatsu *et al.*, 1978; Laflamme and Hites, 1978; Wakeham *et al.*, 1980; Radke *et al.*, 1982b; Simoneit *et al.*, 1986; Alexander *et al.*, 1992b; Heppenheimer *et al.*, 1992). The structure and numbering system of 1-methyl-7-isopropylphenanthrene (retene) is shown in Figure 1.4.

Bacteriohopanoids have been suggested as a major source of 1,2,8-TMP in lacustrine sediments (Killops, 1991). Multivariate analysis has recently been

used to define origin/maturity markers based on MP, DMP, TMP distributions (Budzinski *et al.*, 1995b). From their work 1-MP, 9-MP, 1,9-DMP, 1,8-DMP, 1,2-DMP, 1,7-DMP, 1,2,7-TMP, 1,2,8-TMP, 1,2,6-TMP, 1,2,9-TMP, 1,6,9-TMP, 1,7,9-TMP + 1,3,8-TMP and 1,3,9-TMP were suggested as biomarkers originating from natural products.

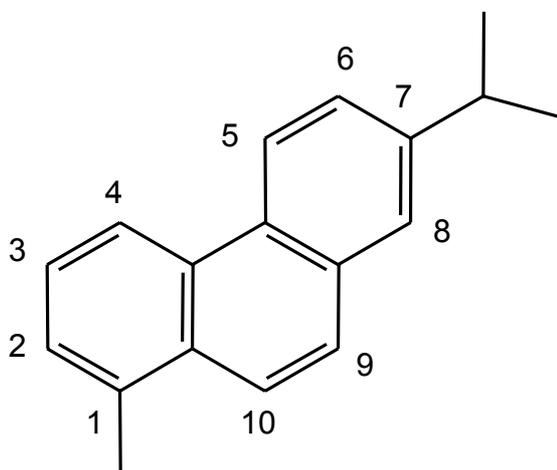


Figure 1.4. The structure and numbering system of 1-methyl-7-isopropyl phenanthrene (retene).

1.5 Geosynthesis of aromatic compounds

The occurrence of aromatic hydrocarbons in sediments that have non-isoprenoidal carbon skeletons has led to the suggestion that such compounds are the products of sedimentary reactions (Radke *et al.*, 1982a, 1982b, 1993; Smith *et al.*, 1994). Sedimentary methylation processes have been proposed to account for the occurrence of numerous methyl-substituted aromatic hydrocarbons that occur in low maturity samples that have carbon skeletons that are not obviously derived from common natural product precursors (Radke *et al.*, 1982a).

Phenanthrenes and perylenes were first suggested as compound classes to undergo alkylation in early thermal processes in sedimentary organic matter (Ishiwatari and Fukushima, 1979; Radke *et al.*, 1982a; Louda and Baker, 1984). From their work, phenanthrene and perylene alkylation indices were formulated by Ishiwatari and Fukushima (1979) and Louda and Baker (1984)

respectively. However, these indices were produced as a measure of maturity and not as an empirical measure of the amount of alkylation the sedimentary organic matter had undergone.

The likelihood of formation of alkylaromatics in sediments by alkylation processes has been demonstrated for phenols (Ioppolo-Armanios, 1995), benzenes (He *et al.*, 1995; Ellis *et al.*, 1995b), naphthalenes (He *et al.*, 1992, 1994), phenanthrenes (Smith *et al.*, 1994, 1995), anthracenes (Smith *et al.*, 1994, 1995) and pyrene (Derbyshire and Whitehurst, 1981; Smith *et al.*, 1994, 1995).

1.6 Effects of maturity on aromatic compounds

The chemical and physical properties of sedimentary organic matter change with the influence of temperature and time during burial (Tissot and Welte, 1984). These changes can be categorized into three stages, diagenesis, catagenesis and metagenesis (Tissot and Welte, 1984). Diagenesis starts during shallow burial and is thought to be a stage of equilibration in the sediments (Tissot and Welte, 1984). During early diagenesis, microbial activity destroys biogenic polymers (proteins and carbohydrates) and their constituents undergo chemical transformation perhaps forming kerogen (Tissot and Welte, 1984). Although, microscopical and chemical studies have recognised entities in the kerogen that represent preserved resistant biomacromolecules from original extant organisms (Tegelaar *et al.*, 1989). Catagenesis results from increasing temperature and pressure during deeper burial of sediments causing thermal breakdown of kerogen producing first liquid petroleum and then “wet gas” and condensate (Tissot and Welte, 1984). Metagenesis refers to evolution of organic matter in sedimentary basins at higher temperatures and pressures where organic material is converted to methane and graphite (Tissot and Welte, 1984). These stages are illustrated with reference to a scheme of hydrocarbon formation as given in Figure 1.5.

Thermal maturity describes the extent of heat-driven reactions which convert sedimentary organic matter into petroleum, according to Peters and Moldowan (1993). Thermal maturity can be classified as immature, mature and post mature stages with respect to oil formation (Peters and Moldowan, 1993). For example, immature source rocks refer to those source rocks in which the organic matter has undergone diagenetic effects without a significant effect from temperature (Peters and Moldowan, 1993). The second category, mature source rocks, refer to those source rocks in which the organic matter has undergone significant thermal breakdown. This generally occurs during catagenesis (Peters and Moldowan, 1993) and represents the major zone of oil production. Post-mature source rocks refer to the thermal destruction of organic matter into graphite capable of only generating gas (Peters and Moldowan, 1993), brought about by the stage of metagenesis.

Maturity can be determined by the measurements made on either the solid (kerogen) or liquid (bitumen) components of sedimentary organic matter. Biomarker maturity parameters based on hopanes, steranes and their aromatic counterparts are widely used to assess the thermal maturity of sedimentary hydrocarbons (e.g. Mackenzie and McKenzie, 1983; Peters and Moldowan, 1993) and are used as standard tools in oil exploration. Commonly used types of maturity parameters include isomerisation and aromatisation reactions (e.g. Radke *et al.*, 1982a,b, 1983, 1986, 1987, 1990a; Mackenzie and McKenzie, 1983; Alexander *et al.*, 1985, 1994; Abbott *et al.*, 1990; Rullkötter and Marzi, 1988; Strachan *et al.*, 1988; Marzi and Rullkötter, 1992; Yawanarajah and Kruege, 1994; Sosrowidjojo *et al.*, 1996), cyclisation of alkylbiphenyls (Alexander *et al.*, 1988, 1989; Kagi *et al.*, 1990), dealkylation (Sosrowidjojo *et al.*, 1996) and ester elimination reactions (Alexander *et al.*, 1991, 1997; Kralert *et al.*, 1995).

Aromatic maturity parameters based on molecular ratios of alkylnaphthalenes and alkylphenanthrenes are the most commonly used. One of the first molecular maturity parameter proposed was based on methylphenanthrenes and was developed from a detailed investigation of the aromatic compound

distribution in rock samples from a Western Canada Basin (Radke *et al.*, 1982a). It was from this work that the development of the methylphenanthrene ratios, MPR 1, MPR 2, MPR 3, MPR 9 came (Radke *et al.*, 1982a), MPR (Radke *et al.*, 1982b), PP-1 (Alexander *et al.*, 1986), methylphenanthrene indices, MPI 1, MPI 2 (Radke *et al.*, 1982a) and MPI 3

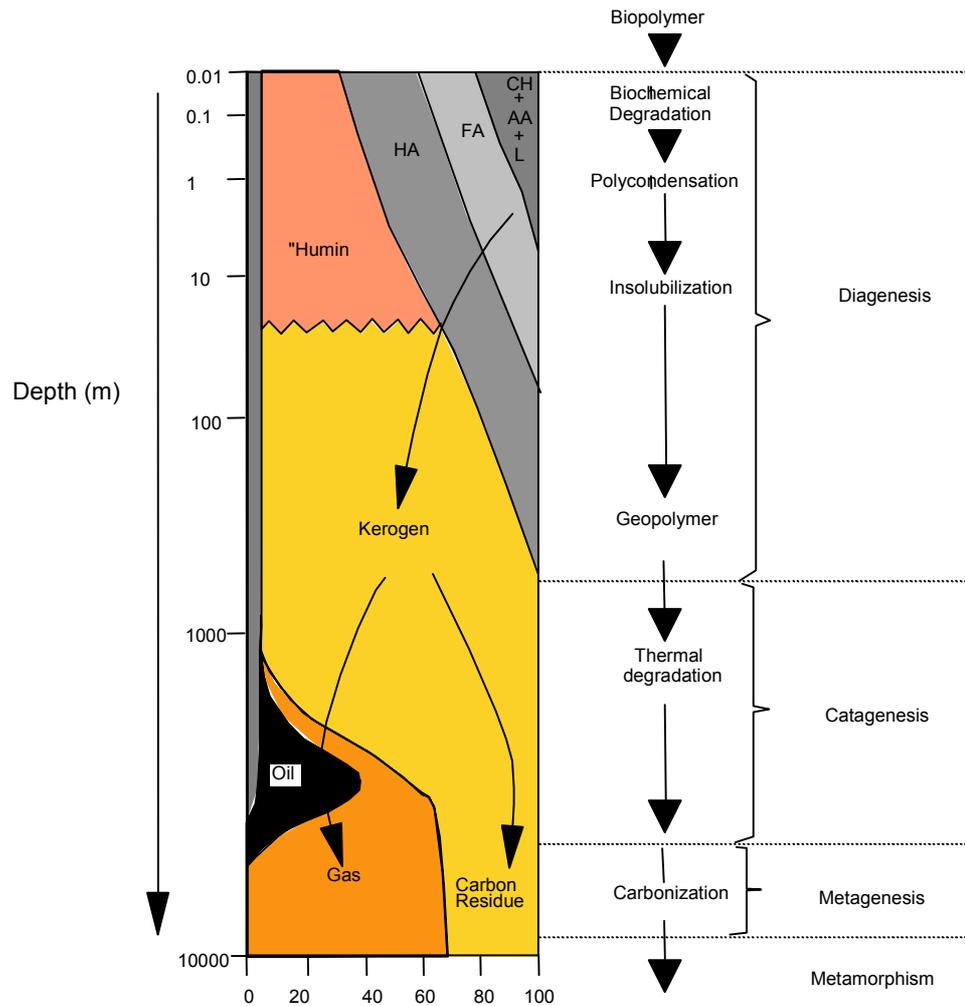


Figure 1.5. General scheme of evolution of the organic matter, from freshly deposited sediment to the metamorphic zone. *CH*: carbohydrates, *AA*: amino acids, *FA*: Fulvic acids, *HA*: humic acids, *L*: Lipids, *HC*: hydrocarbons, *N, S, O*: N,S,O compounds (non-hydrocarbons) (adapted from Tissot and Welte, 1984).

(Angelin *et al.*, 1983), calculated vitrinite reflectance based on MPI 1 (Radke and Welte, 1983), dimethylphenanthrenes to phenanthrene ratios, DPR I

(Radke *et al.*, 1982a), dimethylphenanthrene ratio, DPR (Radke *et al.*, 1982b) and the dimethylphenanthrene index, DPI (Radke *et al.*, 1982a). These maturity indicators are characterised by a relative increase in the abundance of the most stable isomers, which are usually based on compounds substituted in the α position being less stable than its β substituted isomers. Artificial maturation experiments in which the changes in MPs and DMPs have been studied and compared to phenanthrene maturity parameters (Garrigues *et al.*, 1990). Multivariate analysis was also used to investigate MP, DMP and TMP distributions to distinguish isomers as origin or maturity markers in an attempt to make better maturity parameters (Budzinski *et al.*, 1995b). Multivariate analysis has also been used to show maturity effects and used to develop a dimethylphenanthrene ratio, DMP (Yawanarajah and Kruge, 1994). Molecular mechanics (MM) methods have also been used to predict the most stable alkylphenanthenes (Budzinski *et al.*, 1993b) and DMPs using experimental and sedimentary data (van Duin *et al.*, 1997).

Alkyl-naphthalene ratios have also been used extensively as measures of maturity. These maturity ratios include methyl-naphthalene ratio, MNR (Radke *et al.*, 1982b), ethyl-naphthalene ratio, ENR (Radke *et al.*, 1982b), dimethyl-naphthalene ratios, DNR 1 (Radke *et al.*, 1982b), DNR 2, DNR 3, DNR 4, DNR 5 (Alexander *et al.*, 1985), 1,8-DMN ratio (Alexander *et al.*, 1984c), DP-1 (Alexander *et al.*, 1986), trimethyl-naphthalene ratios, TNR 1, (Alexander *et al.*, 1985), TNR 2 (Radke *et al.*, 1986) and TP-1 (Alexander *et al.*, 1986). These maturity parameters are based on an increase in the relative abundance of the most stable isomers with increased maturity. Compounds substituted in the α position are usually less stable than those with β substituents. Molecular mechanics have also been used to calculate the enthalpies of formation for DMNs, TMNs and TeMNs to predict the most stable alkyl-naphthalene in each class (Budzinski *et al.*, 1993b; van Duin *et al.*, 1997). Recently, Yawanarajah and Kruge (1994) used multivariate analysis on a sample set to produce maturity parameters such as, dimethyl-naphthalene ratio (DMN), trimethyl-naphthalene ratio (TrMN) and a

tetramethylnaphthalene ratio, (TeMN). Cadalene has also been demonstrated to isomerise to isocadalene (3-isopropyl-1,6-dimethylnaphthalene) in the laboratory and a maturity parameter has also been developed from this for sedimentary organic matter (Alexander *et al.*, 1994). This last maturity parameter shows the development of an aromatic maturity parameter based on a clear precursor/product relationship.

1.7 Effects of biodegradation on aromatic compounds

Biodegradation and water washing substantially alters the chemical compositions of crude oils (Tissot and Welte, 1984; Connan, 1984; Peters and Moldowan, 1993). The effects of biodegradation have been studied for many classes of compounds, such as n-alkanes (Winters and Williams, 1969; Heath *et al.*, 1997), isoprenoids (Seifert and Moldowan, 1979), the unresolved complex mixture (UCM) (Deroo *et al.*, 1974; Gough and Rowland, 1990; Killops and Al-Juboori, 1990; Volkman *et al.*, 1983a,b, 1984; Connan, 1984; Gough *et al.*, 1992), steranes (Seifert and Moldowan, 1979; Mackenzie and McKenzie, 1983; Seifert *et al.*, 1984; Dajiang *et al.*, 1988), hopanes (Reed, 1977; Seifert and Moldowan, 1979; Alexander *et al.*, 1983c; Philp, 1983; Volkman *et al.*, 1983b; Hoffmann and Strausz, 1986; Peters and Moldowan, 1991; Peters *et al.*, 1996; Blanc and Connan, 1994; Chosson *et al.*, 1992), terpanes (Reed, 1977; Seifert and Moldowan, 1979; Seifert *et al.*, 1984), alkylbenzenes (Connan, 1984; Kelly *et al.*, 1997), alkylnaphthalenes (Solanas *et al.*, 1984; Volkman *et al.*, 1984; Rowland *et al.*, 1986; Budzinski *et al.*, 1995a, 1998; Fisher *et al.*, 1996a, 1998), alkylphenanthrenes (Rowland *et al.*, 1986; Garrigues *et al.*, 1987; Budzinski *et al.*, 1995a, 1998; Fisher *et al.*, 1998), aromatic steranes (Wardroper *et al.*, 1984; Connan, 1984; Peters and Moldowan, 1993), porphyrins (Barwise and Park, 1983; Lin *et al.*, 1989; Strong and Filby, 1987) and carboxylic acids (Mackenzie and McKenzie, 1983; McMahon and Chappelle, 1991; Jaffe and Gallardo, 1993). The effect of biodegradation of crude oils often results in the depletion of biomarkers in the order n-paraffins, acyclic isoprenoids, hopanes (25-norhopanes present), steranes, hopanes (no 25-norhopanes)/diasteranes, aromatic steroids and porphyrins (Peters and Moldowan, 1993).

Various scales for assessing the extent to which an oil has been biodegraded based on the relative abundances of various hydrocarbon classes have been constructed by Volkman *et al.* (1983a,b), Cassani and Eglinton (1991), Peters and Moldowan (1991) and Fisher *et al.* (1998).

Of the aromatic compounds, the mono and triaromatic steroids often appear to be untouched, except in extreme cases of degradation, after the steranes and hopanes have been completely destroyed. Preferential depletion of the 20*R* isomer in the mono- and triaromatic steroids has also been observed in some extreme cases (Wardroper *et al.*, 1984). Recently, 6-isopropyl-2, 4-dimethyl-1-(4-methylpentyl)naphthalene has been tentatively identified and found to survive in the most extreme of biodegraded oils (Ellis 1994; Ellis *et al.*, 1996b), and may be a useful biomarker for future study. Biodegradation of aromatic hydrocarbons has the following broad rate of susceptibility: monoaromatics > diaromatics > triaromatics (Connan, 1984). Within classes of compounds the susceptibility to biodegradation decreases as the number of alkylsubstituents increases, i.e. unsubstituted > mono > di > tri. The differential biodegradation of certain isomers has been demonstrated for dimethylnaphthalenes, trimethylnaphthalenes, methylphenanthrenes, dimethylphenanthrenes and trimethylphenanthrenes (Connan, 1984; Volkman *et al.*, 1984; Rowland *et al.*, 1986; Budzinski *et al.*, 1995a, 1998; Fisher *et al.*, 1998). Alkylphenanthrenes with substituents in the 9 or 10 positions have been reported to degrade more slowly than the other alkylphenanthrene isomers (Rowland *et al.*, 1986; Budzinski *et al.*, 1995a). More extensive studies of alkylnaphthalenes and alkyphenanthrenes using GC-MS and GC-FTIR have shown the order of susceptibility to biodegradation of alkylnaphthalenes and alkyphenanthrenes (Fisher *et al.* 1996a, 1998) (Figure 1.6). Fisher *et al.* (1996a, 1998) also noted that isomers with 1,6-substituents are more susceptible to biodegradation than are other polymethylnaphthalene isomers. This work has led to the comparison of saturated and aromatic hydrocarbons shown in Table 1.1.

Dimethylnaphthalenes

1,6 > 2,7 > 1,3 > 1,7 ~ 1,4 ~ 1,2 ~ 2,6 > 1,5 > 2,3 (Coastal)
1,6 > 2,7 >> 1,3 ~ 1,7 ~ 1,4 ~ 1,2 ~ 2,6 >> 1,5 >> 2,3 (Seafloor)
1,6 >> 1,5 > 2,3 (Reservoirs)

Trimethylnaphthalenes

1,3,6 > **1,2,6** > **1,2,5** > **1,6,7** ~ **1,4,6** > 2,3,6 > 1,2,7 ~ 1,3,5 > 1,3,7 > 1,2,4 (Coastal)
1,3,6 ~ **1,6,7** > **1,2,6** > **1,2,5** > **1,4,6** >> 2,3,6 > 1,3,7 ~ 1,2,7 >> 1,3,5 >> 1,2,4 (Seafloor)
1,3,6 >> 1,2,4 (Reservoirs)

Tetramethylnaphthalenes

1,3,6,7 >> 1,3,5,7 (Coastal)
1,3,6,7 > **1,2,5,6** > **1,2,6,7** >> **1,2,4,6**; **1,2,4,7** > **1,4,6,7**; **1,2,5,7** > **1,2,3,5** > **1,2,3,6** > 1,2,3,7; 2,3,6,7 > 1,3,5,7 (Reservoirs)

Methylphenanthrenes

2 > 9 > 1~3 (Coastal)

Dimethylphenanthrenes

1,7~2,6~3,10~2,10 > 1,6~2,9~3,9 > 2,3~1,9~1,8~1,2~2,7~ 3,6~1,3 (Coastal)

Figure 1.6. A summary of the order of susceptibility to microbial attack of polymethylnaphthalenes in three environments, namely coastal sediments (coastal), seafloor sediments (seafloor) and reservoir oils (reservoirs). The position of methyl substituents on the naphthalene nucleus are referred to by numbers : polymethylnaphthalenes with a 1,6-disubstitution pattern are shown in bold text. The order of susceptibility to biodegradation of alkylphenanthrene in coastal sediments is also shown (after Fisher *et al.*, 1996a, 1998).

Table 1.1. The effects of biodegradation on the composition of a condensate spilled in coastal sediments in north Western Australia, (After Fisher *et al.*, 1998).

Level of biodegradation	Chemical composition	
	Saturated hydrocarbons	Aromatic hydrocarbons
1	Light-end n-alkanes depleted	MNs depleted
2	General depletion of n-alkanes	DMNs altered
3	>90% n-alkanes absent Alkylcyclohexanes affected	DMNs altered TMNs altered
3-4 (Moderate)	No n-alkanes Acyclic isoprenoids affected Alkylcyclohexanes absent	DMNs altered TMNs altered MPs altered
5	Acyclic isoprenoids absent	DMNs severely depleted TMNs altered TeMNs altered DMPs altered
5-6	Bicyclic alkanes affected	DMNs absent TMNs severely depleted TeMNs altered MPs depleted DMPs altered
6 (Extensive)	Bicyclic alkanes severely depleted Steranes affected	TMNs absent MPs absent TeMNs depleted DMPs altered EPs unaltered

1.8 Analysis of sedimentary aromatic compounds

The analysis of aromatic hydrocarbons in sedimentary organic matter began with the use of gas chromatography (GC) using a flame ionising detector (FID). However, due to the complexity of the hydrocarbon mixtures it was necessary to carry out separations using column chromatography or thin layer chromatography to obtain less complicated fractions (Hirsch *et al.*, 1972; Sawatzky *et al.*, 1976; Seifert and Moldowan, 1978; Radke *et al.*, 1982a,b; Alexander *et al.*, 1983b). More specific aromatic hydrocarbon fractions have

been obtained using high pressure liquid chromatography (HPLC) (Garrigues and Ewald, 1983a,b; Radke *et al.*, 1984b, 1988; Budzinski *et al.*, 1993b).

Capillary gas chromatography-FID has been used to analyse aromatic hydrocarbons in sedimentary matter (e.g. Alexander *et al.*, 1983b; Radke *et al.*, 1982a,b, 1983, 1984a,b; Rowland *et al.*, 1984), capillary gas chromatography-mass spectrometry (GC-MS) (e.g. Forster *et al.*, 1989b; Hartgers *et al.*, 1992, 1994a,b; Budzinski *et al.*, 1992, 1993a,b; Dong *et al.*, 1993; Sinninghe Damsté *et al.*, 1993b; Radke *et al.*, 1993; Hoefs *et al.*, 1995; Ellis *et al.*, 1995a,b, 1996a,b; Clegg *et al.*, 1997) high performance liquid chromatography (HPLC) (e.g. Garrigues and Ewald, 1983a,b; Garrigues *et al.*, 1984, 1987) and capillary gas chromatography-Fourier transform infrared (GC-FTIR) (Fisher *et al.*, 1996a,b, 1998). Of these techniques GC-MS is the most widely used technique for the analysis of most aromatic hydrocarbons. However, capillary gas chromatography can not always separate all the isomers of benzenes, naphthalenes and phenanthrenes. This causes an analytical problem for GC-MS because mass spectrometry cannot be used to distinguish the overlapping isomers of these classes.

Other techniques have been used for the analysis of alkylphenanthrenes in sedimentary matter. These techniques include high resolution Shpol'skii luminescence spectroscopy (HRS) of very specific HPLC fractions (Garrigues and Ewald, 1983a,b; Garrigues *et al.*, 1984, 1987; Radke *et al.*, 1984b, 1990b) and GC-MS with specialised capillary gas chromatography columns (Budzinski *et al.*, 1992, 1993a,b). Budzinski *et al.* (1992, 1993a,b) were successful in resolving alkylated phenanthrenes using a GC-MS with a smectic liquid crystalline phase. However, the recent use of capillary gas Fourier transform infrared (GC-FTIR) has improved the identification of alkylated phenanthrenes in sedimentary (Radke *et al.*, 1993) and even more so when GC-FTIR was used together with GC-MS (Alexander *et al.*, 1995; Fisher *et al.*, 1996a,b, 1998).

The complexity of mixtures of aromatic hydrocarbons has been decreased with the use of dealuminated mordenite molecular sieves. Molecular sieving

of mono aromatic fractions showed that n-alkyl toluenes were all selectively sorbed, n-alkyl xylenes with methyl substituents in positions 2,3-; 2,4-; 2,5-; 3,4- were sorbed, and n-alkyl xylenes with substituents in positions 3,5-; 2,6- were partially sorbed (Ellis *et al.*, 1992). Molecular sieving of alkylnaphthalenes has shown that isomers with 1,3- substituents are partially sorbed and isomers with 1,4- substituents were not sorbed (Ellis *et al.*, 1994). The molecular sieving of alkylphenanthrenes were shown to partially sorb isomers with 4 and 1,3 substituents, however, 9 substituted isomers were not sorbed (Ellis *et al.*, 1994).

1.9 Chiral gas chromatography phases

The first chiral phases were based on amino acid derivatives (Frank *et al.*, 1977) or chiral metal complexes (Schurig and Nowotny, 1988). However, these show enantioselectivity for only a small range of functional groups because they rely on the chemical interaction that forms a diastereomeric association with the analyte and the chiral phase. The ability of cyclodextrin phases to separate analytes is based primarily on shape recognition, giving them a wide range of applications.

Cyclodextrins are oligomers of 1,4-linked α -D-glucose units that contain a large number of chiral centres with the overall shape of a truncated cone that results in a cavity. Cyclodextrins are classified by the number of glucose units they contain. α -Cyclodextrins have 6 units, β -cyclodextrins have 7 units and γ -cyclodextrins have 8 units (Fig 1.7). Cyclodextrins are prepared by the enzymatic degradation of starch with cyclodextrin-glucanotransferase from *Bacillus macerans*, *Bacillus megaterium*, and other bacterial strains (König, 1992). Cyclodextrins are readily acylated and can be acylated with different groups due to the different reactivity of the hydroxyl groups in the glucose units (König, 1992). These differences in chemical reactivity (OH acidity decreases from the 6-position to 2- and 3- positions) can allow the hydroxyl groups to be chemically modified in a stepwise fashion. The cyclodextrin's macrocyclic conformation changes with acylation, generally making the angle of the cone larger (Harata *et al.*, 1987). This in turn changes the size of the

internal cavity and the extent interaction between the modified cyclodextrin and the analyte, which is responsible for the observed differences in enantioselectivity brought about by minor differences in the substitution of the modified cyclodextrins (König, 1992). The structure of heptakis(2,3,6-tri-O-methyl) β -cyclodextrin (permethyl- β -cyclodextrin) is shown in Figure 1.8.

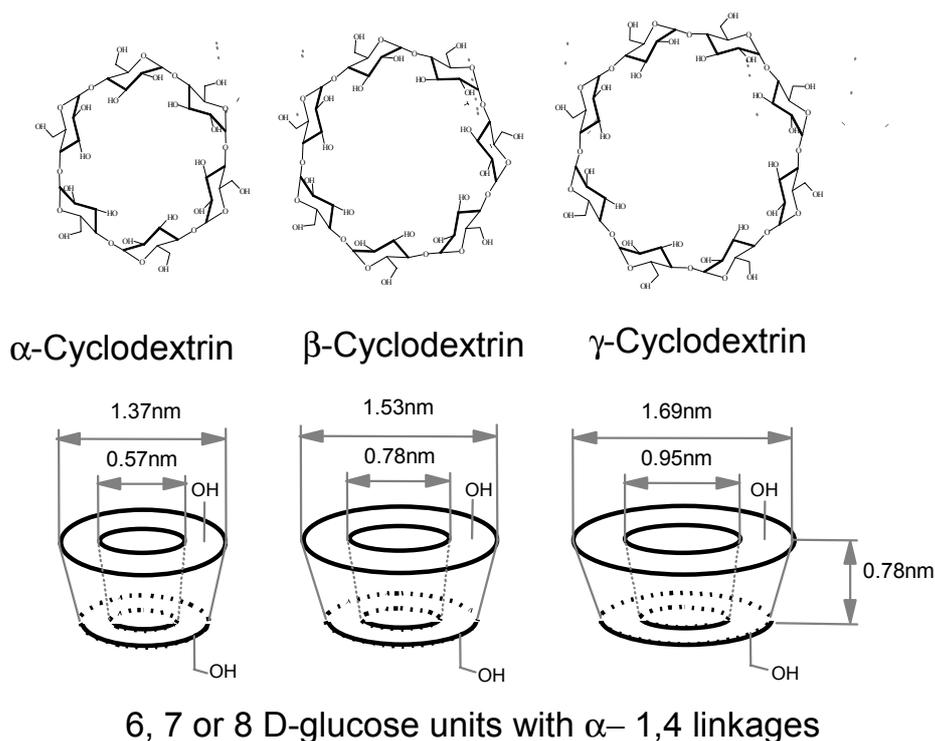


Figure 1.7. Structures and dimensions of α -, β -, γ -cyclodextrins.

The first application of a cyclodextrin in gas chromatography was to separate optical isomers of α -pinene using a α -cyclodextrin packed column (Koscielski *et al.*, 1983). Capillary gas chromatography using cyclodextrins was started by Juvancz *et al.* (1987) who coated a capillary column with molten permethylated β -cyclodextrin and then by Schurig and Nowotny (1988) who dissolved the permethylated β -cyclodextrin in OV-1701 silicone phase.

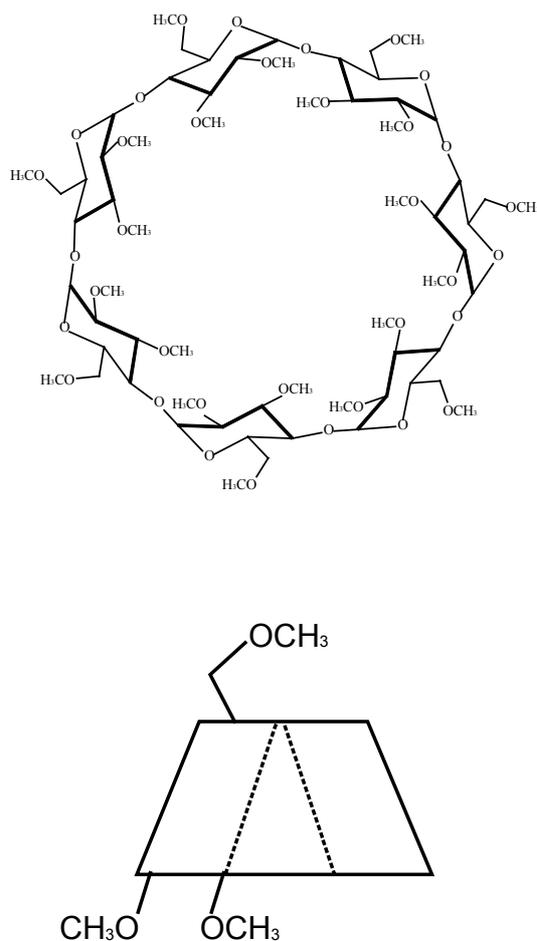


Figure 1.8. The molecular structure of heptakis(2,3,6-tri-O-methyl) β -cyclodextrin and its geometric shape.

Further work immobilised the permethylated β -cyclodextrin through chemical binding to a polydimethylsiloxane phase to increase the thermal stability (Schurig *et al.*, 1990; Fischer *et al.*, 1990). With increasing interest other modified cyclodextrins were introduced such as dipentylacetyl cyclodextrin (König *et al.*, 1988), dipentyl butyryl γ -cyclodextrin (König *et al.*, 1989a), tripentyl α - and β -cyclodextrin, dipentylated methyl-, butyryl- and acetyl- α , β and γ -cyclodextrins (Wenz *et al.*, 1990), butyryl dipentyl γ -cyclodextrin (König, 1993) and trichloroacetyl pentyl β -cyclodextrin (Shitangkoon and Vigh, 1994). However, permethylated β -cyclodextrin in a polysiloxane phase was found to have the widest applicability (Keim *et al.*, 1991; Lipkowitz *et al.*, 1997; Vetter

and Schurig, 1997), the permethylated β -cyclodextrin phases showed chiral selectivity with a wide range of compounds such as hydrocarbons, bicyclic acetals, cyclic ethers, cyclic carbonates, lactones, esters, ketones, aldehydes, alcohols, halocarbons and some nitrogen containing compounds (Schurig and Nowotny, 1988; Schurig *et al.*, 1989; König *et al.*, 1989a,b,c,d; Nowotny *et al.*, 1989; Keim *et al.*, 1991; Mayer *et al.*, 1992).

1.10 Chiral biomarkers in organic geochemistry

Evidence for petroleum containing molecules that are optically active was first provided by measuring the optical rotation of different fractions of sedimentary organic matter or from compounds isolated from sedimentary organic matter (Fenskie *et al.*, 1942; Oakwood *et al.*, 1952; Silverman, 1967, 1971; Louis, 1968; Williams, 1974; Hills *et al.*, 1966; Maxwell *et al.*, 1973; Philippi, 1977). The optical rotation of fractions of lubricating oils from US oil refinery blends was shown to increase with molecular weight (Fenskie *et al.*, 1942). Further work attributed the optical rotation of petroleum to steranes and triterpanes, based on mass spectrometry and nuclear magnetic resonance techniques (Whitehead, 1971). High optical rotation has also been observed from hydrocarbons isolated from crude oils, suggesting that these types of compounds were responsible for most of the optical activity in sedimentary hydrocarbons (Hills *et al.*, 1966). Silverman (1967, 1971) measured the carbon isotope compositions of crude oil fractions and their optical rotation, and observed that the fractions with the highest concentration of biogenic material also had the highest optical activity. Thermal maturity was also studied with respect to optical rotation which showed a decrease in optical rotation with maturity (Louis, 1968; Winters and Williams, 1969; Williams *et al.*, 1974). Microbial degradation has been shown to increase optical rotation due to selective degradation (Philippi, 1977; Winters and Williams, 1969; Williams, 1974).

Hydrocarbon enantiomers of farnesane (2,6,10-trimethyldodecane) and pristane (2,6,10,14-tetramethylpentadecane) isolated from crude oils have been analysed by Brooks *et al.* (1977) and Patience *et al.* (1978)

respectively. The analysis of these enantiomers in crude oils involved their oxidation to carboxylic acids and subsequent derivatisation with chiral alcohols and analysis by achiral gas chromatography of the diastereomers.

Enantiomers of amino acids have also been studied in order to provide an understanding of the origin and diagenetic histories of amino acids in geological and biological systems (Macko *et al.*, 1986, 1991, 1994; Serban *et al.*, 1988; Silfer *et al.*, 1992, 1994). These methods required derivatisation of the amino acids and analysis by gas chromatography using a phase based on chiral amino acid derivatives. Modified cyclodextrin phases have been used to separate enantiomeric hydrocarbons standards, such as alkyltetralins, alkylindanes and isoprenoidal, that have been reported in sedimentary organic matter (Armstrong *et al.*, 1991).

Achiral stationary phases that are typically used in gas chromatography do not separate enantiomers. However, most biologically formed compounds in nature have chiral centers and this material is the basis of sedimentary organic material deposited in the geological record. Therefore, the occurrence of enantiomers in sedimentary matter is important to organic geochemistry in understanding sedimentary processes.

1.11 Scope and framework of the thesis

The aim of this work is to establish the processes involved in the change of hydrocarbons that have obvious natural product precursors into hydrocarbons without obvious natural product precursors and to apply these processes to oil exploration. To obtain this result an understanding of the first stages of change that the hydrocarbons with natural product precursors underwent was needed. Therefore, oils and sediments were investigated for the presence of hydrocarbon compounds that showed similarities to hydrocarbons with obvious natural product precursors.

In chapter 2 hydrocarbon racemates were separated using β -cyclodextrin and γ -cyclodextrin gas chromatography columns. The structures resolved in

this chapter gave an insight in to the capabilities of the cyclodextrin phases to separate hydrocarbons in sedimentary organic matter. Chapter 3 is the first reported occurrence of two hydrocarbon racemates, 1,2,2,5-tetramethyltetralin and 1,2,2,5,6-pentamethyltetralin, in crude oil. Bicyclic hydrocarbon natural products that give rise to drimanes are suggested to be a possible source for the two hydrocarbons 1,2,2,5-tetramethyltetralin and 1,2,2,5,6-pentamethyltetralin. These two hydrocarbons are also suggested to be intermediates in the formation of alkylnaphthalenes discussed in chapters 6 and 7. In chapter 4 isodihydro-*ar*-curcumene was identified in petroleum using GC-MS techniques. Its co-occurrence in crude oil with dihydro-*ar*-curcumene gives rise to the suggestion that it may originate from this compound *via* a sedimentary rearrangement process. Enantiomers of both compounds have also been shown to occur in crude oils using chiral GC-MS techniques. In chapter 5 the effects of maturity and biodegradation on dihydro-*ar*-curcumene and isodihydro-*ar*-curcumene enantiomers have been assessed by applying chiral GC-MS techniques to aromatic hydrocarbon fractions obtained from crude oils and sedimentary rocks. Dihydro-*ar*-curcumene of natural origins undergoes rapid racemisation in the subsurface, yielding a racemic mixture before the onset of significant oil formation. Laboratory experiments using proton catalysts and clay (Lewis acid catalyst) show that the alkyl shift reaction is catalysed by proton and Lewis acids and racemisation is only catalysed by Lewis acids.

In chapter 6, 1,2,3,5-tetramethylnaphthalene (TeMN) was identified in petroleum using GC-MS techniques by comparing its retention time and mass spectrum with those of a synthetic reference compound. Bicyclic compounds of microbial origins or their precursors *via* a tetralin intermediate (reported in chapter 3) are proposed as the source of 1,2,3,5-TeMN in crude oil. 1,2,3,5-TeMN was previously unidentified due to its co-chromatography with 1,2,5,6-TeMN. In chapter 7, four previously unreported pentamethylnaphthalenes have been synthesised, characterised and identified in a number of crude oils and sediments, ranging in age from Proterozoic to Tertiary. 1,2,3,5,6-Pentamethylnaphthalene (PMN) is suggested to form predominantly from the aromatisation of drimanoid

precursors *via* 1,2,2,5,6-pentamethyltetralin (reported in chapter 3). Laboratory heating experiments showed that the proportions of PMNs change, reflecting the relative stability of the isomers. This suggested that the other pentamethylnaphthalenes form *via* acid-catalysed isomerisation or transalkylation processes. A maturity parameter has been developed from these observations. In chapters 8, 9 and 10, crude oils and several shales which contain anomalously high concentrations of alkylphenanthrenes and alkylnaphthalenes derived from obvious natural product precursors have been shown to contain relatively high concentrations of their corresponding methyl counterparts. In laboratory experiments carried out under mild conditions, each of the alkylphenanthrenes and alkylnaphthalenes has been shown to be substituted preferentially when a methyl donor was heated with the substrate in the presence of a clay catalyst. These observations have been interpreted as evidence for a sedimentary methylation process. Also in these chapters, previously co-chromatographing TeMN isomers 1,2,4,6-TeMN, 1,2,4,7-TeMN and 1,4,6,7-TeMN are resolved using two different capillary gas chromatography phases. Also three previously unreported compounds (1,2,3,5,6,7-hexamethylnaphthalene; 9-methylretene and 6-isopropyl-2,4-dimethyl-1-(4-methylpentyl)naphthalene) are reported as methylation products in sedimentary organic matter. In chapter 11, compounds originating from land-plants (identified in chapters 8 and 10) which are the result of reactions of the natural products involving aromatisation, rearrangement and methylation in the sediments are suggested as markers for land-plants in severely biodegraded oils in which few other biologically derived compounds can still be recognised. The order of biodegradability of these compounds has been assessed relative to their non-methylated counterparts. In both cases the methylated homologue is less susceptible to biodegradation. These results have been used to assess that a crude oil is a mixture of a severely biodegraded and less biodegraded crude oil.

2.0 Separation of petroleum hydrocarbon racemates using capillary gas chromatography

2.1 Introduction

The recent introduction of modified cyclodextrins stationary phases for capillary gas chromatography has enabled hydrocarbon enantiomers to be separated (König *et al.*, 1989b; König *et al.*, 1989c; König *et al.*, 1989d; Stoev, 1992; Alexander *et al.*, 1992a). In this chapter is reported the results obtained when racemic mixtures of alkylcyclohexylbenzenes, phenylalkanes and alkyltetralins were subjected to high resolution capillary gas chromatography using a commercially available column coated with a permethylated β -cyclodextrin.

2.2 Results

All gas chromatography was carried out using a 50 m x 0.22 i.d. SGE cydex- β capillary gas chromatography column (permethylated β -cyclodextrin phase). The range of compounds used in the study is shown in Table 2.1 together with the abbreviations used.

Table 2.1. Names, abbreviations and structures of the racemates used in the study.

Name	Abbreviations	Structures
<i>cis</i> -3'-methylcyclohexylbenzene	<i>cis</i> 3'-MCHB	
<i>trans</i> -3'-methylcyclohexylbenzene	<i>trans</i> 3'-MCHB	
<i>cis</i> -4,3'-dimethylcyclohexylbenzene	<i>cis</i> 4,3'-DMCHB	
<i>trans</i> -4,3'-dimethylcyclohexylbenzene	<i>trans</i> 4,3'-DMCHB	
<i>cis</i> -3,3'-dimethylcyclohexylbenzene	<i>cis</i> 3,3'-DMCHB	
<i>trans</i> -3,3'-dimethylcyclohexylbenzene	<i>trans</i> 3,3'-DMCHB	
4-phenyloctane	4-PhO	
3-phenyloctane	3-PhO	
2-phenyloctane	2-PhO	
5-phenyldecane	5-PhD	
4-phenyldecane	4-PhD	
3-phenyldecane	3-PhD	
2-phenyldecane	2-PhD	
1-methyl-7-isopropyltetralin	1-M-7iPT	
1,5,6,7-tetramethyltetralin	1,5,6,7-TeMT	
1,2,2,5-tetramethyltetralin	1,2,2,5-TeMT	
1,2,2,5,6-pentamethyltetralin	1,2,2,5,6-PMT	

* denotes chiral centres.

Alkylcyclohexylbenzenes

Figure 2.1 shows the partial gas chromatograms of the racemates of alkylcyclohexylbenzenes used in this study. Of these compounds only the *trans* racemates were separated except for *trans* 3,3'-dimethylcyclohexylbenzene which was not.

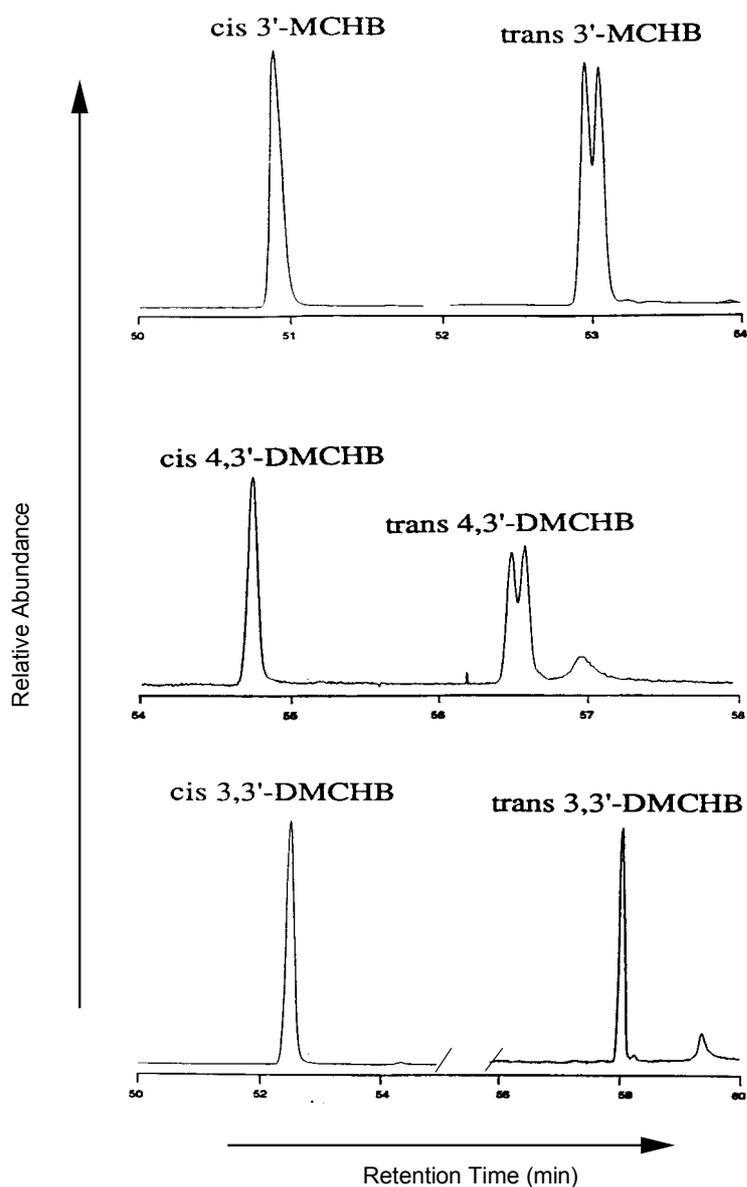


Figure 2.1. Partial gas chromatograms showing results for the attempted separation of racemic alkylcyclohexylbenzenes. Hydrogen was used as the carrier gas at a head pressure of 25 psi. The oven was programmed from 50 to 220 °C at 2 °C min⁻¹.

Phenylalkanes

Figure 2.2 shows the partial gas chromatograms of the phenylalkane racemates investigated. Of these phenylalkane racemates only 3-phenyloctane and 3-phenyldecane were partially separated.

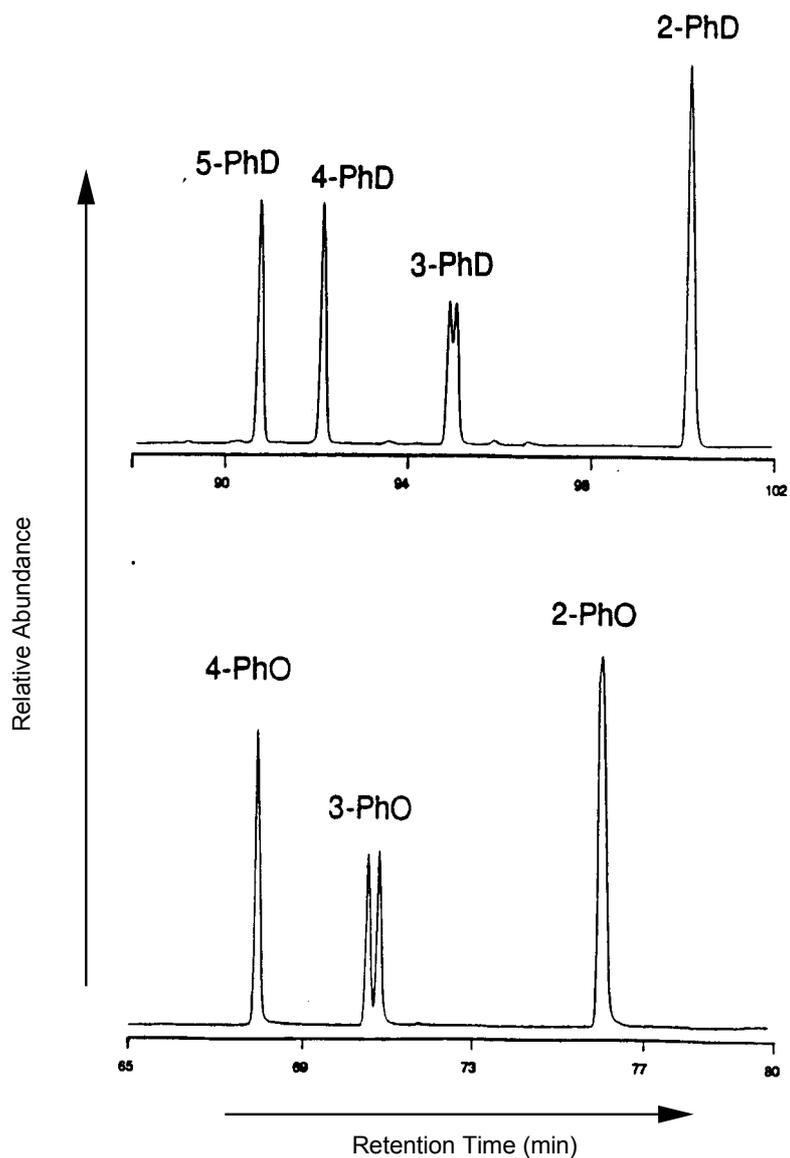


Figure 2.2. Partial gas chromatograms showing results for the attempted separation of racemic phenylalkanes. Hydrogen was used as a carrier gas at a head pressure of 25 psi. The oven was programmed from 50 to 220 °C at 2 °C min⁻¹.

Alkyltetralins

Figures 2.3 and 2.4 show the racemates of the alkyltetralins used in this study. All of the alkyltetralin racemates investigated were separated. Of the compounds investigated only 1,2,2,5-tetramethyltetralin and 1,2,2,5,6-pentamethyltetralin were identified in petroleum as racemates (Figure 2.4).

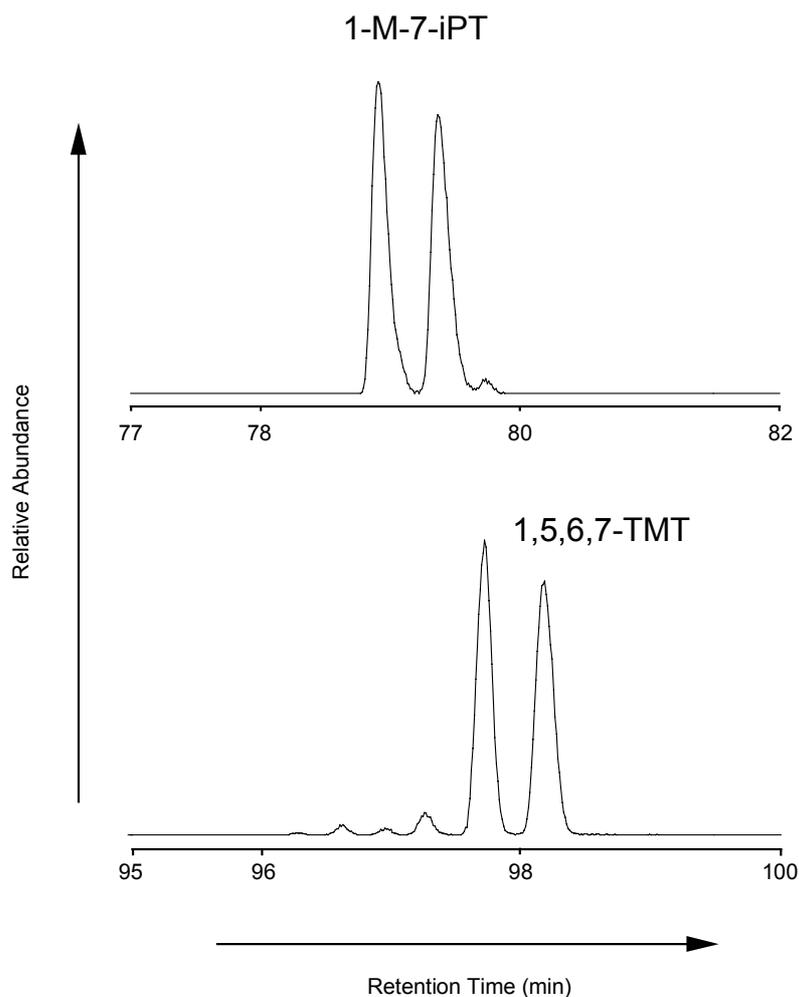


Figure 2.3. Partial gas chromatograms showing results for the attempted separation of racemic alkylcyclohexylbenzenes. Helium was used as a carrier gas at a flow velocity of 26 cm s^{-1} . The oven was programmed from 50 to $220 \text{ }^\circ\text{C}$ at $1 \text{ }^\circ\text{C min}^{-1}$.

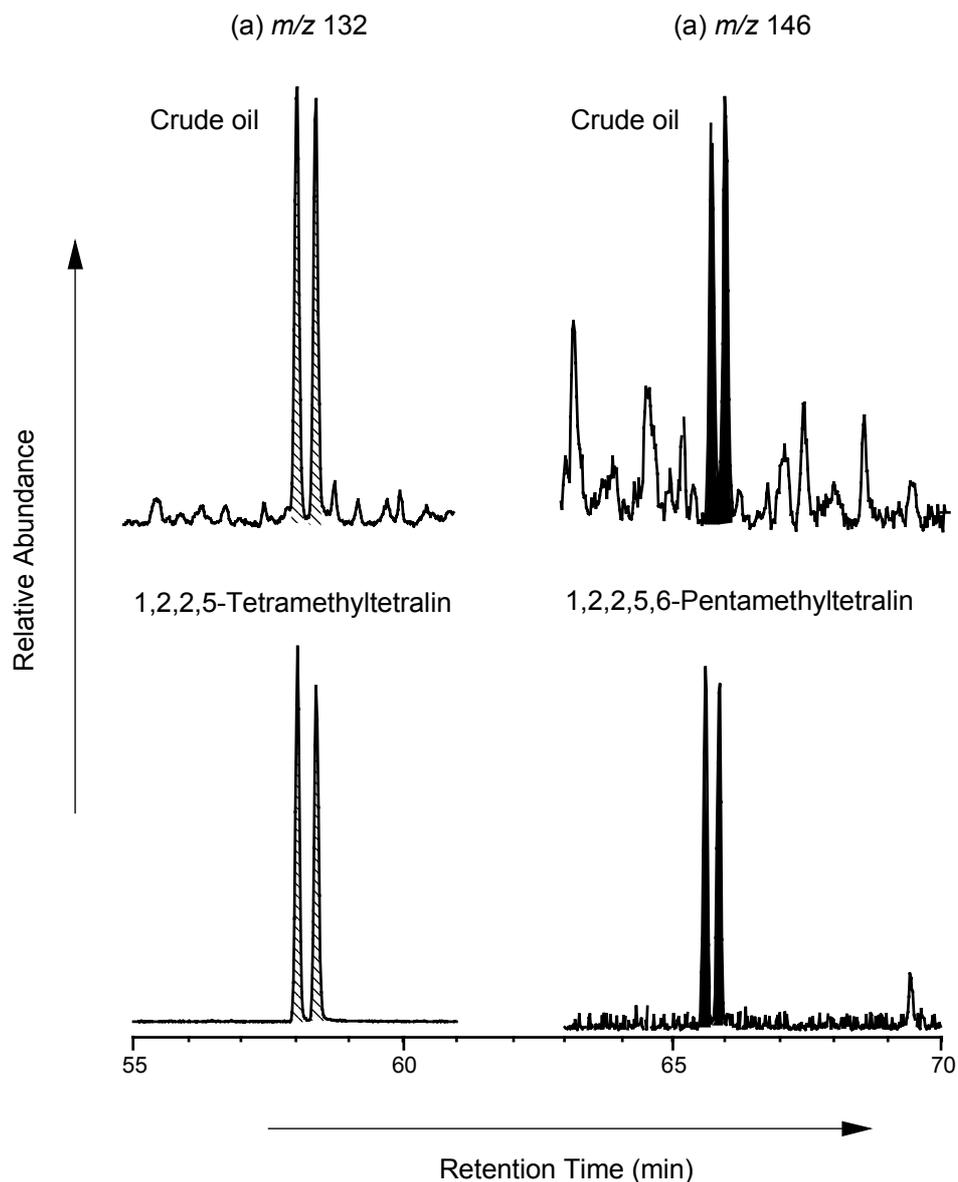


Figure 2.4. Partial m/z 132 (a) and 146 (b) mass chromatograms of the monoaromatic fraction from a crude oil from the Eromanga Basin, Australia and the racemates of 1,2,2,5-tetramethyltetralin and 1,2,2,5,6-pentamethyltetralin. Helium was used as a carrier gas at a flow velocity of 26 cm s^{-1} . The oven was programmed from 50 to $240 \text{ }^\circ\text{C}$ at $2 \text{ }^\circ\text{C min}^{-1}$ (after Alexander *et al.*, 1992a).

2.3 Conclusion

This investigation has shown the application of a cyclodextrin phase for the separation of racemic mixtures of hydrocarbons in petroleum and that the

mechanism by which the enantiomer separations occur is shape and size selective.

2.4 Acknowledgements

Technical assistance from Mr G Chidlow in the operation of the GC-MS system was greatly appreciated. Mr Ben Warton and Mr Damien McGrath are thanked for providing samples of alkylcyclohexylbenzenes. Dr P. Forster is thanked for providing a sample of 1-methyl-7-isopropyltetralin.

3.0 Identification of 1,2,2,5-tetramethyltetralin and 1,2,2,5,6-pentamethyltetralin as racemates in petroleum

3.1 Abstract

1,2,2,5-tetramethyltetralin and 1,2,2,5,6-pentamethyltetralin have been synthesized and identified as racemates in crude oil and are proposed from dehydrogenation experiments as intermediates in the formation of alkylnaphthalenes in crude oil.

