School of Applied Chemistry

# STUDIES OF THE SATURATE AND AROMATIC HYDROCARBON

# UNRESOLVED COMPLEX MIXTURES IN PETROLEUM

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That which doesn't kill me, makes me stronger

Friedrich Nietzsche

# ABSTRACT

This thesis reports the results of investigations carried out into the composition of the saturate and aromatic unresolved complex mixtures (UCMs) in crude oils. It is divided into two sections. Section A reports on studies of the saturate UCM and Section B reports on studies of the aromatic UCM. UCMs are mixtures of very large numbers of compounds of low individual abundances, hence structural information on individual components is difficult or impossible to obtain using conventional GC or GC-MS techniques. The investigations reported in this thesis used a combination of GC-MS techniques (Section A) and oxidations of UCMs followed by GC-MS characterisation of the oxidation products (Section B) to develop a more detailed picture of the structures of components of the saturate and aromatic UCMs. UCMs are present in all crude oils, and may account for the vast majority of the material present in heavily weathered or biodegraded oils. An understanding of the types of compounds present may have a bearing on the refining processes an oil is subjected to, as well as assessing its potential environmental and toxicological effects.

Single branched $C_{18}$ isomers were prepared to establish the chromatographic behaviour and mass spectral fragmentation patterns of open chain compounds with ethyl-, propyl-, butyl- and pentyl- substituents. All open chain structural isomers with a single $n$-alkyl branch larger than methyl in the range $C_{10}$ to $C_{20}$ were identified at each carbon number in a series of crude oils of varying ages, source types and depositional environments. Also, $C_{21}$ to $C_{25}$ structural isomers containing an ethyl branch were identified in all of these samples. This represents a total of 163 compounds. These monoalkylalkanes comprise approximately 3 % of the alkanes in these oils, with the $n$-alkanes (35-60 %) and methylalkanes (10 %) being the most abundant compound classes present. Isoprenoids, alkylcyclohexanes and other branched and/or cyclic alkanes make up the remainder of the material.

Rock samples from a sedimentary sequence of Late Cretaceous age were analysed for ethylalkanes using GC-MS techniques. In the less mature samples, 3- and 5-ethylalkanes were in higher abundance relative to the other isomers at odd carbon numbers from $C_{17}$ to $C_{23}$. In the more mature samples, this odd preference was no longer apparent. Several other low maturity sediment samples were analysed and found to have a similar ethylalkane distribution to the shallow sample from the sedimentary sequence. A mature crude oil which was also analysed exhibited a distribution similar to the deeper sample from the sedimentary sequence. Tetralin pyrolysis of a low maturity lignite sample yielded only the 3- and 5-substituted ethylalkanes, providing supporting evidence that the initial odd preference is the result of defunctionalisation of specific natural product precursors containing a 3- or 5-ethylalkyl structural moiety. With increasing maturity this preference is diluted by input of ethylalkanes without preference. A mechanism involving acid-catalysed rearrangement of *n*-alkenes to form monoalkylalkanes is proposed to account for the loss of preference in ethylalkane relative abundances.

Investigations into the composition of aromatic unresolved complex mixtures were conducted by oxidising the total aromatic fraction of a moderately biodegraded crude oil (biodegradation level 4) using potassium permanganate. This reagent cleaves the alkyl substituents attached to aromatic rings between the $\alpha$ and $\beta$ carbons and oxidises the $\alpha$ carbon to a carboxylate group. A biodegraded crude oil was chosen because the majority of the resolved components have been removed by biodegradation, leaving a complex mixture of componds almost completely unresolved by gas chromatography. The oxidation product was separated into dichloromethane-soluble monocarboxylic acids (both aliphatic and aromatic) and water-soluble polycarboxylic acids (aromatic only). GC-MS analysis of these oxidation products gave the proportions of monosubstituted:disubstituted:trisubstituted: tetrasubstituted monoaromatic rings as 29:59:12:0.1, of which from one to three substituents were carboxylic acid groups with the remainder of the substituents being unoxidised methyl groups. Of the disubstituted monoaromatic oxidation products, 53 % were dicarboxylic acids

with the most sterically hindered 1,2-disubstitution pattern. This observation was interpreted as evidence for the presence of significant amounts of naphthenoaromatic systems such as tetralins and indanes in the crude oil aromatic fraction. Analysis of the permanganate oxidation products also enabled a quantitative measure of the proportion of methyl substituents to be made. It was found that methyl groups accounted for a significant proportion of the alkyl substituents attached to aromatic systems. Of the disubstituted monoaromatic oxidation products, 59 % had a methyl group as one of the substituents, while of trisubstituted monoaromatic systems, 41 % had one methyl and 37 % had two methyls. Compounds containing a biphenyl carbon skeleton comprised 3 % of the aromatic oxidation products, with isomers containing from one to four substituents of which one was a carboxylic acid group and the remainder were unoxidised methyls. This indicates that biphenyls containing more than one alkyl ($> C_1$) substituent were not present. Of the monosubstituted biphenylcarboxylic acids, the ratio of ortho:meta:para substituted isomers was 0:65:35, which correlates well with literature reports of the relative abundances of methylbiphenyl isomers, and suggests that the overall distribution of all monosubstituted biphenyls has not been significantly affected by biodegradation to level 4.

The monoaromatic, diaromatic and triaromatic fractions of the same biodegraded crude oil (level 4) were separately treated with ruthenium tetroxide, which cleaves aromatic rings so that the ring carbon bearing the substituent is oxidised to become the carbonyl carbon of a carboxylic acid. These oxidation products represent the alkyl moieties that were attached to aromatic rings in the initial crude oil aromatic fractions. Identification of these alkyl side chains provides an insight into the nature of the components of the aromatic crude oil UCM. The oxidation products were separated into dichloromethane-soluble monocarboxylic acids, which were subsequently reduced to monodeuterated hydrocarbons for characterisation using gas chromatography-mass spectrometry (GC-MS) techniques, and water-soluble dicarboxylic acids which were analysed as dimethyl esters. n-Alkanes, methylalkanes, alkylalkanes, alkylcyclohexanes, methylalkylcyclohexanes, isoprenoids and bicyclic alkanes were identified in the

monodeuterated hydrocarbon samples derived from all three aromatic fractions. Most of these compounds had carbon skeletons strikingly similar to those observed in the saturate fractions of unbiodegraded crude oils, with the only differences being the addition of a carbon from the aromatic ring, and the presence of a deuterium atom attached to that carbon. Because the electron-withdrawing nature of carboxylic acid groups prevents further aromatic ring oxidation, numerous aromatic monocarboxylic acids were also identified in the acidic products of the oxidation of the crude oil diaromatic and triaromatic fractions. These included $C_1$ to $C_3$ alkyl-substituted benzoic acids derived from compounds containing a biphenyl or phenylnaphthalene structural moiety, as well as $\omega$-phenylalkanoic acids with chain lengths up to $C_{11}$, derived from compounds in which two aromatic systems are connected by an alkyl chain. The main components of the dicarboxylic acid oxidation products of all three aromatic fractions were $\alpha,\omega$-dicarboxylic acids and alkylcyclopentane-dicarboxylic acids and alkylcyclohexane-dicarboxylic acids, with phthalic acids also present in the oxidation products of the diaromatic and triaromatic fractions. The observation that 1,5-pentanedicarboxylic acids and 1,6-hexanedicarboxylic acids were the only $\alpha,\omega$-dicarboxylic acids in the oxidation products of the crude oil monoaromatic fraction, and were present in high abundance relative to other $\alpha,\omega$-dicarboxylic acids in the oxidation products of the diaromatic and triaromatic fractions indicated that substituted indanes and/or tetralins were quantitatively important constituents of the overall crude oil aromatic fraction. This finding is supported by the results of the analysis of the mass spectra of the crude oil aromatic fractions.

These studies of aromatic UCMs have provided new insights into the origins of the aromatic components of petroleum. The presence of a pronounced odd-over-even predominance in the $C_{25}$, $C_{27}$ and $C_{29}$ monodeuterated $n$-alkanes (CPI = 1.07), which corresponds to the odd-over-even predominance observed in the $n$-alkane components of unbiodegraded crude oils from the same basin, suggests that the $n$-alkyl side chains and the $n$-alkanes have a common source. Evidence is presented to support the hypothesis that the $n$-alkylaromatics are formed in part by

geosynthetic processes involving alkylation of aromatic systems by electrophilic species such as carbocations and acylium ions formed from carboxylic acids. This hypothesis is then extended to explain the formation of other groups of compounds, including aromatic systems with isoprenoidal-, methylalkyl- and monoalkyl-branched side chains. Because isoprenoids, methylalkanes and alkylalkanes are well-known components of petroleum, these results suggest that these alkylaromatic components of petroleum may share a common source with the corresponding alkane components. It is suggested that aromatic unresolved complex mixtures arise due to the very large number of structurally related compounds present, which are formed by geosynthetic processes such as alkylation of aromatic rings.

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# CHAPTER ONE

# INTRODUCTION

## 1.1 THE UNRESOLVED COMPLEX MIXTURE IN PETROLEUM

The compounds which make up the hydrocarbon fraction of crude oils can be grouped into two broad categories: saturated hydrocarbons (alkanes) and hydrocarbons which contain one or more aromatic rings. Analysis of the hydrocarbon fraction of petroleum by gas chromatography reveals that it is a very complex mixture containing literally thousands of individual components. Compounds which are usually clearly resolved in a crude oil gas chromatogram, and have been extensively studied, include the $n$-alkanes, methylalkanes, acyclic isoprenoids and alkylated (usually methyl) benzenes, naphthalenes and phenanthrenes (for general reviews see Tissot and Welte, 1984; Petrov, 1984; Killops and Killops, 1993). The remaining material appears as a "hump" under the peaks corresponding to the resolved components of the chromatogram, and is usually referred to as the unresolved complex mixture, or UCM (Blumer *et al.,* 1973). Examples of UCMs are shown in Figure 1.1. Despite modern analytical instrumentation, little is known about the composition of crude oil UCMs (c.f. Killops and Al-Juboori, 1990; Gough and Rowland, 1990, 1991; Thomas, 1995).

Processes such as biodegradation, weathering and refining result in a relative enrichment of the UCM by removal of the resolved components and/or formation of new compounds (eg. Palmer, 1993; Peters and Moldowan, 1993). $n$-Alkanes are usually the most prominent peaks in the gas chromatograms of mature crude oils (Peters and Moldowan, 1993), and as a result, the UCM is often overlooked in such samples. As can be seen in the GC-MS total ion chromatogram (TIC) shown in Figure 1.1$a$, the presence of large peaks corresponding to individual components such as the $n$-alkanes gives the appearance that this sample contains only a very small UCM. This is an artefact of the analytical technique, and the area of the unresolved portion of the chromatogram is often much larger than would be expected from a casual inspection. Figure 1.1$b$ shows the TIC of a crude oil which has been subjected to moderate biodegradation. It can clearly be seen that the UCM is a more prominent feature of the chromatogram than in Figure 1.1$a$, with the $n$-alkanes having been removed and the light isoprenoids
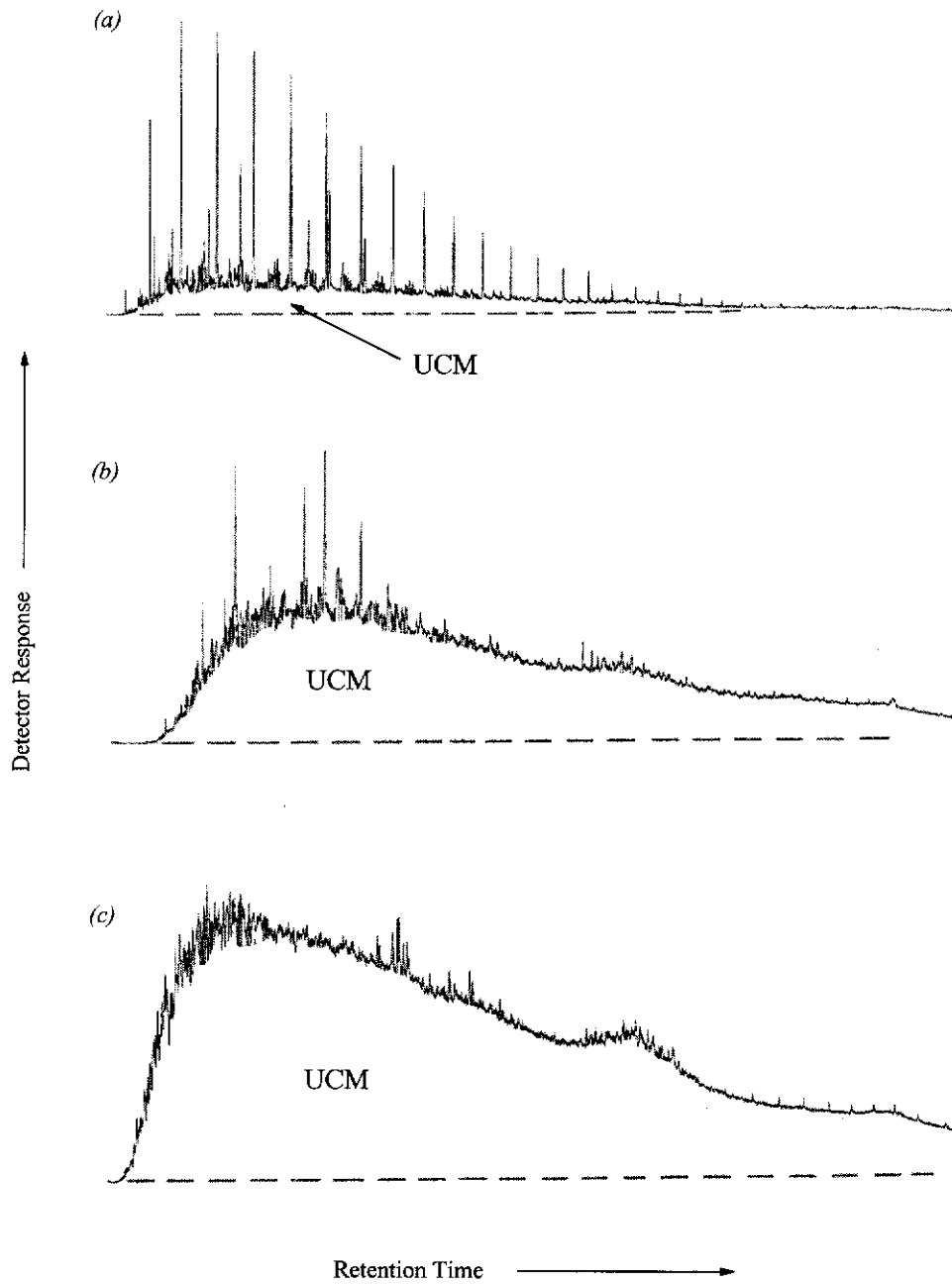
Figure 1.1. Gas chromatograms for three related Gulf Coast oils with differing levels of biodegradation: (a) nonbiodegraded; (b) moderately degraded; and, (c) very heavily degraded (adapted from Peters and Moldowan, 1993).

depleted. The TIC of a crude oil which has been subjected to extreme biodegradation is given in Figure 1.1c. In this case, the UCM is the dominant feature of the chromatogram, with practically all of the material present being unresolved by gas chromatography.

### 1.1.1 Characterisation of the UCM

Early attempts to characterise the UCM involved interpretation of the elemental composition and physical properties (Rossini *et al.*, 1953) and analysis by mass spectrometry (Clerc *et al.*, 1955; Hood and O'Neal, 1959; Hood *et al.*, 1959). On the basis of these studies, it was proposed that UCMs are comprised largely of condensed polycyclic ring systems containing a mixture of saturated and aromatic rings. Hood *et al.*, (1959) concluded that there was generally only one such ring system per molecule, with only a small contribution from compounds containing two ring systems at either end of an alkyl chain. The proposal that the UCM consists of naphthenoaromatic species was later supported by Farcasiu and Rubin (1987), who used a procedure which involved the dehydrogenation of petroleum residues to fully aromatised polycyclic compounds, followed by analysis of the reaction product by UV spectroscopy. They observed that the dehydrogenation reaction proceeded very easily, a characteristic of the dehydrogenation of hydroaromatic species, and resulted in an increase in the average number of aromatic rings per molecule. Thus they concluded that much of the UCM is comprised of compounds containing naphthenoaromatic structures.

Killops and Al-Juboori (1990) used a combination of instrumental and chemical degradation techniques in order to obtain compositional information about the UCM of biodegraded crude oils. Analyses using FT-IR, ^{1}H and ^{13}C FT-NMR, UV, elemental analysis, EI-MS and CI-MS, TLC and various size fractionation techniques were performed on the combined saturate and aromatic fraction of a biodegraded crude oil (almost completely unresolved by GC, and therefore referred to as a UCM). They concluded that this UCM was comprised mainly of aliphatic structures, with the proportion of carbon atoms that are aromatic

estimated at 10 %. They also found that 30 % of the total UCM material was associated with aromatic structures, suggesting that aromatic compounds contained significant aliphatic moieties. Oxidation of a total UCM was carried out using chromic acid, with the oxidation products analysed by GC-MS. *n*-Alkanoic acids were the major products, comprising nearly half of the identified material. Other compound classes identified included *n*-alkane-α,ω-dioic acids, isoprenoid and other branched chain alkanoic acids, cycloalkanecarboxylic acids and aromatic acids. The homologous series of *n*-alkanoic acids to $C_{19}$ indicates that *n*-alkyl chains were present in the original UCM. The presence of the homologous series of *n*-alkane-α,ω-dioic acids was attributed to either the presence of polymethylene bridges in the UCM or further oxidation of the *n*-alkanoic acids already produced. The aromatic components were mainly alkyl substituted benzenoid species, with an average of 2.3 substituents per ring. Cycloalkanecarboxylic acids comprised a significant proportion of the oxidation products, with the majority being monocyclic. The authors concluded that either fused polycyclic systems are rare in the UCM, or that polycyclic structures are more susceptible to oxidation. $^1$H NMR and elemental analysis of the UCM suggested a value of ~2.5 rings per molecule for an average saturated component of the UCM. These workers also observed that the oxidation products of the crude oil UCM were similar in both type and distribution to those obtained from the oxidation of a kerogen sample similar to that which produced the oxidised oil. It was concluded that, rather than the components of the UCM being the products of biodegradation within the reservoir, it is in fact comprised of material expelled from kerogen during petroleum formation, and is therefore present but usually not observed in all non-biodegraded oils.

### 1.1.2   The saturate unresolved complex mixture

Oxidation using chromium trioxide has been used to characterise isolated saturate UCMs from lubricating oils, environmental oil spill samples and a heavily biodegraded crude oil (Gough and Rowland, 1990; 1991). The products identified were similar for all samples, and included homologous series of normal and

branched carboxylic acids, *n*-alkane-α,ω-dioic acids, γ-lactones and ketones. *n*-Alkanoic acids were the principal resolved products, with cyclic products being present in minor quantities only. This last observation contrasts with the assertion that UCMs most likely contains significant amounts of cyclic material (Killops and Al-Juboori, 1990). A process of ring opening during oxidation is invoked to account for the discrepancy between the observed amount of cyclic oxidation products and the expected amount of cyclic material present in the UCM.

Gough and Rowland (1990, 1991) also oxidised model saturated compounds with chromium trioxide. They observed that the major oxidation products from the monoalkylalkane 7-hexylnonadecane (Figure 1.2) were straight chain *n*-alkanoic acids, generated *via* cleavage at the tertiary centre (Figure 1.3). Straight chain *n*-alkanoic acids were also produced from the oxidation of 9-(2-cyclohexylethyl)-heptadecane. As *n*-alkanoic acids were also the major products of the oxidation of saturate UCMs, they thus concluded that the saturate UCMs contained, "in part, monoalkyl-substituted T-branched alkanes."
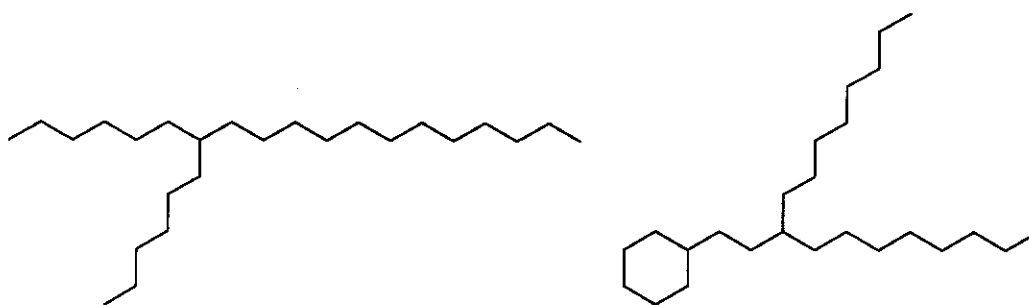


Figure 1.2. Examples of model compounds subjected to oxidation with chromium trioxide by Gough and Rowland (1990): 7-hexylnonadecane and 9-(2-cyclohexylethyl)nonadecane.
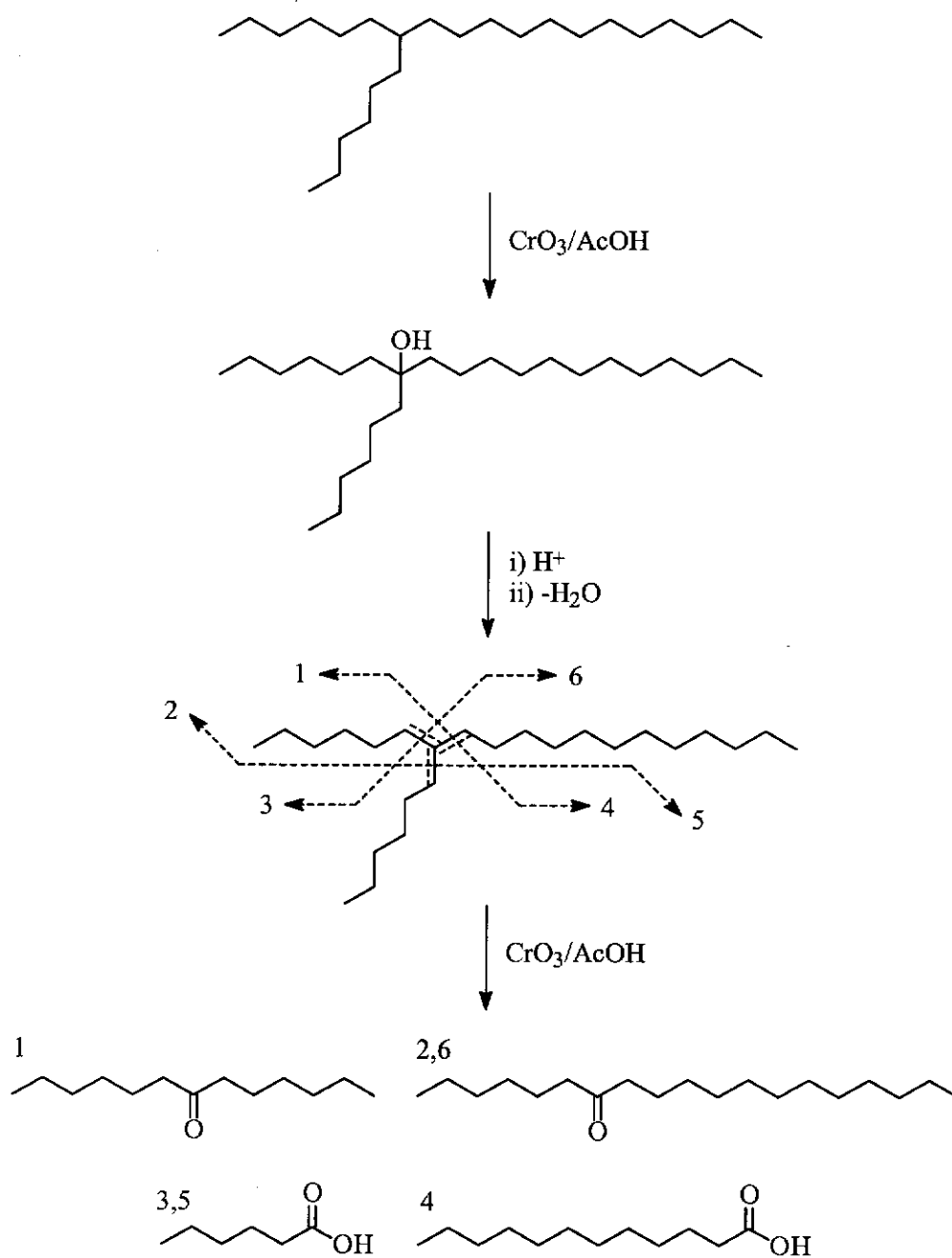
Figure 1.3. Mechanism of the chromium trioxide oxidation of branched alkanes (Cainelli and Cardillo, 1984)

Branched acyclic hydrocarbons comprise a significant proportion of the saturated hydrocarbon fraction of crude oils (Tissot and Welte, 1984). One of the most widely studied classes of these compounds are the monomethylalkanes, which occur in crude oils from a wide range of source types and depositional environments (c.f. Tissot and Welte, 1984; Klomp, 1986; Fowler and Douglas, 1987; Hoffmann et al., 1987; Kissin, 1987; Summons, 1987; Summons et al., 1988a, b); however, information in the literature concerning compounds with n-alkyl side chains longer than methyl is extremely scarce.

There have been a small number of reports in the literature of compounds in which the side chain is greater than one carbon atom in length. Using $^{13}$C NMR, Musayev et al. (1985) identified structural fragments containing ethyl groups in the branched and cyclic alkane portion of the 350°-540°C distillate fraction from a mixture of West Siberian crude oils, and Kurashova et al. (1989) identified ethyl-containing structural fragments in a crude oil from the Khar'yag field (Arkhangel'sk region, U.S.S.R.) using a similar method. In another study (Smirnov, 1990), structural moieties containing propyl and butyl groups were identified by $^{13}$C NMR in the 350°-530°C fractions of Khar'yaginsk and West Siberian crude oils; however, in all of these studies, the molecular structures of the compounds containing these moieties were not determined.

## 1.1.3 The aromatic unresolved complex mixture

While there have been relatively few studies conducted of whole UCMs and the UCMs of isolated saturate fractions, even less has been reported concerning investigation of unresolved complex mixtures of aromatic compounds. Revill (1992) studied the bulk characteristics of the aromatic fraction of a biodegraded crude oil (5 % resolved by GC-FID; level of biodegradation not given). Field desorption mass spectrometry (FDMS) indicated a molecular weight range for the sample of 200-1200 a.m.u. Ultraviolet fluorescence (UVF) spectroscopy suggested that compounds with up to five aromatic rings were present, although infrared (IR) spectroscopy indicated that the sample was highly aliphatic in nature.

Absent from the IR spectrum was any indication of monosubstituted monoaromatic systems, and it was concluded that the majority of the aromatic compounds were *ortho-* or *para*-disubstituted benzenes, or substituted naphthalenes, with decreasing amounts of components with higher numbers of aromatic rings. Proton nuclear magnetic resonance ($^1$H NMR) analysis confirmed that the sample was highly aliphatic in nature, with $^{13}$C NMR suggesting a high proportion of naphthenoaromatic components, in particular alkyltetralins.

Revill (1992) also used oxidation, with both chromium trioxide and ruthenium tetroxide, in order to characterise the aromatic UCM. The principal resolved components of the chromium trioxide oxidation were *n*-alkanoic acids, with alkylketones also identified. These compound classes are proposed to derive from the oxidation of alkyl-branched alkylbenzenes, similar to the reaction scheme outlined in Fig. 1.3, with an aromatic system attached to one of the terminal carbons. The identification of phthalic acid as a minor oxidation product was interpreted as evidence for the presence of naphthenobenzenes such as tetralins in the original UCM.

The major products of the ruthenium tetroxide oxidation of the crude oil aromatic fraction, as was the case with chromium trioxide, were *n*-alkanoic acids (Revill, 1992). Investigation of the GC-MS *m/z* 74 mass chromatogram (characteristic of carboxylic acid methyl esters) revealed a large amount of unresolved material, attributed to the presence of significant quantities of branched alkanoic acids. $^1$H NMR analysis of the acidic oxidation products suggested that the average component was a dicarboxylic acid derived from a $C_{32}$ monoaromatic precursor, such as an alkyltetralin. 1,6-Hexanedioic acid is the major product of the ruthenium tetroxide oxidation of tetralin (Stock and Tse, 1983), hence the presence of branched $\alpha,\omega$-dicarboxylic acids provides further evidence of naphthenoaromatic compounds as abundant components of the aromatic UCM studied.

Rowland *et al.* (1995) also oxidised aromatic UCMs using ruthenium tetroxide, and quantified the identified oxidation products. *n*-Alkanoic acids and branched alkanoic acids were the major products, with significant quantities of cyclic monocarboxylic acids and carbon dioxide (from the oxidation of unsubstituted aromatic carbon atoms) also formed. A generalised molecular structure proposed by Rowland *et al.,* (1995) indicating the precursor structural moieties of the various oxidation products is given in Figure 1.4. A more quantitative picture of the general classes of alkyl substituents attached to the aromatic rings of GC-unresolved crude oil components was thus obtained.

Thomas (1995) conducted a similar study into the UCMs of the aromatic fractions obtained from refinery products (the "post-distillation products produced by vacuum distillation and cracking of the atmospheric distillation residue") and also



|  *n*-alkanoic acids and branched alkanoic acids | cycloalkane carboxylic acids | dicarboxylic acids | carbon dioxide |
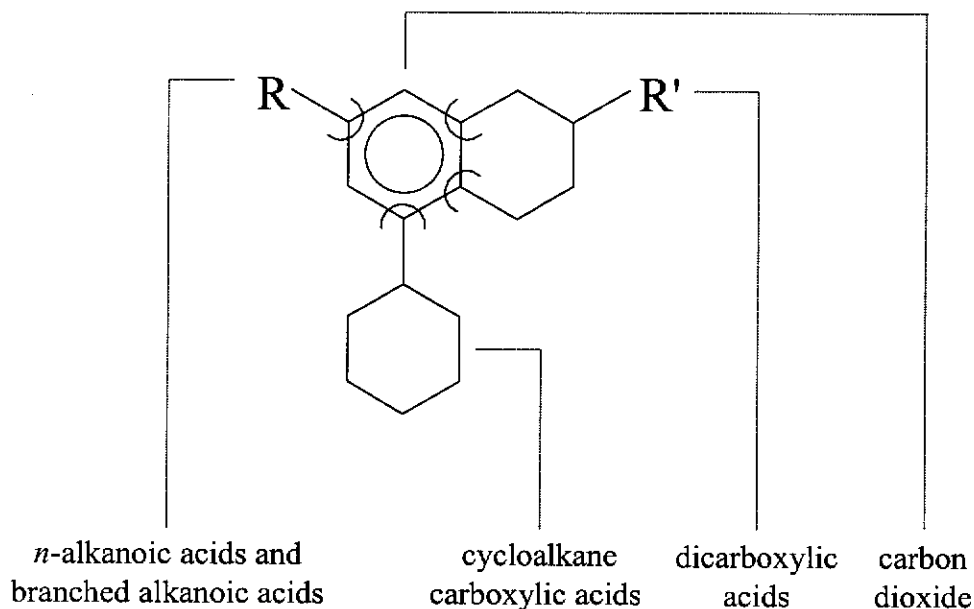
Figure 1.4. Generalised structure representing aromatic UCM components, showing the origin of carboxylic acids generated by oxidation using ruthenium tetroxide (adapted from Rowland *et al.,* 1995).

found that *n*-alkanoic acids were the principal oxidation products. Also identified were iso- and anteiso-monocarboxylic acids and 1,5- and 1,6-dicarboxylic acids, which may arise from oxidation of naphthenoaromatic structures or bridging methylene units. Other compounds which were also identified include cyclohexanecarboxylic acid (from cyclohexylbenzenes) and phenylacetic acid (from diphenylmethanes).

## 1.2  SCOPE AND SIGNIFICANCE OF THE PRESENT STUDY

This thesis describes investigations undertaken into the nature of components of both the aliphatic and aromatic UCMs of sedimentary organic matter. The GC-unresolved component of sedimentary hydrocarbon mixtures often comprises a significant proportion of the material present in the case of crude oils which have been subjected to biodegradation or weathering (eg. Peters and Moldowan, 1993). Many higher boiling refinery fractions are also principally unresolved. The molecular structures of the vast majority of the components of these mixtures are unknown at present.

The results and discussion part of this thesis is divided into two sections. Section One comprises Chapters Four and Five, and reports on the results of investigations into the monoalkylalkanes, a class of compounds proposed as components of the saturate UCMs of crude oils. Section Two comprises Chapters Six and Seven, and reports on the results of investigations into the aromatic fraction of a biodegraded crude oil using two oxidative degradation techniques.

Chapter Four of this thesis describes the identification and analysis of the monoalkylalkanes, a class of compounds which it has been proposed are contributors to aliphatic UCMs. All open chain structural isomers with a single *n*-alkyl branch larger than methyl in the range $C_{10}$ to $C_{20}$, as well as $C_{21}$ to $C_{25}$ structural isomers containing an ethyl branch have been identified in a series of crude oils of different source types and depositional environments.

Chapter Five reports the distribution of individual monoalkylalkane isomers in samples with different levels of thermal maturity. The change in the relative abundance of certain isomers with increasing depth in a sedimentary sequence is investigated and the potential for application to thermal maturity studies is assessed. The possible biological and geosynthetic origins of the monoalkylalkanes is also discussed.

Chapter Six describes the results of the potassium permanganate oxidation of a crude oil aromatic fraction. Analysis of the substitution patterns of the aromatic carboxylic acids formed has enabled a clear picture of the overall substitution patterns of the entire suite of original UCM components to be developed. The relative proportions of compounds with different positions of substituents is discussed. This information is consistent with the results given in the literature for the known members of specific compound classes. The relative proportions of compounds with different numbers of substituents has also been used to infer the nature of some components of the original aromatic fraction.

Chapter Seven describes the results of the oxidation of the monoaromatic, diaromatic and triaromatic fractions of a crude oil aromatic UCM with ruthenium tetroxide in order to gain information on the nature of the compounds present in these aromatic fractions. The organic-soluble oxidation products are reduced to deuterated hydrocarbons to allow a much more detailed investigation of the structure of these compounds than has previously been achieved. Detailed analysis of the water-soluble oxidation products was also performed. The possible origins of the aromatic compounds from which the oxidation products are formed is discussed.

# CHAPTER TWO


# EXPERIMENTAL

## 2.1    MATERIALS AND REAGENTS

*Solvents*

All general purpose solvents were purified and dried where necessary according to the procedures outlined in Perrin *et al.* (1983). Solvent purity was checked by concentration (100 mL sample evaporated to 50 $\mu$L) and analysis by capillary gas chromatography.

*Reagents*

Commercially available analytical grade reagents (AR) were used without further purification. Laboratory grade (LR) and technical grade reagents were purified where appropriate according to the procedures outlined in Perrin *et al.* (1983) prior to use.

*Silica gel*

Silica gel 60 [Merck; particle size 0.063-0.200 mm (70-230 mesh)] for column chromatography was extracted with dichlormethane and stored in an oven (120°C) prior to use. Silica gel 60 $PF_{254}$ (Merck) was used as obtained for thin layer chromatography.

*Alumina*

Aluminium oxide 60 $GF_{254}$ neutral (Merck, type E) was used as obtained for thin layer chromatography.

*Molecular sieves*

ZSM-5 molecular sieve powder (100 mesh) was obtained as CBV 2802 (PQ Corporation). It was activated before use by heating under vacuum at 120°C for 24 h in a Gallenkamp drying pistol.

*Copper (precipitated)*

Elemental copper powder (BDH) was activated by stirring with 3 M hydrochloric acid (5 min) and then washing sequentially with de-ionised water ($\times$ 2), acetone ($\times$ 2) and dichloromethane ($\times$ 2) immediately before use.

*Anhydrous magnesium sulfate*

Magnesium sulfate (BDH) was dried (600°C, 12 h) in a ventilated oven and stored in an airtight container prior to use.

*Glassware*

Glassware was cleaned by soaking in a solution of Pyroneg (Diversey Australasia, Pty Ltd; 2-3 g L$^{-1}$) in warm water (*ca.* 40°C) for 2 h and then rinsed thoroughly with de-ionised water. Items were heated at 600°C in a ventilated oven overnight, and rinsed with dichloromethane ($\times$ 2) before use.

## 2.2 GEOCHEMICAL TECHNIQUES

### 2.2.1 Extraction of soluble organic matter from sediments and coals

Powdered sedimentary rocks and coals (5-100 g) were placed in a conical flask fitted with a reflux condenser and extracted with a dichloromethane/methanol (95:5 v/v) solvent mixture (50-250 mL depending on sample size; $\times$ 3) using an ultrasonic bath (2-3 h). The mixture was filtered under vacuum through a sintered glass funnel and the residue washed with additional solvent ($\times$ 3). The combined filtrate was then concentrated by distillation (to *ca.* 5 mL) and passed through a column containing activated copper and anhydrous magnesium sulfate to remove any water and elemental sulphur present. The solvent was carefully removed from the filtrate by fractional distillation to afford the soluble organic matter (SOM).

## 2.2.2 Fractionation of crude oils and sedimentary rock and coal extracts by column chromatography

Crude oils and sediment and coal extracts were separated into aliphatic, aromatic and polar fractions by liquid chromatography. Glass columns (40 cm × 1.0 cm i.d.) were packed with activated silica gel (6 g) as a slurry in $n$-pentane. The sample (50-80 mg) in $n$-pentane (0.2 mL) was introduced to the top of the column. Aliphatic hydrocarbons were eluted under gravity with $n$-pentane (35 mL), aromatic hydrocarbons with $n$-pentane/dichloromethane (9:1 v/v; 40 mL) and polar compounds with dichloromethane/methanol (1:1 v/v; 30 mL). Neat fractions were obtained by careful removal of the solvent on a steam and/or sand bath (100°C).

For larger scale separations, the sample (~ 900 mg) in $n$-pentane (0.5 mL) was introduced into a glass column (80 cm × 2.0 cm i.d.) packed with silica gel (60 g). The saturated hydrocarbons were eluted with $n$-pentane (250 mL) and the aromatic hydrocarbons with $n$-pentane/dichloromethane (9:1 v/v; 600 mL).

## 2.2.3 Separation of saturated and aromatic hydrocarbons using preparative thin layer chromatography (TLC)

Crude oil and sediment and coal extract saturated and aromatic hydrocarbons were separated by preparative thin layer chromatography (TLC). The crude oil or extract (20-80 mg, in a minimum volume of hexane where necessary) was applied to silica gel TLC plates (20 × 20 cm or 20 × 10 cm, 0.75 mm thickness) activated at 120°C for 12 h prior to use. A standard reference mixture of tridecylbenzene, naphthalene and anthracene was applied to one end of the plate, and the plate was developed with hexane. The aromatic ($R_f$ <0.8) hydrocarbon fraction was visualised using short wavelength ultraviolet light (254 nm). The saturate and aromatic fractions were recovered by scraping the appropriate sections of silica from the TLC plate into filter funnels and washing with dichloromethane

(3 × 15 mL). The solvent was evaporated on a sand bath and the residue dissolved in hexane for GC and/or GC-MS analysis.

### 2.2.4 Isolation of branched and cyclic alkanes using ZSM-5 molecular sieves

Branched and cyclic hydrocarbons were isolated from the saturate fractions (Section 2.2.2) by column chromatography using ZSM-5 molecular sieves according to the procedure of West *et al.* (1990). A 10 mg sample dissolved in the minimum volume of n-pentane was placed on a column of ZSM-5 molecular sieve (2 g) packed in a Pasteur pipette and allowed to stand for 2 min. The excluded fraction was eluted using pentane (10 mL) as the mobile phase using a positive pressure of nitrogen to maintain a flow rate of *ca.* 0.5 mL min$^{-1}$. The solvent was removed and the residue was dissolved in hexane to provide a sample ready for GC-MS analysis.

### 2.2.5 Isolation of monoaromatic, diaromatic and triaromatic hydrocarbons using preparative thin layer chromatography (TLC)

Aromatic hydrocarbon fractions (Section 2.2.4; 5-40 mg) in a minimum volume of dichloromethane were applied to preparative alumina TLC plates (20 × 20 cm or 20 × 10 cm, 0.75 mm thickness) activated at 120°C for 12 h prior to use. A standard reference mixture of tridecylbenzene, naphthalene and anthracene was applied to one end of the plate, and the plate was developed with hexane. The monoaromatic ($R_f$ >0.6), diaromatic ($R_f$ = 0.4-0.6) and triaromatic ($R_f$ = 0.2-0.4) hydrocarbon fractions were visualised using short wavelength ultraviolet light (254 nm). The individual fractions were recovered by scraping the appropriate sections of alumina from the TLC plate into filter funnels and washing with dichloromethane (3 × 15 mL). The solvent was evaporated on a sand bath and the residue dissolved in hexane for GC and/or GC-MS analysis.

## 2.3    ANALYTICAL METHODS AND INSTRUMENTATION

### 2.3.1    Gas chromatography (GC)

GC analyses were performed using a Hewlett-Packard (HP) 5890 Series II gas chromatograph fitted with a 30 m × 0.53 mm i.d. wall-coated open tubular (WCOT) fused silica capillary column coated with a 1.5 µm methylsilicone stationary phase (BP-1, SGE Australia). The GC oven was programmed from 50-300°C at 6°C $min^{-1}$. Samples were dissolved in hexane and injected on-column using a HP 7673 autosampler at an oven temperature of 50°C. Hydrogen was used as the carrier gas operating at a linear velocity of 20 cm $s^{-1}$. Data acquisition and processing was performed by DAPA software (DAPA Scientific Software, Australia).