3.2 Introduction

Alkylnaphthalenes are major components of the aromatic hydrocarbon fraction of most crude oils and of solvent extracts from organic-rich sediments (Tissot and Welte, 1984). Although some of the trimethylnaphthalenes and one tetramethylnaphthalene have been shown to be formed from triterpenoid precursor natural products *via* intermediate tetralins, the formation mechanisms of many of the alkylnaphthalenes remains obscure (Stachan *et al.*, 1988; Püttmann and Villar, 1987; Forster *et al.*, 1989b). In order to elucidate further the origins and formation of alkylnaphthalenes in petroleum we have synthesised 1,2,2,5-tetramethyltetralin **1** and 1,2,2,5,6-pentamethyltetralin **2** and have identified them as racemic mixtures in crude oil using gas chromatography techniques with a capillary column coated with a chiral stationary phase. These compounds have been further dehydrogenated and yielded

trimethylnaphthalenes and tetramethylnaphthalenes which also co-occur in the crude oil with the tetralins **1** and **2**.

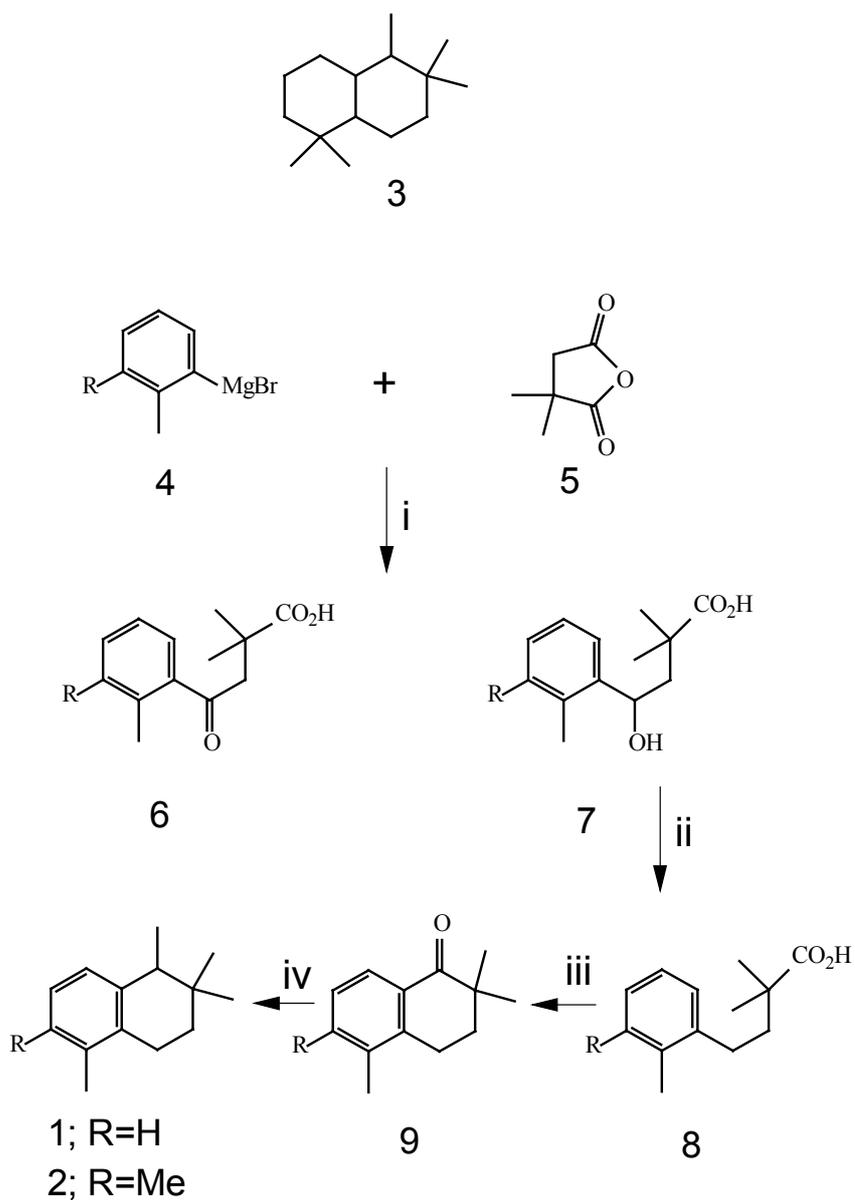
The similarity in carbon skeletons of the tetralins and the rearranged drimane **3**, a common constituent of crude oil, suggests that there may well be a link between the naphthalenes on the one hand and the drimanes on the other in that these two groups of compounds may share common precursors, most probably of microbial origin (Alexander *et al.*, 1983a)

3.3 Experimental

The tetralins **1** and **2** were conveniently prepared in good yield using the sequence of reactions shown in scheme 3.1. 2,2-Dimethylsuccinic anhydride **5** was reacted with the appropriate Grignard reagent **4** to form a mixture of the keto and hydroxy carboxylic acids **6** and **7** in the proportion 4:1 (Edwards and Kammanann, 1964). This mixture was subjected to hydrogenolysis (Augustine, 1965) to afford a mixture of carboxylic acids **6** and **8** that was then reduced using zinc amalgam (Martin, 1942) to give the acid **8**. The acid was cyclised to the ketone **9** on treatment with polyphosphoric acid (Synder and Werber, 1955). The ketone was methylated using methylmagnesium iodide and the resultant tertiary alcohol function removed by hydrogenolysis. The NMR data for the alkyltetralins are given and the mass spectrum for each compound is given in Figure 3.1.

NMR data for alkyltetralins

¹H NMR spectral data for **1** δ (CDCl₃) 0.92, 0.94 (2 x s, 3H, *gem*-dimethyl), 1.16 (d *J* 7.1 Hz, 3H, ArCH-CH₃), 1.4 (m, 1H, CHa-CMe₂), 1.7 (m, 1H, CHb-CMe₂), 2.20 (s, 3H, Ar-CH₃), 2.49 (q, *J* 7.1 Hz, 1H, Ar-CH-Me), 2.6 (m, 2H, Ar-CH₂), 7.0 (m, 3H, Ar-H). For **2** δ (CDCl₃) 0.91, 0.92 (2 x s, 3H, *gem*-dimethyl), 1.16 (d, *J* 7.1 Hz, 3H, ArCH-CH₃), 1.4 (m, 1H, CH-CMe₂), 1.8 (m, 1H, CHb-CMe₂), 2.11 (s, 3H, Ar-Me), 2.25 (s, 3H, Ar-Me), 2.46 (q, *J* 7.2 Hz, 1H, Ar-CH-Me), 2.6 (m, 2h, Ar-CH₂), 6.93 (AB q, *J* 7.3 Hz, 2H, Ar-H).



Scheme 3.1 Reagents and conditions: i. dry diethyl ether, $-20\text{ }^{\circ}\text{C}$; ii. H_2 (1 atm) Pd/C, EtOH, Zn-Hg, HCl, reflux; iii. polyphosphoric acid, $90\text{ }^{\circ}\text{C}$; iv. MeMgI, dry diethyl ether, H_2 (1 atm) Pd/C, EtOH.

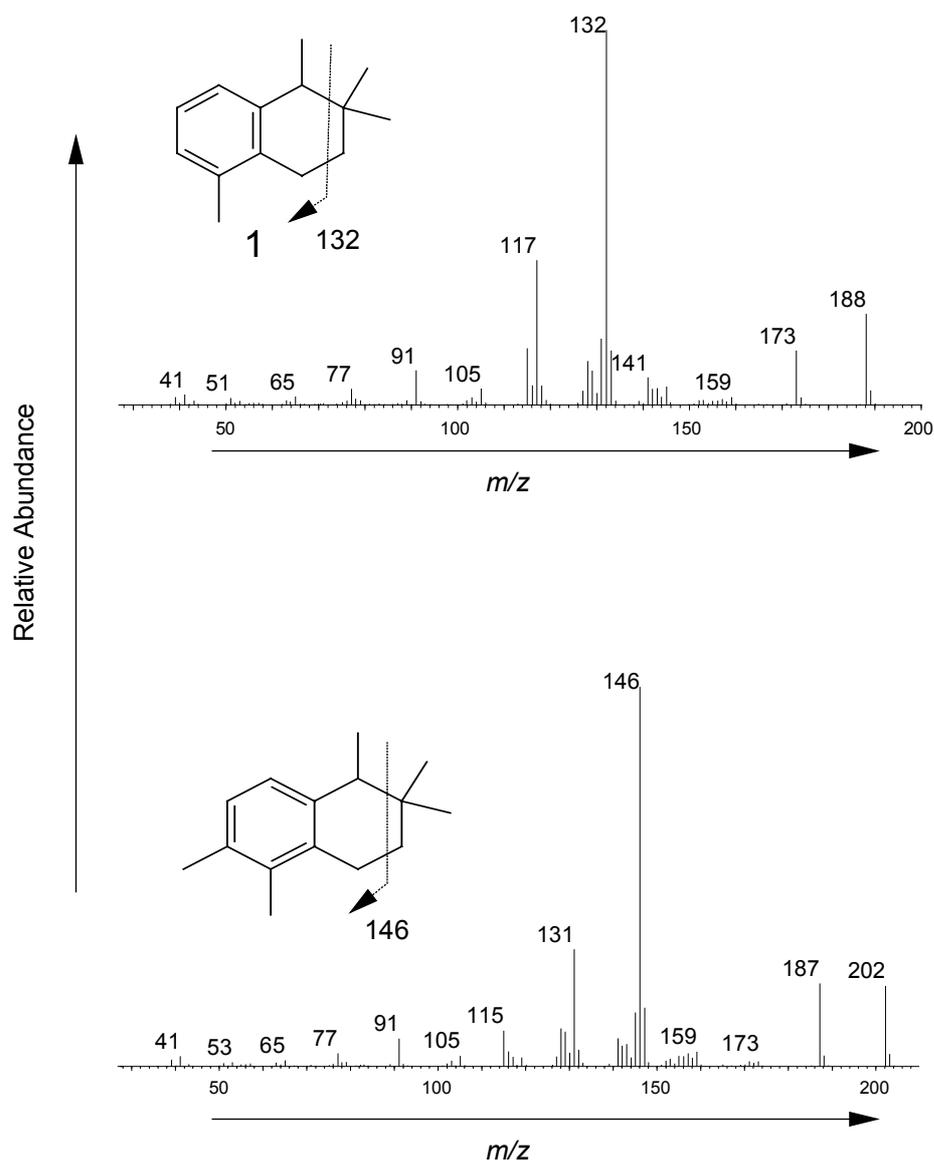


Figure 3.1. Mass spectra of tetralins **1** and **2**.

3.4 Results and discussion

Figure 3.2 shows m/z 132 and 146 partial mass chromatograms of the monoaromatic fraction of a crude oil and of racemic synthetic alkyltetralins obtained using GC-MS techniques with a capillary column coated with a chiral stationary phase. Two peaks of approximately the same area (shaded) were observed in each case representing the separate enantiomers. The compounds represented by the shaded peaks had similar mass spectra to the alkyltetralins with the same retention time. When analysed using capillary columns coated with the commonly used non-chiral phases BP-1

and BP-5 (SGE, Australia), both of the synthetic alkyltetralins gave a single peak in the chromatogram as did the corresponding components from the crude oil. These results show that 1,2,2,5-tetramethyltetralin **1** and 1,2,2,5,6-pentamethyltetralin **2** occur as racemic mixtures in the crude oil using direct gas chromatography techniques. It has been shown previously by less direct methods that certain non-aromatic isoprenoid alkanes in crude oils are also racemic mixtures (Brooks *et al.*, 1977; Patience *et al.*, 1978).

A possible source of these sedimentary alkyltetralins is the bicyclic natural products that give rise to drimane and other bicyclic hydrocarbons with skeletons that appear to be derived by rearrangement of the drimanes (Kagramanova *et al.*, 1976; Alexander *et al.*, 1983a). We have also observed that the abundances of these alkyltetralins decreases with increase in sample maturity, and suggest that they are intermediates in the formation of alkylnaphthalenes. Furthermore, treatment of 1,2,2,5-tetramethyltetralin with palladium on carbon at 300°C gave 1,2,3,5-tetramethylnaphthalene and 1,2,5-trimethylnaphthalene, two alkylnaphthalenes that are abundant in low maturity sediment samples (Stachan *et al.*, 1988; Alexander *et al.*, 1993). Under the same conditions 1,2,2,5,6-pentamethyltetralin gave 1,2,5,6-tetramethylnaphthalene and 1,2,6-trimethylnaphthalene as major products. Again, all these alkylnaphthalenes have been reported to occur in crude oil (Stachan *et al.*, 1988; Alexander *et al.*, 1993). Of special interest is the co-occurrence of 1,2,3,5-tetramethylnaphthalene and 1,2,2,5-tetramethyltetralin

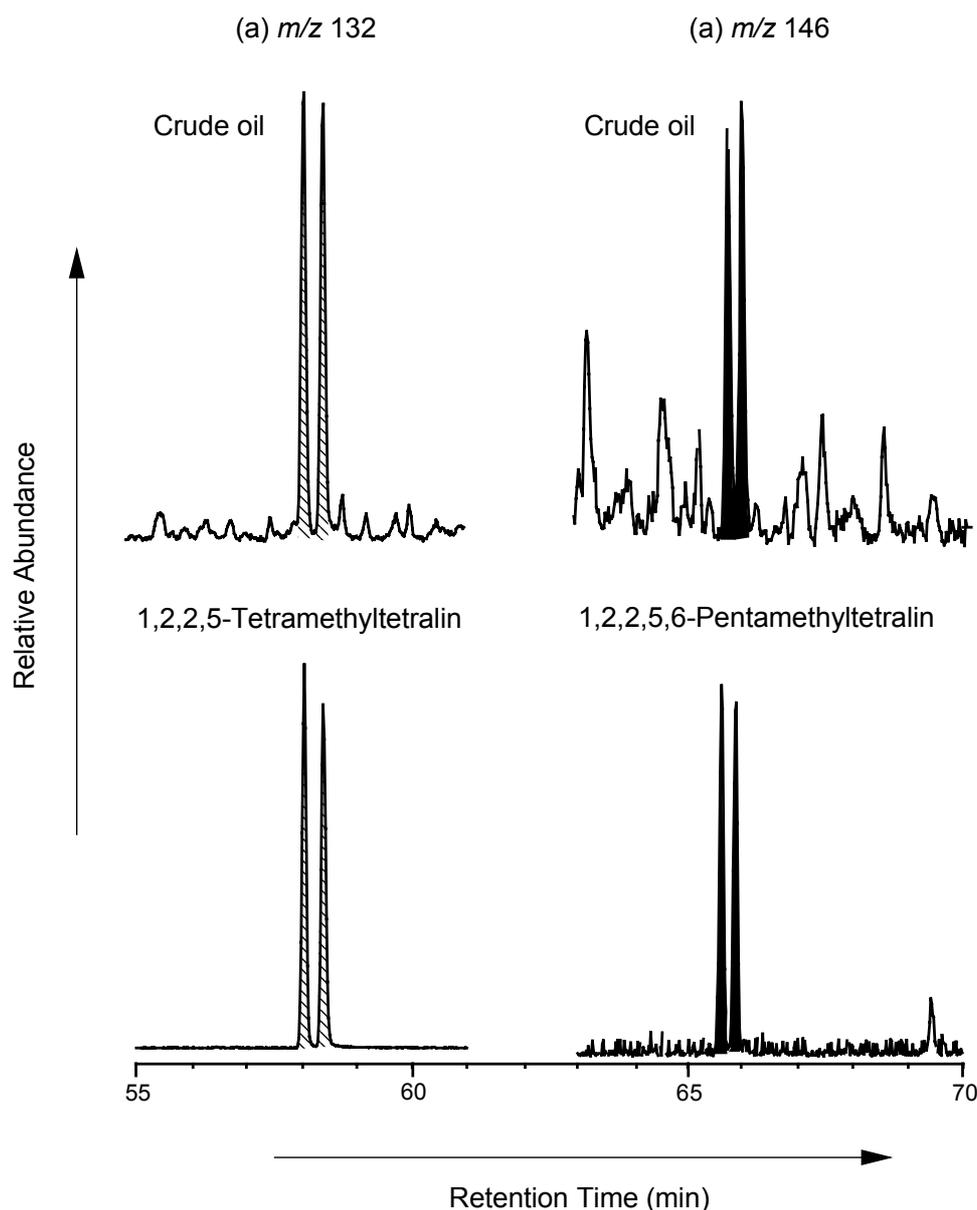


Figure 3.2. Partial m/z 132 (a) and 146 (b) mass chromatograms of the monoaromatic fraction from a crude oil from the Eromanga Basin, Australia and the alkyltetralins. A Hewlett-Packard 5890 Series II Gas Chromatograph equipped with a 5971 Series MSD was used. The chromatograph was fitted with a 50 m x 0.22 mm internal diameter fused silica capillary column with CYDEX-B (chiral) stationary phase (SGE, Australia). Helium was used as a carrier gas at a flow velocity of 26 cm s^{-1} . The oven was programmed from 50 to $240 \text{ }^\circ\text{C}$ at $2 \text{ }^\circ\text{C min}^{-1}$. The MSD was operated with a 70 eV electron beam energy, a source temperature of $240 \text{ }^\circ\text{C}$ and 1680 V EM voltage.

in the crude oil used in this study, suggesting that there may be a product-precursor relationship between these two compounds in the natural system, similar to that shown in our laboratory dehydrogenation experiments.

3.5 Acknowledgements

Geoff Chidlow is thanked for his assistance with GC-MS analysis. Financial support was provided by the Australian Petroleum Co-operative Research and from a Curtin University Postgraduate Scholarship.

4.0 Identification and analysis of dihydro-*ar*-curcumene enantiomers and related compounds in petroleum

4.1 Abstract

Isodihydro-*ar*-curcumene [1-(1,5-dimethylhexyl)-3-methylbenzene] has been identified in petroleum using GC-MS techniques by comparing its retention time and mass spectrum with the same parameters from a synthetic reference compound. It co-occurs in crude oil with dihydro-*ar*-curcumene [1-(1,5-dimethylhexyl)-4-methylbenzene] and is suggested to originate from this compound *via* a sedimentary rearrangement process. Enantiomers of both compounds have also been shown to occur in crude oils using chiral GC-MS techniques. The elution order of the enantiomers has been established using reference compounds of known configuration.

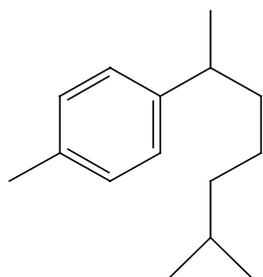
4.2 Introduction

Separation and analysis of trace amounts of hydrocarbon enantiomers in complex mixtures has been made possible by recent developments in chiral gas chromatography. The most widely used chiral stationary phases employ derivatised cyclodextrins as the enantioselective component. A recent review of chiral phases and their applications is given by Schurig (1994) and references contained therein. Briefly, the α -, β - and γ -cyclodextrins provide a macrocyclic system comprising of 6, 7 or 8 D-glucose units respectively. The

molecular structures are such that they appear as truncated cones with internal diameters ranging from 0.57, 0.78 and 0.95 nm. Capillary columns prepared with stationary phases from derivatives of these cyclodextrins have been shown to effectively separate enantiomers of a range of hydrocarbons of the types that occur in geological and environmental samples. These include branched alkanes, alkylbenzenes, alkylbiphenyls (Kobor *et al.*, 1993), tricyclic alkanes and branched aromatic hydrocarbons (Tang *et al.*, 1994). Recently, we have reported successfully applying these techniques to the analysis of sedimentary hydrocarbon mixtures (Alexander *et al.*, 1992a; Bastow *et al.*, 1993).

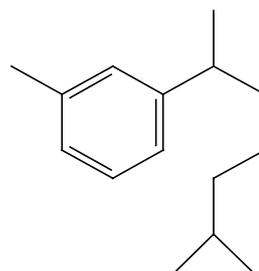
Dihydro-*ar*-curcumene (I) occurs widely in sedimentary rocks. Ellis *et al.*, (1995a) have shown that it occurs in crude oils and sediments with a wide range of depositional settings and ages. Its association with biomarkers of higher plant origin led them to suggest that it too was primarily derived from higher plants. Because of its widespread distribution in sedimentary rocks it is a worthwhile candidate for more detailed geochemical investigation as the properties of its various isomeric forms could provide insights into geochemical processes in a similar manner, for example, the isomeric steranes (Peters and Moldowan, 1991).

Monocyclic sesquiterpenoids with the same carbon skeleton as dihydro-*ar*-curcumene are common constituents of essential oils derived from the wood resins of extant higher plants (Dev, 1989). Natural products with this carbon skeleton (bisabolane) are usually unsaturated and a few are aromatic (Figure 4.1). For example, curcumene (III) isolated from turmeric oil (Simonsen, 1952) and nuciferal (V), obtained from the wood oil of the Kaya tree (Dev, 1989), are two natural plants of this structural type.



(I)

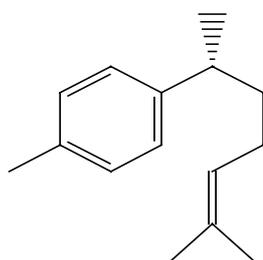
Dihydro-*ar*-curcumene



(II)

Isodihydro-*ar*-curcumene

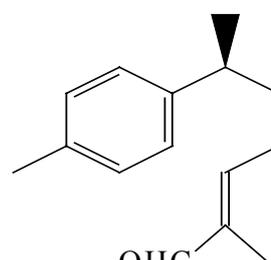
R Enantiomers



(III)

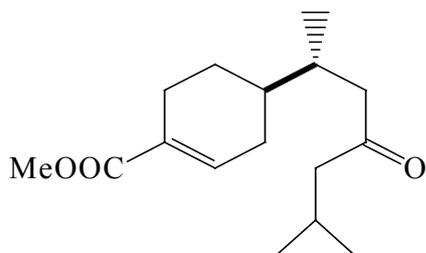
(-)- α -Curcumene
(Turmeric oil)

S Enantiomers



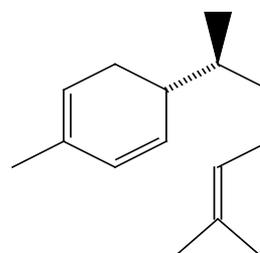
(V)

Nuciferal
(Conifer)



(IV)

(+)-Juvabione
(Insect juvenile hormone
from conifers)



(VI)

(-)-Zingiberene
(Angiosperm)

Figure 4.1. Molecular structures and chirality of some natural products with bisabolane skeletons.

The stereochemistry of this group of monocyclic sesquiterpenoids is based on the reported absolute stereochemistry of (-)- α -curcumene (III) established using material isolated from turmeric oil (Honward and Rao, 1965). Most of the natural products have a chiral centre adjacent to the ring. Natural products derived from higher plants have been reported with both the R and S configurations at this position. (-)- α -Curcumene (III) and (+)-juvabione (IV) are examples of the R configuration and nuciferal (V) and zingiberene (VI) have the S configuration.

In this chapter is reported the identification of a ring isomer of dihydro-*ar*-curcumene in crude oils, the application of chiral GC-MS for the separation of the enantiomers of this compound and of dihydro-*ar*-curcumene in hydrocarbon fractions from petroleum.

4.3 Experimental

Sample

MD-8 is an Indonesian crude oil of Miocene age. The sterane 20S/20R value of 1.1 indicates that it was released from mature source rocks.

Isolation of monoaromatic fractions

In a typical separation, a crude oil or sediment extract (100 mg) was subjected to preparative thin layer chromatography on glass plates spread with silica gel (Merck Silica Gel 60 PF₂₅₄: 0.6 mm, activated at 140°C for at least 12h). The plates were developed with hexane and the required band comprising mononuclear aromatic compounds was located relative to reference compounds (hexamethylbenzene, naphthalene, phenanthrene) using UV light (254 nm). The organic material was recovered using a Kuderna-Danish apparatus, and the residue was taken up in hexane (300 μ L) to provide a sample for GC-MS analysis.

Isolation of (-)- α -curcumene from turmeric

Ground turmeric was steam distilled and the oil recovered. Turmeric oil was subjected to liquid chromatography using alumina and pentane as solvent. The fraction containing alkenes and monoaromatic compounds was isolated and then hydrogenated using 10% palladium-carbon catalyst and ethanol as solvent. The reaction products were then separated using alumina TLC plates and hexane as solvent. Pure dihydro-*ar*-curcumene (R) was recovered from the plate.

Preparation of isodihydro-ar-curcumene

The Grignard reagent prepared from 4-methylpentyl bromide (8 mmol) and magnesium (10 mmol) (Furniss *et al.*, 1989) was added to 3-methylacetophenone (8 mmol) in ether. The resulting benzylic alcohol was extracted and subjected to hydrogenolysis using palladium/carbon (10%) as a catalyst in glacial acetic acid with a hydrogen pressure of 1.5 atm for 48 h. The crude hydrocarbon product (75%) was further purified by chromatography using activated alumina with *n*-hexane as solvent to obtain isodihydro-*ar*-curcumene [1-(1,5-dimethylhexyl)-3-methylbenzene]: $^1\text{H-NMR}$ (200 Mhz, CDCl_3) δ (ppm relative to TMS): 0.83 (*dd*, J_1 1.7, J_2 6.6, 6H, $\text{CH}(\text{CH}_3)_2$), 1.09-1.29 (*m*, 4H, $\text{Ar-CHR-CH}_2\text{-CH}_2\text{-CH}_2\text{-R}'$), 1.215 (*d*, J 7.0, 3H, Ar-CHR-CH_3), 1.40-1.60 (*m*, 3H, $\text{R-CH}(\text{CH}_3)_2$, $\text{Ar-CHR-CH}_2\text{-CH}_2\text{-CH}_2\text{R}'$), 2.31 (*s* 3H, Ar-CH_3), 2.53-2.71 (*m*, 1H, $\text{Ar-CHRR}'$), 6.93-7.21 (*m*, 4H, Ar-H); $^{13}\text{C-NMR}$ (50 MHz, CDCl_3) δ (ppm relative to TMS): 21.51 (1C, Ar-CH_3), 22.35, 22.61, 22.72 (3C, R-CH_3), 25.57 (1C, $\text{Ar-CHR-CH}_2\text{-CH}_2\text{-CH}_2\text{R}'$), 27.91 (1C, $\text{RCH}(\text{CH}_3)_2$), 38.75, 39.15 (2C, $\text{Ar-CHR-CH}_2\text{-CH}_2\text{-CH}_2\text{R}'$), 39.96 (1C, $\text{Ar-CHRR}'$), 124.01, 126.54, 127.82, 128.20 (4C, unsub-Ar) 137.76, 147.97 (2C, sub-Ar).

The mass spectrum of this compound is shown in Figure 4.2

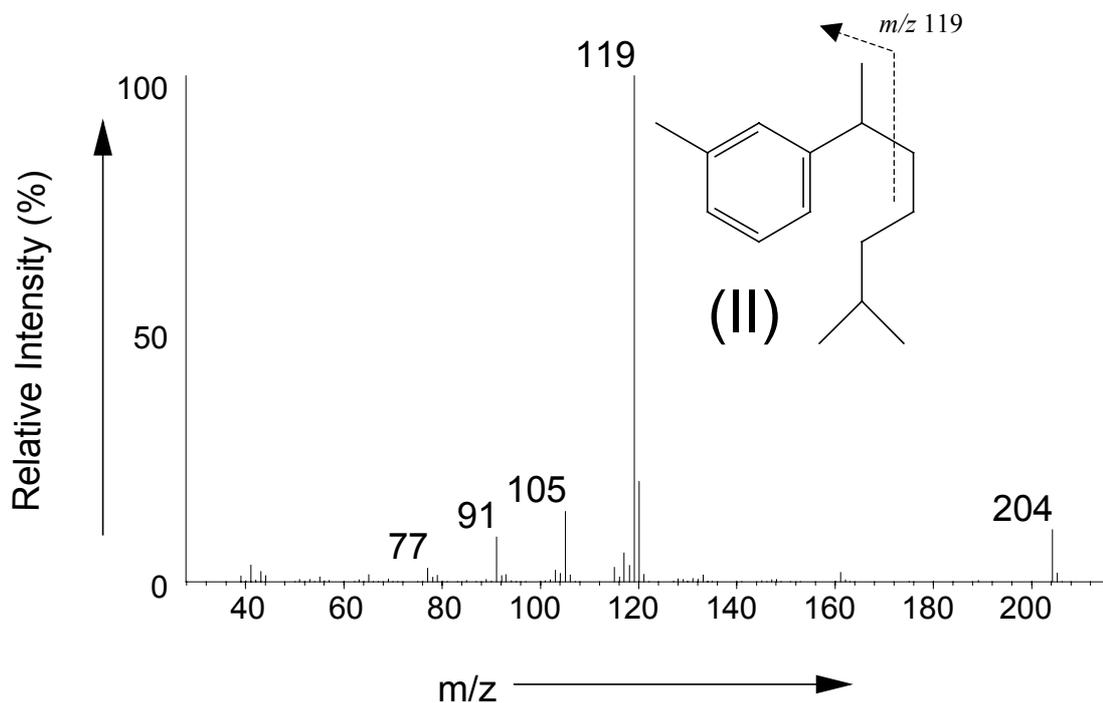


Figure 4.2. Mass spectrum of isodihydro-*ar*-curcumene.

Heating experiments

The aluminium montmorillonite used in the study was material prepared for an earlier study (Alexander *et al.*, 1984a). Dihydro-*ar*-curcumene (R) (1 mg), and biphenyl (1 mg) and finely divided aluminium montmorillonite (10 mg) were placed in small (3 mL) Pyrex glass ampoules and flushed with dry nitrogen. The ampoules were evacuated, sealed and then heated at various times and temperatures. The contents were extracted with dichloromethane (2 mL), the solvent was removed by careful distillation, and hexane (1.8 mL) containing the external standard hexamethylbenzene (1 mg) was added. An aliquot was then subjected to analysis using GC-MS techniques.

Gas chromatography-mass spectrometry (GC-MS)

A Hewlett-Packard 5971 MSD interfaced with a 5890 Series II gas chromatograph fitted with a DB-5 (40 m x 0.18 mm i.d., J&W Scientific) or a DB-1 (60 m x 0.25 mm i.d., J&W Scientific) fused silica open tubular column was used. The GC oven was temperature programmed from 70-300°C at 3°C min⁻¹. Samples for analysis were dissolved in hexane and injected on-column using a HP 7673 auto sampler at an oven temperature of 70°C. Helium was used as a carrier gas at a linear velocity of 25 cm s⁻¹. Typical MSD conditions were: ionisation energy 70 eV; source temperature 200°C; electron multiplier voltage 2200 V. Dihydro-*ar*-curcumene and isodihydro-*ar*-curcumene were assigned by mass chromatography of their parent ions and by comparison of retention times with reference compounds.

Chiral gas chromatography-metastable reaction monitoring (MRM-Q)

A Fisons VG-Autospec Ultima Q interfaced with a Hewlett-Packard 5890 Series II gas chromatograph was used. The GC was fitted with a 50 m x 0.22 mm i.d. fused silica capillary column custom coated (SGE, Australia) with 6% permethyl- γ -cyclodextrin in DB1701 stationary phase. Hydrogen was used as a carrier gas at a linear flow velocity of 46 cm s⁻¹. The oven temperature was programmed from 50-230°C at 1°C min⁻¹. The mass spectrometer was operated in MRM mode monitoring the parent-daughter transition of *m/z* 204 to 119.

4.4 Results and discussion

Reference compounds

A racemic mixture of dihydro-*ar*-curcumene (I) was available from a previous study (Ellis *et al.*, 1995a). The optically pure R enantiomer was prepared by partial hydrogenation of (-)- α -curcumene (III) isolated from turmeric oil (Simonsen, 1952). The compound described as isodihydro-*ar*-curcumene [1-(1,5-dimethylhexyl)-3-methylbenzene (II)] was also prepared as a racemate in an analogous manner to dihydro-*ar*-curcumene but starting with *m*-methylacetophenone.

Identification and analysis

Dihydro-*ar*-curcumene and isodihydro-*ar*-curcumene were identified in crude oils by comparison of GC-retention time and mass spectral data with those of the reference compounds. Data for dihydro-*ar*-curcumene has recently been reported (Ellis *et al.*, 1995a). In the case of isodihydro-*ar*-curcumene, GC-MS analysis of petroleum fractions using columns with non-chiral stationary phases (DB-1 and DB-5) resulted in co-elution of the reference compound with a compound from petroleum that also gave a similar mass spectrum to the reference compound. Figure 4.3 shows partial mass chromatograms (*m/z* 119) for the synthetic isodihydro-*ar*-curcumene and for a crude oil fraction. It is apparent from these chromatograms that isodihydro-*ar*-curcumene is well separated from dihydro-*ar*-curcumene and that it has a retention time approximately 40 seconds less than dihydro-*ar*-curcumene. Retention indices relative to PAH standards for dihydro-*ar*-curcumene and isodihydro-*ar*-curcumene are listed in Table 4.1. The retention indices were calculated using the method of Lee *et al.* (1979).

Table 4.1. Retention indices for dihydro-*ar*-curcumene and isodihydro-*ar*-curcumene.

Compound	Structure	Retention Index I ^{a)}	
		DB-1	DB-5
dihydro- <i>ar</i> -curcumene	I	250.03	245.40
isodihydro- <i>ar</i> -curcumene	II	247.98	243.24

a) $I = I_{(\text{naphthalene})} + (t_R - t_{R(\text{naphthalene})}) / (t_{R(\text{phenanthrene})} - t_{R(\text{naphthalene})}) \times 100$, where, $I_{(\text{naphthalene})} = 200$ and t_R is the retention time.

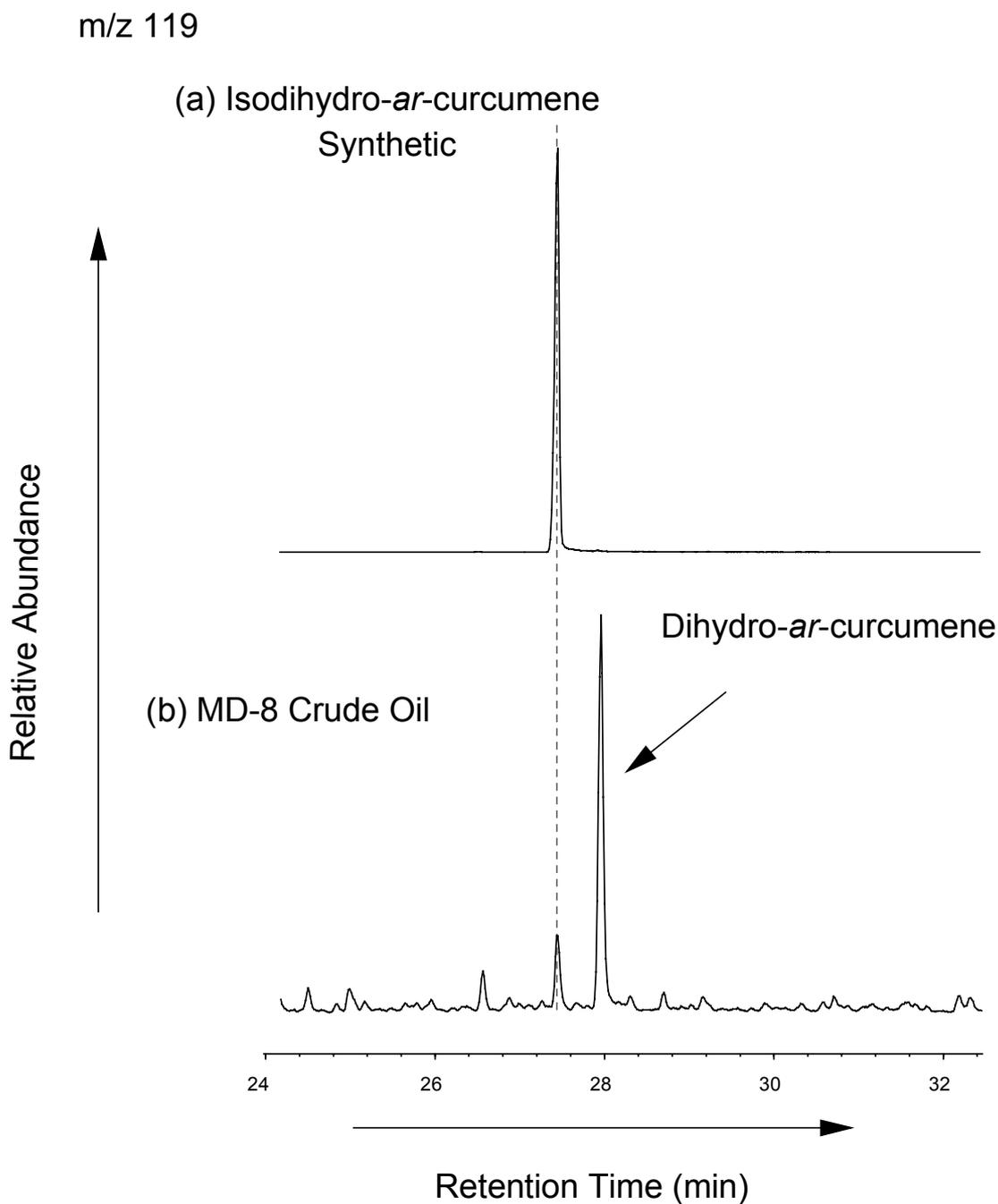


Figure 4.3. Mass chromatograms (m/z 119) from (a) isodihydro-*ar*-curcumene and (b) a crude oil fraction, using a DB-5 capillary column.

Separation of enantiomers was achieved using a high performance capillary column coated with a mixture of permethyl- γ -cyclodextrin and cyanopropylphenyldimethylsiloxane. This type of stationary phase has been shown to effectively separate monoaromatic hydrocarbon racemates with

benzylic chiral centres (Alexander *et al.*, 1992a; Bastow *et al.*, 1993). Using a column of this type the enantiomers of dihydro-*ar*-curcumene and isodihydro-*ar*-curcumene could be separated. Figure 4.4 shows mass chromatograms from the reference compounds and from a crude oil using MRM techniques (m/z 204- \rightarrow 119). A single peak was obtained from the dihydro-*ar*-curcumene (R) that had been prepared from the optically pure natural product (-)- α -curcumene. The two synthetic reference compounds prepared as racemates gave doublets indicating that partial resolution of enantiomers had been achieved. It is apparent from these chromatograms that the enantiomers of both compounds have been separated and that these compounds are present in the crude oil.

The elution order of the enantiomers of each compound has been determined. In the case of dihydro-*ar*-curcumene the R enantiomer was shown to have the shorter retention time. Figure 4.4 shows mass chromatograms using MRM techniques for the reference compound derived from a natural product with the R configuration, and the racemic mixture from synthesis. The first eluting enantiomer in the synthetic product had the same retention time as the single enantiomer of natural origin, and was therefore assigned as the R enantiomer. For isodihydro-*ar*-curcumene no optically pure reference compounds were available. However a laboratory heating experiment in which optically pure dihydro-*ar*-curcumene (R) was isomerised to isodihydro-*ar*-curcumene with substantial retention of configuration provided a reference compound that was used to assign the elution order of the enantiomers. Figure 4.5 shows mass chromatograms (MRM) for the transition m/z 204 \rightarrow 119 for the products obtained from heating dihydro-*ar*-curcumene (R) with a Lewis acid catalyst (aluminium montmorillonite). It is clear from the experiment at 140°C that the initially formed isodihydro-*ar*-curcumene is predominantly one enantiomer. We

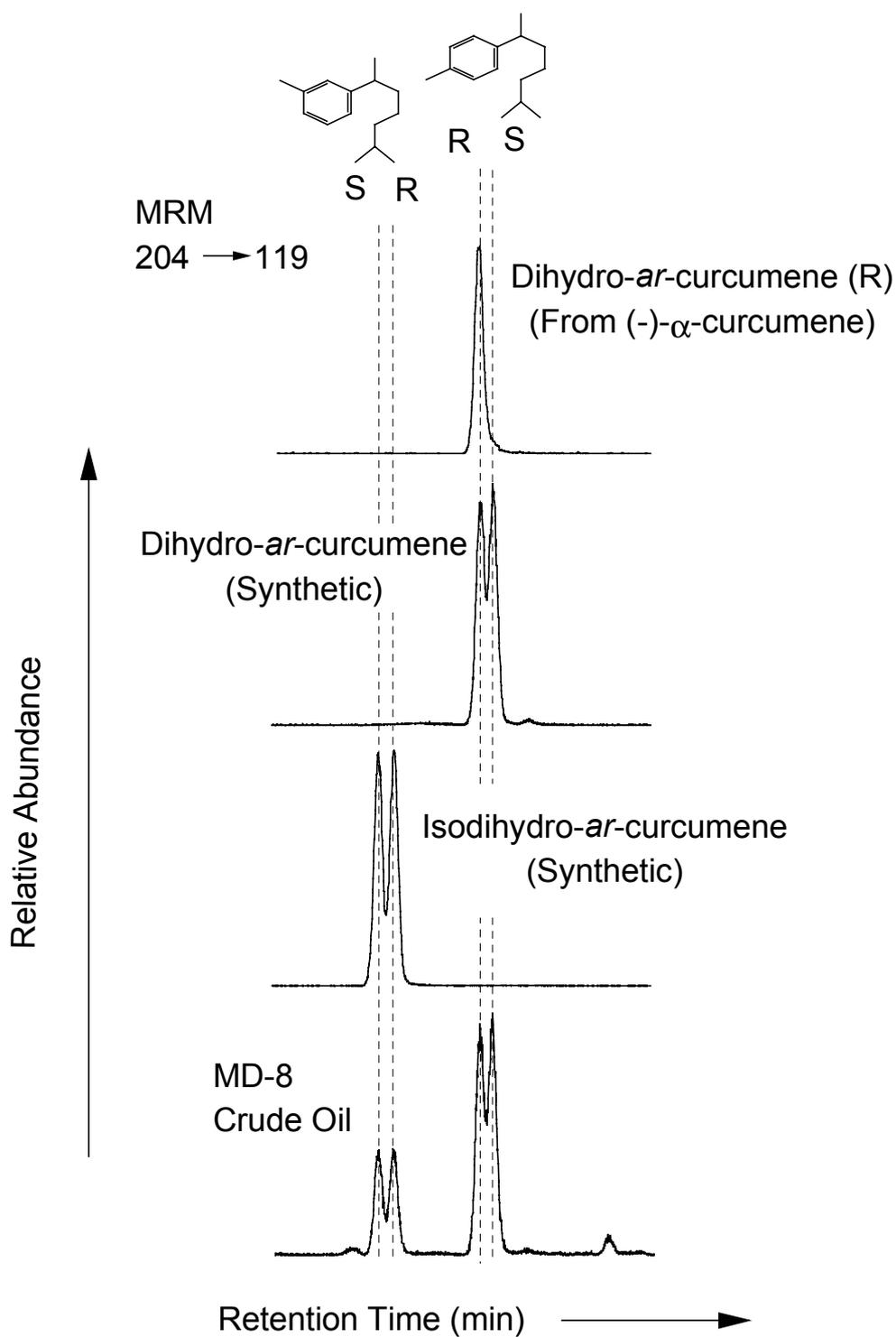


Figure 4.4. Mass chromatograms (MRM-Q) for m/z 204 \rightarrow 119 transitions showing the enantiomeric compositions of dihydro-*ar*-curcumene (R), dihydro-*ar*-curcumene (synthetic), isodihydro-*ar*-curcumene (synthetic), and a crude oil fraction (MD-8).

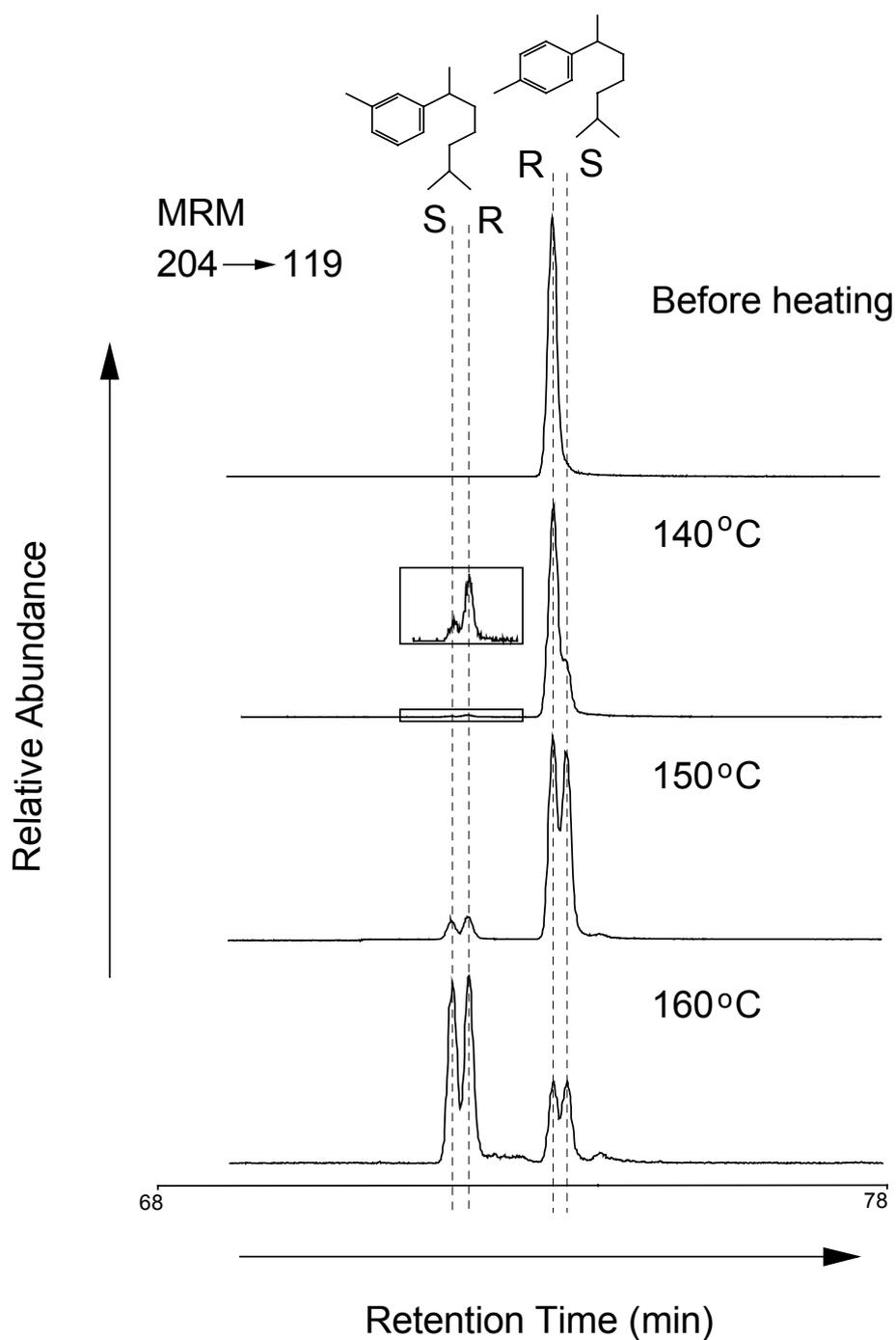


Figure 4.5. Partial mass chromatograms (m/z 204→119) showing the relative abundances of dihydro-*ar*-curcumene and isodihydro-*ar*-curcumene for reaction products from heating dihydro-*ar*-curcumene (R) with aluminium montmorillonite for two hours.

suggest that this major enantiomer has retained the configuration of the substrate and therefore has the R configuration. The results from higher temperature experiments show that racemisation of both compounds occurs under more forcing conditions.

Isodihydro-*ar*-curcumene is probably derived from dihydro-*ar*-curcumene by a sedimentary rearrangement reaction. Although dihydro-*ar*-curcumene is derived from natural product precursors, the carbon skeleton of isodihydro-*ar*-curcumene has not been reported in natural products. Furthermore, the facile rearrangement of dihydro-*ar*-curcumene when treated with acid clays under laboratory conditions suggests that it could form in sediments by similar processes. The relative abundance of the two compounds and the extent of racemisation in crude oils and sediments may therefore depend on a combination of heating and the catalytic effects of minerals and macerals.

4.5 Conclusions

Isodihydro-*ar*-curcumene [1-(1,5-dimethylhexyl)-3-methylbenzene (II)] has been shown to occur in petroleum. This compound may be derived from dihydro-*ar*-curcumene of natural origins by a sedimentary rearrangement reaction. Dihydro-*ar*-curcumene and isodihydro-*ar*-curcumene have been shown to occur as racemates in petroleum. The first eluting enantiomer has the R configuration in the case of dihydro-*ar*-curcumene, however the first eluting enantiomer of isodihydro-*ar*-curcumene has the S configuration.

4.6 Acknowledgements

Geoff Chidlow is thanked for his assistance with GC-MS analysis. Financial support was provided by the Australian Petroleum Co-operative Research and from a Curtin University Postgraduate Scholarship.

5.0 The effect of maturity and biodegradation on the enantiomeric composition of sedimentary dihydro-*ar*-curcumene and related compounds

5.1 Abstract

The effects of maturity and biodegradation on dihydro-*ar*-curcumene and isodihydro-*ar*-curcumene enantiomers have been assessed by applying chiral GC-MS techniques to aromatic hydrocarbon fractions obtained from crude oils and sedimentary rocks. Dihydro-*ar*-curcumene of natural origins undergoes rapid racemisation in the subsurface, yielding a racemic mixture before the onset of significant oil formation. 1,2-Alkyl shifts on the aromatic ring also begin at an early stage to yield isodihydro-*ar*-curcumene and continue with increasing maturity. Laboratory experiments using proton catalysts and clay (Lewis acid catalyst) show that the alkyl shift reaction is catalysed by proton and Lewis acids and racemisation is only catalysed by Lewis acids. A moderately biodegraded crude oil has been shown to be depleted in the *R* enantiomer of dihydro-*ar*-curcumene and an extensively degraded oil has dihydro-*ar*-curcumene depleted relative to isodihydro-*ar*-curcumene. These results indicate preferential metabolism of both enantiomers and positional isomers by degrading micro organisms.

5.2 Introduction

Dihydro-*ar*-curcumene has recently been reported to occur widely in sedimentary organic matter that postdates the evolution of higher plants (Ellis *et al.*, 1995a). The occurrence of this compound in association with other biomarkers of land-plant origin, and the widespread occurrence of compounds with this carbon skeleton in plant natural products, led to the suggestion that it too was derived from higher plants (Ellis *et al.*, 1995a). However, a natural product with this carbon skeleton (bisabolane) has also been reported in an Australian marine sponge (Fraga, 1993). A related compound, isodihydro-*ar*-curcumene, in which the methyl substituent on the aromatic ring is *meta* to the alkyl group, has also been identified in sedimentary organic matter (Bastow *et al.*, 1997). Because this compound has a carbon skeleton not reported in natural products it was suggested to be a rearrangement product of dihydro-*ar*-curcumene or its precursors (Bastow *et al.*, 1997).

Chiral GC-MS techniques have recently been used to analyse for enantiomers of both dihydro-*ar*-curcumene and isodihydro-*ar*-curcumene. Bastow *et al.*, (1997) have shown that both compounds occur as racemic mixtures in mature samples. However samples of low maturity, such as lignite, contain predominantly one enantiomer reflecting the configuration of the precursor natural product.

In this paper we report on the change in composition of enantiomers and in positional isomers of dihydro-*ar*-curcumene with maturity and biodegradation. A reaction mechanism for racemisation and positional isomerisation is also suggested.

5.3 Experimental

Samples

The locations of the samples and their ages, or in the case of crude oils the probable ages of their source rocks, are shown in Table 5.1. Typical ash (dry basis) values for the HCH samples were 10-20 % for the lignites and 55-75 % for the shales. Some geochemical data for the samples are given in Table 5.2.

Table 5.1. Location, lithology and age of samples.

Sample		Country	Basin	Probable age of source rocks
Oils				
Leatherjacket-1		Australia	Gippsland	Cretaceous
Moorari-4		Australia	Eromanga	Jurassic
Roller-1		Australia	Carnarvon	Jurassic
MD-8		Indonesia	Mahakam Delta	Tertiary
Wirrah-1		Australia	Gippsland	Cretaceous
Sediments	Lithology			
GK	Shale/Sand/Coal	Indonesia	S.Sumatara	Tertiary
HCH 28 (19.10 m)	Shale	Australia	Bremer	Miocene
HCH 28 (19.30 m)	Shale	Australia	Bremer	Miocene
HCH 28 (19.55 m)	Lignite	Australia	Bremer	Miocene
HCH 34 (21.8 m)	Lignite	Australia	Bremer	Miocene
Volador-1	Shale/Sand/Coal	Australia	Gippsland	Cretaceous

Volador-1; for detailed description of the tectonic history and basin development refer to Thompson (1985), Hegarty *et al.* (1985) and references therein. GK; for detailed description of the tectonic history, basin development and geochemical data refer to Sosrowidjojo *et al.* (1996) and references therein.

Reference compounds

A racemic mixture of dihydro-*ar*-curcumene (D) was available from a previous study (Ellis *et al.*, 1995a). Optically pure dihydro-*ar*-curcumene (R) and a racemic mixture of isodihydro-*ar*-curcumene (ID) were also available from a previous study (Bastow *et al.*, 1997). Compound ratios i.e. ID/(ID +

D), Figure 5.1, were calculated from m/z 119 responses using a response factor of 1.28 for isodihydro-*ar*-curcumene.

Table 5.2. Maturity and biodegradation data for samples.

Sample	$\frac{20S}{20R}$	$\frac{T_s}{T_s + T_m}$	MPI-1	Degree of biodegradation
Oils				
Leather Jacket-1	0.9	0.4	0.4	extensive
Moorari-4	0.6	0.1	0.2	none
Roller-1	0.6	0.6	0.7	moderate
MD-8	1.1	0.4	0.7	none
Wirrah-1	0.8	0.4	0.7	none
Sediments				
HCH 28 (19.10 m)	<0.1	<0.1	0.3	none
HCH 28 (19.30 m)	<0.1	<0.1	0.2	none
HCH 28 (19.55 m)	<0.1	<0.1	0.1	none
HCH 34 (21.8 m)	<0.1	<0.1	0.4	none

Definitions and methods of measurements: 20S/20R, 20S and 20R diastereomers of 5 α (H), 14 α (H), 17 α (H)-ethylcholestane (m/z 217) (Mackenzie and McKenzie, 1983; Marzi and Rullkötter, 1992); Ts/(Ts + Tm), 17 α (H)-trisnorhopane (Tm) and 18 α (H)-trisnorhopane (Ts) (m/z 191) (Seifert and Moldowan, 1978); MPI-1. GC-MS data corrected to FID response (Radke *et al.*, 1982a,b); degree of biodegradation (Volkman *et al.*, 1983a,b).

Sample preparation

Rock samples were air dried, crushed to a fine powder using a Tema mill and extracted ultrasonically with dichloromethane/methanol (95:5). The solvent extract was recovered by filtration and the solvent was carefully distilled to yield the soluble organic matter.

Isolation of monoaromatic fractions

In a typical separation, a crude oil or sediment extract (100 mg) was subject to preparative thin layer chromatography on glass plates spread with

aluminium oxide (Merck 60 GF₂₅₄: 0.6 mm, activated at 140°C for at least 12h). The resultant residue was again subjected to preparative thin layer chromatography on glass plates spread with silica gel (Merck Silica Gel 60 PF₂₅₄: 0.6 mm, activated at 140°C for at least 12h). The plates were developed with *n*-hexane and the required band comprising of mononuclear aromatic compounds was located relative to reference compounds using UV light (254 nm). The residue was taken up in *n*-hexane (300 µL) to provide a sample ready for GC-MS analysis.

Aluminium montmorillonite heating experiments

The aluminium montmorillonite used in the study was material prepared for an earlier study (Alexander *et al.*, 1984a). Dihydro-*ar*-curcumene (*R*) (1 mg), biphenyl (1 mg) and finely divided aluminium montmorillonite (10 mg) were placed in small (3 mL) Pyrex glass ampoules and flushed with dry nitrogen. The ampoules were evacuated, sealed and then heated at various times and temperatures. The contents were extracted with dichloromethane (2 mL), the solvent was removed by careful distillation, and *n*-hexane (1.8 mL) containing the external standard hexamethylbenzene (1 mg) was added. An aliquot was then subjected to analysis using GC-MS techniques.

Trifluoroacetic acid heating experiments

Dihydro-*ar*-curcumene (*R*) (1 mg) and trifluoroacetic acid (100 µL) were placed in small (3 mL) Pyrex glass ampoules and flushed with dry nitrogen. The ampoules were cooled with liquid nitrogen, evacuated, sealed and then heated at various times and temperatures. The contents were neutralised with aqueous sodium hydroxide, extracted with *n*-hexane (4 x 500 µL), the *n*-hexane washed with water (1 x 1000 µL) and dried with anhydrous magnesium sulphate. Hexamethylbenzene (1 mg) was added as an external standard. An aliquot was then analysed using GC-MS techniques.

Chiral gas chromatography-metastable reaction monitoring (MRM)

A Fisons VG-Autospec Ultima Q interfaced with a Hewlett-Packard 5890 Series II gas chromatograph was used. The GC was fitted with a 50 m x 0.22 mm i.d. fused silica capillary column custom coated with a 0.25 µm

thick, 6% permethyl- γ -cyclodextrin in DB1701, stationary phase (SGE, Australia). Hydrogen was used as a carrier gas at a velocity of 46 cm s⁻¹. The oven was programmed from 50-230°C at 1°C min⁻¹. The mass spectrometer was operated in MRM mode monitoring the parent-daughter transition (m/z 204 to 119).

Gas chromatography-mass spectrometry (GC-MS)

A Hewlett-Packard 5971 MSD interfaced with a 5890 Series II gas chromatograph fitted with a DB-5 (40 m x 0.18 mm i.d., phase thickness 0.40 μ m, J&W Scientific) fused silica open tubular column was used. The GC oven was temperature programmed from 70-300°C at 3°C min⁻¹. Samples for analysis were dissolved in hexane and injected on-column using a HP 7673 auto sampler at an oven temperature of 70°C. Helium was used as a carrier gas at a velocity of 25 cm s⁻¹. Typical MSD conditions were: ionisation energy 70 eV; source temperature 200°C; electron multiplier voltage 2200 V.

5.4 Results and discussion

Maturity effects

Sedimentary organic matter contains both racemic and non-racemic mixtures of the enantiomers of dihydro-*ar*-curcumene. Figure 5.2 shows the mass chromatograms obtained from monitoring the m/z 204 \rightarrow 119 transition for the monoaromatic hydrocarbon fractions from a lignite and three crude oils. The pair of peaks with the greater retention times represent enantiomers of dihydro-*ar*-curcumene (Bastow *et al.*, 1997). The relative maturity of each sample can be inferred from the 20S/20R ethylcholestane diastereomer values (Table 5.2). The very low maturity lignite (20S/20R < 0.1) contains both enantiomers of dihydro-*ar*-curcumene, although the S enantiomer is the

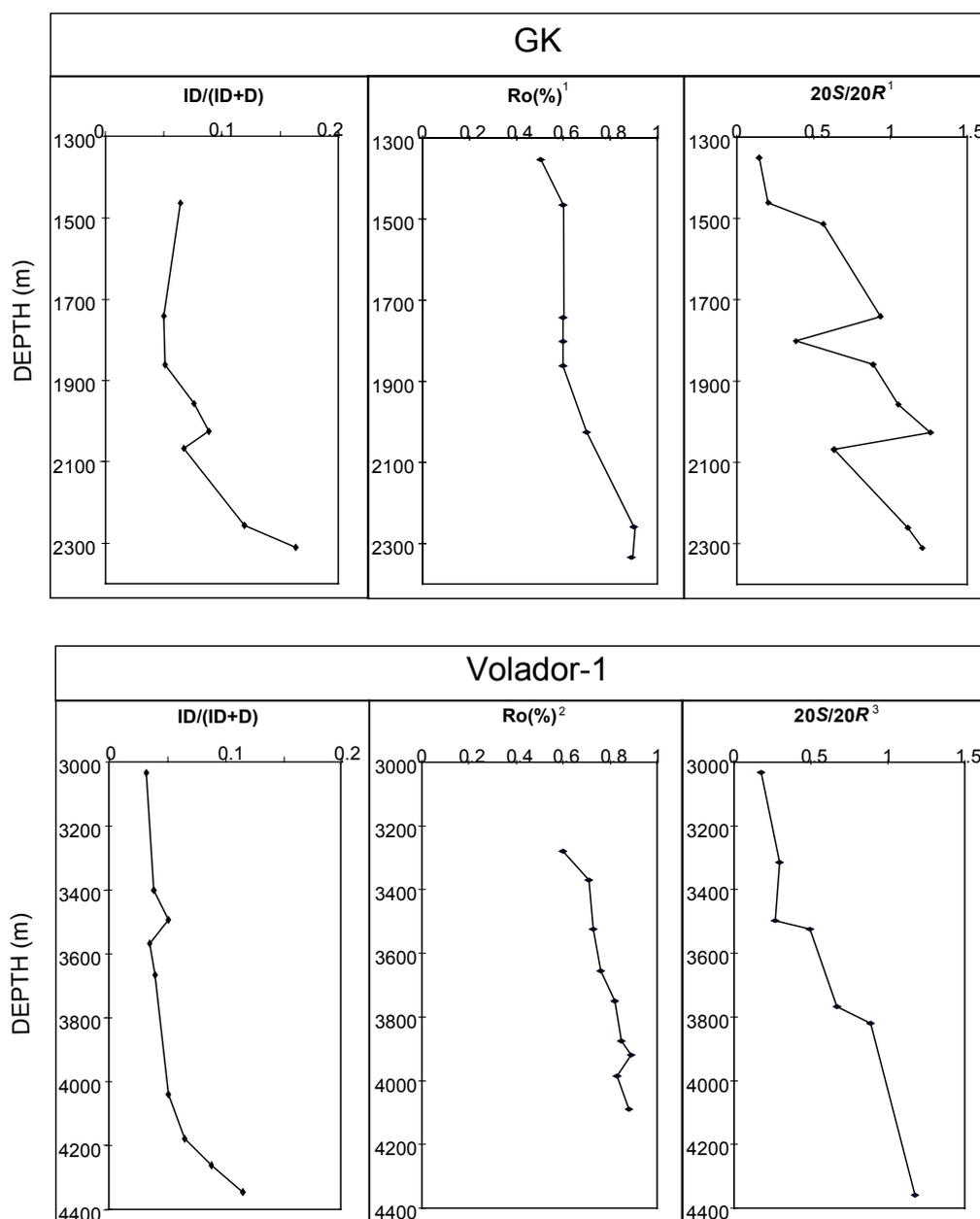


Figure 5.1. Plots showing changes in the relative abundance of isodihydro-*ar*-curcumene and dihydro-*ar*-curcumene, vitrinite reflectance (R_o) and ethylcholestane diastereomers with depth for (a) GK and (b) Volador-1 wells. 1. Data from Sosrowidjojo *et al.* (1996), 2. Data from Stainforth (1984), 3. Data from Alexander *et al.* (1991), Definitions and methods of measurement: ID/(ID+ D), isodihydro-*ar*-curcumene/(isodihydro-*ar*-curcumene + dihydro-*ar*-curcumene) (molecular responses); R_o , vitrinite reflectance; steranes, 20S and 20R diastereomers of 5 α (H), 14 α (H), 17 α (H)-ethylcholestane.

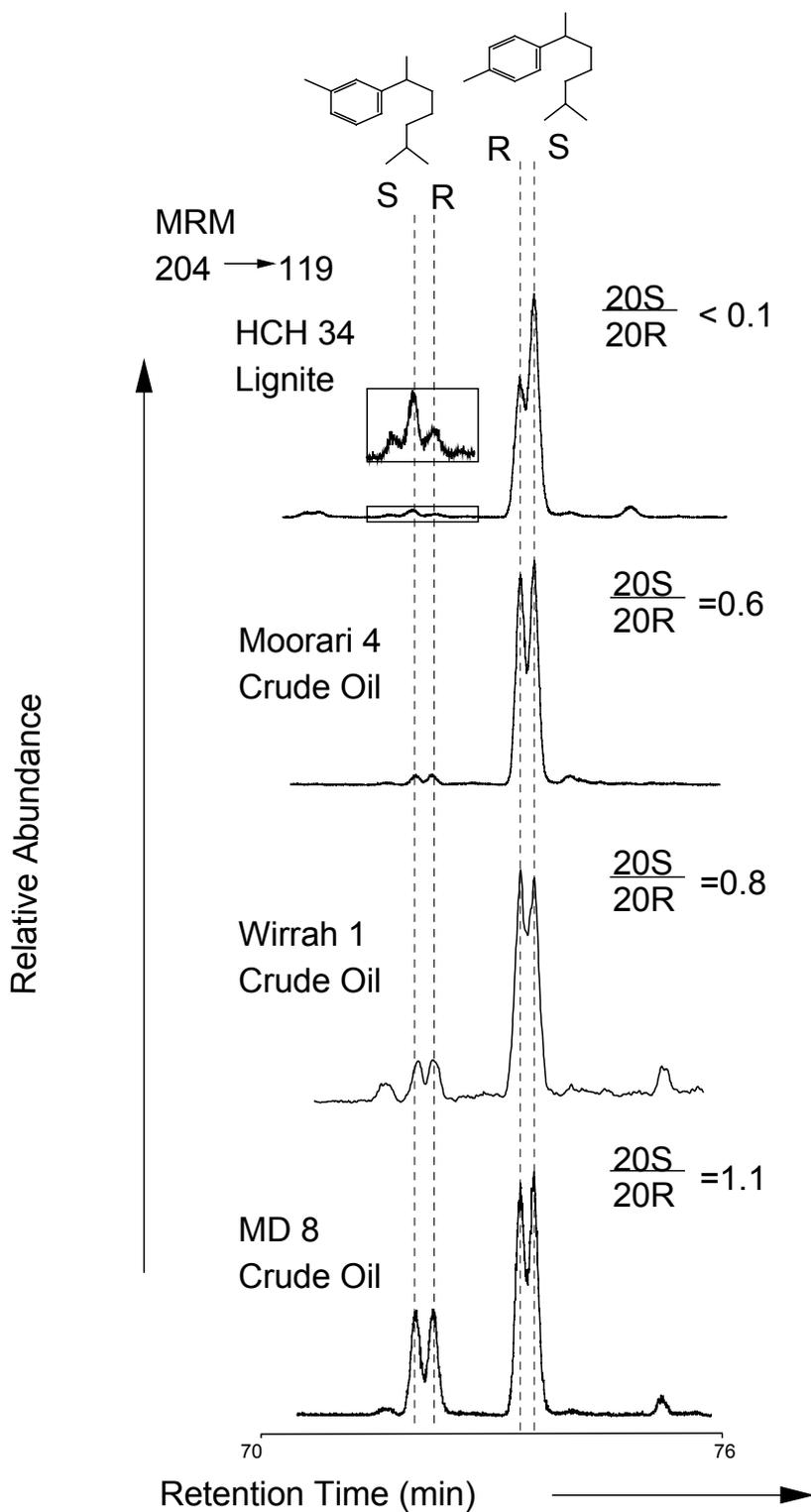


Figure 5.2. Partial mass chromatograms for the m/z 204 \rightarrow 119 transition showing the dihydro-*ar*-curcumene and isodihydro-*ar*-curcumene enantiomeric compositions of samples with different maturities.

more abundant. In the case of the crude oils it is apparent that they all contain the *R* and *S* enantiomers in equal amounts and are therefore racemic mixtures. These results suggest that the composition of enantiomers in low maturity sediments reflect the configurations of the natural products from which dihydro-*ar*-curcumene originates and that racemisation processes occur at an early stage of maturation, so that even low maturity crude oils such as Moorari (20*S*/20*R* = 0.6) contain racemic mixtures.

The conversion of dihydro-*ar*-curcumene to isodihydro-*ar*-curcumene occurs after racemisation. Figure 5.1 shows plots of the relative abundances of isodihydro-*ar*-curcumene and dihydro-*ar*-curcumene, expressed as a fraction of reaction for the conversion of dihydro-*ar*-curcumene to isodihydro-*ar*-curcumene, against sample depth for the GK well located in Indonesia (South Sumatara Basin) and for the Volador-1 well located in Australia (Gippsland Basin). The results show that with increase in sample depth an increase occurs in the value of the isodihydro-*ar*-curcumene/ dihydro-*ar*-curcumene ratio in both wells, as does vitrinite reflectance and the sterane diastereomer ratio of 20*S*/20*R*.

Isodihydro-*ar*-curcumene is apparently derived from dihydro-*ar*-curcumene and also occurs in racemic and non-racemic mixtures. Isodihydro-*ar*-curcumene has a retention time slightly less than dihydro-*ar*-curcumene and is also readily resolvable into its enantiomers using the same chiral GC-MS techniques as those used to analyse dihydro-*ar*-curcumene (Bastow *et al.*, 1997). From Figure 5.2 it is apparent that samples of higher maturity also have a higher relative abundance of isodihydro-*ar*-curcumene and all but the most immature sample are racemic. Although racemisation is complete before oil formation, the isomerisation reaction that results in formation of isodihydro-*ar*-curcumene continues into more mature zones and is potentially useful as an indicator of maturity for higher plant derived hydrocarbons in crude oils and possibly condensates. The non-racemic isodihydro-*ar*-curcumene in the low maturity lignite sample shown in the expanded window of the chromatogram (Figure 5.2) suggests that if conversion of dihydro-*ar*-

curcumene to isodihydro-*ar*-curcumene occurs in sediments it is a different process to that which results in racemisation.

Reaction mechanism

The observation that low maturity samples contain a predominance of one enantiomer (*S*) of dihydro-*ar*-curcumene and mature samples contain racemic mixtures of dihydro-*ar*-curcumene and isodihydro-*ar*-curcumene suggests that sedimentary racemisation and isomerisation processes occur. In order to further investigate these processes the reactions of dihydro-*ar*-curcumene with proton and clay catalysts were examined under laboratory conditions. Figure 5.3 shows mass chromatograms (m/z 204 \rightarrow 119) obtained from chiral GC-MS analysis of the reaction products from heating optically pure dihydro-*ar*-curcumene (*R*) with trifluoroacetic acid at 160 °C for various times. Blank experiments without an acid catalyst resulted in no change in concentration of dihydro-*ar*-curcumene (*R*) up to temperatures of 400°C. With increase in heating time a higher relative abundance of isodihydro-*ar*-curcumene occurs in the reaction mixture, however no appreciable amount of the *S* enantiomers of either compound was formed. Clearly proton catalysis results in an alkyl shift on the aromatic ring, without significant racemisation. Because positional isomerisation of the substituents on the aromatic ring is most likely to occur via a 1,2-migration of the tertiary (chiral) carbon of the alkyl group rather than via a methyl shift, the new bond must form before the old one has broken in order to preserve configuration at the chiral carbon.

The reaction products obtained from heating optically pure dihydro-*ar*-curcumene (*R*) with a clay catalyst are apparent from the mass chromatograms (m/z 204 \rightarrow 119) shown in Figure 5.4. Heating the substrate under increasingly vigorous conditions promoted both racemisation and 1,2-alkyl shifts. Because acidic clays such as the aluminium montmorillonite used in these experiments are Lewis acids, they are also proton acids in the presence of moisture. The mechanism of 1,2-alkyl shifts may therefore be similar to that occurring under conditions of proton catalysis. However these results suggest that it is the Lewis acid properties of the clay that cause

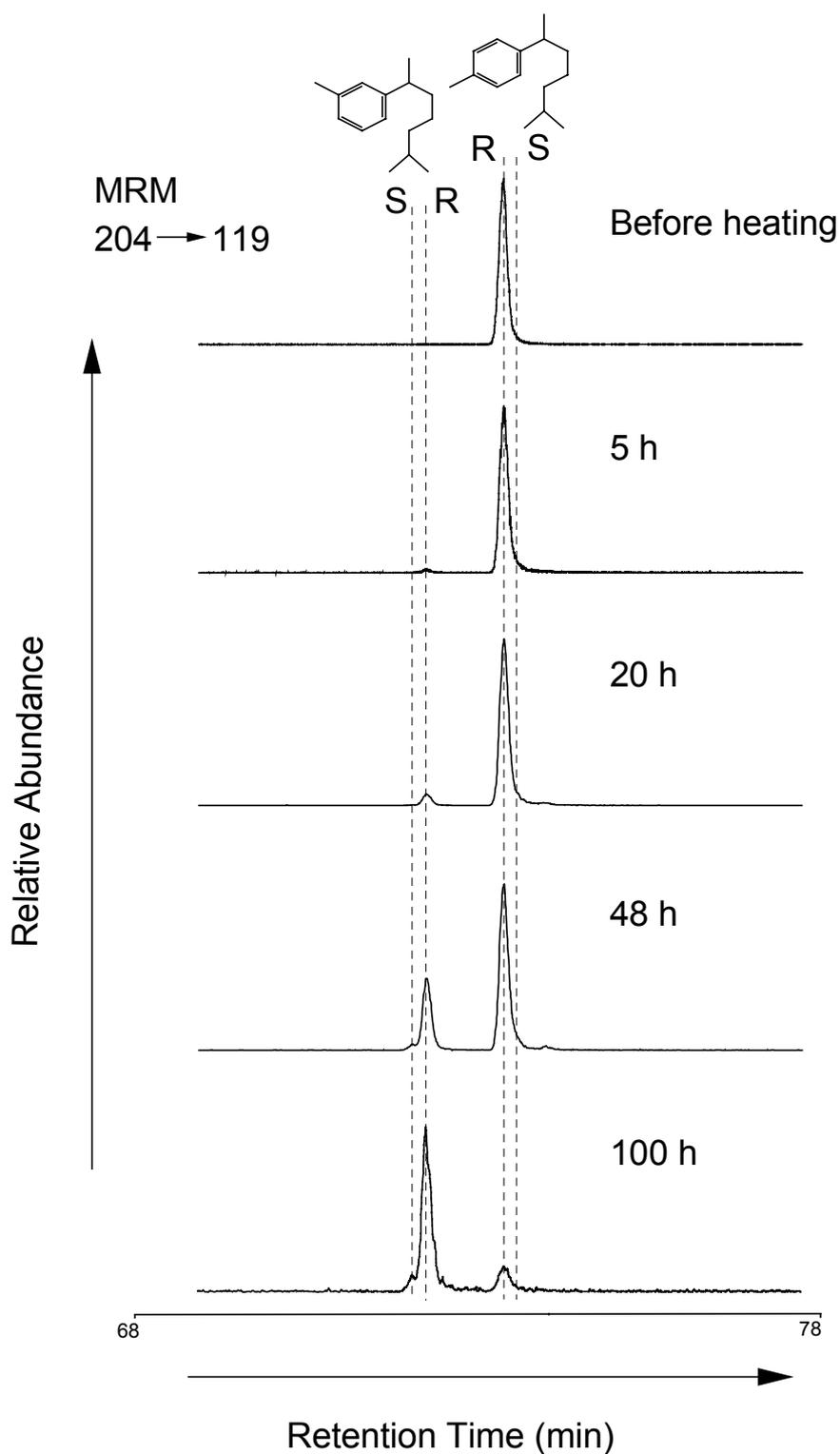


Figure 5.3. Partial mass chromatograms (m/z 204 \rightarrow 119) showing the relative abundances of dihydro-*ar*-curcumene and isodihydro-*ar*-curcumene for reaction products from heating dihydro-*ar*-curcumene (*R*) with trifluoroacetic at 160°C for various times.

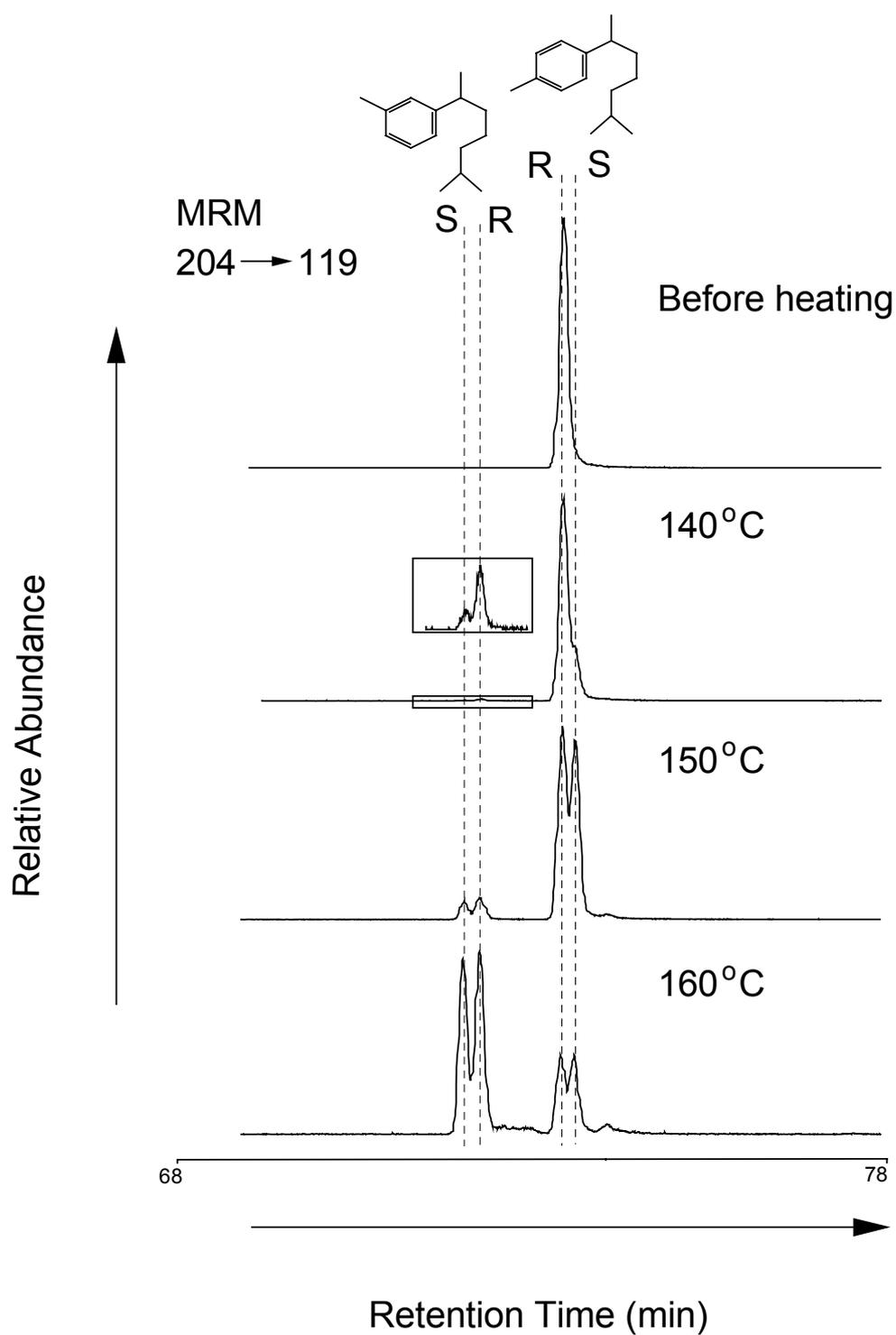
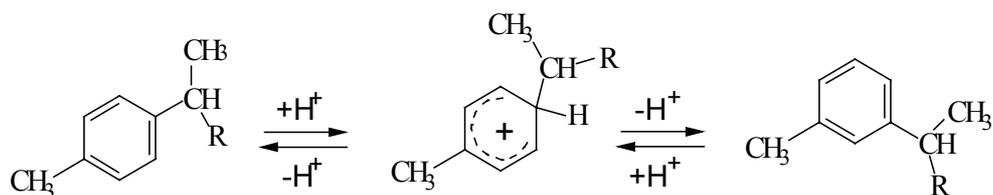


Figure 5.4. Partial mass chromatograms (m/z 204 \rightarrow 119) showing the relative abundances of dihydro-*ar*-curcumene and isodihydro-*ar*-curcumene for reaction products from heating dihydro-*ar*-curcumene (*R*) with aluminium montmorillonite for 2 hours at various temperatures (after Bastow *et al.*, 1997).

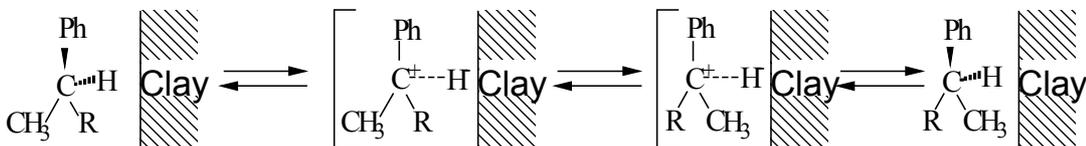
racemisation. Racemisation and positional isomerisation therefore appear to be separate processes.

Reaction Mechanisms

1,2-Alkyl shift: proton catalysed without racemisation



Racemisation: Lewis acid catalysed hydrogen exchange



The reaction mechanisms that would allow both processes to occur independently of one another are shown (Reaction Mechanisms). The mechanism of proton catalysed 1,2-alkyl shifts which result in positional isomerisation of groups on aromatic rings occur without the complete detachment of the migrating tertiary carbon atom (chiral) from the aromatic ring have been suggested to occur via a π -complex type intermediate (Olah and Molnár, 1995). In this mechanism a sufficiently strong bond is maintained at all times during the migration process between the aromatic carbons and the chiral centre such that inversion of configuration is prevented. However, in the case of Lewis acid catalysed racemisation, the C-H bond involving the chiral benzylic carbon is polarised and weakened by

interaction with the Lewis acid to such an extent that inversion of configuration can occur. This is a similar process to that which was suggested to account for the exchange of benzylic hydrogens of clay-sorbed aromatic compounds from a study of H-exchange using isotopically labelled compounds (Alexander *et al.*, 1984a).

The Lewis acid catalytic effect of clay under natural conditions on the racemisation and positional isomerisation reactions in sedimentary rocks can be seen by comparing the isomers and enantiomers present in a coal and a shale that have been subjected to the same thermal histories. The coal and shale samples from the Bremer Basin were obtained from the same (HCH 28) or adjacent (HCH 34) boreholes and from similarly shallow depths (Table 5.1).

The mass chromatograms (m/z 204 \rightarrow 119) in Figure 5.5 shows that racemisation (relative abundance of *R* enantiomers) is more advanced in the shale than in the coal. The enhanced reactivity of the shale is attributed to the catalytic effects of clay minerals. At this time we do not have an example from the natural system which corresponds to proton catalysis without Lewis acid catalysis.

Biodegradation effects

Selective depletion of enantiomers and positional isomers of dihydro-*ar*-curcumene has been observed in biodegraded crude oils. Figure 5.6a shows gas chromatograms of the three crude oils selected for this study. They represent a non-biodegraded (Wirrah-1, with an intact distribution of *n*-alkanes) and two biodegraded oils (Roller-1 and Leatherjacket-1, with depleted *n*-alkanes). The gas chromatograms of the two biodegraded oils suggest that Roller-1 is less biodegraded than Leatherjacket-1 because of a higher relative abundance of early eluting compounds in the former (Tissot and Welte, 1984). The mass chromatograms in Figure 5.6b show that the non-biodegraded crude oil contains racemic dihydro-*ar*-curcumene and isodihydro-*ar*-curcumene in proportions typical of a mature crude oil. In the

case of the Roller-1 crude oil a small but significant reduction in the amount of

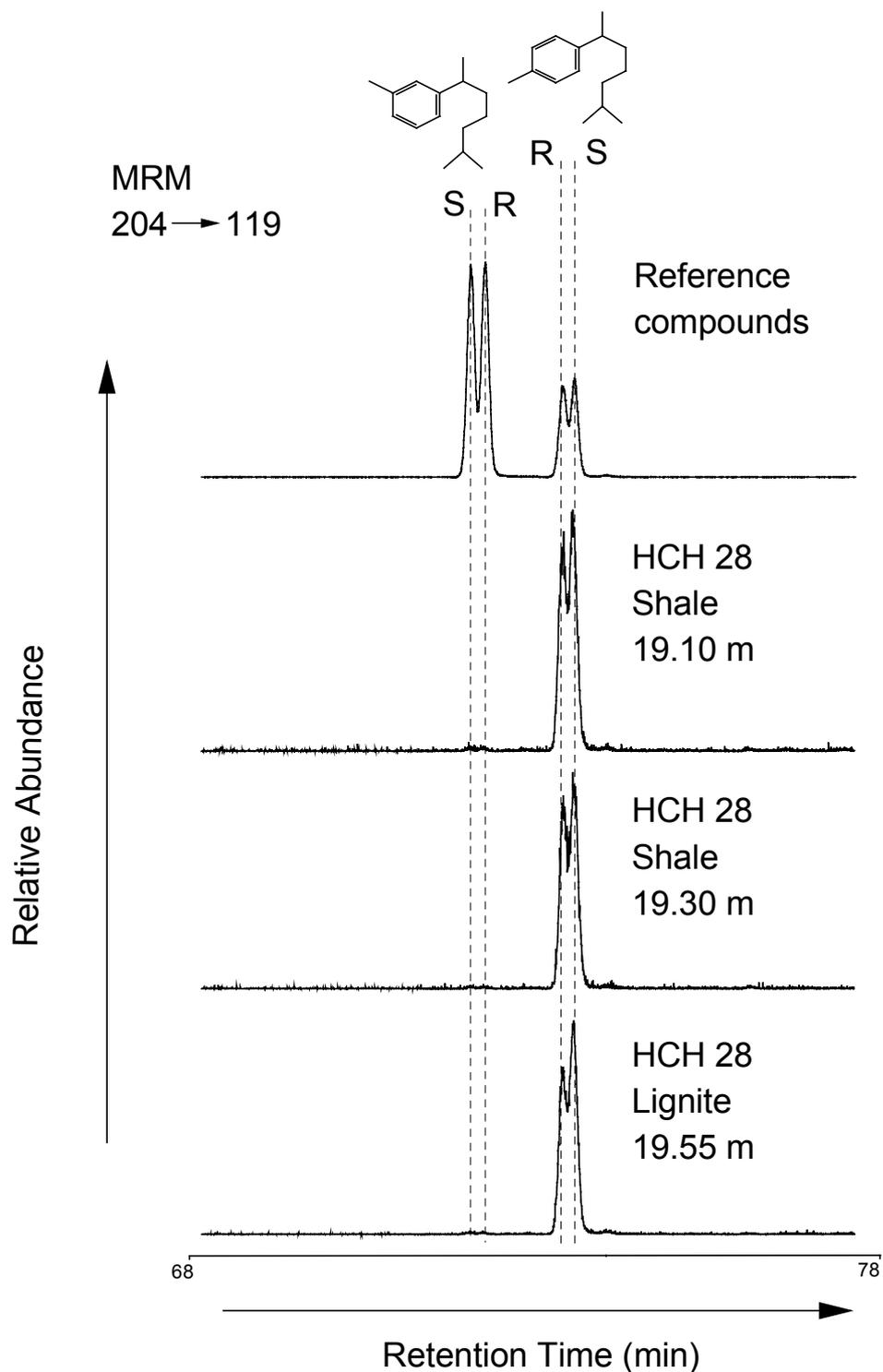


Figure 5.5. Partial mass chromatograms for the m/z 204 \rightarrow 119 transitions showing dihydro-*ar*-curcumene and isodihydro-*ar*-curcumene enantiomeric compositions of the reference compounds, HCH 28 (19.10 m) shale, HCH 28 (19.30 m) shale and HCH 28 (19.55 m) shale.

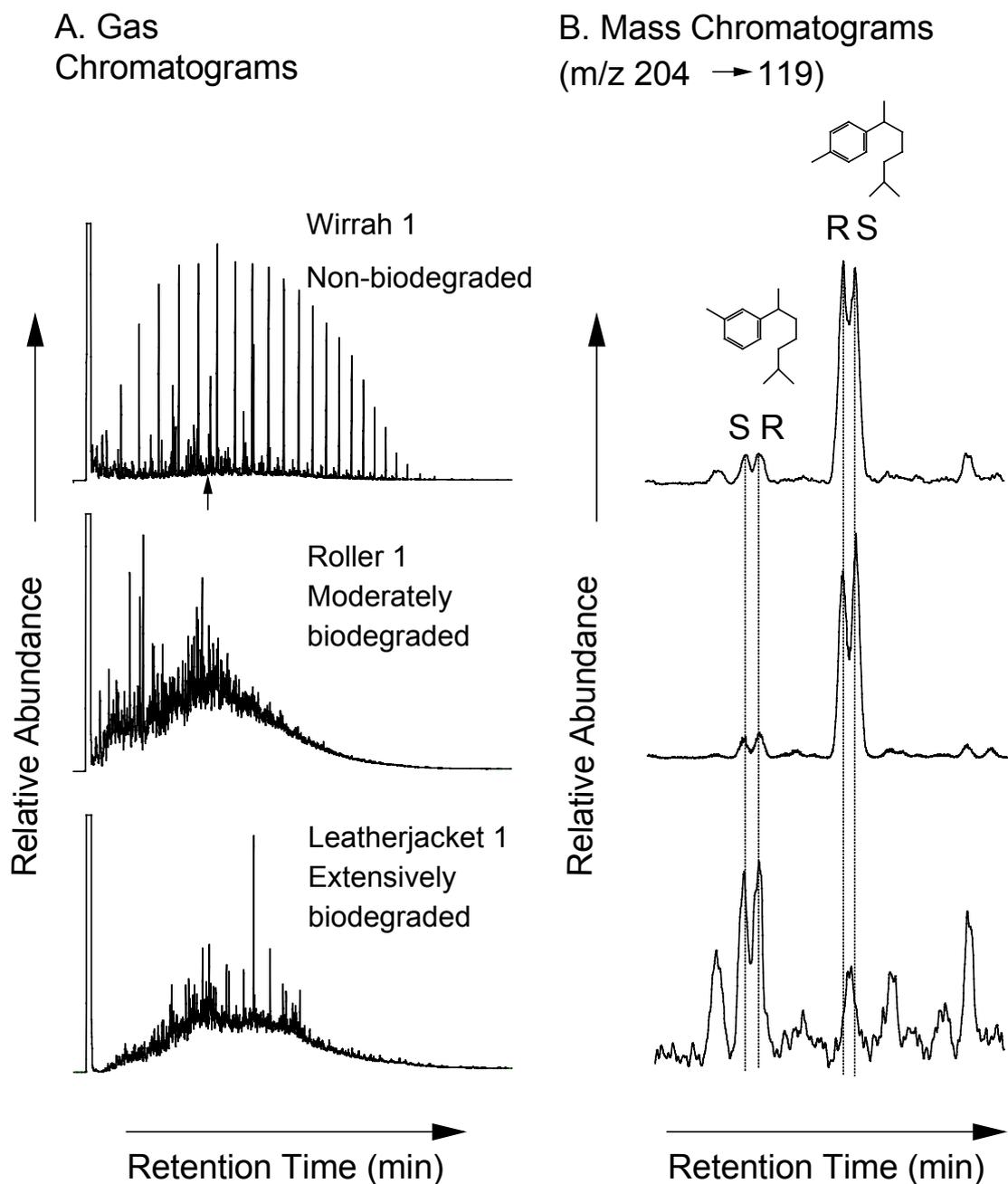


Figure 5.6. (a) Gas chromatograms and (b) partial mass chromatograms (m/z 204 \rightarrow 119) showing the relative abundances of dihydro-*ar*-curcumene and isodihydro-*ar*-curcumene and their enantiomers in non-biodegraded and biodegraded crude oils.
 ↑ Approximate retention time of dihydro-*ar*-curcumene.

the dihydro-*ar*-curcumene (*R*) enantiomer is apparent. The proportions of the positional isomers and isodihydro-*ar*-curcumene enantiomers suggest that little, if any, selective degradation of these isomers has taken place in this sample. In the case of the Leatherjacket-1 crude oil, both enantiomers of the dihydro-*ar*-curcumene have been severely depleted relative to the isodihydro-*ar*-curcumene. However, there is no evidence of selective depletion of enantiomers of the more refractory positional isomer. The persistence of isodihydro-*ar*-curcumene in an extensively biodegraded oil makes it a potentially useful biomarker for the former presence of dihydro-*ar*-curcumene, and consequently a higher plant input, in the source rocks of extensively biodegraded crude oils.

5.5 Conclusions

This study of sedimentary rocks and crude oils has shown that racemisation of dihydro-*ar*-curcumene occurs in the maturity zone before crude oil formation. However, the 1,2-alkyl shift transforming dihydro-*ar*-curcumene to isodihydro-*ar*-curcumene begins before oil formation and continues to occur with increase in maturity. Laboratory experiments show that racemisation is catalysed by acid clays (Lewis acids) and the 1,2-alkyl shift by proton acids. The faster rate of racemisation in a shale compared with a coal of similar maturity is attributed to the catalytic effects of minerals in the shale sample. Dihydro-*ar*-curcumene is more susceptible to biodegradation than isodihydro-*ar*-curcumene. The *R* enantiomer of dihydro-*ar*-curcumene is depleted at a faster rate than the *S* enantiomer.

5.6 Acknowledgements

Geoff Chidlow is thanked for his assistance with the GC-MS analyses. Financial support was provided by the Australian Petroleum Co-operative Research Centre and from a Curtin University Postgraduate Research Scholarship.

6.0 Tetramethylnaphthalenes in crude oils

6.1 Introduction

Tetramethylnaphthalenes (TeMNs) have long been recognised as constituents of petroleum. Isomers such as 1,2,5,6-TeMN and 1,2,3,4-TeMN have been reported by Ptitsyna (1959) and later 1,3,5,7-TeMN (Yew and Mair, 1966), 1,3,6,7-TeMN and 2,3,6,7-TeMN (Duswalt and Mayer, 1971) were also reported in crude oils. There have also been reports of TeMNs in other sedimentary materials such as oil shales (Radke *et al.*, 1984b) and coals (Ouchi and Imuta, 1963; Yew and Mair, 1966). Püttmann and Villar (1987) have recently reported 1,2,5,6-TeMN in coals and shales and have proposed the formation of the TeMN *via* the appropriate tetralins from triterpenoids from microbial and higher plant sources.

In this paper we report on the analysis of the isomeric TeMNs in a range of crude oils and show that the results are consistent with a derivation from natural products of microbial origin.

6.2 Materials and methods

Isolation of dinuclear and trinuclear aromatic fraction from crude oils and sediment extracts.

Crude oils or sediment extracts were subjected to column chromatography using silicic acid and pentane eluent to obtain an aromatic fraction. This fraction was then subjected to TLC using alumina plates and hexane solvent to obtain a combined dinuclear and trinuclear aromatic fraction (Forster *et al.*, 1989b).

GC-MS analysis

GC-MS analysis was carried out using a Hewlett-Packard (HP) 5987B GC-MS system equipped with a 60 m x 0.25 mm i.d. fused silica column coated with DB1701 (J & W Scientific). Hydrogen was used as carrier at a flow rate of 40 $\text{cm}^3\text{min}^{-1}$. The oven temperature was programmed from 50 °C to 110 °C at 2 °C per minute, then isothermal at 110 °C for 50 minutes and finally to 180 °C at 1 °C per minute.

Further details of analytical procedures can be found in published procedures (Forster *et al.*, 1989b).

Tetramethylnaphthalenes

Tetramethylnaphthalenes (TeMNs) were identified by comparing their retention times and mass spectra with synthetic reference compounds. TeMNs were available from a previous study (Forster *et al.*, 1989b).

6.3 Results and discussion

Analysis of crude oils using capillary GC-MS

Figure 6.1 shows the results obtained from the analysis of a mixture of standard TeMN isomers and from the dinuclear and trinuclear aromatic fraction of a crude oil sample. A column coated with DB1701 was used because it enables separation of the maximum number of isomers.

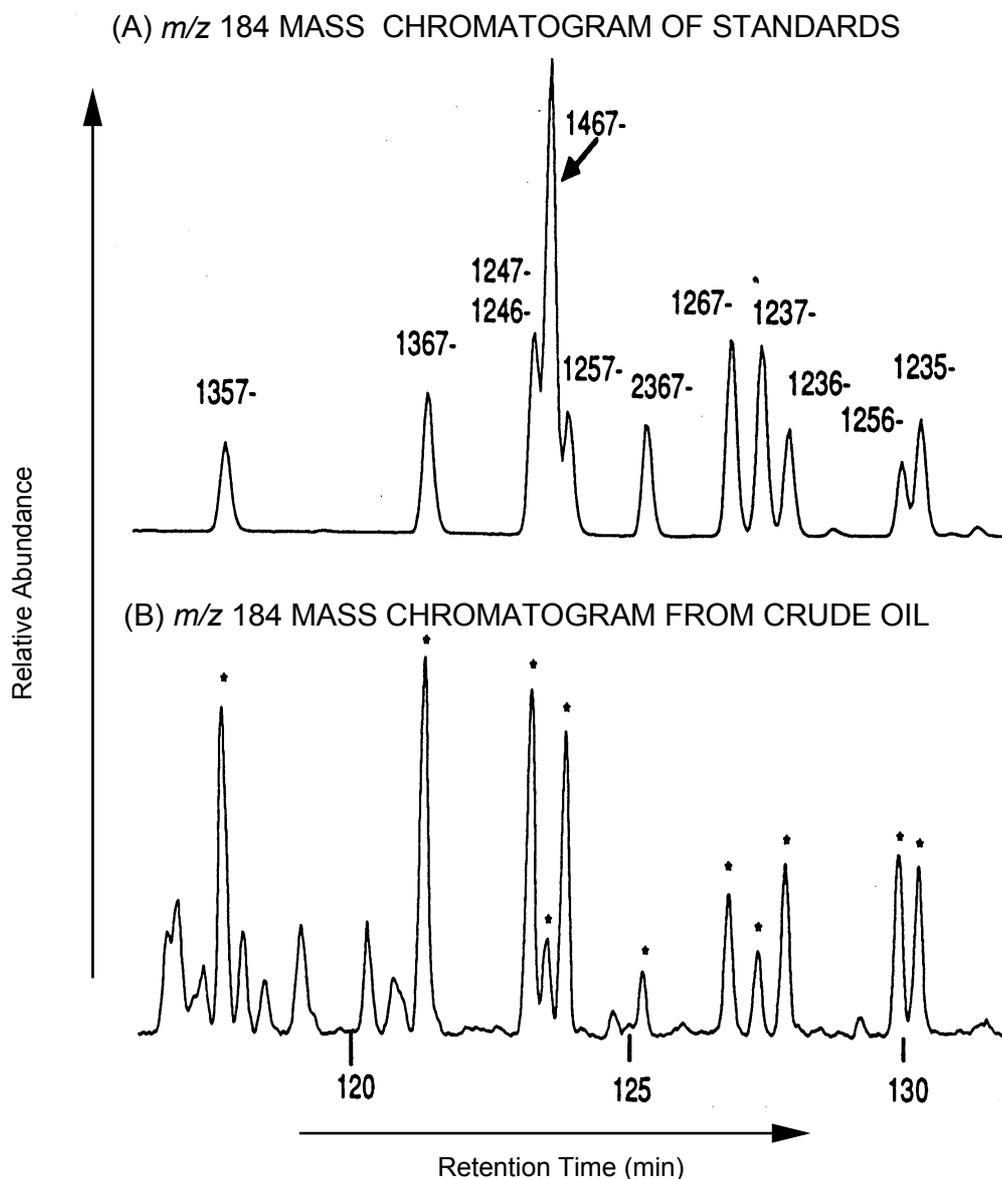


Figure 6.1. (A) Partial gas chromatogram of a mixture of TeMN isomers and (B) m/z 184 mass chromatogram of the aromatic fraction of a crude oil. Peaks shown by (*) co-eluted with standards.

Under the analytical conditions used in this study only two isomers co-chromatographed, namely 1,2,4,6-TeMN and 1,2,4,7-TeMN. Previously reported analytical conditions using columns coated with BP5 (Forster *et al.*, 1989b) resulted in coelution of these two isomers together with 1,4,6,7-TeMN. One additional benefit of using a DB1701 column coating is that it enabled resolution of 1,2,3,5-TeMN and 1,2,5,6-TeMN.

Table 6.1. Age and locations of crude oils.

Sample Name	Location	Probable age of source
Barrow	Carnarvon Basin, Australia	Jurassic
Fly Lake	Cooper Basin, Australia	Permian
Great Sandy	Canning Basin, Australia	Ordovician
McManus	McArthur Basin, Australia	Proterozoic
Moorari	Eromanga Basin, Australia	Jurassic
Safanyia	Saudi Arabia	Cretaceous

The locations and the ages of the crude oil samples are given in Table 6.1. Table 6.2 shows the distributions of TeMN isomers in each crude oil. Although the crude oils are from a range of locations and the age of the source material is very different, some common features can be recognised in the distributions of the TeMNs. Firstly, some isomers are absent or present at very low concentrations. These are the compounds with substituents in positions 1 and 8. It has been shown previously that dimethylnaphthalenes and trimethylnaphthalenes with substituents in these two positions are unstable and occur in low concentrations in crude oils (Radke *et al.*, 1982b; Alexander *et al.*, 1983b, 1985). Again in the case of the TeMNs this is an unfavourable substitution pattern. Another feature of the distributions is the relatively low concentration of 2,3,6,7-TeMN. Although this compound has the stable all- β substitution pattern, it is not the dominant isomer in any of the crude oils. Rather, 1,3,5,7-TeMN and 1,3,6,7-TeMN that occur in highest concentrations in most samples.

The Moorari sample is especially interesting because of its low maturity. The low maturity of this crude oil was inferred from the value of 0.6 for the ratio of the 20S/20R diastereomers of ethylcholestane compared with the lowest value of 0.8 for the other crude oils. It has a distribution dominated by 1,2,5,6-TeMN and 1,2,3,5-TeMN while the more mature samples have more even distributions of isomers with much lower relative abundances of these

two compounds. In our experience, this behaviour is typical of the changes in the TeMN distribution with increase in sample maturity. It suggests that 1,2,5,6-TeMN and 1,2,3,5-TeMN may be the predominant sources of the TeMNs in sediments and that the other isomers are derived from them by processes such as isomerisation as maturation progresses.

Table 6.2. Relative concentrations (%) of TeMN isomers in crude oils.

Sample	Isomer Substitution Pattern										
	1357	1367	1247 + 1246	1467	1257	2367	1267	1237	1236	1256	1235
Barrow	15.2	31.5	11.0	5.6	8.4	5.3	7.3	2.7	3.6	5.7	3.6
Fly Lake	18.6	42.0	9.3	6.5	7.5	7.0	3.4	1.6	1.0	2.2	0.9
Great Sandy	13.3	39.0	2.9	4.7	10.6	6.3	8.3	2.5	3.6	6.5	2.3
McManus	15.4	32.4	12.4	5.0	8.4	5.3	7.9	2.6	3.6	3.8	3.2
Moorari	3.5	5.5	6.6	2.8	12.4	0.5	4.9	1.7	3.3	37.3	21.5
Safaniya	14.3	17.3	16.1	4.3	12.9	2.6	6.2	3.8	7.5	7.8	7.2

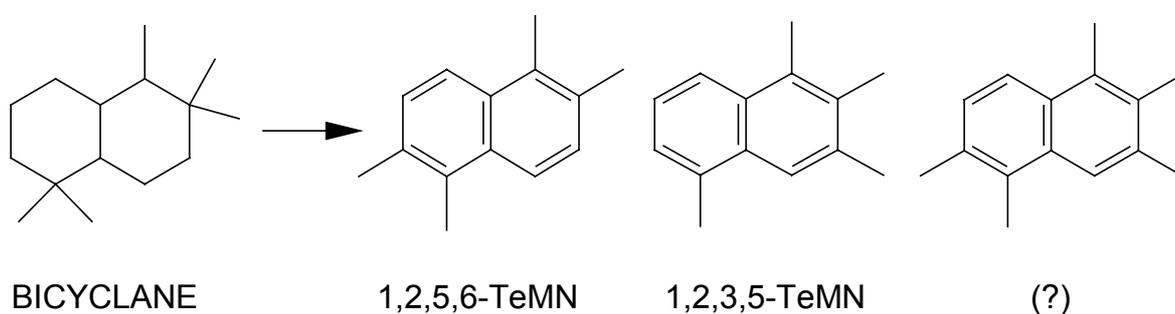
The occurrence of TeMNs in samples of Proterozoic and Ordovician ages shows that they have been formed from natural products derived from primitive organisms. These samples predate the evolution of higher plants and other complex life forms and therefore show that the TeMNs were most likely to have been derived from organic matter of bacterial or algal origins. Furthermore, the occurrence of TeMNs in crude oils from widely different locations and depositional conditions points to an origin from a ubiquitous organism. A previous report (Püttmann and Villar, 1987) proposed that one source of 1,2,5,6-TeMN was bacterially derived sedimentary hopanoids. These observations are consistent with this proposal since hopanoids are ubiquitous in their occurrence in sediments.

Tetramethylnaphthalenes in crude oil

The previously unreported 1,2,3,5-TeMN must also be derived from a ubiquitous organism. Examination of its methyl substitution pattern suggests that the sedimentary bicyclanes (**2**) or their precursors might undergo aromatisation processes according to Reaction Scheme 6.1 to yield this isomer. Such aromatisation processes commonly result in migration of one

of the *gem*-dimethyls to the adjacent ring position during aromatisation under laboratory conditions and in sediments (Püttmann and Villar, 1987 and references therein). The bicyclanes have a wide distribution in sedimentary organic matter of all ages, further supporting their likely involvement in the formation of 1,2,3,5-TeMN.

REACTION SCHEME 6.I



6.4 Conclusions

Crude oils contain at least 12 isomeric TeMNs. Samples of low maturity have 1,2,5,6-TeMN and 1,2,3,5-TeMN as the most abundant isomers where as 1,3,5,7-TeMN and 1,3,6,7-TeMN are the most abundant isomers in mature samples.

The bicyclic compounds of microbial origin or their precursors, are proposed as the source of the previously unreported 1,2,3,5-TeMN component of crude oil.

6.6 Acknowledgements

Technical assistance from Mr G. Chidlow in operation of the GC-MS system was greatly appreciated. Dr P. Forster is thanked for providing a sample of 1,2,3,5-TeMN. Mr Steve Fisher is thanked for providing a sample of 1,2,4,6-TeMN. Mr M. Audino is thanked for providing a sample of 1,4,6,7-TeMN and for his help with gas chromatography.

7.0 Pentamethylnaphthalenes and related compounds in sedimentary organic matter

7.1 Abstract

The isomeric pentamethylnaphthalenes (PMNs) 1,2,3,5,6-PMN, 1,2,3,5,7-PMN, 1,2,3,6,7-PMN and 1,2,4,6,7-PMN have been synthesised, characterised and identified in a number of crude oils and sediments, ranging in age from Proterozoic to Tertiary. We suggest that 1,2,3,5,6-PMN forms predominantly from the aromatisation of trimanoid precursors *via* 1,2,2,5,6-pentamethyltetralin. When 1,2,3,5,6-PMN was heated in laboratory experiments, the proportions of PMNs changed in a manner which reflected the relative stability of the isomers. The other PMNs in sediments are therefore suggested to arise *via* acid catalysed isomerisation or transalkylation processes. A conveniently measured pentamethylnaphthalene ratio $PNR = \frac{1,2,4,6,7-PMN}{1,2,3,5,6-PMN + 1,2,4,6,7-PMN}$ has been defined as a maturity parameter. In an Indonesian sedimentary sequence, the values of PNR changed smoothly with increasing depth with values ranging from 0.1 to 0.4. Values in crude oils range from 0.15 to 0.85, suggesting a value of approximately 0.15 for the beginning of the oil window.

7.2 Introduction

Alkylnaphthalenes are common constituents of Ancient sedimentary organic matter. Methyl-naphthalenes (MNs), dimethyl-naphthalenes (DMNs) and trimethyl-naphthalenes (TMNs) have been reported to occur in shales (Radke *et al.*, 1982a; Alexander *et al.*, 1985; Radke *et al.*, 1986; Strachan *et al.*, 1988; Budzinski *et al.*, 1993a), coal (Radke *et al.*, 1982b, 1984a, 1990b) and in crude oils (Mair and Mayer, 1964; Yew and Mair, 1966; Alexander *et al.*, 1983b; Alexander *et al.*, 1984c; Rowland *et al.*, 1984; Alexander *et al.*, 1985; Radke *et al.*, 1990a; Alexander *et al.*, 1993). Tetramethyl-naphthalenes (TeMNs) have also been reported to occur in sedimentary rocks and petroleum (Püttmann and Villar, 1987; Forster *et al.*, 1989b; Alexander *et al.*, 1993).

Some polymethyl-naphthalenes have been reported to occur in high relative abundances in sediments and crude oils of low maturity. Examples include 1,2,5-TMN (Püttmann and Villar, 1987; Strachan *et al.*, 1988), 1,2,7-TMN (Püttmann and Villar, 1987; Strachan *et al.*, 1988; Forster *et al.*, 1989a), 1,2,5,6-TeMN (Püttmann and Villar, 1987; Alexander *et al.*, 1993) and 1,2,3,5-TeMN (Alexander *et al.*, 1992a, 1993). The presence of these alkylnaphthalenes in sediments and crude oils of low maturity has led to the suggestion that they originate from triterpenoids derived from microbial and land-plant sources (Püttmann and Villar, 1987; Strachan *et al.*, 1988; Forster *et al.*, 1989a; Alexander *et al.*, 1992a, 1993).

Numerous studies of sedimentary organic matter have shown that proportions of the less stable α -methyl substituted alkylnaphthalenes decrease with increasing maturity relative to the more stable β -substituted isomers. (Radke *et al.*, 1982a; Alexander *et al.*, 1984c, 1985, 1986; Radke *et al.*, 1986; Strachan *et al.*, 1988). This change in the relative abundances of isomers with increasing maturity has been attributed to isomerisation and transalkylation processes (Strachan *et al.*, 1988; Alexander *et al.*, 1993).

In this paper we report on the identification and occurrence of pentamethylnaphthalenes (PMNs) in sedimentary organic matter and present evidence for their formation from bicyclic precursor natural products.

7.3 Experimental

Samples

The locations of the samples and their ages, or in the case of crude oils the probable age of their source rock, together with two commonly used maturity parameters (MPI-1; 20S/20R), are shown in Table 7.1.

Sample preparation

Rock samples were air dried and crushed to a fine powder using a Tema mill and extracted ultrasonically with dichloromethane/methanol (95:5). The solvent extract was recovered by filtration and the solvent was carefully removed to yield the soluble organic matter (SOM).

Isolation of aromatic hydrocarbon fractions

In a typical separation, a crude oil or sediment extract was subjected to liquid chromatography as follows. Glass columns (40 cm x 1.2 cm i.d.) were packed with activated silica gel (6 g, Merck, particle size 0.063 – 0.200 mm for chromatography) as a slurry in *n*-pentane. The sample (50-100 mg) in *n*-pentane was introduced to the top of the column to produce a concentrated band. Aliphatic hydrocarbons were then eluted under gravity with *n*-pentane (40 mL), aromatic hydrocarbons with *n*-pentane/dichloromethane (35:5, 40 mL), and polar compounds with dichloromethane/methanol (1:1, 30 mL). The aromatic fractions were obtained by careful removal of the solvent in a Kuderna-Danish apparatus, the residue was dissolved in hexane to provide a sample ready for GC-MS analysis.

Table 7.1. Location, age and maturity data for crude oils and source rock samples.

Samples	Type ^a	Location		Probable age of source rocks	MPI-1	<u>20S</u> / <u>20R</u>
		Country	Basin			
Iron Duke	C	Brunei	N/A	Tertiary	0.8	0.6
Basker-1	C	Australia	Gippsland	Cretaceous	1.2	1.1
Barrow Island	C	Australia	Carnarvon	Up. Jurassic	1.3	1.2
Moorari-4	C	Australia	Eromanga	Jurassic	0.2	0.6
Fly Lake-1	C	Australia	Cooper/Erom	Permian	1.4	-----
East Dollahide-1	C	Texas, USA	N/A	Cambro-Ordo	0.7	-----
Acacia-1, 779 m	S	Australia	Canning	Ordovician	0.3	0.6
Acacia-1, 782 m	S	Australia	Canning	Ordovician	0.4	0.6
Acacia-1, 974 m	S	Australia	Canning	Ordovician	0.5	0.8
Acacia-1, 1032 m	S	Australia	Canning	Ordovician	0.6	1.0
Great Sandy-1	C	Australia	Canning	Ordovician	1.0	1.1
Blina-1	C	Australia	Canning	Devonian	1.4	1.0
Eremophila	S	Australia	Canning	Devonian	1.3	0.7
PD 130A	S	Australia	Canning	Devonian	0.3	0.5
Jamison	S	Australia	McArthur	Proterozoic	1.0	-----
McManus	S	Australia	McArthur	Proterozoic	0.7	1.1

^a C= crude oil; S= sedimentary rock

Definitions and methods of measurements: 20S/20R, 20S and 20R diastereomers of 5 α (H),14 α (H),17 α (H)-ethylcholestane (*m/z* 217) (Mackenzie and McKenzie, 1983); MPI-1. GC-MS data corrected to FID response (Radke *et al.*, 1982a,b).