### 2.3.2    Gas chromatography-mass spectrometry (GC-MS)

Early in the study, GC-MS analysis was performed using a HP 5971 MSD interfaced with a HP 5890 Series II gas chromatograph. A 60 m × 0.25 mm i.d. WCOT fused silica capillary column coated with a 0.25 µm 5 % phenylmethyl-silicone stationary phase (DB-5, J & W Scientific) was used. The GC oven was programmed from 50-300°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 50°C. Helium was used as the carrier gas at a linear velocity of 28 cm $s^{-1}$. Typical mass spectrometer conditions were: ionisation energy 70 eV, source temperature 200°C, electron multiplier voltage 2200 V.

Later in the study, GC-MS analysis was performed using a HP 5973 MSD interfaced with a HP 6890 gas chromatograph fitted with a 60 m × 0.25 mm i.d. WCOT fused silica capillary column coated with a 0.25 µm 5 % phenylmethyl-silicone stationary phase (DB-5, J & W Scientific). The GC oven was programmed from 50-300°C at 3°C $min^{-1}$. Samples for analysis were dissolved in hexane and injected on-column using a HP 6890 auto sampler at an oven

temperature of 50°C. Helium was used as the carrier gas at a linear velocity of 28 cm s$^{-1}$. Typical mass spectrometer conditions were: ionisation energy 70 eV, source temperature 230°C, electron multiplier voltage 1800 V.

GC-MS analysis was also performed using a Fisons Autospec Ultima Q MS-MS interfaced with a Hewlett-Packard 5890 Series II gas chromatograph fitted with a 60 m × 0.25 mm i.d. fused silica open tubular column coated with a 0.25 $\mu$m DB-5 stationary phase (J & W Scientific). The GC oven was programmed from 50-300°C at 4°C min$^{-1}$ with the transfer line held at 290°C. Samples for analysis were dissolved in hexane and injected on-column using a HP 7673 auto sampler at an oven temperature of 50°C. Helium was used as the carrier gas at a linear velocity of 23 cm s$^{-1}$. Typical mass spectrometer source conditions were an ionisation energy of 70 eV and a temperature of 200°C.

Magnet scans were performed by scanning from 50-450 a.m.u. with a scan time of 700 ms and an accelerating voltage of 8000 V. The instrument was tuned to a resolution of 1000. Magnet selected ion recording was typically performed by monitoring 14 masses with a dwell time of 30 ms each, with a total scan time of approximately 1060 ms. For these experiments, the instrument was tuned to a resolution of 600.

### 2.3.3   GC-MS metastable reaction monitoring (MRM)

GC-MS MRM analyses were performed using a Fisons Autospec Ultima Q MS-MS interfaced with a Hewlett-Packard 5890 Series II gas chromatograph as described in Section 2.3.2. Analyses were typically performed on 12-14 parent-daughter transitions with a dwell time of 20 ms per transition, giving a total scan time of approximately 1000 ms. The instrument was tuned to a resolution of 600.

### 2.3.4 Nuclear magnetic resonance (NMR) spectroscopy

Proton nuclear magnetic resonance ($^1$H NMR) measurements of crude oil aromatic fractions and $^{13}$C NMR measurements of reference compounds were carried out using a Varian Gemini 200 instrument. The instrument was operated at 200 MHz for $^1$H NMR and 50 MHz for $^{13}$C NMR measurements. Typically, a 50 mg sample of the analyte was dissolved in deuterochloroform for analysis.

### 2.3.5 Infrared (IR) spectroscopy

Transmission infrared spectroscopy of crude oil aromatic fractions was performed using a Bruker IFS66 Fourier transform infrared spectrometer. Samples (20 % in dichloromethane) were spotted onto a zinc selenide plate, the solvent evaporated under an infrared lamp, and scanned from 4000 to 600 wavenumbers at a resolution of 4 wavenumbers using a mercury-cadmium-telluride (MCT) detector.

## 2.4  PREPARATION OF REFERENCE COMPOUNDS

3-Ethylhexadecane, 4-propylpentadecane , 5-butyltetradecane  and 6-pentyltridecane  were prepared by the following general method as described by Furniss *et al.* (1989): an ethyl ester (15 mmol in 50 mL diethyl ether) was added to the appropriate Grignard reagent (30 mmol in 60 mL ether). The resulting tertiary alcohol (75-85% yield) was dehydrated to a mixture of alkenes by refluxing with 85% phosphoric acid. The alkene mixtures from each alcohol were then hydrogenated using 10% palladium on carbon catalyst to afford the monoalkylalkane (80-90%), which was recovered in pure form after subjecting the reaction product to column chromatography using activity III alumina (Brockmann scale) with pentane as the solvent.

## 2.5    DEGRADATION OF CRUDE OIL AROMATIC FRACTIONS

### 2.5.1    Ruthenium tetroxide oxidation

Oxidation of crude oil monoaromatic, diaromatic and triaromatic fractions was carried out using a modification of the procedure of Ilsley *et al.* (1986). Briefly, the aromatic fraction (200-600 mg) was added to a mixture of acetonitrile (10 mL), dichloromethane (10 mL) and water (15 mL). With rapid stirring, enough sodium periodate to give a co-oxidant to substrate ratio of 8 (calculated by assuming an average molecular weight of 300 for the aromatic fraction) was added. After the sodium periodate had dissolved, 25 mg of ruthenium dioxide trihydrate ($RuO_2.3H_2O$) was added and the mixture stirred for 72 h. Methanol (0.5 mL) was added to quench the reaction mixture. The organic layer of the reaction mixture was removed and the aqueous phase extracted with dichloromethane (3 × 5 mL). The aqueous layer was evaporated to dryness (rotary evaporator) to yield a sample containing dicarboxylic acids. The combined organic fractions were evaporated on a sand bath to *ca.* 10 mL and then extracted with potassium hydroxide (1 M; 3 × 10 mL). The remaining organic fraction was dried over anhydrous magnesium sulfate to yield a sample of non-acidic compounds for analysis by GC-MS. Dichloromethane (10 mL) was added to the combined potassium hydroxide extracts, which were then acidified to litmus with hydrochloric acid (3 M; *ca.* 10 mL). The organic layer was removed and the aqueous layer extracted with dichloromethane (3 × 10 mL). The combined dichloromethane fractions were dried over anhydrous magnesium sulfate and the volume carefully reduced by evaporation on a sand bath to yield a sample containing monocarboxylic acids.

### 2.5.2    Esterification of carboxylic acids using boron trifluoride/methanol complex

A sample of carboxylic acids (see section 2.5.1) was methylated with boron trifluoride/methanol complex using a modification of the procedure of Watson

(1993). The carboxylic acid sample (20 mg) was dissolved in dichloromethane (5 mL) and boron trifluoride/methanol complex (3 mL) was added. The reaction vessel was then sealed and heated at 60°C for 30 minutes. Water (3 mL) was added to the reaction mixture and the organic layer removed. The aqueous layer was extracted with dichloromethane (2 × 3 mL) and pentane (1 × 3 mL) and the combined organic layers dried (anhydrous magnesium sulfate) and evaporated on a sand bath. The resultant mixture of methyl esters was dissolved in hexane for analysis by GC and/or GC-MS techniques.

## 2.5.3 Reduction of carboxylic acids using lithium aluminium hydride

A sample of carboxylic acids (see section 2.5.1) was reduced using lithium aluminium hydride in a modification of the procedure of Monson (1971). In a typical experiment, lithium aluminium hydride (50 mg, $1.3 \times 10^{-3}$ mol) was stirred in anhydrous diethyl ether (10 mL) for 10 min. The carboxylic acid sample (100 mg, $6.7 \times 10^{-4}$ mol assuming an average molecular weight of 150) was dissolved in anhydrous diethyl ether (1 mL) and added to the previous mixture dropwise and the resulting solution stirred for 1 h. Saturated ammonium chloride solution (ca. 0.5 mL) was added to the reaction mixture to quench excess hydride reagent and sulphuric acid (3 M, 2 mL) added to dissolve the solid residue. The organic layer was removed and the aqueous layer extracted with dichloromethane (2 × 4 mL) and pentane (1 × 4 mL). The combined organic extracts were dried (anhydrous magnesium sulfate) and carefully evaporated on a steam bath. The resultant mixture of primary alcohols was dissolved in hexane for analysis by GC and/or GC-MS techniques.

## 2.5.4 Derivatisation of alcohols using *p*-toluenesulfonyl chloride

The mixture of primary alcohols (see section 2.5.3) was converted into tosyl esters using *p*-toluenesulfonyl chloride in a modification of the procedure of Kabalka *et al.* (1986). In a typical procedure, the alcohol mixture (100 mg, $6.7 \times 10^{-4}$ mol assuming an average molecular weight of 150) was dissolved in dichloromethane

(7 mL) and the mixture cooled to 0°C in an ice bath. Pyridine (160 μL; 2.0 × 10⁻³ mol) and *p*-toluenesulfonyl chloride (250 mg, 1.3 × 10⁻³ mol) were added consecutively with constant stirring, the *p*-toluenesulfonyl chloride slowly in small portions. The conditions were maintained for 4 h, after which time water (4 mL) and diethyl ether (5 mL) were added to the reaction flask. The organic layer was removed and the aqueous layer extracted with diethyl ether (3 × 5 mL). The combined organic layers were washed successively with hydrochloric acid (2 M; 5 mL), sodium bicarbonate (5%; 5 mL) and water (5 mL), dried (anhydrous magnesium sulfate) and the solvent carefully removed on a steam bath. The resultant mixture of tosylates was dissolved in hexane for analysis by GC and/or GC-MS techniques.

## 2.5.5 Reduction of *p*-toluene sulfonate derivatives using lithium aluminium deuteride

A mixture of tosylate derivatives was reduced using lithium aluminium deuteride according to the procedure outlined in section 2.5.3. Again, the ratio of reductant to substrate was 2, assuming an average molecular weight of tosylate derivative of 300. The resultant mixture of deuterated hydrocarbons was dissolved in hexane for analysis by GC and/or GC-MS techniques.

## 2.5.6 Potassium permanganate oxidation

Oxidation of crude oil aromatic fractions was carried out using a modification of the procedure of Tietze and Eicher (1989). Briefly, the aromatic fraction (40 mg) was added to a refluxing mixture of water (20 mL) and pyridine (10 mL). Potassium permanganate (0.8 g) was added in small portions to the refluxing mixture over four hours. The mixture was allowed to reflux for a further 15 h, and was then cooled. The precipitated manganese dioxide was filtered off and washed copiously with boiling water, with the washings added to the filtrate. The combined filtrate and washings were reduced to 100 mL and extracted with dichloromethane (3 × 20 mL) to yield a sample containing monocarboxylic acids.

The remaining aqueous fraction containing polycarboxylic acids was evaporated to dryness and the residue methylated using boron trifluoride/methanol complex as described in Section 2.5.2. The combined organic fractions were dried (anhydrous magnesium sulfate), the volume was reduced to 5 mL and methylated using boron trifluoride/methanol complex as described in Section 2.5.2.

## 2.6     HEATING EXPERIMENTS WITH STANDARD COMPOUNDS

The aluminium montmorillonite clay used in this study was material prepared for an earlier study (Alexander *et al.*, 1984b). 1-Octadecene (1 mg) and aluminium montmorillonite (10 mg) were placed in 8 cm × 2 mm i.d. glass ampoules and flushed with dry nitrogen. The ampoules were evacuated, sealed, and heated at 180°C for 20 hours. The contents were extracted with hexane (3 × 0.5 mL) and filtered through a plug of anhydrous magnesium sulfate in a Pasteur pipette to afford a sample for GC and/or GC-MS analysis.

# CHAPTER THREE

# GEOLOGICAL AND GEOCHEMICAL DESCRIPTION OF SAMPLES

## 3.1 GEOLOGICAL DESCRIPTION OF SAMPLES

Crude oils from Australia and Indonesia, and sediment samples from Australia were used in this study. A total of seven crude oils, and sedimentary rocks from six locations, were studied. Geological data relating to the crude oil samples are given in Table 3.1, and to the sediment samples in Table 3.2.

Table 3.1.  Geological data relating to the crude oil samples used in this study.

| Name | Basin | Country | Inferred age of source | Depositional environment |
|---|---|---|---|---|
| Petapahan | - | Indonesia | Tertiary | Deltaic |
| Tuna-4, 2919.5m | Gippsland | Australia | Late Cretaceous | Terrestrial |
| Leatherjacket 788.5m | Gippsland | Australia | Cretaceous | Terrestrial |
| Barrow Deep | Carnarvon | Australia | Jurassic | Marine |
| Dongara 17 | Perth | Australia | Triassic | Marine |
| Horse Creek | Cooper | Australia | Permian | Terrestrial |
| Great Sandy | Canning | Australia | Ordovician | Marine |

Table 3.2.  Geological data relating to the sediment samples used in this study.

| Name | Depth (m) | Basin | Country | Inferred age of source | Depositional environment |
|---|---|---|---|---|---|
| Loy Yang | 67-68 | Gippsland | Australia | Eocene | Freshwater swamp/marsh |
| Grunter-1 | 2290 | Gippsland | Australia | Paleocene | Terrestrial |
| Grunter-1 | 2385 | Gippsland | Australia | Paleocene | Terrestrial |
| Grunter-1 | 2390 | Gippsland | Australia | Paleocene | Terrestrial |
| Grunter-1 | 3775 | Gippsland | Australia | Late Cretaceous | Terrestrial |
| Grunter-1 | 3125 | Gippsland | Australia | Late Cretaceous | Terrestrial |
| Grunter-1 | 3260 | Gippsland | Australia | Late Cretaceous | Terrestrial |
| Grunter-1 | 3450 | Gippsland | Australia | Late Cretaceous | Terrestrial |
| Grunter-1 | 3540 | Gippsland | Australia | Late Cretaceous | Terrestrial |
| Grunter-1 | 3676 | Gippsland | Australia | Late Cretaceous | Terrestrial |
| Mercury | 2060 | Carnarvon | Australia | Late Cretaceous | Marine |
| Paqualin-1 | 2799 | Bonaparte | Australia | Upper Jurassic | Marine |
| PD 26 | 338.8 | Canning | Australia | Devonian | Marine |
| ID-13 | 470 | Canning | Australia | Ordovician | Marine |

## 3.2    GEOCHEMICAL DATA

Geochemical data relating to the crude oil and sediment samples used in this study
are given in Tables 3.3 and 3.4 respectively.

Table 3.3.   Geochemical data relating to the crude oil samples used in this study.

| Name | Pristane/ Phytane | $20S^a$/ $(20S + 20R)$ | Diasterane[b]/ Sterane | $C_{29}$ Sterane[c]/ $C_{27}$ Sterane |
|---|---|---|---|---|
| Petapahan | 2.3 | 0.56 | 0.67 | 1.9 |
| Tuna-4, 2820m [d] | 5.8 | 0.74 | 2.8 | 10 |
| Leatherjacket 788.5m [e] | 2.3 | 0.47 | 0.9 | 5.4 |
| Barrow Deep | 2.5 | 0.56 | 1.4 | 1.8 |
| Dongara 17 | 1.1 | 0.45 | 0.4 | 1.2 |
| Horse Creek | 4.2 | 0.54 | 0.48 | 1.2 |
| Great Sandy | 1.9 | 0.46 | 1.14 | 1.3 |

a: $20S/(20S+20R) = (20S)-14\alpha,17\alpha(H)$-Ethylcholestane/$(20S+20R)-14\alpha,17\alpha(H)$-Ethylcholestane;
b: Diasterane/Sterane $=(20S+20R)-13\beta,17\alpha(H)$-Ethyldiacholestane/$(20S+20R)-14\alpha,17\alpha(H)$-Ethylcholestane;
c: $C_{29}$ Sterane/$C_{27}$ Sterane $=(20S+20R)-14\alpha,17\alpha(H)$-Ethylcholestane/$(20S+20R)-14\alpha,17\alpha(H)$-Cholestane.
d: Ioppolo-Armanios, 1995.
e: Ellis, 1994.

Table 3.4.  Geochemical data relating to the sediment samples used in this study.

| Name | Depth (m) | CPI[a] | 20S[b]/ (20S + 20R) |
|------|-----------|--------|---------------------|
| Loy Yang | 67-68 | 5.80 | - |
| Grunter-1 | 2290 | 1.26[c] | 0.15[c] |
| Grunter-1 | 2385 | - | - |
| Grunter-1 | 2390 | 1.16[c] | 0.26[c] |
| Grunter-1 | 2775 | - | 0.20[c] |
| Grunter-1 | 3125 | 2.01[c] | 0.38[c] |
| Grunter-1 | 3260 | 1.67[c] | 0.45[c] |
| Grunter-1 | 3450 | 1.44[c] | 0.49[c] |
| Grunter-1 | 3540 | 1.3[c] | 0.52[c] |
| Grunter-1 | 3676 | 1.36[c] | 0.53[c] |
| Mercury | 2060 | - | 0.38 |
| Paqualin-1 | 2799 | 1.24 | 0.19 |
| PD 26 | 338.8 | - | 0.27 |
| ID-13 | 470 | 1.0 | 0.16 |

a: $CPI = 0.5(\Sigma C_{24} \dots C_{32}/\Sigma C_{25} \dots C_{33} + \Sigma C_{25} \dots C_{33}/\Sigma C_{26} \dots C_{34})$

b: $20S/(20S+20R) = (20S)$-$14\alpha,17\alpha$-Ethylcholestane/$(20S+20R)$-$14\alpha,17\alpha$-Ethylcholestane;

c: Kralert, 1995

**SECTION ONE**

# CHAPTER FOUR

# IDENTIFICATION OF NOVEL MONOALKYLALKANES IN CRUDE OILS

## 4.1 INTRODUCTION

The saturated hydrocarbon fractions of crude oils contain normal, branched and cyclic hydrocarbons as their main components (Tissot and Welte, 1984). Branched hydrocarbons comprising a linear carbon chain with a single branch, or monoalkylalkanes have been referred to as T-branched alkanes (e.g. Gough and Rowland, 1990; Gough, *et al.*, 1992). Compounds in which the side chain is a methyl group are well-known components of crude oils and sediments and are commonly known as monomethylalkanes (e.g. Tissot and Welte, 1984; Klomp, 1986; Fowler and Douglas, 1987; Hoffmann *et al.*, 1987; Kissin, 1987; Summons, 1987; and Summons *et al.*, 1988a, b; Gurgey, 1998; Thiel *et al.*, 1999).

*Monomethylalkanes*
Monomethylalkanes and their functionalised counterparts have been reported to occur in biological systems. Monomethylalkane isomers with the methyl group in positions 2 and 3, also known as iso-alkanes and anteiso-alkanes, respectively, have been reported as natural products (e.g. Eglinton and Hamilton, 1967; Tornabene *et al.*, 1970; Kolattukudy, 1976). Iso- and anteiso-carboxylic acids have also been identified in biological systems (Kaneda, 1977; Dowling *et al.*, 1986; Shiea *et al.*, 1991; Gunstone, 1996), as have iso- and anteiso-alcohols (Downing, 1976). There have also been numerous reports of the occurrence of specific methylalkanes with mid-chain branching both in plants and animals (e.g. Nelson, 1978). Mid-chain monomethylalkanes in the range $C_{16}$-$C_{21}$ have also been reported to occur in cyanobacteria (Han *et al.*, 1968; Gelpi *et al.*, 1970; Robinson and Eglinton, 1990; Shiea *et al.*, 1990, 1991; Dachs *et al.*, 1998) with 7- and 8-methylheptadecane being the most common constituents. Mid-chain monomethyl acids (Dowling *et al.*, 1986) and alcohols (Pond *et al.*, 1986) have also been identified in bacteria.

Monomethylalkanes are also present in sedimentary organic matter. In the immature Green River Shale of North America, it is apparent from the chromatogram published by Hoering and Freeman (1984) that the 2- and

3-methylalkanes are by far the major monomethylalkane isomers present. Hoffmann *et al.* (1987) reported that 2- and 3-methylalkanes were slightly more prominent than other isomers in a series of Ordovician sediments. This preference was also reported by Summons *et al.* (1988b) at carbon numbers 16 and 17 for Middle Proterozoic sediments, but from carbon numbers 22 to 25 the mid-chain isomers became predominant. Mid-chain monomethylalkanes have also been reported to occur in crude oils (Jackson *et al.*, 1986; Klomp, 1986; Fowler and Douglas, 1987; Hoffmann *et al.*, 1987; Kissin, 1987; Summons, 1987; Summons *et al.*, 1988a, b; Thiel *et al.*, 1999). Kissin (1987) has observed that isomers with methyl substitution at all possible positions on the carbon chain are present in crude oils in approximately equal amounts.

Several origins have been proposed to account for the monomethylalkanes found in crude oils and sediments. These include: (i) direct biological input of monomethylalkanes (Fowler and Douglas, 1987); (ii) transformation of functionalised precursors such as carboxylic acids (Summons, 1987; Summons *et al.*, 1988a); (iii) long-term equilibration of a limited number of isomers (Hoering, 1980; Klomp, 1986); and, (iv) acid-catalysed reactions of alkenes formed by thermal cracking (Kissin, 1987).

*Monoalkylalkanes*

There have also been some reports in the literature of compounds in which the side chain is greater than one carbon atom in length. Using $^{13}C$ NMR, Musayev *et al.* (1985) identified structural fragments containing ethyl groups in the branched and cyclic portion of the 350°-540°C distillate fraction from a mixture of West Siberian crude oils, and Kurashova *et al.* (1989) identified ethyl-containing structural fragments in a crude oil from the Khar'yag field (Arkhangel'sk region, U.S.S.R.) using a similar method. In another study (Smirnov, 1990), structural moieties containing propyl and butyl groups were identified by $^{13}C$ NMR in the 350°-530°C fractions of Khar'yaginsk and West Siberian crude oils. In all of these studies, the molecular structures of the compounds containing these moieties were not determined. It has also been suggested on the basis of the results of

oxidative cleavage experiments that the aliphatic unresolved complex mixture (UCM) in crude oils may be comprised partly of monoalkyl- ("T-branched") alkanes (Gough and Rowland, 1990; Killops and Al-Juboori, 1990).

In this chapter, the identification of 163 novel branched alkanes in a series of crude oils is reported. Four single branched $C_{18}$ alkanes, one each with an ethyl, propyl, butyl and pentyl substituent, were synthesised in order to establish their gas chromatographic retention behaviour and mass spectral fragmentation patterns. A series of crude oils of varying ages, source types and depositional environments were analysed for branched alkanes by comparison of mass spectra and gas chromatographic retention behaviour with these authentic reference compounds. Using magnet selected ion recording mass spectrometry, all open chain structural isomers with a single $n$-alkyl branch larger than methyl in the range $C_{10}$ to $C_{20}$ were identified at each carbon number in the crude oils. Also, $C_{21}$ to $C_{25}$ structural isomers containing an ethyl branch were identified in all of these samples. It was found that monoalkylalkanes comprise approximately 3% of the saturated hydrocarbons with GC retention times between $n$-$C_{19}$ and $n$-$C_{20}$ for the series of oils examined in this study.

## 4.2    RESULTS AND DISCUSSION

### 4.2.1    Preparation of reference compounds

Four single-branched alkanes, *viz* 3-ethylhexadecane, 4-propylpentadecane, 5-butyltetradecane and 6-pentyltridecane, were prepared for use as authentic reference compounds. The general reaction scheme followed is given in Figure 4.1. These four compounds were prepared according to the general procedure of Furniss *et al.* (1989) for the addition of a Grignard reagent to a carboxylic ester. Briefly, the ethyl ester of the appropriate carboxylic acid was added to a solution of an alkylmagnesium halide which upon acidification yielded a tertiary alcohol. Subsequent dehydration and hydrogenation of the resultant alkene mixture afforded the desired monoalkylalkanes. All compounds were obtained in good yield (> 70 %) and high purity (> 95 %). The mass spectra of the compounds are given in Figure 4.2 and are consistent with the stated identifications.

The mass spectral diagnostic ions for these compounds are the fragments generated by cleavage at the tertiary carbon, with the charge retained at the tertiary centre. These ions are typically present in mass spectra as an odd/even pair, corresponding to products of simple cleavage and cleavage with hydrogen rearrangement (Biemann, 1962; McCarthy *et al.*, 1968; Holzer *et al.*, 1979). The mass spectrum of a monoalkylalkane may contain from one to three such diagnostic ions depending on the lengths of the alkyl groups present in the molecule. Aside from these characteristic ions, the mass spectra of single branched alkanes resemble those of *n*-alkanes. The diagnostic ions of all 16 of the $C_{18}$ structural isomers are summarised in Table 4.1, with the general fragmentation pattern for the monoalkylalkanes shown in Figure 4.3.

The four reference compounds were also characterised by carbon-13 nuclear resonance spectrometry ($^{13}C$ NMR). The chemical shifts of each of the carbon atoms in each compound are given in Table 4.2, together with the chemical shifts calculated using the equation given in Silverstein *et al.* (1981). In all cases, the
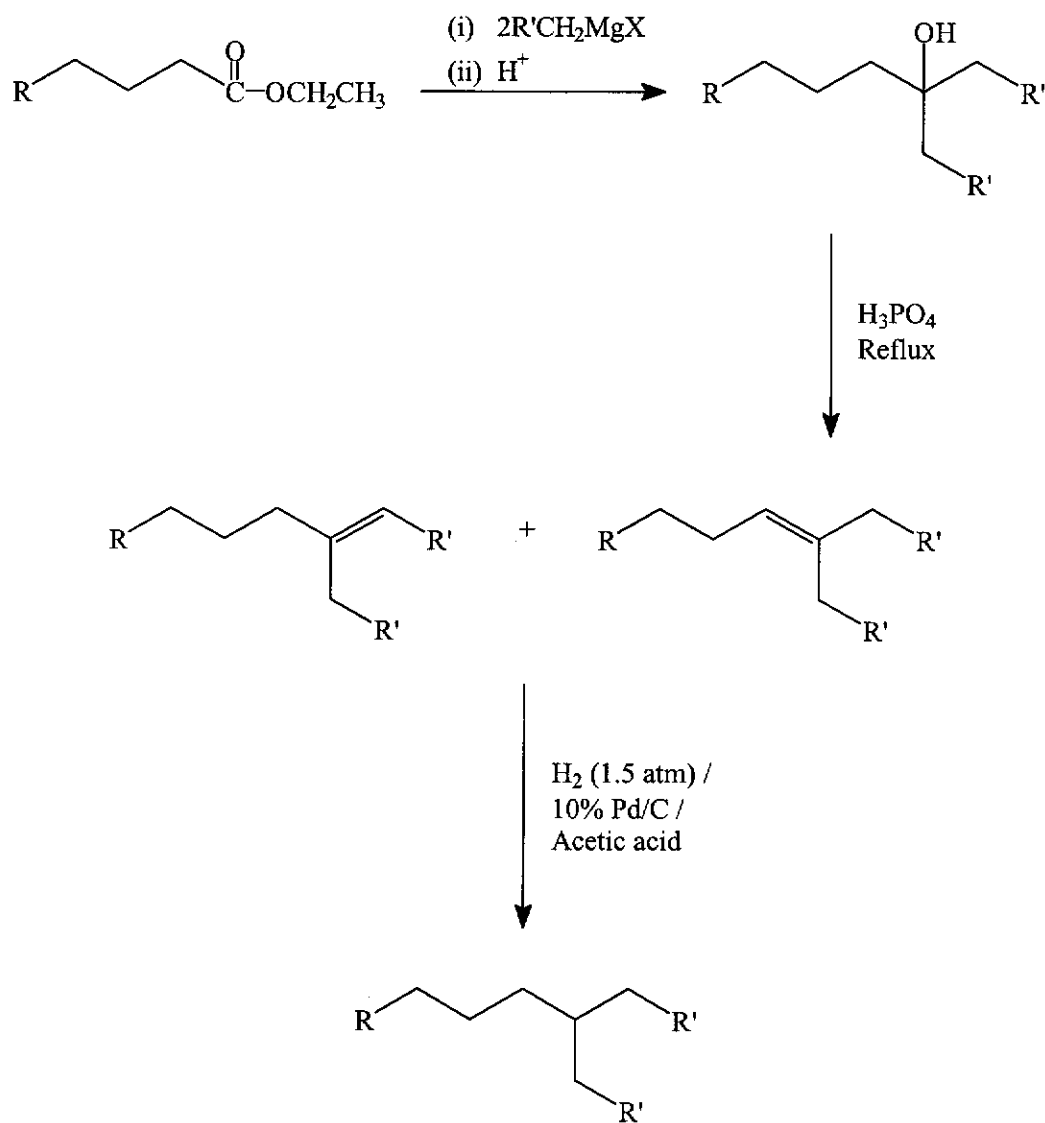
Figure 4.1. General reaction scheme showing the reaction sequence used for the preparation of single-branched alkane reference compounds.
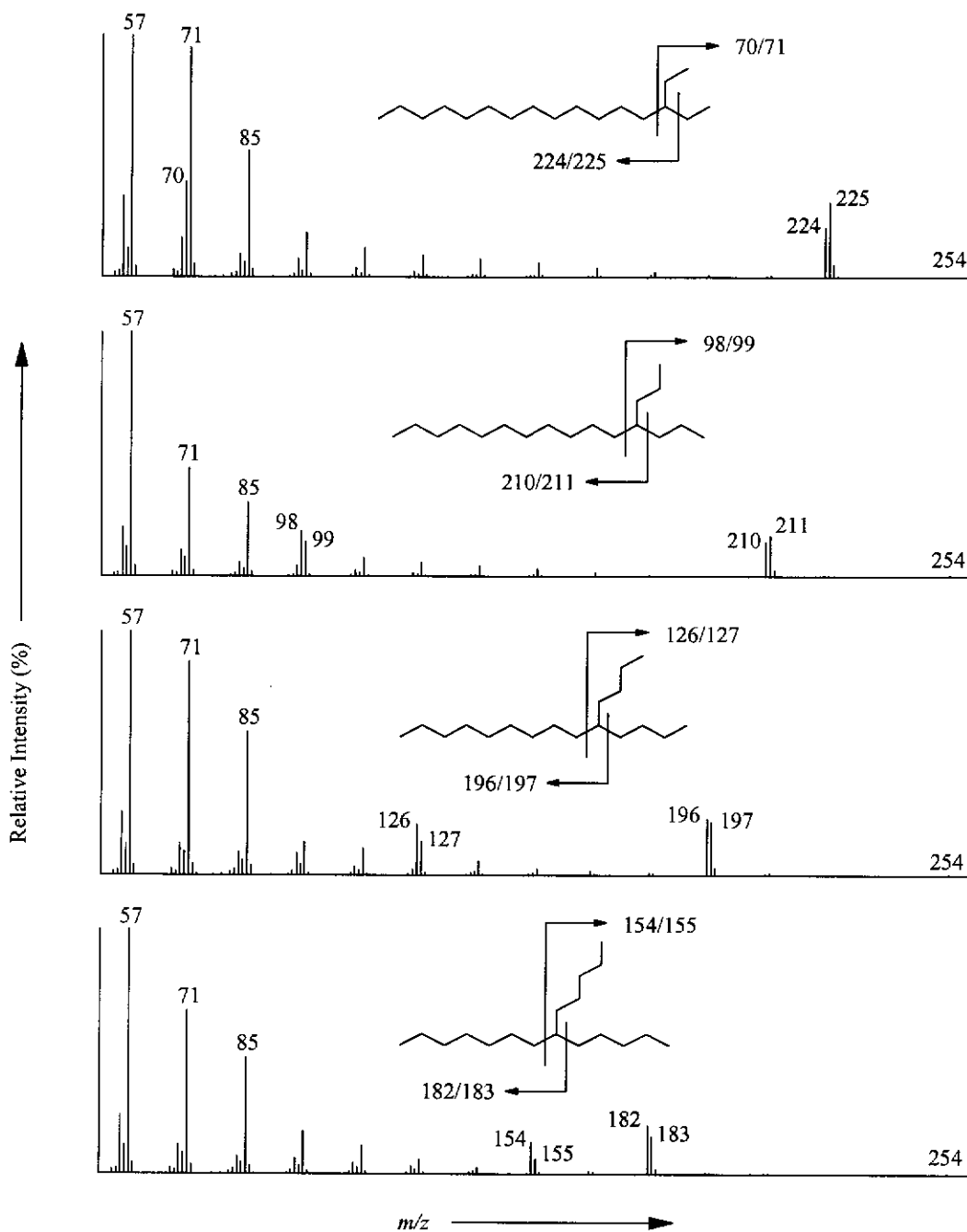
Figure 4.2. Electron impact mass spectra (70 eV) and structures of the monoalkylalkane reference compounds 3-ethylhexadecane, 4-propylpentadecane, 5-butyltetradecane and 6-pentyltridecane. The characteristic mass spectral cleavage patterns about the tertiary carbon are shown on each structure.

experimentally determined chemical shift is within 2.2 ppm of the calculated value.

Table 4.1. Names and mass spectral diagnostic ions of $C_{18}$ monoalkylalkanes.

| | | Diagnostic Ions | | | | | |
|---|---|---|---|---|---|---|---|
| Abbreviations Used | Compound Name | R | m/z | R' | m/z | R" | m/z |
| 8Et | 8-Ethylhexadecane | $C_2$ | 224 | $C_7$ | 154 | $C_8$ | 140 |
| 7Et | 7-Ethylhexadecane | $C_2$ | 224 | $C_6$ | 168 | $C_9$ | 126 |
| 6Et | 6-Ethylhexadecane | $C_2$ | 224 | $C_5$ | 182 | $C_{10}$ | 112 |
| 5Et | 5-Ethylhexadecane | $C_2$ | 224 | $C_4$ | 196 | $C_{11}$ | 98 |
| 4Et | 4-Ethylhexadecane | $C_2$ | 224 | $C_3$ | 210 | $C_{12}$ | 84 |
| 3Et | 3-Ethylhexadecane | $C_2$ | 224 | $C_2$ | 224 | $C_{13}$ | 70 |
| 8Pr | 8-Propylpentadecane | $C_3$ | 210 | $C_7$ | 154 | $C_7$ | 154 |
| 7Pr | 7-Propylpentadecane | $C_3$ | 210 | $C_6$ | 168 | $C_8$ | 140 |
| 6Pr | 6-Propylpentadecane | $C_3$ | 210 | $C_5$ | 182 | $C_9$ | 126 |
| 5Pr | 5-Propylpentadecane | $C_3$ | 210 | $C_4$ | 196 | $C_{10}$ | 112 |
| 4Pr | 4-Propylpentadecane | $C_3$ | 210 | $C_3$ | 210 | $C_{11}$ | 98 |
| 7Bu | 7-Butyltetradecane | $C_4$ | 196 | $C_6$ | 168 | $C_7$ | 154 |
| 6Bu | 6-Butyltetradecane | $C_4$ | 196 | $C_5$ | 182 | $C_8$ | 140 |
| 5Bu | 5-Butyltetradecane | $C_4$ | 196 | $C_4$ | 196 | $C_9$ | 126 |
| 7Pe | 7-Pentyltridecane | $C_5$ | 182 | $C_6$ | 168 | $C_7$ | 154 |
| 6Pe | 6-Pentyltridecane | $C_5$ | 182 | $C_5$ | 182 | $C_8$ | 140 |
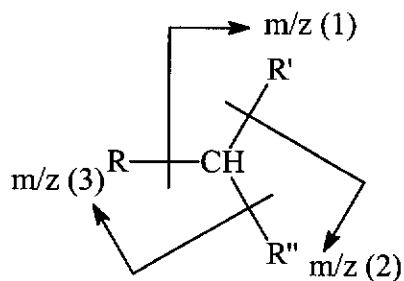


Figure 4.3. Predicted mass fragmentation pattern of monoalkylalkanes.

Table 4.2. Experimental and calculated $^{13}$C NMR chemical shifts (ppm) for 3-ethylhexadecane, 4-propylpentadecane, 5-butyltetradecane and 6-pentyltridecane.

| Carbon | 3-ethylhexadecane | | 4-propylpentadecane | | 5-butyltetradecane | | 6-pentyltridecane | |
|---|---|---|---|---|---|---|---|---|
| | Experimental | Calculated | Experimental | Calculated | Experimental | Calculated | Experimental | Calculated |
| 1 | 10.9 | 11.7 | 14.5 | 14.3 | 14.2 | 14.0 | 14.1 | 13.9 |
| 2 | 25.5 | 27.4 | 19.8 | 20.9 | 23.2 | 23.4 | 22.8 | 23.1 |
| 3 | 40.4 | 39.8 | 36.2 | 37.1 | 29.8 | 30.3 | 32.5 | 32.8 |
| 4 | 32.8 | 34.3 | 37.0 | 34.8 | 33.4 | 34.7 | 26.2 | 27.8 |
| 5 | 26.8 | 28.0 | 33.7 | 34.9 | 37.2 | 35.4 | 33.7 | 35.0 |
| 6 | 30.2 | 30.7 | 26.7 | 28.2 | 33.8 | 35.1 | 37.5 | 35.6 |
| 7 | 29.8 | 30.4 | 30.2 | 30.7 | 26.8 | 28.2 | 33.7 | 35.1 |
| 8 | 29.8 | 30.3 | 29.8 | 30.4 | 30.2 | 30.7 | 26.8 | 28.2 |
| 9 | 29.8 | 30.3 | 29.8 | 30.3 | 29.8 | 30.4 | 30.2 | 30.6 |
| 10 | 29.8 | 30.3 | 29.8 | 30.3 | 29.4 | 30.2 | 29.5 | 30.0 |
| 11 | 29.8 | 30.3 | 29.8 | 30.2 | 29.0 | 29.9 | 32.0 | 32.4 |
| 12 | 29.8 | 30.2 | 29.4 | 29.9 | 32.0 | 32.4 | 22.8 | 23.0 |
| 13 | 29.5 | 29.9 | 32.0 | 32.4 | 22.7 | 23.0 | 14.1 | 13.9 |
| 14 | 32.0 | 32.4 | 22.8 | 23.0 | 14.2 | 13.9 | | |
| 15 | 22.8 | 23.0 | 14.1 | 13.9 | | | | |
| 16 | 14.1 | 13.9 | | | | | | |

### 4.2.2 Identification of monoalkylalkanes in crude oils

To facilitate the analyses, the saturated hydrocarbons were isolated from several crude oils using silica gel column chromatography. The branched/cyclic fractions were then isolated from the saturate fractions by removal of most of the $n$-alkanes and monomethylalkanes by sorption using ZSM-5 molecular sieves (West et al., 1990). The $C_{18}$ monoalkylalkanes in the crude oils were identified by recording the four mass chromatograms ($m/z$ 224, 210, 196 and 182), with the results from the Horse Creek crude oil shown in Figure 4.4. These correspond to the characteristic fragment ions of compounds with ethyl-, propyl-, butyl- and pentyl-substituent groups, respectively. For comparison, Figure 4.4e shows the summed mass chromatogram ($m/z$ 224 + 210 + 196 + 182) for the mixture of four authentic monoalkylalkane reference compounds.

The $C_{18}$ ethylalkane structural isomers were identified in the crude oils by co-chromatography and comparison of mass spectra with the authentic standard for 3-ethylhexadecane, and by comparison of predicted mass fragmentation patterns and GC retention behaviour for the remaining five isomers. As an example, the $m/z$ 224 mass chromatogram in Figure 4.4a shows $C_{18}$ compounds containing an ethyl substituent in the Horse Creek crude oil. The peak labelled 3Et was identified as 3-ethylhexadecane by co-chromatography and comparison of its mass spectrum with the authentic standard (the peak labelled 3Et in Figure 4.4e). The remaining five possible ethylhexadecane isomers were also identified in the chromatogram on the basis of their retention times relative to that of 3-ethylhexadecane: it is expected that, in an analogous manner to the monomethylalkanes, isomers with the side chain closer to the centre of the molecule will have shorter GC retention times (Kissin and Feulmer, 1986). The mass spectra of all these compounds were examined and found to contain all of the ions characteristic of branched alkanes, corresponding to "cleavage and charge retention at the branched carbon" (McLafferty and Turecek, 1993). The predicted diagnostic $m/z$ values for these ions are listed in Table 4.1.
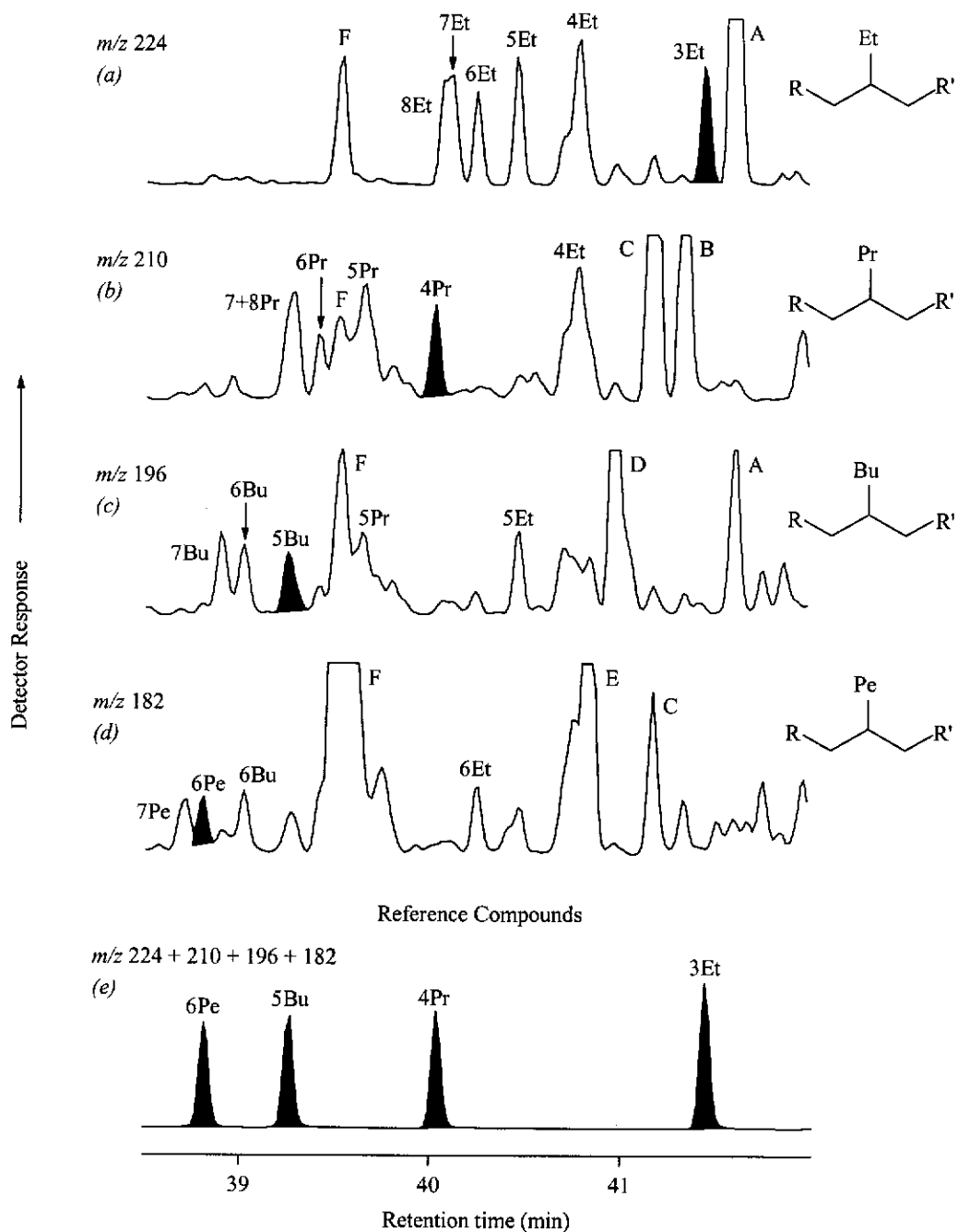
Figure 4.4. Partial mass chromatograms ($m/z$ 224, 210, 196, 182) of the branched/cyclic hydrocarbon fraction from the Horse Creek crude oil ($a$ - $d$) and the corresponding combined partial mass chromatogram ($m/z$ 224+210+196+182) from the mixture of the $C_{18}$ monoalkylalkane reference compounds ($e$). (A: 3-methylheptadecane; B: 2-methylheptadecane; C: 4-methylheptadecane; D: 5-methylheptadecane; E: 6-methylheptadecane; F: pristane; refer to Table 4.1 for other peak labels.)

The $C_{18}$ monoalkylalkanes with a propyl-, butyl- or pentyl- substituent were also identified in the crude oils. Using the Horse Creek crude oil as an example, 4-propylpentadecane (4Pr), 5-butyltetradecane (5Bu) and 6-pentyltridecane (6Pe) were identified in Figures 4.4*b*, 4.4*c* and 4.4*d* respectively by co-chromatography and comparison of mass spectra with the authentic standards (shown in Figure 4.4*e*). The remaining four propylpentadecane isomers, two butyltetradecane isomers and one pentyltridecane isomer were identified in Figures 4.4*b*, 4.4*c* and 4.4*d* respectively on the basis of their GC retention behaviour relative to the appropriate authentic standard in a similar manner to the ethylhexadecane isomers. Again, as with the ethylhexadecane isomers, the mass spectra of all these compounds were examined and found to contain all of the predicted diagnostic ions as listed in Table 4.1.

This approach demonstrated that all 16 possible $C_{18}$ monoalkylalkanes containing side chains ranging from two to five carbon atoms in length are present in the crude oils investigated. It was also observed that all isomers with a given side-chain length are present in similar relative concentrations. In the Horse Creek crude oil, the ethylalkanes accounted for 51 % of the $C_{18}$ monoalkylalkanes present, the propylalkanes 28 %, the butylalkanes 14 % and the pentylalkanes 7 %, with the other crude oils giving similar values. Partial mass chromatograms showing the $C_{18}$ monoalkylalkanes in the other crude oils studied are shown in Figure 4.5. A summary of all of the monoalkylalkanes identified in the crude oils is given in Table 4.3.

Further analogous investigations of the crude oils indicated that similar series of monalkylalkanes ranging from $C_{10}$ to $C_{20}$ were also present. Some isomers could not be positively identified on the basis of mass spectra, owing to their co-elution with other compounds present, including isoprenoids and alkylcyclohexanes. However, in these cases, diagnostic ions for the particular monoalkylalkane were always observed in the mass spectra at the retention time of the combined peak formed by monoalkylalkanes and co-eluting compounds.
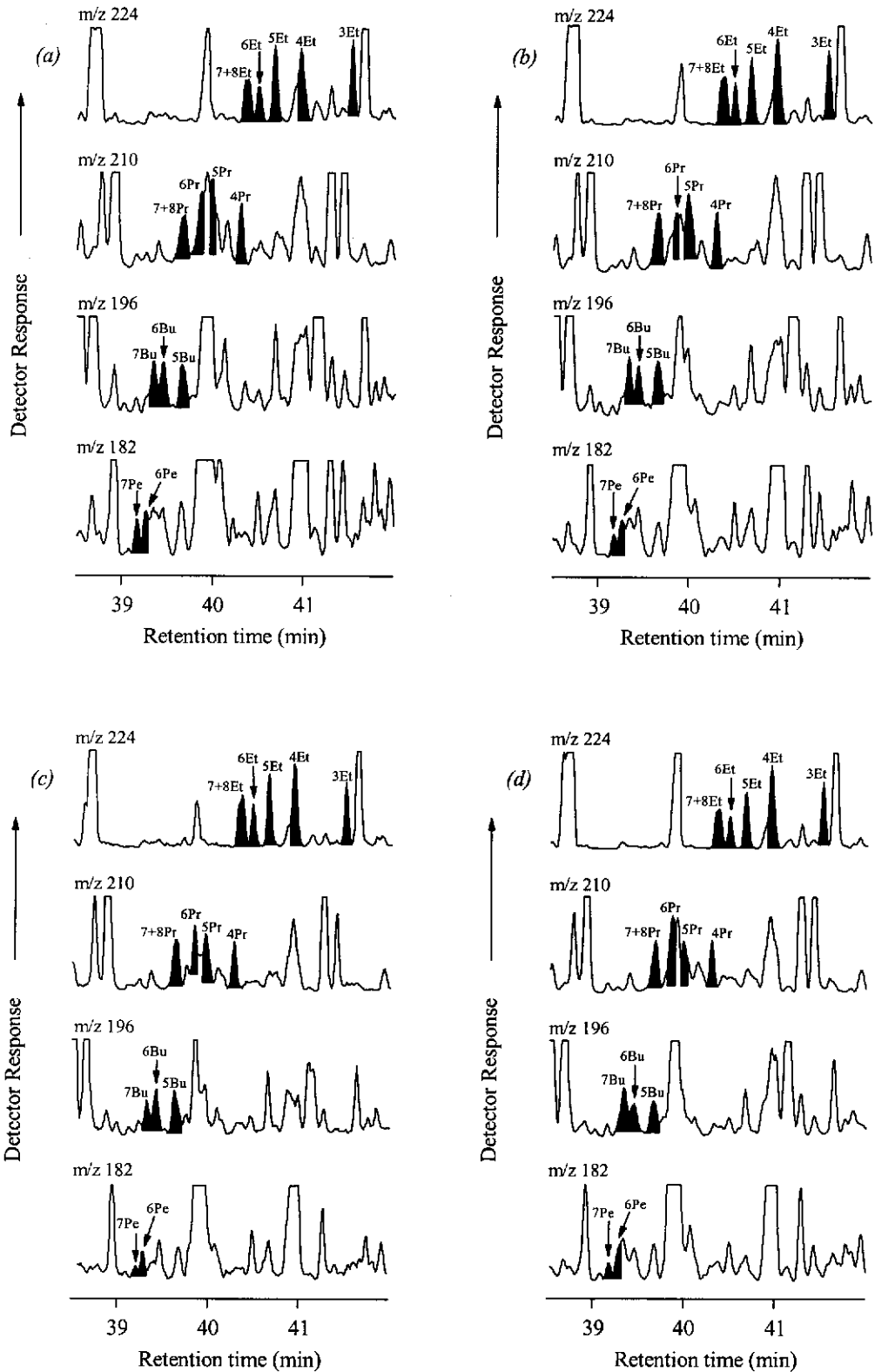
Figure 4.5. Partial mass chromatograms (*m/z* 224, 210, 196 and 182) showing the C₁₈ monoalkylalkane components of (a) Great Sandy; (b) Dongara; (c) Barrow Deep; and, (d) Petapahan crude oils (For peak identifications see Figure 4.4).

For monoalkylalkanes with carbon numbers above about 20, the analysis becomes difficult owing to the large number of isomers present and co-elution with other compounds; however, the series appear to extend over the carbon number range of the oil. For example, the ethylalkane isomers from $C_{21}$ to $C_{25}$ were also tentatively identified in the crude oils on the basis of mass spectra and expected GC retention behaviour (see Table 4.3). In each case, isomers with substituents in positions 3 to 7 were resolved, and a peak was observed corresponding to each isomer with a substituent in a position more central than the 7 position. In a situation analogous to the monomethylalkanes, these centrally-branched isomers were not resolved by gas chromatography under the conditions used. However, in all cases, mass spectra obtained at the retention time of the peak formed by co-eluting isomers contained all of the diagnostic ions corresponding to the isomers expected to be present.

Crude oils analysed ranged in age from Ordovician to Tertiary, and source types included both marine and non-marine. As with the Horse Creek crude oil, within each set of isomers the relative concentrations of the individual isomers with a given side-chain length is broadly similar. This suggests that a formation mechanism involving equilibration of intermediate species may have been involved, perhaps reflecting their origins from geosynthetic processes. However, the presence of 4- and 6-ethyl-branched fatty acids in biological systems has been reported (Sugiyama *et al.*, 1981, 1986; Birch *et al.*, 1989; Hillbrick *et al.*, 1995), and defunctionalisation of such compounds may account for the presence of some monoalkylalkanes in crude oils.

Table 4.3. All $C_{10}$ to $C_{20}$ monoalkylalkanes (branch greater than methyl), and $C_{21}$ to $C_{25}$ ethylalkanes identified in crude oils.

| Compound Name | Barrow Deep | Dongara | Great Sandy | Horse Creek | Petapahan |
|---|---|---|---|---|---|
| $C_{10}$ | | | | | |
| 4-ethyloctane | ✓ | ✓ | A | ✓ | ✓ |
| 3-ethyloctane | B | ✓ | B | ✓ | B |
| 4-propylheptane | ✓ | ✓ | ✓ | ✓ | ✓ |
| $C_{11}$ | | | | | |
| 5-ethylnonane | ✓ | ✓ | ✗ | ✓ | ✓ |
| 4-ethylnonane | ✓ | ✓ | ✗ | ✓ | ✓ |
| 3-ethylnonane | ✓ | ✓ | ✗ | ✓ | ✓ |
| 4-propyloctane | ✗ | ✗ | ✗ | ✓ | ✗ |
| $C_{12}$ | | | | | |
| 5-ethyldecane | A | A | A | ✓ | A |
| 4-ethyldecane | A | ✓ | ✓ | ✓ | A |
| 3-ethyldecane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 5-propylnonane | ✓ | ✓ | ✗ | ✓ | ✓ |
| 4-propylnonane | ✓ | ✓ | ✗ | ✓ | ✓ |
| $C_{13}$ | | | | | |
| 6-ethylundecane | B | B | A | ✓ | B |
| 5-ethylundecane | A | A | ✓ | ✓ | A |
| 4-ethylundecane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 3-ethylundecane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 5-propyldecane | A | A | ✓ | ✓ | A |
| 4-propyldecane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 5-butylnonane | A | ✓ | A | ✓ | ✓ |
| $C_{14}$ | | | | | |
| 6-ethyldodecane | A | A | ✓ | ✓ | A |
| 5-ethyldodecane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 4-ethyldodecane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 3-ethyldodecane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 6-propylundecane | A | ✓ | A | ✓ | A |
| 5-propylundecane | B | B | A | ✓ | B |
| 4-propylundecane | ✓ | ✓ | A | ✓ | ✓ |
| 5-butyldecane | A | B | A | ✓ | B |
| $C_{15}$ | | | | | |
| 7-ethyltridecane | A | A | A | ✓ | B |
| 6-ethyltridecane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 5-ethyltridecane | ✓ | ✓ | ✓ | ✓ | A |
| 4-ethyltridecane | B | A | A | ✓ | B |
| 3-ethyltridecane | A | ✓ | A | ✓ | ✓ |
| 6-propyldodecane | A | ✓ | ✓ | ✓ | B |
| 5-propyldodecane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 4-propyldodecane | A | ✓ | ✓ | ✓ | ✓ |
| 6-butylundecane | B | B | A | A | B |
| 5-butylundecane | A | ✓ | ✓ | ✓ | A |
| $C_{16}$ | | | | | |
| 7-ethyltetradecane | A | A | ✓ | ✓ | ✓ |
| 6-ethyltetradecane | A | ✓ | ✓ | ✓ | A |
| 5-ethyltetradectane | ✓ | ✓ | ✓ | ✓ | ✓ |

Table 4.3 (continued).  All $C_{10}$ to $C_{20}$ monoalkylalkanes (branch greater than methyl), and $C_{21}$ to $C_{25}$ ethylalkanes identified in crude oils.

| | Crude Oil | | | | |
|---|---|---|---|---|---|
| Compound Name | Barrow Deep | Dongara | Great Sandy | Horse Creek | Petapahan |
| $C_{16}$ (continued) | | | | | |
| 4-ethyltetradecane | ✓ | ✓ | A | ✓ | ✓ |
| 3-ethyltetradecane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 7-propyltridecane | B | A | A | ✓ | A |
| 6-propyltridecane | B | A | A | ✓ | B |
| 5-propyltridecane | ✓ | ✓ | ✓ | ✓ | A |
| 4-propyltridecane | A | A | A | ✓ | B |
| 6-butyldodecane | B | ✗ | B | ✓ | B |
| 5-butyldodecane | A | A | B | A | B |
| 6-pentylundecane | B | B | B | ✓ | B |
| $C_{17}$ | | | | | |
| 8-ethylpentadecane | A | A | A | ✓ | A |
| 7-ethylpentadecane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 6-ethylpentadecane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 5-ethylpentadecane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 4-ethylpentadecane | ✓ | ✓ | A | ✓ | ✓ |
| 3-ethylpentadecane | A | A | A | ✓ | A |
| 7-propyltetradecane | A | A | A | ✓ | A |
| 6-propyltetradecane | A | ✓ | A | ✓ | A |
| 5-propyltetradecane | A | A | A | ✓ | A |
| 4-propyltetradecane | A | A | A | A | A |
| 7-butyltridecane | B | B | B | ✓ | B |
| 6-butyltridecane | A | ✓ | A | ✓ | A |
| 5-butyltridecane | A | ✓ | A | ✓ | B |
| 6-pentyldodecane | A | ✓ | ✓ | ✓ | A |
| $C_{18}$ | | | | | |
| 8-ethylhexadecane | ✓ | ✓ | ✓ | ✓ | A |
| 7-ethylhexadecane | A | A | ✓ | ✓ | A |
| 6-ethylhexadecane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 5-ethylhexadecane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 4-ethylhexadecane | ✓ | A | A | ✓ | A |
| 3-ethylhexadecane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 8-propylpentadecane | A | A | A | ✓ | A |
| 7-propylpentadecane | A | A | A | ✓ | A |
| 6-propylpentadecane | A | A | B | ✓ | B |
| 5-propylpentadecane | A | A | A | ✓ | A |
| 4-propylpentadecane | A | A | A | ✓ | A |
| 7-butyltetradecane | A | ✓ | A | ✓ | A |
| 6-butyltetradecane | A | ✓ | A | ✓ | ✓ |
| 5-butyltetradecane | A | A | A | ✓ | A |
| 7-pentyltridecane | A | ✓ | A | ✓ | ✓ |
| 6-pentyltridecane | ✓ | ✓ | A | ✓ | A |
| $C_{19}$ | | | | | |
| 9-ethylheptadecane | ✓ | A | A | ✓ | ✓ |
| 8-ethylheptadecane | ✓ | A | ✓ | ✓ | ✓ |
| 7-ethylheptadecane | A | A | ✓ | ✓ | A |
| 6-ethylheptadecane | ✓ | ✓ | A | ✓ | A |
| 5-ethylheptadecane | ✓ | ✓ | ✓ | ✓ | ✓ |

Table 4.3 (continued). All $C_{10}$ to $C_{20}$ monoalkylalkanes (branch greater than methyl), and $C_{21}$ to $C_{25}$ ethylalkanes identified in crude oils.

| Compound Name | Crude Oil | | | | |
|---|---|---|---|---|---|
| | Barrow Deep | Dongara | Great Sandy | Horse Creek | Petapahan |
| $C_{19}$ (continued) | | | | | |
| 4-ethylheptadecane | ✓ | ✓ | A | ✓ | A |
| 3-ethylheptadecane | ✓ | ✓ | ✓ | ✓ | A |
| 8-propylhexadecane | A | ✓ | A | ✓ | A |
| 7-propylhexadecane | ✓ | ✓ | A | ✓ | ✓ |
| 6-propylhexadecane | A | A | A | A | B |
| 5-propylhexadecane | ✓ | A | A | ✓ | A |
| 4-propylhexadecane | A | A | ✓ | A | A |
| 8-butylpentadecane | A | A | A | ✓ | A |
| 7-butylpentadecane | ✓ | A | ✓ | ✓ | A |
| 6-butylpentadecane | ✓ | ✓ | ✓ | ✓ | A |
| 5-butylpentadecane | A | A | A | ✓ | A |
| 7-pentyltetradecane | ✓ | ✓ | ✓ | ✓ | A |
| 6-pentyltetradecane | ✓ | A | A | ✓ | A |
| 7-hexyltridecane | A | B | B | A | B |
| $C_{20}$ | | | | | |
| 9-ethyloctadecane | A | A | A | A | A |
| 8-ethyloctadecane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 7-ethyloctadecane | ✓ | ✓ | ✓ | ✓ | A |
| 6-ethyloctadecane | ✓ | ✓ | A | ✓ | A |
| 5-ethyloctadecane | A | A | A | A | A |
| 4-ethyloctadecane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 3-ethyloctadecane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 9-propylheptadecane | A | B | A | ✓ | B |
| 8-propylheptadecane | A | B | B | ✓ | B |
| 7-propylheptadecane | A | B | A | ✓ | B |
| 6-propylheptadecane | ✓ | ✓ | A | ✓ | A |
| 5-propylheptadecane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 4-propylheptadecane | ✓ | ✓ | ✓ | ✓ | A |
| 8-butylhexadecane | A | A | B | ✓ | B |
| 7-butylhexadecane | ✓ | ✓ | ✓ | ✓ | A |
| 6-butylhexadecane | ✓ | A | B | ✓ | B |
| 5-butylhexadecane | × | × | × | A | × |
| 8-pentylpentadecane | × | × | × | A | × |
| 7-pentylpentadecane | × | × | × | A | × |
| 6-pentylpentadecane | B | A | A | ✓ | B |
| 7-hexyltetradecane | A | ✓ | A | ✓ | B |
| $C_{21}$ | | | | | |
| 10-ethylnonadecane | A | A | A | ✓ | A |
| 9-ethylnonadecane | A | A | ✓ | ✓ | A |
| 8-ethylnonadecane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 7-ethylnonadecane | A | A | A | A | A |
| 6-ethylnonadecane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 5-ethylnonadecane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 4-ethylnonadecane | ✓ | ✓ | ✓ | ✓ | A |
| 3-ethylnonadecane | ✓ | ✓ | ✓ | A | ✓ |

Table 4.3 (continued).  All $C_{10}$ to $C_{20}$ monoalkylalkanes (branch greater than methyl), and $C_{21}$ to $C_{25}$ ethylalkanes identified in crude oils.

| Compound Name | Barrow Deep | Dongara | Great Sandy | Horse Creek | Petapahan |
|---|---|---|---|---|---|
| **$C_{22}$** | | | | | |
| 10-ethylicosane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 9-ethylicosane | ✓ | A | ✓ | A | ✓ |
| 8-ethylicosane | A | A | A | ✓ | A |
| 7-ethylicosane | ✓ | ✓ | A | ✓ | ✓ |
| 6-ethylicosane | A | A | A | ✓ | A |
| 5-ethylicosane | ✓ | ✓ | ✓ | ✓ | A |
| 4-ethylicosane | ✓ | A | ✓ | ✓ | A |
| 3-ethylicosane | ✓ | A | ✓ | ✓ | ✓ |
| **$C_{23}$** | | | | | |
| 11-ethylhenicosane | ✓ | ✓ | A | A | A |
| 10-ethylhenicosane | ✓ | A | A | A | A |
| 9-ethylhenicosane | ✓ | ✓ | A | ✓ | ✓ |
| 8-ethylhenicosane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 7-ethylhenicosane | ✓ | A | A | A | B |
| 6-ethylhenicosane | ✓ | ✓ | A | ✓ | ✓ |
| 5-ethylhenicosane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 4-ethylhenicosane | A | A | A | ✓ | A |
| 3-ethylhenicosane | ✓ | ✓ | A | ✓ | ✓ |
| **$C_{24}$** | | | | | |
| 11-ethyldocosane | A | A | A | A | A |
| 10-ethyldocosane | A | A | ✓ | A | A |
| 9-ethyldocosane | ✓ | A | A | ✓ | A |
| 8-ethyldocosane | A | ✓ | ✓ | A | A |
| 7-ethyldocosane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 6-ethyldocosane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 5-ethyldocosane | A | ✓ | ✓ | ✓ | ✓ |
| 4-ethyldocosane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 3-ethyldocosane | ✓ | ✓ | ✓ | ✓ | ✓ |
| **$C_{25}$** | | | | | |
| 12-ethyltricosane | ✓ | A | A | A | A |
| 11-ethyltricosane | A | A | A | A | B |
| 10-ethyltricosane | ✓ | A | A | A | B |
| 9-ethyltricosane | ✓ | A | A | A | A |
| 8-ethyltricosane | ✓ | ✓ | ✓ | ✓ | A |
| 7-ethyltricosane | A | A | A | A | A |
| 6-ethyltricosane | A | A | A | A | A |
| 5-ethyltricosane | A | A | A | A | A |
| 4-ethyltricosane | ✓ | ✓ | ✓ | ✓ | ✓ |
| 3-ethyltricosane | ✓ | ✓ | ✓ | ✓ | A |

✓ = full mass spectrum obtained with little or no interference from co-eluting compounds;

A = mass spectrum containing all diagnostic ions and minor amounts of interfering ions obtained;

B = mass spectrum containing all diagnostic ions and major amounts of interfering ions obtained;

X = mass spectrum did not contain all diagnostic ions.

### 4.2.3 Abundance of monoalkylalkanes in crude oils

The abundances of the monoalkylalkanes as a percentage of the saturated hydrocarbon fraction of the crude oils investigated are given in Table 4.4, together with the percent abundances of some other major components determined using GC-FID and GC-MS. The percent abundances of the sum of the $C_{20}$ monoalkylalkanes in a one-carbon-number-wide window of the saturated hydrocarbon chromatogram range from 2.9% in the Petapahan crude oil sample to 3.8% in the Horse Creek and Dongara-17 crude oil samples. The abundance of the monoalkylalkanes as a percentage of the total saturated hydrocarbons thus exhibits little variation in the series of crude oils examined in this study.

Table 4.4. Relative concentrations of the various classes of compounds identified in the crude oil samples investigated.

| Crude Oil | Concentration (%) [a] | | | |
| | $n$-$C_{20}$ [b] | Methylalkanes [c] | Monoalkylalkanes [d] | Other [e] |
|---|---|---|---|---|
| Horse Creek | 62.3 | 11.5 | 3.8 | 22.4 |
| Barrow Deep | 34.3 | 9.3 | 3.1 | 53.3 |
| Great Sandy | 34.0 | 10.2 | 3.4 | 52.4 |
| Dongara-17 | 45.4 | 11.3 | 3.8 | 39.5 |
| Petapahan | 59.9 | 8.7 | 2.9 | 28.5 |

a: Percent concentration of components eluting on a gas chromatograph in a one-carbon-number-wide window between $n$-$C_{19}$ and $n$-$C_{20}$ (including $n$-$C_{20}$); b: $C_{20}$ normal alkane; c: sum of all $C_{20}$ methylalkanes; d: sum of all $C_{20}$ monoalkylalkanes; e: sum of all other saturated hydrocarbons with GC retention times between $n$-$C_{20}$ and $n$-$C_{20}$, including isoprenoids, alkylcyclohexanes, etc.

## 4.3    CONCLUSIONS

Crude oils of various ages and source types have been shown to contain monoalkylalkanes as mixtures of all side chain position and length isomers where the relative concentrations of individual isomers with a given side chain length at a given carbon number are similar.  This observation suggests that in mature hydrocarbon mixtures these compounds are formed predominantly *via* geosynthetic processes.  The concentration of the $C_{20}$ monoalkylalkanes range from 2.9% to 3.8% of the total saturated hydrocarbons with retention times on a gas chromatogram between $n$-$C_{19}H_{40}$ and $n$-$C_{20}H_{42}$ for the crude oils analysed.

# CHAPTER FIVE

# THE EFFECT OF MATURATION ON THE DISTRIBUTION OF MONOETHYLALKANES IN SEDIMENTARY ROCKS AND CRUDE OILS

## 5.1 INTRODUCTION

Branched hydrocarbons comprise a significant proportion of the saturated hydrocarbon fraction of crude oils (Tissot and Welte, 1984). Those compounds comprising a linear carbon chain with a single branch have been referred to as T-branched alkanes (cf. Gough and Rowland, 1990; Gough, et al., 1992). The most widely studied of these compounds are the monomethylalkanes, although compounds with ethyl-, propyl-, and butyl-containing structural fragments have also been reported. It has been suggested that the aliphatic unresolved complex mixture (UCM) in crude oils may be comprised partly of monoalkylalkanes (Gough and Rowland, 1990; Killops and Al-Juboori, 1990). In Chapter Four, all possible monoalkylalkane structural isomers with a single $n$-alkyl branch larger than methyl in the range $C_{10}$ to $C_{20}$ as well as all ethylalkanes from $C_{21}$ to $C_{25}$ were identified in a series of crude oils of varying source types and depositional environments. It was observed that monoalkylalkanes comprise from 2.9% to 3.8% of the saturated hydrocarbons with retention times on a gas chromatogram between $n$-$C_{19}H_{40}$ and $n$-$C_{20}H_{42}$ for the series of crude oils investigated.

Several origins of the monomethylalkanes have been proposed to account for their presence in crude oils and sediments. These include: (i) direct biological input of monomethylalkanes (Fowler and Douglas, 1987); (ii) transformation of functionalised precursors such as carboxylic acids (Summons, 1987; Summons et al., 1988a); (iii) long-term equilibration of a limited number of isomers (Hoering, 1980; Klomp, 1986); and, (iv) acid-catalysed reactions of alkenes formed by thermal cracking (Kissin, 1987). Much less has been reported in the literature on the origins of monoalkylalkanes with the side chain greater than a methyl group. Gough and Rowland (1990) have proposed that these compounds may be produced from polymethylene precursors by chain scission and recombination. On the other hand, the presence of 4- and 6-ethyl-branched fatty acids in wool wax has been reported, (Sugiyama et al., 1981, 1986; Birch et al., 1989; Hillbrick et al., 1995), indicating that a biosynthetic pathway exists for the formation of some of these compounds. Defunctionalisation of such compounds from more

ancient, as yet unidentified source organisms, may account for the presence of some of the monoalkylalkanes found in crude oils.

In this chapter, the occurrence of monoethylalkanes in a crude oil, a lignite and in a sedimentary sequence is reported. The possible origins of these compounds is discussed, along with the effect of thermal maturity on their distribution in sedimentary organic matter. Rock samples from a sedimentary sequence of Late Cretaceous age were analysed for ethylalkanes using GC-MS techniques. In the less mature samples, 3- and 5-ethylalkanes were in higher abundance relative to the other isomers at odd carbon numbers from $C_{17}$ to $C_{23}$. In the more mature samples, this odd preference was no longer apparent. Several other lower maturity samples were analysed and found to have a similar ethylalkane distribution to the shallow sample from the sedimentary sequence. A mature crude oil which was also analysed exhibited a distribution similar to the deeper sample from the sedimentary sequence. It is suggested that the initial odd preference is the result of defunctionalisation of specific natural product precursors, and that with increasing maturity this preference is diluted by input of ethylalkanes without preference. A mechanism involving carbocation rearrangement of n-alkenes to form monoalkylalkanes is proposed to account for the change in relative ethylalkane abundances.

## 5.2 RESULTS AND DISCUSSION

### 5.2.1 Analysis of ethylalkanes in sedimentary organic matter

The analysis of ethylalkane isomers in sedimentary organic matter was made on the basis of interpretation of mass spectra and gas chromatographic retention characteristics as described in Chapter Four. Briefly, the samples were analysed using gas chromatography-mass spectrometry (GC-MS) with the mass spectrometer in magnet selected ion recording mode. The ethylalkane isomers were analysed by monitoring the M–30 even-mass ion which corresponds to loss of the ethyl portion of the molecule. In a manner analogous to the monomethylalkanes, the GC elution order of the compounds is determined by the position of the ethyl branch - the closer the branch to the end of the molecule, the longer the retention time.

### 5.2.2 Relative abundances of ethylalkanes in a sedimentary sequence

Low maturity samples from the Grunter-1 sedimentary sequence were analysed for ethylalkanes. In Figure 5.1, the mass chromatograms show responses for the ethylalkanes from $C_{19}$ to $C_{22}$ in the sample obtained from a depth of 2285-95m. In the mass chromatograms for the $C_{19}$ and $C_{21}$ ethylalkanes, it is apparent that the isomers substituted in positions 3 and 5 are in higher abundances relative to the other ethylalkane isomers. On the other hand, in the mass chromatograms for the $C_{20}$ and $C_{22}$ ethylalkanes, the isomers substituted in positions 3 and 5 are present in similar abundances to the other isomers. This apparent preference for ethylalkane isomers substituted at positions 3 and 5 was also observed with the $C_{17}$ and $C_{23}$ homologues, but not with the $C_{16}$, $C_{18}$ and $C_{24}$ homologues. It was also observed that ethylalkanes with the substituent at the 4- and 6-positions on the molecule were in slightly higher relative abundance in the mass chromatograms with responses from the even carbon numbered homologues. Other major peaks in the mass chromatograms of Figure 5.1 correspond to methylalkanes and other branched alkanes as indicated.
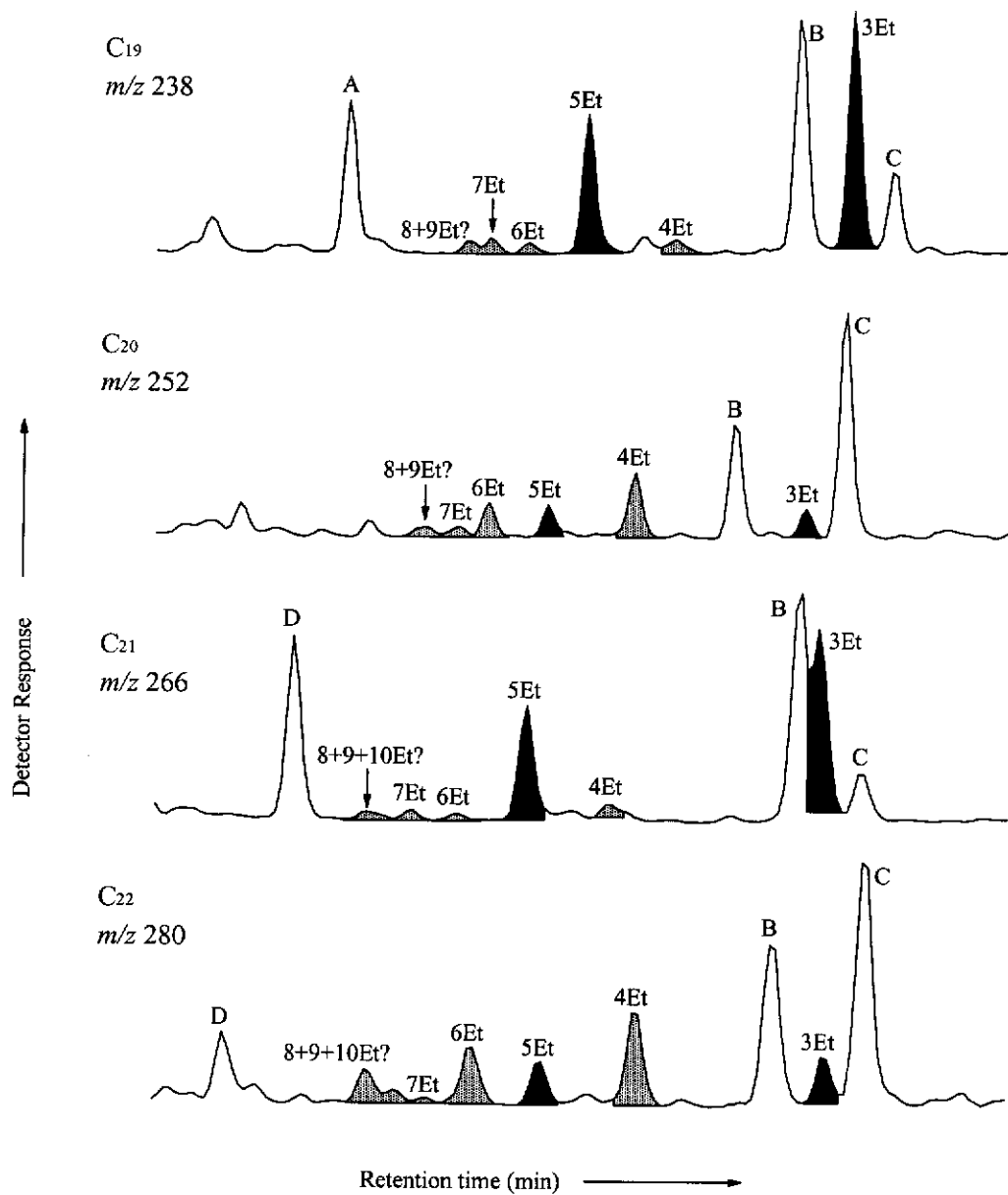
Figure 5.1. Partial mass chromatograms of the saturated hydrocarbon fraction from the low maturity 2285-95m Grunter-1 sediment sample showing the $C_{19}$ to $C_{22}$ ethylalkanes (Et: ethylalkane; A: phytane; B: unknown but mass spectra indicate probably branched alkanes); C: 3-methylalkane; D: unknown but mass spectra indicate probably polymethylalkanes).
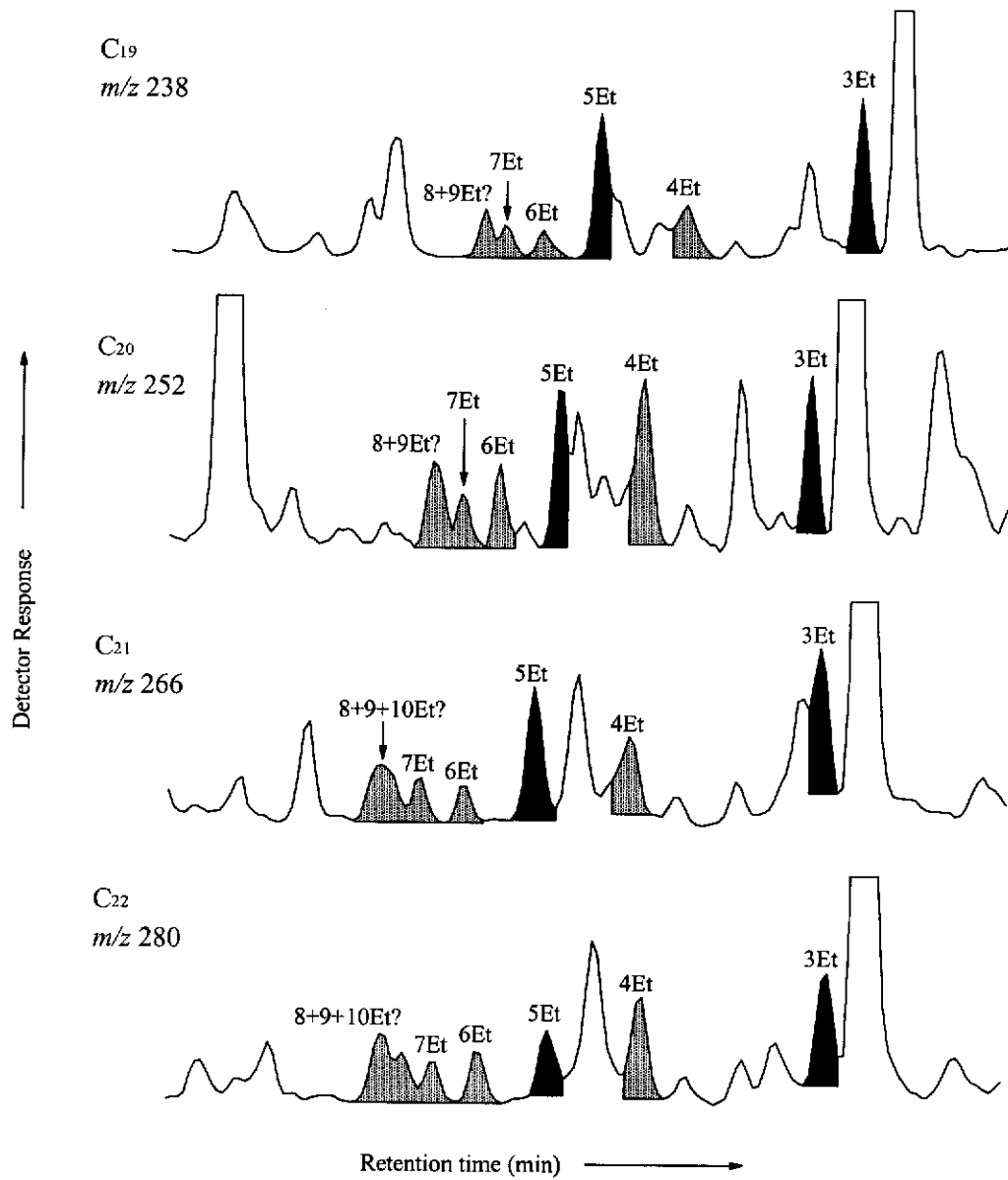
Figure 5.2. Partial mass chromatograms of the saturated hydrocarbon fraction from the high maturity 3676m Grunter-1 sediment sample showing the $C_{19}$ to $C_{22}$ ethylalkanes (Et: ethylalkane; A: phytane; B: unknown but mass spectra indicate probably branched alkanes); C: 3-methylalkane; D: unknown but mass spectra indicate probably polymethylalkanes).

High maturity samples were also analysed for ethylalkanes. Figure 5.2 shows the mass chromatograms for the ethylalkanes from $C_{19}$ to $C_{22}$ in the Grunter-1 sedimentary sequence sample obtained from a depth of 3676m. It is apparent that, when compared with the mass chromatograms in Figure 5.1, the odd carbon number isomers substituted at positions 3 and 5 exhibit a much less significant preference over the other isomers. This was also the case at $C_{17}$ and $C_{23}$. For even carbon number isomers, all ethylalkane isomers were present in similar abundances (varying by not more than a factor of two).

The abundance of the ethylalkanes relative to the total saturated hydrocarbons is demonstrated in Figures 5.3 and 5.4 respectively. The total ion chromatogram obtained from analysis of the lower maturity sedimentary sequence sample, together with the m/z 238, 252, 266 and 280 mass chromatograms from Figure 5.1, are given in Figure 5.3, while the corresponding chromatograms for the higher maturity sample are given in Figure 5.4. It is apparent from Figures 5.3 and 5.4 that the ethylalkanes are significant components of the saturated hydrocarbon fraction of the immature sedimentary sequence sample, and are relatively less abundant in the higher maturity sample. It was stated in Chapter Four that the monoalkylalkanes comprise approximately 3-4% of the crude oil saturated hydrocarbons eluting between $n$-$C_{19}H_{40}$ and $n$-$C_{20}H_{42}$ on a gas chromatograph. In the higher maturity sedimentary sequence samples examined in this study, it was estimated that the ethylalkanes comprised approximately 0.5-1.5 % of the extracted saturated hydrocarbons.