GC-MS analysis

Two systems were used for GC-MS analysis. One, a Hewlett-Packard 5970 MSD interfaced with a 5890 gas chromatograph was fitted with a fused-silica open tubular column (SGE, Australia, 50 m x 0.22 mm i.d.) coated with BP-20 stationary phase (0.25 μ m). The GC oven temperature was programmed from 70 to 270°C at 3°C min⁻¹. Samples for analysis were dissolved in hexane and injected on-column using a HP 7673 autosampler at an oven temperature of 70°C. Helium was used as carrier gas at a velocity of 27 cm s⁻¹ at 70°C. Typical MSD conditions were: ionisation energy 70 eV; source temperature 200°C; electron multiplier voltage 2200 V. The other system was a Hewlett-Packard 5971 MSD interfaced with a 5890 Series II gas

chromatograph fitted with a DB-5 (60 m x 0.25 mm i.d., phase thickness 0.25 μm , J&W Scientific) or a HP-1 (50 m x 0.20 mm i.d., phase thickness 0.33 μm , Hewlett Packard) fused silica open tubular column. Helium was used as carrier gas at a constant flow of 1.28 mL min^{-1} (DB-5), 0.77 mL min^{-1} (HP-1) and the GC oven was temperature programmed from 60-300°C at 3°C min^{-1} . Other conditions were as described above. Individual alkylnaphthalenes in crude oils and sediment isolates were identified by mass chromatography on the basis of mass spectra and by co-chromatography with reference compounds.

Reference compounds

Authentic 1,2,3,4,6-PMN (**5**) was obtained from Professor L. H. Klemm (Klemm *et al.*, 1968). The remaining compounds were prepared using a well-documented general synthetic scheme (Araki and Mukaiyama, 1974) (Reaction Scheme 7.1). 1,2,3,5,6-PMN (**1**) was prepared using the following procedure. 1,2,3-Trimethylbenzene (13 mmol) in glacial acetic acid (50 mL) at 0°C, was treated with bromine (15 mmol), and the mixture was allowed to warm to room temperature over a period of 1h yielding 1-bromo-2,3,4-trimethylbenzene. The Grignard reagent prepared from 1-bromo-2,3,4-trimethylbenzene (8 mmol) and magnesium (10 mmol) was added to methylsuccinic anhydride (8 mmol) in THF. The resulting two keto carboxylic acids 2,3,4, α -tetramethyl- γ -oxophenylbutanoic acid (80%, mp 117-120 °C) and 2,3,4, β -tetramethyl- γ -oxophenylbutanoic acid (20%, 122-126 °C) were isolated using fractional crystallisation (ethanol and water). The major keto carboxylic acid (2,3,4, α -tetramethyl- γ -oxophenylbutanoic acid 4 mmol) was and then reduced using zinc amalgam (Martin, 1942) to give the carboxylic acid. The carboxylic acid (2 mmol) was cyclised to 2,5,6,7-tetramethyltetralone by treatment with polyphosphoric acid (PPA). The tetralone (1 mmol) was methylated using methylmagnesium iodide and the resultant tertiary alcohol (0.8 mmol) was converted to the unsaturated alkyltetralin by dehydration in a mixture of sulfuric acid and glacial acetic acid. This alkyltetralin was then treated with activated platinum on carbon at 280°C to yield 1,2,3,5,6-PMN (**1**) as a white solid (mp 92-94°C) with an

overall yield of 17 %. The mass spectrum and proton chemical shifts (δ , ppm) of the product are shown in Figure 7.1.

Preparation of 1,2,3,5,7-PMN (3), 1,2,3,6,7-PMN (2) and 1,2,4,6,7-PMN (4).

The Grignard reagent (in THF) from the appropriate bromobenzene (1-bromo-2,4-dimethylbenzene, 1-bromo-2,3,4-trimethylbenzene, 1-bromo-2,4,5-trimethylbenzene, respectively) were added to dimethylsuccinic anhydride (in THF). The resulting keto carboxylic acids were isolated and the keto functionality reduced using zinc amalgam (Martin, 1942) to give the carboxylic acids. The carboxylic acids were cyclised to the appropriate alkyltetralone by treatment with PPA.

In the case of 1,2,3,5,7-PMN (**3**), the tetralone (2,3,5,7-tetramethyltetralone) was methylated using methylmagnesium iodide and the resultant tertiary alcohol was converted to the unsaturated alkyltetralin by dehydration in a mixture of sulfuric acid and glacial acetic acid. This alkyltetralin was then treated with activated platinum on carbon at 280°C to yield 1,2,3,5,7-PMN (**3**) as a white solid (mp 98-100°C) with an overall yield of 24 %.

In the cases of 1,2,3,6,7-PMN (**2**) and 1,2,4,6,7-PMN (**4**), the alkyltetralones were reduced using zinc amalgam (Martin, 1942) to give the alkyltetralins. The alkyltetralins were then treated with activated platinum on carbon at 280°C to yield the appropriate pentamethylnaphthalenes as white solids (1,2,3,6,7-PMN (**2**), mp 113-116°C, overall yield of 12%; 1,2,4,6,7-PMN (**4**) overall yield of 29 %, m.p. 56-59°C).

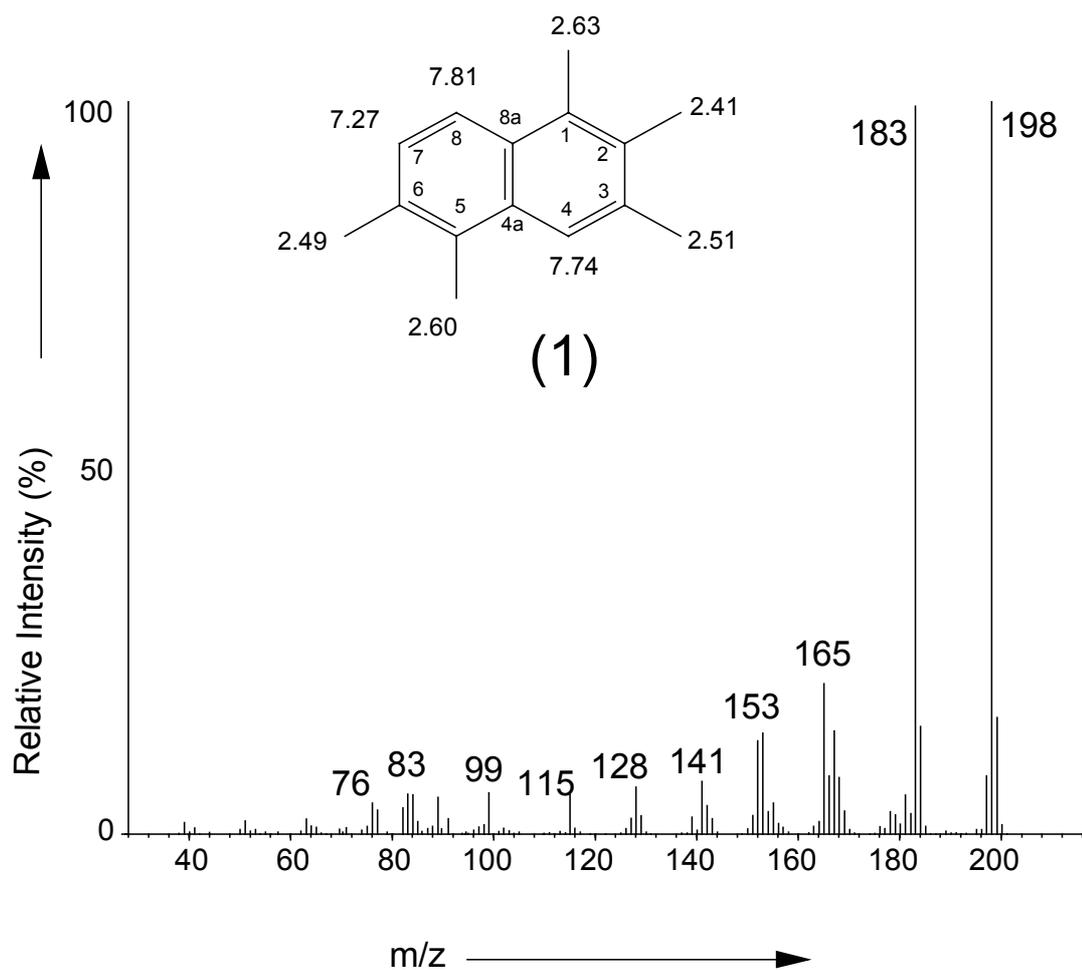


Figure 7.1. Mass spectrum and ^1H nmr chemical shifts (δ) of 1,2,3,5,6-pentamethylnaphthalene (**1**).

NMR data

All measurements were carried out in a solution of chloroform-d using a Varian Gemini-200 spectrometer operating at 200 MHz for ^1H (ppm relative to CDCl_3 at 7.25 ppm) and 50 MHz for ^{13}C (ppm relative to CDCl_3 at 77.0 ppm).

^1H nmr data for the intermediates for the synthesis of reference compounds:

2,3,4, α -tetramethyl- γ -oxophenylbutanoic acid: δ 1.29, *d*, J 7.2 Hz, 3H, RCH(CH₃)R; 2.20, 2.31, 2.31, 3s, 9H, Ar(C2, C3, C4)-CH₃; 2.89, *dd*, J 17.2, 5.2 Hz, 1H, RCH_aH_bR'; 3.04-3.21, *m*, 1H, RCH(CH₃)R'; 3.32, *dd*, J 17.2, 7.6 Hz, 1H, RCH_aH_bR'; 7.03, *d*, J 7.8 Hz, 1H, Ar(C5)-H; 7.28, *d*, J 7.8 Hz, 1H, Ar(C6)-H.

2,3,4, β -tetramethyl- γ -oxophenylbutanoic acid: δ 1.13, *d*, J 7.2 Hz, 3H, RCH(CH₃)R; 2.19, 2.26, 2.30, 3s, 9H, Ar(C2, C3, C4)-CH₃; 2.42, *dd*, J 17.0, 5.6 Hz, 1H, RCH_aH_bR'; 2.94, *dd*, J 17.0, 8.2 Hz, 1H, RCH_aH_bR'; 3.56-3.73, *m*, 1H, RCH(CH₃)R'; 7.03, *d*, J 8 Hz, 1H, Ar(C5)-H; 7.30, *d*, J 7.8 Hz, 1H, Ar(C6)-H.

¹³C nmr data for the intermediates for the synthesis of reference compounds:

2,3,4, α -tetramethyl- γ -oxophenylbutanoic acid: \square 15.50, 16.76, 16.83, 21.04, Ar(C2, C3, C4)-CH₃, RCH(CH₃)R'; 35.03, RCH(CH₃)R'; 45.35, RCH₂R'; 124.81, Ar(C6); 126.95, Ar(C5); 135.29, 136.86, 137.56, 139.88, Ar(C1, C2, C3, C4); 181.85, RCO₂H; 203.86, ArC(O)R.

2,3,4, β -tetramethyl- γ -oxophenylbutanoic acid: δ 15.49, 16.61, 16.79, 20.97, Ar(C2, C3, C4)-CH₃, RCH(CH₃)R'; 36.61, RCH₂R'; 41.07, RCH(CH₃)R'; 124.53, Ar(C6); 126.96, Ar(C5); 135.35, Ar(C1); 136.69, Ar(C2); 137.32, Ar(C3); 139.53, Ar(C4); 177.93, RCO₂H; 208.04, ArC(O)R.

¹H nmr data for the pentamethylnaphthalene reference compounds prepared:

1,2,3,5,6-PMN (1): δ 2.41, *s*, 3H, Ar(C2)-CH₃; 2.49, *s*, 3H, Ar(C6)-CH₃; 2.51, *s*, 3H, Ar(C3)-CH₃; 2.60, *s*, 3H, Ar(C5)-CH₃; 2.63, *s*, 3H, Ar(C1)-CH₃; 7.27, *d*, J 8.6 Hz, 1H, Ar(C7)-H; 7.74, *s*, 1H, Ar(C4)-H; 7.81, *d*, J 8.8 Hz, 1H, Ar(C8)-H.

1,2,3,6,7-PMN (**2**): δ 2.37, s, 3H, Ar(C2)-CH₃; 2.40, 2.43, 2s, 6H, Ar(C6)-CH₃, Ar(C7)-CH₃; 2.45, s, 3H, Ar(C3)-CH₃; 2.60, s, 3H, Ar(C1)-CH₃; 7.39, 7.46, 2s, 2H, Ar(C4)-H, Ar(C5)-H; 7.74, s, 1H, Ar(C8)-H.

1,2,3,5,7-PMN (**3**): δ 2.44, s, 3H, Ar(C2)-CH₃; 2.53, 2.54, 2s, 6H, Ar(C3)-CH₃, Ar(C7)-CH₃; 2.66, s, 3H, Ar(C1)-CH₃; 2.69, s, 3H, Ar(C5)-CH₃; 7.15, s, 1H, Ar(C6)-H; 7.68, s, 1H, Ar(C4)-H; 7.71, s, 1H, Ar(C8)-H.

1,2,4,6,7-PMN (**4**): δ 2.42, s, 3H, Ar(C2)-CH₃; 2.45, 2.46, 2s, 6H, Ar(C6)-CH₃, Ar(C7)-CH₃; 2.54, s, 3H, Ar(C1)-CH₃; 2.60, s, 3H, Ar(C4)-CH₃; 7.06, s, 1H, Ar(C3)-H; 7.69, 7.78, 2s, 2H, Ar(C5)-H, Ar(C8)-H.

¹³C nmr data for the pentamethylnaphthalene reference compounds prepared:

1,2,3,5,6-PMN (**1**): δ 14.56, 15.02, 16.07, 20.48, 22.05, Ar(C1, C2, C3, C5, C6)-CH₃; 121.53, 121.83, 127.96, Ar(C4, C7, C8); 130.35, 130.62, 131.37, 131.37, 131.63, 131.73, 134.92, Ar(C1, C2, C3, C4a, C5, C6, C8a).

1,2,3,6,7-PMN (**2**): δ 14.80, 16.12, 19.79, 20.50, 21.49, Ar(C1, C2, C3, C6, C7)-CH₃; 123.62, 124.98, 127.27, Ar(C4, C5, C8); 130.09, 130.49, 131.06, 132.11, 133.96, 134.22, 134.26, Ar(C1, C2, C3, C4a, C6, C7, C8a).

Table 7.2. Calculated^a and observed ¹H NMR chemical shifts NMR (ppm relative to CDCl₃ at 7.25 ppm) for pentamethylnaphthalene isomers

Methyl position	1,2,3,5,6-PMN(1)			1,2,3,6,7-PMN(2)			1,2,3,5,7-PMN(3)			1,2,4,6,7-PMN(4)			1,2,3,4,6-PMN(5)		
	calc	obs	Δ												
1	2.53	2.63	0.1	2.53	2.60	0.07	2.53	2.66	0.13	2.47	2.54	0.07	2.56	2.50	0.06
2	2.32	2.41	0.09	2.32	2.37	0.05	2.32	2.44	0.12	2.31	2.42	0.11	2.33	2.31	0.02
3	2.37	2.51	0.14	2.37	2.45	0.08	2.37	2.54	0.17	-	-	-	2.33	2.31	0.02
4	-	-	-	-	-	-	-	-	-	2.52	2.60	0.08	2.56	2.50	0.06
5	2.51	2.60	0.09	-	-	-	2.56	2.69	0.13	-	-	-	-	-	-
6	2.35	2.49	0.14	2.35	2.43	0.08	-	-	-	2.35	2.45	0.1	2.44	2.48	0.04
7	-	-	-	2.35	2.40	0.05	2.40	2.53	0.13	2.35	2.46	0.11	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

^a Yew *et al.*, (1964)

1,2,3,5,7-PMN (**3**): δ 15.11, 16.24, 19.46, 21.87, 21.96, Ar(C1, C2, C3, C5, C7)-CH₃; 121.38, 121.96, 127.80, Ar(C4, C6, C8); 129.34, 130.87, 131.87, 132.77, 133.57, 133.75, 133.93, Ar(C1, C2, C3, C4a, C5, C7, C8a).

1,2,4,6,7-PMN (**4**): δ 14.26, 19.09, 20.15, 20.42, 20.42, Ar(C1, C2, C4, C6, C7)-CH₃; 124.10, 124.31, 129.16, Ar(C3, C5, C8); 128.22, 130.23, 130.60, 131.70, 131.88, 133.64, 134.78, Ar(C1, C2, C4, C4a, C6, C7, C8a).

Heating experiments

(a) 1,2,3,5,6-PMN and trifluoroacetic acid.

1,2,3,5,6-PMN (1 mg), and trifluoroacetic acid (100 μ L) were placed in small Pyrex glass ampoules (3 mL). The ampoules were flushed with dry nitrogen, cooled with liquid nitrogen, evacuated, sealed and then heated at 200°C.

Ampoules were removed after 20h, 48h, and 100h. The tubes were cooled and opened, the contents were neutralised with aqueous sodium hydroxide, and the hydrocarbons were recovered by extraction with hexane (4 x 500 μ L). The extract was dried with anhydrous magnesium sulphate and an aliquot was subjected to analysis for PMNs using GC-MS.

(b) 1,2,2,5,6-Pentamethyltetralin (**6**) and platinised carbon.

1,2,2,5,6-Pentamethyltetralin (**6**) (1 mg) and Pt/C (10 mg, 5%) were placed in small Pyrex glass ampoules (3 mL). The ampoules were flushed with dry nitrogen, evacuated, sealed and then heated at 300°C for 30 h. The contents were recovered in dichloromethane (2 mL), then the solvent was removed by careful distillation, and hexane (1.8 mL) containing *n*-pentadecane (0.5 mg) was added. An aliquot was then subjected to analysis for polymethylnaphthalenes using GC-MS.

(c) 1,2,5,6-TeMN and aluminium montmorillonite.

The aluminium montmorillonite used in the study was material prepared for an earlier study (Alexander *et al.*, 1984a). 1,2,5,6-TeMN (0.2 mg) and finely divided aluminium montmorillonite (5 mg) were placed in small Pyrex glass ampoules (3 mL). The ampoules were flushed with dry nitrogen, evacuated,

sealed and then heated at 150°C for 1h. The contents were recovered with dichloromethane (2 mL), then the solvent was removed by careful distillation. An aliquot was then subjected to analysis using GC-MS techniques.

7.4 Results and discussion

Reference compounds

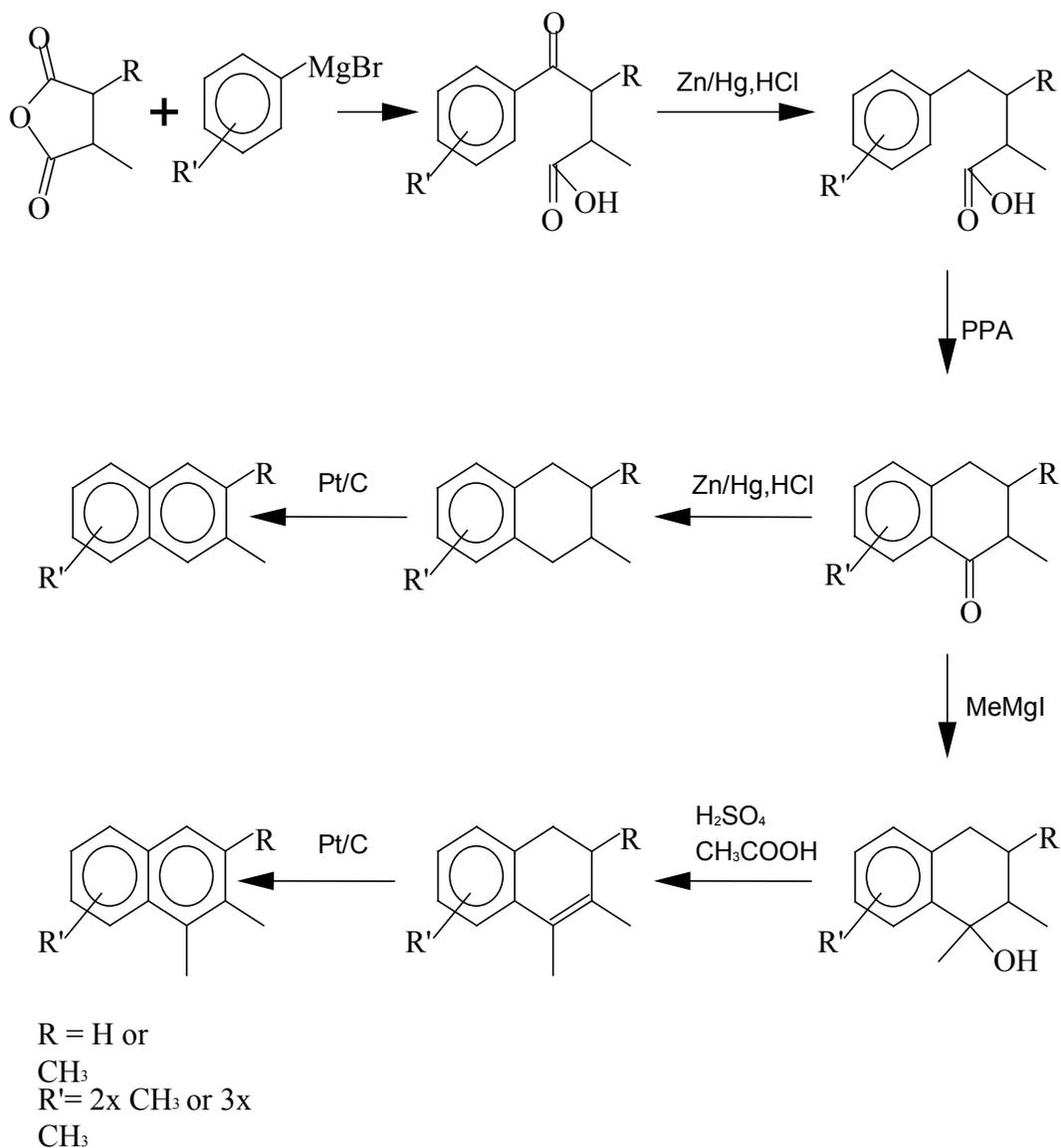
The pentamethylnaphthalenes were prepared from the appropriate bromo alkylbenzenes and methylsuccinic anhydride or dimethylsuccinic anhydride *via* an intermediate tetralone. This reaction scheme (Reaction Scheme 7.1) ensured that methyl substituents were located at the appropriate positions in the pentamethylnaphthalene.

The structures of the five reference compounds used in this study are based on synthetic grounds. Authentic 1,2,3,4,6-PMN (**5**) was purchased from the original workers (Klemm *et al.* 1968). In the case of 1,2,3,5,6-PMN (**1**) two keto acids were obtained from the reaction of the Grignard reagent of 1-bromo-2,3,4-trimethylbenzene and methylsuccinic anhydride. These compounds were isolated and characterised by NMR. The major product was used to prepare 1,2,3,5,6-PMN (**1**). The route to prepare 1,2,3,6,7-PMN (**2**), 1,2,3,5,7-PMN (**3**) and 1,2,4,6,7-PMN (**4**) is unambiguous. The remaining nine isomers were not prepared because they contain the very unstable 1,8 substitution pattern and are unlikely to occur in sedimentary rocks.

Further evidence is available from simple NMR considerations. Only 1,2,3,5,6-PMN (**1**) which has a 7,8 hydrogen substitution, which showed splitting of resonances due to ring hydrogens. Also using the relation published by Yew and Mair (1966), chemical shifts have been calculated for the methyl groups in each of the five isomers. The calculated versus observed values are shown in Table 7.2.

Reaction Scheme 7.1

Reaction scheme for the synthesis of the pentamethylnaphthalenes



These results show a good agreement with calculated and observed chemical shifts for the five isomers. We attributed the systematically higher values we observed to the fact that our measurements were carried out on much more dilute solutions than would have been possible 30 years ago.

Yew *et al.*, (1964) measured a decrease in δ values of approximately 0.5 ppm with a ten fold increase in concentration.

Table 7.3. Retention indices for the pentamethylnaphthalenes.

Compound	Retention index (I*)					
	HP-1	(Std. Dev.) n=10	DB-5	(Std. Dev.) n=10	BP-20	(Std. Dev.) n=10
1,2,3,5,6-PMN (1)	316.28	0.03	314.27	0.02	288.09	0.03
1,2,3,6,7-PMN (2)	313.61	0.03	311.37	0.02	284.84	0.02
1,2,3,5,7-PMN (3)	310.06	0.03	307.89	0.02	280.71	0.03
1,2,4,6,7-PMN (4)	308.89	0.03	306.58	0.03	278.78	0.03
1,2,3,4,6-PMN (5)	316.62	0.03	314.65	0.02	288.87	0.03
1,2,2,5,6-PMT (6)	271.76	0.02	269.69	0.01	225.22	0.02

*I = $I_{(\text{naphthalene})} + (t_r - t_{r(\text{naphthalene})}) / (t_{r(\text{phenanthrene})} - t_{r(\text{naphthalene})}) \times 100$, where $I_{(\text{naphthalene})} = 200$ and t_r is the retention time (Lee *et al.*, 1979).

Identification of pentamethylnaphthalenes in sedimentary organic matter

Aromatic hydrocarbon fractions isolated from crude oils and sedimentary rock extracts were analysed using GC-MS and shown to contain four isomeric pentamethylnaphthalenes. Figure 7.2 shows partial m/z 198 mass chromatograms for the Acacia-1 1032 m sample and a mixture of the five reference compounds. For purposes of clarity of presentation, the chromatograms shown in this figure were obtained using a BP-20 capillary column: use of DB-1 and DB-5 columns results in the methylidibenzothiophenes eluting in the same retention time zone as the pentamethylnaphthalenes, but the elution order of the PMNs is the same (retention indices for the pentamethylnaphthalenes using these columns are given in Table 7.3). In all samples examined, these four compounds (1-4) were the major PMN components. All theoretically possible isomers other than those shown have either 1,2,3,4 or 1,8 substitution patterns which are evidently less stable and were never observed. The peaks with shorter retention times in the mass chromatogram shown in Figure 7.2a are due to C_5 naphthalenes other than PMNs.

m/z 198 Mass Chromatograms (BP 20)

(a) Acacia #1 1032m Aromatic Fraction

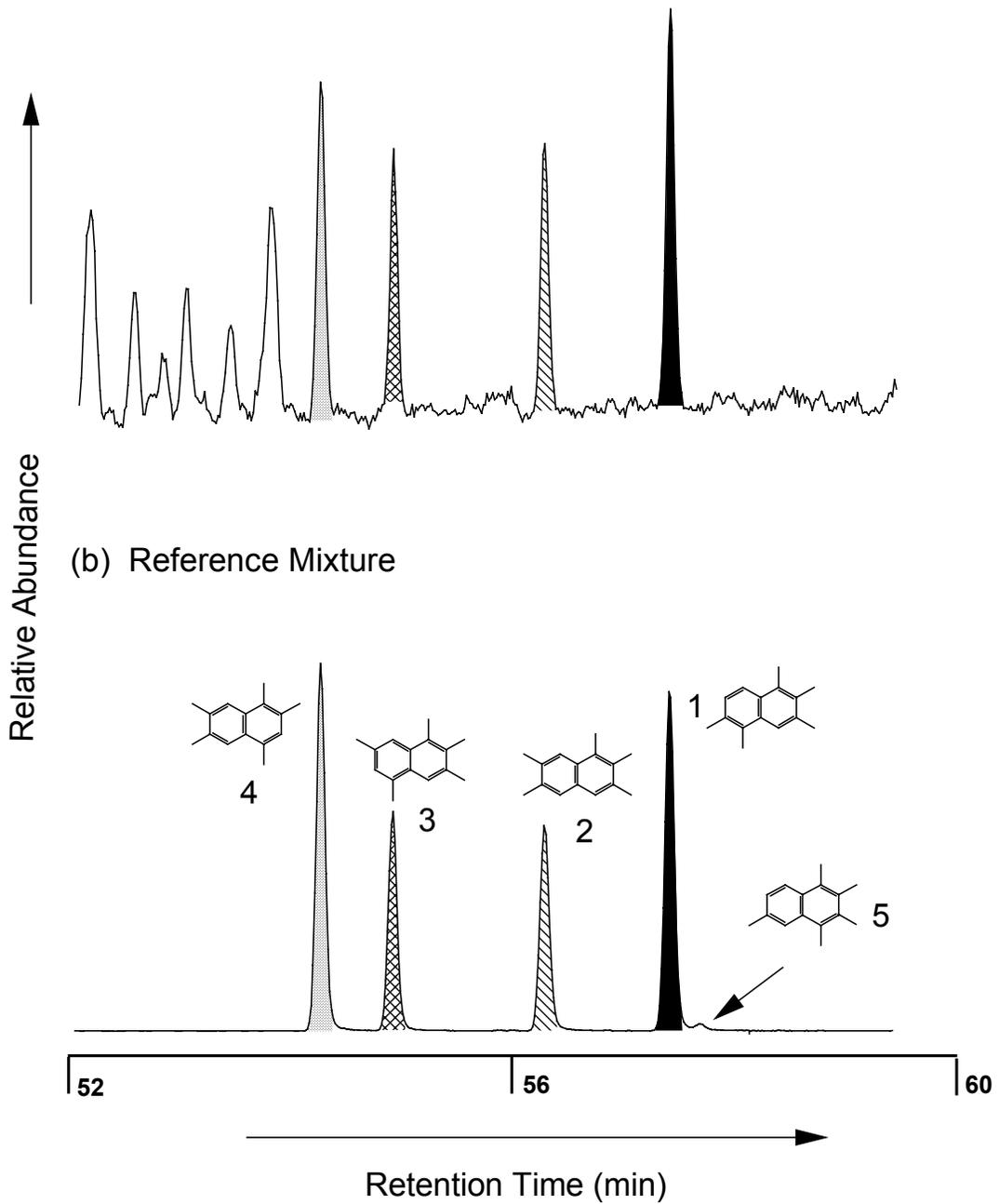


Figure 7.2. Partial m/z 198 mass chromatograms of (a) the aromatic hydrocarbon fraction from the Acacia-1, 1032 m sediment and (b) a mixture of reference compounds.

Occurrence of pentamethylnaphthalenes

Pentamethylnaphthalenes occur in sedimentary rocks and crude oils with wide geographical and temporal distributions. Table 7.1 shows the location and age of the crude oils and sedimentary rock samples and Table 7.4 shows the relative abundances of the PMN isomers in each sample. The abundances of pentamethylnaphthalenes in the older Proterozoic samples, which predate the evolution of higher plants, were similar to those in all the other samples, which indicates that PMNs can be derived from micro-organisms. It is apparent from the data in Table 7.1 and 7.4 that in the least mature samples (e.g. Moorari-4, Acacia 779 m) 1,2,3,5,6-PMN (**1**) is the major PMN component, and in the more mature samples (e.g. Barrow Island, Fly Lake) 1,2,4,6,7-PMN (**4**) is the major PMN component. In our experience, crude oils unaltered by post-migrational effects such as water washing and biodegradation, the PMNs are invariably less abundant than the lower molecular weight polymethylnaphthalenes. Figure 7.3 is a reconstructed ion chromatogram which indicates that the approximate relative abundances of each group of the polymethylnaphthalenes in the Jamison crude oil decrease with increase in the number of methyl substituents. This effect is sometimes less pronounced in carbonates (cf. peaks for DMNs vs TMNs in chromatograms in Figure 7.3 in Chakhmakhchev and Suzuki, 1995).

Formation

Figure 7.4 shows partial m/z 146 and m/z 198 mass chromatograms for the Acacia-1 782 m sample. The m/z 146 mass chromatogram shows that the tetralin (**6**) is the major C_5 -tetralin and 1,2,3,5,6-PMN (**1**) is the major pentamethylnaphthalene isomer. The conversion of the tetralin (**6**) into 1,2,3,5,6-PMN (**1**) was demonstrated in a laboratory experiment in which the tetralin was heated with a dehydrogenation reagent (Pt/C). The reaction mixture contained 1,2,3,5,6-PMN (**1**) and 1,2,5,6-tetramethylnaphthalene in the proportion of 1:4.

We believe that the PMNs may share common precursors with their saturated analogues the sedimentary drimanenes, which are ubiquitous in

sediments and appear to be of microbial origin (Alexander *et al.*, 1984b). Reaction Scheme 7.2 is a proposed formation pathway for the formation of PMT (6) along with a number of saturated drimanoids from some reactive precursor, then, by analogy with our laboratory observation, the PMT may undergo methyl migration and aromatisation to give 1,2,3,5,6-PMN (1).

Table 7.4. Relative abundance data for pentamethylnaphthalenes and values for PNR, a proposed pentamethylnaphthalene maturity ratio.

Sample	Pentamethylnaphthalenes (Rel. %)				PNR
	1,2,3,5,6 (1)	1,2,3,6,7 (2)	1,2,3,5,7 (3)	1,2,4,6,7 (4)	
Iron Duke	44	13	12	30	0.41
Basker-1	38	15	21	26	0.41
Barrow Island	17	20	21	43	0.71
Moorari-4	72	5	10	13	0.15
Fly Lake-1	12	18	17	53	0.81
East Dollarhide-1	24	12	16	48	0.67
Acacia-1, 779	72	11	8	8	0.10
Acacia-1, 782	63	11	12	14	0.18
Acacia-1, 974	28	22	22	8	0.32
Acacia-1, 1032	30	26	19	25	0.45
Great Sandy-1	31	23	25	21	0.40
Blina-1	25	17	22	36	0.58
Eremophila	30	18	25	27	0.47
PD 130A	65	13	12	10	0.13
Jamison	10	13	22	55	0.85
McManus	14	14	16	56	0.80

a. m/z 142 + 156 + 170 + 184 + 198 (DB 5)

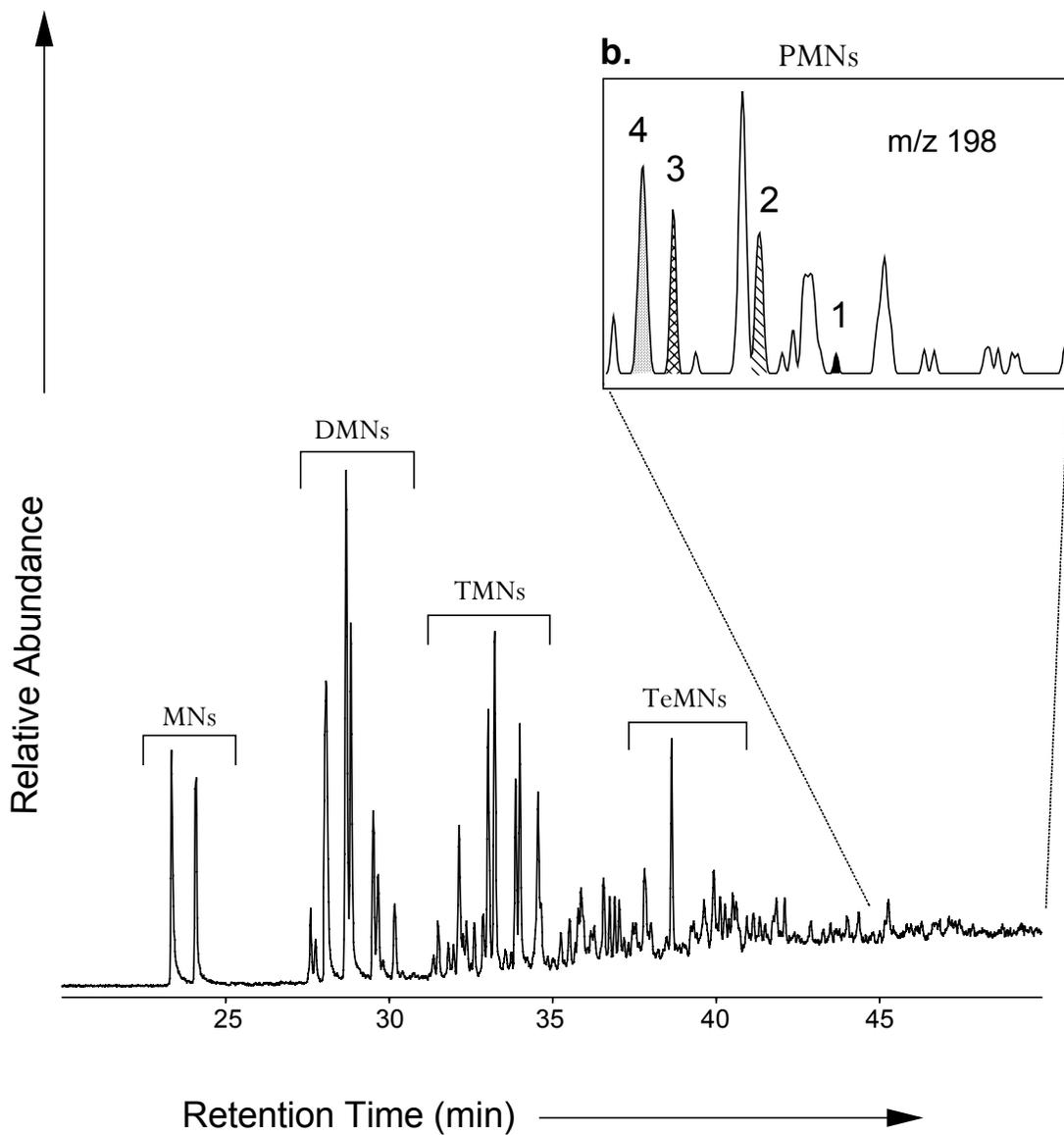


Figure 7.3. Partial mass chromatograms of the aromatic hydrocarbon fraction from Jamison showing (a) the m/z 142 + 156 + 170 + 184 + 198 mass chromatograms and (b) the m/z 198 mass chromatogram for the retention time zone where PMNs elute.

Acacia #1 782m Aromatic Fraction

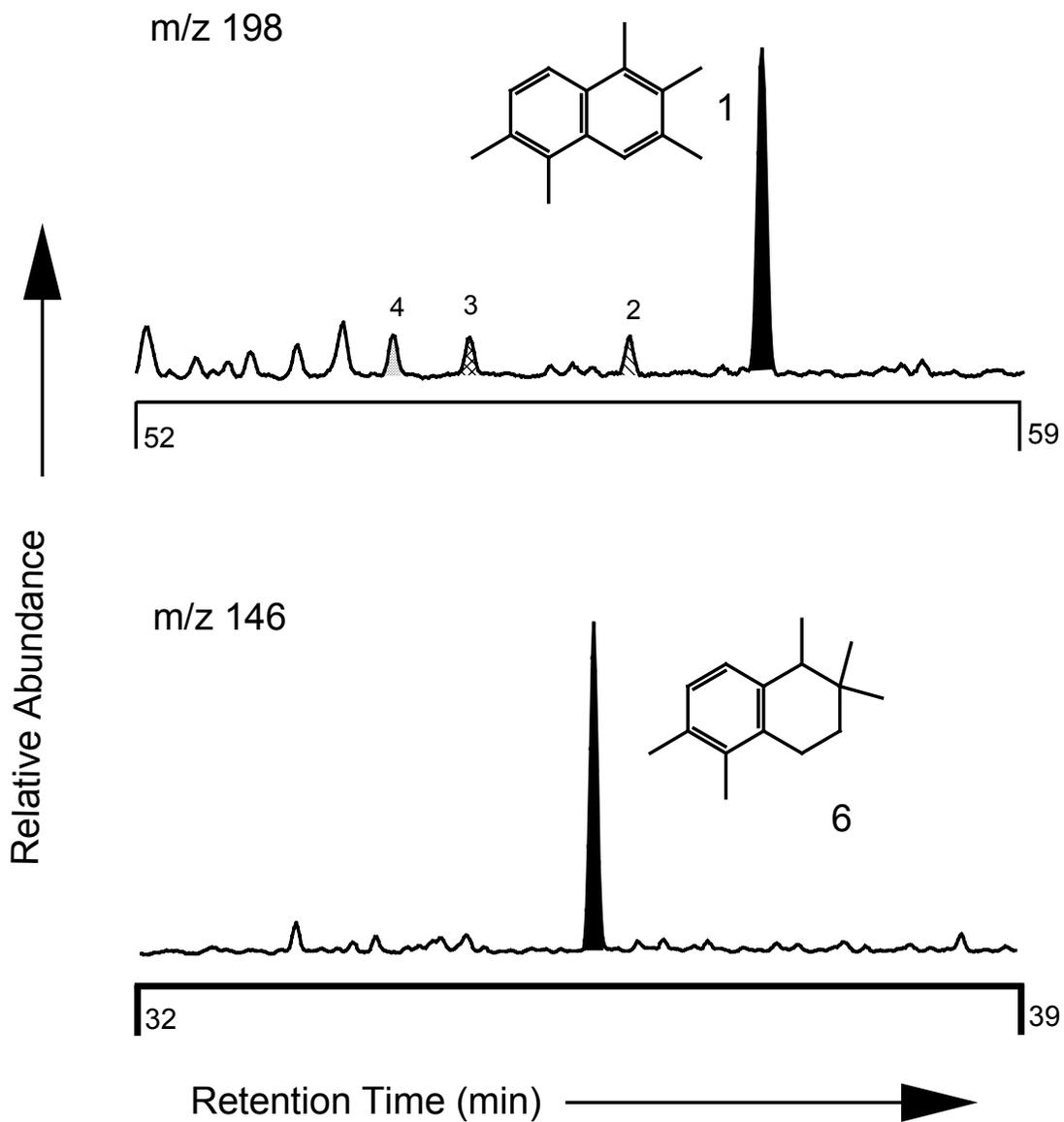
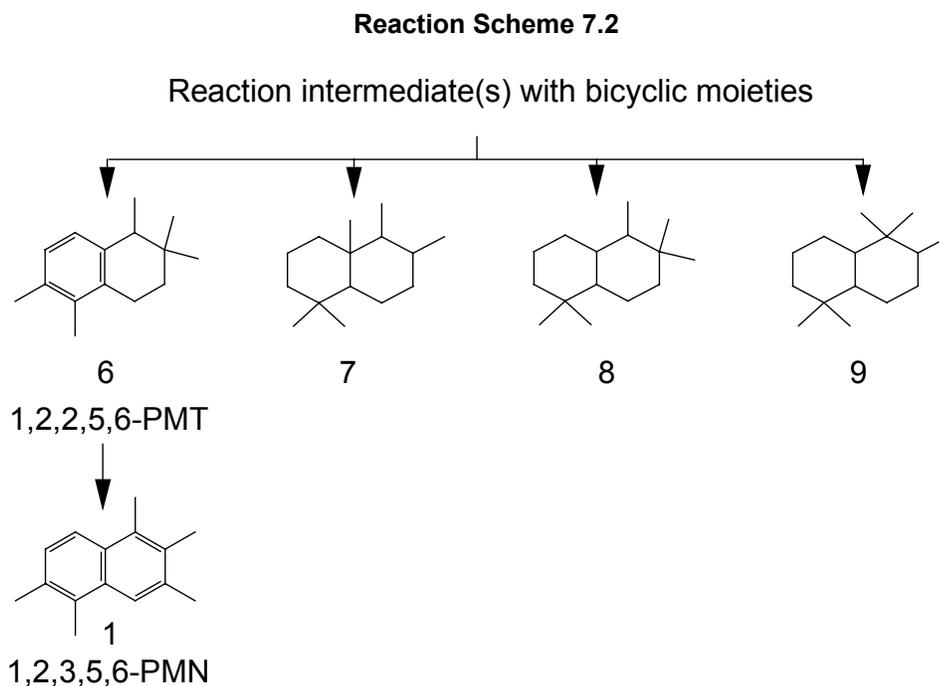


Figure 7.4. Partial mass chromatograms of the aromatic hydrocarbon fraction from Acacia-1, 782 m sediment showing (a) the m/z 198 ion (pentamethylnaphthalenes) and (b) the m/z 146 ion (pentamethyltetralins).

The scheme proposed for the formation mechanism of 1,2,3,5,6-PMN (**1**) is consistent with its occurrence in samples of Proterozoic age. These samples all contain rearranged drimanes which have previously been attributed to microbially derived natural products (Alexander *et al.*, 1984b). Apparently, even the earliest biomass capable of producing hydrocarbons, through sedimentary processes, contained these biogenic natural products (rearranged drimanes).



An alternative mechanism of formation of 1,2,3,5,6-PMN could involve sedimentary methylation of 1,2,5,6-TeMN, which is the major TeMN isomer in low maturity organic matter (Püttmann and Villar, 1987). To test this possibility we heated 1,2,5,6-TeMN with an active clay catalyst (aluminium montmorillonite) at 150 °C for 1h (cf. Bastow *et al.*, 1996), and examined the products. The extent of reaction had reached about 10% and the only pentamethylnaphthalene was 1,2,3,5,6-PMN (**1**). This observation suggests that 1,2,3,5,6-PMN can be formed in sediments from methylation of 1,2,5,6-TeMN; however we believe that for the crude oils and sediments examined in this study methylation has not been an important process. For example, 1,2,5-TMN is a prominent trimethylnaphthalene; methylation of this would give 1,2,5,7-TeMN but this TeMN is present in normal abundance. We

therefore infer that in the systems we have examined, the formation of 1,2,3,5,6-PMN (**1**) from the tetralin is the major source of this compound.

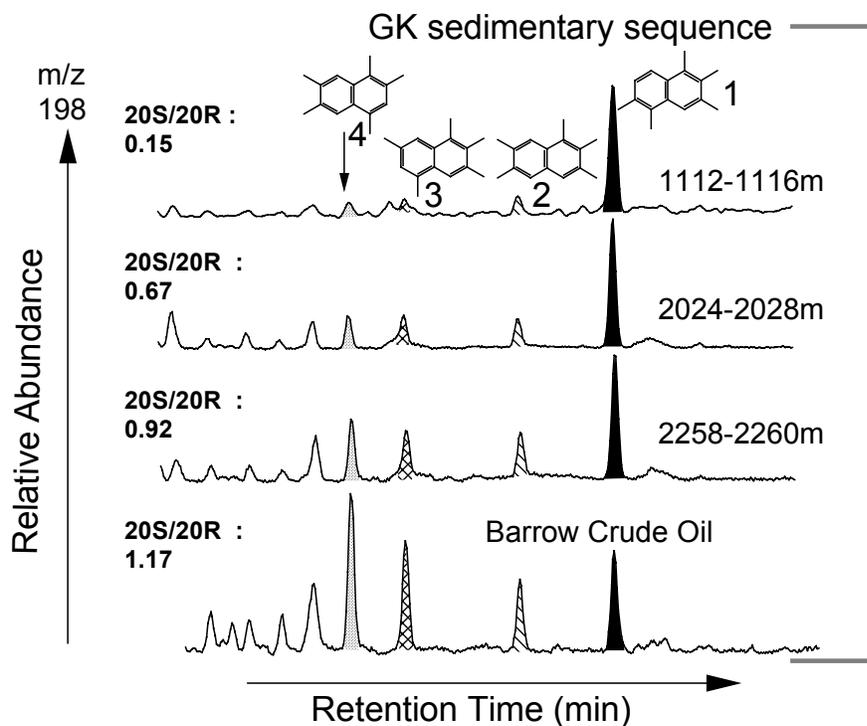
Maturity effects

1,2,3,5,6-PMN (**1**) is the most abundant pentamethylnaphthalene in low maturity sediments, but becomes relatively less abundant with increases in maturity. Figure 7.5(a) shows *m/z* 198 mass chromatograms for three samples from a sedimentary sequence and a crude oil. The maturity of the sediment sample and the crude oil can be inferred from the 20S/20R sterane ratios. It is apparent that as the sterane maturity values increase there is a corresponding decrease in relative abundance of 1,2,3,5,6-PMN (**1**) and an increase in the other isomers.

The relative stabilities of pentamethylnaphthalenes was investigated using laboratory heating experiments, and the results compared with changes in the relative abundance of isomers in samples heated under natural conditions in sediments. Figure 7.5b shows the change in isomer distribution that occurs with increase in reaction time when 1,2,3,5,6-PMN (**1**) was heated with trifluoroacetic acid at 200°C. These conditions cause predominantly 1,2-methyl shift reactions to occur, and at sufficiently long reaction times, will produce a mixture of compounds whose relative abundances reflect their stability. From the reaction products after extended heating (100 h) it is apparent that the order of isomer stability is as follows: 1,2,4,6,7-PMN > 1,2,3,6,7-PMN > 1,2,3,5,7-PMN > 1,2,3,5,6-PMN. Little if any 1,2,3,4,6-PMN and isomers with *peri* methyl groups were detected, showing that these isomers are all less stable than 1,2,3,5,6-PMN.

That the least stable of the sedimentary pentamethylnaphthalenes is the most abundant isomer in sediments of low maturity demonstrates that its relative abundance is sensitive to sediment maturity. A maturity-sensitive parameter can therefore be devised using the most stable and least stable of the common sedimentary isomers. The pentamethylnaphthalene ratio (PNR)

a. Samples from GK well and Barrow crude oil



b. Products from heating 1,2,3,5,6-PMN with trifluoroacetic acid at 200°C.

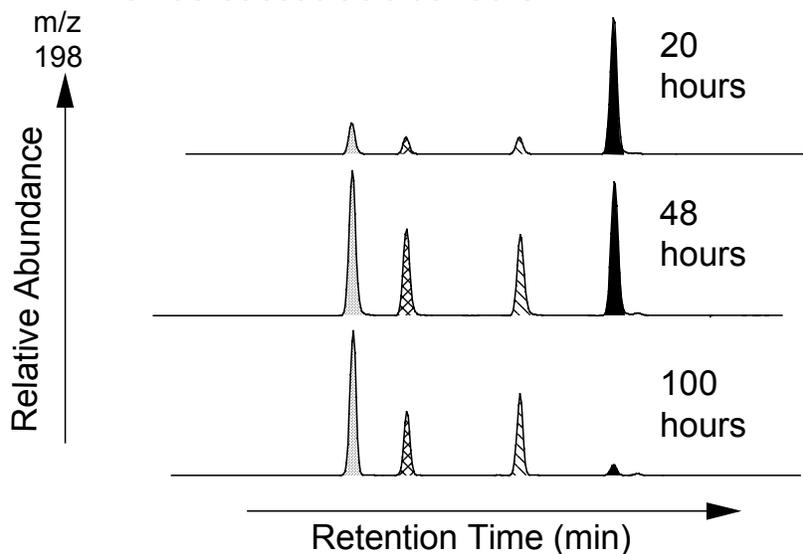


Figure 7.5. Partial m/z 198 mass chromatogram of (a) the aromatic hydrocarbon fractions from the GK well and Barrow crude and (b) products from heating 1,2,3,5,6-PMN with trifluoroacetic acid at 200°C for various times.

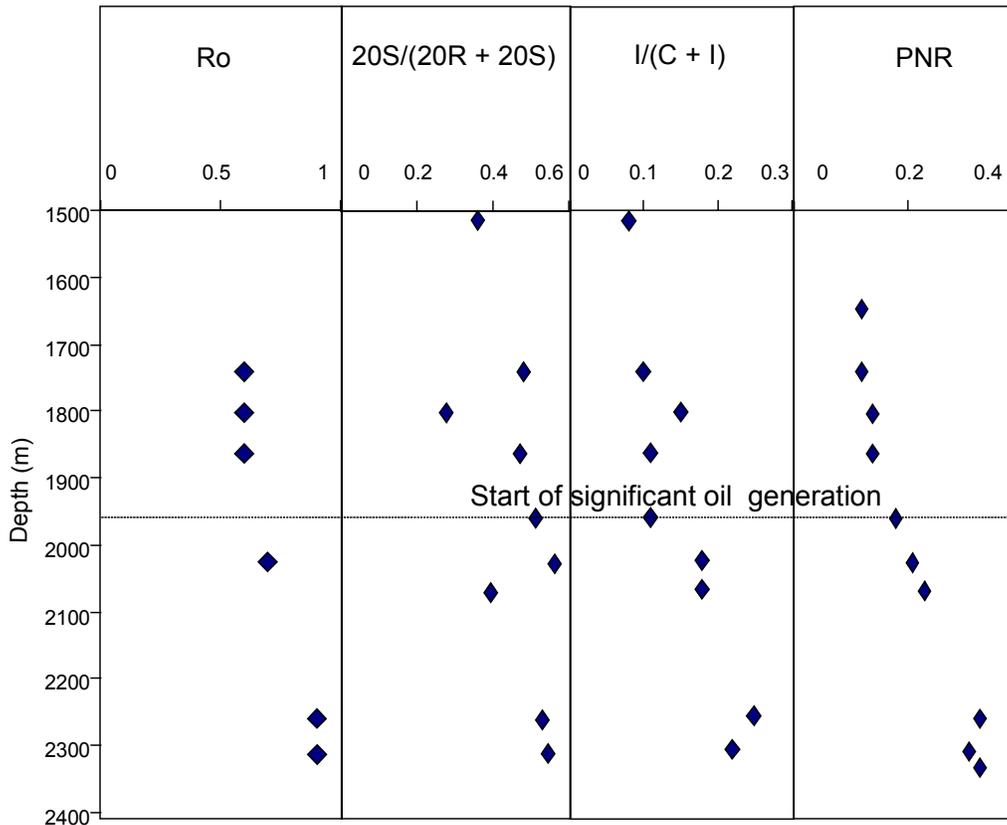


Figure 7.6. Plots showing measured changes in PNR and various other maturity parameters vs depth for the GK well. (PNR = 1,2,4,6,7-PMN/(1,2,3,5,6-PMN + 1,2,4,6,7-PMN); adapted from Sosrowidjojo *et al.*, 1996). Definitions: R_o , vitrinite reflectance; steranes, 20S and 20R diastereomers of 5 α (H), 14 α (H), 17 α (H)-ethylcholestane.

expressed as 1,2,4,6,7-PMN/(1,2,3,5,6-PMN + 1,2,4,6,7-PMN) is conveniently measured along with the methyl dibenzothiophene maturity parameter in a single m/z 198 mass chromatogram using a DB-5 column. Figure 7.6, which is adapted from Sosrowidjojo *et al.* (1996) shows values for this PNR parameter as a plot against depth for the GK sedimentary sequence. It is apparent from this plot that a systematic increase in PNR occurs with increase in sample depth (maturity). Three other measures of maturity: R_o , 20S/(20S + 20R), and a ratio involving cadalene(C) and isocadalene(I) (cf. Alexander *et al.*, 1994) are also shown in this plot.

Relative abundances of PMNs for sedimentary organic matter and crude oils used in this study are shown in Table 7.4. These measurements show that oils can have PNR values as low as 0.15, suggesting that this is the value of PNR at the commencement of the oil window. The systematic trend observed in this sedimentary sequence suggest that this parameter may potentially be useful for maturity assessment. The values in the above example were obtained from a sequence whose maturity was difficult to assess using conventional parameters (Sosrowidjojo *et al.*, 1996), indicating that the PNR parameter may prove to be a more robust measure of maturity than some of the previously used indicators.

7.5 Conclusions

Four stable pentamethylnaphthalenes have been synthesised identified and shown to occur widely in petroleum and in sediments. With increasing maturity the relative proportion of these isomers changes with the more stable isomers becoming more abundant. This allows definition of a pentamethylnaphthalene maturity parameter (PNR), which shows promise as a conveniently measured and robust indicator of the maturity of crude oils and petroleum source rocks.

7.6 Acknowledgements

Geoff Chidlow is thanked for his assistance with GC-MS analysis. Financial support was provided by the Australian Petroleum Co-operative Research and from a Curtin University Postgraduate Scholarship.

8.0 Geosynthesis of organic compounds. II - Methylation of phenanthrene and alkylphenanthrenes

8.1 Abstract

A crude oil and several shales which contain anomalously high concentrations of phenanthrene, 1-methylphenanthrene, 1,7-dimethylphenanthrene and retene have been shown to contain relatively high concentrations of their corresponding 9-methyl counterparts. In laboratory experiments carried out under mild conditions each of the phenanthrenes has been shown to be substituted preferentially at the 9-position when a methyl donor was heated with the substrate in the presence of a clay catalyst. These observations have been interpreted as evidence for a sedimentary methylation process.

8.2 Introduction

The widespread occurrence of aromatic hydrocarbons whose carbon skeletons are non-isoprenoidal in sediments has led to the suggestion that such compounds are the products of sedimentary reactions (Radke *et al.*, 1982a, b, 1993; Smith *et al.*, 1994). Phenanthrenes with methyl substituents in ring positions 9 and 10 are examples of such compounds (Radke *et al.*, 1982a, 1984a, 1984b, 1993).

Substituted phenanthrenes with methyl substituents in positions 9 or 10 have been reported to occur in sedimentary material including crude oil (Lekveishvili *et al.*, 1980; Melikadze *et al.*, 1985; Radke *et al.*, 1993), oil shales (Radke and Welte, 1983 and Radke *et al.*, 1982b) and coals (Radke *et al.*, 1982b, 1990b). 9-Methylphenanthrene has been shown to have a high relative abundance compared to the other methylphenanthrene isomers in low rank coals (Radke *et al.*, 1982b, 1990b) and lower maturity petroleum source rocks (Radke and Welte, 1983). With increase in coal rank or sediment maturity a relative decrease in the 9-methylphenanthrene abundance commonly occurs (Radke *et al.*, 1982b; Radke and Welte, 1983 and Radke *et al.*, 1990b). A similar observation has been made for the dimethylphenanthrenes with substituents in the 9 or 10 positions compared with the more stable isomers. Both compound classes have been used to develop maturity indicators (Radke *et al.*, 1982a, 1982b, 1988) based on the lower stability of the 9 or 10 substituted isomers.