The branched/cyclic (ZSM-5 excluded) fractions of the immature Loy Yang lignite and the Tuna-4 crude oil (both from the Gippsland Basin, Australia) were also analysed for ethylalkanes. As was observed in the immature sediment sample shown in Figure 5.1, the isomers substituted in positions 3 and 5 were in high abundance relative to the other ethylalkane isomers for the odd carbon number homologues, but not for the even number homologues in the lignite (cf. Table 5.1). On the other hand, the individual isomers at a given carbon number are present in approximately equal abundances in the higher maturity crude oil (cf.
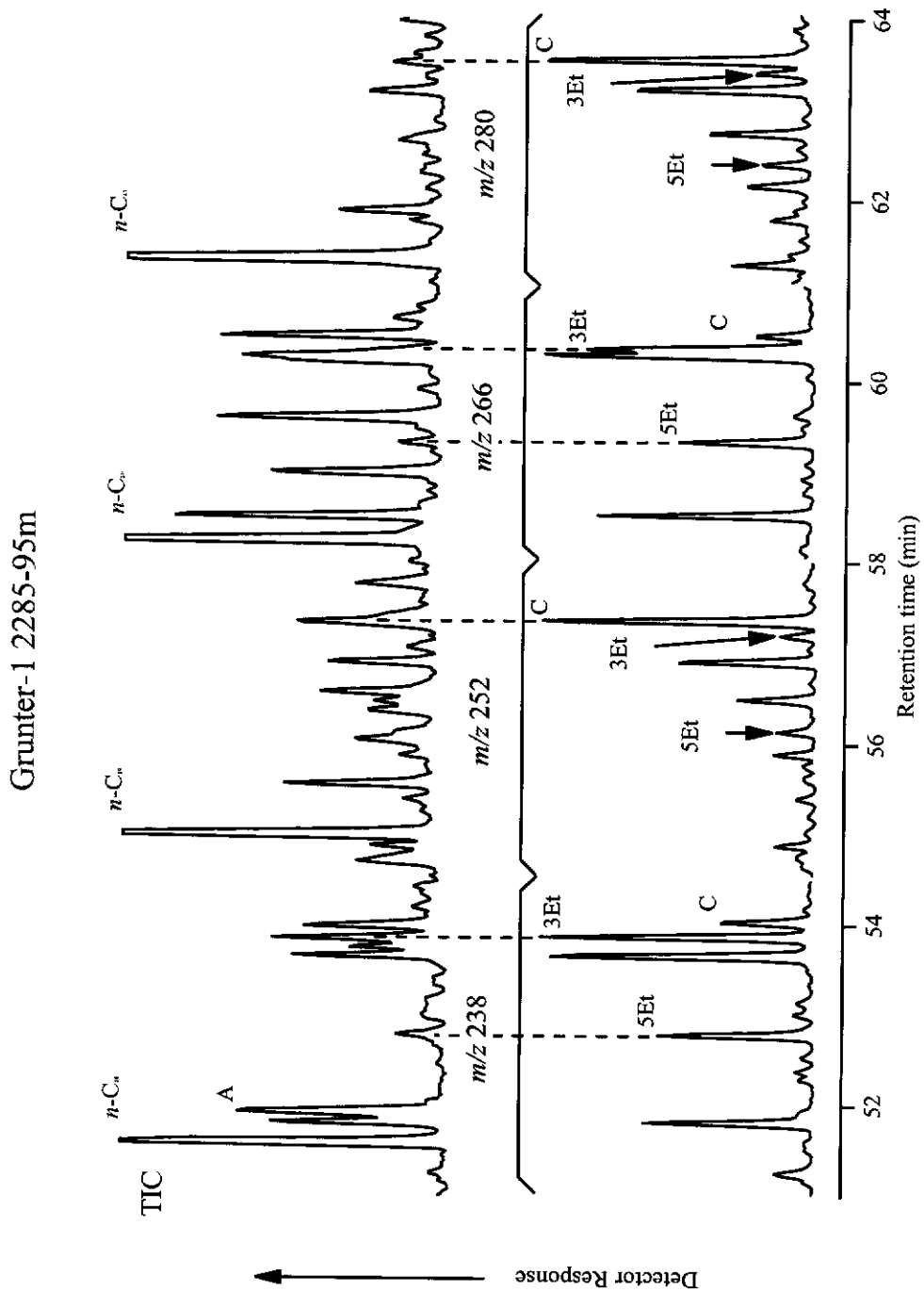
Figure 5.3. Total ion chromatogram of the saturated hydrocarbon fraction from the low maturity 2285-95m Grunter-1 sediment sample, together with the partial mass chromatograms showing the $C_{19}$ to $C_{22}$ ethylalkanes (Et: ethylalkane; A: phytane; B: unknown but mass spectra indicate probably branched alkanes); C: 3-methylalkane; D: unknown but mass spectra indicate probably polymethylalkanes).
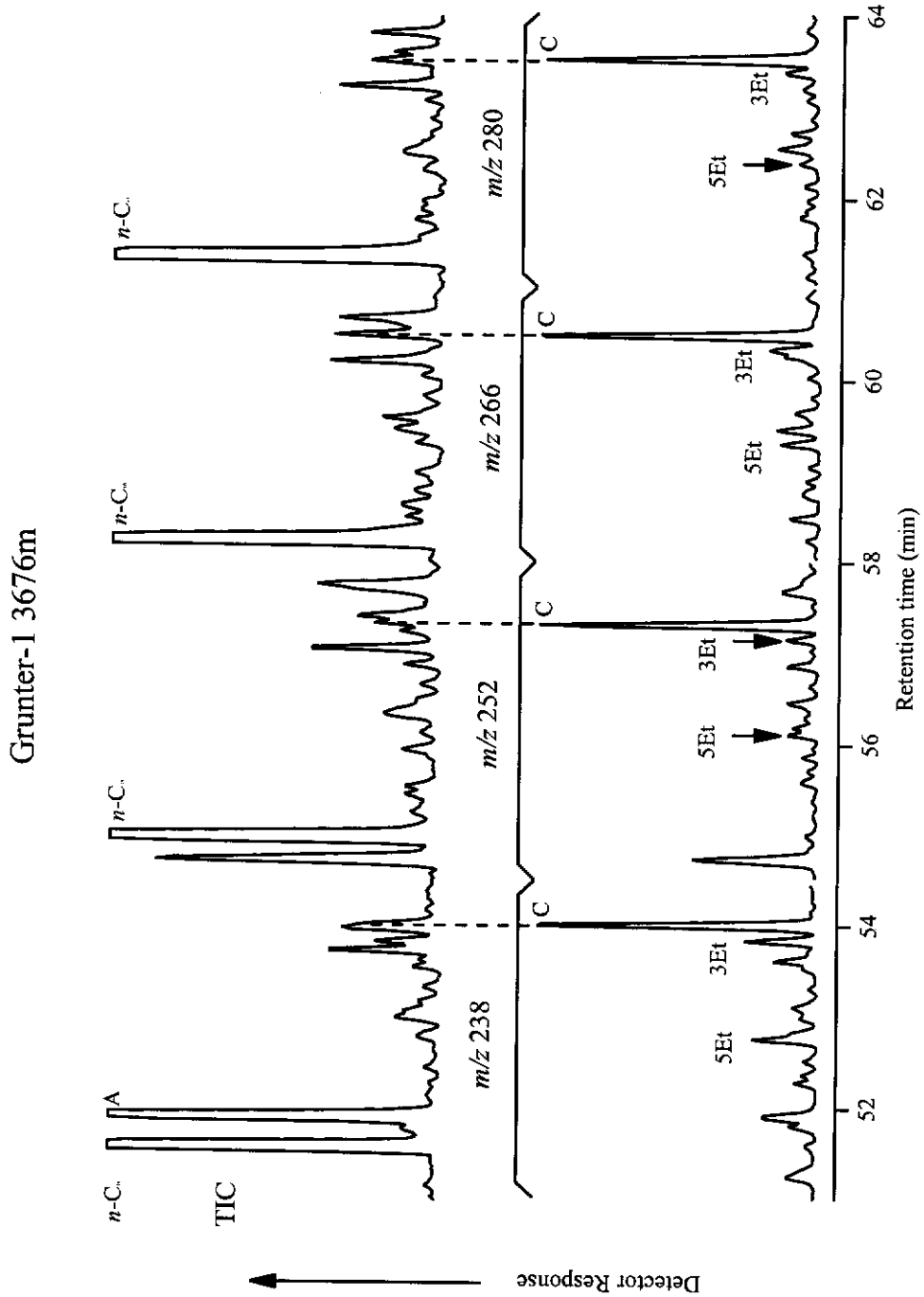
Figure 5.4. Total ion chromatogram of the saturated hydrocarbon fraction from the high maturity 3676m Grunter-1 sediment sample, together with the partial mass chromatograms showing the $C_{19}$ to $C_{22}$ ethylalkanes (Et: ethylalkane; A: phytane; B: unknown but mass spectra indicate probably branched alkanes); C: 3-methylalkane; D: unknown but mass spectra indicate probably polymethylalkanes).

Table 5.1   Values for the ratio 4Et/(4Et + 5Et) at some carbon numbers for the crude oil and sediment samples investigated (4Et: 4-ethylalkane; 5Et: 5-ethylalkane; sample details given in Chapter Three).

| Sample name | Depth (m) | $C_{19}$ | $C_{20}$ | $C_{21}$ | $C_{22}$ |
|---|---|---|---|---|---|
| Grunter-1 | 2290 | 0.05 | 0.74 | 0.12 | 0.75 |
| Grunter-1 | 2385 | 0.07 | 0.72 | 0.15 | 0.72 |
| Grunter-1 | 2395 | 0.10 | 0.71 | 0.18 | 0.72 |
| Grunter-1 | 2775 | 0.09 | 0.72 | 0.18 | 0.76 |
| Grunter-1 | 3125 | 0.18 | 0.69 | 0.27 | 0.73 |
| Grunter-1 | 3260 | 0.19 | 0.66 | 0.32 | 0.69 |
| Grunter-1 | 3455 | 0.21 | 0.66 | 0.27 | 0.67 |
| Grunter-1 | 3540 | 0.26 | 0.59 | 0.26 | 0.60 |
| Grunter-1 | 3676 | 0.35 | 0.59 | 0.46 | 0.58 |
| Loy Yang lignite | 67-68 | 0.07 | 0.71 | 0.08 | 0.71 |
| Tuna-4 oil | 2820 | 0.48 | 0.57 | 0.53 | 0.53 |
| Mercury | 2060 | 0.24 | 0.54 | 0.32 | 0.52 |
| Paqualin-1 | 2799 | 0.38 | 0.64 | 0.29 | 0.58 |
| PD 26 | 338.8 | 0.13 | 0.70 | 0.18 | 0.70 |
| ID-13 | 470 | 0.40 | 0.61 | 0.49 | 0.47 |

Table 5.1). While not having exactly the same ethylalkane distribution as the deeper, more mature sediment sample examined, the distribution much more closely resembled this than the shallower, less mature sample.

In addition to the two low maturity samples already described, low maturity shales of various ages, source types and depositional environments were observed to contain 3- and 5-ethylalkanes in high abundance relative to the other ethylalkane isomers for the odd carbon number homologues, but not for the even number homologues. These included samples of Late Cretaceous age (Exmouth Plateau, Carnarvon Basin, Australia), Upper Jurassic age (Vulcan Sub-basin, Bonaparte Basin, Australia), Devonian age (Canning Basin, Australia) and Ordovician age (Canning Basin, Australia) (see Table 5.1). These samples were also observed to have a slightly higher abundance of 4- and 6-ethylalkanes relative to other isomers at even carbon numbers.

## 5.2.3 Possible sources of ethylalkanes in sedimentary organic matter

The ethylalkanes in sedimentary organic matter have two possible sources: 1) biologically derived precursors bearing the ethylalkane carbon skeleton, but possibly containing some functionality ("biologically derived"), and 2) generation *via* sedimentary reactions from precursors having a different carbon skeleton ("geosynthetically derived").

### 5.2.3.1 Biological sources of ethylalkanes

A preference for odd-carbon-number homologues is frequently observed in the *n*-alkane distribution in immature sediments with a high contribution from land plants and is usually attributed to either the direct synthesis of these compounds by the plants or to defunctionalisation of the predominantly even-carbon-number *n*-alkyl fatty acids, alcohols or esters (eg. Tissot and Welte, 1984). As the sediments increase in maturity, *n*-alkanes without preference are released from kerogen degradation, diluting the initial odd preference until the observed CPI

value approaches 1.0. By analogy, the observed preference of odd-carbon number ethylalkane isomers with the side chain in positions 3 or 5 on the alkyl chain in the immature sediment extract and the lignite may be the result of defunctionalisation of ethyl-branched natural product precursor compounds. Also by analogy with the *n*-alkanes, this preference for certain isomers may be diluted by the addition of ethylalkanes without preference for the position of substitution, possibly *via* some geosynthetic process. Although some biologically occurring ethyl-branched fatty acids have been identified, it appears probable that the precursors to the sedimentary ethylalkanes arise from as yet unidentified source organisms. Such biologically occurring compounds may also account for the slight preference observed for 4- and 6-ethylalkanes at even carbon numbers, by reduction of the functional group without the loss of a carbon atom.

In order to determine whether functionalised ethylalkane isomers were present in sediments during diagenesis, a solvent-extracted sample of the Loy Yang lignite was heated at 330°C in the presence of tetralin, a free radical hydrogen donor (Alexander *et al.*, 1992a). The *m/z* 238, 252, 266 and 280 mass chromatograms of the reaction products are shown in Figure 5.5. It is apparent from Figure 5.5 that the 3- and 5-ethylalkanes are in high abundance relative to the other ethylalkanes for the odd carbon number isomers. This is the same preference that was observed in the immature sediment sample (see Figure 5.1). This strongly suggests that these particular compounds have natural product precursors and that they are incorporated into the higher molecular weight macromolecules formed during diagenesis.

### 5.2.3.2 Geosynthetic sources of ethylalkanes

Laboratory experiments were conducted to show that the monoalkylalkanes can form from *n*-alkenes of the same carbon number by an acid-catalysed rearrangement reaction. A sample of 1-octadecene was heated with aluminium montmorillonite , a strong Lewis acid which can also act as a proton donor, in an evacuated glass ampoule at 180°C for 20 hours. The products were extracted and
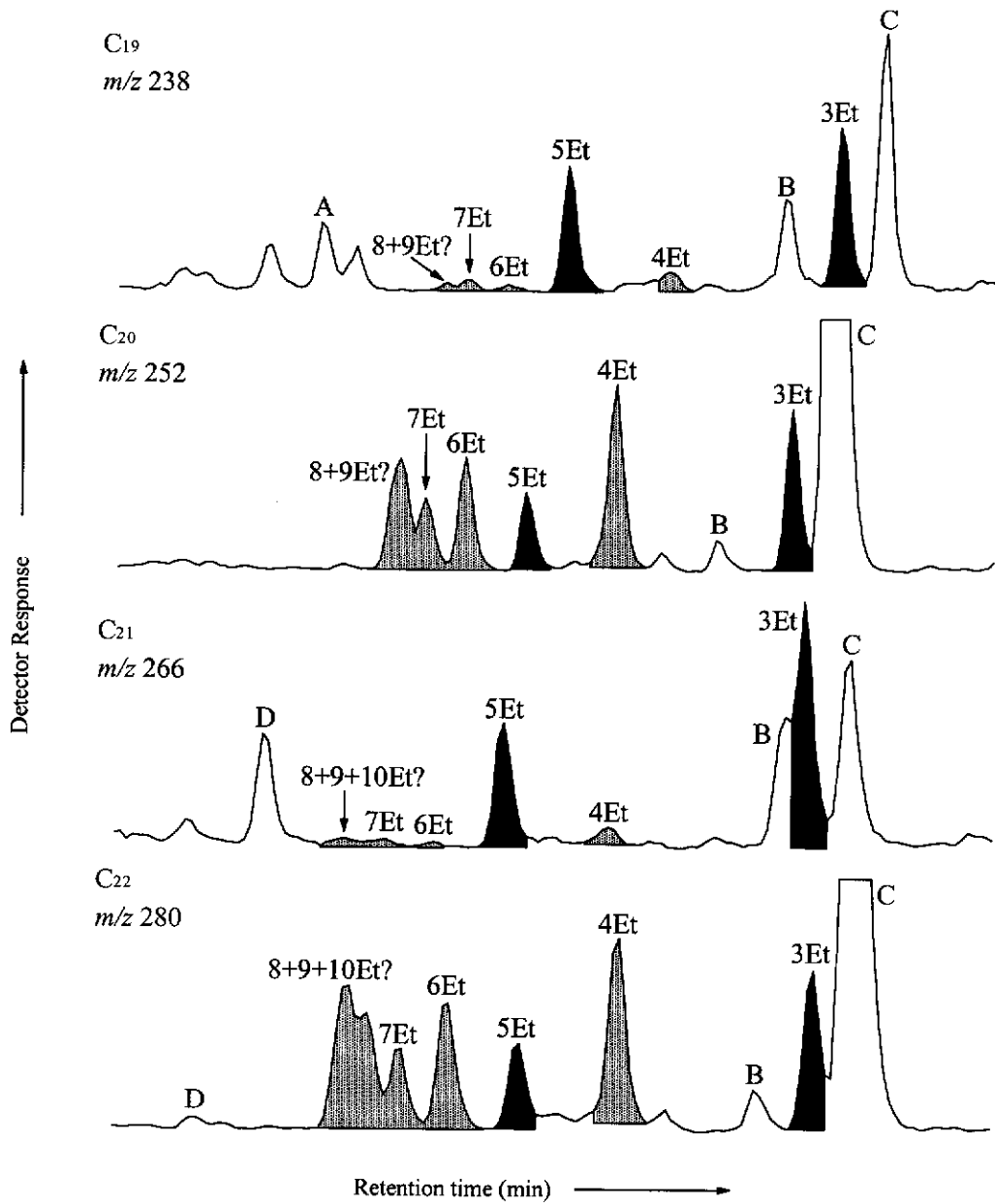
Figure 5.5. Partial mass chromatograms of the saturated hydrocarbon fraction from the tetralin pyrolysate of the solvent-extracted Loy Yang lignite showing the $C_{19}$ to $C_{22}$ ethylalkanes (Et: ethylalkane; A: phytane; B: unknown but mass spectra indicate probably branched alkanes); C: 3-methylalkane; D: unknown but mass spectra indicate probably polymethylalkanes).

subjected to silica gel column chromatography, and the saturated hydrocarbon fraction was analysed by GC-MS. Figure 5.6 shows the m/z 224, 210, 196 and 182 mass chromatograms corresponding to $C_{18}$ monoalkylalkanes with ethyl, propyl, butyl and pentyl side-chain features respectively (chromatograms $a$ - $d$), the total ion chromatogram (chromatogram $e$), and the combined m/z 224 + 210 + 196 + 182 mass chromatogram for the mixture of monoalkylalkane reference compounds (chromatogram $f$).

All of the sixteen $C_{18}$ monoalkylalkane structural isomers are present in the reaction mixture. Identifications were made by comparison of GC retention times and mass spectra with authentic reference compounds for 3-ethylhexadecane, 4-propylpentadecane, 5-butyltetradecane and 6-pentyltridecane. The remaining five ethyl, four propyl, two butyl and one pentyl isomers were identified on the basis of their predicted mass fragmentation patterns and their retention behaviour relative to the reference compound of the same chain length. In all cases, the retention times and mass spectra matched exactly those of the compounds previously identified in the high maturity Horse Creek crude oil (see Chapter Four).

A mechanism involving 1,2-alkyl shifts and 1,2-hydride shifts (Figure 5.7) is proposed to account for the formation of the reaction products observed in these laboratory experiments. The alkene is protonated, producing a secondary carbocation which, by a series of 1,2-hydride shifts, can move along the chain. A 1,2-alkyl shift followed by a 1,2-hydride shift gives the more stable tertiary carbocation via the primary carbocation (Olah and Molnár, 1995). This tertiary carbocation can then be quenched to form the monomethylalkane. The small equilibrium proportion of secondary carbocation may also undergo another 1,2-alkyl shift followed by a 1,2-hydride shift to give a tertiary carbocation containing an ethyl chain. This may be quenched with hydride to give the ethylalkane, or undergo another 1,2-alkyl shift followed by a 1,2-hydride shift to form a propyl-containing tertiary carbocation. A continuation of this series of reactions can lead to the formation of the skeletons of the complete set of isomers,
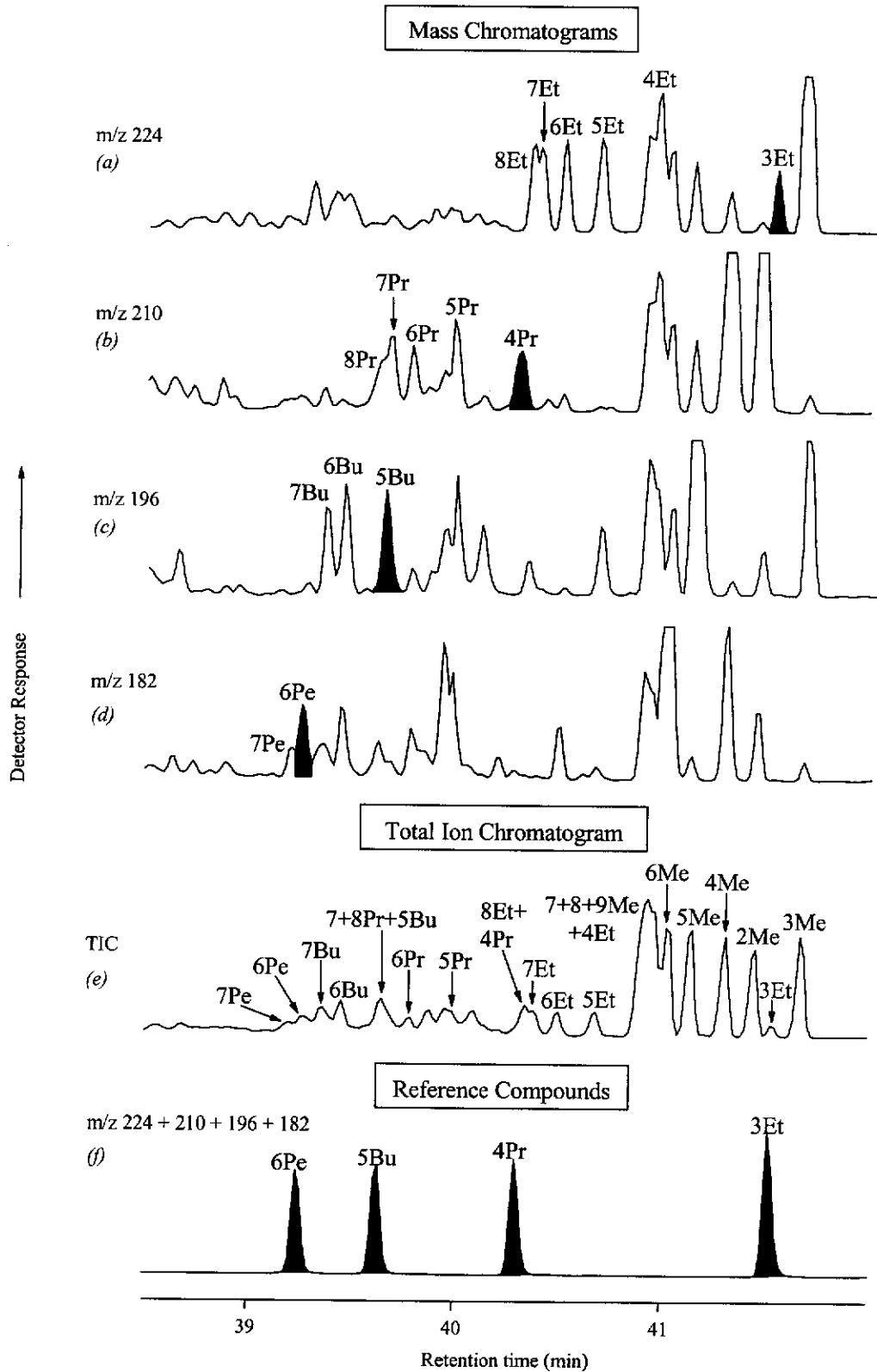
Figure 5.6. Partial mass chromatograms and the total ion chromatogram of the saturated hydrocarbon fraction from the products of heating n-octadecene and aluminium montmorillonite clay conducted in the laboratory, together with the summed ion chromatogram (m/z 224+210+196+182) of the mixture of reference compounds (Pr: propylalkane; Bu: butylalkane; Pe: pentylalkane).
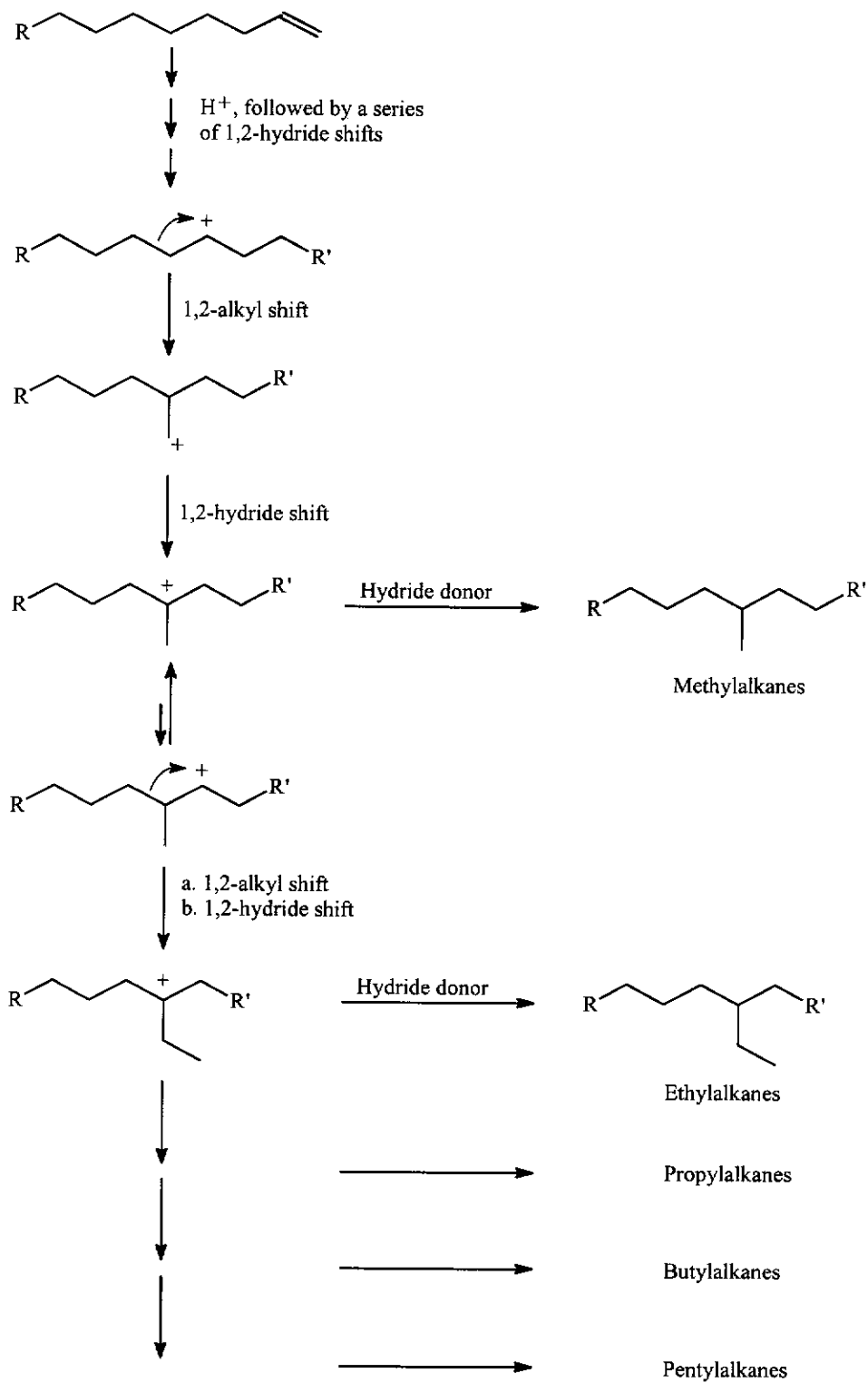
Figure 5.7. Proposed reaction mechanism for the formation of monoalkylalkanes.

which when quenched by hydride would give the hydrocarbons. Because the carbocation in the initial step of the reaction has approximately equal probability of being located on any of the secondary carbon atoms in the $n$-alkyl chain, the quenched reaction products should contain approximately equal numbers of isomers with a given side chain length. The chromatograms given in Figure 4.5, showing the $C_{18}$ monoalkylalkanes from a series of crude oils of differing ages and source types, confirm that this is indeed the case.

The experiment performed here also produced all $C_{18}$ monomethylalkane isomers in approximately equal amounts. It has been reported (Kissin, 1987) that α–olefins are catalytically transformed into monomethylalkanes in the presence of acidic mineral clays. Thus these clays may provide the acidic sites necessary for the conversion of α–olefins into monoalkylalkanes in the subsurface.

The heating experiment was also conducted using trifluoroacetic acid, a strong protic acid, in place of the acidic aluminium montmorillonite clay. The products obtained from this experiment were very similar to those obtained from heating the alkene in the presence of the clay. These results provide strong evidence that the rearrangements occurring here proceed *via* carbocationic intermediates. Skeletal rearrangement of $n$-alkenes when heated with acidic catalysts is well documented and is reported to proceed *via* carbocationic intermediates (Olah and Molnár, 1995). Similar mechanisms for the acid-catalysed rearrangement of straight chain hydrocarbons to branched alkanes *via* carbocationic intermediates have been proposed previously (Greensfelder *et al.*, 1949; Gates *et al.*, 1979). It has also been reported (Kursanov *et al.*, 1967) that when heated with trifluoroacetic acid and a hydride donor, such as a trisubstituted silane, olefins undergo hydrogenation to give alkanes, and "this is sometimes accompanied by isomerisation".

There are at least two potential sources of the $n$-alkenes required for this rearrangement reaction to occur. Alexander *et al.* (1991a) have reported the formation of $n$-alkenes from the thermal decomposition reaction of carboxylic

esters in sediments. It has also been suggested that alkenes are formed by the cracking reactions which occur at higher levels of thermal stress in the subsurface (van de Meent *et al.*, 1980).

### 5.2.4 Effect of maturity on the distribution of ethylalkanes in a sedimentary sequence

The ethylalkane isomers were analysed in a total of nine samples from the Grunter-1 sedimentary sequence. It was observed that there was a gradual change in the distribution of ethylalkanes with increasing depth. In the shallow, less mature samples, there is a strong preference for the 3- and 5-ethylalkanes at odd carbon numbers, with a less pronounced preference for the 4- and 6-ethylalkanes at even carbon numbers, as represented in Figure 5.1. With increasing depth and maturity in the sedimentary sequence, the abundances of odd carbon number isomers substituted at positions 4, 6, 7, and so on increases relative to the 3- and 5-ethylalkanes, as represented in Figure 5.2. In a similar manner, an increase in even carbon number isomers substituted at positions 3, 5, 7 and so on relative to the 4- and 6-ethylalkanes is observed. A numerical measure of these distribution changes was calculated by dividing the area under the peak in the mass chromatogram representing the 4-ethylalkane isomer by the sum of the areas under the peaks representing the 4- and 5-ethylalkanes. Table 5.1 summarises these calculated values at carbon numbers 19 to 22 in the nine samples, and they are plotted against sample depth in Figure 5.8.

From Figure 5.8, it is apparent that there is an increase in the abundance of the 4-ethylalkane isomer relative to the 5-substituted compound at the odd carbon numbers $C_{19}$ and $C_{21}$. This ratio has values of 0.05 to 0.2 in the shallowest samples, and from around 3000m begins to increase significantly, ending with values near 0.4 in the deepest samples. It was also observed that the values for this ratio calculated for the low maturity Loy Yang lignite were similar to the values for the lowest maturity samples from the sedimentary sequence, while the
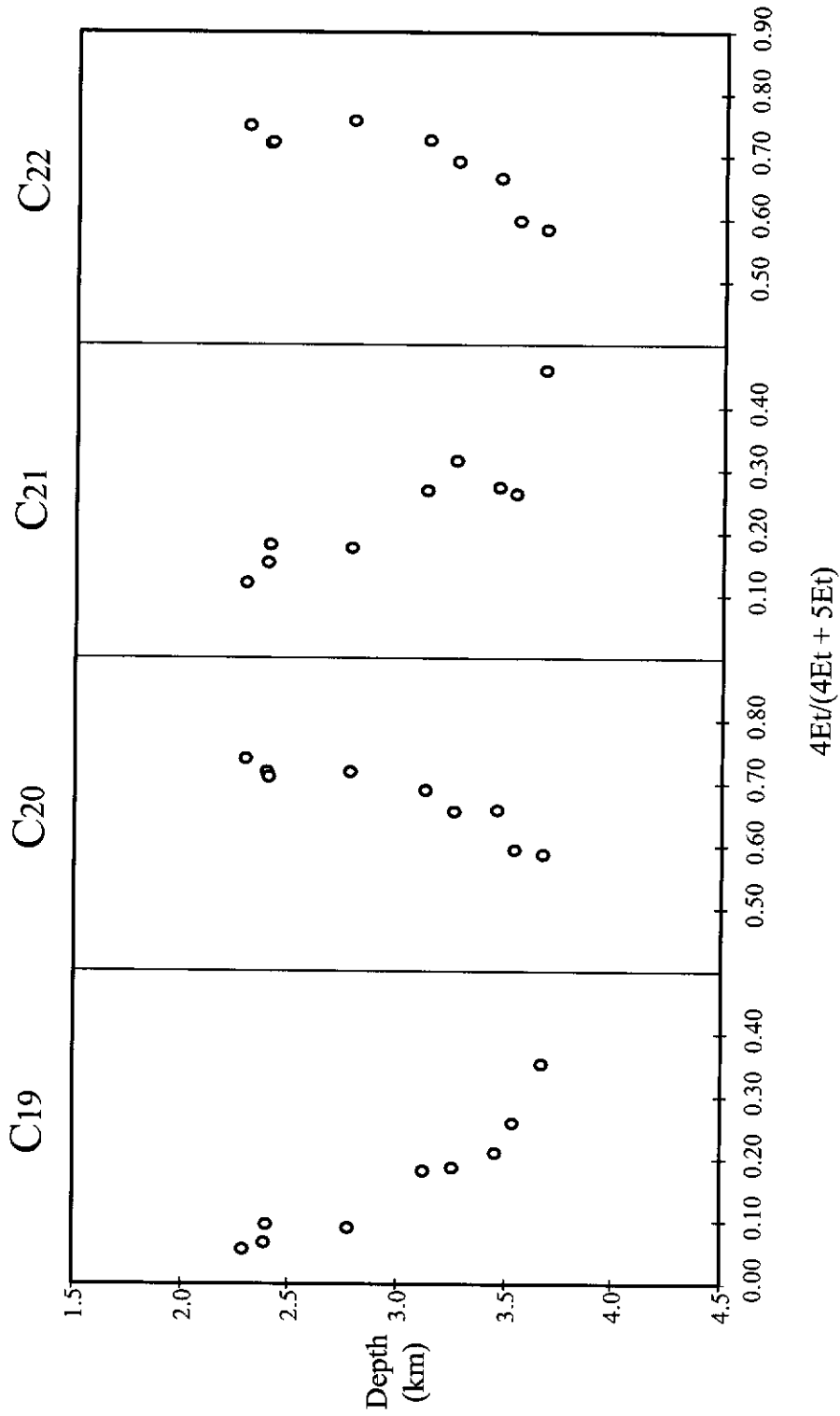
Figure 5.8. Plots showing the change in the ratio 4Et/(4Et +5Et) with increasing depth in the Grunter-1 sedimentary sequence (4Et: 4-ethylalkane; 5Et: 5-ethylalkane).

values for the higher maturity Tuna-4 crude oil approximated those for the most mature sedimentary sequence samples (Table 5.1).

The relative abundances of ethylalkane isomers changes with increasing depth and thermal maturity in the sedimentary sequence investigated. In the shallow, less mature samples, there is a strong preference for the odd carbon number 3- and 5-ethylalkanes. These compounds are probably derived from natural product precursors, possibly carboxylic acids with an ethyl group at positions 4 or 6, *via* decarboxylation. The abundances of isomers substituted at positions 4, 6, 7, and so on increases relative to the 3- and 5-ethylalkanes with increasing depth and maturity in the sedimentary sequence. The same, or similar, natural product precursors could also be reduced to 4- and 6-ethylalkanes, accounting for the observed preference of these compounds at even carbon numbers.

Ethylalkanes (and other T-branched alkanes) can be formed without preference for the position of substitution *via* acid-catalysed rearrangement of *n*-alkenes in the laboratory, and this reaction may be catalysed in the subsurface by acidic clays. The observed loss of preference for the odd carbon number 3- and 5-ethylalkanes (as well as the even carbon number 4- and 6-ethylalkanes) may be as a result of dilution of the initial preference by addition of ethylalkanes without preference for position of substitution, formed by acid-catalysed rearrangement of *n*-alkenes. If these *n*-alkenes are being generated by the thermal decomposition of carboxylic esters in the sediments, then the change in relative abundances of the ethylalkanes may potentially be useful in measuring the progress of the ester decomposition reaction. Kralert (1995) has reported that the progress of this reaction appears to correlate well with the change in carbon preference index (CPI) in two wells in the Gippsland Basin, including Grunter-1. Figure 5.9 shows the ratio 4Et/(4Et+5Et) at $C_{19}$ and $C_{21}$ for this well, and the corresponding CPI values. It is clear from Fig. 5.9 that both the CPI and the ethylalkane ratio change in the same depth range, between approximately 3000m and 3700m.
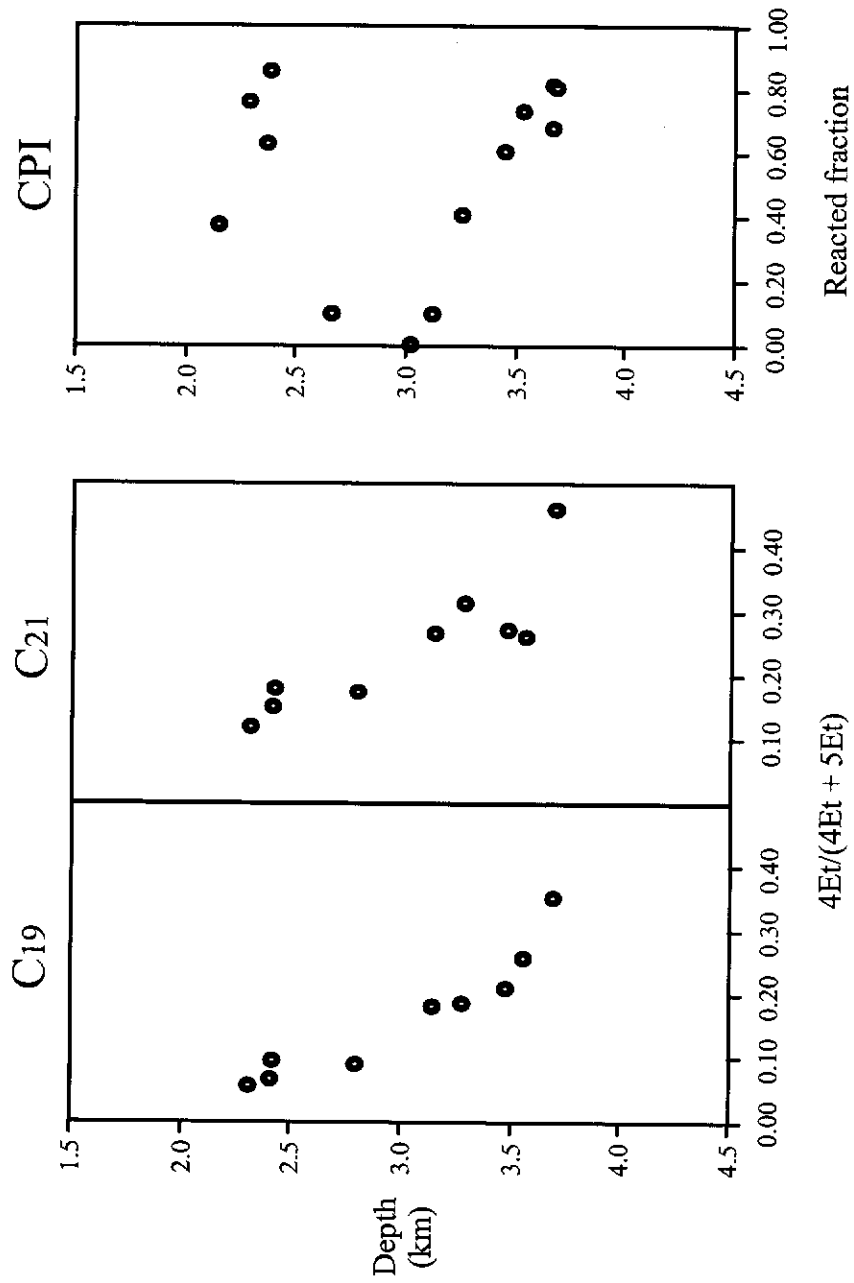
Figure 5.9. Plots showing the change in the ratio 4Et/(4Et +5Et) at $C_{19}$ and $C_{21}$, and CPI values with increasing depth in the Grunter-1 sedimentary sequence (4Et: 4-ethylalkane; 5Et: 5-ethylalkane; plot showing CPI values adapted from Kralert, 1995).

## 5.3 CONCLUSIONS

Hydrocarbon extracts from shallow samples in a sedimentary depth sequence were shown to contain ethylalkanes with a predominance of 3- and 5-substituted isomers at odd carbon numbers, with a lesser preference for 4- and 6-ethylalkanes at even carbon numbers. Extracts from deeper samples in the same depth sequence contained all ethylalkane isomers in broadly similar relative abundances at each carbon number. An immature lignite was shown to contain ethylalkanes in a distribution closely resembling the distribution in the shallow sedimentary sequence sample, with a crude oil being shown to contain a distribution similar to the deeper sedimentary sequence sample. The predominance of odd carbon number 3- and 5-substituted ethylalkanes in the immature samples is probably the result of defunctionalisation of specific natural product precursor compounds, and the predominance of even carbon number 4- and 6-ethylalkanes the result of reduction of the same or similar precursors. The loss of this preference with increasing depth and maturity is likely to be due to the formation of ethylalkanes without preference, possibly *via* some geosynthetic process. 1-Octadecene was heated with aluminium montmorillonite clay and the products included all $C_{18}$ monoalkylalkanes without preference for any isomers at a given branch length. A mechanism involving 1,2-alkyl shifts and 1,2-hydride shifts is proposed to account for the formation of the observed products. It is further suggested that conversion of $\alpha$-olefins into monoalkylalkanes under acidic conditions may account for the observed loss of preference of odd carbon number 3- and 5-substituted ethylalkanes, and even carbon number 4- and 6-ethylalkanes, with increasing depth in the sedimentary sequence studied, by diluting the initial preference.

# SECTION TWO

CHAPTER SIX


INVESTIGATION OF AN AROMATIC UNRESOLVED COMPLEX
MIXTURE BY OXIDATION WITH POTASSIUM PERMANGANATE

## 6.1 INTRODUCTION

Potassium permanganate is a strong oxidising agent which is used in a broad variety of applications (Hudlický, 1990 and references therein), and is frequently employed in high pH aqueous solution. It is capable of oxidising not only methyl groups, but virtually any alkyl group attached to an aromatic ring, to a carboxylic acid (Lee, 1980 and references therein). Indeed, larger alkyl groups are more susceptible to oxidation than methyls, with the exception of alkyl groups containing a benzylic tertiary carbon, in which case, if the side chain is oxidised, then the ring is usually cleaved (March, 1992). With side chains of two or more carbon atoms, the bond between the α and β carbons is cleaved (Lee, 1980). A general reaction scheme is shown in Figure 6.1.

Examples of substrates which have been oxidised to aromatic carboxylic acids using potassium permanganate include toluene to benzoic acid (Sam and Simmons, 1972; Furniss et al., 1989), ethylbenzene to benzoic acid (Furniss et al., 1989) and 6-t-butyltetralin to 4-t-butylphthalic acid (Lee, 1980).

Attempts to oxidise alkylated polynuclear aromatic species almost always leads to either destruction of the compound, attack on the aromatic nucleus or electrophilic substitution (Friedman et al., 1965). Alkylated naphthalenes, phenanthrenes and chrysenes have been oxidised to the corresponding aromatic acids at elevated temperatures and pressures using aqueous sodium dichromate (cf. Newman and Boden, 1961; Friedman et al., 1965; Lee and Spitzer, 1969), however the reaction
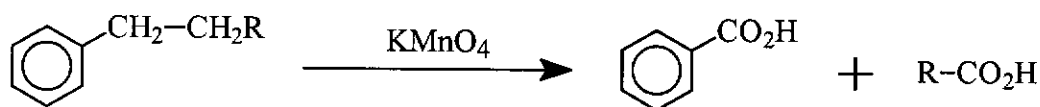


Figure 6.1. General reaction scheme showing the oxidation of an alkylbenzene using potassium permanganate.

is sensitive to the relative molar quantities of the reagents employed (Friedman *et al.*, 1965). It has been reported (Lee, 1980) that permanganate is more vigorous in oxidising alkyl arenes than chromium (VI).

Previous studies have utilised chromium trioxide oxidation of crude oil UCMs as a tool for structural elucidation (Killops and Al-Juboori, 1990; Gough and Rowland, 1990, 1991; Rowland *et al.*, 1995). These studies focussed on the aliphatic components of the UCMs. However, Revill (1992) oxidised a crude oil aromatic UCM using chromium trioxide in order to investigate the structures of the components of the mixture, and reported the occurrence of a series of *n*-alkanoic acids and phthalic acid, an aromatic carboxylic acid, in the oxidation products. Kerogens have also been subjected to structural elucidation by potassium permanganate oxidation, as reviewed by Vitorovic (1980).

This chapter describes the results of the potassium permanganate oxidation of the whole aromatic fraction of a biodegraded crude oil (Leatherjacket-1 788.5m; Gippsland Basin, Australia. This oil has been biodegraded to level 4 (Peters and Moldowan, 1993), with *n*-alkanes and alkylcyclohexanes absent, and acyclic isoprenoids, dimethylnaphthalenes, trimethylnaphthalenes and methylphenanthrenes affected.. The aromatic fraction is predominantly unresolved by GC, and in such unresolved mixtures, while certain specific compounds (most notably the methyl, dimethyl, trimethyl, etc, aromatics) are known, the vast majority of the components are yet to be identified. UCMs are thought to be comprised of large numbers of chemically similar compounds (eg. Thomas, 1995), with similar structures, and hence exhibit similar behaviour when analysed using instrumental techniques. For this reason, even state-of-the-art techniques such as GC-MS metastable reaction monitoring (MRM) are unable to provide structural information on individual components of such complex mixtures (this will be demonstrated in Chapter Seven). Thus other techniques, such as the oxidation procedure described in this chapter, may be utilised in order to gain information about such mixtures.

The aim of the work described in this chapter is to gain an increased understanding into the structures of compounds which comprise the aromatic UCM by oxidation of a UCM followed by analysis of the products. Investigation of the products of the potassium permanganate oxidation of the aromatic fraction provides information about the types of aromatic systems present, and the positions of ring substituents, and therefore also about the nature of the components of the aromatic UCM. While information such as the predominant ring substitution positions are known for those compounds which have been identified, it can by no means be assumed that such information also applies to the rest of the compounds present. The relative proportions of products from oxidation of monosubstituted, disubstituted, trisubstituted and tetrasubstituted benzenoid and biphenyl species were measured and the results are discussed, as is the relative amounts of the different ring substitution patterns within each of these groups. These results are used to infer information concerning the relative amounts of aromatic compounds with different ring substitutions in the initial crude oil aromatic fraction.

## 6.2 POTASSIUM PERMANGANATE OXIDATION OF AN AROMATIC UNRESOLVED COMPLEX MIXTURE

The aromatic UCM from the Leatherjacket-1 788.5 m crude oil (Gippsland Basin, Australia) was obtained according to the procedures described in Section 2.2.2. Briefly, the crude oil was subjected to column chromatography using silica gel as the stationary phase to separate the saturated and aromatic hydrocarbons. The aromatic fraction was then oxidised using potassium permanganate by refluxing in a 1:2 pyridine:water solvent mixture (Section 2.5.6). This reaction product was fractionated into organic-soluble monocarboxylic acids (13 % yield) and water-soluble polycarboxylic acids (20 % yield) by extraction with dichloromethane (cf. Section 2.5.1). Both of these acid fractions were methylated using boron trifluoride/methanol reagent (Section 2.5.2). The remaining unaccounted material was assumed to have been lost as gaseous oxidation products such as carbon dioxide (cf. Gough and Rowland, 1990).

### 6.2.1 Analysis of the monocarboxylic acid methyl esters generated from the permanganate oxidation of the crude oil aromatic fraction

The GC-MS TIC of the methyl esters of the monocarboxylic acids described above is given in Figure 6.2. Many of the components of this sample have been identified and compound classes include straight chain and branched alkanoic acids and various alkyl-substituted benzoic acids and biphenylcarboxylic acids. As has been previously reported for the chromium trioxide oxidations of aromatic UCMs (Revill, 1992), a homologous series of n-alkanoic acids was the dominant features of the GC-MS total ion chromatogram.

*Benzoic acids*

The base peak in the GC-MS TIC (Peak 2, Fig. 6.2) corresponds to benzoic acid methyl ester (Figure 6.3a). This compound must be derived from monosubstituted precursor compounds, in which the alkyl side chain attached to the monoaromatic ring may be straight or branched or contain a cyclic moiety.

Figure 6.2. GC-MS total ion chromatogram showing methyl esters of the dichloromethane-soluble monocarboxylic acids derived from the potassium permanganate oxidation of the aromatic fraction of Leatherjacket 788.5m crude oil (for peak identification refer to Table 6.1).

Table 6.1. Identification of peaks labelled with numbers in figures in this chapter.

| Peak no. | Identification | Peak no. | Identification |
|---|---|---|---|
| 1 | Methyl heptanoate | 32 | Methyl $C_2$-biphenylcarboxylate [b] |
| 2 | Methyl benzoate | 33 | Methyl $C_2$-biphenylcarboxylate [b] |
| 3 | Methyl octanoate | 34 | Methyl $C_2$-biphenylcarboxylate [b] |
| 4 | Methyl 2-methylbenzoate | 35 | Methyl $C_2$-biphenylcarboxylate [b] |
| 5 | Methyl 3-methylbenzoate | 36 | Methyl $C_2$-biphenylcarboxylate [b] |
| 6 | Methyl nonanoate | 37 | Methyl $C_3$-biphenylcarboxylate [c] |
| 7 | Methyl decanote | 38 | Methyl $C_3$-biphenylcarboxylate [c] |
| 8 | Methylundecanoate | 39 | Methyl $C_3$-biphenylcarboxylate [c] |
| 9 | Methyldodecanoate | 40 | Methyl $C_3$-biphenylcarboxylate [c] |
| 10 | Methyltridecanoate | 41 | Dimethyl phthalate |
| 11 | Methyltetradecanoate | 42 | Dimethyl terephthalate |
| 12 | Methyl ester of unknown monoaromatic compound [a] | 43 | Dimethyl isophthalate |
|  |  | 44 | Dimethyl 4-methylphthalate [d] |
| 13 | Methyl biphenyl-3-carboxylate | 45 | Dimethyl 5-methylisophthalate [d] |
| 14 | Methyl ester of unknown monoaromatic compound | 46 | Unknown ($m/z$ 163) [e,f] |
|  |  | 47 | Unknown ($m/z$ 163) [e] |
| 15 | Methyl ester of unknown monoaromatic compound [a] | 48 | Unknown ($m/z$ 177) [e] |
|  |  | 49 | Unknown ($m/z$ 177) [e] |
| 16 | Methyl octadecanoate | 50 | Unknown ($m/z$ 177) [e] |
| 17 | Methyl $C_2$-benzoate | 51 | Trimethyl benzene-1,2,4-tricarboxylate |
| 18 | Methyl 4-methylbenzoate | 52 | Trimethyl benzene-1,3,5-tricarboxylate |
| 19 | Methyl $C_2$-benzoate [b] | 53 | Unknown ($m/z$ 221) [e] |
| 20 | Methyl $C_2$-benzoate [b] | 54 | Unknown ($m/z$ 221) [e,g] |
| 21 | Methyl $C_2$-benzoate [b] | 55 | Unknown ($m/z$ 235) [e] |
| 22 | Methyl biphenyl-4-carboxylate | 56 | Dimethyl 4-methylisophthalate [e] |
| 23 | Methyl $C_1$-biphenylcarboxylate | 57 | Dimethyl 2-methylterephthalate [e] |
| 24 | Methyl $C_1$-biphenylcarboxylate | 58 | Unknown ($m/z$ 177) [e] |
| 25 | Methyl $C_1$-biphenylcarboxylate | 59 | Unknown ($m/z$ 177) [e] |
| 26 | Methyl $C_1$-biphenylcarboxylate | 60 | Unknown ($m/z$ 221) [e] |
| 27 | Methyl $C_1$-biphenylcarboxylate | 61 | Trimethyl 5-methylbenzene-1,2,4-tricarboxylate |
| 28 | Methyl $C_1$-biphenylcarboxylate |  |  |
| 29 | Methyl $C_2$-biphenylcarboxylate [b] | 62 | Unknown ($m/z$ 235) [e] |
| 30 | Methyl $C_2$-biphenylcarboxylate [b] | 63 | Unknown ($m/z$ 235) [e] |
| 31 | Methyl $C_2$-biphenylcarboxylate [b] | 64 | Unknown ($m/z$ 235) [e] |

a: exact structures undetermined, but mass spectra indicate tetralin carbon skeletons; b: may be dimethyl or ethyl; c: may be trimethyl, methyl and ethyl, or propyl; d: tentative identification; e: compounds with very similar mass spectra to the polycarboxylic acid methyl ester with the same diagnostic ion, but lacking an obvious parent ion. The structures of these compounds are discussed in the relevant sections of the text; f: two coeluting compounds with very similar mass spectra; g: three coeluting compounds with very similar mass spectra.

The precursor compounds may be monoaromatic, or the side chain may contain one or more additional aromatic rings.

Three other prominent peaks are apparent, corresponding to the methyl esters of the three toluic acids (Fig. 6.3$b$). The peak corresponding to 3-methylbenzoic acid is the largest of the three, indicating a relative abundance of 78 %, and the 2- and 4-methyl isomers each comprise about 11 % of the toluic acids in the sample. These compounds are derived from the incomplete oxidation of dialkylated precursor compounds similar to those described above for benzoic acids, but with at least one of the alkyl groups being a methyl. A similar group of $C_2$-substituted benzoic acids (Fig. 6.3$c$) was also detected in the sample. The precursors for these compounds are trialkylated monoaromatic structural moieties analogous to those of benzoic acid and the toluic acids, but in which at least two of the alkyl groups are methyls.

As was pointed out in Section 6.1, larger alkyl groups are more susceptible to oxidation than methyl groups. Efforts to conduct the oxidation procedure under more severe conditions in order to oxidise all of the alkyl substituent groups to carboxyl groups were unsuccessful, and resulted in a greatly reduced recovery of oxidation products. The resistance to oxidation of these methylbenzoic acids was demonstrated by the oxidation of pure 1,2,4-trimethylbenzene. Under identical conditions to those used for the oxidation of the crude oil aromatics, this oxidation yielded dimethylbenzoic acids (99.6 %) and methylphthalic acids (0.43 %) as products. No benzenetricarboxylic acid (as trimethyl ester) was identified in the reaction products. These results suggest that under the oxidation conditions employed here, oxidation of a methyl group attached to an aromatic ring already containing a carboxylate functional group occurred to only a very small extent.

The relative proportions of the $C_0$:$C_1$:$C_2$ benzoic acids in the oxidation product are 45:48:7. This suggests that alkylaromatics containing a single methyl and a larger alkyl group are fairly abundant in the crude oil sample, while those containing an alkyl group and two methyls are present in considerably lower abundance. As it is
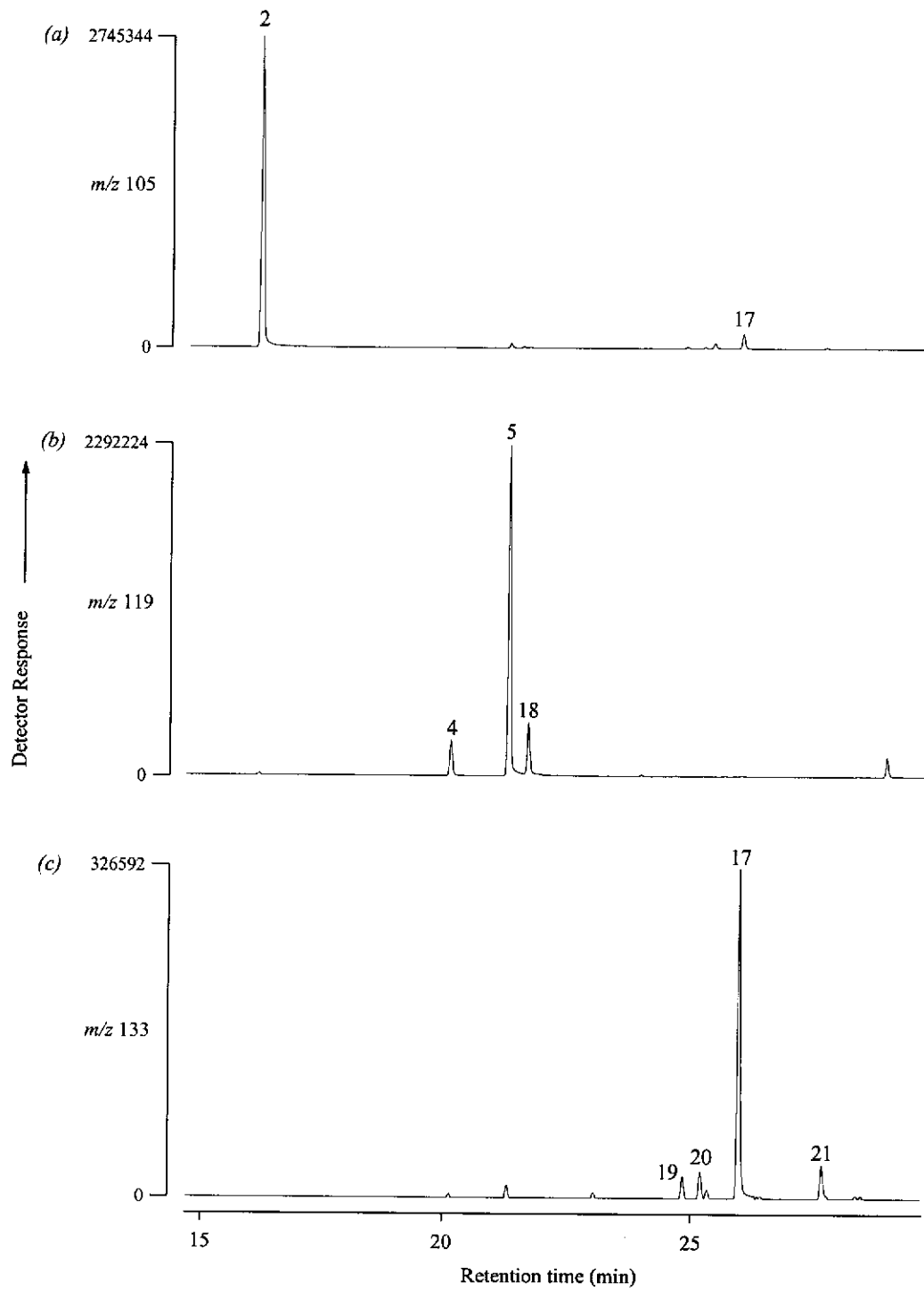
Figure 6.3. GC-MS *m/z* 105, 119 and 133 reconstructed ion chromatogram showing methyl esters of: (a) $C_0$; (b) $C_1$; and, (c) $C_2$ benzoic acids respectively (for peak identification refer to Table 6.1).

possible that a small proportion of the methyl groups of some of these precursor compounds have also been oxidised to yield dicarboxylic acids, the relative amounts of the $C_1$ and $C_2$ benzoic acids should be regarded as minimum values and may be higher than the figures stated.

*Biphenylcarboxylic acids*

Biphenyl-3-carboxylic acid and biphenyl-4-carboxylic acid were identified in the sample in relative proportions of 65:35, as shown in Figure 6.4a. The mass spectrum of the first eluting isomer, biphenyl-3-carboxylic acid, is given in Fig. 6.4b. These compounds are derived from alkyl-substituted biphenyls, in which the alkyl chain may be straight, branched, cyclic or contain further aromatic systems, in a situation analogous to the benzoic acids. The relative proportions indicates that monoalkylated biphenyls substituted in the 3-position are present in approximately double the abundance of those substituted in the 4-position in the crude oil. Biphenyl-2-carboxylic acid was not identified in the sample, most likely reflecting the low relative abundance of alkylbiphenyl precursor molecules bearing substituents in the 2-position (Alexander *et al.*, 1986; Cumbers *et al.*, 1987). Further, Cumbers *et al.* (1987) determined the relative abundances of the three methylbiphenyl and ethylbiphenyl isomers in a series of crude oils including two from the Gippsland Basin, Australia, the source of the crude oil used in the present study. The relative abundances of the 2:3:4 methylbiphenyls observed for the Gippsland Basin oils investigated by Cumbers *et al.* (1987) were 2.3:70:27 and 1.6:74:24, while the ratios of the 2:3:4 ethylbiphenyls were both <0.1:70:30, all very similar to the results of the present study (0:65:35).

The combined *m/z* 226+240+254 mass chromatogram showed peaks corresponding to a series of six monomethyl biphenylcarboxylic acids (peaks 23-28, Figure 6.5). As with the alkyl-substituted benzoic acids described earlier, these compounds are the products of incomplete oxidation of dialkylbiphenyls in which at least one of the alkyl groups is methyl. Again, it is assumed that methyl groups on compounds containing a carboxylate functional group are only oxidised to a very small extent as indicated by the oxidation of the reference compound

Figure 6.4. (a) GC-MS *m/z* 212 reconstructed ion chromatogram of the dichloromethane soluble monocarboxylic acid methyl esters derived from potassium permanganate oxidation of the aromatic fraction of the Leatherjacket-1 788.5 m crude oil (for peak identification refer to Table 6.1); and, (b) mass spectrum of biphenyl-3-carboxylic acid methyl ester (compound 13).

described earlier. As reference compounds were not available, positive identification of substitution patterns was not possible. Likewise, several peaks corresponding to $C_2$- and $C_3$-substituted (presumably dimethyl- and trimethyl-) biphenylcarboxylic acids were also observed in the GC of the oxidation product (peaks 29-40, Fig. 6.5), however the lack of reference compounds once again meant that the identification of specific isomers was not possible. The relative abundances of the $C_0:C_1:C_2:C_3$ biphenylcarboxylic acids was 22:29:28:21. Again, as with the substituted benzoic acids, the methyl groups of some of the precursor compounds may have been oxidised to carboxylic acids, hence these relative abundances for the $C_1$, $C_2$ and $C_3$ biphenylcarboxylic acids may be higher, and the abundances of the UCM hydrocarbon precursors to these oxidation products would therefore also be higher.

*Aliphatic carboxylic acids*

Peaks 1, 3, 6-11 and 16 in the TIC (Fig. 6.2) represent a series of *n*-alkanoic acid methyl esters, which extends from $C_7$ to $C_{25}$. These acids result from the oxidative cleavage of *n*-alkyl side chains attached to aromatic rings, through the bond between the $\alpha$ and $\beta$ carbons (Lee, 1965). This indicates that the original side chains were up to 26 carbon atoms in length. Thus precursor compounds in which an *n*-alkyl chain was attached directly to an aromatic ring were present in the initial crude oil aromatic fraction.

Series of 2- and 3-methylalkanoic acids have also been identified in the oxidation products of the crude oil aromatic fraction (chromatograms not shown). The 2-methyl series extends to $C_{14}$, while 3-methyl substituted isomers to $C_{12}$ have been identified. The precursors from which these compounds are derived are likely to be similar to those described for the *n*-alkanoic acids above, but in which the side chain has a methyl branch. Peaks which may correspond to isomers with the methyl group in different positions along the alkyl chain were observed in the *m/z* 74 mass chromatogram of the sample (not shown). However, the lack of distinguishing features in their mass spectra makes their identification difficult without reference compounds.
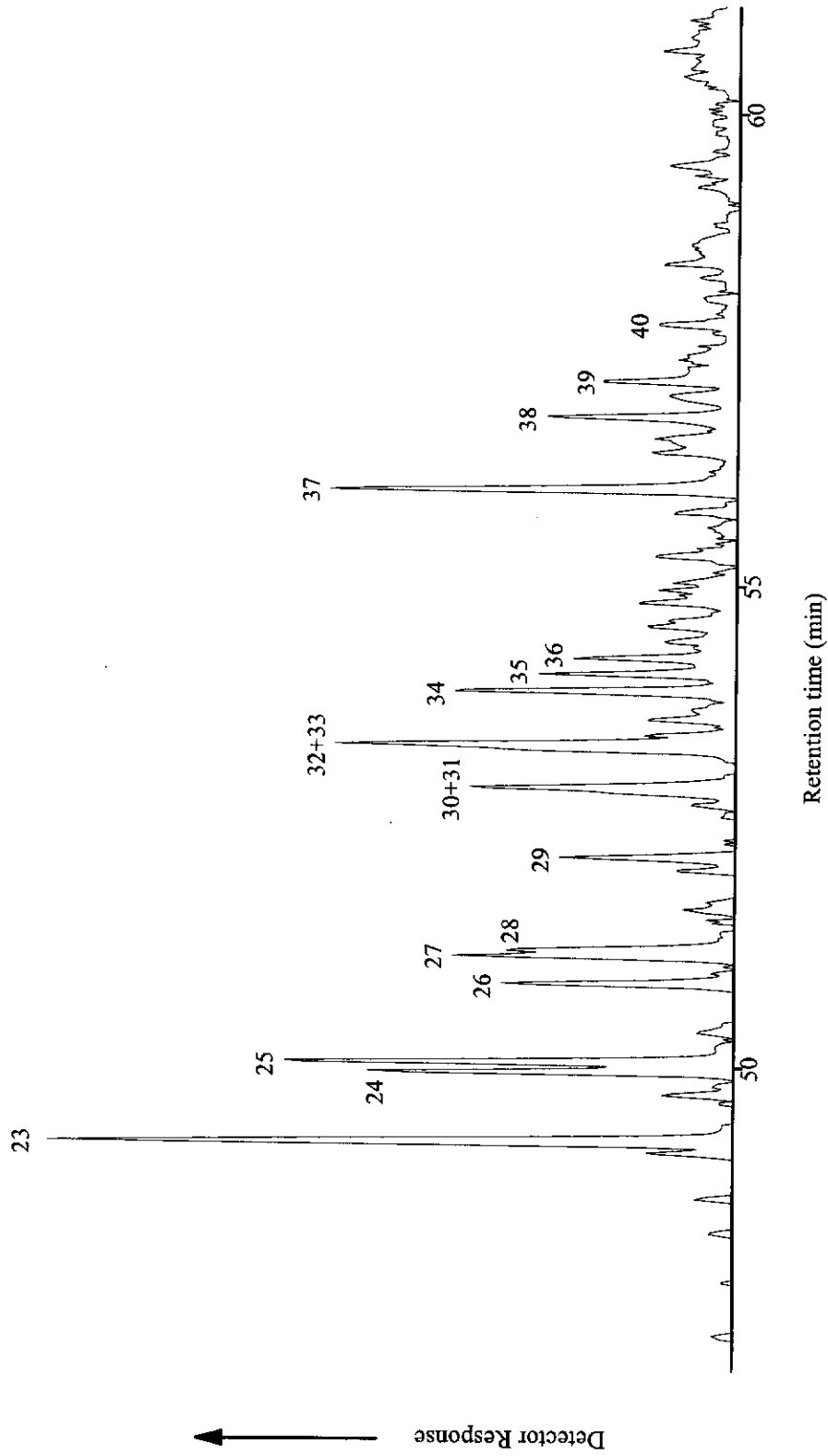
Figure 6.5. Combined *m/z* 226, 240 and 254 mass chromatograms showing the methyl esters of the C₁ to C₃ alkyl substituted biphenylcarboxylic acids from the potassium permanganate oxidation of the aromatic fraction of Leatherjacket 788.5m crude oil (for peak identification refer to Table 6.1).

Five isoprenoid alkanoic acids, *viz* 2,6-dimethylheptanoic acid ($C_9$),

3,7-dimethyloctanoic acid ($C_{10}$), 2,6,10-trimethylundecanoic acid ($C_{14}$),

3,7,11-trimethyldodecanoic acid ($C_{15}$) and 2,6,10,14-tetramethylpentadecanoic

acid ($C_{19}$) were also identified in the oxidation product. The isomers containing a

2-methyl substituent gave ions of *m/z* 88 and 101 corresponding to McLafferty

rearrangement, and a similar process yielded ions of *m/z* 74 and 101 for the

3-methyl substituted compounds. These compounds are the same as the

isoprenoids produced in the ruthenium tetroxide oxidation of aromatic fractions

reported in Chapter Seven; however, the nature of the two oxidation mechanisms

means that a side chain of a given carbon number will yield an acid of two

carbons less from permanganate oxidation (which cleaves the bond between the

carbons $\alpha$ and $\beta$ to the ring) than from ruthenium tetroxide oxidation (which

cleaves at the *ipso* carbon). The longest isoprenoid identified in the potassium

permanganate oxidation was $C_{19}$ which corresponds to $C_{21}$ for ruthenium tetroxide

oxidation of the same precursor, which was the longest isoprenoid identified in

Chapter Seven. Other isoprenoid acids with substituents in the 4- and 5-positions

do not give these characteristic fragmentation patterns, and analogous with the

methyl-substituted alkanoic acids described above, are more difficult to identify

with certainty. The presence of isoprenoid acids indicates that precursor

compounds in which an isoprenoidal side chain was attached directly to an

aromatic ring were present in the initial crude oil aromatic fraction.

### 6.2.2 Analysis of the polycarboxylic acids generated from the potassium permanganate oxidation of the crude oil aromatic fraction

The GC-MS total ion chromatogram of the methyl esters of the water soluble

polycarboxylic acids produced in the potassium permanganate oxidation of the

crude oil aromatic fraction is shown in Figure 6.6. As Fig. 6.6 shows, 100 % of

the material in this sample is resolved by GC.

The largest peak in the TIC has been identified as phthalic acid, with isophthalic

acid and terephthalic acid also present in the sample. The *m/z* 163 mass
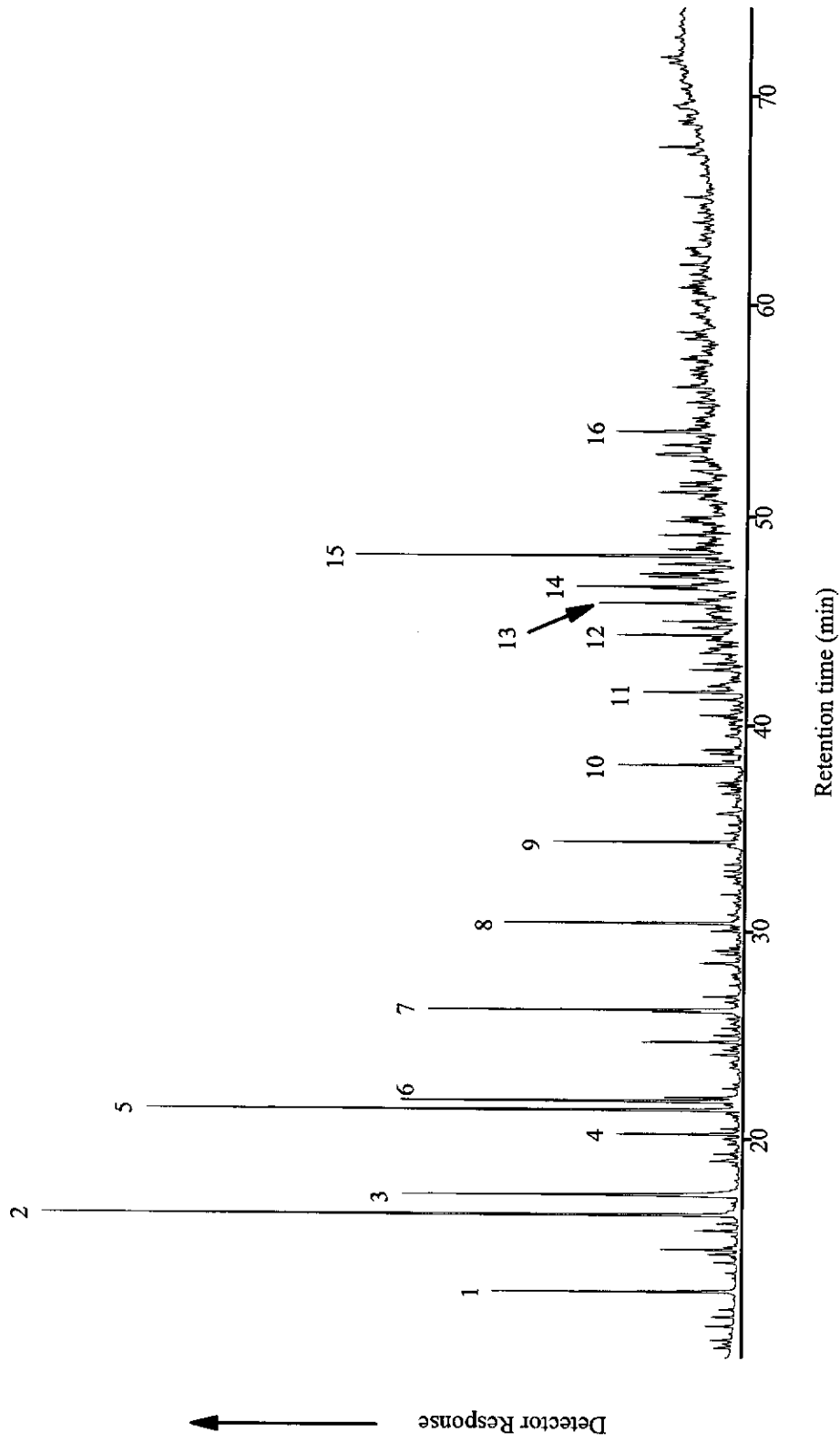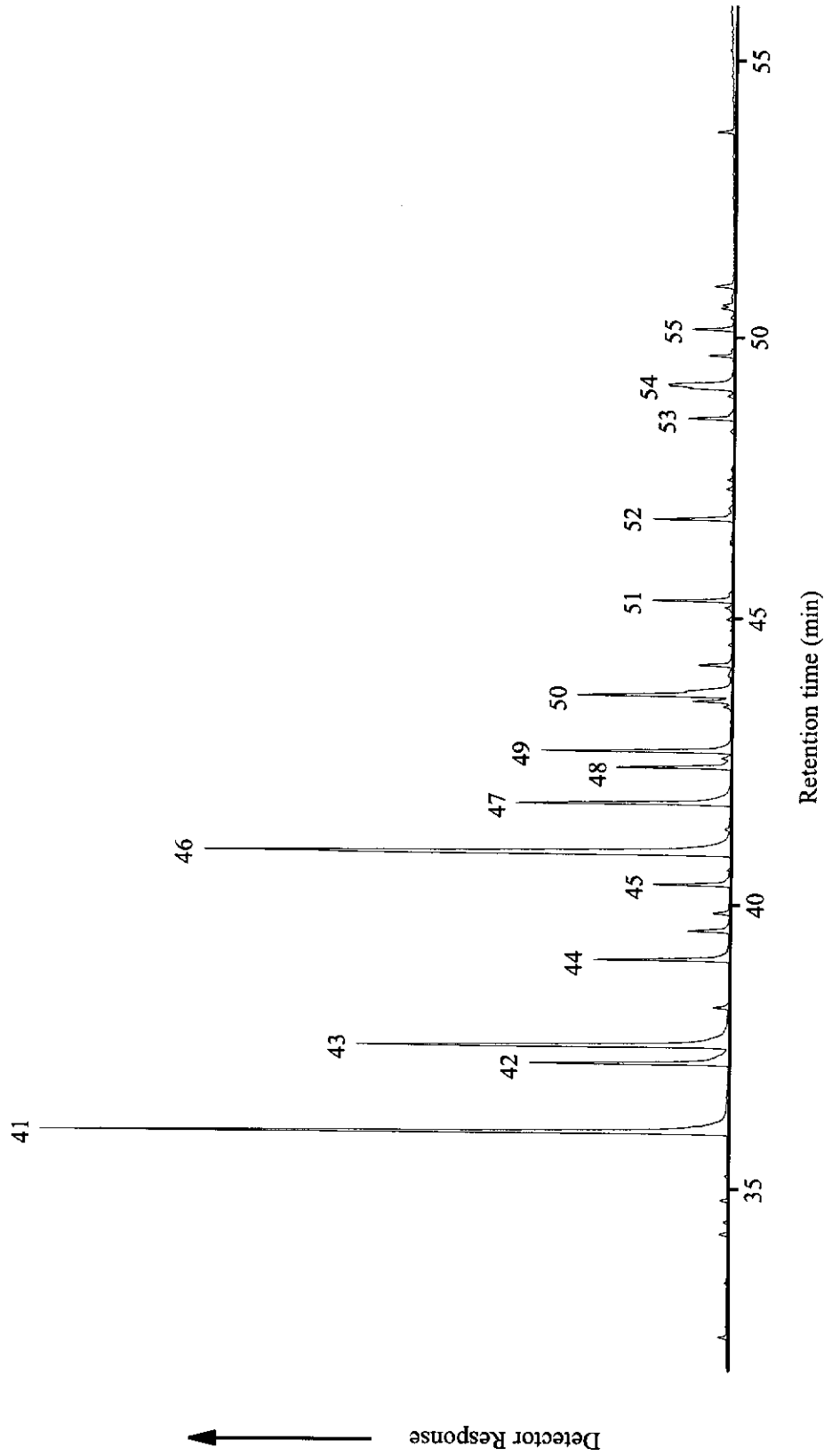
Figure 6.6. GC-MS total ion chromatogram showing the methyl esters of the water-soluble polycarboxylic acids from the potassium permanganate oxidation of the aromatic fraction of Leatherjacket 788.5m crude oil (for peak identification refer to Table 6.1).

chromatogram showing peaks corresponding to these compounds is given in Figure 6.7a, with the mass spectrum of phthalic acid given in Fig. 6.7b. The relative abundance of these acids is 53:31:16, which is therefore an indication of the ratio of 1,2 to 1,3 to 1,4 alkyl disubstitution on monoaromatic rings in the initial aromatic fraction. It has been reported that *meta-* and *para-*alkyltoluenes, an example of disubstituted monoaromatic compounds which are potential precursors for the phthalic acids, are in higher relative abundance than the corresponding *ortho* isomers in mature crude oils (Albaiges *et al.*, 1986). The crude oil used in this study is moderately mature (20S/[20S+20R] sterane ratio = 0.47, moretane/hopane ratio = 0.12; Ellis, 1994), therefore if the disubstituted monoaromatic compounds from which the phthalic acids are produced follow the same trend as the alkyltoluenes reported by Albaiges *et al.* (1986), then the *ortho* isomer would be expected to be the least abundant of the three isomers. Instead, at 53 %, it is the most abundant. The high relative abundance of disubstituted monoaromatic rings with a 1,2 substitution pattern in the permanganate oxidation product is therefore attributed to the presence of a large proportion of tetralin and/or indane moieties in the initial crude oil aromatic fraction, as has been suggested by Revill (1992).

The presence of alkyltetralins and alkylindanes in sedimentary organic matter has previously been reported (eg. Williams *et al.*, 1988; Forster *et al.*, 1989; Alexander *et al.*, 1992b; Koopmans *et al.*, 1997). It has been proposed that particular (polymethylated) isomers have direct natural product origins (Forster *et al.*, 1989; Alexander *et al.*, 1992b). Williams *et al.* (1988) reported the occurrence of long chain alkyltetralins and alkylindanes, and based on evidence presented, concluded that these compounds were formed *via* alkylation of monoaromatic rings followed by ring closure to yield the naphthenoaromatic systems.

There are two possible precursor compounds for the phthalic acids identified in the oxidation product. All three phthalic acids may derive from compounds containing two alkyl chains (which may be straight, branched, cyclic or contain further aromatic systems) attached to an aromatic ring, while the *ortho* isomer
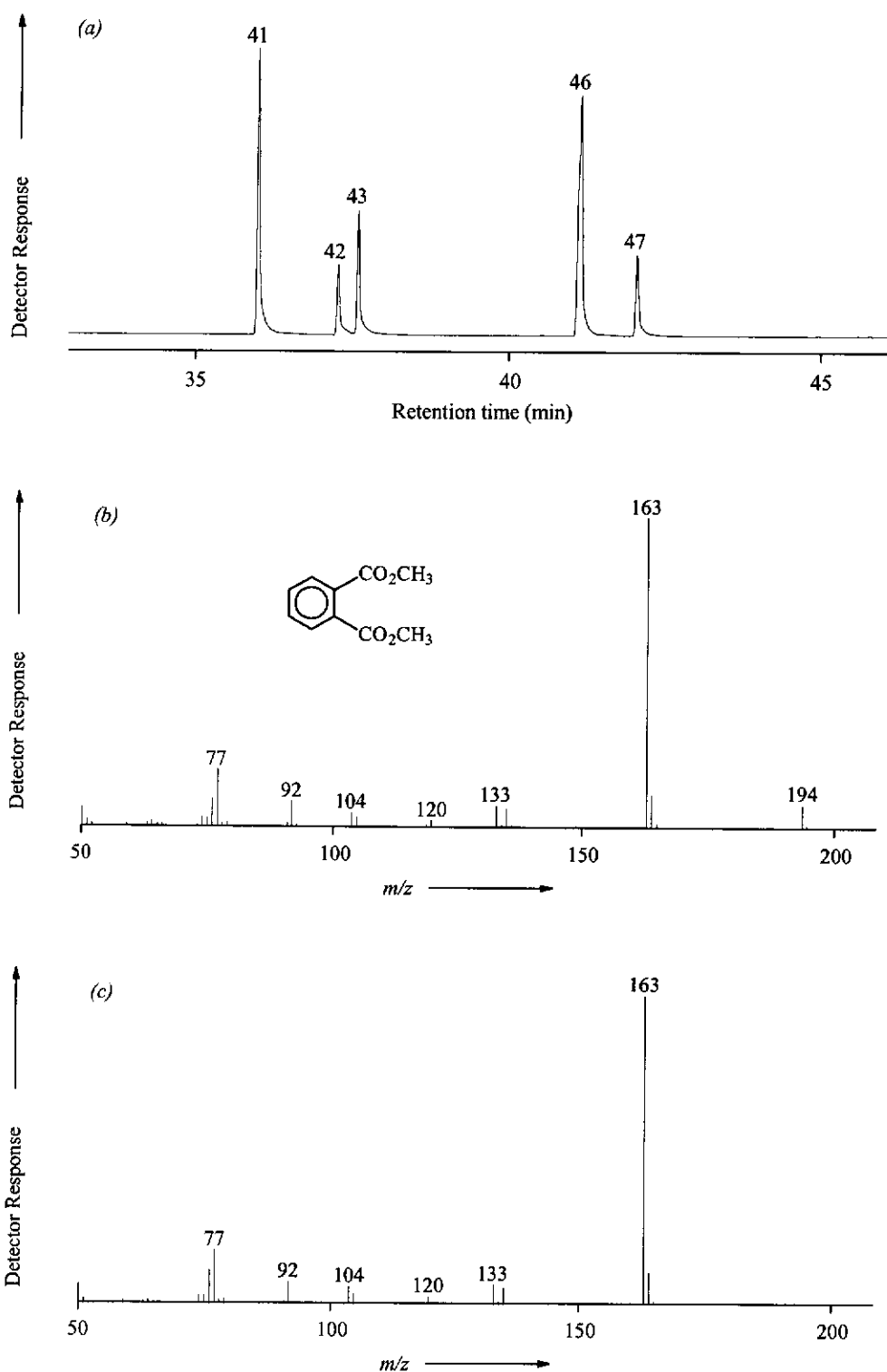
Figure 6.7. (a) GC-MS *m/z* 163 reconstructed ion chromatogram of the water-soluble polycarboxylic acids methyl esters derived from potassium permanganate oxidation of the aromatic fraction of the Leatherjacket-1 788.5 m crude oil (for peak identification refer to Table 6.1); (b) mass spectrum of phthalic acid dimethyl ester (compound 41); and, (c) mass spectrum of compound 46.

may also be sourced from compounds containing alkyltetralin and/or alkylindane structural moieties.