Although sedimentary methylation of phenanthrenes has been proposed as the origin of isomers with methyl groups in positions 9 and 10, the nature and timing of this sedimentary process remains unclear. In this paper we report on the unambiguous identification of 9-methyl substituted alkylphenanthrenes and show that such compounds can be formed in reactions between a phenanthrene or alkylphenanthrenes without 9 or 10 substituents and a methyl donor in the presence of an acidic clay.

8.3 Experimental

Samples

Information about the samples is given in Table 8.1. The Moorari-4 crude oil was recovered from the Birkhead Formation of Jurassic age (Kantsler *et al.*, 1983). Longreach and Mayneside samples were obtained as core samples from the Toolebuc Formation of Cretaceous age located at Julia Creek, (Patterson *et al.*, 1986) and were supplied by the Australian Geological Survey Organisation (sample numbers 1105 and 1256). For a detailed

description of the tectonic history and basin development readers are referred to Price *et al.* (1985) and references therein.

Other sediment samples were obtained as conventional cores.

Table 8.1. Some geological and geochemical data for Australian crude oil and sediment samples

Sample		Location: Basin	Age or Probable Age of Source Rock	Source/ Depositional Environment	TNR-1*	Reference
Name	Type					
Moorari-4	Crude oil	Eromanga	Jurassic	higher plant, lacustrine	0.27	Kantsler <i>et al.</i> , 1983
Sample A	Shale	Otway	Cretaceous	higher plant, lacustrine	0.44	Lovibond <i>et al.</i> , 1995
Mayneside	Shale	Eromanga	Cretaceous	algal, marine	0.77	Patterson <i>et al.</i> , 1986
Longreach	Shale	Eromanga	Cretaceous	algal, marine	0.85	Patterson <i>et al.</i> , 1986
Dampier-1	Shale	Dampier sub-basin	Jurassic	algal, marine	0.92	Brikke, 1982

* TNR-1 = $2,3,6\text{-TMN} / (1,3,5\text{-TMN} + 1,4,6\text{-TMN})$ where TMN = Trimethylnaphthalene

Preparation of alkylphenanthrenes

1-Methylphenanthrene (1-MP), 9-methylphenanthrene (9-MP), 1,7-dimethylphenanthrene (1,7-DMP) and 1,9-dimethylphenanthrene (1,9-DMP) were purchased from Chiron Laboratories, Trondheim, Norway. 1,9-Dimethyl-7-isopropylphenanthrene (9-methylretene) and 1,7,9-trimethylphenanthrene (1,7,9-TMP) were prepared from the appropriate alkylstilbenes according to the method described by Wood and Mallory (1964). Briefly, the Grignard reagent (15 mmol in 50 mL of ether) from the appropriate benzyl bromide was added to an alkylacetophenone (13 mmol in 20 mL ether). The resultant tertiary alcohol (65-75% yield) was recovered in pure form after subjecting the reaction mixture to liquid chromatography procedures using alumina (activity III) with hexane and dichloromethane as solvents. The substituted stilbenes were obtained by dehydration of the tertiary alcohol with a mixture of sulphuric and acetic acids (85-95% yield), and were then photochemically cyclised to phenanthrenes in cyclohexane in the presence of air and iodine.

N.M.R

All measurements were carried out in a solution of chloroform-d using a Bruker ARX-500 operating at 500 MHz for ^1H and 125 MHz for ^{13}C . ^1H NMR data for 9-methylretene:

δ 1.41, d, J 6.9 Hz, 6H, $-\text{CH}(\text{CH}_3)_2$; 2.74, s, 3H, 2.79, s, 3H, Ar(C1)- CH_3 , Ar(C9)- CH_3 ; 3.17, septet, J 6.9 Hz, Ar(C7)- $\text{CH}(\text{CH}_3)_2$; 7.39, d, J 7.0 Hz, 1H, Ar(C2)-H; 7.47, apparent t, J 8.1, 7.24 Hz, 1H, Ar(C3)-H; 7.57, dd, J 1.9, 8.6 Hz 1H, Ar(C6)-H; 7.78, s, 1H, Ar(C10)-H; 7.88, d, J 1.8 Hz, 1H, Ar(C8)-H; 8.52, d, J 8.3 Hz, 1H, Ar(C4)-H; 8.67, d, J 8.6 Hz, 1H; Ar(C5)-H.

^{13}C NMR data for 9-methylretene:

δ 19.91, 20.53, Ar(C1,C9) CH_3 ; 24.19, Ar(C7) $\text{CH}(\text{CH}_3)_2$; 34.39, Ar(C7) $\text{CH}(\text{CH}_3)_2$; 120.51, 121.60, 122.75, 123.37, 125.23, 125.25, 127.31 Ar(C2, C3, C4, C5, C6, C8, C10); 128.91, 129.70, 130.33, 131.71, 132.11, 133.95, 146.91, Ar(C1, C4a, C5a, C7, C8a, C10a).

Isolation of aromatic hydrocarbons

In a typical separation, the crude oil sample (100 mg) was subjected to preparative TLC using glass plates spread with alumina (Merk Alumina G; 0.6 mm, activated at 120°C for at least 12 hours). The plates were developed with n-hexane and the required band comprising the trinuclear aromatic compounds was located relative to reference compounds (naphthalene and phenanthrene) using UV light (254 nm). The organic material was recovered from alumina with dichloromethane and the solvent was removed in a Kuderna-Danish apparatus. The residue was dissolved in hexane to provide a sample ready for GC-MS analysis (or further separations using molecular sieves).

Molecular sieve chromatography

Molecular sieve chromatography was carried out with mordenite and US-Y 400 molecular sieves using a procedure described by Ellis *et al.* (1994). The fraction that was not retained by the sieves was further analysed using gas

chromatography-mass spectrometry (GC-MS) and gas chromatography-Fourier transform infrared spectroscopy (GC-FTIR) techniques.

GC-MS analysis

A Hewlett-Packard 5971 MSD interfaced with a 5890 Series II gas chromatograph fitted with 60 m x 0.25 mm i.d. (J&W Scientific) fused-silica open tubular column containing a 0.25 μm DB-5 or DB-1 stationary phase was used for analysis. The GC oven was temperature programmed from 70 to 300°C at 3°C min⁻¹. Samples for analysis were dissolved in hexane and injected on-column using a HP 7673 auto sampler at an oven temperature of 70°C. Helium was used as a carrier gas at a linear velocity of 30 cm s⁻¹. Typical MSD conditions were: ionisation energy 70 eV; source temperature 200°C; electron multiplier voltage 2200 V. Alkylphenanthrenes were assigned by mass chromatography of their parent ions and by comparison of retention times with those of standards.

GC-FTIR analysis

Infrared spectra were acquired by direct deposition GC-FTIR using a Digilab TRACER(TM) interfaced with a Hewlett Packard 5890A gas chromatograph (GC) and a Digilab FTS-60A Fourier transform infrared spectrometer. The GC was equipped with an OCI-3 on-column injector (SGE Australia) connected to a 50 m x 0.22 mm I.D. fused silica open tubular column coated with a 0.25 μm thick film of 5% phenyl : 95% dimethylsiloxane (BP5, SGE Australia). The oven was temperature programmed from 40 to 290°C at a rate of 3°C min⁻¹. Hydrogen was the carrier gas at a linear flow velocity of 30 cm s⁻¹ at the initial operating temperature. The column effluent was passed through a fused silica transfer line (1 m x 0.15 mm I.D.) to the fused silica deposition tip (1 cm x 0.05 mm I.D.). The temperature of both of these heated transfer zones was maintained at 290°C. The eluants were cryogenically focussed onto a moving zinc selenide plate (60 mm x 30 mm x 2 mm) held at approximately -180°C. The resultant frozen deposits described a continuous square wave pattern of 1.2 mm amplitude and 0.2

mm period. The slide speed which controls the post-GC resolution of compounds was maintained at a nominal setting of two, enabling compounds separated by a minimum of two seconds to be resolved. The infrared beam was focussed by a cassagrainian microscope onto a fast response mercury / cadmium telluride (MCT) detector.

The Gram - Schmitt (total spectral response) chromatogram was scanned in real time between 650 and 4000 cm^{-1} at a resolution of eight wavenumbers and a scan rate of four scans per scan set producing an absorbance spectrum every 0.8 seconds. Functional group chromatograms were similarly collected in selected regions of the infrared spectrum, namely 700 - 920, 1400-1650 and 2800-3000 cm^{-1} .

High resolution infrared spectra of selected alkylphenanthrenes were obtained by re-examining the solid deposited material after the chromatographic analysis was completed. These spectra were obtained by the addition of the interferograms from 256 successive scans at a resolution of 2 cm^{-1} .

Alkylation experiments

The aluminium montmorillonite used in the study was material prepared for an earlier study (Alexander *et al.*, 1984a). Individual alkylphenanthrenes (1 mg), and hexamethylbenzene (3 mg) and finely divided aluminium montmorillonite (10 mg) were placed in small (3 mL) Pyrex glass ampoules and flushed with dry nitrogen. The ampoules were evacuated, sealed and then heated at 100°C for 8 h. The contents were extracted with dichloromethane (2 mL), the solvent was removed by careful distillation, and hexane (1.8 mL) containing the external standard pentadecane (0.5 mg) was added. An aliquot was then subjected to analysis using GC-MS techniques.

8.4 Results

Preparation of reference compounds

9-Methylretene and 1,7,9-trimethylphenanthrene (TMP) were not available from commercial sources and were prepared from the appropriate alkylstilbenes by photochemical cyclisation according to the method described by Wood and Mallory (1964). 9-Methylretene was obtained in good yield and NMR spectra (proton and ^{13}C) were consistent with 9-methylretene. The mass spectrum of 9-methylretene has not previously been published and is shown in Figure 8.1. 1,7,9-TMP was prepared as a mixture with 1,5,9-TMP. It was identified as the first eluting compound from a GC capillary column by comparison with retention data published by Radke *et al.* (1993).

Identification of 9-methyl(alkyl)phenanthrenes in crude oil

A group of phenanthrenes containing 9-methyl substituents were identified in crude oil using GC-MS, GC-FTIR and molecular sieving techniques. Figure 8.2 shows a combined mass chromatogram (m/z 206 + 220 + 234 + 248) for

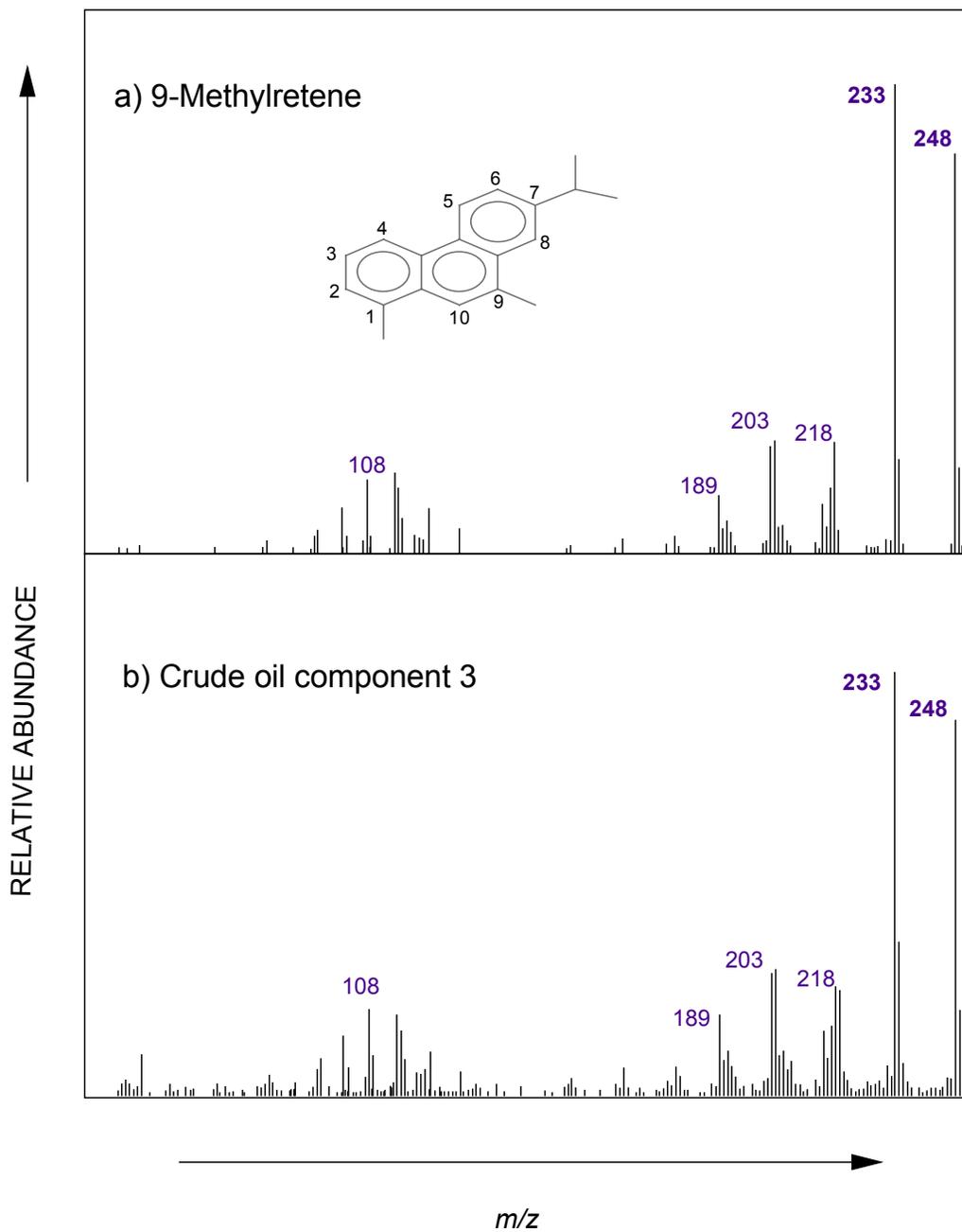


Figure 8.1. (a) Mass spectrum of 9-methylretene (1,9-dimethyl-7-isopropylphenanthrene) and (b) component 3 from the crude oil (cf. Figure 8.2).

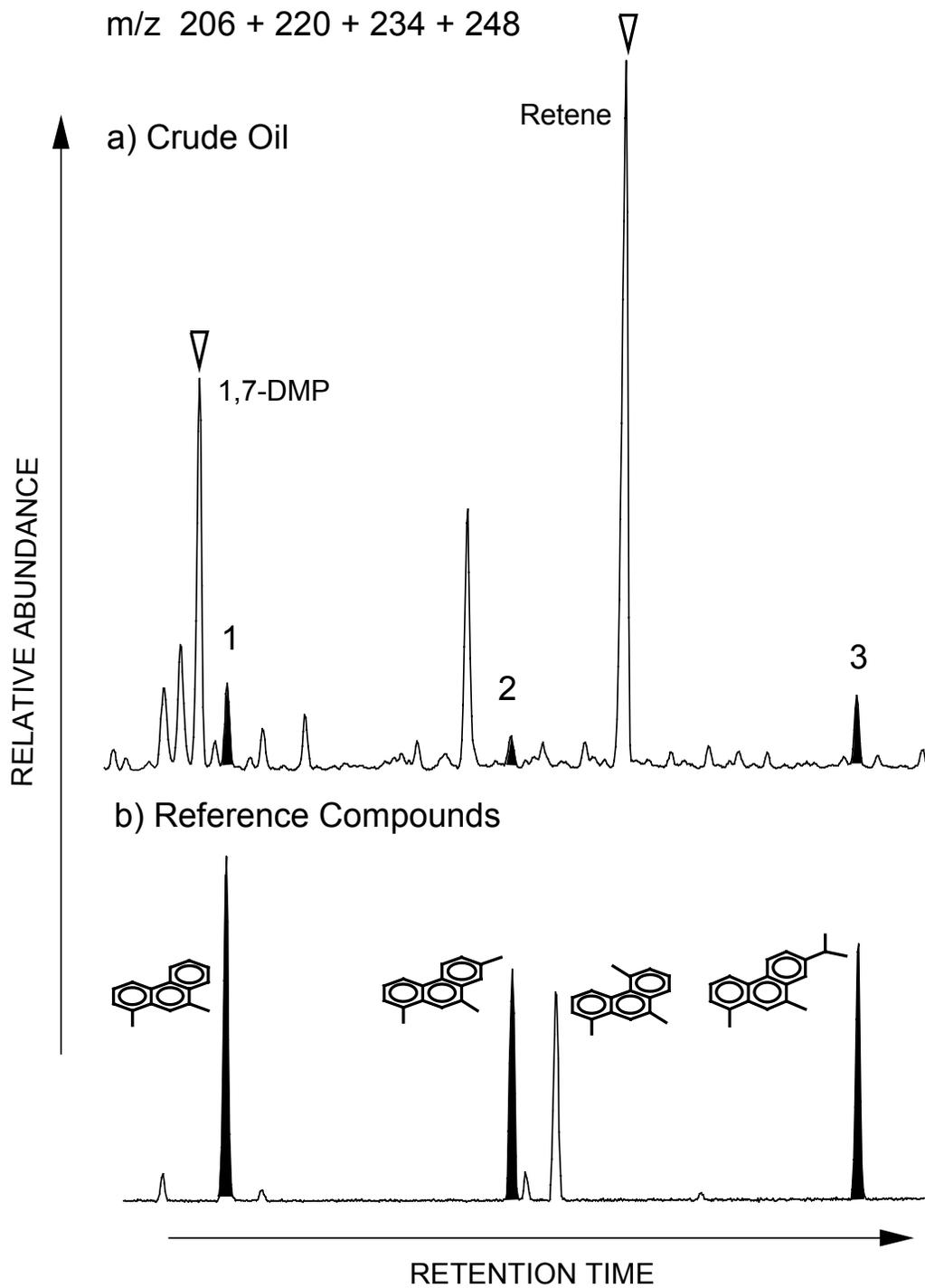


Figure 8.2. Combined partial mass chromatogram of the trinuclear aromatic hydrocarbon fraction from the Moorari-4 crude oil (a) and the corresponding mass chromatogram from a mixture of reference compounds (b).

the trinuclear aromatic fraction of the Moorari-4 crude oil showing responses for the C₂-C₅ alkylphenanthrenes. Also shown is the corresponding mass chromatogram from a mixture of the reference compounds. The peaks labelled 1, 2 and 3 in the chromatogram of the crude oil had the same retention times and similar mass spectra to the reference compounds. In the case of 1,9-dimethylphenanthrene further evidence as to its occurrence in the crude oil was required because it co-elutes with 4,9- and 4,10-dimethylphenanthrene under the analytical conditions used in this study (Radke *et al.*, 1984a, 1984b, 1986). Figure 8.3 shows the solid state infrared spectrum of the compound in the crude oil that co-elutes with 1,9-dimethylphenanthrene (crude oil component 1) together with the spectra of the three co-eluting dimethylphenanthrenes. Clearly, the spectrum obtained from crude oil component 1 and that of 1,9-DMP are very similar and quite different to those of the other co-eluting dimethylphenanthrenes (4,9-DMP and 4,10-DMP) showing that, in the case of this crude oil, the peak represents mainly 1,9-DMP. In fact the absence from the spectra of the crude oil component 1 of peaks prominent in the spectra of 4,10-DMP and 4,9-DMP (733 and 725 cm⁻¹, respectively) places an upper limit on the proportion of these components of a few percent. In this analysis it was also possible to obtain an FTIR spectrum of crude oil component 3 (Figure 8.2). This spectrum, together with that of 9-methylretene are shown in Figure 8.4. The similarities in the spectra support the assignment of component 3 in the crude oil as 9-methylretene and adds further support to the evidence obtained from GC-MS analysis (Figure 8.1) as to the identity of this component.

GC-MS techniques could not be used to identify component 2 (Figure 8.2) in the trinuclear aromatic fraction of the crude oil because several trimethylphenanthrene isomers, all with similar mass spectra, elute with a narrow range of retention times. However, the application of a recently developed separation technique using molecular sieves (Ellis *et al.*, 1994)

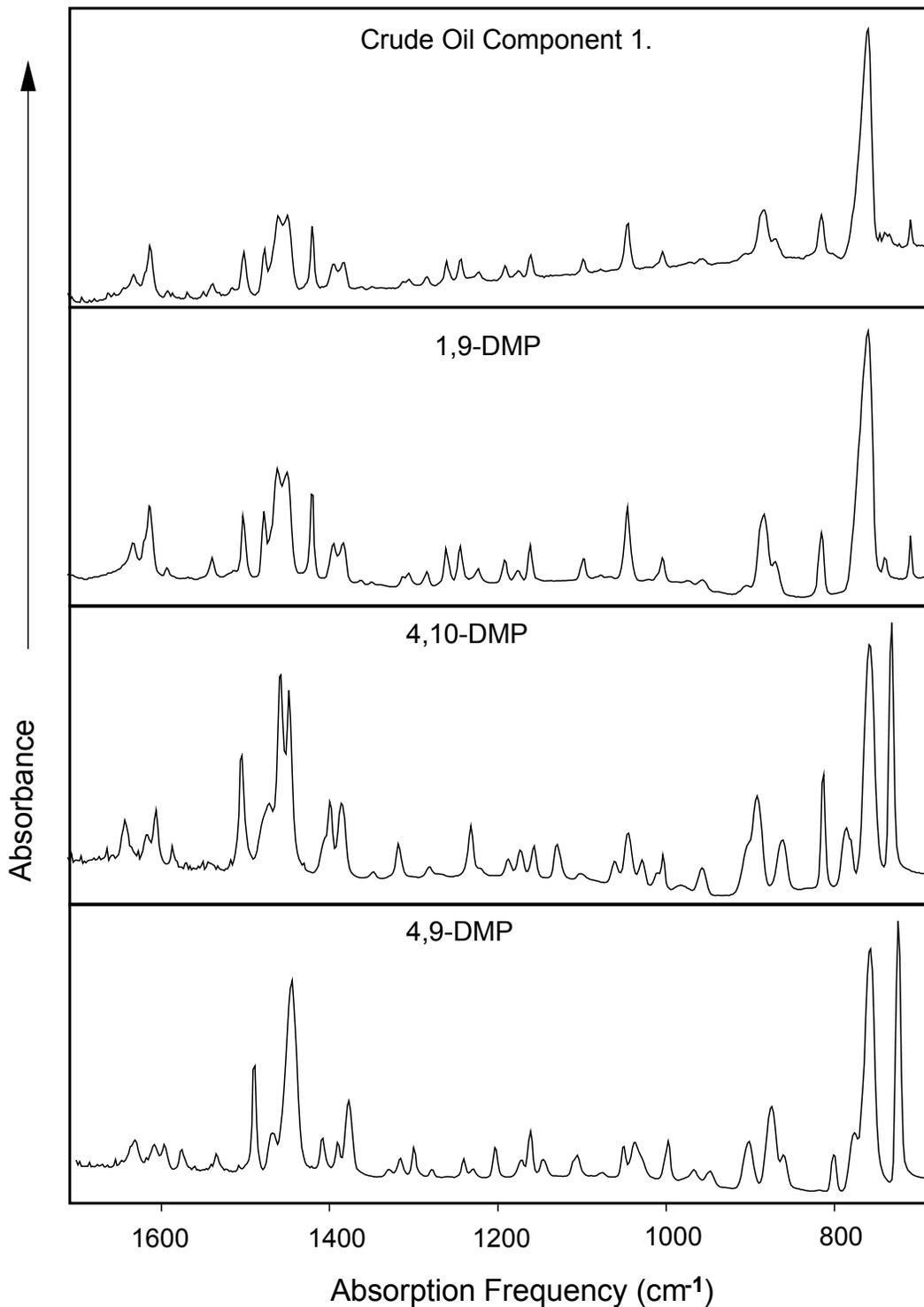


Figure 8.3. Solid state FTIR spectra of crude oil component 2 (cf. Figure 2) and reference dimethylphenanthrene (DMP) isomers measured using direct deposition GC-FTIR techniques. (Data provided by Steve J. Fisher).

enabled a mixture of alkylphenanthrenes to be isolated that was enriched in compounds with 9-methyl substituents. Figure 8.5 shows partial chromatograms of this mixture obtained using direct deposition GC-FTIR and GC-MS techniques. The mass spectrum of the component assigned as 1,7,9-TMP had a mass spectrum consistent with that compound but was indistinguishable from other isomers. The most compelling evidence for its identity was provided by its FTIR spectrum. Figure 8.6 shows the solid state FTIR spectrum obtained from this component of the mixture, together with the corresponding spectrum of 1,7,9-TMP. The similarities of the two spectra show that crude oil component 2 is mainly 1,7,9-TMP. The analytical conditions used for analysis of these samples results in co-elution of 1,7,9-TMP and 2,3,5-TMP. This combined peak is just resolved from the peak for the later co-eluting pair 1,6,9-TMP and 2,3,7-TMP (Radke *et al.*, 1993). Thus, the only isomer in the first pair with a 9-methyl substituent is 1,7,9-TMP and as a result its assignment is unambiguous.

Methylation of phenanthrene and alkylphenanthrenes in sediments

The co-occurrence in sediments of anomalously high abundances of phenanthrene and alkylphenanthrenes with their 9-methyl counterparts of a mixture of trinuclear aromatic compounds isolated using mordenite molecular sieves. This suggests a link between the two compound types, possibly involving sedimentary methylation. To illustrate this relationship we have analysed samples with anomalously high abundances of phenanthrene, 1-MP, 1,7-DMP and retene, and obtained relative abundance data for the corresponding 9-methyl compounds. The results are shown in Figure 8.7 together with the corresponding results for samples without high abundances

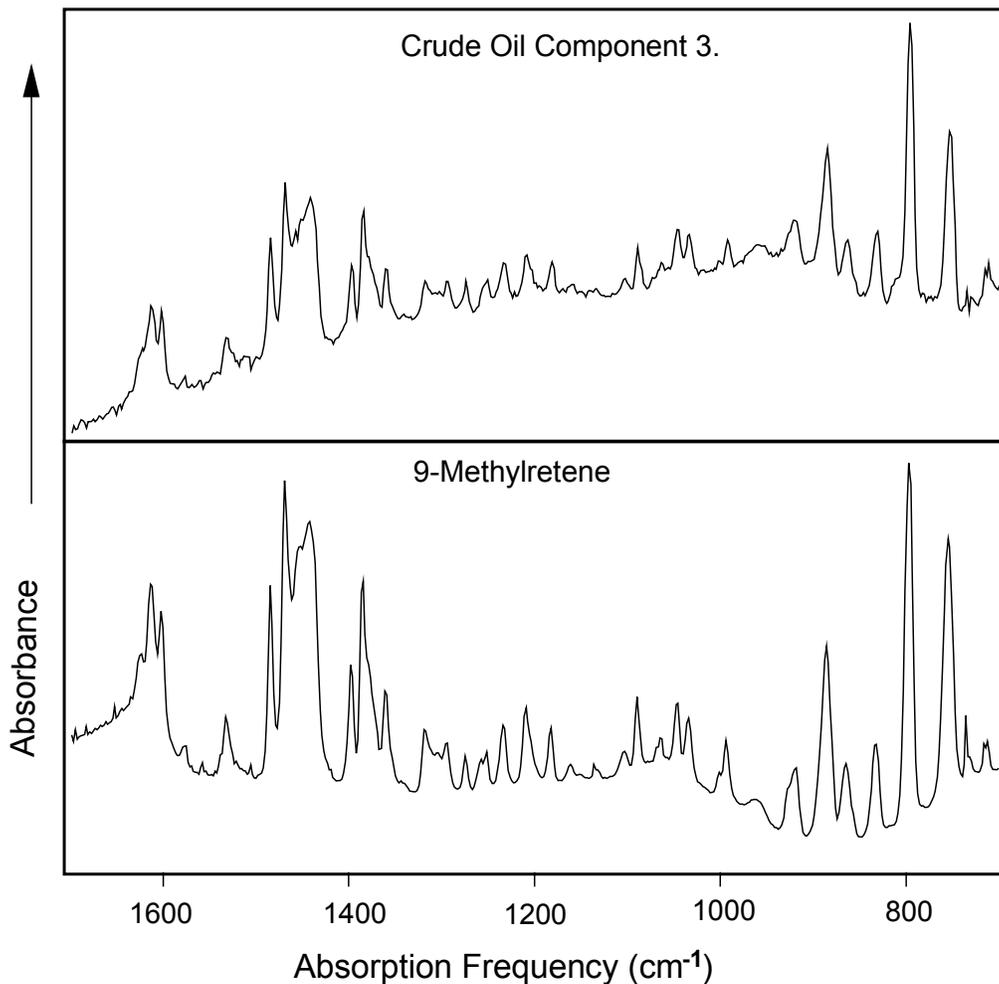


Figure 8.4. Solid state spectra of crude oil component 3 (cf. Fig. 8.2) and 9-methylretene obtained from GC-FTIR measurements. (Data provided by Steve J. Fisher).

of these compounds and with more common distributions of alkylphenanthrenes.

In the case of the phenanthrene and 9-MP pair, two samples with similar maturities both from the Toolebuc Formation, Eromanga Basin, Australia have been selected for comparison. The combined partial mass chromatograms in Figure 8.7a show that the sample with the higher relative

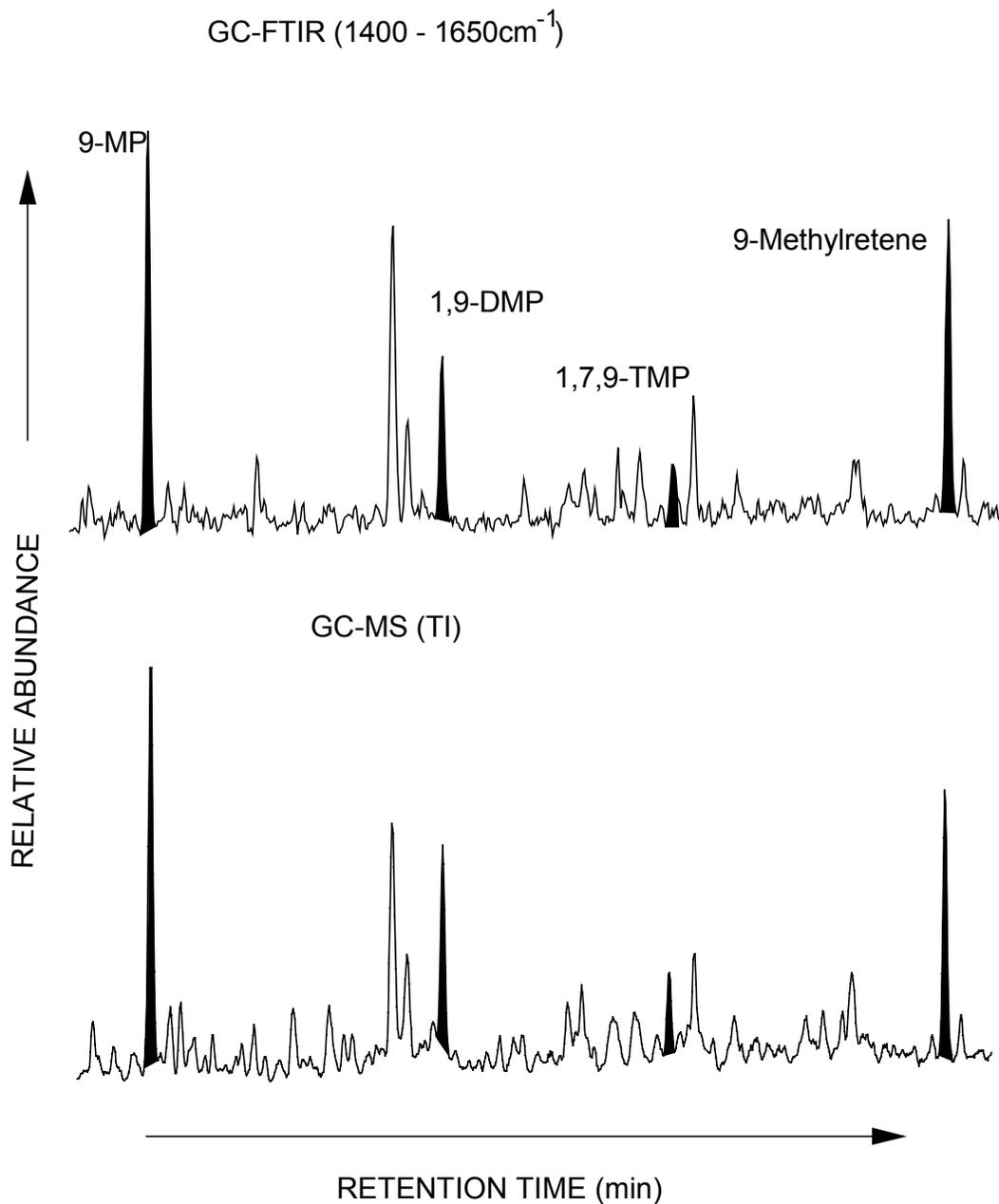


Figure 8.5. Partial GC-FTIR functional group (1400 - 1650 cm^{-1}) chromatogram and corresponding GC-MS (TI) partial mass chromatogram of the trinuclear aromatic compounds that were excluded from mordenite molecular sieves.

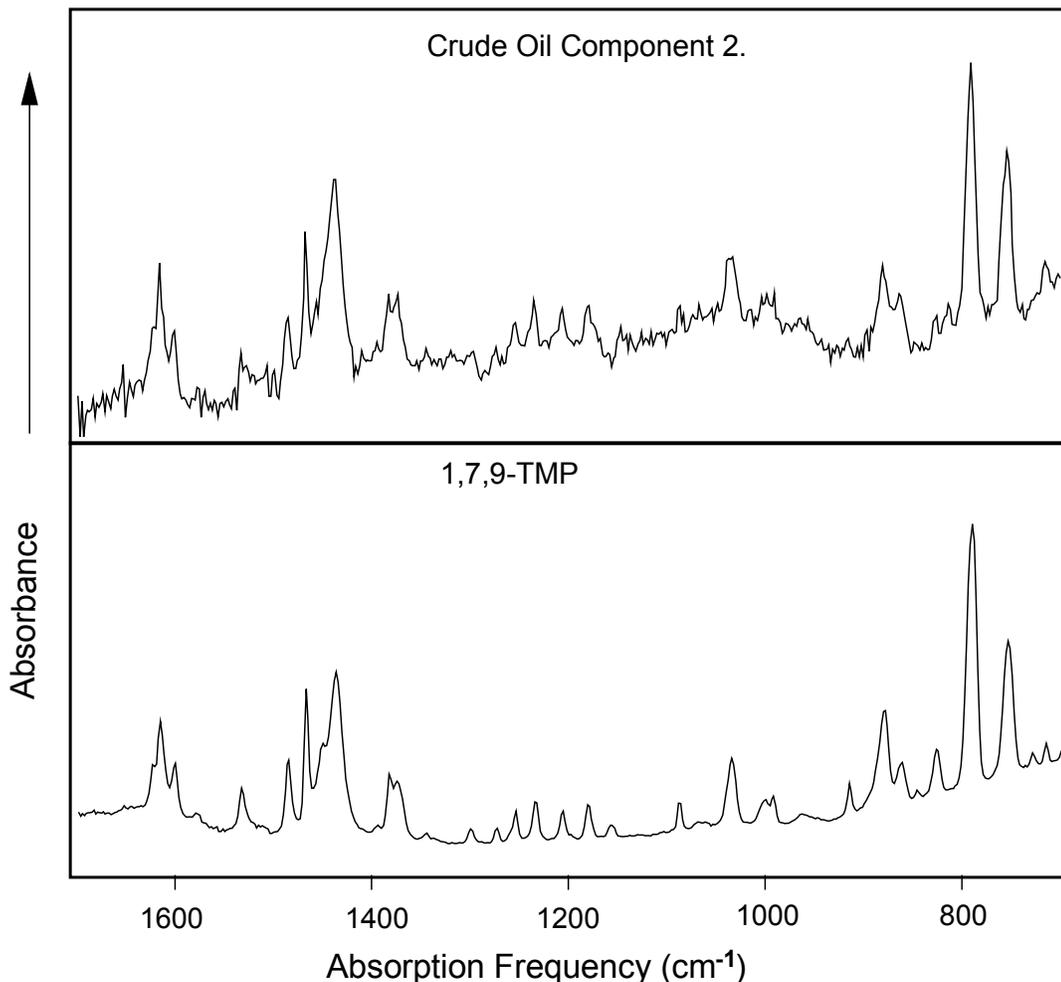


Figure 8.6. Solid state FTIR spectra of crude oil component 2 and 1,7,9-TMP. The spectrum of crude oil compound 2 was obtained from GC-FTIR analysis. (Data provided by Steve J. Fisher).

abundance of phenanthrene (P) also has the highest relative abundance of 9-MP and that the ratio of P/9-MP in the samples are similar. The combined partial mass chromatograms in Figure 8.7b with responses from of phenanthrene (P) also has the highest relative abundance of 9-MP and that the ratio of P/9-MP in the samples are similar. The combined partial mass chromatograms in Figure 8.7b with responses from methylphenanthrenes and dimethylphenanthrenes show that the Moorari-4 sample with unusually abundant 1-MP also has an anomalously high concentration of 1,9-DMP

compared with the more common distributions illustrated in this case by sample A. Similarly, as shown in Figure 8.2c, the high relative abundance of 1,7-DMP in the Moorari-4 sample is accompanied by a very predominant peak for 1,7,9-TMP. In the case of 1,9-DMP and 1,7,9-TMP, GC-FTIR and molecular sieving techniques, as described above, were applied to establish the identities of the compounds in the samples. The samples shown in Figure 8.7 that were used for comparison purposes namely Mayneside, sample A (from the Otway Basin) and Dampier, were chosen because they have similar maturities to the samples with which they are compared and because they have distributions of alkylphenanthrenes similar to those commonly reported for sediments of these maturities (Table 8.1). The Moorari-4 sample, which shows evidence for methylation of 1-MP and 1,7-DMP, also has abundant retene associated with a lesser concentration of 9-methylretene. Figure 8.7d is a partial mass chromatogram (m/z 234 + 248) showing the relative abundances of retene and 9-methylretene. The co-occurrences of 1-MP; 1,7-DMP and retene with lesser proportions of the corresponding 9-methyl species suggests a precursor product relationship between these compounds. That is they are indicators that methylation has taken place in these shales. In the cases of 1-MP and 1,9-DMP; 1,7-DMP and 1,7,9-TMP and retene and 9-methylretene the proportions are similar, however the ratio of phenanthrene to 9-methylphenanthrene is much different. It may be that due to sedimentary methylation is the primary source of the first three mentioned 9-methyl substituted compounds but not the main source of 9-MP.

Laboratory methylation of phenanthrenes

Laboratory experiments were carried out to show that the 9-methyl(alkyl)phenanthrenes can be formed from the corresponding (alkyl)phenanthrene by clay-catalysed methylation reactions. A sample of (alkyl)phenanthrene was heated with hexamethylbenzene (methyl donor) and aluminium smectite at 100 °C for 8 hours and the reaction mixture was

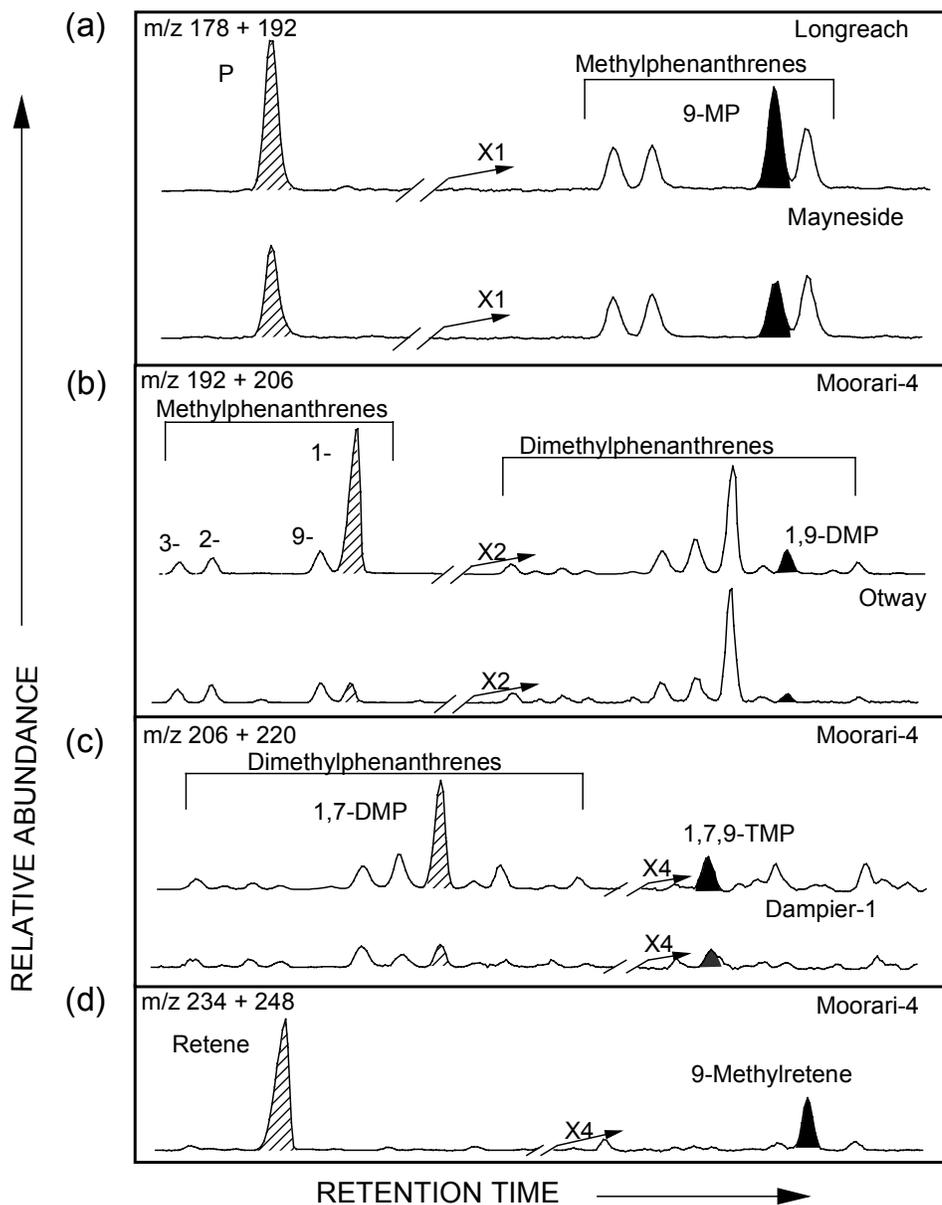


Figure 8.7 Combined partial mass chromatogram showing a comparison of results from samples with anomalously high relative abundances of alkylphenanthrenes and a correspondingly high relative abundance of their 9-methyl counterparts with samples showing the common distribution of isomers (a-c). A similar chromatogram showing the relative abundances of retene and 9-methylretene (d). P = phenanthrene, MP = methylphenanthrene, DMP = dimethylphenanthrene, TMP = trimethylphenanthrene.

analysed using GC-MS techniques. The total ion mass chromatograms of each of the reaction mixtures is shown in Figure 8.8 together with the

percentage conversion of the substrate ((alkyl)phenanthrene) into the compound labelled in the chromatogram. In each case the major methylation product was the isomer with the additional methyl located at the 9 position. These results show that low temperature (100 °C) methylation of (alkyl)phenanthrene gave predominantly the 9-methyl isomers. Similarly methylation processes in sediments involving donation of a methyl group to the 9-position of a phenanthrene could account for the alkylphenanthrene/ 9-methylalkylphenanthrene pairs identified in the crude oils and sediments used in this study.

The presence of a group of alkylphenanthrenes and their 9-methyl counterparts in sediments and crude oil is more likely due to a geosynthesis than to a specific group of natural product precursors. The carbon skeleton of these 9-methylalkylphenanthrenes is uncommon in natural products. The suggestion that sedimentary methylation has occurred is also supported by a recent report that other compound types such as phenols (Ioppolo-Armanios *et al.*, 1995) also show evidence of formation from simpler compounds involving methylation processes. The methylation process may be similar to that occurring during the coalification of lignin in which the methyl of the methoxyl group is released and substitutes the aromatic ring (Botto *et al.*, 1989). The demonstration that anisoles in the presence of clay catalysts readily form products in which the aromatic ring is predominantly substituted in the *ortho* and *para* positions (Carrado *et al.*, 1990) adds further support that such processes could occur in petroleum source rocks and under relatively mild maturation conditions. The depletion of lignin methoxyl groups in the early stages of coalification (Hatcher and Breger, 1982) suggests that if similar processes occur in petroleum source rocks the methylation processes would take place in the immature zone before appreciable oil generation occurs.

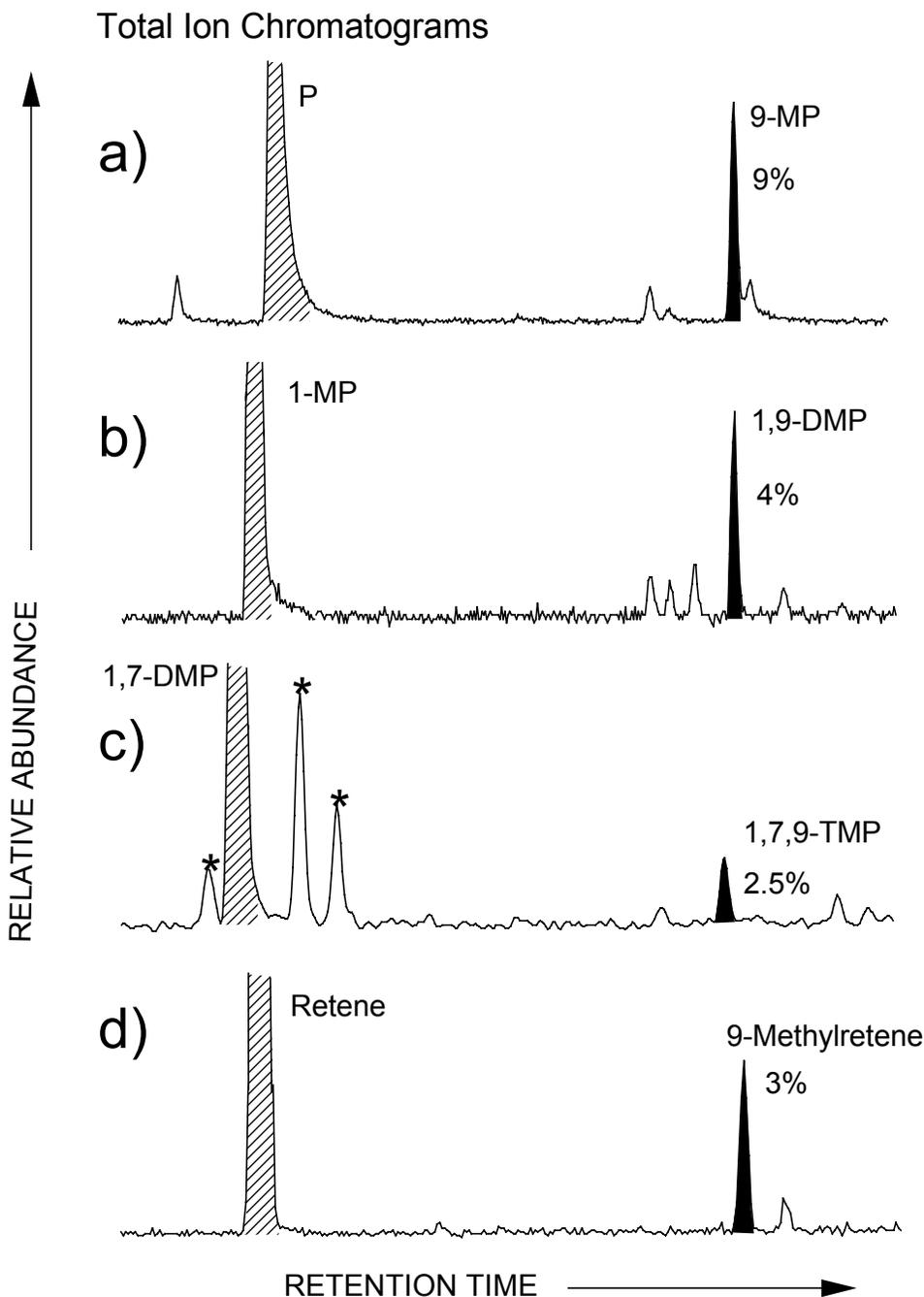


Figure 8.8. Partial total ion mass chromatograms of the reaction mixtures from heating (alkyl)phenanthrenes with a methyl donor (hexamethylbenzene) and aluminium montmorillonite. Percentages refer to the extent of conversion of the substrates phenanthrene; 1-methylphenanthrene; 1,7-dimethylphenanthrene and retene. MP = methylphenanthrene, DMP = dimethylphenanthrene, TMP = trimethylphenanthrene, * indicates minor impurities in the reactant.

8.5 Conclusions

9-MP, 1,9-DMP, 1,7,9-TMP and 9-methylretene have been shown to occur in anomalously high relative abundances in their respective isomer classes in sedimentary samples which also contained high relative abundances of the corresponding compounds without 9-methyl substituents. This observation together with the demonstration that phenanthrene and alkylphenanthrenes are readily methylated in the 9-position when treated with a methyl donor under laboratory conditions, has been interpreted as evidence for a sedimentary methylation reaction.

8.6 Acknowledgements

Geoff Chidlow is thanked for his assistance with GC-MS analysis. Financial support was provided by the Australian Petroleum Co-operative Research and from a Curtin University Postgraduate Scholarship.

9.0 Geosynthesis of organic compounds. IV- Methylation of 1,2,7-trimethylnaphthalene

9.1 Abstract

In this paper we report on the anomalously high relative abundance of 1,2,4,7-tetramethylnaphthalene in sedimentary hydrocarbons that also contain a high relative abundance of 1,2,7-trimethylnaphthalene. Because 1,2,4,7-tetramethylnaphthalene was formed when 1,2,7-trimethylnaphthalene was heated with hexamethylbenzene and a clay catalyst at 120°C in the laboratory, we suggest that similar transalkylation reactions occur under natural heating conditions.

9.2 Introduction

Trimethylnaphthalenes (TMNs) (Rowland *et al.*, 1984; Alexander *et al.*, 1985; Strachan *et al.*, 1988) and tetramethylnaphthalenes (TeMNs) (Forster *et al.*, 1989b; Alexander *et al.*, 1993) are common constituents of petroleum and fossil fuels. The presence of abundant 1,2,5-TMN (Püttmann and Villar, 1987; Strachan *et al.*, 1988), 1,2,7-TMN (Strachan *et al.*, 1988; Forster *et al.*, 1989a), 1,2,5,6-TeMN (Püttmann and Villar, 1987; Alexander *et al.*, 1993) and 1,2,3,5-TeMN (Alexander *et al.*, 1992a, 1993) in low maturity sediments led to the suggestion that these compounds originate from triterpenoids derived from microbial and land-plants sources. With increase in sediment maturity

the relative abundances of these isomers is diminished and oil and condensates typically contain isomer distributions that more closely reflect their relative stabilities (Alexander *et al.*, 1985, 1986, 1993). This change in relative abundances of isomers with increase in maturity has been attributed to ring isomerisation and transalkylation processes (Strachan *et al.*, 1988, Alexander *et al.*, 1993). Some sedimentary samples however, contain tetramethylnaphthalenes that are not easily accounted for by these processes. In particular the anomalously high abundance of 1,2,4,7-TeMN among the TeMN isomers is not obviously derived from either of the initially formed TeMN isomers (1,2,5,6-TeMN and 1,2,3,5-TeMN) by these processes.

In this paper we show that samples with a high relative abundance of 1,2,7-TMN have a correspondingly high abundance of 1,2,4,7-TeMN and suggest that this is caused by sedimentary methylation of 1,2,7-TMN derived from land plant natural products. This adds further support to recent suggestions (Alexander *et al.*, 1995; Ellis *et al.*, 1995b; Iopollo-Armanios *et al.*, 1995; Smith *et al.*, 1995) based on studies of sedimentary alkylphenols, alkylphenanthrenes and alkylxylenes, that sedimentary methylation of aromatic systems is a common and widespread process.

9.3 Materials and methods

Isolation of dinuclear and trinuclear aromatic fractions from crude oils and sediment extracts

Crude oils or sediment extracts were subjected to liquid chromatography using silica gel and pentane/dichloromethane eluent to obtain aromatic fractions. Further TLC of these fractions using alumina plates and hexane solvent gave a combined dinuclear and trinuclear fraction (Forster *et al.*, 1989b).

GC-MS analysis

GC-MS analysis was carried out using a Hewlett-Packard (HP) 5970 GC-MS system with a 50 m x 0.22 mm i.d. fused silica column coated with β -

cyclodextrin phase (β -cydex, SGE, Australia). Helium was used as carrier at a head pressure of 28 psi. The oven temperature was programmed from 70° to 200° at 1° per minute, then to 230° at 5° per minute.

Trimethylnaphthalenes and tetramethylnaphthalenes

Trimethylnaphthalenes and tetramethylnaphthalenes were identified by comparing their retention times and mass spectra with synthetic reference compounds (Rowland *et al.*, 1984; Forster *et al.*, 1989b).

Alkylation experiments

The aluminium montmorillonite used was material prepared for an earlier study (Alexander *et al.*, 1984a). 1,2,7-TMN (1 mg), hexamethylbenzene (3 mg), and finely divided aluminium montmorillonite (10 mg) were placed in small (3 mL) Pyrex glass ampoules and flushed with dry nitrogen. The ampoules were evacuated, sealed, and then heated at 120°C for 8 hr. The contents were extracted with dichloromethane (2 mL), the solvent was removed by careful distillation, and hexane (1.8 mL) containing the external standard pentadecane (0.5 mg) was added. An aliquot was then subjected to analysis using GC-MS techniques.

9.4 Results and discussion

Aromatic fractions obtained from the crude oils by liquid chromatography techniques were further analysed using GC-MS. The results of a typical analysis with the gas chromatograph operating with a β -cydex capillary column are shown in Figure 9.1.

This stationary phase was selected because it enabled separation of 1,2,7-TMN from other TMN isomers and also separated 1,2,4,7-TeMN from the 1,2,4,6-TeMN; 1,4,6,7-TeMN co-eluting pair. The identities of the other TMN and TeMN isomers shown in the partial mass chromatograms are given in Table 9.1.

Table 9.I. Identity of the trimethylnaphthalene and tetramethylnaphthalene isomers shown in Figure 9.1.

Peak N ^o	Name	Peak N ^o	Name
1	1,3,7-TMN	11	1,3,6,7-TeMN
2	1,3,6-TMN	12	1,2,4,6-/1,4,6,7-TeMN
3	1,4,6-/1,3,5-TMN	13	1,2,4,7-TeMN
4	2,3,6-TMN	14	1,2,5,7-TeMN
5	1,2,7-TMN	15	2,3,6,7-TeMN
6	1,2,6-/1,6,7-TMN	16	1,2,6,7-TeMN
7	1,2,4-TMN/C4-N*	17	1,2,3,7-TeMN
8	1,2,5-TMN	18	1,2,3,6-TeMN
9	1,2,3-TMN	19	1,2,5,6-/1,2,3,5-TeMN
10	Cadalene		

* C4-N = C4-naphthalene

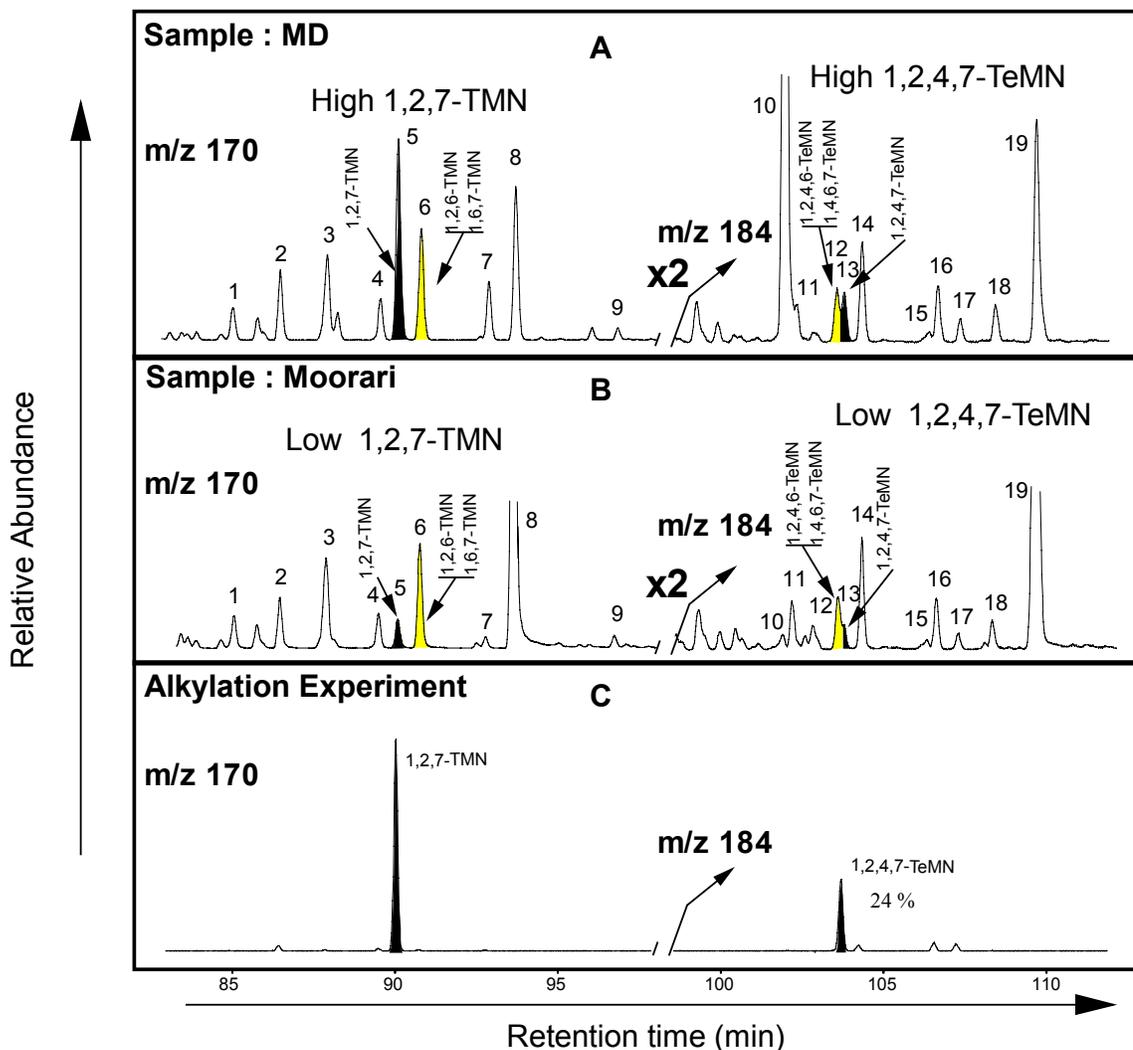


Figure 9.1. Partial m/z 170 and 184 mass chromatogram for (A) a sample (MD) with a high relative abundance of 1,2,7-TMN and 1,2,4,7-TeMN, (B) a crude oil a sample (Moorari) with a low relative abundance of 1,2,7-TMN and 1,2,4,7-TeMN and (C) alkylation experiment for 1,2,7-TMN and hexamethylbenzene heated on aluminium montmorillonite at 120°C for 8 h. For peak identifications refer to Table 9.1.