Also apparent in Fig. 6.7*a* are two peaks with slightly longer retention time than the phthalic acids (peaks 46 and 47). The mass spectrum of the first of these peaks (peak 46) is given in Fig. 6.7*c* and is similar to the spectrum of dimethyl phthalate (Fig. 6.7*b*), having the same base peak at *m/z* 163, but lacking an obvious parent ion. The mass spectra of the three compounds (peak 47 is in fact two peaks representing coeluting compounds) are very similar to each other. The presence of the same *m/z* 163 ion, which corresponds to the loss of a methoxy group from the phthalic acid dimethyl esters, suggests that this second set of compounds contains at least one -COOCH$_3$ group and a benzylic carbonyl group. A very similar mass spectrum has been published (Thomas, 1995) and the compound identified as 2-(1-oxopropyl)benzoic acid methyl ester (Figure 6.8). The base peak (*m/z* 163) is attributed to loss of an ethyl group *via* cleavage adjacent to the benzylic ketone group as shown in Fig. 6.8. This compound has been reported as an oxidation product of both 9-ethylfluorene and 1-ethylnaphthalene (Thomas, 1995). The mass spectra of the compounds represented by peaks 46 and 47 in Fig. 6.7*a* indicate that they may have similar structures to this compound, and would therefore be likely to be derived from similar precursors to those reported by Thomas (1995), ie. naphthalenes and/or fluorenes. The presence of three peaks in the chromatogram may indicate that
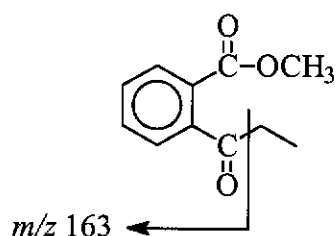


Figure 6.8. Structure and preferred site of mass spectral fragmentation of 2-(1-oxopropyl)benzoic acid methyl ester (from Thomas, 1995).

ortho, meta and para isomers are present in the sample, but it is unclear how the meta and para isomers would be formed.

A group of methyl-substituted monoaromatic dicarboxylic acids has also been identified in the sample, as revealed in the m/z 177 reconstructed ion chromatogram (Figure 6.9a). In all, four of the six possible isomers are present in the sample and have been tentatively identified on the basis of their mass spectra as (in order of increasing GC retention time) 4-methylphthalic acid, 4-methylisophthalic acid, 2-methylterephthalic acid acid and 5-methylisophthalic acid (peaks 44, 56, 57 and 45 respectively, Fig. 6.9a). The mass spectrum of the first of these compounds is given in Fig. 6.9b. The two isomers which are believed not to be present in the sample are the two 1,2,3-trisubstituted compounds, 3-methylphthalic acid and 2-methylisophthalic acid. It is anticipated that steric hindrance associated with a 1,2,3-trisubstituted benzene ring, in which two of the groups are relatively bulky carboxylic acids, may inhibit the formation and/or esterification of these compounds (cf. March, 1992).

The most abundant methyl-substituted dicarboxylic acid in the sample was again that with the carboxylic acid groups *ortho* to each other, and again this observation is interpreted as evidence of the presence of significant amounts of alkyltetralin and/or alkylindane structural moieties in this crude oil sample. The presence of these monoaromatic methylated dicarboxylic acids indicates that trialkyl-substituted monoaromatic compounds, of which at least one of the alkyl groups on the aromatic ring is a methyl, are present in the initial crude oil aromatic fraction.

Also present in the sample is a second, later eluting set of compounds (Fig. 6.9a) with mass spectra similar to the methyl substituted dicarboxylic acids. As was the case with the unsubstituted dicarboxylic acids, the mass spectra of these compounds indicates that they also contain one -$COOCH_3$ group and one other benzylic carbonyl group. They are likely to be similar to the compound shown in Fig. 6.8, with an extra methyl group attached to the aromatic ring, analogous to
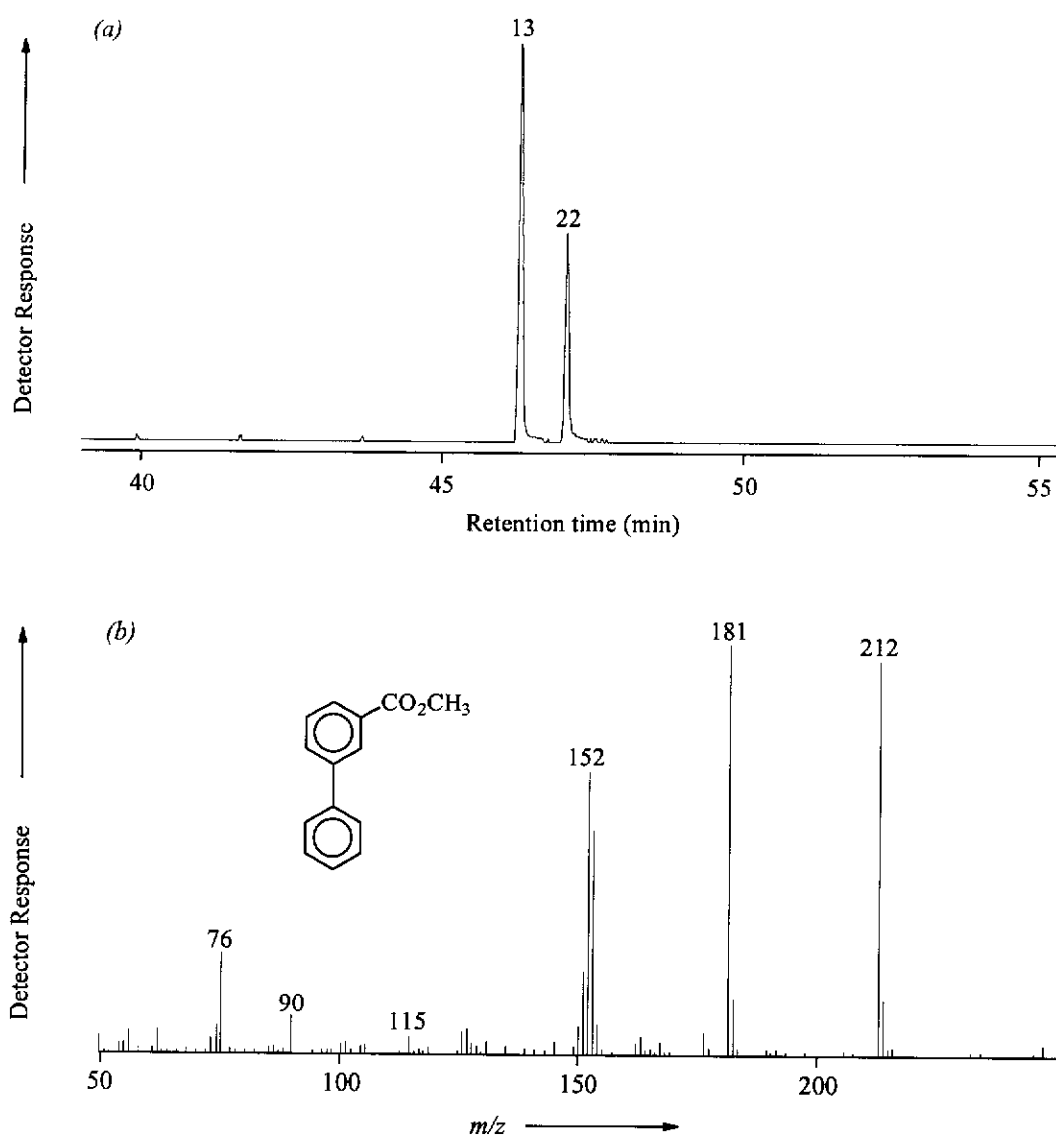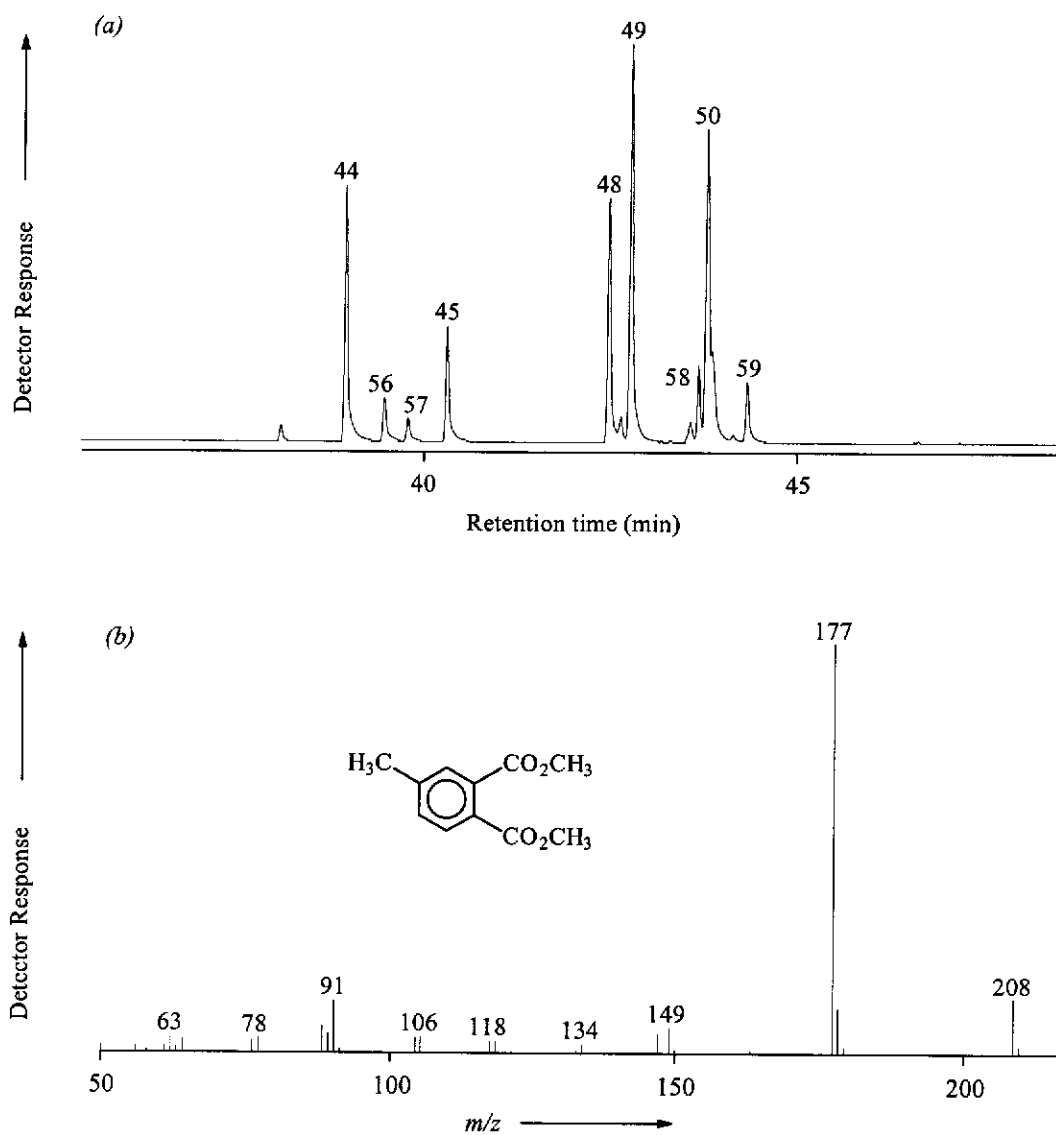
Figure 6.9. (a) GC-MS m/z 177 reconstructed ion chromatogram of the water-soluble polycarboxylic acids methyl esters derived from potassium permanganate oxidation of the aromatic fraction of the Leatherjacket-1 788.5 m crude oil (for peak identification refer to Table 6.1); and, (b) mass spectrum of dimethyl 4-methylphthalate (compound 44).

the compounds observed in the *m/z* 163 mass chromatogram of the phthalic acids (Fig. 6.7*a*). These compounds therefore probably have similar precursor compounds in the crude oil aromatic fraction to the compound shown in Fig. 6.8.

Two monoaromatic tricarboxylic acids in the sample have been identified as benzene-1,2,4 tricarboxylic acid and benzene-1,3,5 tricarboxylic acid, with a relative abundance of 56:44 (Figure 6.10*a*). These compounds derive from trisubstituted monoaromatic systems in the initial crude oil aromatic fraction. In a similar manner to the phthalic acids discussed above, the higher relative abundance of the more sterically hindered 1,2,4 isomer (Fig. 6.10*b*), which has two adjacent carboxylic acid groups, may be as a result of the presence of tetralins and/or indanes with an alkyl substituent attached to the aromatic ring as well as three separate alkyl groups attached to monoaromatic rings. The presence of monoaromatic tricarboxylic acids in the oxidation product indicates that compounds containing trialkyl-substituted monoaromatic structural moieties were present in the initial monoaromatic fraction.

As was the case with the dicarboxylic acids, a second, later eluting set of compounds was observed in the sample (Fig. 6.10*a*) with mass spectra similar to the tricarboxylic acids. In a situation analogous to the compounds observed with mass spectra similar to the phthalic acids, these compounds appear to contain two -COOCH$_3$ groups and one benzylic carbonyl group. Again, these compounds may be structurally similar to the compound shown in Fig. 6.8, with a second carboxylic acid group attached to the aromatic ring. These compounds presumably also have similar precursors to the analogous compounds described earlier, with an extra alkyl group which has also been oxidised.

Also present in the oxidation product is a single compound identified as a methyl-substituted tricarboxylic acid (Figure 6.11*a*). Due to the steric hindrance considerations discussed above for the methyl phthalic acids, this compound is tentatively identified as 5-methyl-benzene-1,2,4-tricarboxylic acid (Fig. 6.11*b*), the only possible isomer without a 1,2,3 trisubstitution pattern. The presence of
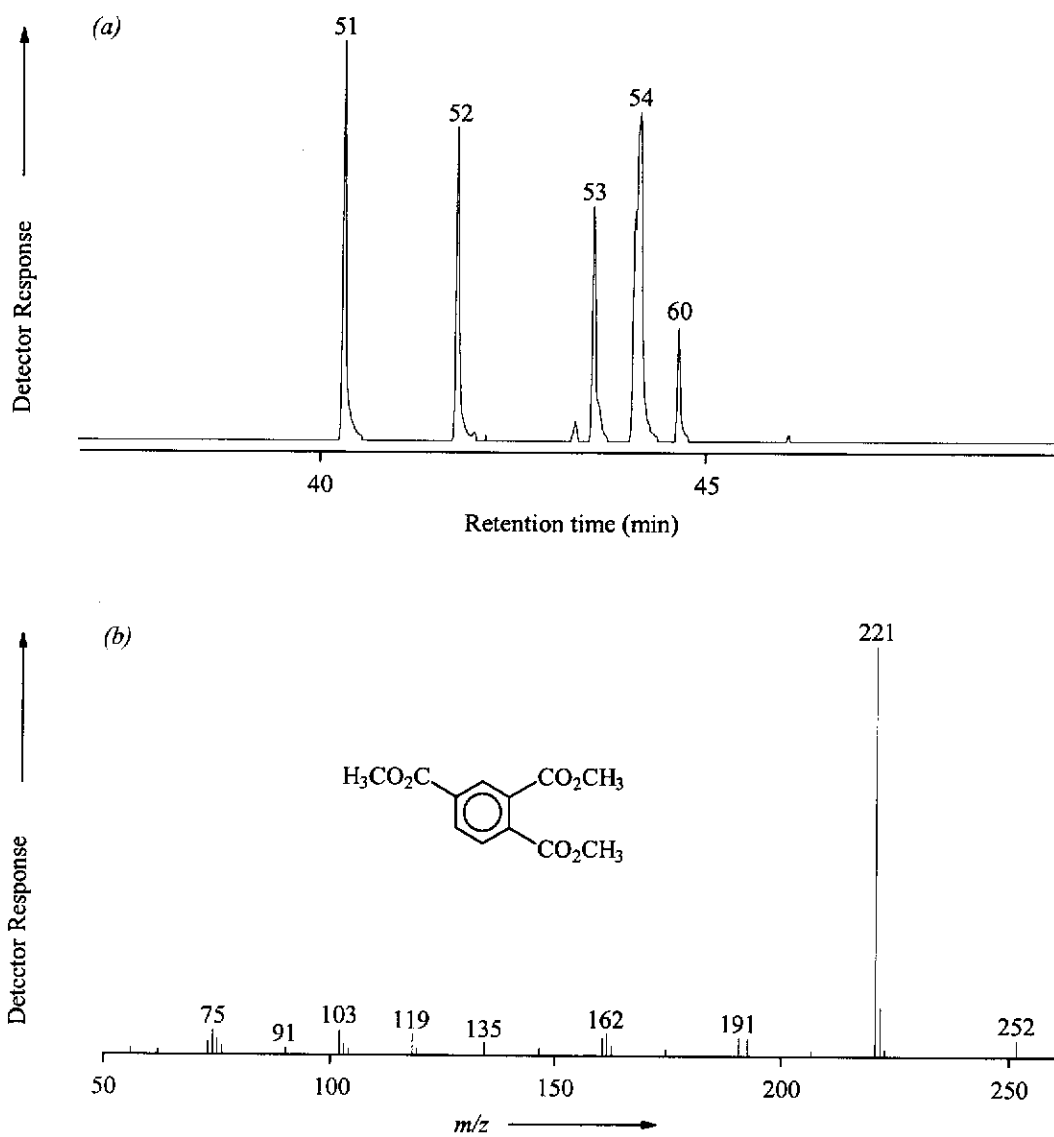
Figure 6.10. (a) GC-MS *m/z* 221 reconstructed ion chromatogram of the water-soluble polycarboxylic acids methyl esters derived from potassium permanganate oxidation of the aromatic fraction of Leatherjacket-1 788.5 m crude oil (for peak identification refer to Table 6.1); and, (b) mass spectrum of trimethyl benzene-1,2,4-tricarboxylate (compound 51).
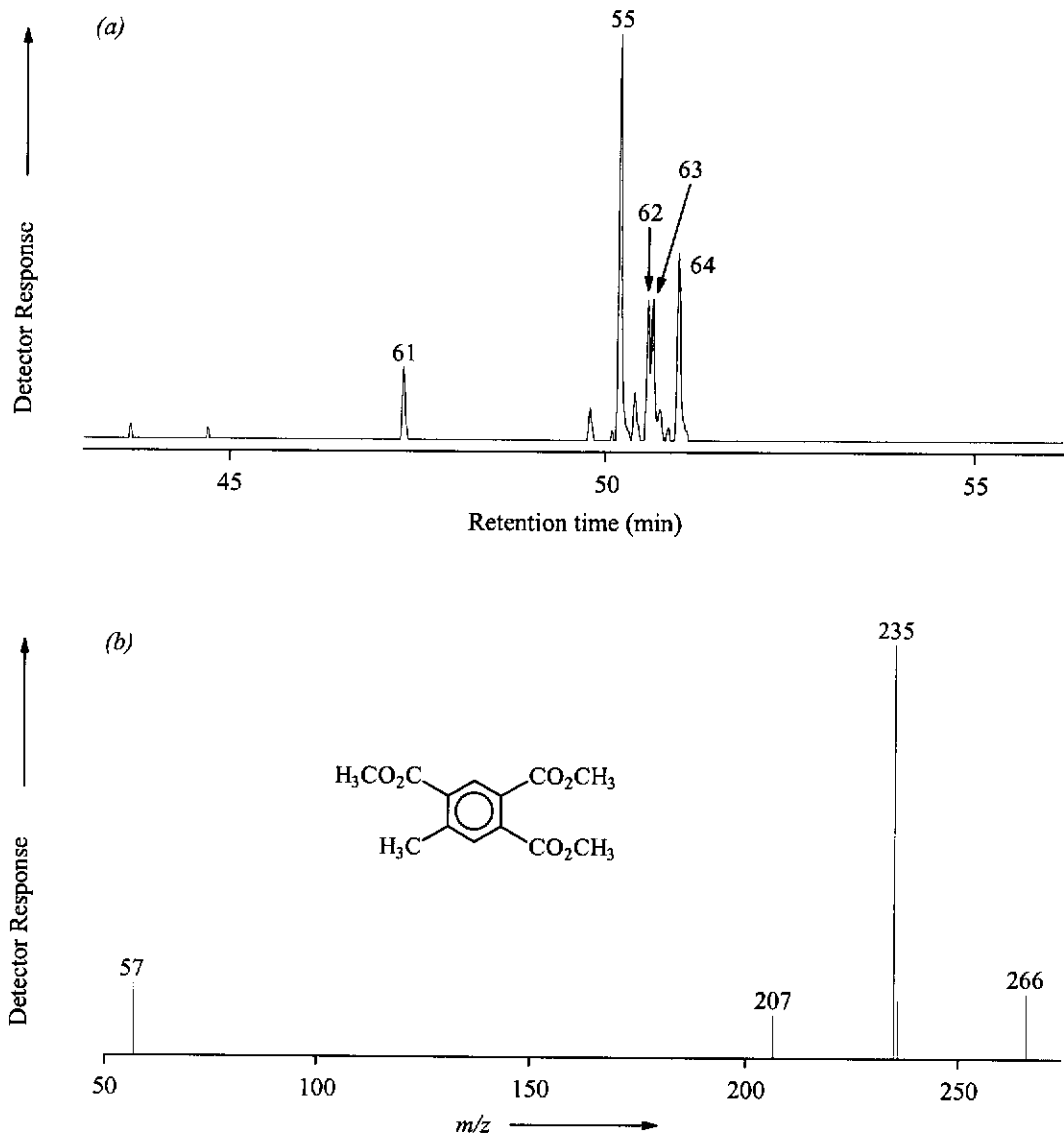
Figure 6.11. (a) GC-MS *m/z* 235 reconstructed ion chromatogram of the water-soluble polycarboxylic acids methyl esters derived from potassium permanganate oxidation of the aromatic fraction of Leatherjacket-1 788.5 m crude oil (for peak identification refer to Table 6.1); and, (b) mass spectrum of trimethyl 5-methylbenzene-1,2,4-tricarboxylate (compound 61).

this compound in the oxidation product indicates the presence of compounds containing tetra-alkyl substituted monoaromatic structural moieties (in which at least one of the alkyl groups is methyl) in the initial crude oil aromatic fraction. Also present in Fig. 6.11a is a group of peaks analogous to those observed with the tricarboxylic acids in Fig. 6.10a, containing the same base peak as the methyl-substituted tricarboxylic acid, but lacking a detectable parent ion under the MS conditions used. Once again, these probably represent compounds similar to the compound shown in Fig. 6.8, but with a second carboxylic acid group and a methyl group attached to the aromatic ring.

A summary of the aromatic carboxylic acids generated by potassium permanganate oxidation of the crude oil aromatic fraction is shown in Figure 6.12, together with the general structures of the original compounds from which the acids are most likely to have been derived. The relative amounts of monoaromatic systems with different numbers and positions of substituents are summarised in Table 6.2.

Table 6.2. Summary of the relative percentages of the monosubstituted, disubstituted, trisubstituted and tetrasubstituted monaromatic rings, and monocarboxylic, dicarboxylic and tricarboxylic acids, in the products of the potassium permanganate oxidation of the Leatherjacket-1 788.5m crude oil aromatic fraction.

|  | Monosub | Disub | Trisub | Tetrasub | Total |
|---|---|---|---|---|---|
| Monoacid | 29 | 35 | 4.4 | - | 68.4 |
| Diacid | - | 24 | 4.9 | - | 28.9 |
| Triacid | - | - | 2.6 | 0.11 | 2.71 |
| Total | 29 | 59 | 11.9 | 0.11 | 100 |

Methylated Carboxylic Acids                    Precursor
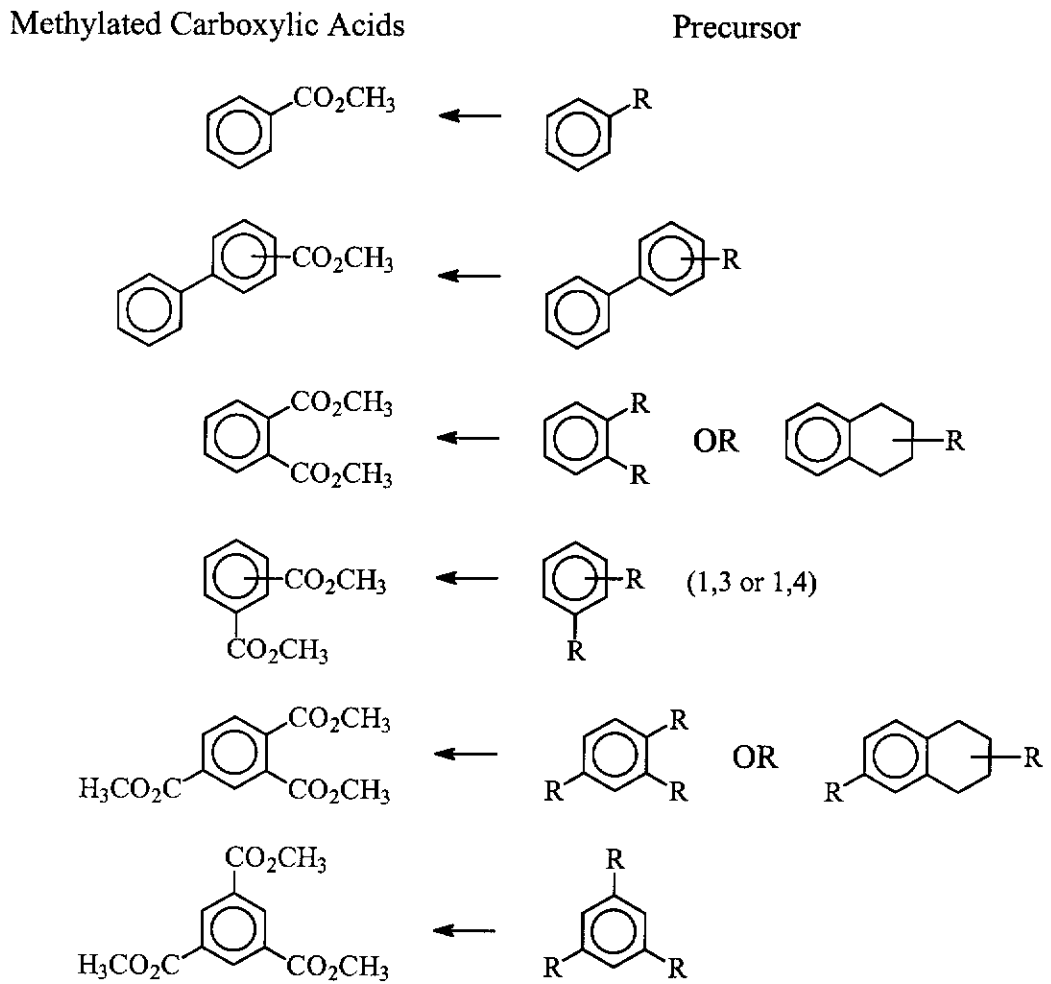


Figure 6.12. A summary of the methyl esters of the aromatic carboxylic acid products obtained from the potassium permanganate oxidation of a crude oil aromatic fraction, and the general structures of the alkylaromatic precursor compounds from which these products are most likely derived (aromatic rings may also contain one or more methyl groups).

## 6.3 CONCLUSIONS

The potassium permanganate oxidation of the crude oil aromatic fraction of a biodegraded crude oil (biodegradation level 4) has enabled a measure of the relative proportions of alkylated monoaromatic rings with different numbers and patterns of substituents to be made, as was summarised in Table 6.2.

The results presented in Table 6.2 demonstrate that over half of the monoaromatic rings in the sample were disubstituted. The proportion of benzenedicarboxylic acids with a 1,2 subtitution pattern was a very high 53 %, and it was proposed that the presence of significant quantities of indanes and tetralins in the initial aromatic fraction accounts for this observation. There is very little in the literature concerning the relative susceptibility to biodegradation of monoaromatic compounds with different substitution patterns. Connan (1984) reports that o-xylene is slightly more resistant to biodegradation than m- and p-xylene. If this trend were to occur with substituents greater than methyl, this observation may also partly explain the high amount of benzenedicarboxylic acids with a 1,2 (relative to 1,3 and 1,4) subtitution pattern. Of the benzenetricarboxylic acids, 56 % had a 1,2,4 substitution pattern as compared with 44 % with a 1,3,5 substitution pattern. The preference for the isomer with adjacent substituents may also be due to the presence of compounds containing tetralin and indane structural moieties. Of the disubstituted monoaromatic compounds formed, 59 % had a methyl group as one of the substituents. As some methyl groups may have also been oxidised, this figure should be considered a minimum value, and the proportion of disubstituted monoaromatic compounds containing at least one methyl group may be higher. It was also observed that 41 % of the trisubstituted monoaromatic compounds formed contained one methyl group, with 37 % having two methyl substituents. Again, these should be regarded as minimum values. These results demonstrate that a significant proportion of the alkyl groups attached to monoaromatic rings in the initial crude oil aromatic fraction are methyls.

Biphenylcarboxylic acids accounted for approximately 3 % of the aromatic carboxylic acids identified in the reaction product, indicating the presence of moderate amounts of compounds containing biphenyl structural moieties in the initial crude oil aromatic fraction. Biphenylcarboxylic acids with a single alkyl substituent comprised 22 % of the biphenylcarboxylic acids identified. The remaining 78 % also contained from one to three methyl groups, at 29 %, 28 % and 21 % respectively. Compounds containing a biphenyl carbon skeleton and more than one carboxylic acid group were not detected in the oxidation product, indicating that biphenyls with two or more long (> $C_1$) alkyl chains are either absent, or present in very low abundance, in the crude oil aromatic fraction. Of the monosubstituted biphenylcarboxylic acids, the ratio of ortho:meta:para substituted isomers was 0:65:35. This correlates well with literature data for the relative abundances of the methylbiphenyls in unbiodegraded crude oils, indicating that the process of biodegradation has not resulted in the preferential removal of isomers with particular substitution patterns.

CHAPTER SEVEN


# CHARACTERISATION OF THE AROMATIC UNRESOLVED COMPLEX MIXTURE OF A BIODEGRADED CRUDE OIL BY OXIDATION WITH RUTHENIUM TETROXIDE

## 7.1   INTRODUCTION

### 7.1.1   Ruthenium tetroxide as a reagent for oxidation in organic chemistry

The first reported use of ruthenium tetroxide as an organic oxidant was by Djerassi and Engle in 1953, who oxidised phenanthrene to 9,10-phenanthrenequinone using carbon tetrachloride as a solvent. Since this time, ruthenium tetroxide has been used in the oxidation of alcohols, aldehydes, alkenes, amides, ethers, esters and other compounds. These applications have been comprehensively reviewed by Courtney (1986). The reagent is typically prepared *in situ* by oxidation of a low-valence precursor, usually ruthenium dioxide or ruthenium trichloride, with a co-oxidant such as sodium periodate. Early workers used a biphasic solvent system consisting of carbon tetrachloride and water until it was realised that the slow rate of reaction was due to the formation of low-valence ruthenium carboxylate complexes which reduced the catalyst activity (Carlsen *et al.*, 1981). These workers found that introducing acetonitrile into the solvent system disrupted the complexes, preventing removal of the catalyst. Nowadays the solvent system used consists of acetonitrile:carbon tetrachloride:water, typically in a ratio of 2:2:3 (cf. Carlsen *et al.*, 1981).

### 7.1.2   Oxidation of aromatic compounds using ruthenium tetroxide

Ruthenium tetroxide oxidises alkylaromatic species at the *ipso* carbon, resulting in the formation of a carboxylic acid from the alkyl part of the molecule, with the unsubstituted aromatic carbon atoms oxidised to carbon dioxide. As described in Chapter Six, this behaviour contrasts with other oxidising agents such as permanganate ion and the various hexavalent chromium species, which oxidise the $\alpha$ carbon and leave the aromatic ring intact. An example of the ruthenium tetroxide oxidation of an alkylbenzene is shown in Figure 7.1.
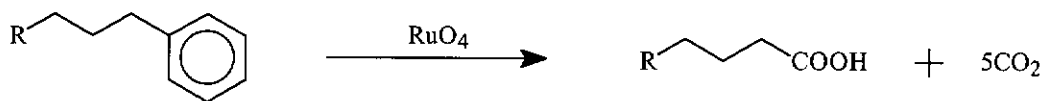
Figure 7.1. General reaction scheme showing the oxidation of an alkylbenzene using ruthenium tetroxide (R = alkyl) (Richnow *et al.*, 1992).

The reaction is not quantitative, with yields of 70 – 80 % reported for simple alkylbenzenes and phenylalkanes (Stock and Tse, 1983). Also there is frequently a mixture of products formed. In the oxidation of 1-phenylpropane, Stock and Tse (1983) reported 76 % conversion (excluding losses of unsubstituted aromatic ring carbons as carbon dioxide), with the products consisting of butyric acid (95 %), propiophenone (5 %) and propanoic acid (trace). In the case of phenyltridecane, they report a yield of 70 %, with the expected acid comprising 91 % of the product and the α-ketone the remainder.

The amount of co-oxidant used can have a significant effect on both the overall yield of the reaction and on the nature and distribution of oxidation products. Ilsley *et al.* (1986) oxidised 1-phenylpentadecane at co-oxidant to substrate (c/s) molar ratios of 2, 4, 6 and 8, where the substrate is the material being oxidised. They reported a yield of oxidised products of < 10 % at c/s = 2, which increased to 85 % at c/s = 8. At lower c/s ratios, the oxidation product was comprised almost exclusively of the carboxylic acid containing the aromatic ring carbon, while at higher c/s ratios, small amounts of shorter chain carboxylic acids were also produced. Thus a c/s ratio of 8 appears to give the highest rate of oxidation while minimising potential side reactions.

The oxidation of many other aromatic hydrocarbons using ruthenium tetroxide have been reported in the literature. The major products of the oxidation of compounds containing saturated rings fused with aromatic rings are α,ω-dicarboxylic acids, such as the oxidation of tetralin to 1,6-hexanedioic acid, with smaller amounts of 1-tetralone and 1,5-pentanedioic acid being formed

(Stock and Tse, 1983; Ilsley *et al.*, 1986). Oxidation of 4-pentylbiphenyl yields the aromatic acids benzoic acid and 4-pentylbenzoic acids as major products, with the oxidation of diphenylmethane forming both benzoic acid and phenylacetic acid. These results suggest that once a ring is cleaved and a carboxylic acid group formed on a compound containing another aromatic ring, this remaining ring is resistant to further oxidation. Oxidation of naphthalene yields phthalic acid as the major product, while the oxidation of methylnaphthalenes results in the formation of both phthalic acid and methylphthalic acid, with the product distribution strongly in favour of the unsubstituted phthalic acid (Spitzer and Lee, 1974). Examples of the oxidation of representative aromatic hydrocarbons by Stock and Tse (1983) are summarised in Table 7.1.

### 7.1.3 Structural elucidation of petroleum unresolved complex mixtures using ruthenium tetroxide

Ruthenium tetroxide has been used to determine the structures of the components of complex mixtures such as coals (Stock and Tse, 1983; Mallaya and Zingaro, 1984; Stock *et al.*, 1985; Stock and Wang, 1985; Blanc and Albrecht, 1990), kerogens (Standen *et al.*, 1991a, b), bitumens (Strausz and Lown, 1991), asphaltenes (Trifilieff *et al.*, 1992; Richnow *et al.*, 1992, 1993), refinery fractions (Thomas, 1995) and crude oils (Revill, 1992).

Revill (1992) oxidised the aromatic fraction of a biodegraded crude oil (Tia Juana Pesado, Venezuela) using ruthenium tetroxide. The acidic portion of the reaction product was 16 % resolved by GC, with the major components being a series of $n$-alkanoic acids from $C_8$ to $C_{22}$. There remained a large amount of unresolved material (84 %), of which branched alkanoic acids and alkyl-$\alpha$,$\omega$-dicarboxylic acids were proposed as the major components. On the basis of the results presented it was suggested that the aromatic UCM was comprised of highly aliphatic naphthenoaromatic compounds such as those shown in Figure 7.2.

Table 7.1. The proportions of the products of the oxidation of various alkylaromatic compounds using ruthenium tetroxide (after Stock and Tse, 1983).

| Compound | Products | Percent |
|---|---|---|
| 1-Phenylpropane | Butyric acid | 95 |
| | Propiophenone | 5 |
| | Propionic acid | trace |
| 2-Phenylbutane | 2-Methylbutyric acid | 95 |
| | 2-Phenyl-2-butanol | 1 |
| Tetralin | 1,6-Hexanedioic acid | 75 |
| | 1,5-Pentanedioic acid | 17 |
| | 1-Tetralone | 8 |
| Diphenylmethane | Phenylacetic acid | 41 |
| | Benzophenone | 32 |
| | Benzoic acid | 4 |
| 4-Pentylbiphenyl | Benzoic acid | 54 |
| | Hexanoic acid | 51 |
| | 4-Pentylbenzoic acid | 38 |
| | (4-Phenylphenyl)butyl ketone | 3 |
| | Pentanoic acid | trace |
| 1-Butylnaphthalene | Phthalic acid | 49 |
| | Pentanoic acid | 48 |
| | 3-Butylphthalic acid | 43 |
| | Butyric acid | trace |
| Phenanthrene | Diphenic acid | 91 |
| | Phthalic acid | 5 |
| | Phenanthrenequinone | 4 |
| | 1,2-Naphthalenedicarboxylic acid | trace |
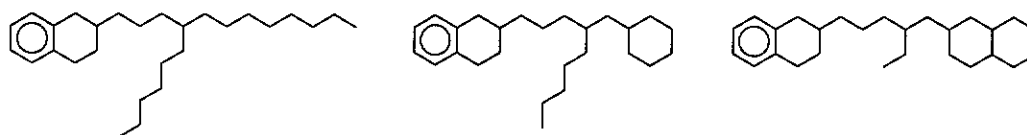
Figure 7.2. General monoaromatic structures proposed by Revill (1992) as constituents of the aromatic UCM.

Thomas (1995) used a similar approach to investigate a series of aromatic refinery fractions of a crude oil (Forties, North Sea) and vacuum distillation fractions of the same oil studied by Revill (1992), all of which were almost entirely unresolved by GC. Two modifications to the procedure were made - the oxidation products were fractionated into monocarboxylic acids, dicarboxylic acids and neutral compounds on the basis of solubility, and the amount of carbon dioxide produced was measured. Overall yields for the oxidation of the refinery fractions ranged from 38 % to 92 %. Of this, the monocarboxylic acids accounted for 6 - 34 %, dicarboxylic acids were 0 - 10 % and neutral compounds were 5 - 15 %. The remainder of the material was accounted for as carbon dioxide, formed from oxidation of unsubstituted aromatic ring carbons (cf. Fig. 7.1). As already noted by Revill (1992), the principal components identified in the oxidation products were $n$-alkanoic acids, iso- and anteisoalkanoic acids and $\alpha,\omega$-dicarboxylic acids. Other specific compounds identified included cyclohexanecarboxylic acid and phenylacetic acid, which are the products of the ruthenium tetroxide oxidation of phenylcyclohexanes and diphenylmethanes respectively (Stock and Tse, 1983), indicating that these compounds were present in the initial aromatic fraction. Ion cyclotron resonance (ICR) mass spectrometry was also used in an attempt to characterise the ruthenium tetroxide oxidation products. As this is a bulk analytical technique, conducted on the entire sample simultaneously, only general conclusions were drawn. The main compound classes identified were alkyl- and cycloalkylmonocarboxylic acids, and alkyldicarboxylic acids.

The aim of this chapter is to investigate the structure of components of an aromatic UCM. The oil selected for the study was Leatherjacket 788.5m, from the Gippsland Basin, Australia. This oil was derived from a terrestrial source, and has a biodegradation level of 4 (Burns *et al.*, 1987; Peters and Moldowan, 1993), with *n*-alkanes and alkylcyclohexanes absent, and acyclic isoprenoids, dimethylnaphthalenes, trimethylnaphthalenes and methylphenanthrenes affected. A biodegraded oil was chosen for the study as most of the GC-resolved components have been removed by biodegradation, leaving a sample with a prominent UCM. In the case of the aromatic fraction of the Leatherjacket crude oil, approximately 80 % of the material present is not resolved by GC. As will be demonstrated later in this chapter (Section 7.2.1), because of the extreme complexity of this mixture and consequent coelutions, the individual components that comprise this material cannot be adequately characterised even using current state-of-the-art techniques such as GC-MS metastable reaction monitoring (MRM). Hence, an alternative technique, utilising an oxidation procedure with modification of the oxidation products, was chosen in order to provide information about these components.

In order to characterise the alkyl side chains attached to aromatic rings, ruthenium tetroxide oxidation was performed separately on the crude oil monoaromatic, diaromatic and triaromatic fractions. A modification to the existing oxidation procedure, which involves reducing the monocarboxylic acids formed to monodeuterated hydrocarbons, is described. Because of their similarity to common constituents of petroleum, these monodeuterated hydrocarbons are readily analysed and identified by GC-MS, thus enabling a far more detailed analysis of the structures of the oxidation products to be performed than has previously been reported. These oxidation products represent the alkyl moieties which were attached to aromatic rings in the initial crude oil aromatic fractions. The results presented in this chapter are used to infer information concerning the nature of the compounds present in the aromatic UCM of this crude oil, and of the origins of these compounds.

## 7.2 RESULTS

### 7.2.1 Characterisation of crude oil aromatic fractions using instrumental techniques

The monoaromatic, diaromatic and triaromatic fractions from the Leatherjacket-1 788.5 m crude oil, Gippsland Basin, Australia; biodegradation level 4 (Burns *et al.*, 1987; Peters and Moldowan, 1993) were obtained according to the procedures described in Sections 2.2.2 and 2.2.5. Briefly, the crude oil was subjected to column chromatography using silica gel as the stationary phase to separate the saturated and aromatic hydrocarbons. The aromatic fraction was separated into monoaromatic, diaromatic and triaromatic fractions by preparative TLC using alumina as the stationary phase. Inspection of the TLC plates under ultraviolet light revealed very good separation of the various aromatic bands, which were identified by comparison of $R_f$ values with monoaromatic, diaromatic and triaromatic reference compounds. The separate aromatic fractions were then analysed using gas chromatography-mass spectrometry (GC-MS), infrared spectroscopy (IR) and $^1H$ nuclear magnetic resonance (NMR) spectroscopic techniques (Figs. 7.3 - 7.8).

*GC-MS Analysis*

GC-MS analysis of the monoaromatic fraction (Fig. 7.3a) shows that approximately 88% of the material present is unresolved. The mass spectrum averaged over the entire mass chromatogram is given in Fig. 7.4a, with a summary of the ions attributed to general structures summarised in Table 7.2.

Analysis of the mass spectrum revealed that monoaromatic mono-, di-, tri-, tetra- and pentacyclic compounds were present in this fraction. Prominent peaks at $m/z$ values of 91, 105, 119, 133 etc. (ie. $C_nH_{2n-7}$) are characteristic of monoaromatic systems with acyclic alkyl substituents. Prominent peaks at values of 131, 145, 159 etc. (ie. $C_nH_{2n-9}$) indicate the presence of alkylated monoaromatic systems with a single cyclic aliphatic moiety, which may or may not be fused to the
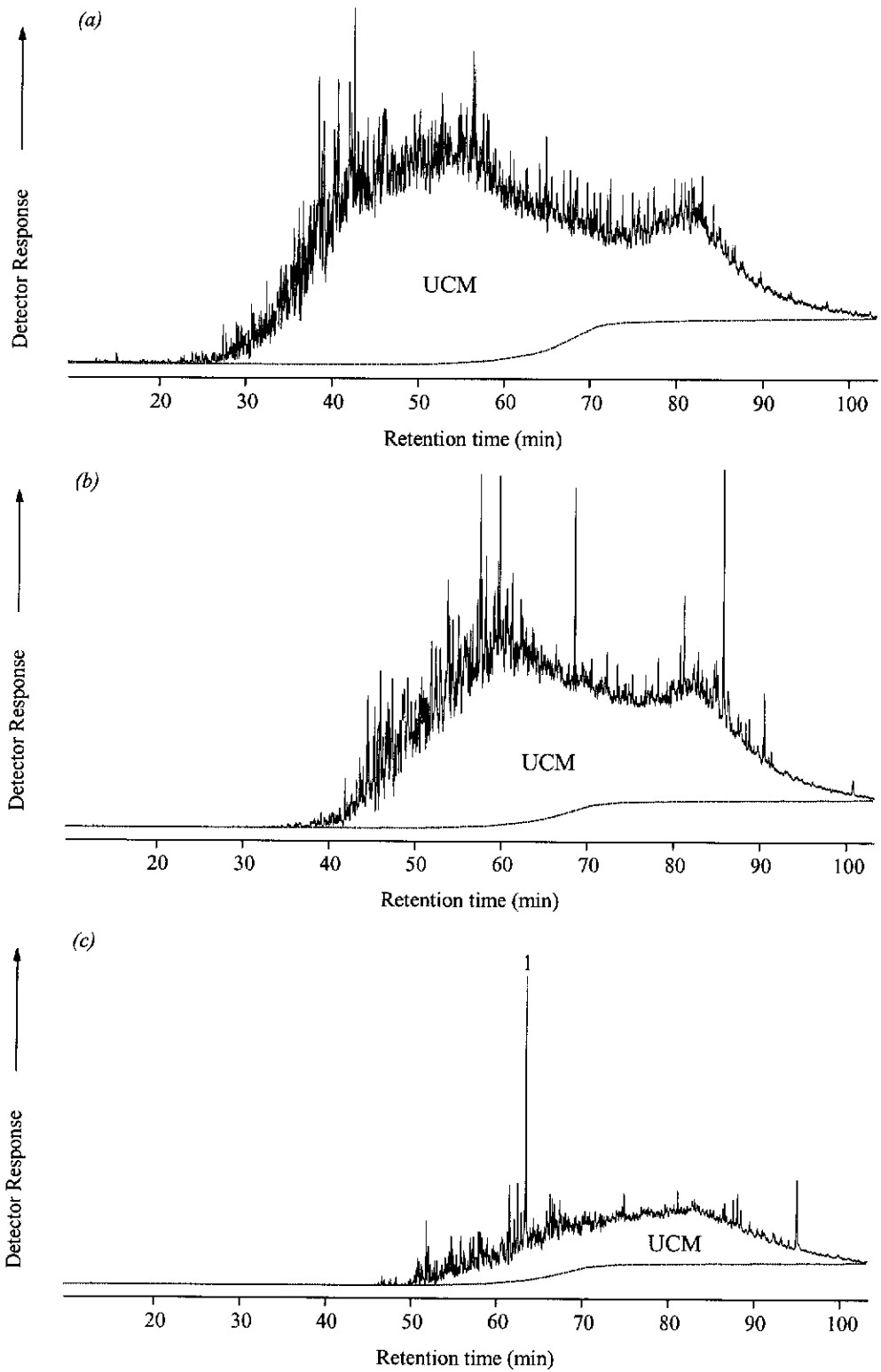
Figure 7.3. GC-MS total ion chromatograms showing the (a) monoaromatic; (b) diaromatic; and, (c) triaromatic fractions from the Leatherjacket-1 788.5m crude oil (for peak identification refer to Table 7.4).

Table 7.2. A summary of the ions attributed to general structures in the GC-MS analysis of the Leatherjacket-1 788.5m crude oil monoaromatic, diaromatic and triaromatic fractions.

| Crude oil fraction | Structure | Fragment ions ($m/z$) |
|---|---|---|
| monoaromatic | monocyclic | 91, 105, 119, etc. ($C_nH_{2n-7}$) |
| | bicyclic | 131, 145, 159, etc. ($C_nH_{2n-9}$) |
| | tricyclic | 185, 199, 213, etc. ($C_nH_{2n-11}$) |
| | tetracyclic | 239, 253, 267, etc. ($C_nH_{2n-13}$) |
| | pentacyclic | 293, 307, 321, etc. ($C_nH_{2n-15}$) |
| diaromatic | bicyclic | 141, 155, 169, etc. ($C_nH_{2n-13}$) |
| | tricyclic | 181, 195, 209, etc. ($C_nH_{2n-15}$) |
| | tetracyclic | 235, 249, 263, etc. ($C_nH_{2n-17}$) |
| | pentacyclic | 289, 303, 317, etc. ($C_nH_{2n-19}$) |
| triaromatic | tricyclic | 191, 205, 219, etc. ($C_nH_{2n-19}$) |
| | tetracyclic | 231, 245, 259, etc. ($C_nH_{2n-21}$) |
| | pentacyclic | 285, 299, 313, etc. ($C_nH_{2n-19}$) |

aromatic system, such as cyclohexylbenzenes, tetralins and indanes. Peaks with values of $C_nH_{2n-11}$ indicate the presence of two saturated cyclic systems in the monoaromatic fraction. In a similar manner, peaks corresponding to three, four and more aliphatic ring systems per molecule were also present. The mass spectra of the resolved peaks indicate that these components have similar structures to those indicated above for the bulk of the unresolved material and are simply present in higher abundance than the majority of components.

The TIC of the diaromatic fraction (Figure 7.3$b$) showed that approximately 85 % of the sample is unresolved by gas chromatography. The mass spectrum of the sample averaged over the entire chromatogram is given in Fig. 7.4$b$. It is clear from comparison of this spectrum with the averaged mass spectrum of the monoaromatic fraction (Fig. 7.4$a$) that the diaromatic fraction contains more higher molecular weight fragments. The base peak ($m/z$ 165) is characteristic of fragments with two degrees of unsaturation, indicating the presence of bicyclic saturated moieties. The peaks at $m/z$ 141, 155 and 169 (ie. $C_nH_{2n-13}$) indicate fragments with seven degrees of unsaturation, or fused ring diaromatic structures.
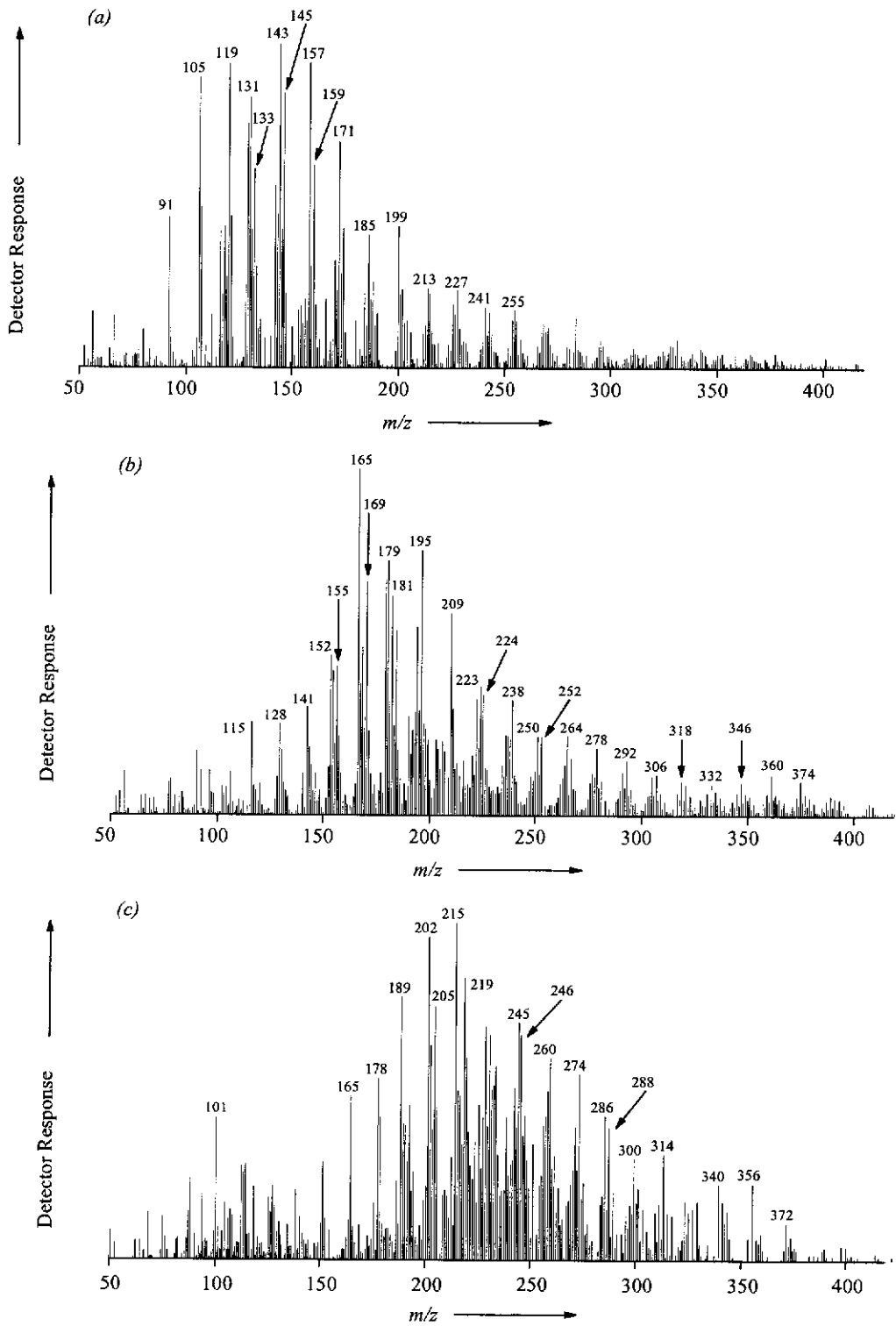
Figure 7.4. Averaged mass spectra of the total ion chromatograms shown in Fig. 7.3: (a) monoaromatic fraction (Fig. 7.3*a*, 30 min to 90 min); (b) diaromatic fraction (Fig. 7.3*b*, 40 min to 90 min); and, : (c) triaromatic fraction (Fig. 7.3*c*, 50 min to 90 min).

The major peaks at $m/z$ 181, 195, 209 and 223 (ie. $C_nH_{2n-15}$) indicate fragments with eight degrees of unsaturation, or fused tricyclic diaromatic systems. Alternatively, these fragments may be due to non-fused diaromatic compounds, such as alkylated biphenyls. The series of even-mass ions at $m/z$ 224, 238 and 252 (ie. $C_nH_{2n-14}$) probably represents the molecular ions of these tricyclic compounds, while the peaks at $C_nH_{2n-16}$ and $C_nH_{2n-18}$ indicate the molecular ions of (fused) diaromatic tetracyclic and pentacyclic compounds respectively.

GC-MS analysis of the triaromatic fraction (Fig. 7.3c) shows that approximately 66 % of the sample is unresolved. The large resolved peak (compound 1) corresponds to retene, a triaromatic compound which occurs widely in crude oils. The averaged mass spectrum of the sample is given in Fig. 7.4c, and shows a base peak of $m/z$ 215. This peak indicates the presence of fragments with five degrees of unsaturation, probably corresponding to a bicyclic monoaromatic fragment. The peak at $m/z$ 245 corresponds to fragments with 11 degrees of unsaturation, and suggests a triaromatic tetracyclic structure, such as a triaromatic sterane. Even-mass ions at $m/z$ 260, 274 and 288 probably represent the parent ions of such species, with varying numbers of aliphatic carbon atoms on the molecule. The series of even-mass ions at $m/z$ 286, 300 and 314 correspond to the parent ions of triaromatic pentacyclic compounds.

MS analysis of all three aromatic fractions indicated that alkyl sulfides ($m/z$ 61, 75, 89 and 103), alkylthiolanes ($m/z$ 87, 101, 115 and 129), alkylthiophenes ($m/z$ 97, 111, 125 and 139), alkylbenzothiophenes ($m/z$ 147, 161, 175 and 189) and alkyldibenzothiophenes ($m/z$ 197, 211, 225 and 239) were not quantitatively significant components in this sample. The presence of alkyl sulfides, alkylthiolanes and alkylthiophenes could not be confirmed in any of the three aromatic fractions. Peaks due to small amounts of alkylbenzothiophenes and alkyldibenzothiophenes were detected in the diaromatic and triaromatic samples respectively, but these were quantitatively minor components (< 5 %) in both cases. The presence of dibenzothiophenes is not surprising as these compounds are resistant to biodegradation, and therefore would be relatively enriched in a

crude oil which has been subjected to moderate biodegradation, such as this one. Moreover, all aromatic sulfur species detected contained only short alkyl chains, and there was no evidence for the presence of isomers of either compound class with side chains greater than $C_3$. As an example, the summed mass chromatograms of the diagnostic ions for alkyl sulfides, alkylthiolanes and alkylthiophenes is given in Figure 7.5, together with the GC-MS TIC of the monoaromatic fraction. Close investigation of the summed mass chromatograms demonstrated that many of the peaks present corresponded to non-sulfur-containing species. Other peaks may correspond to sulfur-containing compounds, however the low abundance of these compounds and the large amount of co-eluting material made unambiguous identification impossible. As Fig. 7.5 clearly shows, if sulfur-containing species are present, their abundance relative to the remainder of the material is very low. Comparison of the GC-MS TICs of the diaromatic and triaromatic fractions with the respective diagnostic ions for the sulfur species which may be present in these fractions as a result of the chromatographic separation technique employed also demonstrated that these compounds, if present, were only minor contributors to the aromatic fractions. The overwhelming majority of the components in the aromatic fractions studied are aromatic hydrocarbons.
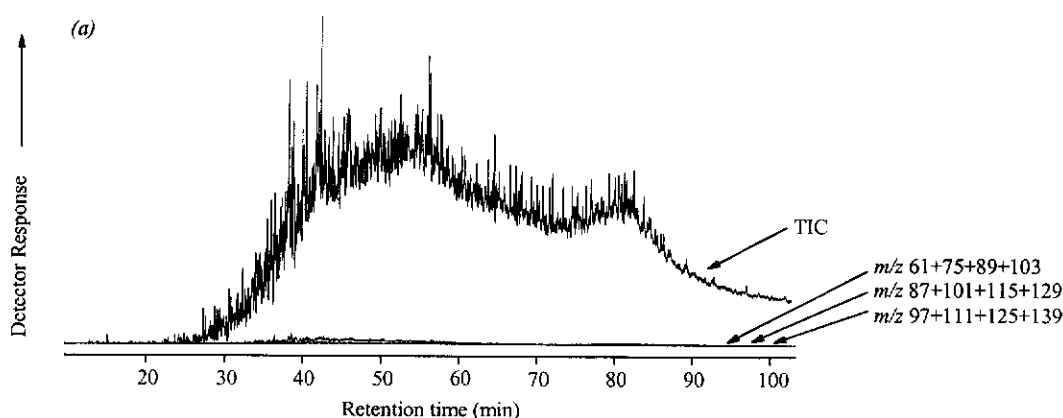


Figure 7.5. GC-MS TIC of Leatherjacket 788.5m monoaromatic fraction, together with the summed mass chromatograms of the diagnostic ions for alkyl sulfides ($m/z$ 61+75+89+103), alkylthiolanes ($m/z$ 87+101+115+129) and alkylthiophenes ($m/z$ 97+111+125+139).

*Metastable reaction monitoring (MRM) analysis*

The monoaromatic, diaromatic and triaromatic fractions of the Leatherjacket-1 788.5m crude oil were analysed by GC-MS using metastable reaction monitoring (MRM). A number of parent-to-daughter transitions were analysed based on the cleavage of various alkyl moieties from aromatic systems, at carbon numbers ranging from $C_{16}$ to $C_{29}$ (see Table 7.3). As an example, the $274 \rightarrow 105$ transition for the monoaromatic fraction is shown in Figure 7.6a. This transition represents the loss of a $C_{12}H_{25}$ group from a $C_{20}H_{34}$ alkylmethylmonoaromatic compound, *via* cleavage β to the aromatic ring, as shown in Figure 7.6b.

If a single branch is present in the alkyl chain, the number of compounds responding to the $274 \rightarrow 105$ transition is 90. The structural similarities between these compounds means that they will all elute from the GC column within a very narrow time window, and their similar mass spectral behaviour makes them virtually impossible to distinguish from each other using MRM. The potential existence of compounds with multiply-branched side chains only complicates matters even further.

The MRM analyses conducted demonstrated that, even when looking at specific parent-to-daughter transitions, large numbers of isomeric compounds in individually low abundance were present in the samples. This modern, state-of-the-art instrumental technique was not powerful enough to elucidate the structures of individual components from complex mixtures of structurally similar compounds. It was for this reason that the oxidation procedure was employed, to provide structural information about the components of these unresolved complex mixtures.

*Infrared analysis*

IR analysis of the monoaromatic fraction (Fig. 7.7a) indicates that the sample is largely aliphatic in nature. The large peaks in the spectrum at 2952, 2924 and 2854 wavenumbers (cm$^{-1}$) correspond to asymmetric and symmetric methyl and methylene stretching, with the peak at 1458 cm$^{-1}$ due to asymmetric methyl

Table 7.3.  A summary of parent-to-daughter transitions analysed using GC-MRM.

| MONOAROMATIC | | DIAROMATIC | |
|---|---|---|---|
| Parent | Daughter | Parent | Daughter |
| 218 | 105, 119, 133, 147, 161, 175 | 212 | 141, 155, 169, 183 |
| 232 | " | 226 | " |
| 246 | " | 240 | " |
| 260 | " | 254 | " |
| 274 | " | 268 | " |
| 288 | " | 282 | " |
| 302 | " | 296 | " |
| 316 | " | 310 | " |
| 330 | " | 324 | " |
| 344 | " | 338 | " |
| 358 | " | 352 | " |
| 372 | " | 366 | " |
| 386 | " | 380 | " |
| 400 | " | 394 | " |
| 216 | 105, 119, 131, 133, 145, 147, 159, 161, 173, 175 | 210 | 105, 119, 133, 141, 155, 167, 169, 181, 183, 195 |
| 230 | " | 224 | " |
| 244 | " | 238 | " |
| 258 | " | 252 | " |
| 272 | " | 266 | " |
| 286 | " | 280 | " |
| 300 | " | 294 | " |
| 314 | " | 308 | " |
| 328 | " | 322 | " |
| 342 | " | 336 | " |
| 356 | " | 350 | " |
| 370 | " | 364 | " |
| 384 | " | 378 | " |
| 398 | " | 392 | " |
| 214 | 105, 119, 133, 147, 161, 175 | 208 | 141, 155, 167, 169, 181, 183, 195 |
| 228 | " | 222 | " |
| 242 | " | 236 | " |
| 256 | " | 250 | " |
| 270 | " | 264 | " |
| 284 | " | 278 | " |
| 298 | " | 292 | " |
| 312 | " | 306 | " |
| 326 | " | 320 | " |
| 340 | " | 334 | " |
| 354 | " | 348 | " |
| 368 | " | 362 | " |
| 382 | " | 376 | " |
| 396 | " | 390 | " |
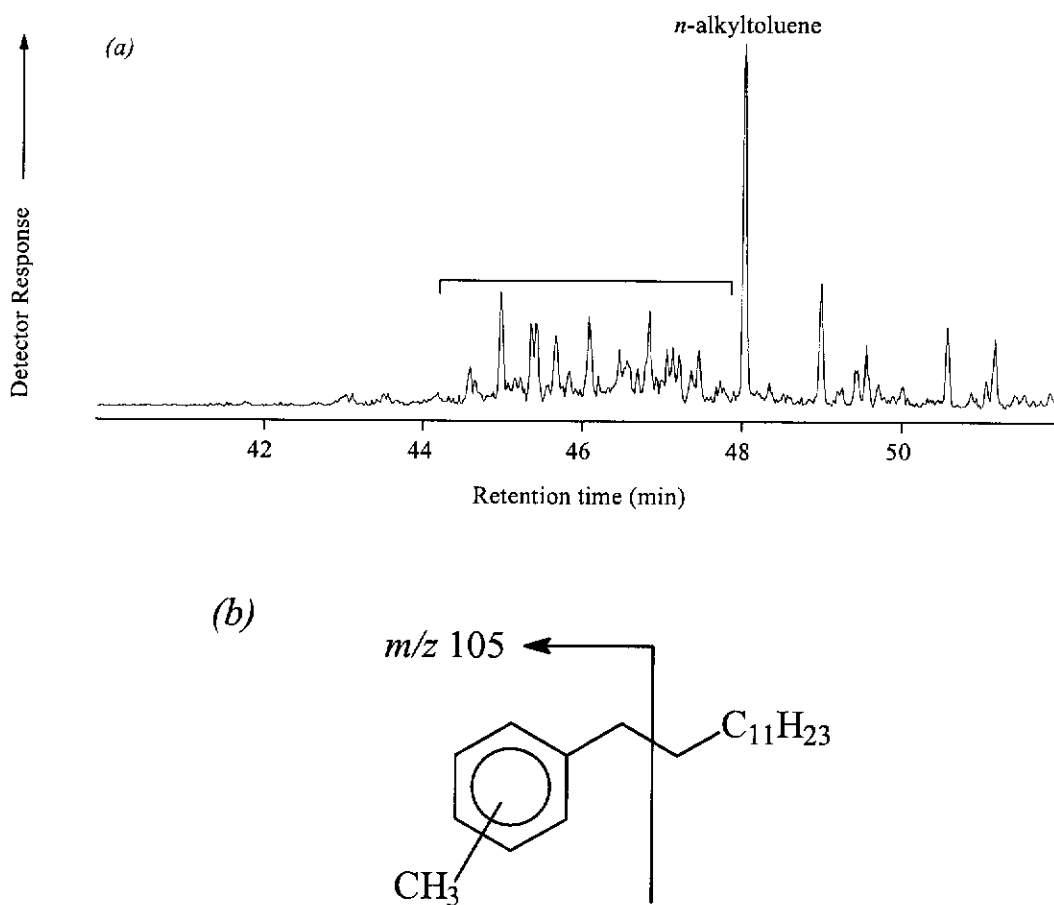
Figure 7.6.  (a) GC-MS MRM 274 → 105 transition for the Leatherjacket 788.5m monoaromatic fraction (the region showing probable branched alkyl-chain compounds is indicated by the square bracket); and, (b) the mass spectral fragmentation pathway for $C_{20}H_{34}$ alkylmethylmonoaromatic compounds.
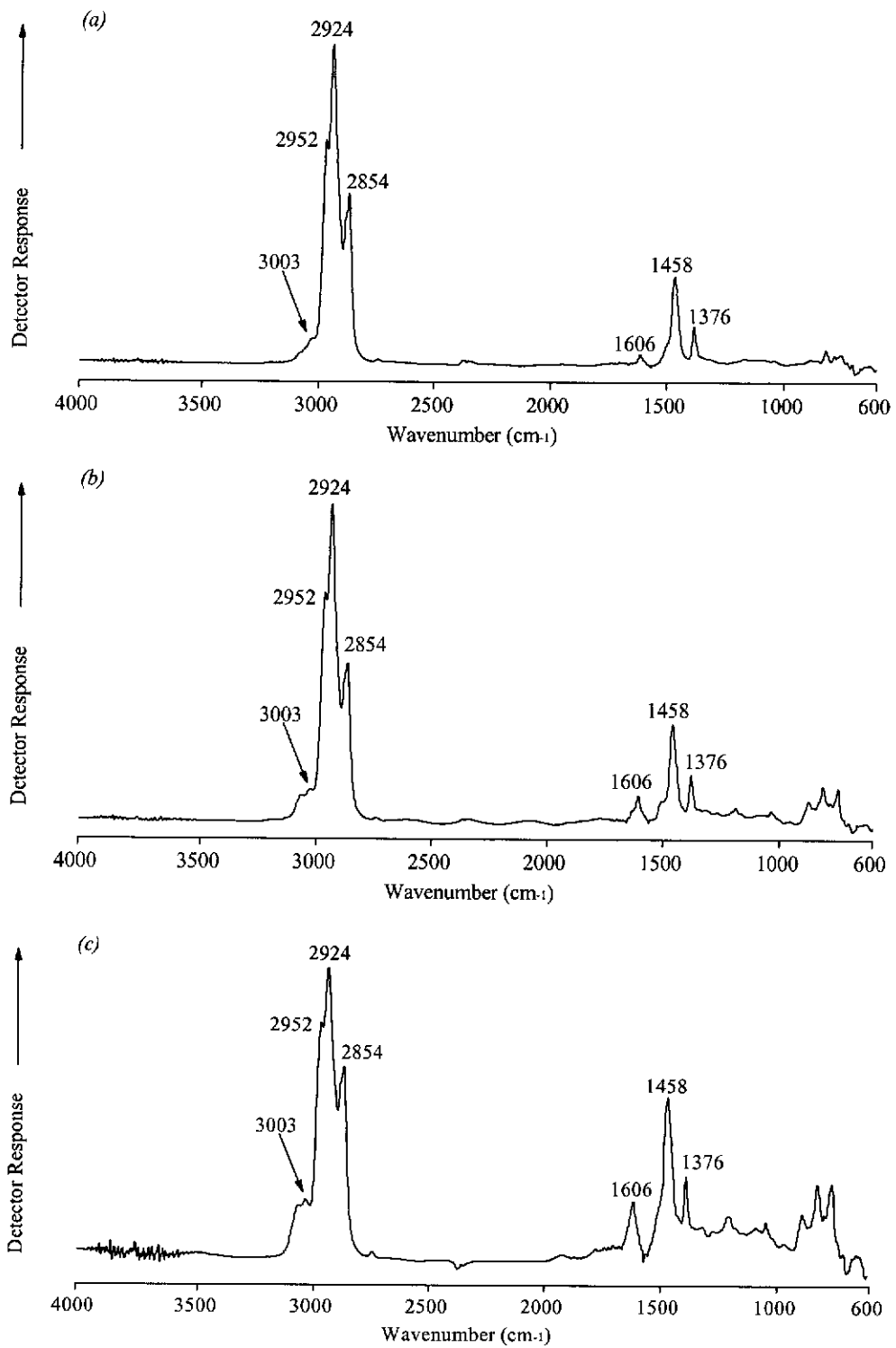
Figure 7.7. Infrared spectra of the (a) monoaromatic; (b) diaromatic; and, (c) triaromatic fractions from the Leatherjacket 788.5m crude oil.

bending and methylene scissoring and the peak at 1376 cm$^{-1}$ due to symmetric methyl bending. The shoulder at about 3000 cm$^{-1}$ indicates aromatic C-H stretching, and the small peak at 1606 and the shoulder at around 1500 cm$^{-1}$ are characteristic of the phenyl nucleus. The peaks at 820-750 cm$^{-1}$ indicate out of plane bending and ring puckering of aromatic systems, and refer to the pattern of alkyl substitution on the aromatic rings. Peaks corresponding to sulfur-containing species (e.g. alkyl sulfides, thiolanes, thiophenes, benzothiophenes and dibenzothiophenes), and oxygen-containing species (e.g. furans, benzofurans, dibenzofurans, alcohols, phenols and carbonyl compounds) were not apparent in the spectrum (Nakanishi and Solomon, 1977). This indicates that such compounds were either absent, or present in low abundance, in the sample.

IR analysis of the diaromatic fraction (Figure 7.7$b$) indicates that, like the monoaromatic fraction, it is also largely aliphatic in nature. The same peaks at 2952, 2924, 2855, 1458 and 1376 wavenumbers corresponding to methyl and methylene stretching and bending vibrations are apparent in the spectrum. The main differences in the spectra are in the peaks at 3020, 1603 and 1500 wavenumbers, corresponding to the aromatic moieties of compounds in the sample, and those at 880-750 wavenumbers corresponding to aromatic substitution patterns. The relative intensities of all of these peaks has increased significantly in the diaromatic fraction relative to the monoaromatic, indicating a higher degree of aromaticity, as expected for the diaromatic sample. As was the case with the monoaromatic fraction, peaks corresponding to sulfur- and oxygen-containing species were not apparent in the spectrum.

IR analysis of the triaromatic fraction (Fig. 7.7$c$) indicates that, like the monoaromatic and diaromatic fractions, it is also largely aliphatic in nature. Peaks at 2952, 2924, 2855, 1458 and 1376 wavenumbers, corresponding to methyl and methylene stretching and bending vibrations are once again present in the spectrum. The peaks at 3020, 1603, 1500 and 880-750 wavenumbers, corresponding to aromatic systems, are much larger relative to the aliphatic peaks than in the spectrum of the diaromatic fraction, which are larger in turn than those

for the monoaromatic fraction, and indicate an increased degree of aromaticity in the triaromatic sample as expected. Peaks corresponding to sulfur- and oxygen-containing species were once again not apparent in the spectrum.

*NMR analysis*

$^1$H NMR analysis of the monoaromatic fraction (Fig. 7.8*a*) supports the conclusion from the IR analysis, that the sample is largely aliphatic in nature. The spectrum shows strong signals below 3 ppm which correspond to aliphatic protons, while the much weaker signals at around 7 ppm indicates protons attached to aromatic rings. Integration of the spectrum shows that the aliphatic:aromatic hydrogen ratio is 11.1:1, or 91.7% of the protons present are attached to aliphatic carbons.

$^1$H NMR analysis also shows that the diaromatic UCM, like the monoaromatic fraction, is largely aliphatic in nature (Fig. 7.8*b*). In the case of the diaromatic fraction, however, it was found that the ratio of aliphatic to aromatic hydrogen atoms was a much lower 5.09:1, or 83.6 % of the protons are attached to aliphatic carbons. This indicates that a greater proportion of the carbons in the sample are part of aromatic ring systems. However, as mentioned previously, much of the sample probably contains fused diaromatic systems, the ring junction carbons of which have no hydrogen atoms attached. Thus the true extent of aromaticity of the sample is likely to be higher than is suggested by $^1$H NMR analysis.

$^1$H NMR analysis of the triaromatic fraction (Fig. 7.8*c*) again suggests that this sample also has a significant aliphatic content. In this case it was found that the ratio of aliphatic to aromatic hydrogens was 3.5:1, or 78 % of the protons in the sample are attached to aliphatic carbons (cf. 92 % and 84 % for the monoaromatic and diaromatic samples respectively). However, much of this sample most likely contains fused triaromatic systems, the ring junction carbons of which have no hydrogen atoms attached. Thus, as with the diaromatic fraction, the true extent of aromaticity of the sample is likely to be higher than is suggested by $^1$H NMR analysis.
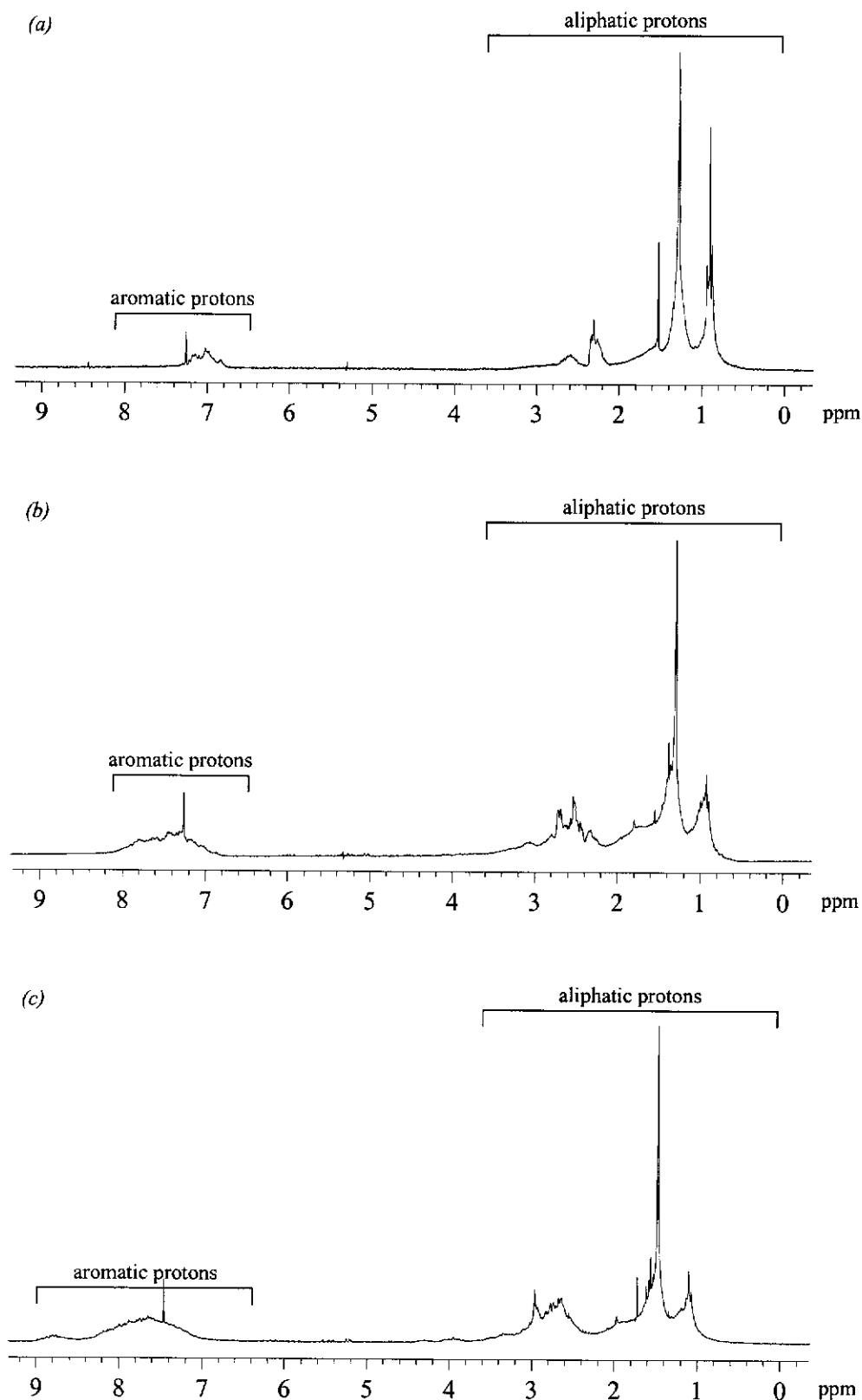
Figure 7.8. Proton NMR spectra of the (a) monoaromatic; (b) diaromatic; and, (c) triaromatic fractions from the Leatherjacket 788.5m crude oil.

### 7.2.2 Oxidation of crude oil aromatic fractions using ruthenium tetroxide

The aromatic fractions of the biodegraded Leatherjacket 788.5m crude oil described in Section 7.2.1 were oxidised using ruthenium tetroxide as described in Section 2.5.1, using a similar procedure to that of Ilsley *et al.* (1986). The oxidation and subsequent work-up procedure is summarised in Figure 7.9. A key feature is that the reaction gave a dichloromethane-soluble fraction mainly comprised of monocarboxylic acids and a water-soluble fraction mainly comprised of dicarboxylic acids. A small sub-sample of the monocarboxylic acid fraction, as well as the dicarboxylic acid sample, were methylated using boron trifluoride/ methanol reagent as described in Section 2.5.2.

The remainder of the monocarboxylic acid sample was subjected to the three-stage procedure detailed in Sections 2.5.3 - 2.5.5. Firstly, the acids were reduced using lithium aluminium hydride to yield primary alcohols. The alcohol functionalities were then converted to tosyl esters using toluene-*p*-sulfonyl chloride. Finally the tosylates were reduced using lithium aluminium deuteride to yield a sample of monodeuterated hydrocarbons in which the deuterium is attached to the carbon which was originally part of the aromatic ring in the initial aromatic compound. Note that the carbon to which the deuterium is attached is always primary by virtue of the fact that an aromatic ring carbon can have no more than one substituent. The TICs of the monocarboxylic acid methyl ester samples and the monodeuterated hydrocarbon samples are shown in Figures 7.10 and 7.11 respectively.

### 7.2.3 Analysis of the monocarboxylic acids generated from the alkyl substituents of crude oil aromatic components

*Monocarboxylic acid methyl esters*
The most prominent peaks in the TIC of the monocarboxylic acid methyl ester sample from the monoaromatic fraction (Fig. 7.10*a*) correspond to a homologous series of *n*-alkanoic acids as indicated. The largest peak in the

Crude oil aromatic
fraction

Stir 100 mg in a solvent mixture containing CH3CN:DCM:
H2O (10 mL:10 mL:15 mL) with NaIO4 (c/s = 8) and
RuO2.3H2O (25 mg) for 72 h. Quench with MeOH (0.5 mL),
add H2O (20 mL), separate organic layer and extract aqueous
layer with DCM (3 x 5 mL). Combine organics.

Dichloromethane (DCM) | H2O

DCM soluble reaction
products

(H2O soluble)
Dicarboxylic acids

Reduce volume to *ca.* 10 mL
on a sand bath and extract with
KOH (1 M; 3 x 10 mL).

Evaporate to dryness
on rotary evaporator
and methylate with
BF3/MeOH reagent.

KOH extract | DCM residue

(DCM soluble)
Monocarboxylic acids

Dimethyl esters of
dicarboxylic acids

Acidify with dil HCl and extract with DCM (3 x 5 mL).
Remove solvent by evaporation and add dry ether (1 mL).
Stir LiAlH4 in dry ether (10 mL) and add the former
solution dropwise, and stir for 1 h. Neutralise xs LiAlH4
with NH4Cl (1 M) and dissolve ppt with H2SO4 (3 M).
Separate organic layer and extract aqueous layer with
DCM (3 x 5 mL). Combine organics and dry (MgSO4).
(Methylate a small subsample for analysis by GC and
GC-MS as per dicarboxylic acids.)

Analysis by GC and GC-MS

Primary alcohols

DCM soluble neutral
compounds

Reduce volume to *ca.* 7 mL and cool in
an ice bath. Add pyridine and
toluene-*p*-sulfonyl chloride with stirring
and maintain conditions for 4 h. Add
water (5 mL) and separate organic layer.
Extract aqueous layer with ether (3 x 5
mL) and combine organics. Wash with
HCl (2 M; 5 mL), NaHCO3 (5 %; 5 mL)
and water (5 mL). Dry with MgSO4.

Analysis by GC and GC-MS

Toluene-*p*-sulfonyl
chloride derivatives

Reduce as per monocarboxylic acids
above, but using LiAlD4 in place of
LiAlH4.