The co-occurrence in sedimentary material of anomalously high relative abundances of 1,2,7-TMN with 1,2,4,7-TeMN suggests a link between the two compounds, possibly involving sedimentary methylation. Partial mass chromatograms are shown in Figure 9.1 for a sample with a high relative abundance of 1,2,7-TMN and a corresponding high relative abundance of 1,2,4,7-TeMN together with a second sample in which 1,2,7-TMN and 1,2,4,7-TeMN are both low relative to other isomers in their respective

classes. In order to show that this relationship holds over a range of concentrations, relative abundance data for the two compounds was obtained from the crude oil and sediment samples given in Table 9.2. A plot of the data is shown in Figure 9.2. Clearly the results from this sample suite show that an enhanced relative abundance of 1,2,7-TMN is also associated with an enhanced relative abundance of 1,2,4,7-TeMN.

Table 9.2 Information about samples.

Sample Name	Type	Basin/Country	Probable age
a. Blina	Crude oil	Canning Basin/Australia	Devonian
b. Bream	Crude oil	Gippsland Basin/Australia	Cretaceous
c. East Dollarhide	Crude oil	USA	Cambr.-Ordov.
d. Fly Lake	Crude oil	Cooper Basin/ Australia	Permian
e. MD	Sed. rock	Indonesia	Miocene
f. Kedidi North	Crude oil	Brunei/Indonesia	Tertiary
g. Moorari	Crude oil	Eromanga Basin/Australia	Jurassic
h. Tuna	Crude oil	Gippsland Basin/Australia	Eocene

The results of heating hexamethylbenzene (methyl donor) with 1,2,7-TMN in the presence of a clay catalyst showed that methylation of 1,2,7-TMN occurs readily. Partial mass chromatograms from the reaction products of the alkylation experiment (Figure 9.1) show that 1,2,4,7-TeMN is the major TeMN isomer produced. This is consistent with an electrophilic aromatic

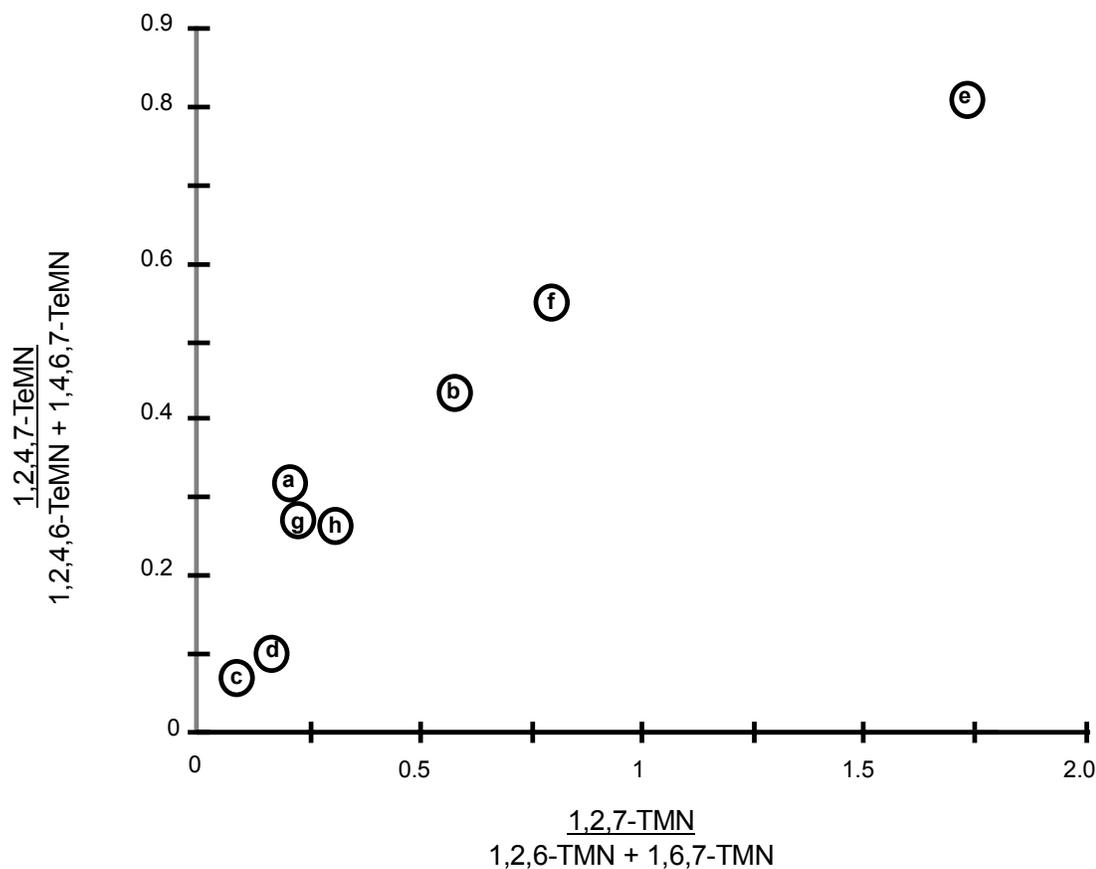
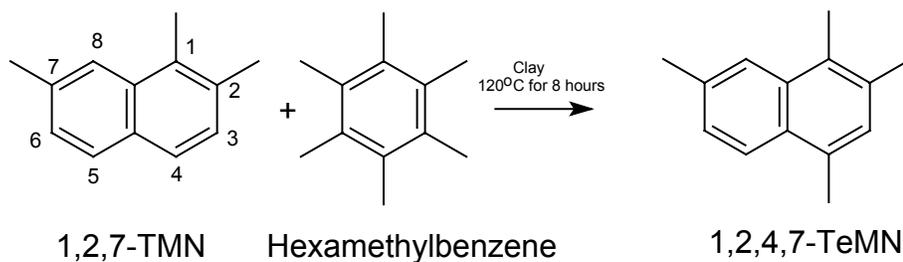


Figure 9.2. Plot of 1,2,7-TMN/(1,2,6- + 1,6,7-TMN) Vs 1,2,4,7-TeMN/(1,2,4,6- + 1,4,6,7-TeMN) for samples in Table 9.2. The size of the circles is used to indicate the uncertainty in integrating overlapping peaks in the chromatograms (cf. Figure 9.1).

substitution reaction resulting in methylation of the most reactive ring position (Reaction Scheme 9.1). In this experiment hexamethylbenzene was used as the methylating agent because it undergoes transalkylation reactions in the presence of acidic clay catalysts with mild heating. We do not suggest this is the actual methylating reagent in nature. Potential methylating reagents in the natural system are natural products with angular methyl and *gem* dimethyl groups on compounds that undergo aromatisation and highly substituted polymethylaromatic compounds such as 1,2,2,5,6-pentamethyltetralin (Alexander *et al.*, 1992a,1993).

Reaction scheme 9.1.



9.5 Conclusions

1,2,4,7-TeMN has been shown to occur in anomalously high relative abundances in sedimentary samples which also contain high relative abundances of 1,2,7-TMN. This observation, together with the demonstration that 1,2,7-TMN is readily methylated to form 1,2,4,7-TeMN when heated with a methyl donor under mild laboratory conditions, has been interpreted as evidence for a sedimentary methylation reaction.

9.6 Acknowledgements

Geoff Chidlow is thanked for his assistance with GC-MS analysis. Financial support was provided by the Australian Petroleum Co-operative Research Centre and from a Curtin University Postgraduate Scholarship.

10.0 Geosynthesis of organic compounds. V- Methylation of alkylnaphthalenes

10.1 Abstract

Several crude oils and rock extracts which contain high concentrations of 1,6-dimethylnaphthalene, 1,2,5-trimethylnaphthalene, 1,2,3,5-tetramethylnaphthalene, 1,2,3,5,6-pentamethylnaphthalene and 6-isopropyl-2-methyl-1-(4-methylpentyl)naphthalene have been shown to contain relatively high concentrations of their corresponding methylated counterparts. In laboratory experiments carried out under mild conditions each of the parent alkylnaphthalenes has been shown to be substituted in preferential positions when a methyl donor was heated with the substrate in the presence of a clay catalyst. The distributions of these products from laboratory methylation experiments are similar to the distributions found in sedimentary material. These observations have been interpreted as evidence for a sedimentary electrophilic aromatic methylation process.

10.2 Introduction

Sedimentary methylation processes have been proposed to account for the occurrence of numerous methyl substituted aromatic hydrocarbons which have carbon skeletons that are not obviously derived from common natural product precursors (Radke *et al.*, 1982a). The formation of sedimentary alkylaromatics by alkylation processes has been demonstrated for alkylphenols (Ioppolo-Armanios, 1995), benzenes (He *et al.*, 1995; Ellis *et al.*, 1995b), naphthalenes (He *et al.*, 1992, 1994, Bastow *et al.*, 1996), phenanthrenes (Smith *et al.*, 1994, 1995; Alexander *et al.*, 1995), anthracenes (Smith *et al.*, 1994, 1995) and pyrene (Derbyshire and Whitehurst, 1981; Smith *et al.*, 1994, 1995). Further evidence to support an alkylation process comes from the tentative identification of 6-isopropyl-2,4-dimethyl-1-(4-methylpentyl)naphthalene (4-Me-iP-iHMN) as a methylation product of 6-isopropyl-2-methyl-1-(4-methylpentyl)naphthalene (iP-iHMN) (Ellis, 1994; Ellis *et al.*, 1996b). In this paper we show evidence for the formation of methylated alkyl naphthalenes via sedimentary methylation processes.

Dimethylnaphthalenes (DMNs), trimethylnaphthalenes (TMNs), tetramethylnaphthalenes (TeMNs) and pentamethylnaphthalenes (PMNs) are common constituents of petroleum and fossil fuels. The presence of abundant 1,6-DMN (van Aarssen *et al.*, 1992), 1,2,5-TMN (Püttmann and Villar, 1987; Strachan *et al.*, 1988; Killops *et al.*, 1991; Ellis *et al.*, 1996b), 1,2,7-TMN (Strachan *et al.*, 1988; Forster *et al.*, 1989a), 1,2,5,6-TeMN (Püttmann and Villar, 1987; Killops *et al.*, 1991; Ellis *et al.*, 1996b; Alexander *et al.*, 1993), 1,2,3,5-TeMN (Alexander *et al.*, 1992a, 1993), 1,2,3,5,6-PMN (Bastow *et al.*, 1998b) and iP-iHMN (Ellis *et al.*, 1996b) in low maturity sedimentary matter has led to the suggestion that these compounds originate from terpenoids derived from microbial and land plant sources. However with increasing maturity the relative abundances of these isomers is diminished until at high maturity the isomer distributions reflect their relative stabilities (Alexander *et al.*, 1985, 1986, 1993; Strachan *et al.*, 1988). This change in the relative abundances of isomers with increasing maturity has been attributed to ring isomerisation and transalkylation processes (Strachan

et al., 1988; Alexander *et al.*, 1993; Smith *et al.*, 1994, 1995; Bastow *et al.*, 1998b).

In this paper we show that samples with a high relative abundance in alkylnaphthalenes likely to be derived from natural product origins have a correspondingly high abundance of their methylated counterparts, and suggest that this is caused by sedimentary methylation of these alkylnaphthalenes.

10.3 Materials and methods

Samples

Information about the samples is given in Table 10.1 and 10.2.

Table 10.1. Geological data for the crude oils and rock extracts.

Sample		Location		Age or Probable Age of Oil Source Rock
Name	Type	Country	Basin	
Dullingari-26	Crude oil	Australia	Eromanga	Jurassic
GK 1959m	Rock extract	Indonesia	S.Sumatra	Tertiary
GK 2026m	Rock extract	Indonesia	S.Sumatra	Tertiary
Moorari-4	Crude oil	Australia	Eromanga	Jurassic
Moorari-3 7108'	Rock extract	Australia	Eromanga	Jurassic
PD130A	Rock extract	Australia	Canning	Devonian

Reference compounds

1,6-DMN was purchased from Sigma. 1,2,3,5-TeMN; 1,2,3,5,6-PMN; and iP-iHMN were available from previous studies (Alexander *et al.*, 1993; Bastow *et al.*, 1998b; Ellis *et al.*, 1996b). A sample of 1,2,5-TMN was provided by Dr P. Forster.

Table 10.2. Geochemical maturity data for the crude oils and rock extracts.

Sample Name	20S/20R	MPI-1	TNR-1	PNR	MDR
Dullingari-26	1.0	0.4	0.4	0.21	1.5
GK 1959m	1.0	0.6	0.6	0.17	2.1
GK 2026m	1.2	0.7	0.6	0.20	2.3
Moorari-4	0.6	0.2	0.3	0.15	1.5
Moorari-3 7108'	1.1	0.3	0.4	0.12	2.4
PD 130A	0.5	0.3	0.5	0.13	0.8

Parameters and methods of measurement: 20S/20R, sterane diastereomer ratio (m/z 217) (Mackenzie and McKenzie, 1983); MPI-1. GC-MS data corrected to FID response (Radke *et al.*, 1982a,b); TNR-1 (m/z 170) (Alexander *et al.*, 1985); PNR (m/z 198) (Bastow *et al.*, 1998b); MDR (m/z 198) (Radke *et al.*, 1986).

1,2,3,5,6,7-Hexamethylnaphthalene (1,2,3,5,6,7-HMN) was prepared using the synthetic scheme reported by Araki and Mukaiyama (1974). 1,2,3-Trimethylbenzene (13 mmol) in glacial acetic acid (50 mL) at 0°C, was treated with bromine (15 mmol), and the mixture was allowed to warm to room temperature over a period of 1h yielding 1-bromo-2,3,4-trimethylbenzene. The Grignard reagent prepared from 1-bromo-2,3,4-trimethylbenzene (8 mmol) and magnesium (5 mmol) was added to 2,3-dimethylsuccinic anhydride (4 mmol) in tetrahydrofuran (THF). The resulting keto carboxylic acid (2 mmol) was reduced using zinc amalgam (Martin, 1942) to give the carboxylic acid. The carboxylic acid (1 mmol) was cyclised to 2,3,5,6,7-pentamethyltetralone by treatment with polyphosphoric acid (PPA). The tetralone (0.5 mmol) was methylated using methylmagnesium iodide and the resultant tertiary alcohol (0.4 mmol) was converted to the alkyltetralin by dehydration in a mixture of sulfuric acid and glacial acetic acid. This alkyltetralin was then treated with activated platinum on carbon at 280 °C to yield 1,2,3,5,6,7-PMN as a white solid (m.p. 172-174 °C) with an overall yield of 8 %. The ¹H-NMR spectra and melting point of this compound correspond well with that published by Chen *et al.* (1973).

4-Me-iP-iHMN was prepared using a general synthetic scheme (Araki and Mukaiyama, 1974). The Grignard reagent prepared from 1-bromo-3-isopropylbenzene (20 mmol) and magnesium (19 mmol) was added to methylsuccinic anhydride (20 mmol) in THF. The resulting mixture of two keto carboxylic acids (10 mmol) was methylated using methylmagnesium iodide (25 mmol) (Adachi *et al.*, 1981). The resultant lactones were reduced using zinc amalgam (Martin, 1942) to give the carboxylic acids. The carboxylic acids (4 mmol) were cyclised to the alkyltetralones by treatment with PPA. The tetralones (2 mmol) were added to isohexylmagnesium bromide and the resultant tertiary alcohols (1 mmol) were converted to the alkyltetralins by dehydration in a mixture of sulfuric acid and glacial acetic acid. These alkyltetralins were then treated with activated platinum on carbon at 280 °C to yield 4-Me-iP-iHMN as the major product (80%) with an overall yield of 19 %. 4-Me-iP-iHMN was further purified to a purity of greater than 95 % using liquid chromatography methods to obtain NMR data. The synthesis of 4-Me-iP-iHMN is shown in Reaction Scheme 10.1.

NMR data

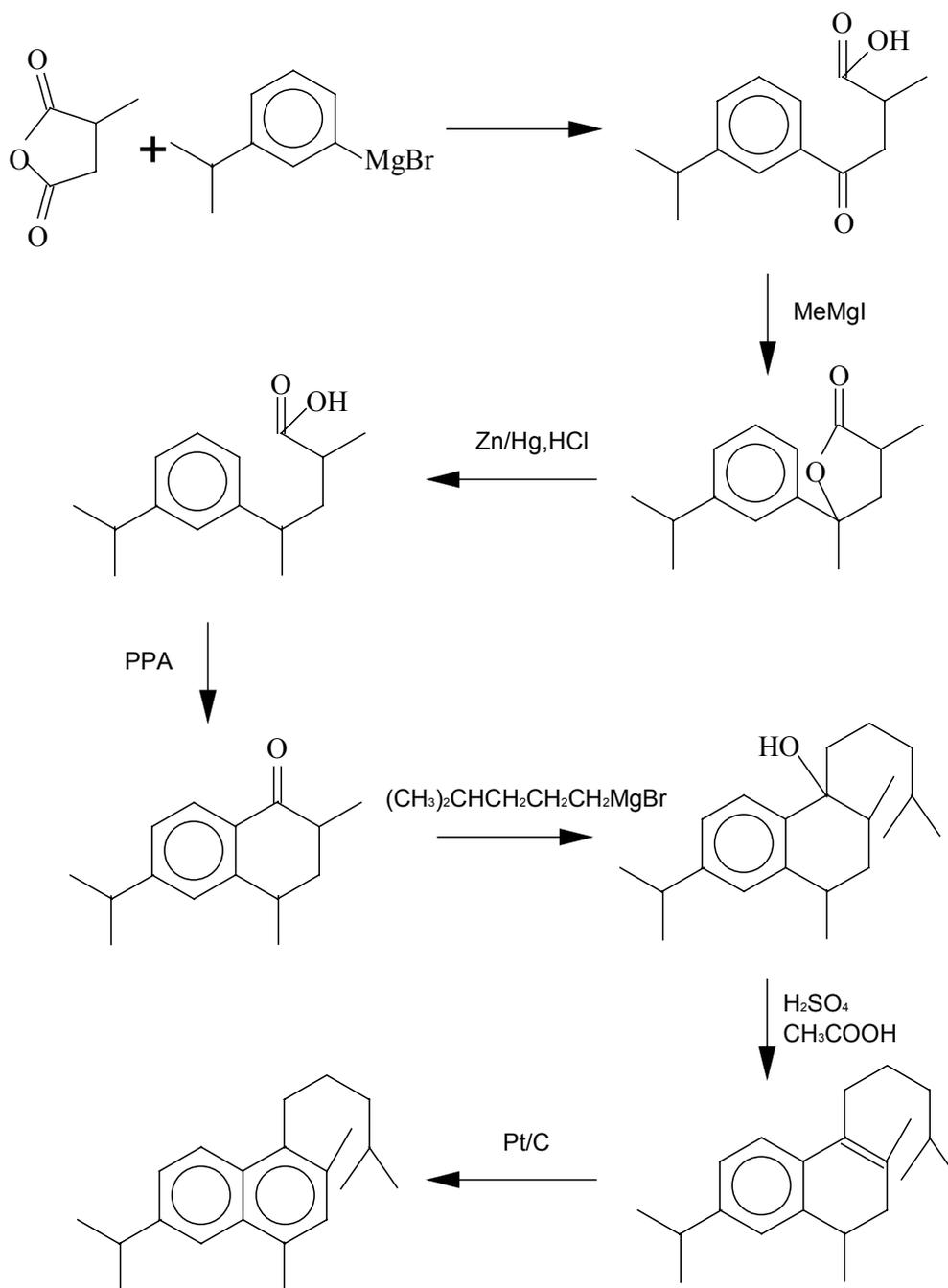
All measurements were carried out in a solution of chloroform-*d* using a Varian Gemini-200 spectrometer operating at 200 MHz for ¹H (ppm relative to CDCl₃ at 7.25 ppm) and 50 MHz for ¹³C (ppm relative to CDCl₃ at 77.0 ppm).

¹H-NMR data of reference compounds;

4-Me-iP-iHMN: δ 0.89, *d*, J 6.4 Hz, 6H, RCH(CH₃)₂; 1.17-1.73, *m*, 5H, ArCH₂CH₂CH₂CHRR'; 1.34, *d*, J 7.0 Hz, 6H, ArCH(CH₃)₂; 2.42, *s*, 3H, Ar(C2)-CH₃; 2.61, *s*, 3H, Ar(C4)-CH₃; 2.91-3.17, *m*, 3H, ArCH₂R, ArCH(CH₃)₂; 7.11, *s*, 1H, Ar(C3)-H; 7.40, *dd*, J 1.8, 8.8 Hz, 1H, Ar(C7)-H; 7.73, *d*, J 1.8 Hz, 1H, Ar(C5)-H; 7.95, *d*, J 8.8 Hz, 1H, Ar(C8)-H.

Reaction Scheme 10.1

Reaction scheme for the synthesis of the 4-Me-iP-iHMN



1,2,3,5,6,7-HMN: δ 2.37, s, 6H, Ar(C2, C6)- CH_3 ; 2.47, s, 6H, Ar(C3, C7)- CH_3 ; 2.57, s, 6H, Ar(C1, C5)- CH_3 ; 7.67, s, 2H, Ar(C4, C8)-H.

¹³C-NMR data of reference compounds prepared:

4-Me-iP-iHMN: δ 19.23, 19.83, Ar(C2, C4)-CH₃; 22.59, 22.59, RCH-(CH₃)₂; 24.03, 24.03, ArCH(CH₃)₂; 27.90, RCH-(CH₃)₂; 28.15, 28.86, ArCH₂CH₂CH₂ CHRR'; 34.26, ArCH(CH₃)₂; 39.55, ArCH₂CH₂CH₂ CHRR'; 121.11, 124.28, 124.97, 130.23, Ar(C3, C5, C7, C8); 130.85, 131.27; 131.35, 131.77, 134.18, 144.58, Ar(C1, C2, C4, C6, C4a, C8a).

1,2,3,5,6,7-HMN: δ 14.98, 16.11, 22.05, Ar(C1, C2, C3, C5, C6, C7)-CH₃; 121.95, Ar(C4, C8); 130.05, 130.56, 131.67, 133.93, Ar(C1, C2, C3, C4a, C5, C6, C7, C8a).

Isolation of aromatic hydrocarbon fractions

In a typical separation, a crude oil or sediment extract was subjected to liquid chromatography as follows. Glass columns (40 cm x 1.2 cm i.d.) were packed with activated silica gel (6 g, Merck, particle size 0.063 – 0.200 mm for chromatography) as a slurry in *n*-pentane. The sample (50-100 mg) in *n*-pentane was introduced to the top of the column to produce a concentrated band. Aliphatic hydrocarbons were then eluted under gravity with *n*-pentane (40 mL), aromatic hydrocarbons with *n*-pentane/dichloromethane (35:5, 40 mL), and polar compounds with dichloromethane/methanol (1:1, 30 mL). The aromatic fractions were obtained by careful removal of the solvent, the residue was dissolved in hexane to provide a sample ready for GC-MS analysis.

GC-MS analysis

Two systems were used for GC-MS analysis. One, a Hewlett-Packard 5973 MSD, interfaced with a 6890 gas chromatograph was fitted with a fused-silica open tubular column (SGE, Australia, 50 m x 0.22 mm i.d.) coated with BP-20 stationary phase (0.25 μ m). The GC oven temperature was programmed from 50 to 260°C at 3°C min⁻¹. Samples for analysis were dissolved in hexane and injected on-column using a HP 6890 autosampler at an oven temperature of 50°C. Helium was used as carrier gas at a constant flow of 1.00 mL min⁻¹. Typical MSD conditions were: ionisation energy 70 eV;

source temperature 230°C; electron multiplier voltage 1800 V. The other system was a Hewlett-Packard 5971 MSD interfaced with a 5890 Series II gas chromatograph fitted with a DB-5 (60 m x 0.25 mm i.d., phase thickness 0.25 µm, J&W Scientific) fused silica open tubular column. Samples for analysis were dissolved in hexane and injected on-column using a HP 5890 autosampler at an oven temperature of 60°C. Helium was used as carrier gas at a constant flow of 1.28 mL min⁻¹ (DB-5), and the GC oven was temperature programmed from 60-300°C at 3°C min⁻¹. Typical MSD conditions were: ionisation energy 70 eV; source temperature 200°C; electron multiplier voltage 2200 V. Individual alkylnaphthalenes in crude oils and sediment isolates were identified by mass chromatography on the basis of mass spectra and by co-chromatography with reference compounds.

Methylation experiments

The aluminium montmorillonite used in the study was material prepared for an earlier study (Alexander *et al.*, 1984a). Individual alkylnaphthalenes (1 mg), and hexamethylbenzene (3 mg), pentadecane (0.5 mg)(normalisation standard) and finely divided aluminium montmorillonite (10 mg) were placed in small (3 mL) Pyrex glass ampoules and flushed with dry nitrogen. The ampoules were evacuated, sealed and then heated to 120 °C for various times. The contents were extracted with dichloromethane (2 mL), the solvent was removed by careful distillation, and hexane (1.8 mL) containing the external standard hexadecane (0.5 mg) was added. An aliquot was then subjected to analysis using GC-MS techniques.

10.4 Results and discussion

Laboratory methylation of alkylnaphthalenes

Laboratory experiments were carried out to show that the methylated form of the alkylnaphthalenes can be produced from the corresponding alkylnaphthalene by clay-catalysed methylation. Samples of alkylnaphthalenes were heated with hexamethylbenzene (methyl donor) and aluminium montmorillonite at 120 °C for various times and the reaction mixtures analysed using GC-MS techniques. The partial mass

chromatograms of each of the reaction mixtures are shown in Figure 10.1 together with the percentage conversion of alkylnaphthalene into the methylated counterpart. Low yields for the conversion of the alkylnaphthalene into their methylated counterparts were produced in order to show only the first products of alkylation without other reactions such as isomerisation and transalkylation interfering. In each case the major methylation product was the isomer with the additional methyl located at a position on the ring that was activated to electrophilic aromatic substitution (EAS) and in the position with the least steric hindrance from adjacent methyl groups (Figure 10.2).

These results show that low temperature (120 °C) methylation of alkylnaphthalenes gave predominantly the methylated homologue in the described positions. Similar methylation processes occurring in sediments involving donation of a methyl group to the described position of the naturally abundant alkylnaphthalenes could account for the pairs of alkylnaphthalenes and methylalkylnaphthalenes identified in the following crude oils and sediments used in this study.

Methylation of alkylnaphthalenes in crude oils and sediments

The co-occurrence in sedimentary matter of anomalously high abundances of certain alkylnaphthalenes and their methyl counterparts suggests a link

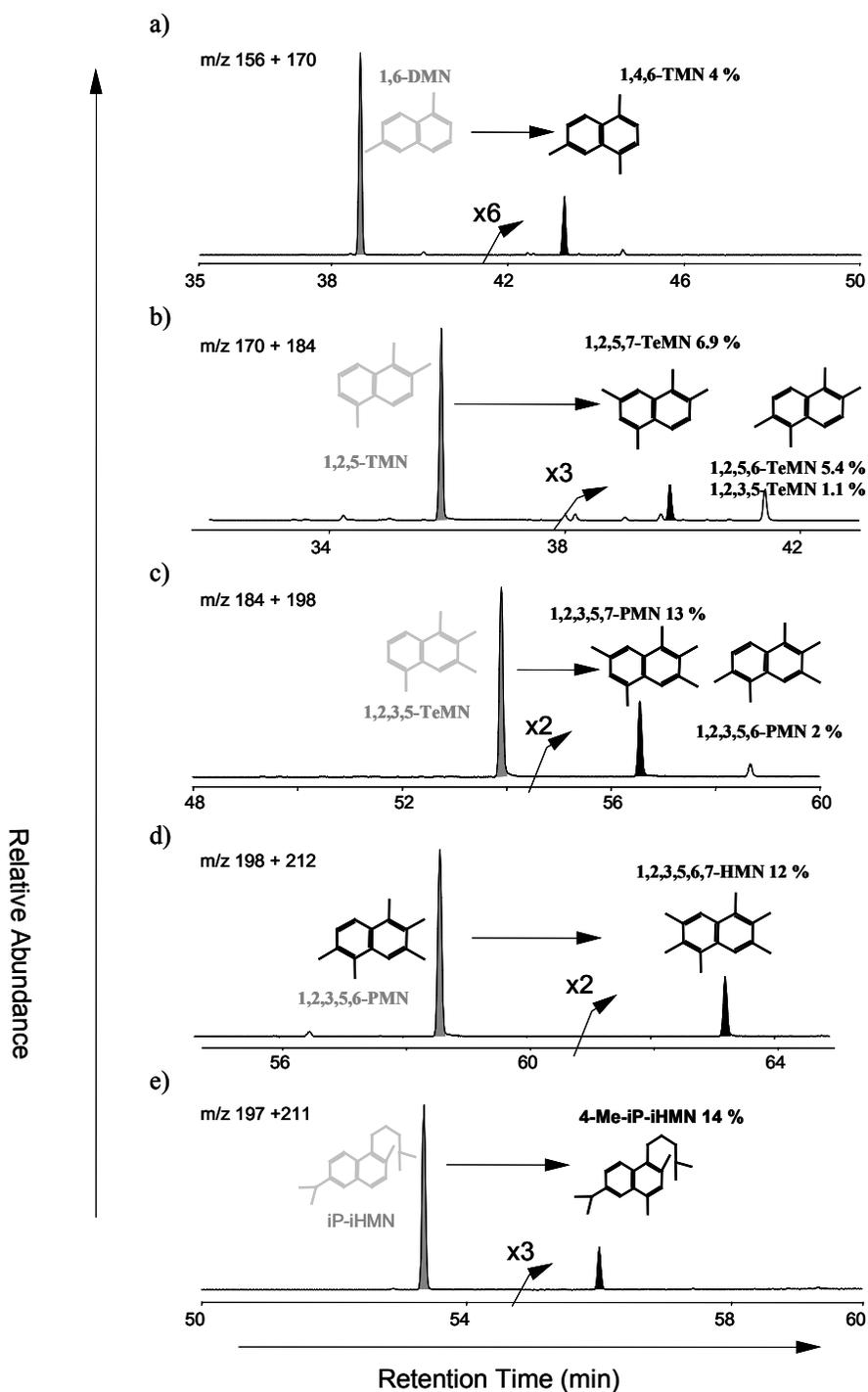
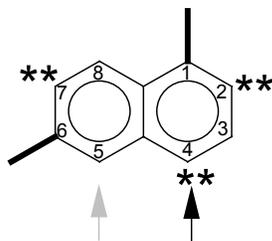


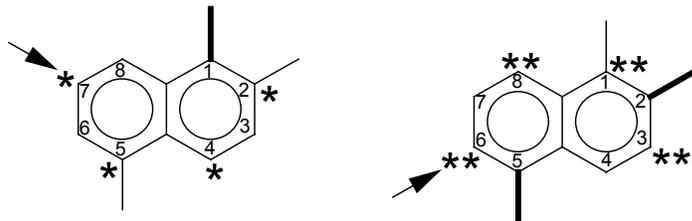
Figure 10.1. Partial mass chromatograms for laboratory methylation experiments where a methyl donor (HMB) and aluminium montmorillonite were heated at 120 °C with (a) 1,6-DMN for 8h. (BP-20, GC column); (b) 1,2,5-TMN for 8h. (DB-5, GC column); (c) 1,2,3,5-TeMN for 8h. (BP-20, GC column); (d) 1,2,3,5,6-PMN for 1h. (BP20, GC column) and (e) iP-iHMN for 2 h. (DB-5, GC column). Percentages refer

to extent of conversion of the substrate into the identified methylated counterparts.

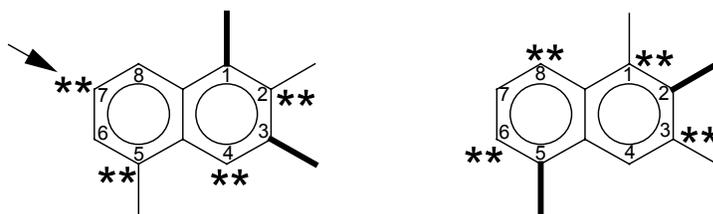
(a) 1,6-DMN



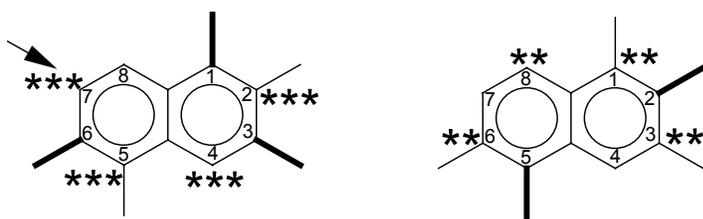
(b) 1,2,5-TMN



(c) 1,2,3,5-TeMN



(d) 1,2,3,5,6-PMN



(e) iP-iHMN

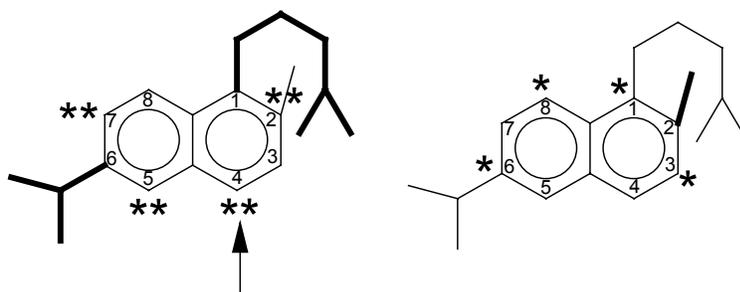


Figure 10.2 The position of alkylnaphthalenes activated to EAS (*) in relation to the alkyl groups highlighted in bold and the preferred positions of methylation are denoted by a bold arrow for (a) 1,6-DMN, (b) 1,2,5-TMN, (c) 1,2,3,5-TeMN, (d) 1,2,3,5,6-PMN and (e) iP-iHMN.

between the two compound types, possibly involving sedimentary methylation. To illustrate this relationship we have analysed samples of low maturity with anomalously high abundances of 1,6-DMN; 1,2,5-TMN; 1,2,3,5-TeMN; 1,2,3,5,6-PMN and 4-Me-iP-iHMN to obtain the relative abundance data for their corresponding methyl compounds.

In the case of the 1,6-DMN two samples with similar maturities (GK 1959 m and PD 130A, see Table 10.2) have been selected for comparison. The partial mass chromatograms in Figure 10.1a show that in laboratory experiments 1,6-DMN has been methylated predominantly to give 1,4,6-TMN. Figure 10.3a and 10.3b show that GK 1959 m, the sample with the high relative abundance of 1,6-DMN, also has the highest relative abundance of 1,4,6-TMN when compared to PD 130A, the sample with a low relative abundance of 1,6-DMN.

The partial mass chromatograms in Figure 10.1b show in the laboratory experiment that 1,2,5-TMN has been methylated to 1,2,5,7-TeMN and 1,2,5,6-TeMN. Figure 10.4a and 10.4b shows the responses from trimethylnaphthalenes and tetramethylnaphthalenes for a sample with abundant 1,2,5-TMN which also has a high relative abundance of 1,2,5,7-TeMN compared to a sample with a lower relative abundance of 1,2,5-TMN (1,2,5,6-TeMN in these samples is mainly derived from natural products). Similarly, the partial mass chromatograms in Figure 10.1c shows the alkylation experiment for 1,2,3,5-TeMN giving mainly 1,2,3,5,7-PMN. Figure 10.5a and 10.5b show the high relative abundance of 1,2,3,5-TeMN in the Moorari-4 sample is accompanied by a predominant peak 1,2,3,5,7-PMN compared to a sample with a lower relative abundance of 1,2,3,5-TeMN. Figure 10.1d shows the partial mass chromatogram of the product from the methylation of 1,2,3,5,6-PMN which shows a high proportion of 1,2,3,5,6,7-HMN in the products. Figure 10.6a shows a high relative abundance of 1,2,3,5,6-PMN co-occurring with 1,2,3,5,6,7-HMN in a rock extract, GK 1959 m. Figure 10.6b shows the partial m/z 212 mass chromatogram for the same

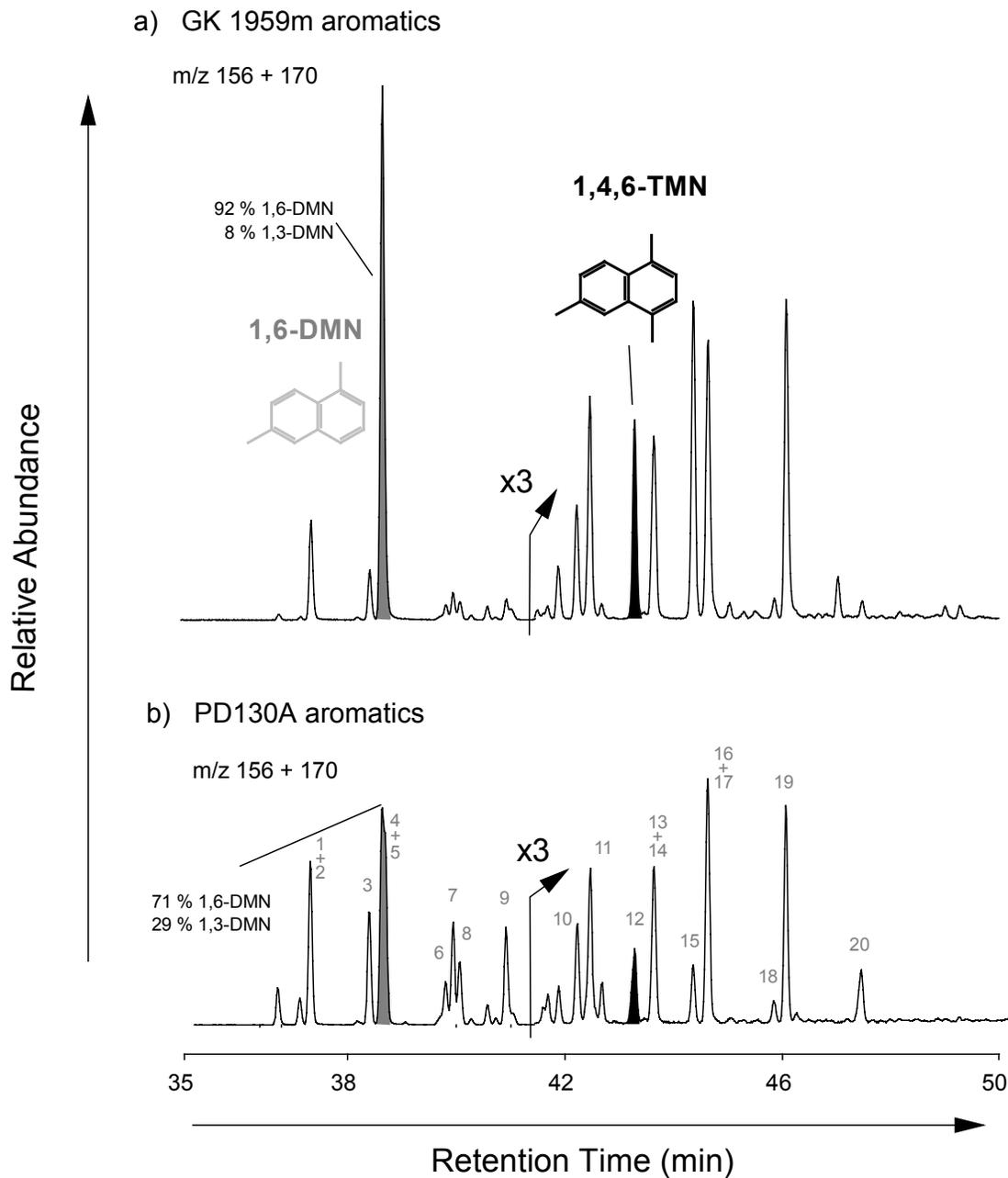


Figure 10.3. Partial m/z (170 + 184) mass chromatograms, using a BP-20 GC-column, of (a) an aromatic fraction of GK 1959 m with a high relative abundance of 1,6-DMN and (b) an aromatic fraction of PD 130A without a high relative abundance of 1,6-DMN. For peak identifications refer to Table 10.3. Percentages of 1,6-DMN and 1,3-DMN were obtained from separate analysis using a BP-20 and DB-5 GC-columns.

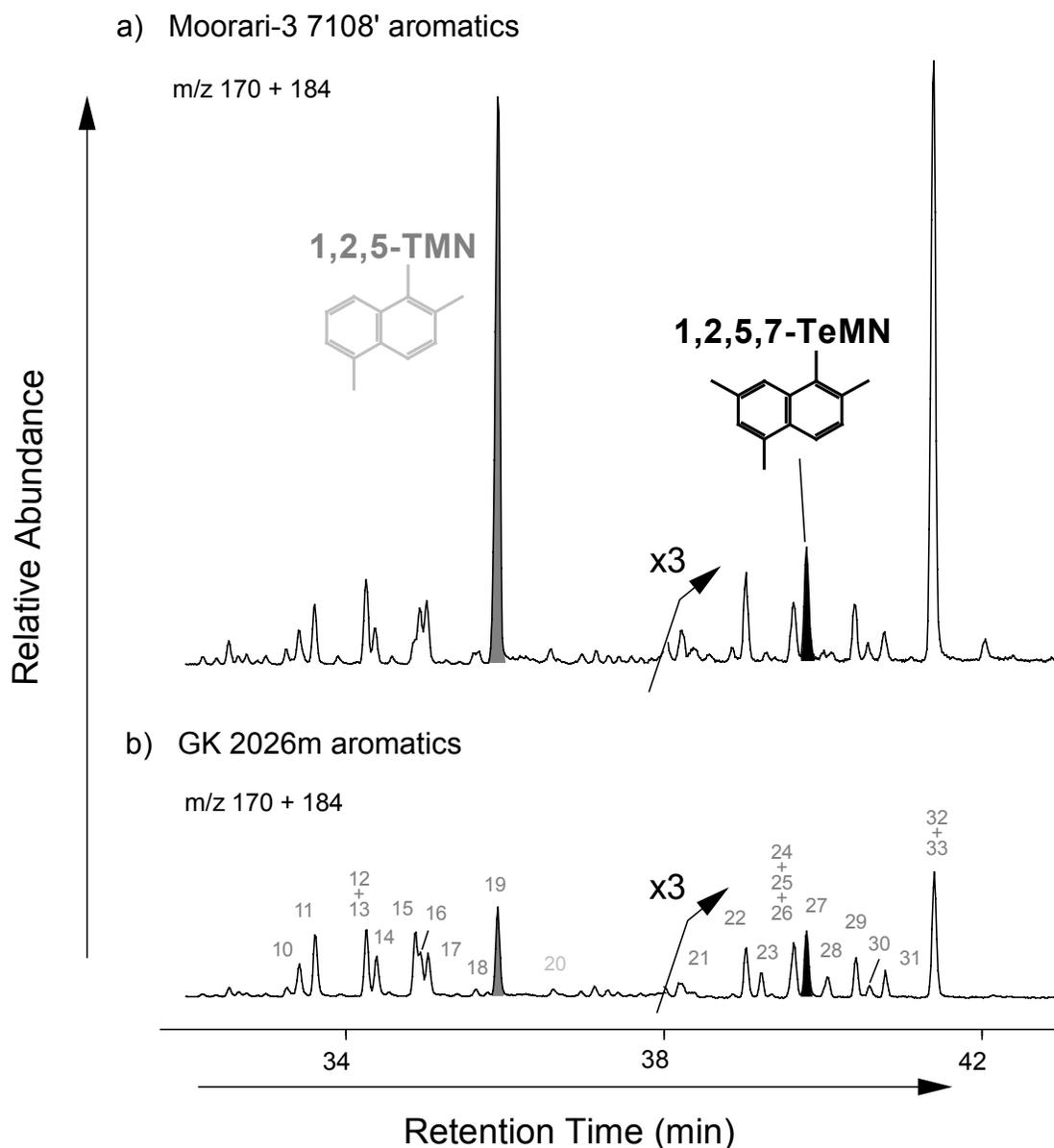


Figure 10.4. Partial m/z (170 + 184) mass chromatograms, using a BP-5 GC-column, of (a) an aromatic fraction of Moorari-3 7108' with a high relative abundance of 1,2,5-TMN and (b) an aromatic fraction of GK 2026 m without a high relative abundance of 1,2,5-TMN. For peak identifications refer to Table 10.3.

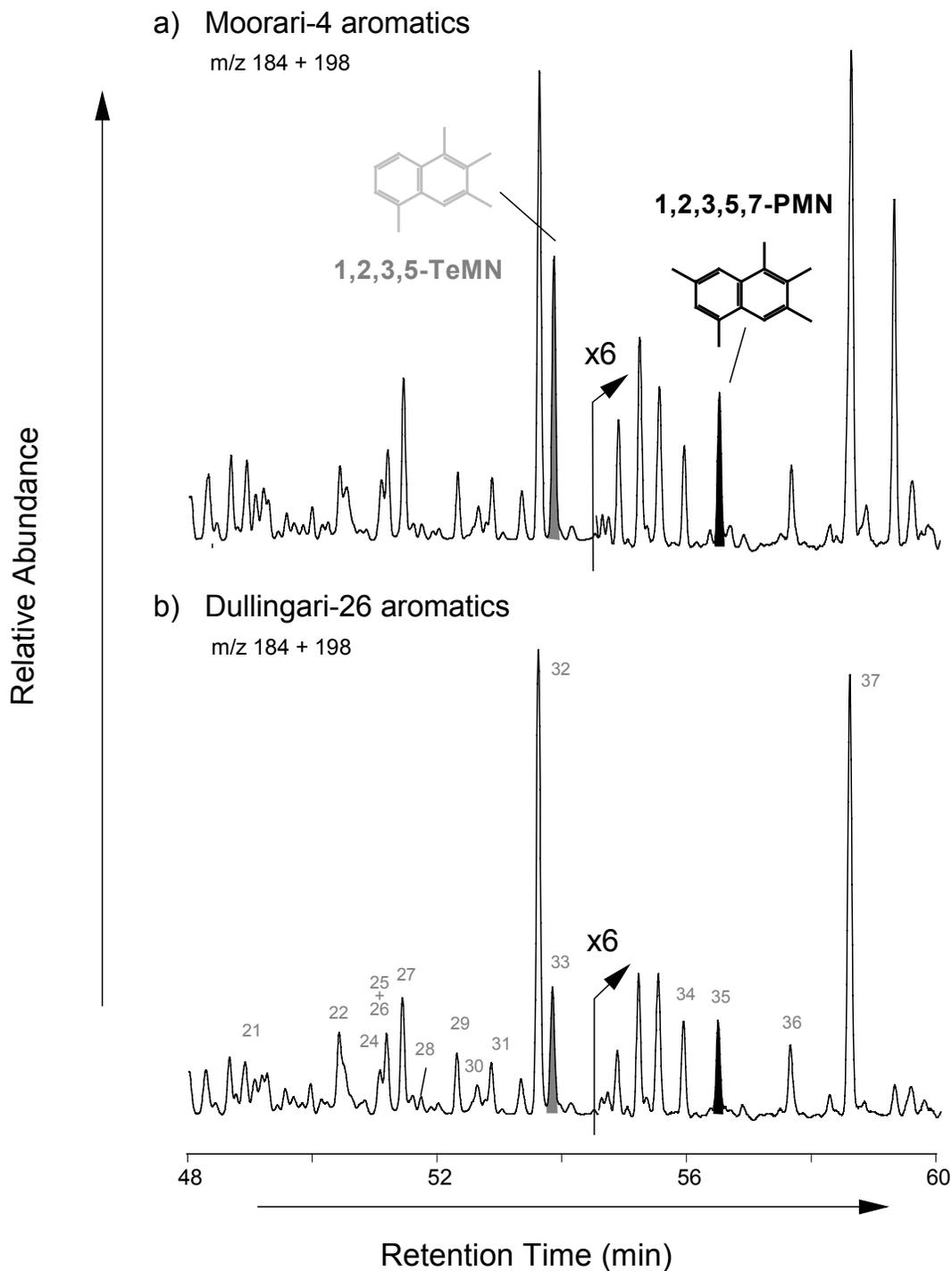


Figure 10.5. Partial m/z (184 + 198) mass chromatograms, using a BP-20 GC-column, of (a) an aromatic fraction of Moorari-4 with a high relative abundance of 1,2,3,5-TeMN and (b) an aromatic fraction of Dullingari-26 sample without a high relative abundance of 1,2,3,5-TeMN (c). For peak identifications refer to Table 10.3.

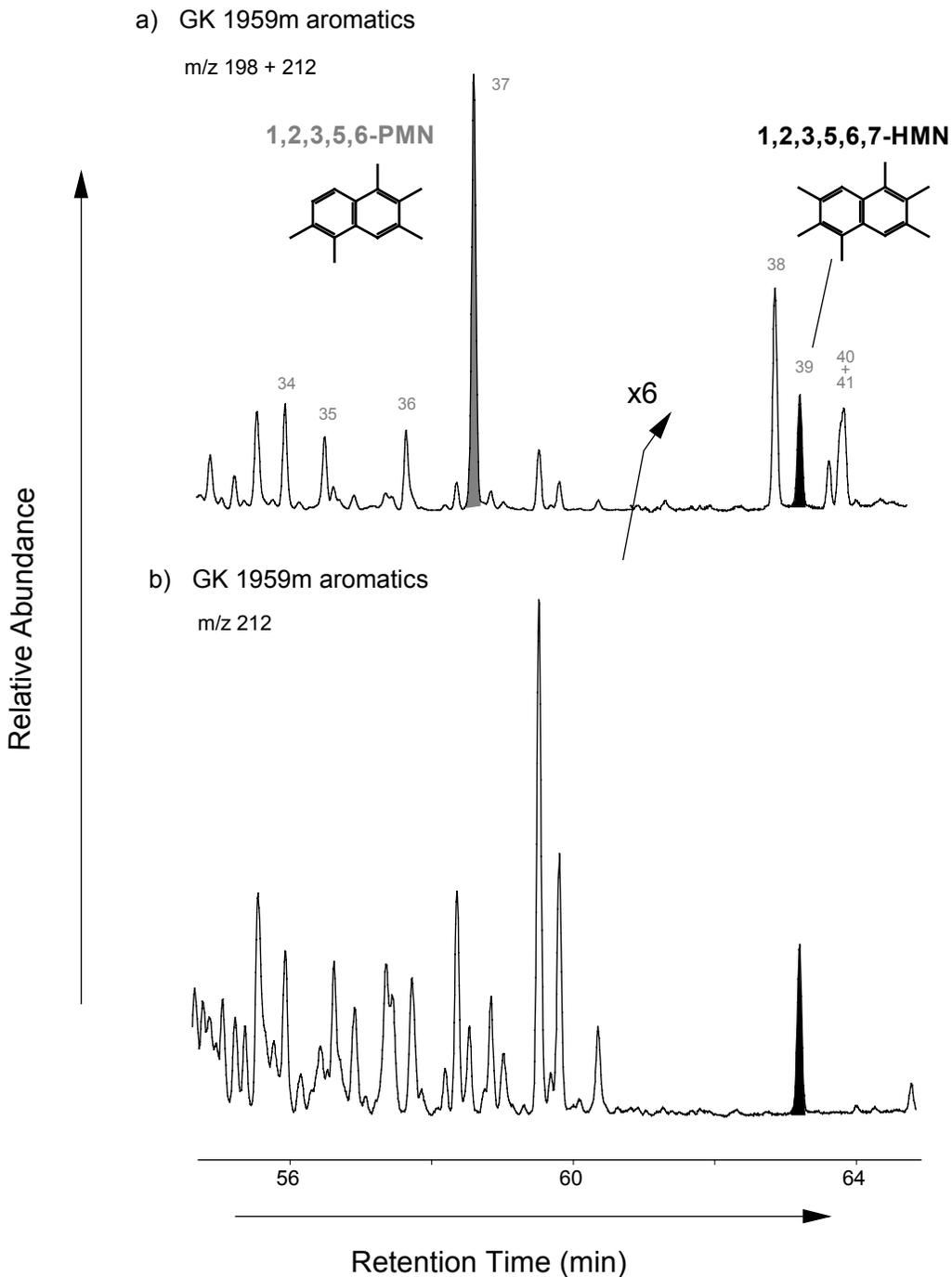


Figure 10.6. Partial m/z (198 + 212) mass chromatograms, using a BP-20 GC-column, of (a) an aromatic fraction of GK 1959 m with a high relative abundance of 1,2,3,5,6-PMN and (b) the partial m/z 212 mass chromatogram for an aromatic fraction of GK 1959 m with a high relative abundance of 1,2,3,5,6-PMN (c). For peak identifications refer to Table 10.3.

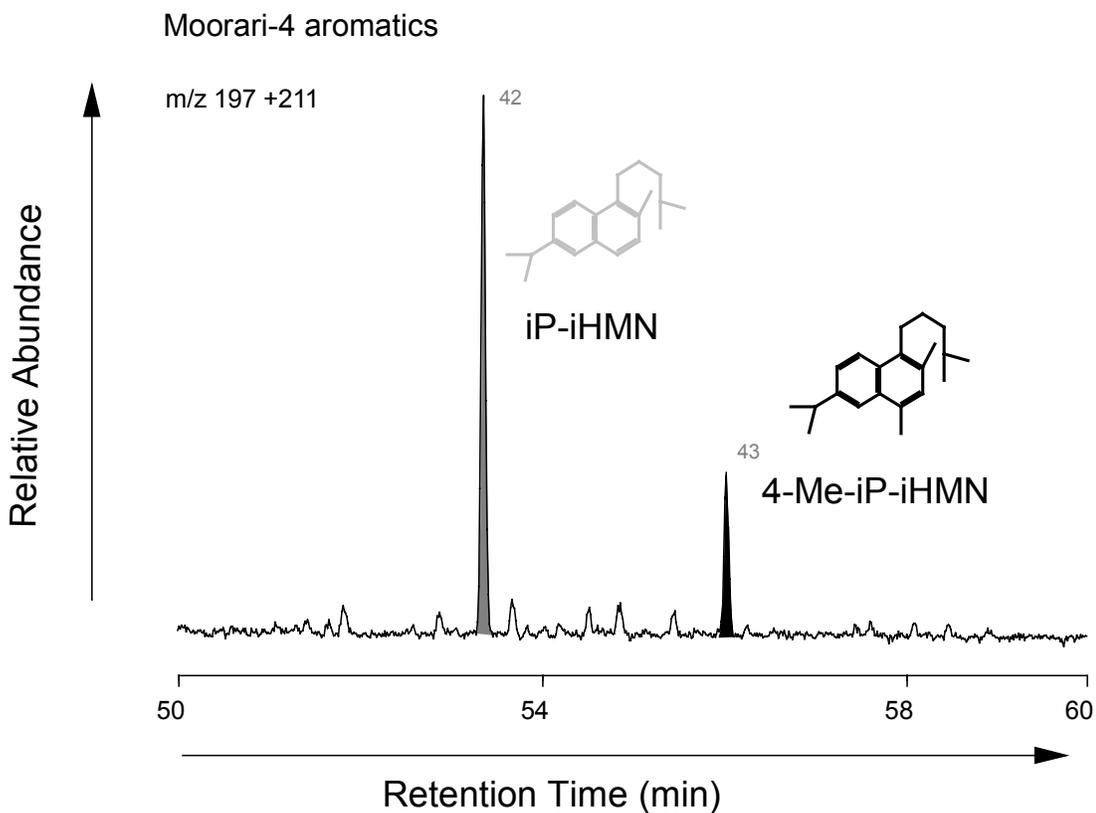


Figure 10.7. Partial m/z (197 + 211) mass chromatograms, using a DB-5 GC-column, of an aromatic fraction of Moorari-4 where iP-iHMN co-occurs with its methylated counterpart 4-Me-iP-iHMN. For peak identifications refer to Table 10.3.

sample showing 1,2,3,5,6,7-HMN with little else eluting in the same region when the m/z 198 is not used. The partial mass chromatogram in Figure 10.1e shows the methylation experiment for iP-iHMN with HMB giving 4-Me-iP-iHMN. The Moorari-4 sample which shows evidence for the methylation of 1,2,3,5-TeMN also has abundant iP-iHMN associated with its methylated counterpart 4-Me-iP-iHMN shown in Figure 10.7. The co-occurrences of

Table 10.3. Compounds identified in crude oils and rock extracts. Peak assignments for alkylnaphthalenes.

Peak No.	Compound	Abbreviation
1	2,6-Dimethylnaphthalene	2,6-DMN
2	2,7-Dimethylnaphthalene	2,7-DMN
3	1,7-Dimethylnaphthalene	1,7-DMN
4	1,3-Dimethylnaphthalene	1,3-DMN
5	1,6-Dimethylnaphthalene	1,6-DMN
6	1,4-Dimethylnaphthalene	1,4-DMN
7	2,3-Dimethylnaphthalene	2,3-DMN
8	1,5-Dimethylnaphthalene	1,5-DMN
9	1,2-Dimethylnaphthalene	1,2-DMN
10	1,3,7-Trimethylnaphthalene	1,3,7-TMN
11	1,3,6-Trimethylnaphthalene	1,3,6-TMN
12	1,4,6-Trimethylnaphthalene	1,4,6-TMN
13	1,3,5-Trimethylnaphthalene	1,3,5-TMN
14	2,3,6-Trimethylnaphthalene	2,3,6-TMN
15	1,2,7-Trimethylnaphthalene	1,2,7-TMN
16	1,6,7-Trimethylnaphthalene	1,6,7-TMN
17	1,2,6-Trimethylnaphthalene	1,2,6-TMN
18	1,2,4-Trimethylnaphthalene	1,2,4-TMN
19	1,2,5-Trimethylnaphthalene	1,2,5-TMN
20	1,2,3-Trimethylnaphthalene	1,2,3-TMN
21	1,3,5,7-Tetramethylnaphthalene	1,3,5,7-TeMN
22	1,3,6,7-Tetramethylnaphthalene	1,3,6,7-TeMN
23	Cadalene	Cad
24	1,4,6,7-Tetramethylnaphthalene	1,4,6,7-TeMN
25	1,2,4,7-Tetramethylnaphthalene	1,2,4,7-TeMN
26	1,2,4,6-Tetramethylnaphthalene	1,2,4,6-TeMN
27	1,2,5,7-Tetramethylnaphthalene	1,2,5,7-TeMN
28	2,3,6,7-Tetramethylnaphthalene	2,3,6,7-TeMN
29	1,2,6,7-Tetramethylnaphthalene	1,2,6,7-TeMN
30	1,2,3,7-Tetramethylnaphthalene	1,2,3,7-TeMN
31	1,2,3,6-Tetramethylnaphthalene	1,2,3,6-TeMN
32	1,2,5,6-Tetramethylnaphthalene	1,2,5,6-TeMN
33	1,2,3,5-Tetramethylnaphthalene	1,2,3,5-TeMN
34	1,2,4,6,7-Pentamethylnaphthalene	1,2,4,6,7-PMN
35	1,2,3,5,7-Pentamethylnaphthalene	1,2,3,5,7-PMN
36	1,2,3,6,7-Pentamethylnaphthalene	1,2,3,6,7-PMN
37	1,2,3,5,6-Pentamethylnaphthalene	1,2,3,5,6-PMN
38	4-Methyldibenzothiophene	4-MDBT
39	1,2,3,5,6,7-Hexamethylnaphthalene	1,2,3,5,6,7-HMN
40	3-Methyldibenzothiophene	3-MDBT
41	2-Methyldibenzothiophene	2-MDBT
42	6-Isopropyl-2-methyl-1-(4-methylpentyl)naphthalene	iP-iHMN
43	6-Isopropyl-2,4-dimethyl-1-(4-methylpentyl)naphthalene	4-Me-iP-iHMN

Peak numbers refer to Figure 10.1-10.7.

1,6-DMN, 1,2,5-TMN, 1,2,3,5-TeMN, 1,2,3,5,6-PMN and iP-iHMN with their methylated counterparts suggests a precursor-product relationship between these compounds, and is strong evidence that methylation is a geosynthetic process.

Further supporting evidence is provided by the fact that the carbon skeleton of these methylated alkylnaphthalenes is uncommon in natural products and the occurrence of 1,2,3,5,6,7-HMN, due to its high level of substitution, has no obvious natural product precursor and is difficult to explain without a methylation process. The suggestion that sedimentary methylation has occurred is also supported by a recent report that other compound types such as phenols (Ioppolo-Armanios *et al.*, 1995), alkylphenathrenes (Smith *et al.*, 1994,1995; Alexander *et al.*, 1995), anthracenes (Smith *et al.*, 1994,1995), alkylbenzenes (Ellis *et al.*, 1995b), pyrene (Derbyshire and Whitehurst, 1981, Smith *et al.*, 1994, 1995) and alkylnaphthalenes (Bastow *et al.*, 1996) also show evidence of formation from simpler compounds involving methylation processes. Table 10.4 shows the percentage of 9-methylretene compared to retene for the samples used in this study. 9-Methylretene was identified by Alexander *et al.* (1995) as a methylation product of retene and its presence in all the sample, except one Devonian sample with no detectable retene, used in this study is supporting evidence that methylation has occurred in these samples.

It is evident from these observations that alkylnaphthalenes involved in this methylation process can produce high concentrations of the methylated alkylnaphthalene. Moreover, in cases where 1,6-DMN occurs in high relative abundances an increase in the abundance of 1,4,6-TMN would be expected and could cause a distortion in the TNR-1 maturity parameter, since this parameter is based on the ratio of 1,4,6-TMN and 1,3,5-TMN to 2,3,6-TMN.

Table 10.4. Percentage of 9-methylretene compared to retene (%MR) identified for the crude oils and rock extracts.

Sample Name	%MR
Dullingari-26	7.3
GK 1959m	13.0
GK 2026m	12.4
Moorari-4	8.0
Moorari-3 7108'	11.8
PD 130A	-

10.5 Conclusions

1,6-DMN; 1,2,7-TMN; 1,2,5-TMN; 1,2,3,5-TeMN; 1,2,3,5,6-PMN and iP-iHMN have been shown to occur in high relative abundances in sedimentary samples together with high relative abundances of their corresponding methyl counterparts. This observation, together with the demonstration that alkylnaphthalenes are readily methylated in specific positions consistent with EAS when treated with a methyl donor under laboratory conditions, has been interpreted as evidence for a sedimentary methylation process.

10.6 Acknowledgements

Geoff Chidlow is thanked for his assistance with GC-MS analysis. Dr Iman B. Sosrowidjojo and Lemigas are thanked for the GK well samples used in this paper. Financial support was provided by the Australian Petroleum Co-operative Research Centre and from a Curtin University Postgraduate Scholarship.

11.1 Biodegradation of some aromatic land-plant biomarkers in some Australian crude oils

11.1 Abstract

Land-plant-derived aromatic hydrocarbons with a range of susceptibilities to reservoir biodegradation have been identified in crude oils from two Australian sedimentary basins. The compounds used in this study (retene, 9-methylretene, 6-isopropyl-2-methyl-1-(4-methylpentyl)naphthalene and 6-isopropyl-2,4-dimethyl-1-(4-methylpentyl)naphthalene) are suggested to originate from land-plants and are the result of reactions of their natural products precursors, involving aromatisation, rearrangement and methylation in the sediments. They are therefore suggested as markers for land-plants in severely biodegraded oils in which other biologically derived compounds cannot be recognised.

The order of biodegradability of the methylated compounds has been assessed relative to their non-methylated counterparts namely 6-isopropyl-2-methyl-1-(4-methylpentyl)naphthalene and retene. In both cases the methylated homologue is less susceptible to biodegradation.

These compounds have been used to assess the composition of a crude oil that has previously been reported to contain a mixture of a severely degraded and a less degraded crude oil.

11.2 Introduction

Biodegradation of reservoired crude oil is a well-documented process that results in the change of the physical and chemical properties of the residual hydrocarbons (Volkman *et al.*, 1983a,b; Connan, 1984; Tissot and Welte, 1984; Peters and Moldowan, 1993). Biodegradation proceeds along a path of stepwise depletion of compound classes. Among the saturated hydrocarbons the linear alkanes are often depleted before polycyclic hydrocarbons. Rearranged compounds derived from natural products are biodegraded less rapidly. Conversion of compound classes (e.g. 17 α -hopanes to 17 α -25-norhopanes) has also been suggested to occur during the later stages of the process (Volkman *et al.*, 1983b; Peters and Moldowan, 1991, 1993; Peters *et al.*, 1996).

Among the aromatic hydrocarbons the lower molecular weight and more water soluble compounds usually degrade in preference to higher molecular weight and less water soluble compounds (Connan, 1984; Tissot and Welte, 1984; Lafargue and Barker, 1988; Lafargue and Le Thiez, 1996). Within compound classes however some isomers are more susceptible to biodegradation than others. In crude oils degraded under laboratory and natural conditions the most resistant dimethylnaphthalenes (Rowland *et al.*, 1986; Volkman *et al.*, 1984; Fisher *et al.*, 1996a, 1998), trimethylnaphthalenes (Rowland *et al.*, 1986; Fisher *et al.*, 1996a, 1998) and tetramethylnaphthalenes (Fisher *et al.*, 1996a, 1998) have been identified. Alkylphenanthrenes with substituents at the 9 or 10 positions have been reported to be more resistant to biodegradation than alkylphenanthrenes with substituents at other positions (Rowland *et al.*, 1986, Budzinski *et al.*, 1995a).

In the present study we have examined the effects of biodegradation on the abundance of 6-isopropyl-2-methyl-1-(4-methylpentyl)naphthalene (iP-iHMN), formed from a natural product *via* a rearrangement and aromatisation process (Ellis, 1994; Ellis *et al.*, 1996b) as well as retene formed by aromatisation of a natural product. In addition, both of these compounds undergo sedimentary methylation (Ellis, 1994; Ellis *et al.*, 1996b; Alexander

et al., 1995; Bastow *et al.*, 1998a) to yield 6-isopropyl-2,4-dimethyl-1-(4-methylpentyl)naphthalene (4-Me-iP-iHMN) and 9-methylretene. iP-iHMN has also been found to survive in a severely biodegraded crude oil (Ellis, 1994; Ellis *et al.*, 1996b).

In the present study we have examined the effects of biodegradation on two higher-plant derived compounds and their methylated counterparts.

11.3 Experimental

Samples

The locations of the samples, the biodegradation level, together with some maturity parameters (20S/20R; MPI-1; PNR), are shown in Table 11.1.

Isolation of aromatic hydrocarbon fractions

In a typical separation, a crude oil or sediment extract was subjected to liquid chromatography. Glass columns (11 cm x 0.4 cm i.d.) were packed with activated silica gel (0.4 g) and the column flushed with 4 bed volumes of pentane. The sample (5-10 mg) in *n*-pentane was introduced to the top of the column to produce a concentrated band. Aliphatic hydrocarbons were then eluted under gravity with *n*-pentane (800 μ L), aromatic hydrocarbons with *n*-pentane/dichloromethane (80:20, 2 mL), and polar compounds with dichloromethane/methanol (1:1, 30 mL). The aromatic fractions had hexane (500 μ L) added and their volume reduced carefully (200 μ L), which had a further volume of hexane (1500 μ L) added to provide a sample ready for GC-MS analysis.

Reference compounds

D8-naphthalene (d8-nap) and d10-phenanthrene (d10-phen) were purchased from Sigma Chemicals. Retene was purchased from Chiron Laboratories, Trondheim, Norway. 9-Methylretene (Alexander *et al.*, 1995), iP-iHMN (Ellis *et al.*, 1996b), 4-Me-iP-iHMN (Bastow *et al.*, 1998a) were available from previous studies. Table 11.2 shows the response factors for the various techniques used for the analysis of the above compounds.

Table 11.1 Geological and some geochemical data for the samples.

Crude Oil Name	Location Australian Basin	Biodegradation Level ^a	Maturity		
			$\frac{20S}{20R}$ ^b	MPI-1 ^c	PNR ^d
Non-biodegraded					
Barracouta-A5	Gippsland	None, 1	0.4	0.4	0.17
Blackback-1	Gippsland	None, 1	0.7	1.1	0.44
Bream-2	Gippsland	None, 1	0.6	0.9	0.38
Cobia-2	Gippsland	None, 1	0.6	1.1	0.44
Halibut-A1	Gippsland	None, 1	0.6	1.1	0.45
Kingfish-1	Gippsland	None, 1	0.6	1.1	0.43
Mackerel A-8	Gippsland	None, 1	0.6	1.1	0.43
Marlin-A1	Gippsland	None, 1	0.3	0.4	0.38
Marlin-A24	Gippsland	None, 1	0.8	0.6	0.32
Rough Range-1	Carnarvon	None, 1	1.0	0.7	0.12
Snapper-5	Gippsland	None, 1	0.5	1.1	0.30
Sunfish-1	Gippsland	None, 1	0.6	0.8	0.37
Tuna-4	Gippsland	None, 1	1.0	1.0	0.52
Turrum-3	Gippsland	None, 1	0.6	0.7	0.37
Wirrah-1	Gippsland	None, 1	0.8	0.7	0.45
Wirrah-3	Gippsland	None, 1	0.8	0.8	0.43
Biodegraded					
Eaglehawk-1	Carnarvon	Moderate, 4	1.2	0.7	0.45
Lakes Entrance	Gippsland	Very extensive, 7	3.1	-	-
Leatherjacket-1	Gippsland	Moderate, 5	0.9	0.4	0.42
Mardie-1a	Carnarvon	Severe, 8	6.2	-	-
Rankin-1	Carnarvon	None + Severe	1.0	1.5	0.50

Definitions and methods of measurements: $\frac{20S}{20R}$, 20S and 20R diastereomers of $5\alpha(H)$, $14\alpha(H)$, $17\alpha(H)$ -ethylcholestane (m/z 217) (MacKenzie and McKenzie, 1983); MPI-1. GC-MS data corrected to FID response (Radke *et al.*, 1982a,b); PNR (m/z 198) (Bastow *et al.*, 1998b).

Table 11.2. Response factors for reference compounds using various analytical techniques.

Method	Response factors					
	D8-nap	D10-phen	Retene	9-MR	iP-iHMN	4-Me-iP-iHMN
GC-MS	3.01 (m/z 136)	3.09 (m/z 188)	1.00 (m/z 234)	1.11 (m/z 248)	1.94 (m/z 197)	2.15 (m/z 211)
GC-MS (MRM)	-	-	1.00 (m/z 234-219)	1.06 (m/z 248-233)	1.16 (m/z 268-197)	1.40 (m/z 282-211)

Analysis and quantification

In a typical separation, a crude oil (approx. 10 mg) was placed on a small column packed with silica gel (0.4 g) and eluted with 2 mL of

hexane/dichloromethane (8:2), which afforded a hydrocarbon fraction containing both saturate and aromatic compounds.

Quantitative analysis was carried out on the crude oil using external and normalisation (d10-phen) standards. External standards were prepared with concentrations of 5, 50, 100, 250, 500, 1000, 2500 $\mu\text{g/g}$ of 9-methylretene, retene, 4-Me-iP-iHMN and iP-iHMN. D10-phen was used as a normalisation standard and added to the samples in known concentration (2500 $\mu\text{g/g}$). 9-Methylretene, retene, 4-Me-iP-iHMN and iP-iHMN concentrations were calculated by measuring, respectively the m/z 248, 234, 211 and 197 abundances of the standards in the samples relative to the abundance of the standards normalised to the abundance of d10-phen (m/z 188).

Gas chromatography-metastable reaction monitoring (MRM)

A Fisons VG-Autospec Ultima Q interfaced with a Hewlett-Packard 5890 Series II gas chromatograph was used. The GC was fitted with a DB-5 (60 m x 0.25 mm i.d., phase thickness 0.25 μm , J&W Scientific) fused silica open tubular column. Helium was used as carrier gas at a constant flow of 1.00 mL min^{-1} (DB-5), and the GC oven was temperature programmed from 60-300°C at 3°C min^{-1} . The mass spectrometer was operated in MRM mode monitoring the parent-daughter transitions (m/z 282 to 211, m/z 268 to 197, m/z 248 to 233, m/z 234 to 219).

GC-MS analysis

A Hewlett-Packard 5971 MSD interfaced with a 5890 Series II gas chromatograph fitted with a DB-5 (60 m x 0.25 mm i.d., phase thickness 0.25 μm , J&W Scientific) fused silica open tubular column. Helium was used as carrier gas at a constant flow of 1.28 mL min^{-1} and the GC oven was temperature programmed from 60-300°C at 6°C min^{-1} . Samples for analysis were dissolved in hexane and injected on-column using a HP 7673 autosampler. Typical MSD conditions were: ionisation energy 70 eV; source temperature 200°C; electron multiplier voltage 2200 V.

11.4 Results and discussion

Analysis

The target compounds were analysed using a combination of liquid chromatography and GC-MS techniques. The aromatic fractions obtained from liquid chromatography of the crude oil were analysed by GC-MS (MRM) and the transitions that were monitored are shown in a typical chromatogram (Figure 11.1). GC-MS (MRM) was used in this study to determine compound ratios due to its high selectivity and sensitivity for the compounds in this study.

Gippsland Basin crude oils

Oils from the Gippsland Basin were chosen because they occur in a relatively simple petroleum system where oils reservoired predominantly in the non-marine Latrobe group of Late Cretaceous to Eocene age (Burns *et al.*, 1987) generated from the source rocks of the Upper Cretaceous, overlaying basement rocks (Shibaoka *et al.*, 1978). Consequently, it is less likely that multiple generations and accumulation events have occurred in this Basin. Table 11.3 shows the results for the percentage of 9-methylretene compared to retene and percentage 4-Me-iP-iHMN compared to iP-iHMN for the oils used in this study. These oils include 15 non-degraded oils from the Gippsland Basin, which show an average percentage of 8.6 % (std. dev. 2.6 %, n=15) for 9-methylretene compared to retene and a average percentage of 15.0 % (std. dev. 2.0 %, n=15) for 4-Me-iP-iHMN compared to iP-iHMN. These results show that the methylation process has occurred in all of these samples and that the extent of methylation indicated by these compounds has been relatively constant for these oils.

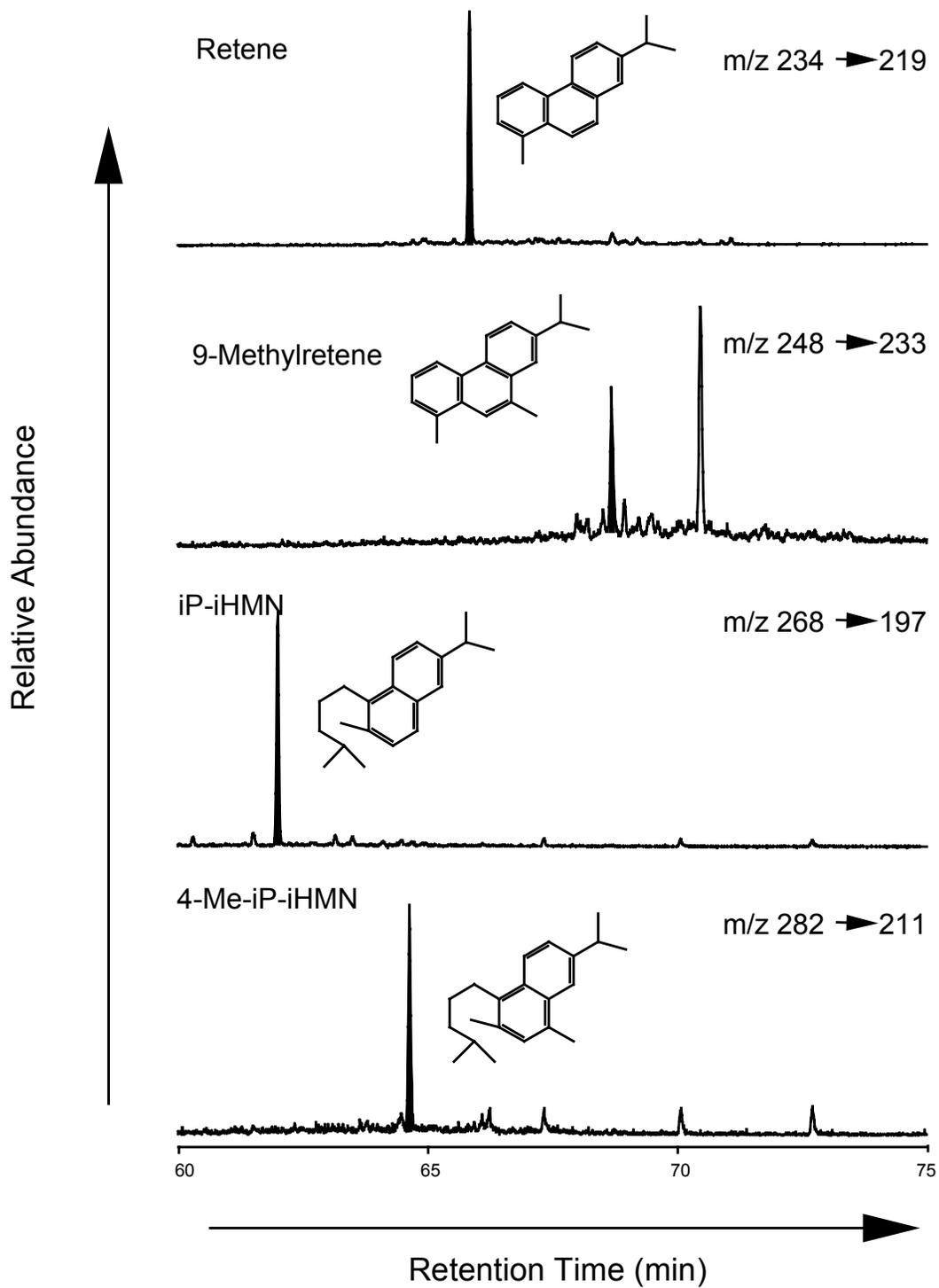


Figure 11.1. Partial mass chromatograms showing the mrm transitions $m/z\ 234$ to 219 , $m/z\ 248$ to 233 , $m/z\ 268$ to 197 and $m/z\ 282$ to 211 for Rough Range-1 crude oil.

Table 11.3. Percentage of methylated compounds relative to their non-methylated counterparts for samples.

Crude oil	% 9-MR ^a	% 4-Me-iP-iHMN ^b
Non-biodegraded		
Barracouta-A5	4.9	11.1
Blackback-1	10.7	15.3
Bream-2	9.2	14.2
Cobia-2	8.7	17.0
Halibut-A1	8.4	16.6
Kingfish-1	9.2	16.6
Mackerel A-8	9.4	16.7
Marlin-A1	3.0	15.1
Marlin-A24	7.1	14.8
Rough Range-1	7.1	16.6
Snapper-5	9.2	13.0
Sunfish-1	8.2	14.3
Tuna-4	10.5	18.3
Turrum-3	8.2	15.4
Wirrah-1	10.5	13.6
Wirrah-3	11.7	15.0
Biodegraded		
Eaglehawk-1	32.4	19.6
Lakes Entrance	92.1	65.9
Leatherjacket-1	83.1	24.3
Mardie-1a	83.6	65.5
Rankin-1	6.7	30.1

^a % 9-MR = 100 x (9-MR/ 9-MR+R), 4-Me-iP-iHMN = 100 x (4-Me-iP-iHMN/4-Me-iP-iHMN + iP-iHMN).

Three crude oils that represent different levels of biodegradation were selected from the Gippsland Basin. Gas chromatograms of the combined saturate and aromatic fractions for these oils are shown in Figure 11.2.

It is evident that Wirrah-1 is non-degraded with its *n*-alkanes intact.

Leatherjacket-1 shows is a large unresolved complex mixture (UCM) with its *n*-alkanes removed, and its isoprenoidal and bicyclic components almost removed, giving this oil an extensive level of biodegradation. Lakes Entrance has also had *n*-alkanes, isoprenoids and bicyclic compounds removed and steranes altered, giving this oil a very extensive level of biodegradation.

The results of these three Gippsland Basin crude oils suggest that reservoir biodegradation depletes the retenes at a faster rate than the iP-iHMNs. Figure 11.3a shows a diagrammatic representation of these compositional

changes for the three Gippsland Basin crude oils. With increasing biodegradation the retenes (retene + 9-methylretene) decrease relative to the

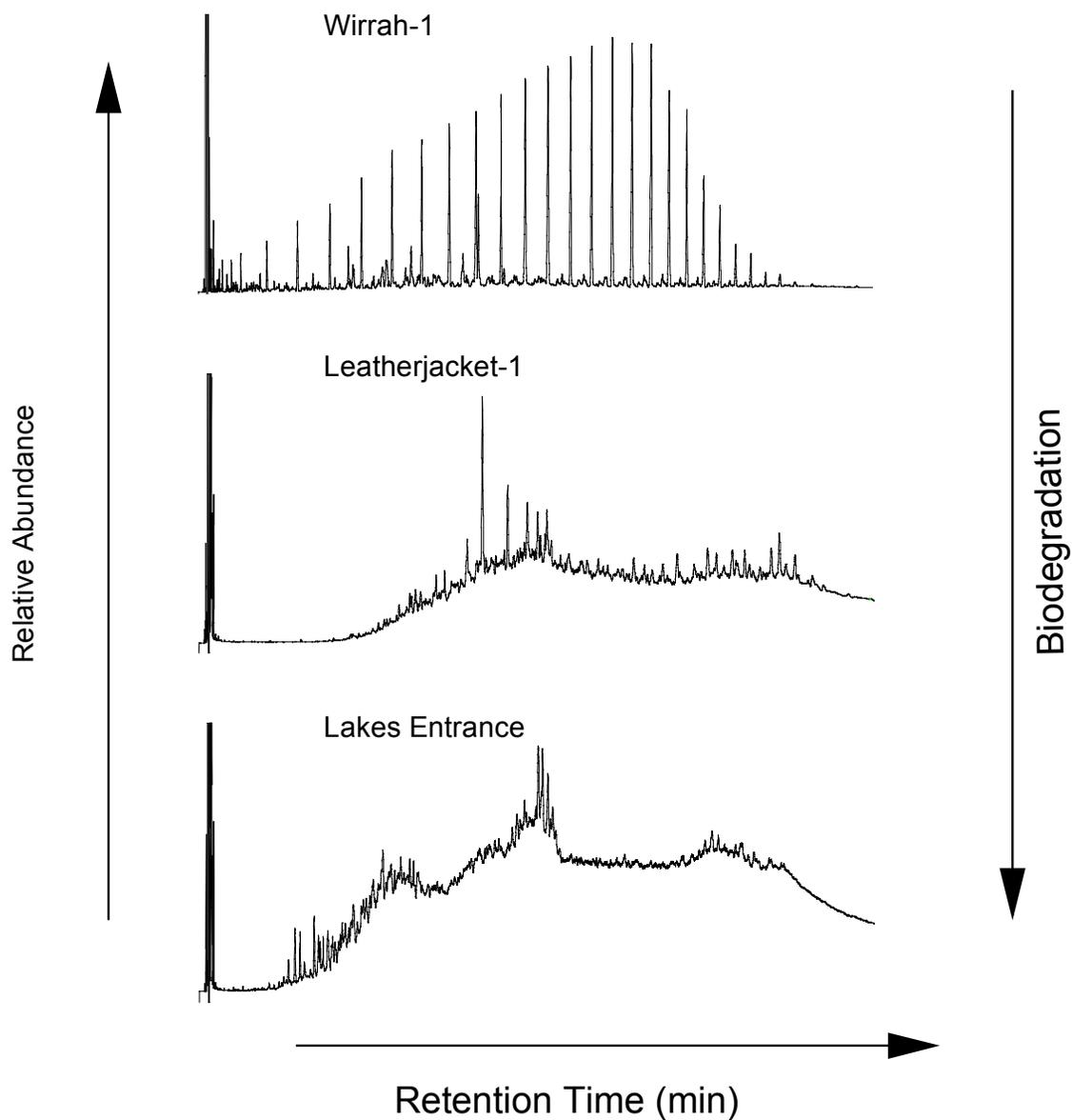
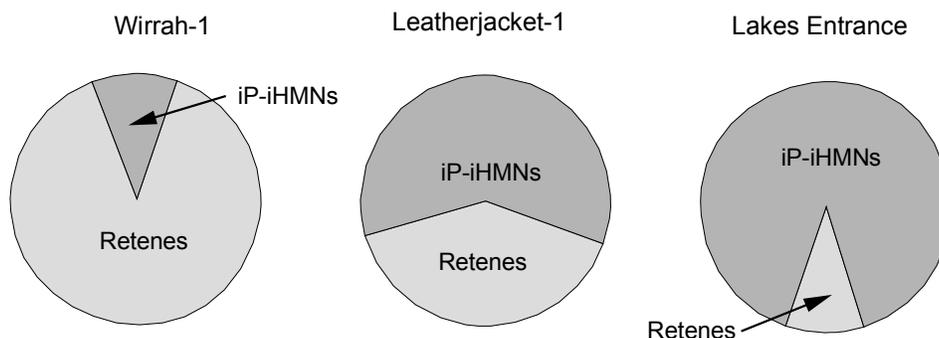


Figure 11.2. Gas chromatograms for Wirrah-1, Leatherjacket-1, and Lakes Entrance-1.

iP-iHMNs (iP-iHMN + 4-Me-iP-iHMN). These results show a progressive depletion of the retenes compared with the more resistant iP-iHMNs with increasing biodegradation.

a) Compositional changes with biodegradation of Retenes and iP-iHMNs



b) Changes in the percentage of the methylated compounds

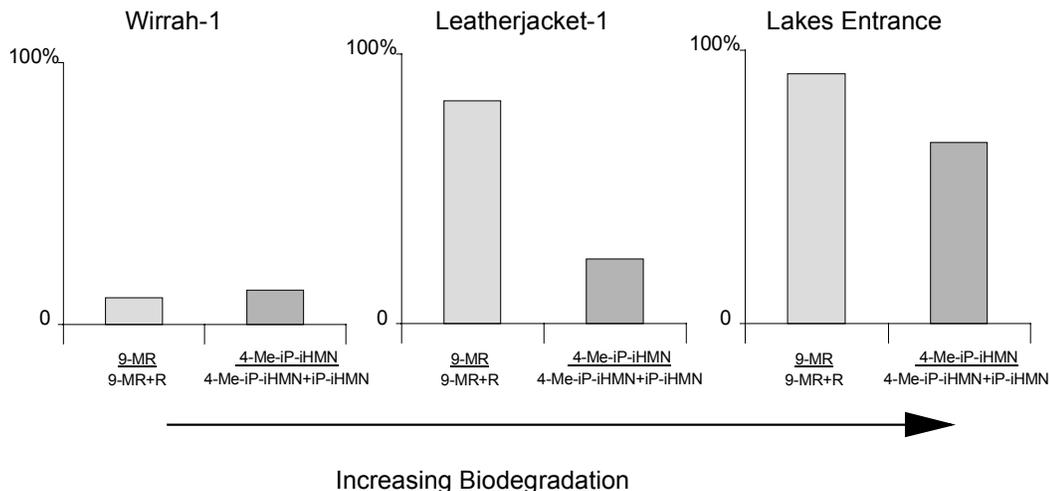


Figure 11.3. a) Pie diagrams showing the composition of retenes (retene + 9-methylretene) relative to iP-iHMNs (iP-iHMN + 4-Me-iP-iHMN) and b) histograms showing the percentage of 9-methylretene (9-MR) compared to retene (R) and the percentage 4-Me-iP-iHMN compared to iP-iHMN for Wirrah-1, Leatherjacket-1 and Lakes Entrance crude oils.

Figure 11.3b shows a set of histograms illustrating the changes in relative abundances of the two compounds with biodegradation. The percentage of 9-methylretene compared to retene has increased significantly in the oil for Leatherjacket-1 (moderately biodegraded) relative to that of Wirrah-1 (non-degraded). This suggests that biodegradation affects the methylated compound to a lesser extent than its parent compound. The ratio of 4-Me-iP-iHMN to iP-iHMN has increased significantly in the oil for Lakes Entrance (very extensively biodegraded) relative to that of Wirrah-1 (non-degraded) and Leatherjacket-1 (moderately degraded). This suggests that biodegradation affects the methylated compound 4-Me-iP-iHMN to a lesser extent than its parent compound. From this data it is apparent that the order of susceptibility to biodegradation is: retene > 9-methylretene ~ iP-iHMN > 4-Me-iP-iHMN. Table 11.4 lists the measured concentrations of these compounds in the crude oils used in this study.

Carnarvon Basin crude oils

Three crude oils have been selected from this basin to represent a non-biodegraded and two biodegraded crude oils. Mardie-1a and Flinders Shoal-1 crude oils have previously been described in detail (Alexander *et al.*, 1983c; Volkman *et al.*, 1984; Rowland *et al.*, 1986). The crude oils are thought to be sourced from Upper Jurassic siltstones and claystones (Crank, 1973; Volkman *et al.*, 1983a). Rough Range-1 is a non-degraded oil with its *n*-alkanes intact. Flinders Shoal-1 is a crude oil moderately biodegraded to level 5 (Volkman *et al.*, 1984) whereas Mardie-1a crude oil is biodegraded severely to level 8 (Volkman *et al.*, 1984). The Flinders Shoal-1 crude oil is thought to be a mixture of a moderately biodegraded oil with a small amount of severely biodegraded since it contains 25-norhopanes and other saturated hydrocarbons indicative of less severe biodegradation (Alexander *et al.*, 1983c; Volkman *et al.*, 1984). Mardie-1a crude is the most degraded crude oil in this sample suite, and very few biomarkers can be recognised. 25-Norhopanes have been reported (Alexander *et al.*, 1983c; Volkman *et al.*, 1984) as well as 4-Me-iP-iHMN (Ellis, 1994; Ellis *et al.*, 1996b).

The identification of 4-Me-iP-iHMN in the Mardie-1a crude oil indicates the high resistance of this compound to biodegradation. It is therefore a potentially useful biomarker for land-plants in such severely biodegraded crude oils. The aromatic land-plant markers in the Flinders Shoal-1 crude oil are also consistent with an oil formed from mixing crude oils with differing biodegradation histories.

Table 11.4. The abundance data for aromatic hydrocarbons used.

Crude oil	Absolute Concentration ($\mu\text{g/g}$ Crude oil)			
	Retene	9-Methylretene	iP-iHMN	4-Me-iP-iHMN
Non-biodegraded				
Barracouta-A5	30	a	30	a
Blackback-1	160	20	25	a
Bream-2	140	15	50	10
Cobia-2	270	30	55	10
Halibut-A1	310	35	65	15
Kingfish-1	210	25	45	10
Mackerel A-8	250	30	50	10
Marlin-A1	65	a	60	10
Marlin-A24	350	30	50	10
Rough Range-1	50	a	30	a
Snapper-5	110	10	75	10
Sunfish-1	160	20	80	10
Tuna-4	110	15	a	a
Turrum-3	420	45	30	a
Wirrah-1	140	15	15	a
Wirrah-3	190	30	35	a
Biodegraded				
Eaglehawk-1	100	45	140	35
Lakes Entrance	a	a	15	25
Leatherjacket-1	20	85	110	30
Mardie-1a	a	a	15	25
Rankin-1	105	10	20	10

a) concentration less than 10 $\mu\text{g/g}$. Values based on GC-MS analysis of combined saturate and aromatic fractions.

Figure 11.4 shows histograms of the relative abundances of the methylated compounds relative to their parent hydrocarbons for Rough Range (non-degraded), Mardie-1a (degraded) and Flinders Shoal-1 (mixture) crude oils. Comparison of these histograms with those obtained from the Gippsland Basin crude oils (Figure 11.3) shows that Flinders Shoal-1 has a percentage 9-methylretene compared to retene similar to the non-degraded Wirrah-1 and

to the non-degraded Rough Range-1 crude oil from the Carnarvon Basin. In contrast to the non-degraded oils, Flinders Shoal-1 has an elevated percentage of 4-Me-iP-iHMN compared to iP-iHMN similar to the extensively biodegraded Gippsland Basin sample (Leatherjacket-1) in Figure 11.3b. Clearly, the simplest explanation for this apparent conflict is a mixture of two oils, one slightly degraded with abundant retenes, the other a more severely degraded oil with abundant iP-iHMNs (enriched relative to retenes by the biodegradation process). Mixing the two, results in the retenes predominating from the non-degraded, with a percentage 9-methylretene compared to retene indicative of a non-degraded oil and the iP-iHMNs predominantly from the degraded oil with a 4-Me-iP-iHMN to iP-iHMN ratio indicative of severe biodegradation. A diagrammatic representation of these processes is given in Figure 11.5.

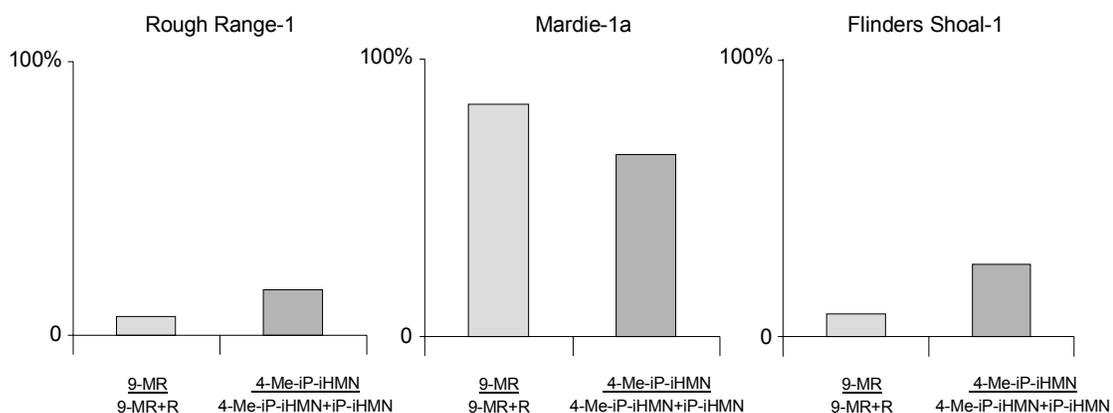


Figure 11.4. Histograms showing the percentage 9-methylretene (9-MR) compared to retene (R) and the percentage 4-Me-iP-iHMN compared to iP-iHMN for Rough Range-1, Mardie-1 and Flinders Shoal-1 crude oils.

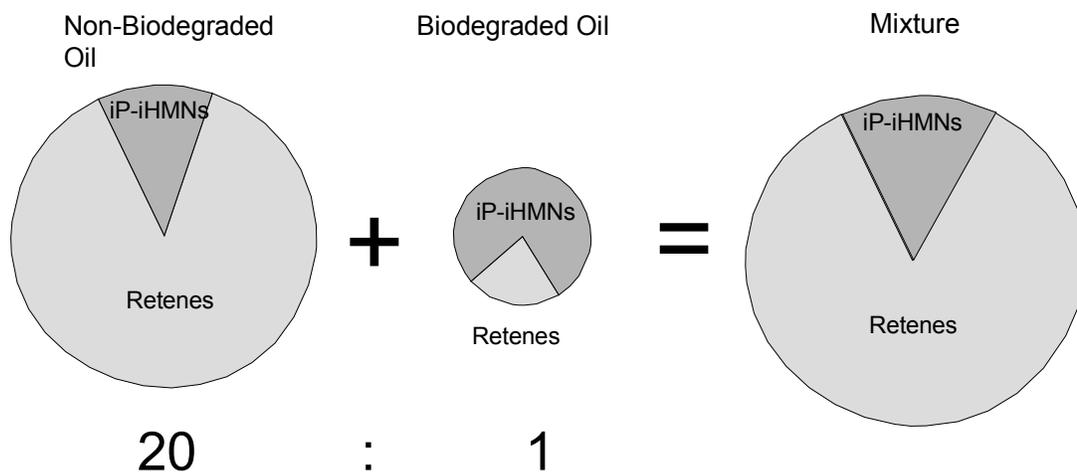
11.5 Conclusions

The order of biodegradability of these aromatic hydrocarbons 6-isopropyl-2,4-dimethyl-1-(4-methylpentyl)naphthalene and 9-methylretene has been assessed relative to their non-methylated counterparts namely 6-isopropyl-2-

methyl-1-(4-methylpentyl)naphthalene and retene, using crude oils from the Gippsland Basin with varying degrees of biodegradation. The order of degradation of the four compounds is: retene > 9-methylretene ~ 6-isopropyl-2-methyl-1-(4-methylpentyl)naphthalene > 6-isopropyl-2,4-dimethyl-1-(4-methylpentyl)naphthalene.

These biodegradation effects have been applied to a crude oil from the Barrow Sub-basin to show that its composition is consistent with a crude oil resulting from mixing of severely degraded and less degraded crude oils.

a) Compositional changes of Retenes and iP-iHMNs for mixtures of non-degraded and degraded crude oils.



b) Changes in the percentage of methylated compounds for mixtures of non-degraded and degraded crude oils.

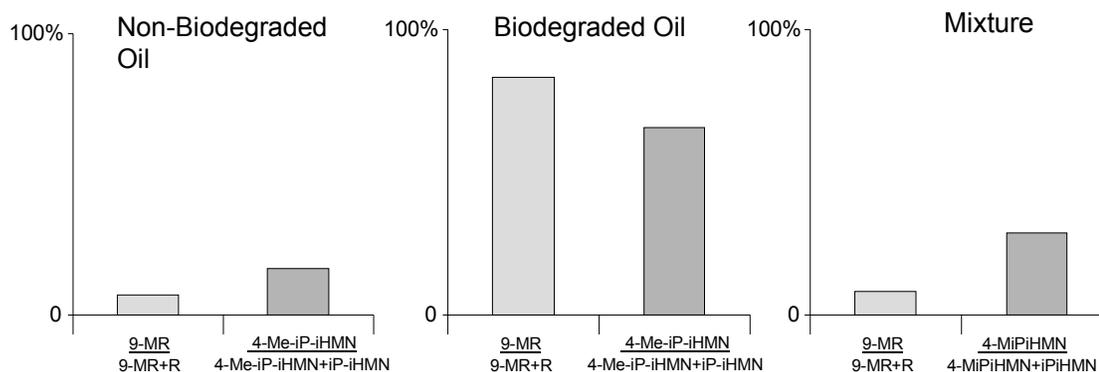


Figure 11.5. Pie diagrams showing the composition of retenes (retene + 9-methylretene) relative to iP-iHMNs (iP-iHMN + 4-Me-iP-iHMN) a), and histograms showing the percentage of 9-methylretene (9-MR) compared to retene (R) and the percentage 4-Me-iP-iHMN compared to iP-iHMN b), for a non-degraded crude oil, degraded crude oil and a mixture of these crude oils.

11.6 Acknowledgements

Geoff Chidlow is thanked for his assistance with GC-MS analysis. Financial support was provided by the Australian Petroleum Co-operative Research Centre and from a Curtin University Postgraduate Scholarship.

References

- van Aarssen B. G. K., Cox, H. C., Hoogendoorn, P. and de Leeuw J. W. (1990) A Cadinene biopolymer in fossil and extant dammar resins as a source for cadinanes and bicadinanes in crude oils from South East Asia. *Geochimica et Cosmochimica Acta* **54**, 3021-3031.
- van Aarssen B. G. K., Horsfield, B. and de Leeuw J. W. (1991) A comparative study of three different pyrolysis methods used to characterise a biopolymer isolated from fossil and extant dammar resins. *Journal of Analytical and Applied Pyrolysis* **20**, 125-139.
- van Aarssen B. G. K., Hessels J. K. C., Abbink O. A. and de Leeuw J. W. (1992) The occurrence of polycyclic sesqui-, tri- and oligoterpenoids derived from a resinous polymeric cadinene in crude oils from South East Asia. *Geochimica et Cosmochimica Acta* **56**, 1231-1246.
- 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 processes during the hydrous pyrolysis of vitrinite kerogen. *Geochimica et Cosmochimica Acta* **54**, 2451-2461.
- Achari, R. G., Shaw, G. and Holleyhead R. (1979) Identification of ionene and other carotenoid degradation products from the pyrolysis of sporopollenis derived from some pollen exines, a spore coal and the Green River Shale. *Chemical Geology* **12**, 229-234.
- Adachi K., Yokota S. and Tanaka J. (1981) Synthesis and characterization of alkylnaphthalenes III. 1-isopropylnaphthalene and its methyl derivatives. *Memoirs of the Osaka Institute of Technology, Series A26*, 1-26.

- Albaiges, J., Algaba, J., Clavell, E. and Grimalt, J. (1986) Petroleum geochemistry of the Taragona Basin (Spanish Mediterranean off-shore). *Organic Geochemistry* **10**, 441-450.
- Alexander, R. Kagi, R. I. and Larcher, A. V. (1982) Clay catalysis of aromatic hydrogen-exchange reactions. *Geochimica et Cosmochimica Acta* **46**, 219-222.
- Alexander, R. Kagi, R. I. and Noble, R. (1983a) Identification of the bicyclic sesquiterpenes drimane and eudesmane in petroleum. *Journal of the Chemical Society, Chemical Communications*, 226.
- Alexander, R., Kagi, R. I. and Sheppard, P. N. (1983b) Relative abundance of dimethylnaphthalene isomers in crude oils. *Journal of Chromatography* **267**, 367-372.
- Alexander, R., Kagi, R. I., Woodhouse, G. W. and Volkman, J. K. (1983c) The geochemistry of some biodegraded Australian oils, *The APEA Journal* **23**, 53-63.
- Alexander R., Kagi R. I. and Larcher A. V. (1984a) Clay catalysis of alkyl hydrogen exchange reactions - reaction mechanisms. *Organic Geochemistry* **6**, 755-760.
- Alexander, R., Kagi, R. I., Noble, R. and Volkman, J. K. (1984b) Identification of some bicyclic alkanes in petroleum. *Organic Geochemistry* **6**, 63-70.
- Alexander, R., Kagi, R. I. and Sheppard, P. N. (1984c) 1,8-Dimethylnaphthalene as an indicator of petroleum maturity. *Nature* **308**, 442-443.

- 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 petroleums. *Geochimica et Cosmochimica Acta* **49**, 385-395.
- Alexander, R., Strachan, M. G., Kagi, R. I. and van Bronswijk, W. (1986) Heating rate effects on aromatic maturity indicators. *Geochimica et Cosmochimica Acta* **52**, 1255-1264.
- Alexander, R., Fisher, S. J. and Kagi, R. I. (1988) 2,3-Dimethylbiphenyl: Kinetics of its cyclisation reaction and effects of maturation upon its relative concentration in sediments. In *Advances in Organic Geochemistry 1987*, eds. L. Mattavelli and L. Novelli, pp. 833-837. Pergamon Press, Oxford.
- Alexander, R., Kagi, R. I. and Toh, E. (1989) Organic reaction kinetics and the thermal histories of sediments. *Chemistry in Australia* **56**, 74-76.
- Alexander, R., Kralert, P. G., Marzi, R., Kagi, R. I. and Evans, E. J. (1991) A geochemical method for assessment of the thermal histories of sediments: a two-well case study from the Gippsland Basin, Australia. *The APEA Journal* **31**, 325-332.
- Alexander, R., Bastow, T. P., Kagi, R. I. and Singh, R. K. (1992a) Identification of 1,2,2,5-tetramethyltetralins and 1,2,2,5,6-pentamethyltetralin as racemates in petroleum. *Journal of the Chemical Society, Chemical Communications* **23**, 1712-1714.
- Alexander, R., Larcher, A. V. and Kagi, R. I. (1992b) An oil-source correlation study using age specific plant-derived aromatic biomarkers. In *Biological Markers in sediments and Petroleum*, eds. J. M. Moldowan, P. Albrecht P. and R. P. Philp, pp. 201-221. Prentice Hall, Englewood Cliffs, N. J., P.

- Alexander R., Bastow T. P., Fisher S. J. and Kagi R. I. (1993)
Tetramethylnaphthalenes in crude oils. *Journal of polycyclic aromatic compounds* **3**, 629-634.
- Alexander, R., Kagi, R. I., Singh, R. K. and Sosrowidjojo, I. B. (1994) The effect of maturity on the relative abundances of cadalene and isocadalene in sediments from the Gippsland Basin, Australia. *Organic Geochemistry* **21**, 115-120.
- Alexander, R., Bastow, T. P., Fisher, S. J. and Kagi, R. I. (1995)
Geosynthesis of organic compounds: II. Methylation of phenanthrene and alkylphenanthrenes. *Geochimica et Cosmochimica Acta* **59**, 4259-4266.
- Alexander, R., Kralert, P.G., Sosrowidjojo, I.B. and Kagi, R.I. (1997) Kinetics and mechanism of the thermal elimination of alkenes from secondary stanyl and triterpenyl esters - Implications for sedimentary processes. *Organic Geochemistry* **26**, 391-398.
- Anders, D. E., Doolittle, F. G. and Robinson, W. E. (1973) Analysis of some aromatic hydrocarbons in a benzene soluble bitumen from Green River Shale. *Geochimica et Cosmochimica Acta* **37**, 1213-1228.
- Angelin, M. L., Collignan, A., Bellocq, J. and Ewald, M. (1983) Empreinte par degré d'aromaticité des hydrocarbures aromatiques polycycliques extraits de la matière organique d'une roche mère Pétrole obtenue par chromatographie liquide haute performance. *Comptes Rendus de l'Académie des Sciences de Paris* **296**, 705-708.
- Araki, M. and Mukaiyama, T. (1974) Reaction of mixed carboxylic anhydrides with Grignard reagents. A useful method for the preparation of ketones. *Chemistry Letters* **7**, 663-666.

- Armstrong, D.W., Tang, Y. and Zukowski, J. (1991) Resolution of enantiomeric hydrocarbon biomarkers of geochemical importance. *Analytical Chemistry* **63**, 2858-2861.
- Audino, M., Alexander R. and Kagi R. I. (1996) Ethylmethylnaphthalenes in crude oils. *Polycyclic Aromatic Compounds* **8**, 93-103.
- Augustine, R. L. (1965) *Catalytic Hydrogenation: Techniques and applications in organic synthesis*. Marcel Dekker inc., New York, 135 pp.
- Barwise, A. J. G. and Park, P. J. D. (1983) Petroporphyrin fingerprinting as a geochemical parameter. In *Advances in Organic geochemistry 1981*, eds. Bjørøy *et al.*, pp. 181-192. J. Wiley and Sons, New York.
- Baset, Z. H., Pancirov, R. J. and Ashe, T. R. (1980) Organic compounds in coal; structure and origins. In *Advances in Organic Geochemistry 1979*, eds. A. G. Douglas and J. R. Maxwell, pp. 619-630. Pergamon, Oxford.
- Bastow, T. P., Singh, R. K., Ellis, L., Alexander, R. and Kagi, R. I. (1993) Separation of petroleum hydrocarbon racemates using capillary gas chromatography. In *Organic Geochemistry-Poster sessions from the 16th International Meeting on Organic Geochemistry, Stavanger, 1993* eds K. Øygard, pp. 754-757. Falch Hurtigtrykk, Oslo.
- Bastow, T. P., Alexander, R. and Kagi, R. I. (1996) Geosynthesis of organic compounds IV. Methylation of 1,2,7-trimethylnaphthalene. *Polycyclic Aromatic Compounds* **9**, 177-183.
- Bastow, T. P., Alexander, R. and Kagi, R. I. (1997) Identification and analysis of dihydro-*ar*-curcumene enantiomers and related compounds in petroleum. *Organic Geochemistry* **26**, 79-83.

- Bastow, T. P., Alexander, R., Fisher, S. J., Singh, R. K. and Kagi, R. I., (1998a) Geosynthesis of organic compounds: V- Methylation of alkylnaphthalenes. *Geochimica et. Cosmochimica Acta* (in press).
- Bastow, T. P., Alexander, R., Sosrowidjojo, I. B. and Kagi, R. I., (1998b) Pentamethylnaphthalenes and related compounds in sedimentary organic matter. *Organic Geochemistry* **28**, 585-596.
- Bendoraitis, J. G. (1974) Hydrocarbons of biogenic origin in petroleum-aromatic triterpenes and bicyclic sesquiterpenes. In *Advances in Organic Geochemistry 1973*, eds. B. Tissot and F. Bienner, pp. 209-224. Technip, Paris.
- Blanc, P. and Connan, J. (1994) Preservation, degradation and destruction of trapped oil. In *The Petroleum System-from source to trap*, eds. L. B. Magoon and W. G. Dow, pp. 237-247. A.A.P.G., Tulsa, Oklahoma.
- Bordoloi, M., Shukla, V. S., Nath, S. C. and Sharma, R. P. (1989) Naturally occurring cadinenes. *Phytochemistry* **28**, 2007-2037.
- 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. *Organic Geochemistry* **12**, 289-294.
- Borrego, A.G., Blanco, C.G. and Püttmann, W. (1997) Geochemical significance of the aromatic hydrocarbon distribution in the bitumens of the Puertollano oil shales, Spain. *Organic Geochemistry* **26**, 219-228.
- Botto, R.E., Hayatsu, R., Carrado, K.A. and Winans, R.E. (1989) Isotope labelling investigations in simulated coalification: A view of geological metamorphism. *Proceedings, 1989 international conference on coal science* **1**, 1-4.

- Brikke, I. (1982) Geochemical interpretation of some oils and condensates from the Dampier Sub-basin of Western Australia. *The APEA Journal* **22**, 179-187.
- Brooks, P. W., Maxwell, J. R., Cornforth, J. W., Butlin, A. G. and Milne, C. B. (1977) Stereochemical studies of acyclic isoprenoid compounds VI. The stereochemistry of farnesane from crude oil. In *Advances in Organic Geochemistry 1975*, eds. R. Campos and J. Goni, pp 81-97. Pergamon, Oxford.
- Budzinski, H., Radke, M., Garrigues, P., Wise, S. A., Bellocq, J. and Willsch, H. (1992) Gas chromatographic retention behaviour of alkylated phenanthrenes on a smectic liquid crystalline phase- Application to organic geochemistry. *Journal of Chromatography* **627**, 227-239.
- Budzinski, H., Garrigues, P., Connan, J. and Bellocq, J. (1993a) Chromatographic fractionation of aromatic compounds from Ancient and recent sediments for access to alkylated PAH by GC-FID and GC-MS. *Quimica Analitica* **12**, 69-74.
- Budzinski, H., Garrigues, P., Radke, M., Connan, J. and Oudin, J. L. (1993b) Thermodynamic calculations on alkylated phenanthrenes - Geochemical applications to maturity and origin of hydrocarbons. *Organic Geochemistry* **20**, 917-926.
- Budzinski, H., Baumard, P., Rivet, L., Lacotte, D., Mille, G., Acquaviva, M., Bertrand, J. C. and Garrigues, P. (1995a) Aerobic biodegradation of alkylated aromatic hydrocarbons. In *Proceedings from the 17th International Meeting on Organic Geochemistry*, eds. J.O. Grimalt and C. Dorronsoro, pp. 588-590. A.I.G.O.A., San Sebastian, Spain.
- Budzinski, H., Garrigues, P., Connan, J., Devillers, J., Domine, D., Radke, M. and Oudin, J.L. (1995b) Alkylated phenanthrene distributions as maturity and origin indicators in crude oils and rock extracts. *Geochimica et*

- Budzinski, H., Raymond, N., Nadalig, T., Gilewicz, M., Garrigues, P., Bertrand, J. C. and Caumette, P. (1998) Aerobic biodegradation of alkylated aromatic hydrocarbons by a bacterial community. *Organic Geochemistry* **28**, 337-348.
- Burns, B. J., Bostwick, T. R. and Emmett, J. K. (1987) Gippsland terrestrial oils-recognition of compositional variations due to maturity and biodegradation effects. *The APEA Journal* **27**, 73-84.
- Carrado, K. A., Hayatsu, R., Botto, R.E. and Winans, R.E. (1990). Reactivity of anisoles on clay and pillared clay surfaces. *Clays and Clay Minerals* **38**, 250-256.
- Carruthers, W. and Douglas, A. G. (1955) The constituents of high-boiling petroleum distillates. II. Trimethylnaphthalenes in a Trinidad oil. *Journal of the Chemical Society* **49**, 1847-1850.
- Carruthers, W. and Douglas, A. G. (1957) The constituents of high-boiling petroleum distillates. IV. Some polycyclic aromatic hydrocarbons in a Trinidad oil. *Journal of the Chemical Society* **50**, 278-281.
- Cassani, F. and Eglinton, G. (1991) Organic geochemistry of Venezuelan extra-heavy oils. 2. Molecular assessment of biodegradation. *Chemical Geology* **91**, 315-333.
- Chaffee, A. L. and Johns, R. B. (1983) Polycyclic aromatic hydrocarbons in Australian coals. I. Angularly fused pentacyclic tri- and tetraaromatic components of Victorian brown coal. *Geochimica et Cosmochimica Acta* **47**, 2141-2155

- Chaffee, A. L., Strachan, M. G. and Johns, R. B. (1984) Polycyclic aromatic hydrocarbons in Australian coals. II. Novel tetracyclic components from Victorian brown coal. *Geochimica et Cosmochimica Acta* **48**, 2037-2043.
- Chakhmakhchev, A. and Suzuki, N. (1995) Saturated biomarkers and aromatic sulfur compounds in oils and condensates from different source rock lithologies of Kagakhan, Japan and Russia. *Organic Geochemistry* **23**, 289-299.
- Chang, T. C. L. and Karr, Jr., C. (1962) Gas-liquid chromatographic analysis of C₁₀-C₁₆ normal paraffins, isoparaffins, and α -olefins in a low temperature coal tar. *Analitica Chimica Acta* **24**, 410-418.
- Chen, T. S., Canonne, P. and Leitch, L.C. (1973) Synthesis of 1,2,3-trimethyl-, 1,2,3,7-tetramethyl-, and 1,2,3,5,6,7-hexamethylnaphthalenes. *Synthesis* **10**, 620-621.
- Chosson, P., Connan, J., Dessort, D. and Lanau, C. (1992) In *vitro* biodegradation of steranes and terpanes: A clue to understanding geological situations. In *Biological Markers in Sediments and Petroleum*. eds. J. M. Moldowan, P. Albrecht, and R. P. Philp, pp. 320-349, Prentice Hall, Englewood Cliffs, N. J.
- Clegg, H., Horsfield, B., Stasiuk, L., Fowler, M. and Vliex, M. (1997) Geochemical characterisation of organic matter in Keg River formation (Elk point group, middle Devonian), La Crete basin, Western Canada. *Organic Geochemistry* **26**, 627-643.
- Connan, J. (1984) Biodegradation of crude oils in reservoirs. In *Advances in Petroleum Geochemistry*, eds. J. Brooks and D.H. Welte, pp. 299-335. Academic Press, London.

- Connan, J., Bouroullec, J., Dessort, D. and Albrecht, P. (1986) The microbial input in carbonate-anhydrite facies of a sabkha palaeoenvironment from Guatemala: A molecular approach. *Organic Geochemistry* **10**, 224-236.
- Crank, K. (1973) Geology of Barrow Island oil Field. *The APEA Journal* **13**, 49-57.
- Dajiang, Z., Difan, H. and Jinchao, L. (1988) Biodegraded sequence of Karamay oils semi-quantitative estimation of their biodegraded degrees in Junggar Basin, China. *Organic Geochemistry* **13**, 1-3.
- Derbyshire, F.J. and Whitehurst, D.D. (1981) Study of coal conversion in polycondensed aromatic compounds. *Fuel* **60**, 655-662.
- Deroo, G., Tissot, B., McCrossan, R. G. and Per, F. (1974) Geochemistry of the heavy oils of Alberta. In *Oil Sands-Fuel of the Future*, eds. L. V. Hills, pp. 148-167. Memoir 3, Canadian Society of Petroleum Geologists.
- Dev, S. (1989) Terpenoids. In *Natural Products of Woody Plants II*, eds. J. W. Rowe, pp. 691-807. Springer-Verlag, Berlin.
- Dong, J., Vorkink, W. P. and Lee, M. L. (1993) Origin of long-chain alkylcyclohexanes and alkylbenzenes in a coal-bed wax. *Geochimica et Cosmochimica Acta* **57**, 837-849.
- Douglas, A. G., Sinninghe Damsté, J. S., Fowler, M. G., Eglinton, T. I. and de Leeuw, J. W. (1991) Unique distributions of hydrocarbons and sulphur compounds released by flash pyrolysis from the fossilized alga *Gloecapsomorpha prisca*, a major constituent in one of four Ordovician kerogens. *Geochimica et Cosmochimica Acta* **55**, 275-291.
- van Duin, A. C. T., Baas, J. M. A., van Degraaf, B., de Leeuw, J. W., Bastow, T. P. and Alexander, R. (1997) Comparison of calculated equilibrium mixtures of alkylnaphthalenes and alkylphenanthrenes with experimental

and sedimentary data - The importance of entropy calculations. *Organic Geochemistry* **26**, 275-280.

Duswalt, T. J. and Mayer, T. J. (1971) Characterisation of dinuclear aromatics in the 275 to 295 °C fraction of catalytic gas oil. *Preprints, Division of Petroleum Chemistry, ASC* **16**, A17-A35.

Edwards, Jr., W. R. and Kammanann, Jr., K. P. (1964) Mixed carboxylic anhydrides in the grignard reaction. I. Synthesis of aldehydes from formic acetic anhydride. *Journal of Organic Chemistry* **29**, 913-915.

Ellis, L., Kagi, R. I. and Alexander, R. (1992) Separation of petroleum hydrocarbons using dealuminated mordenite molecular sieve. I. Monoaromatic hydrocarbons. *Organic Geochemistry* **18**, 587-593.

Ellis, L. (1994) Aromatic hydrocarbons in crude oil and sediments: Molecular sieve separations and biomarkers. Ph. D. Thesis, Curtin University of Technology.

Ellis, L., Alexander, R. and Kagi, R.I. (1994) Separation of petroleum hydrocarbons using dealuminated mordenite molecular sieve. II Alkyl-naphthalenes and alkylphenanthrenes. *Organic Geochemistry* **21**, 849-855.

Ellis, L., Alexander, R., Singh, R.K. and Kagi, R.I. (1995a) Occurrence of dihydro-*ar*-curcumene in sediments and crude oil. *Organic Geochemistry* **23**, 197-203.

Ellis, L., Singh, R. K., Alexander, R. and Kagi, R. I. (1995b) Geosynthesis of organic compounds: III. Formation of alkyltoluenes and alkylxylenes in sediments. *Geochimica et Cosmochimica Acta* **59**, 5133-5140.

- Ellis, L., Singh, R. K., Alexander, R. and Kagi, R. I. (1995c) Formation of long-chain alkylnaphthalenes in sediments via alkylstyrene precursors. In *Proceedings from the 17th International Meeting on Organic Geochemistry*, eds. J. O. Grimalt and C. Dorronsoro, pp. 588-590. A.I.G.O.A., San Sebastian, Spain.
- Ellis, L., Langworthy, T.A. and Winans, R. (1996a) Occurrence of phenylalkanes in some Australian crude oils and sediments. *Organic Geochemistry* **24**, 57-69.
- Ellis, L., Singh, R. K., Alexander, R. and Kagi, R. I. (1996b) Formation of isoheptyl alkylaromatic hydrocarbons from aromatization-rearrangement of terpenoids in the sedimentary environment: A new class of biomarker. *Geochimica et Cosmochimica Acta* **60**, 4747-4763.
- Erdman, J. G. (1961) Some chemical aspects of petroleum genesis as related to the problem of source bed recognition. *Geochimica et Cosmochimica Acta* **22**, 16-36.
- Fenske, M. R., Carnahan, F. L., Breston, J. N., Caser, A. H. and Rescorla, A. R. (1942) Optical rotation of petroleum fractions. *Industrial and Engineering Chemistry* **34**, 638-646.
- Fischer, P., Aichholz, R., Boelz, S., Juza, M. and Krimmer, S. (1990) Chiral recognition in capillary gas chromatography. 3. Polysiloxane-bound permethyl- β -cyclodextrin – a chiral stationary phase with broad application in gas-chromatographic enantiomer separation. *Angewandte Chemie International Edition in English* **29**, 427.
- Fisher, S. J., Alexander, R. and Kagi, R. I. (1996a) Biodegradation of alkylnaphthalenes in sediment adjacent to an off-shore petroleum production platform. *Polycyclic Aromatic Compounds* **11**, 35-42.

- Fisher, S. J., Alexander, R., Ellis, L. and Kagi, R. I. (1996b) The analysis of alkylphenanthrenes by direct deposition gas chromatography-Fourier transform infrared spectroscopy (GC-FTIR). *Polycyclic Aromatic Compounds* **9**, 257-264.
- Fisher, S. J., Alexander, R., Kagi, R. I. and Oliver, G. A. (1998) Aromatic hydrocarbons as indicators of petroleum biodegradation in North Western Australian reservoirs. In *The Sedimentary Basins of western Australia 2, Proceedings Western Australian Petroleum Symposium Perth, Western Australia*, eds. P.G. and R.R. Purcell, pp. 185-194. Frank Daniels Pty Ltd.
- Forster, P. G. Alexander, R. and Kagi, R. I. (1989a) Identification of 2,2,7,8-tetramethyl-1,2,3,4-tetrahydronaphthalene in petroleum. *Journal of the Chemical Society. Chemical Communications* **5**, 274-276.
- Forster, P. G., Alexander, R. and Kagi, R. I. (1989b) Identification and analysis of tetramethylnaphthalenes in petroleum. *Journal of Chromatography* **483**, 384-389.
- Fraga, B. M. (1993) Natural Sesquiterpenoids. *Natural Products Reports* **10**, 397-419.
- Frank, H., Nicholson, G. J. and Bayer, E. (1977) Rapid gas chromatographic separations of amino acid enantiomers with a novel chiral stationary phase. *Journal of Chromatographic Science* **15**, 174-176.
- Furniss, B., Hannford, A. J., Smith, P. W. G. and Tatchell, A. R. (1989) In *Vogel's Textbook of Practical Organic Chemistry*, 5th edn, pp. 538 Longman Group UK Limited, U.K.

- Gallegos, E. J. (1971) Identification of new steranes, terpanes and branched paraffins in Green River shale by combined capillary gas chromatography and mass spectrometry. *Analytical Chemistry*, 1151-1160.
- Garrigues, P., and Ewald, M. (1983a) Identification of monomethylated polycyclic aromatic hydrocarbons in crude oils by liquid chromatography and high-resolution Shpol'skii effect fluorescence spectrometry. *Analytical Chemistry* **55**, 2155-2159.
- Garrigues, P. and Ewald, M. (1983b) Natural occurrence of 4-methylphenanthrene in petroleums and recent marine sediments. *Organic Geochemistry* **5**, 54-56.
- Garrigues, P., Sury, R. de, Angelin, M. I., Oudin, J. L., Ewald, M. and Connan, J. (1984) Analysis of a series of aromatic isomers by high resolution spectrofluorimetry and capillary gas chromatography in HPLC fractions of crude petroleums and sedimentary rock extracts. *Organic Geochemistry* **6**, 829-837.
- Garrigues, P., Parlanti, E., Radke, M., Bellocq, J., Willsch, H. and Ewald, M. (1987) Identification of alkylphenanthrenes in shale oil and coal by liquid and capillary gas chromatography and high-resolution spectrofluorimetry (Shpol'skii effect). *Journal of Chromatography* **395**, 217-228.
- Garrigues, P., Sury, R. de, Angelin, M. I., Bellocq, J., Oudin, J. L. and Ewald, M. (1988) Relation of the methylated aromatic hydrocarbon distribution pattern to maturity of organic matter in ancient sediments from the Mahakam delta. *Geochimica et Cosmochimica Acta* **52**, 375-384.
- Garrigues, P., Radke, M., Druetz, O., Willsch, H. and Bellocq, J. (1989) Reversed-phase liquid chromatographic retention behaviour of dimethylphenanthrene isomers. *Journal of Chromatography* **473**, 207-213.

- Garrigues, P., Oudin, J. L., Parlanti, E., Monin, J. C., Robcis, S. and Bellocq, J. (1990) Alkylated phenanthrene distribution in artificially matured kerogens from Kimmeridge clay and the Brent Formation (North Sea). *Organic Geochemistry* **16**, 167-173.
- Gavât, I. and Irmescu, I. (1942) Aromatische kohlenwasser-stoffe aus rumänischem erdäl, II. Mitteil.: Isolierung von trimethylnaphthalinen und phenanthren. *Berichte de Deutschen Chemischen Gesellscaft* **B75**, 820-826.
- Gontcharov, I. (1993) Long-chain alkylnaphthalenes in crude oils and rock extracts. In *Organic Geochemistry-Poster sessions from the 16th International Meeting on Organic Geochemistry, Stavanger 1993*, eds. K. Øygard, pp. 375-377. Falch Hurtigtrykk, Oslo.
- Gough, M. A. and Rowland, S. J. (1990) Characterisation of unresolved complex mixtures of hydrocarbons in petroleum. *Nature* **344**, 648-650.
- Gough, M. A., Rhead, M. M. and Rowland, S. J. (1992) Biodegradation studies of unresolved complex mixtures of hydrocarbons: model UCM and the aliphatic UCM. *Organic Geochemistry* **18**, 17-22.
- Grantham, P. J., Posthuma, J. and Baak, A. (1983) Triterpanes in a number of Far-Eastern crude oils. In *Advances in Organic Geochemistry 1981*, eds. M. Bjoroy *et al.*, pp. 675-683. John Wiley and Sons, New York.
- Harata, K., Uekama, K., Otagiri, M. and Hirayama, F. (1987) The structures of the cyclodextrin complex. XIX. Crystal structures of hexakis (2,3,6-tri-O-methyl- α -cyclodextrin complexes with (S)- and (R)- mandelic acid. Chiral recognition through the induced-fit conformational change of the macrocyclic ring. *Bulletin of the Chemical Society of Japan* **60**, 497.

- Hartgers, W. A., Sinninghe Damsté, J. S. and de Leeuw, J. W. (1992)
Identification of C₂-C₄ alkylated benzenes in flash pyrolysates of kerogens,
coals and asphaltenes. *Journal of Chromatography* **606**, 211-220.
- Hartgers, W. A., Sinninghe Damsté, J. S. and de Leeuw, J. W. (1994a)
Geochemical significance of alkylbenzene distributions in flash pyrolysates
of kerogens, coals, and asphaltenes. *Geochimica et Cosmochimica Acta*
58, 1759-1775.
- Hartgers, W. A., Sinninghe Damsté, J. S., Requejo, A. G., Allan, J., Hayes, J.
M., Ling, Y., Xie, T. M., Primack, J. and de Leeuw, J. W. (1994b) A
molecular and carbon isotopic study towards the origin and diagenetic fate
of diaromatic carotenoids. *Organic Geochemistry* **22**, 703-725.
- Hatcher, P. G. and Breger, I. A. (1982) Nuclear magnetic resonance studies
of ancient buried wood II. Observations on the origin of coal from lignite to
bituminous coal. *Organic Geochemistry* **4**, 9-18.
- Hayatsu, R., Winans, R. E., Scott, R. G., Moore, R. L. and Studier, M. H.
(1978) Trapped organic compounds and aromatic units in coals. *Fuel* **57**,
541-548.
- He, S. J. X., Long, M. A., Attalla, M. I. and Wilson, M. A. (1992) Methylation
of naphthalene by methane over substituted aluminophosphate molecular
sieves. *Energy and Fuels* **6**, 498-502.
- He, S. J. X., Long, M. A., Attalla, M. I. and Wilson, M. A. (1994) Methylation
of naphthalene by methane-C¹³ over copper-exchanged
silicoaluminophosphate. *Energy and Fuels* **8**, 286-287.
- He, S. J. X., Long, M. A., Wilson, M. A. and Gorbaly, M. L. (1995) Methylation
of benzene by methane-C¹³ over zeolitic catalysts at 400°C. *Energy and
Fuels* **9**, 616-619.

- Heath, D.J., Lewis, C.A. and Rowland, S.J. (1997) The use of high temperature gas chromatography to study the biodegradation of high molecular weight hydrocarbons. *Organic Geochemistry* **26**, 769-785.
- Hegarty, K. A., Duddy, I. A., Green, P. F., Gleadow, A. J. W., Fraser, I. and Weissel, J. K. (1985) Regional evaluation of the tectonic and thermal history of the Gippsland Basin. In *Proceedings of the Second South-eastern Australia Oil Exploration Symposium*, eds. R. C. Glenie, pp. 65-74. Petroleum Exploration Society of Australia, Melbourne.
- Heppenheimer, H., Steffens, K., Püttmann, W. and Kalkreuth, W. (1992) Comparison of resinite-related aromatic biomarker distributions in Cretaceous-Tertiary coals from Canada and Germany. *Organic Geochemistry* **18**, 273-287.
- Hills, I. R., Whitehead, E. V., Anders, D. E., Cummins, J. J. and Robinson, W. E. (1966) An optically active triterpane, gammacerane in Green River, Colorado, oil shale bitumen. *Journal of the Chemical Society, Chemical Communications* **20**, 752-754.
- Hirsch, D. E., Hopkins, R. L., Coleman, H. J., Cotton, F. O. and Thompson, C. J. (1972) Separation of high-boiling petroleum distillates using gradient elution through dual-packed (silica gel-alumina gel) adsorption columns. *Analytical Chemistry* **44**, 915-919.
- Hoefs, M. J. L., Vanheemst, J. D. H., Gelin, F., Koopmans, M. P., Vankaampeters, H. M. E., Schouten, S., de Leeuw, J. W. and Sinninghe Damsté, J.S. (1995) Alternative biological sources for 1,2,3,4-tetramethylbenzene in flash pyrolysates of kerogen. *Organic Geochemistry* **23**, 975-979.
- Hoffmann, C. F. and Strausz, O. P. (1986) Bitumen accumulation in Grosmont Platform Complex, Upper Devonian, Alberta, Canada. *The American Association of Petroleum Geologists Bulletin* **70**, 113-1128.

- Honward, V. K. and Rao, A. S. (1965) Absolute configuration of (-)- α -curcumene. *Tetrahedron* **2**, 2593-2604.
- Hostettler, F. D., Rapp, J. B., Kvenvolden, K. A. and Luoma, S. N. (1989) Organic markers as source discriminants and sediment transport indicators in south San Francisco Bay, California. *Geochimica et Cosmochimica Acta* **53**, 1563-1576.
- Ioppolo-Armanios M., Alexander R. and Kagi R.I. (1995) Geosynthesis of organic compounds. I - Alkylation of sedimentary phenols. *Geochimica et Cosmochimica Acta* **59**, 3017-3027.
- Ishiwatari, R. and Fukushima, K. (1979) Generation of unsaturated and aromatic hydrocarbons by thermal alteration of young kerogen. *Geochimica et Cosmochimica Acta* **43**, 1343-1349.
- Jaffé, R. and Gallardo, M.T. (1993) Application of carboxylic acid biomarkers as indicators of biodegradation and migration of crude oils from the Maracaibo basin, Western Venezuela. *Organic Geochemistry* **20**, 973-984.
- Johnstone, M. H. (1979) A case history of Rough Range, *The APEA Journal* **19**, 1-6.
- Juvancz, Z., Alexander, B. and Bzejtll, S. (1987) Permethylated β -cyclodextrin as stationary phase in capillary gas chromatography. *Journal of High Resolution Chromatography* **10**, 105-107.
- Kagi, R. I., Alexander, R. and Toh, E. (1990) Kinetics and mechanism of the cyclisation reaction of *ortho*-methyl-biphenyls. In *Advances in Organic Geochemistry 1989*, eds. B. Durand and F. Behar, pp. 161-166, Pergamon Press, Oxford.

- Kagramanova, G. R., Pustil'nikova, S. D., Pehk, T.I., Denisov, U. V. and Petrov, A. A. (1976) Sesquiterpenane petroleum hydrocarbons. *Neftekhimiya* **16**, 18-22.
- Kantsler, A. J., Prudence, T. J. C., Cook, A. C. and Zwigulis, M. (1983) Hydrocarbon habitat of the Cooper/Eromanga Basin, Australia. *The APEA Journal* **23**, 75-92.
- Karr, Jr., C. (1960) Chemical thermodynamic equilibriums and free valence indices as applied to low-temperature bituminous coal pyrolyzate. *Journal of Physical Chemistry* **64**, 462-464.
- Keim, W., Köhnes, A. and Meltzow, W. (1991) Enantiomer separation by gas chromatography on cyclodextrin chiral stationary phases. *Journal of High Resolution Chromatography* **14**, 507-529.
- Kelly, W. R., Herman, J. S. and Mills, A. L. (1997) The geochemical effects of benzene, toluene and xylene (BTX) biodegradation. *Applied Geochemistry* **12**, 291-303.
- Killops, S. D. and Al-Juboori, M. A. H. A. (1990) Characterisation of the unresolved complex mixture (UCM) in the gas chromatograms of biodegraded petroleums. *Organic Geochemistry* **15**, 147-160.
- Killops, S. D. (1991) Novel aromatic hydrocarbons of probable bacterial origin in a Jurassic lacustrine sequence. *Organic Geochemistry* **17**, 25-36.
- Klemm, L. H., Shabtai, R. I. and Talyor, D. R. (1968) Alumina-catalyzed reactions of hydroxyarenes and hydroaromatic ketones. *Journal of Organic Chemistry* **33**, 1480-1488.
- Kobor, F., Angermund, K. and Schomburg, G. (1993) Molecular modelling experiments on chiral recognition in GC with specially derivatized

- cyclodextrins as selectors. *Journal of High Resolution Chromatography* **16**, 299-311.
- Kolesnikova, L. P. (1972) Gas chromatography in the study of natural gases, petroleum and condensates. *Nedra*, Moscow, 135.
- König, W. A., Lutz, S., Wenz, G. and von der Bey, E. (1988) Cyclodextrins as chiral stationary phases in capillary gas chromatography-Part II: Heptakis(3-O-acetyl-2,6-di-O-pentyl)- β -cyclodextrin. *Journal of High Resolution Chromatography* **11**, 506-509.
- König, W. A., Krebber, R. and Mischnick, P. (1989a) Cyclodextrins as chiral stationary phases in capillary gas chromatography-Part II: Octakis(3-O-butyryl-2,6-di-O-pentyl)- γ -cyclodextrin. *Journal of High Resolution Chromatography* **12**, 732-738.
- König, W. A., Krebber, R. and Wenz, G. (1989b) Enantoselektive capillary gas chromatography on the basis of host-guest interactions with modified Cyclodextrins. *Journal of High Resolution Chromatography* **12**, 641-644.
- König, W. A., Krebber, R. and Wenz, G. (1989c) Cyclodextrins as chiral stationary phases in capillary gas chromatography-Part VI: Octakis(2,3,6-tri-O-pentyl)- γ -cyclodextrin. *Journal of High Resolution Chromatography* **12**, 790-792.
- König, W. A., Lutz, S., Hagen, M., Krebber, R., Wenz, G., Baldenius, K., Ehlers, J. and Diek, H. (1989d) Cyclodextrins as chiral stationary phases in capillary gas chromatography-Part IV: Heptakis(2,3,6-tri-O-pentyl)- β -cyclodextrin. *Journal of High Resolution Chromatography* **12**, 35-39.
- König, W. A. (1992) *Gas Chromatographic Enantiomeric Separation with Modified Cyclodextrins*. Hüthig, Buch Verlag, Heidelberg.

- König, W. A., and Gehrcke, B. (1993) Gas chromatographic enantiomer separation with modified cyclodextrins: carboxylic acid esters and epoxides. *Journal of High Resolution Chromatography* **16**, 175-181.
- Koopmans, M.P., de Leeuw, J.W. and Sinninghe Damsté, J.S. (1997) Novel cyclised and aromatised diagenetic products of *beta*-carotene in the Green River Shale. *Organic Geochemistry* **26**, 451-466.
- Koscielski, T., Sybilska, D. and Jurczak, J. (1983) Separation of α - and β -pinene into enantiomers in gas-liquid chromatography systems via α -cyclodextrin inclusion complexes. *Journal of Chromatography* **280**, 131-134.
- Kralert, P. G., Alexander, R. and Kagi, R.I. (1995) An investigation of polar constituents in kerogen and coal using pyrolysis-gas chromatography-mass spectrometry with *in situ* methylation. *Organic Geochemistry* **23**, 627-639.
- Kruber, O. and Raeithel, A. (1954) Coal-tar anthracene oil. *Chemische Berichte* **87**, 1469-1478.
- Lafargue, E. and Barker, C. (1988) Effect of water washing on crude oil compositions. *The American Association of Petroleum Geologists Bulletin* **72**, 263-276.
- Lafargue, E. and Le Thiez, P. (1996) Effect of water washing on light ends compositional heterogeneity. *Organic Geochemistry* **24**, 1141-1150.
- Laflamme, R. E. and Hites, R. A. (1978) The global distributions of polycyclic aromatic hydrocarbons in recent sediments. *Geochimica et Cosmochimica Acta* **42**, 289-303.
- Lee, M. L., Vassilaros, D. L., White, C. M. and Novotny, M. (1979) Retention indices for programmed-temperature capillary-column gas

chromatography of polycyclic aromatic hydrocarbons. *Analytical Chemistry* **51**, 768-773.

Lekveishvili, E. G., Melikadze, L. D., Tevdorashvili, M. N. and Kartvelishvili, E. V. (1980) Study of phenanthrene hydrocarbons of petroleum. *Pet. Chem. U.S.S.R.* **19**, 171-177.

Lin, L. H., Michael, G. H., Kovachev, G., Zhu, H., Philp, R. P. and Lewis, C. A. (1989) Biodegradation of tar-sands bitumens from the Ardmore and Anadarko Basins, Carter County, Oklahoma. *Organic Geochemistry* **14**, 511-523.

Lipkowitz, K. B., Coner, B., Peterson, M. A. and Morreale, A. (1997) Enantioselective binding in gas chromatography- A computational study of chiral selection by permethyl-beta-cyclodextrin. *Journal of Physical Organic Chemistry* **10**, 311-322.

Louda, J. W. and Baker, E. W. (1984) Perylene occurrence, alkylation and possible sources in deep-ocean sediments. *Geochimica et Cosmochimica Acta* **48**, 1043-1058.

Louis, M. (1968) Optically active substances and the origin of petroleum. *Revue de l'Institut. Francais Petrole* **23**, 299-314.

Lovibond, R., Suttill, R.J., Skinner, J.E. and Aburas, A.N. (1995). The hydrocarbon potential of the Penola Trough, Otway Basin. *The APEA Journal* **35**, 358-371.

Mackenzie, A. S. and McKenzie, D. (1983) Isomerisation and aromatisation of hydrocarbons in sedimentary basins formed by extension. *Geology Magazine* **120**, 417-470.

- Macko, S. A., Estep, M. L. F., Engel, M. H. and Hare, P. E. (1986) Kinetic fractionation of stable nitrogen isotopes during amino acid transamination. *Geochimica et Cosmochimica Acta* **50**, 2143-2146.
- Macko, S. A., Engel, M. H. Hartley, G., Hatcher, P., Helleur, R., Jackman, P., and Silfer, J. A. (1991) Isotopic compositions of individual carbohydrates as indicators of early diagenesis of organic matter. In *Organic Geochemistry of Hydrocarbon basins*, eds. J. A. Curiale, R. Alexander and P. W. Brooks, pp 147-161. *Chemical Geology* .
- Macko, S. A., Engel, M. H. and Qian, Y. (1994) Early diagenesis and organic matter preservation – a molecular stable carbon isotope perspective. *Chemical Geology* **114**, 365-379.
- Mair, B. J. and Martinez-Pico, J. L. (1962) Composition of the trinuclear aromatic portion of the heavy gas oil and light lubricating distillate. *Proceedings of the American Petroleum Institute* **42**, 173-185.
- Mair, B. J. and Barnewall, J. M. (1964) Composition of mononuclear aromatic material in the light gas oil range, low-refractive index portion, 203° to 305°. *Journal of Chemical Engineering Data* **9**, 282-292.
- Mair, B. J. and Mayer, T. J. (1964) Composition of the dinuclear aromatics, C₁₂ to C₁₄, in the light gas oil fraction of petroleum. *Analytical Chemistry* **36**, 351-362.
- Martin, E. L. (1942) *Organic Reactions*. Vol. 1, Wiley, New York, 155-209 pp.
- Martin, R. L., Winters, J. C. and Williams, J. A. (1963) Composition of crude oils by gas chromatography: Geological significance of hydrocarbon distribution. *Proceedings of the 6th World Petroleum Congress, Frankfurt* **5**, 231-260.

- Marzi, R. and Rullkötter, J. (1992) Qualitative and quantitative evolution and kinetics of biological marker transformations – Laboratory experiments and application to the Michigan Basins. In *Biological Markers in Sediments and Petroleum*, eds. J. M. Moldowan *et al.*, pp. 18-41. Prentice Hall, Englewood Cliffs N.J.
- Maxwell, J. R., Cox, R. E., Eglinton, G., Pillinger, C. T., Ackman. R. G., Hooper, S. N. and Ackman. R. G. (1973) Stereochemical studies of acyclic isoprenoid compounds-II. The role of chlorophyll in the derivation of isoprenoid-type acids in a Lacustrine sediment. *Geochimica et Cosmochimica Acta* , 297-313.
- Mayer, S., Schmalzing, D., Jung, M. and Schleimer, M. (1992) A chiral test mixture for permethylated β -cyclodextrin –polysiloxane gas-liquid chromatography phases: The Schurig test mixture. *LC-GC* **10**, 784-785.
- McMahon, P. B. and Chapelle, F. H. (1991) Microbial production of organic acids in aquitard sediments and its role in aquifers geochemistry. *Nature* **349**, 233-235.
- McKirdy, D. M., Aldridge, A. K. and Ypma, P. J. M. (1983) A geochemical comparison of some crude oils from Pre-ordivician carbonate rocks. In *Advances in Organic Geochemistry 1981*, eds. M. Bjorøy *et al.*, pp. 99-107. J. Wiley and Sons, New York.
- Melikadze, L. D., Lekveishvili, E. G., Tevdorashvili, M. N. and Dznaparidze K.D. (1985) Vydelenie i izuchenie fenantrenovykh uglevodorodov iz vysokokipyashchikh maslyanykh fraktsiye Mirzaanskoye nefti fotokondensatsiye s maleinovym angidridom. (Isolation and study of phenanthrene hydrocarbons from high boiling oil fractions of Mirzaani oil by photocondensation with maleic anhydride). *Soobshcheniya Akademii Nauk Gruzinskoi SSR* **119**, 309-312.

- Mosby, W. L. (1952) The ultraviolet absorption spectra of some polymethylnaphthalenes. *Journal of the American Chemical Society* **74**, 2564-2569.
- Noble, R. A., Alexander, R., Kagi, R. I. and Knox, J. (1986) Identification of some diterpenoid hydrocarbons in petroleum. *Organic Geochemistry* **10**, 825-829.
- Noble, R. A., Wu, C. H. and Atkinson, C. D. (1991) Petroleum generation and migration from Talang Akar coals and shales offshore N.W. Java, Indonesia. *Organic Geochemistry* **17**, 363-374.
- Nowotny, D., Schmalzing, D., Wistuba, D. and Schurig, V. (1989) Extending the scope of enantiomer separation on diluted methylated β -cyclodextrin derivatives by high-resolution gas chromatography. *Journal of High Resolution Chromatography* **12**, 383-393.
- Oakwood, T. S., Shriver, D. S., Fall, H. H., McAleer, W. J. and Wunz, P. R. (1952) Optical activity of petroleum. *Industrial and Engineering Chemistry* **44**, 2568-2570.
- Olah, G. A. and Molnár, A. (1995) *Hydrocarbon Chemistry*, Vol. 4 John Wiley & Sons. Inc. New York, 113 pp.
- Ostroukhov, S. B., Aref'yev, O. A., Makushina, V. M., Zabrodina, M. N. and Petrov, A. A. (1982) Monocyclic aromatic hydrocarbons with isoprenoid chains. *Neftekhimiya* **22**, 723-728.
- Ostroukhov, S. B., Aref'yev, O. A., Pustil'nikova, S. D., Zabrodina, M. N. and Petrov, A. A. (1983) C₁₂-C₃₀ n-alkylbenzenes in crude oils. *Neftekhimiya* **23**, 20-30.

- Otto, A., Walther, H. and Püttmann, W. (1997) Sesqui- and diterpenoid biomarkers preserved in taxodium-rich Oligocene Oxbow Lake clays, Weissensteiner basin, Germany. *Organic Geochemistry* **26**, 105-115.
- Ouchi, K. and Imuta, K. (1963) Analysis of benzene extracts of Yubari coal. II. Analysis by gas chromatography. *Fuel* **42**, 445-456.
- Patience, R. L., Rowland, S. J. and Maxwell, J. R. (1978) The effect of maturation on the configuration of pristane in sediments and petroleum. *Geochimica et Cosmochimica Acta* **42**, 1871-1875.
- Patterson, J. H., Ramsden, A. R., Dale, L. S. and Fardy, J. J. (1986) Geochemistry and mineralogical residences of trace elements in oil shale from Julia Creek Queensland, Australia. *Chemical Geology* **55**, 1-16.
- Peters, K. E. and Moldowan, J. M. (1991) Effects of source, thermal maturity, and biodegradation on the distribution and isomerization of homohopanes in petroleum. *Organic Geochemistry* **17**, 47-61.
- Peters, K. E. and Moldowan, J. M. (1993) *The Biomarker Guide. Interpreting molecular fossils in petroleum and Ancient sediments*. Prentice-Hall Inc., Englewood Cliffs, New Jersey.
- Peters, K. E., Moldowan, J. M., McCaffrey, M. A. and Fago, F. J. (1996) Selective biodegradation of extended hopanes to 25-norhopanes in petroleum reservoirs. Insights from molecular mechanics. *Organic Geochemistry* **24**, 765-783.
- Petrov, A. A. (1984) *Petroleum Hydrocarbons*. Springer-verlag, Nauka, Moscow. 138-164 pp.
- Philippi, G. T. (1977) On the depth, time and mechanism of origin of the heavy to medium-gravity naphthenic crude oils. *Geochimica et Cosmochimica Acta* **41**, 33-52.

- Philp, R. P. (1983) Correlation of crude oils from the San Jorge basin, Argentina. *Geochimica et Cosmochimica Acta* **47**, 267-275.
- Price, P. L., Filatoff, J., Williams, A. J., Pickering, S. A. and Wood, G. R. (1985). Late Palaeozoic and Mesozoic palynostratigraphical units. CSR Oil & Gas Division Palynological Facility Report No. 274/25 1.20 (Queensland Department of Mines Open File Report No 14012)
- Ptitsyna, N. V. (1959) Pitannya Geol. Ta Fiz-Khim. *Zbirnik Disertatsiynikh Prats*, 84-95.
- Püttmann, W. and Villar, H. (1987) Occurrence and geochemical significance of 1,2,5,6-tetramethylnaphthalene. *Geochimica et Cosmochimica Acta* **51**, 3023-3029.
- 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. *Geochimica et Cosmochimica Acta* **46**, 1-10.
- Radke, M., Willsch, H. and Leythaeuser, D. (1982b) Aromatic components of coal: Relation of distribution pattern to rank. *Geochimica et Cosmochimica Acta* **46**, 1831-1848.
- Radke, M. and Welte, D.H. (1983). The Methylphenanthrene Index (MPI): A maturity parameter based on aromatic hydrocarbons. In *Advances in Organic Geochemistry 1981*, eds. M. Bjoroy et al., pp. 504-512. J. Wiley & Sons.
- Radke, M., Leythaeuser, D. and Teichmüller, M. (1984a) Relationship between rank and composition of aromatic hydrocarbons for coals of different origins. *Organic Geochemistry* **6**, 423-30.

- Radke, M., Willsch, H. and Welte, D. H. (1984b) Class separation of aromatic compounds in rock extracts and fossil fuels by liquid chromatography. *Analytical Chemistry* **56**, 2538-46.
- Radke, M., Willsch, H. and Welte, D. H. (1986) Maturity parameters based on aromatic hydrocarbons: Influence of the organic matter type. *Organic Geochemistry* **10**, 51-63.
- Radke, M. (1987) Organic geochemistry of aromatic hydrocarbons. In *Advances in Petroleum Geochemistry*, eds. J. Brooks and D. H. Welte, pp141-207. Academic Press, London.
- Radke, M., Willsch, H. and Welte, D.H. (1988) Applications of aromatic compounds as maturity indicators in source rocks and crude oils. *Marine Petroleum Geology* **5**, 224-236.
- Radke, M., Garrigues, P. and Willsch, H (1990a) Methylated dicyclic and tricyclic aromatic hydrocarbons in crude oils from the Handil field, Indonesia. *Organic Geochemistry* **15**, 17-34.
- Radke, M., Willsch, H. and Teichmüller, M. (1990b) Generation and distribution of aromatic hydrocarbons in coals of low rank. *Organic Geochemistry* **15**, 539-563.
- Radke, M., Budzinski, H., Pierard, C., Willsch, H. and Garrigues, P. (1993). Chromatographic retention behaviour of trimethylphenanthrenes and their identification by GC-FTIR in geological samples. *Journal of polycyclic aromatic compounds* **3**, 467-474.
- Radke, M., Rullkötter, J. and Vriend, S.P. (1994) Distribution of naphthalenes in crude oils from the Java sea - Source and maturation effects. *Geochimica et Cosmochimica Acta* **58**, 3675-3689.

- Radke, M. and Willsch, H. (1994) Extractable alkylthiophenes in Posidonia shale (Toarcian) source rocks - Relationship of yields to petroleum formation and expulsion. *Geochimica et Cosmochimica Acta* **58**, 5223-5244.
- Reed, W. E. (1977) Molecular compositions of weathered petroleum and comparison with its possible source. *Geochimica et Cosmochimica Acta* **41**, 237-247.
- Rossini, F. D. (1960) Hydrocarbons in petroleum. *Journal of Chemical Education* **37**, 554-561.
- Rowland, S. J., Alexander, R. and Kagi, R. I. (1984) Analysis of trimethylnaphthalenes in petroleum by capillary gas chromatography. *Journal of Chromatography* **294**, 407-412.
- Rowland, S.J., Alexander, R., Kagi, R. I. and Jones, D. M. (1986) Microbial degradation of aromatic components of crude oils: A comparison of laboratory and field observations. *Organic Geochemistry* **9**, 153-161.
- Rullkötter, J. and Marzi, R. (1988) Natural and artificial maturation of biological markers in a Toarcian Shale from northern Germany. *Organic Geochemistry* **13**, 639-645.
- Saiz-Jimenez, C. (1995) The origin of alkylbenzenes and thiophenes in pyrolysates of geochemical samples. *Organic Geochemistry* **23**, 81-85.
- Sawatzky, H., George, A. E., Smiley, G. T. and Montgomery, D. S. (1976) Hydrocarbon-type separation of petroleum fractions. *Fuel* **55**, 16-20.
- Schurig, V. and Nowotny, P. (1988) Separation of enantiomers on diluted permethylated β -cyclodextrin by high-resolution gas chromatography. *Journal of Chromatography* **441**, 155-163.

- Schurig, V., Nowotny, P. and Schmaizing, D. (1989) Gas-chromatographic enantiomer separation of unfunctionalised cycloalkanes on permethylated β -cyclodextrins. *Angewandte Chemie International Edition in English* **28**, 736-737.
- Schurig, V., Schmalzing, U., Mühleck, U., Jung, M., Schleimer, M., Mussche, P., Duvekot, C. and Buyten, J. C. (1990) *Journal of High Resolution Chromatography* **13**, 713.
- Schurig V. (1994) Review: Enantiomer separation by gas chromatography on chiral stationary phases. *Journal of Chromatography A* **666**, 111-129.
- Seifert, W. K. and Moldowan, J. M. (1978) Applications of steranes, terpanes and monoaromatics to the maturation, migration and source of crude oils. *Geochimica et Cosmochimica Acta* **42**, 77-95.
- Seifert, W. K. and Moldowan, J. M. (1979) The effect of biodegradation on steranes and terpanes in crude oils. *Geochimica et Cosmochimica Acta* **43**, 111-126.
- Seifert, W. K., Moldowan, J. M. and Demaison, G. J. (1984) Source correlation of biodegraded oils. *Organic Geochemistry* **6**, 633-643.
- Serban, A., Engel, M. H. and Macko, S. A. (1988) The distribution, stereochemistry and stable isotopic composition of amino acid constituents of fossil and modern mollusk shells. *Organic Geochemistry* **13**, 1123-1129.
- Shibaoka, M., Saxby, J. D. and Talyor, G. H. (1978) Hydrocarbon generation in Gippsland Basin, Australia-Comparison with Cooper basin, Australia. *The American Association of Petroleum Geologists Bulletin* **62**, 1151-1158.

- Shitangkoon, A. and Vigh, Gy. (1994) Trichloroacetyl pentyl β -cyclodextrin as a chiral stationary phase for gas chromatography. *Journal of High Resolution Chromatography* **17**, 727-728.
- Silfer, J. A., Engel, M. H. and Macko, S. A. (1992) Kinetic fractionation of stable carbon and nitrogen isotopes during peptide bond hydrolysis: Experimental evidence and geochemical implications. *Chemical Geology* **101**, 211-221.
- Silfer, J. A., Qian, Y., Macko, S. A. and Engel, M. H. (1994) Stable carbon isotope compositions of individual amino acid enantiomers in mollusc shell by Gc C Irms. *Organic Geochemistry* **21**, 603-609.
- Silverman, S. R. (1967) Carbon isotope evidence for the role of lipids in petroleum formation. *Journal of the American Oil Chemical Society* **44**, 691-695.
- Silverman, S. R. (1971) Influence of petroleum origin and transformation on its distribution and redistribution in sedimentary rocks. In *The Proceedings of the eighth World Petroleum Congress*, Applied Science Publications, pp. 47-54.
- Simoneit, B. R. T. (1986) Cyclic terpenoids in the geosphere. In *Biological Markers in the Sedimentary Record*, eds. R. B. Johns, pp. 43-100. Elsevier, Amsterdam.
- Simoneit, B. R. T., Grimalt, J. O., Wang, T. G., Cox, R. E., Hatcher, P. G. and Nissenbaum, A. (1986) Cyclic terpenoids of contemporary resinous plant detritus and of fossil woods, ambers and coals. *Organic Geochemistry* **10**, 877-889.
- Simonsen J. (1952) *The Terpenes*. Vol III. Cambridge University Press.

- Singh, R. K., Alexander, R. and Kagi, R. I. (1994) Identification and occurrence of norcadalenes and related compounds in crude oils and sediments. *Organic Geochemistry* **21**, 249-256.
- Sinninghe Damsté, J. S., Rijkstra, W. I. C., de Leeuw, J. W. and Schenck, P. A. (1988) Origin of organic sulfur compounds and sulfur-containing high molecular weight substances in sediments and immature crude oils. *Organic Geochemistry* **13**, 593-606.
- Sinninghe Damsté, J.S., Rijkstra, W. I. C., Kock-van Dalen, A. C., de Leeuw, J. W. and Schenck, P. A. (1989) Quenching of labile functionalised lipids by inorganic sulphur species: Evidence for the formation of sedimentary organic sulphur compounds at the early stages of diagenesis. *Geochimica et Cosmochimica Acta* **53**, 1343-1355.
- Sinninghe Damsté, J.S., Kock-van Dalen, A. C., Albrecht, P. A. and de Leeuw, J. W. (1991) Identification of long-chain 1,2-di-*n*-alkyl-benzenes in Amposta crude oil from the Tarragona Basin, Spanish Mediterranean: Implications for the origin and fate of alkylbenzenes. *Geochimica et Cosmochimica Acta* **55**, 3677-3683.
- Sinninghe Damsté, J.S., Hartgers, W.A., Baas, M. and de Leeuw, J. W. (1993a) Characterization of high-molecular-weight organic matter in Marls of the salt-IV. Formation of the Mulhouse basin. *Organic Geochemistry* **20**, 1237-1251.
- Sinninghe Damsté, J.S., Keely, B.J., Betts, S.E., Baas, M., Maxwell, J.R. and de Leeuw, J.W. (1993b) Variations in abundances and distributions of isoprenoid chromans and long-chain alkylbenzenes in sediments of the Mulhouse basin - A molecular sedimentary record of palaeosalinity. *Organic Geochemistry* **20**, 1201-1215.

- Smith, H. M. (1967) The hydrocarbon constituents of petroleum and some possible lipid precursors. *The American Oil Chemical Society Journal* **44**, 680-690.
- Smith, J. W., George, S. C. and Batts, B. D. (1994) Pyrolysis of aromatic compounds as a guide to synthetic reactions in sediments. *The APEA Journal* **34**, 231-240.
- Smith, J. W., George, S. C. and Batts, B. D. (1995) The geosynthesis of alkylaromatics. *Organic Geochemistry* **23**, 71-80.
- Sokolov, V. A., Bestuzhev, M. A. and Tikhomolova, T. V. (1972) Chemical composition of crudes and natural gases in connection with their origin. *Nedra, Moscow*. 276.
- Solanas, A. M., Parés, R., Bayona, J. M. and Albaigés, J. (1984) Degradation of aromatic petroleum hydrocarbons by pure microbial cultures. *Chemosphere* **13**, 593-601.
- Solli, H., Larter, S. and Douglas, A. (1979) Analysis of kerogens by pyrolysis-gas chromatography-mass spectrometry using selective ion monitoring III. Long-chain alkylbenzenes. In *Advances in Organic Geochemistry: physics and chemistry of the earth*, eds. pp. 591-597, Pergamon, Newcastle.
- Sosrowidjojo, I. B., Murray, A. P., Alexander, R., Kagi, R. I. and Summons R. E. (1996) Bicinanes and related compounds as maturity indicators for oils and sediments. *Organic Geochemistry* **24**, 43-55.
- Stainforth, J. G. (1984) The Gippsland hydrocarbons-a perspective from the basin edge. *The APEA Journal* **24**, 91-100.

- Stoev, G. (1992) Applications and chiral recognition of heptakis (2,6-di-O-methyl-3-O-trifluoroacetyl)- β -cyclodextrin. *Journal of Chromatography* **589**, 257-263.
- Strachan, M. G., Alexander, R. and Kagi, R. I. (1988) Trimethylnaphthalenes in crude oils and sediments: Effects of source and maturity. *Geochimica et Cosmochimica Acta* **52**, 1255-1264.
- Strong, D. and Filby, R. H. (1987) Vanadyl porphyrin distribution in the alberta oil-sand bitumens. In *Metal Complexes in Fossil Fuels*, eds. R. H. Filby and J. F. Branthaver, pp 154-172. American Chemical Society Symposium Series 344, Washington D. C.
- Summons, R. E. and Powell, T. G. (1987) Identification of arylisoprenoids in a source rock and crude oils: Biological markers for the green sulfur bacteria. *Geochimica et Cosmochimica Acta* **51**, 557-566.
- Snyder, H. R. and Werber, F. X. (1955) *Organic Synthesis*. eds. E. C. Horning, Wiley, New York.
- Tang Y., Zhou Y. and Armstrong D. W. (1994) Examination of the enantioselectivity of wall-immobilized cyclodextrin copolymers in capillary gas chromatography. *Journal of Chromatography A* **666**, 147-159.
- Tegelaar, E. W. de Leeuw, J. W. Derenne, S. and Largeau, C. (1989) A reappraisal of kerogen formation. *Geochimica et Cosmochimica Acta* **53**, 3103-3106.
- Thompson, B. R. (1985) The Gippsland basin-development and stratigraphy. In *Second South-Eastern Australian Oil Exploration Symposium*, eds. R.C. Glenie, pp. 57-64. Australian Exploration Society of Australia, Melbourne.
- Tissot, B. P. and Welte, D. H. (1984) *Petroleum formation and occurrence*, 2nd edn. Springer, Berlin.

- Vetter, W. and Schurig, V. (1997) Enantioselective determination of chiral organochlorine compounds in biota by gas chromatography on modified cyclodextrins. *Journal of Chromatography A* **774**, 143-175.
- 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. *Geochimica et Cosmochimica 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. *Geochimica et Cosmochimica Acta* **47**, 785-794.
- Volkman, J. K., Alexander, R., Kagi, R. I., Rowland, S. J. and Sheppard, P. N. (1984) Biodegradation of aromatic hydrocarbons in crude oils from the Barrow Sub-basin of Western Australia. *Organic Geochemistry* **6**, 619-632.
- Wang, T. G. and Simoneit, B. R. T. (1990) Organic geochemistry and coal petrology of Tertiary brown coal in the Zhoujing mine, Baise basin, South China. 2. Biomarker assemblage and significance. *Fuels* **69**, 12-20.
- Wang, T. G., Simoneit, B. R. T., Philp, R. P. and Yu, C. P. (1990) Extended 8 β (H)-drimane and 8,14-secohopane series in a Chinese Boghead coal. *Energy and Fuels* **4**, 177-183.
- Wakeman, S. G., Schaffner, C. and Giger, W. (1980) Polycyclic aromatic hydrocarbons in Recent lake sediments-II. Compounds derived from biogenic precursors during early diagenesis. *Geochimica et Cosmochimica Acta* **44**, 415-429.
- Wardroper, A. M. K., Hoffmann, C. F., Maxwell, J. R., Barwise, A. J. G., Goodwin, N. S. and Park, P. J. D. (1984) Crude oil biodegradation under

- simulated and natural conditions-II. Aromatic steroid hydrocarbons. *Organic Geochemistry* **6**, 605-617.
- Wenz, G., Mischnick, P., Krebber, R., Richters, M. and König, W. A. (1990) Preparation and characterisation of per-O-pentylated cyclodextrins. *Journal of High Resolution Chromatography* **13**, 724-727.
- Whitehead, E. V. (1971) Chemical clues to petroleum origin. *Chemistry and Industry* **27**, 1116-1118.
- Williams, J. A. (1974) Characterisation of oil types in Williston Basin. *The American Association of Petroleum Geologists Bulletin* **58**, 1243-1252.
- Williams, J. A., Dolcater, D. L., Torkelson, B. E. and Winters, J. C. (1988) Anomalous concentrations of specific alkylaromatic and alkylcycloparaffin components in West Texas and Michigan crude oils. *Organic Geochemistry* **13**, 47-59.
- Winters, J. C. and Williams, J. A. (1969) Microbial alteration of crude oil in the reservoir. *American Chemical Society, Division of Petroleum Chemistry, New York* **14**, E22-E31.
- Wood, C.S. and Mallory, F.B. (1964). Photochemistry of stilbenes. IV. The preparation of substituted phenanthrenes. *Journal of Organic Chemistry* **29**, 3373-3377.
- Yawanarajah, S. R. and Kruger, M. A. (1994) Lacustrine shales and oil shales from Stellarton-basin, Nova-Scotia, Canada - Organofacies variations and use of polyaromatic hydrocarbons as maturity indicators. *Organic Geochemistry* **21**, 153-170.
- Yew, F. F., Kurland, R. J. and Mair, B. J. (1964) Chemical shifts of methyl protons in polymethylnaphthalenes. *Analytical Chemistry* **36**, 843-845.

Yew, F. F. and Mair, B. J. (1966) Isolation and identification of C₁₃ to C₁₇ alkylnaphthalenes, alkylbiphenyls and alkylidibenzofurans from the 275° to 305°C. Dinuclear aromatic fraction of petroleum. *Analytical Chemistry* **38**, 231-237.

List of journal publications

- Alexander, R., **Bastow, T. P.**, Kagi, R. I. and Singh, R. K. (1992)
Identification and separation of racemic hydrocarbons in crude oils.
Journal of the Chemical Society, Chemical Communications **23**, 1712-1714.
- Alexander, R., **Bastow, T. P.**, Fisher, S. J. and Kagi, R. I. (1993)
Tetramethylnaphthalenes in crude oils. *Journal of Polycyclic Aromatic Compounds* **3**, 629-634.
- Alexander, R., **Bastow, T. P.**, Fisher, S. J. and Kagi, R. I. (1995)
Geosynthesis of organic compounds. II - Methylation of phenanthrene and alkylphenanthrenes. *Geochimica et Cosmochimica Acta* **59**, 4259-4266.
- Bastow, T. P.**, Singh, R. K., Alexander, R., Kagi, R. I. and Ellis, L. (1993)
Separation of petroleum hydrocarbon racemates using capillary gas chromatography. In *Organic Geochemistry, Poster sessions from the 16th international meeting on Organic Geochemistry, Stavanger, 1993*. eds. K. Øygard, pp. 754-757. Norway.
- Bastow, T. P.**, Alexander, R. and Kagi, R. I. (1997) Identification and analysis of dihydro-*ar*-curcumene enantiomers and related compounds in petroleum *Organic Geochemistry* **26**, 79-83.
- Bastow, T. P.**, Alexander, R. and Kagi, R. I. (1996) Geosynthesis of organic compounds IV. Methylation of 1,2,7-trimethylnaphthalene. *Journal of Polycyclic Aromatic Compounds* **9**, 177-183.

Bastow, T. P., Durnie, W.H., Jefferson, A. and Pang, J. (1997) Ultraviolet spectroscopy for the analysis of oil-in-water effluent using isopropanol as co-solvent. *Applied Spectroscopy* **51**, 318-322.

Bastow, T. P., Alexander, R., Kagi R. I. and Sosrowidjojo, I. B. (1998) The effect of maturity and biodegradation on the enantiomeric composition of sedimentary dihydro-*ar*-curcumene. *Organic Geochemistry*. (In press).

Bastow, T. P., Alexander, R., Kagi R. I. and Sosrowidjojo, I. B. (1998) Pentamethylnaphthalenes and related compounds in sedimentary organic matter. *Organic Geochemistry* **28**, 585-596.

Bastow, T. P., Alexander R., van Aarssen, B.G.K. and Kagi, R. I. (1998) The biodegradation of aromatic land-plant biomarkers. *Organic Geochemistry*. (to be submitted).

van Duin, A.C.T., Baas, J.M.A., van Degraaf, B., de Leeuw, J.W., **Bastow, T.P.** and Alexander, R. (1997) Comparison of calculated equilibrium mixtures of alkylnaphthalenes and alkylphenanthrenes with experimental and sedimentary data – The importance of entropy calculations. *Organic Geochemistry* **26**, 275-280.

List of conference presentations

- Alexander, R., **Bastow T.P.**, Fisher S.J. and Kagi R.I. (1991)
Tetramethylnaphthalenes in crude oils. 13th International Symposium on Polynuclear Aromatic Hydrocarbons. Bordeaux, France.
- Bastow, T. P.**, Singh, R. K., Alexander, R., Kagi R.I. and Ellis, L. (1993)
Separation of petroleum hydrocarbon racemates using capillary gas chromatography. European Association of Organic Geochemistry. Stavanger, Norway.
- Bastow, T.P.**, Singh, R.K., Alexander, R., Kagi, R.I., and Ellis, L. (1993)
Separation of petroleum hydrocarbon racemates using capillary gas chromatography. 12th Analytical Conference/3rd Environmental Conference. Perth, Western Australia.
- Bastow, T. P.**, Alexander, R., Fisher, S. J. and Kagi, R. I. (1994)
Geosynthesis of organic compounds. II - Methylation of phenanthrene and alkyphenanthrenes. Australian Organic Geochemists Conference. Sydney, N.S.W.
- Bastow, T. P.**, Alexander, R., and Kagi, R. I. (1995)
Pentamethylnaphthalenes and related compounds in sedimentary organic matter. Australian Organic Geochemists Conference. Adelaide, S.A.
- Bastow, T. P.**, Alexander, R. and Kagi, R.I. (1995) Dihydro-*ar*-curcumene in sediments and crude oil. Australian Organic Geochemists Conference. Adelaide, S.A.
- Alexander, R., **Bastow, T. P.** and Kagi, R. I. (1995) Dihydro-*ar*-curcumene enantiomers in sediments and crude oils. 17th International Meeting on Organic Geochemistry. Donostia-San Sebastian, Spain.

Alexander, R., **Bastow, T. P.** and Kagi, R.I. (1995) Geosynthesis of organic compounds IV. Methylation of 1,2,7-trimethylnaphthalene. 13th International Symposium on Polynuclear Aromatic Hydrocarbons. Italy.

Bastow, T. P., Alexander, R. and Kagi, R. I. (1996) Geosynthesis of alkylnaphthalenes. Australian Organic Geochemists Conference. Perth, W.A.

Bastow, T. P., Alexander, R., van Aarssen, B.G.K. and Kagi, R.I. (1997) The biodegradation of aromatic land-plant biomarkers. Production geochemistry at the 5th chemical congress of North America. Cancún, Mexico.