Monodeuterated
hydrocarbons

Analysis by GC and GC-MS

Figure 7.9. Flowchart showing the procedure used in the ruthenium tetroxide oxidation of crude
oil aromatic fractions.
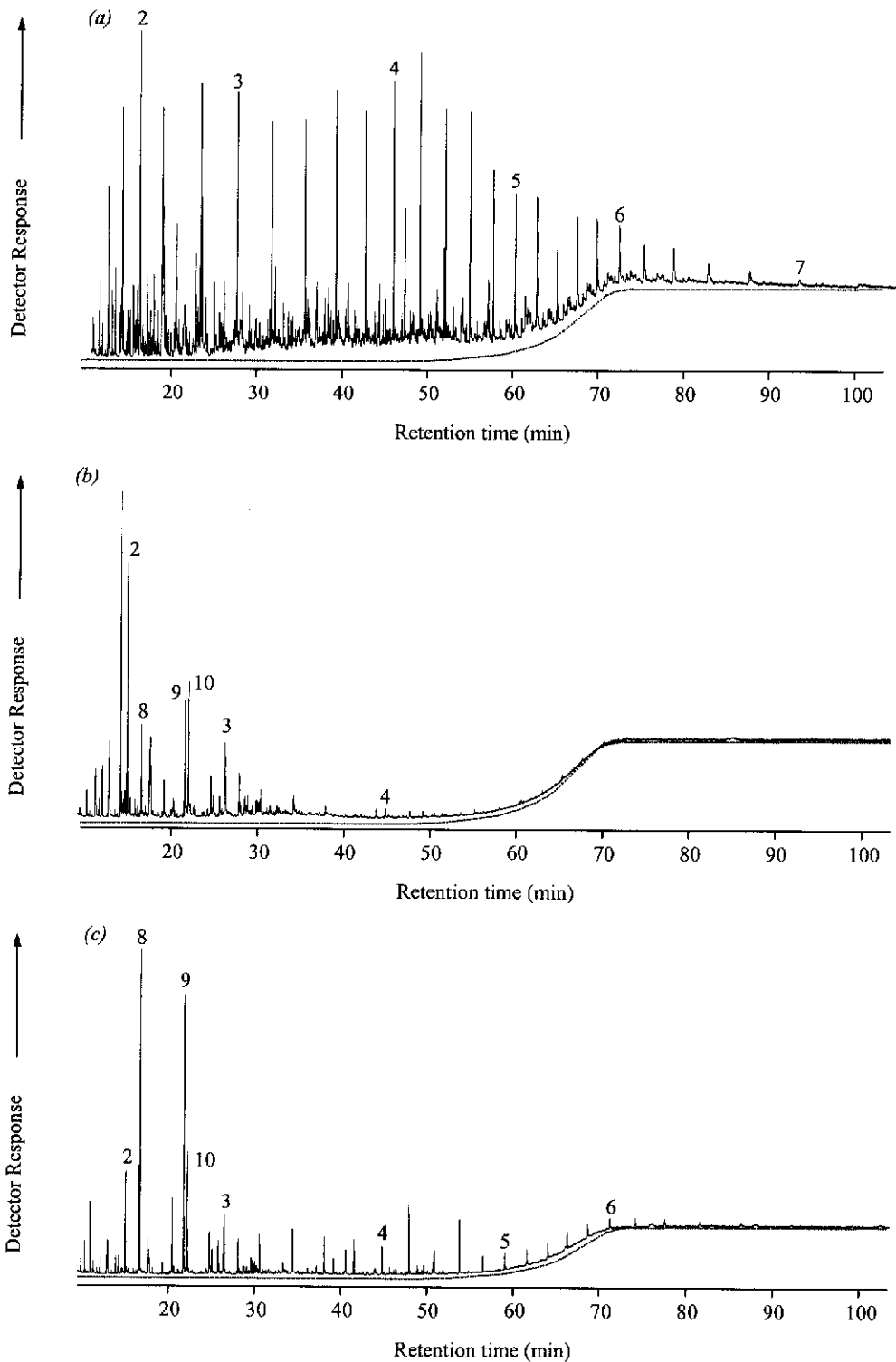
Figure 7.10. GC-MS total ion chromatograms showing the methyl esters of the dichloromethane soluble monocarboxylic acids from the ruthenium tetroxide oxidation of the (a) monoaromatic; (b) diaromatic; and, (c) triaromatic fractions from Leatherjacket 788.5m crude oil (baselines shown for reference purposes; for peak identification refer to Table 7.4).

Table 7.4. Identification of peaks labelled with numbers in figures in this chapter.

| Peak no. | Identification * | Peak no. | Identification * |
|---|---|---|---|
| 1 | Retene [a] | 44 | 7-Ethylhexadecane |
| 2 | Methyl cyclohexanecarboxylate | 45 | 6-Ethylhexadecane |
| 3 | Methyl decanoate | 46 | 5-Ethylhexadecane |
| 4 | Methyl pentadecanoate | 47 | 4-Ethylhexadecane |
| 5 | Methyl eicosanoate | 48 | 3-Ethylhexadecane |
| 6 | Methyl pentacosanoate | 49 | 6-Propylpentadecane |
| 7 | Methyl triacontanoate | 50 | 5-Propylpentadecane |
| 8 | Methyl benzoate | 51 | 4-Propylpentadecane |
| 9 | Methyl 3-methylbenzoate | 52 | 7-Butyltetradecane |
| 10 | Methyl 4-methylbenzoate | 53 | 6-Butyltetradecane |
| 11 | $n$-$C_{10}$ | 54 | 7-Pentyltridecane |
| 12 | $n$-$C_{15}$ | 55 | 6-Pentyltridecane |
| 13 | $n$-$C_{16}$ | 56 | Pentylcyclohexane |
| 14 | $n$-$C_{17}$ | 57 | Dectylcyclohexane |
| 15 | Pristane | 58 | Pentadecylylcyclohexane |
| 16 | $n$-$C_{18}$ | 59 | Eicosylcyclohexane |
| 17 | Phytane | 60 | Pentacosylcyclohexane |
| 18 | $n$-$C_{20}$ | 61 | Butylmethylcyclohexane [b] |
| 19 | $n$-$C_{25}$ | 62 | Methylnonylcyclohexane [b] |
| 20 | $n$-$C_{30}$ | 63 | Methytetradecylcyclohexane [b] |
| 21 | 2,6-Dimethylnonane | 64 | Methylnonadecylcyclohexane [b] |
| 22 | 2,6-Dimethyldecane | 65 | $C_{17}$ Bicyclic compound |
| 23 | 2,6-Dimethylundecane | 66 | Methyldecalin [c] |
| 24 | 2,6,10-Trimethylundecane | 67 | Ethyldecalin [c] |
| 25 | 2,6,10-Trimethyldodecane | 68 | Propyldecalin [c] |
| 26 | 2,6,10-Trimethyltridecane | 69 | Butyldecalin [c] |
| 27 | 2,6,10-Trimethyltetradecane | 70 | Methyl benzeneacetate |
| 28 | 2,6,10-Trimethylpentadecane | 71 | Methyl 2-methylbenzoate |
| 29 | 2,6,10,14-Tetramethylheptadecane | 72 | Methyl $C_1$-benzeneacetate |
| 30 | 3-Methylheptadecane | 73 | Methyl $C_1$-benzeneacetate |
| 31 | 2-Methylheptadecane | 74 | Methyl $C_2$-benzoate |
| 32 | 4-Methylheptadecane | 75 | Methyl $C_2$-benzoate |
| 33 | 5-Methylheptadecane | 76 | Methyl $C_2$-benzoate |
| 34 | 6-Methylheptadecane | 77 | Methyl $C_2$-benzeneacetate |
| 35 | 7-Methylheptadecane | 78 | Methyl $C_2$-benzeneacetate |
| 36 | 8-Methylheptadecane | 79 | Methyl $C_3$-benzoate |
| 37 | 9-Methylheptadecane | 80 | Methyl $C_2$-benzoate |
| 38 | Methyl 2-methylhexanoate | 81 | Methyl $C_2$-benzoate |
| 39 | Methyl 2-methyldecanoate | 82 | Methyl $C_2$-benzeneacetate |
| 40 | Methyl 2-methylpentadecanoate | 83 | Methyl $C_2$-benzoate |
| 41 | Methyl 2-methyleicosanoate | 84 | Dimethyl pentanedioate |
| 42 | Methyl 2-methylpentacosanoate | 85 | Dimethyl 3-methylpentanedioate |
| 43 | 8-Ethylhexadecane | 86 | Dimethyl 2-methylpentanedioate |

Table 7.4 (cont'd). Identification of peaks labelled with numbers in figures in this chapter.

| Peak no. | Identification * | Peak no. | Identification * |
|---|---|---|---|
| 87 | Dimethyl 3,3-dimethylpentanedioate | 111 | Dimethyl methylphthalate |
| 88 | Dimethyl 2,2-dimethylpentanedioate | 112 | Dimethyl ester of unidentified monoaromatic diacid |
| 89 | Dimethyl ester of $C_7$ diacid | | |
| 90 | Dimethyl 2-ethylpentanedioate | 113 | Dimethyl ester of aromatic $C_{10}$ diacid |
| 91 | Dimethyl ester of $C_7$ diacid | 114 | Dimethyl ester of unidentified monoaromatic diacid |
| 92 | Dimethyl ester of $C_8$ diacid | | |
| 93 | Dimethyl ester of $C_8$ diacid | 115 | Dimethyl 3-ethyl-3-methylpentanedioate |
| 94 | Dimethyl ester of $C_8$ diacid | | |
| 95 | Dimethyl ester of $C_8$ diacid | 116 | Dimethyl 2-methylhexanedioate |
| 96 | Dimethyl ester of $C_8$ CP-diacid [c,d] | 117 | Dimethyl 3-methylhexanedioate |
| 97 | Dimethyl ester of $C_9$ CA-diacid [e] | 118 | Dimethyl heptanedioate |
| 98 | Dimethyl ester of $C_9$ CA-diacid [e] | 119 | Dimethyl octanedioate |
| 99 | Dimethyl ester of $C_9$ CA-diacid [e] | 120 | Dimethyl nonanedioate |
| 100 | Dimethyl ester of $C_9$ CA-diacid [e] | 121 | Dimethyl decanedioate |
| 101 | Dimethyl ester of $C_{10}$ CH-diacid [f] | 122 | Dimethyl ester of $C_9$ CA-diacid [e] |
| 102 | Dimethyl ester of $C_9$ CH-diacid [f] | 123 | Dimethyl ester of $C_{10}$ CH-diacid [f] |
| 103 | Dimethyl ester of $C_{10}$ CH-diacid [f] | 124 | Dimethyl ester of $C_{10}$ CH-diacid [f] |
| 104 | Dimethyl ester of $C_9$ CA-diacid [e] | 126 | Dimethyl ester of aromatic $C_{11}$ diacid |
| 105 | Dimethyl ester of $C_{10}$ CH-diacid [f] | 127 | Dimethyl ester of aromatic $C_{11}$ diacid |
| 106 | Dimethyl ester of $C_{10}$ CH-diacid [f] | 128 | Dimethyl ester of aromatic $C_{11}$ diacid |
| 107 | Dimethyl butanedioate | 129 | Dimethyl ester of aromatic $C_{12}$ diacid |
| 108 | Dimethyl methylbutanedioate | 130 | Dimethyl ester of aromatic $C_{12}$ diacid |
| 109 | Dimethyl hexanedioate | 131 | Dimethyl ester of aromatic $C_{12}$ diacid |
| 110 | Dimethyl phthalate | | |

* - compounds listed as hydrocarbons contain one deuterium atom; a: not deuterated; b: four isomers; c: tentative identification; d: CP = cyclopentane; e: CA = cycloalkane; f: CH = cyclohexane.

chromatogram, however, is due to methyl cyclohexanecarboxylate. The TICs of the monocarboxylic acid methyl ester samples from the diaromatic and triaromatic fractions (Figs. 7.10*b* and *c* respectively) appear quite different to Fig. 7.10*a*. The most prominent peaks in both of these chromatograms correspond to methyl cyclohexanecarboxylate, and methyl benzoate and its 3- and 4-methyl substituted isomers. The homologous series of *n*-alkanoic acids is also visible in the chromatograms, but individual isomers are in low abundance relative to these major peaks.

*Monodeuterated hydrocarbons*

The TICs of the monodeuterated hydrocarbon samples from the monoaromatic, diaromatic and triaromatic fractions are given in Figures 7.11*a, b* and *c* respectively. Approximately 60 % of the sample from the monodeuterated hydrocarbons from the monoaromatic fraction is resolved by GC. The proportion of resolved material in the diaromatic and triaromatic fractions respectively is 60 % and 75 %.

A significant proportion of each of the deuterated hydrocarbon samples have been identified using GC-MS techniques. Major components identified in all three samples include *n*-alkanes, monomethylalkanes, monoalkylalkanes, acyclic isoprenoids, alkylcyclohexanes and bicyclic compounds. The relative proportions of these compound classes in the samples are given in Table 7.5. The following is a discussion of the individual compound classes identified in the monocarboxylic acid methyl ester and monodeuterated hydrocarbon samples derived from the three aromatic fractions.
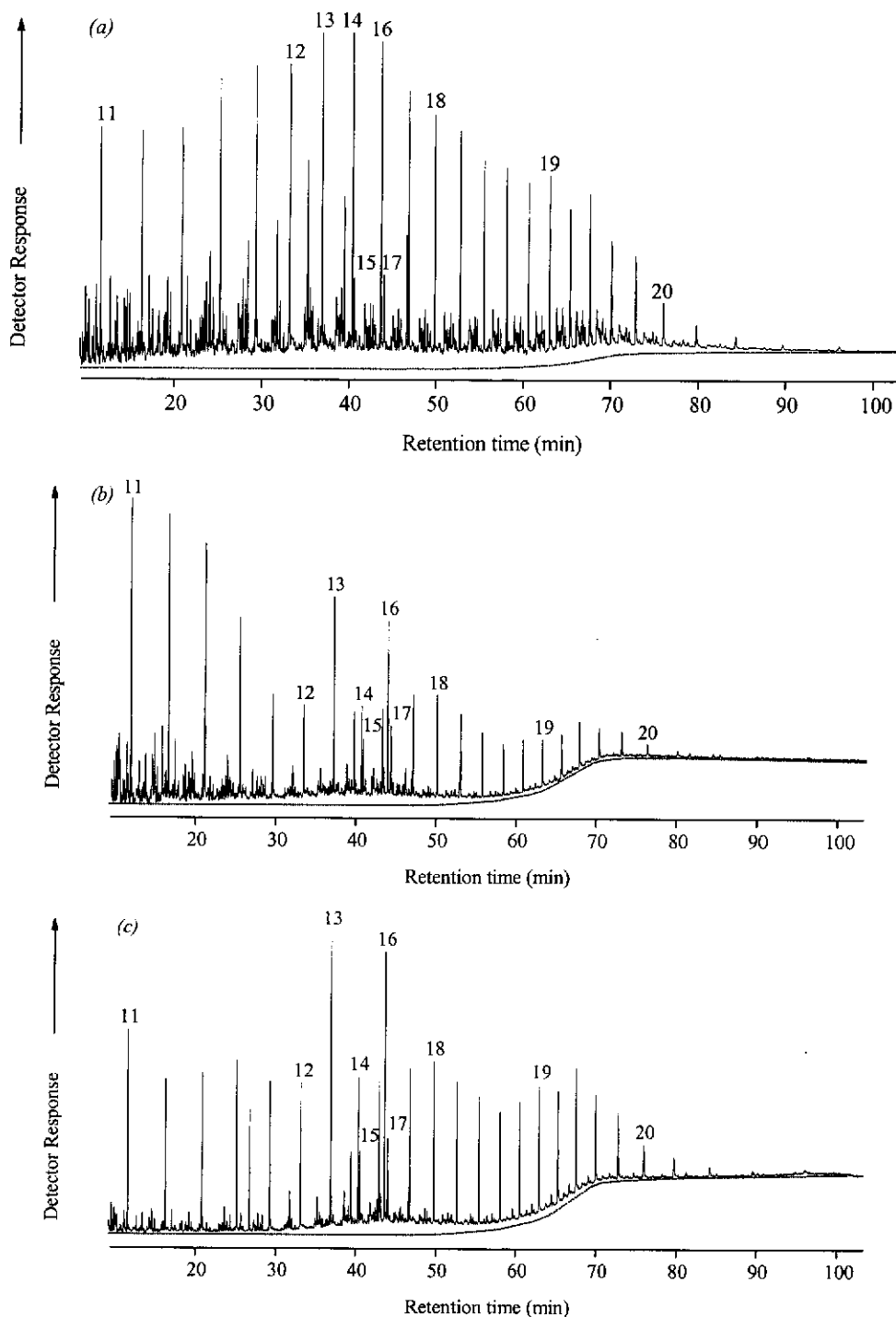
Figure 7.11. GC-MS total ion chromatograms showing the monodeuterated hydrocarbons derived from the monocarboxylic acids from the ruthenium tetroxide oxidation of the (a) monoaromatic; (b) diaromatic; and, (c) triaromatic fractions from Leatherjacket 788.5m crude oil (for peak identification refer to Table 7.4).

Table 7.5. Relative proportions of the various compound classes identified in the monodeuterated hydrocarbon samples derived from the monoaromatic, diaromatic and triaromatic fractions of the Leatherjacket-1 788.5 m crude oil.

| Compound class | Relative proportion (%) | | |
|---|---|---|---|
| | Monoaromatic | Diaromatic | Triaromatic |
| n-Alkanes | 15.0 | 16.1 | 26.6 |
| Isoprenoids | 3.5 | 3.7 | 3.3 |
| Methylalkanes | 17.0 | 13.5 | 9.0 |
| Monoalkylalkanes | 5.5 | 4.3 | 2.8 |
| (Methyl)alkylcyclohexanes | 2.1 | 2.3 | 1.4 |
| Bicyclic compounds | 1.7 | 1.2 | 0.8 |
| Other * | 55.2 | 58.9 | 56.1 |

* analysis of mass spectra indicates that this material is mainly polyalkylated acyclic compounds and other cyclic species. This includes aromatic compounds for the samples derived from the diaromatic and triaromatic fractions.

### 7.2.3.1 n-Alkanes

*Identification of monodeuterated n-alkanes*

The most apparent feature of the TIC of the deuterated hydrocarbons from the monoaromatic fraction (Fig. 7.11$a$) is the prominent set of peaks due to the presence of a homologous series of $n$-alkanes extending from $C_{10}$ to $C_{34}$. This distribution is fairly smooth with a slight preference at $C_{25}$, $C_{27}$ and $C_{29}$ (CPI = 1.07). As an example, the mass spectrum of the $C_{18}$ homologue is shown in Figure 7.12. In this mass spectrum, the peak at $m/z$ 255 represents the molecular ion. As $n$-$C_{18}$ has a molecular weight of 254, it is clear that the compound contains a single deuterium atom. As mentioned previously, the nature of the procedure used to generate the compound dictates that the deuterium must be attached to a primary carbon atom. Also shown in Fig. 7.12 is the mass spectrum of methyl $n$-octadecanoate, the corresponding compound from the monocarboxylic acid methyl ester sample.
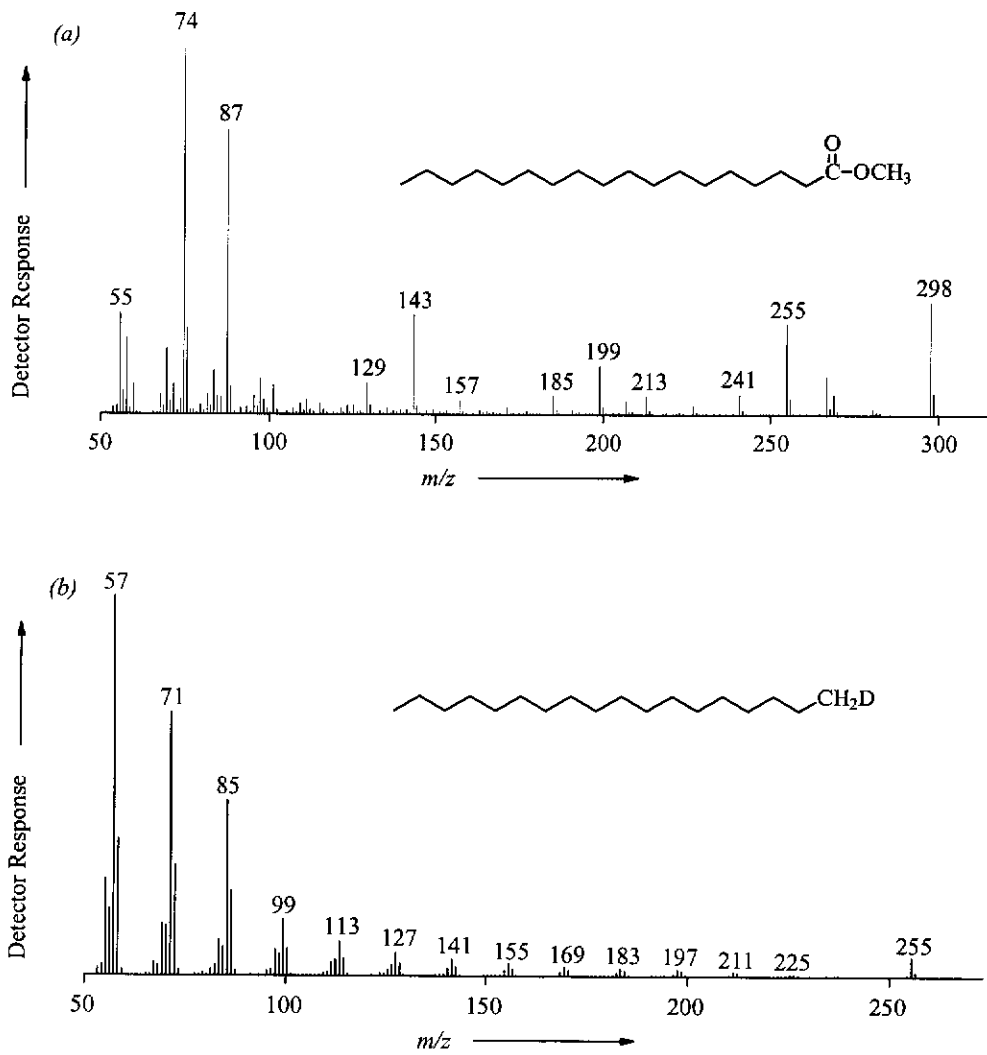
Figure 7.12. Mass spectra of (a) methyl *n*-octanoate; and, (b) monodeuterated *n*-octadecane, from the ruthenium tetroxide oxidation products of the Leatherjacket-1 788.5m monoaromatic fraction.

The most prominent peaks in the chromatogram for the monodeuterated hydrocarbon sample from the diaromatic fraction (Fig. 7.11$b$) were also monodeuterated $n$-alkanes. In this sample, they ranged from $C_{10}$ to $C_{31}$, with a strong preference for the $C_{16}$ and $C_{18}$ homologues. As with the sample from the monoaromatic fraction, there is also a slight preference for the $C_{25}$, $C_{27}$ and $C_{29}$ homologues (CPI = 1.04). The monodeuterated hydrocarbon sample from the triaromatic fraction (Fig. 7.11$c$) appears similar to that from the diaromatic fraction, with a prominent series of monodeuterated $n$-alkanes, ranging from $C_{10}$ to $C_{31}$. Once again, a strong preference for the $C_{16}$ and $C_{18}$ homologues is apparent, together with a slight preference for the $C_{25}$, $C_{27}$ and $C_{29}$ homologues (CPI = 1.09).

*Origins of* n-*alkanes and their aromatic precursors*

The presence of $n$-alkanes in the monodeuterated hydrocarbon samples indicates that species with $n$-alkyl groups directly attached to the aromatic ring were present in the initial aromatic fractions. The monodeuterated $n$-alkanes are formed from the reduction of the $n$-alkanoic acids generated by oxidation of the crude oil aromatic fractions. The TICs of the methyl esters of the monocarboxylic acid oxidation products (Figs. 7.10$a$-$c$) contain series of $n$-alkanoic acids, starting at $C_7$ (the GC method used precluded the analysis of lower molecular weight compounds). The results of the analysis of both the monocarboxylic acid methyl esters and the monodeuterated hydrocarbons suggest that the range of these $n$-alkyl moieties extend from at least $C_7$. The formation of $n$-alkanoic acids from the ruthenium tetroxide oxidation of crude oil monoaromatic and diaromatic fractions has previously been reported (Thomas, 1995), as well as from a total aromatic fraction (Revill, 1992). $n$-Alkanoic acids have also been reported from the ruthenium tetroxide oxidation of aromatic structural units of kerogen (Richnow *et al.*, 1992) and asphaltenes (Trifilieff *et al.*, 1992; Richnow *et al.*, 1993).

An odd-over-even predominance is observed for the $C_{25}$, $C_{27}$ and $C_{29}$ saturate $n$-alkanes in the gas chromatograms of whole unbiodegraded crude oils from the same geographic location (Gippsland Basin, Australia) published by Burns *et al.*

(1987). The odd-over-even predominance observed in crude oils for $n$-alkanes around $C_{25}$ - $C_{33}$ is characteristic of higher-plant input into the sediments (e.g. Eglinton and Hamilton, 1967; Rieley *et al.*, 1991a, b; Killops and Killops, 1993). The odd-carbon-number predominance is due to either direct synthesis of those $n$-alkanes within the plants or to defunctionalisation during diagenesis of the even-carbon-number acids, alcohols or esters from these plants (Tissot and Welte, 1984).

The observed odd-carbon-number predominance in the monodeuterated $n$-alkanes implies that there is a predominance of even-carbon-number alkyl chains attached to aromatic rings in the monoaromatic fraction of the Leatherjacket crude oil. Either the even-carbon-number $n$-alkanoic acids or $n$-alcohols could add to the monoaromatic rings to form $n$-alkylaromatic compounds with even carbon number side chains. These compounds, when subjected to ruthenium tetroxide oxidation, would yield odd carbon number $n$-alkanoic acids, subsequently reduced to odd carbon number monodeuterated $n$-alkanes. These oxidation products would have two carbons more than the corresponding saturate $n$-alkanes, thus also resulting in an odd-over-even predominance. Williams *et al.* (1988) reported the occurrence of specific alkylbenzenes, alkyltoluenes and alkylxylenes all with $n$-$C_{15}$ side chains in two crude oils, and this, together with the results of laboratory heating experiments which demonstrated that benzene undergoes alkylation by primary alcohols to give $n$-alkylbenzenes, led these workers to conclude that these alkylaromatics were formed *via* sedimentary alkylation reactions. Wilkes *et al.* (1998) have reported the occurrence of alkylaromatic compounds bearing an $\alpha$-carbonyl group in Posidonia shale bitumens, and propose that these compounds arise *via* addition of acylium carbocations (from carboxylic acids) onto aromatic rings.

It is proposed that in the current study, the preference for $C_{25}$, $C_{27}$ and $C_{29}$ $n$-alkanes in both the monodeuterated oxidation products and in unbiodegraded crude oils from the same geographic location is evidence for sedimentary alkylation by even carbon number long chain carboxylic acids or alcohols onto

aromatic rings, leading to the formation of monoaromatic compounds with $n$-alkyl side chains. Further evidence for this conclusion is provided by the similar carbon number range observed for the monodeuterated $n$-alkanes (up to $C_{34}$, indicating the presence of alkyl side chains to $C_{33}$) and the $n$-alkanes (up to $C_{35}$) in unbiodegraded Gippsland Basin crude oils (cf. Burns et al., 1987).

If alkylation of monoaromatic species accounts for the presence of alkylmonoaromatic compounds in the crude oil aromatic fraction, then this alkylation process would also occur with diaromatic and triaromatic species. The odd-over-even predominance also observed at $C_{25}$, $C_{27}$ and $C_{29}$ in the monodeuterated hydrocarbon samples from both of these fractions provides strong evidence to support this proposal. The high relative abundance of monodeuterated $n$-$C_{16}$ and $n$-$C_{18}$ (corresponding to $C_{15}$ and $C_{17}$ side chains) observed in the products from the diaromatic and triaromatic fractions therefore suggests that these compounds have, at least partly, a different origin. The specificity of the carbon chain lengths indicates a possible biological origin for these side chains. Richnow et al. (1993) also observed a strong predominance of $C_{16}$ and $C_{18}$ alkanoic acids in the products from the ruthenium tetroxide oxidation of the aromatic structural units of the resin fraction of Rozel Point oil, suggesting that this was the result of early diagenetic incorporation of bacterial biolipids.

The occurrence in sedimentary organic matter of alkylaromatic compounds, such as alkylbenzenes, alkyltoluenes and alkylxylenes, are widely reported in the literature (Solli et al., 1980; Ostroukhov et al., 1983; Albaigés et al., 1986; Williams et al., 1988; Sinninghe Damsté et al., 1988, 1991, 1993; Dong et al., 1993; Ellis et al., 1992, 1995). Sinninghe Damsté et al. (1991) also reported the occurrence of homologous series of 1,2-di-$n$-alkylbenzenes in a Miocene oil from the Tarragona Basin, Spain. Suggested origins of long-chain alkylaromatic compounds include: (i) direct biosynthesis (Connan et al., 1986); (ii) diagenesis of functionalised precursors such as $\omega$-cyclohexylalkanoic acids (Sinninghe Damsté et al., 1991); (iii) alkylation of aromatics (Rigby et al., 1986; Williams et al.,

1988), and; (iv) diagenetic cyclisation and aromatisation of acyclic precursors (Ostroukhov *et al.*, 1983, Reed *et al.*, 1986; Rullkötter *et al.*, 1986).

Diaromatic hydrocarbons can be divided into two broad classes, those in which the aromatic rings are fused (naphthalenes) and those in which the aromatic rings are separate but connected. There have been a limited number of reports in the literature of the occurrence of long chain alkylnaphthalenes in sedimentary organic matter (Connan *et al.*, 1986; Williams *et al.*, 1988; Gontcharov, 1993; Ellis *et al.*, 1995). Compounds containing two non-fused aromatic rings, such as biphenyls and diphenylmethanes, and bearing long chain *n*-alkyl substituents represent potential sources of the monodeuterated *n*-alkanes. However, such compounds have not to date been reported to occur in sedimentary organic matter. The occurrence in crude oils of triaromatic compounds bearing long-chain alkyl substituents has not been reported to date.

## 7.2.3.2    Isoprenoids

*Identification of monodeuterated isoprenoids*

Peaks corresponding to the monodeuterated isoprenoid hydrocarbons pristane and phytane are prominent in the GCs of the deuterated hydrocarbon fractions derived from the ruthenium tetroxide oxidations of the crude oil aromatic fractions (peaks 15 and 17 respectively in Figs. 7.11*a-c*). Investigation of the *m/z* 113 mass chromatograms of the deuterated hydrocarbons from the monoaromatic, diaromatic and triaromatic fractions (Figure 7.13*a-c*) shows the presence of a homologous series of isoprenoid alkanes from $C_{11}$ to $C_{21}$ (identified by gas chromatographic retention behaviour and mass spectra). As mentioned previously, the deuterium in these compounds must be attached to a primary carbon atom, but unlike the *n*-alkanes, where the deuterium must be on one of the two terminal carbons, in the case of the isoprenoids there are several carbons to which the deuterium atom could be attached. In the case of phytane (see Fig. 7.14*a* for mass spectrum), five different primary carbon atoms are present in the molecule. Figure 7.15 shows the structure of the phytane molecule, with the five
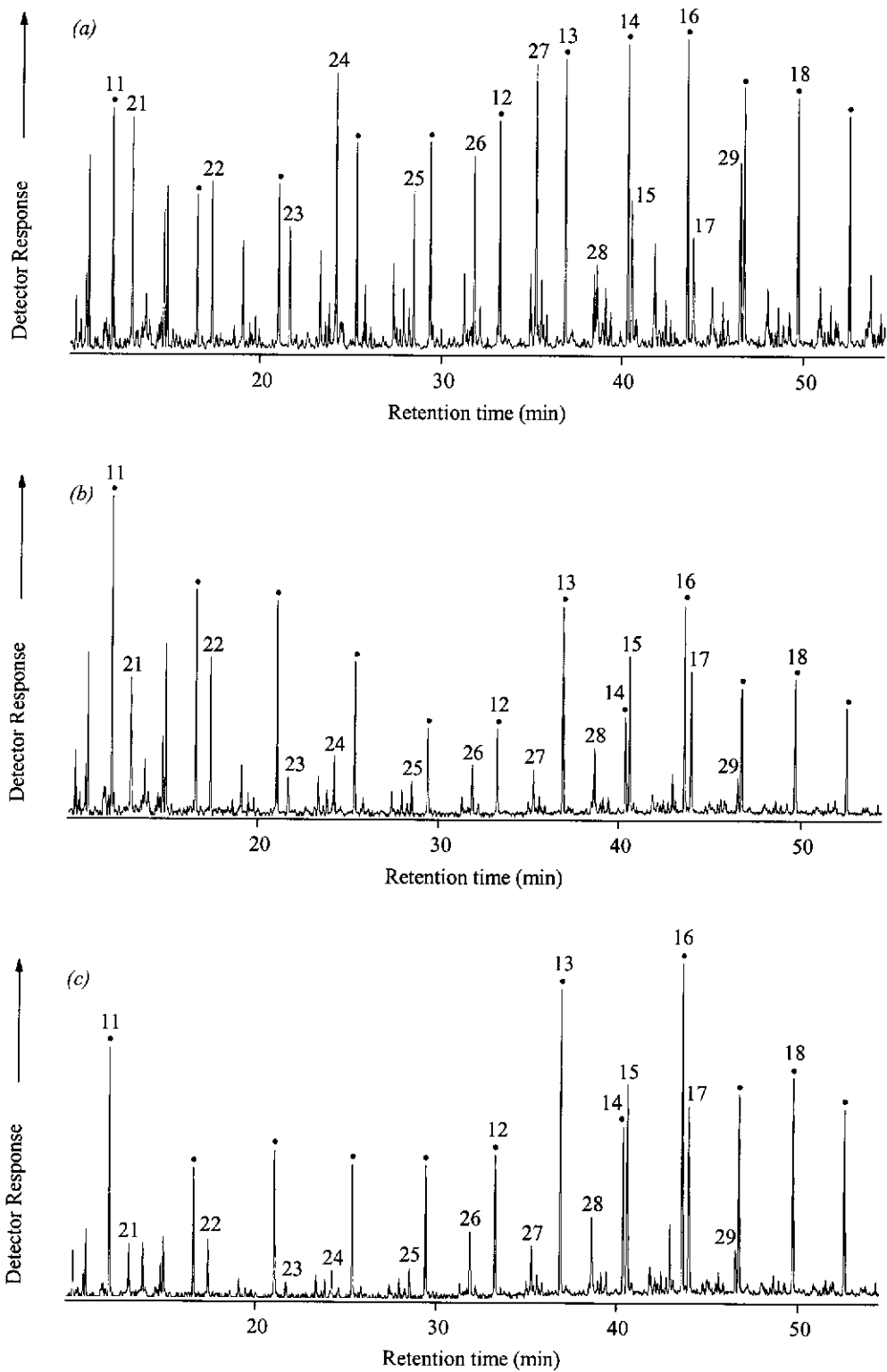
Figure 7.13.  Mass chromatograms (*m/z* 113) showing monodeuterated isoprenoids from the ruthenium tetroxide oxidation of the (a) monoaromatic;  (b) diaromatic; and, (c) triaromatic fractions from Leatherjacket-1 788.5m crude oil (for peak identification refer to Table 7.4; peaks labelled with • correspond to monodeuterated *n*-alkanes).

primary carbon atoms (ie. potential sites of deuterium attachment) numbered 1 through 5. A procedure was developed which involved a detailed examination of the diagnostic ions generated by cleavage of the molecule at the tertiary centres, in order to determine the position of the deuterium atom.

In the case of the phytane molecule, eight diagnostic fragment ions result from cleavage at the four tertiary centres, at $m/z$ values of 267, 253, 197, 183, 127, 113, 57 and 43 (the last is excluded for the purposes of this study, as the mass spectrum was acquired down to $m/z$ 50). The corresponding ions with values one unit greater than these values are generated in the same manner, but contain a $^{13}C$ atom in place of one of the $^{12}C$ atoms. The theoretical abundance of the higher to lower mass fragments (e.g. $m/z$ 268 to 267) can thus be calculated by simply multiplying the abundance of $^{13}C$ versus $^{12}C$ atoms (1.1 %) by the number of carbon atoms in the fragment. These values are given in Table 7.6, together with the corresponding values for the mass spectrum of the peak corresponding to deuterated phytane in this sample. From Table 7.6 it is clear that the ratios of the fragments 268/267, 198/197, 128/127 and 58/57 have increased significantly in the deuterated pristane. From Figure 7.15 it can be seen that position 5 is the only position that occurs in all four of the fragments represented by the increased ratios given above. Therefore the deuterium atom must be located at this position in the deuterated pristane molecule.

The ratios of the fragments 254/253, 184/183 and 114/113 have also increased, but to a much lesser extent than those mentioned above. There are two possible explanations for this observation. It has been reported (e.g. Stevenson and Wagner, 1951; Schissler et al., 1951; Condon, 1951; Condon et al., 1951) that rearrangements of hydrogen atoms occur in the mass spectrometer, which may lead to deuterium being exchanged to fragments derived from parts of the molecule which initially did not contain deuterium (deuterium scrambling). The other possibility is that the deuterium atoms are attached to more than one position on the molecule.
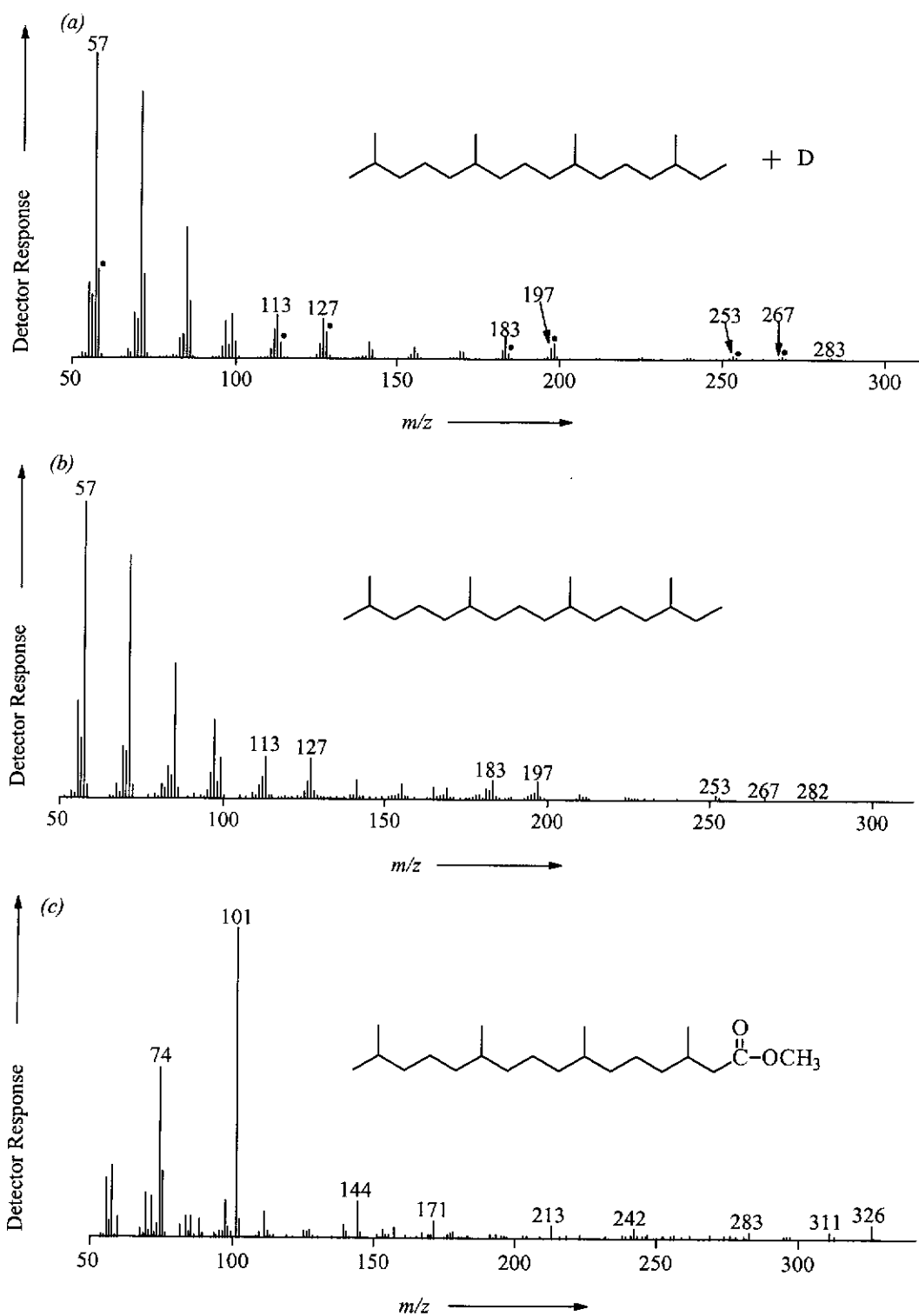
Figure 7.14. (a) mass spectrum of monodeuterated phytane (the peaks indicated by • are those referred to as (m + 1) in Table 7.6; (b) mass spectrum of undeuterated phytane for comparison; and, (c) mass spectrum of methyl 3,7,11,15-tetramethylhexadecanoate. The m/z 101 ion is characteristic of alkanoic acids with a methyl substituent in position 3, and is generated via a McLafferty rearrangement.

Table 7.6. Calculated (based on the natural abundance of $^{13}C$ versus $^{12}C$) and experimental ratios of (m + 1)/m where m is the *m/z* value of the ion generated *via* cleavage at a tertiary centre (see Fig. 7.15) for the peak corresponding to monodeuterated phytane in the deuterated hydrocarbon sample derived from the monoaromatic fraction of the Leatherjacket-1 788.5 m crude oil.

| Diagnostic ion (m) | Experimental ratio (m + 1)/m | Calculated ratio (m + 1)/m | % Difference |
|---|---|---|---|
| 267 | 1.76 | 0.209 | + 843 |
| 253 | 0.51 | 0.198 | + 258 |
| 197 | 1.33 | 0.154 | + 865 |
| 183 | 0.26 | 0.143 | + 183 |
| 127 | 0.64 | 0.099 | + 642 |
| 113 | 0.36 | 0.088 | + 409 |
| 57 | 0.30 | 0.044 | + 677 |
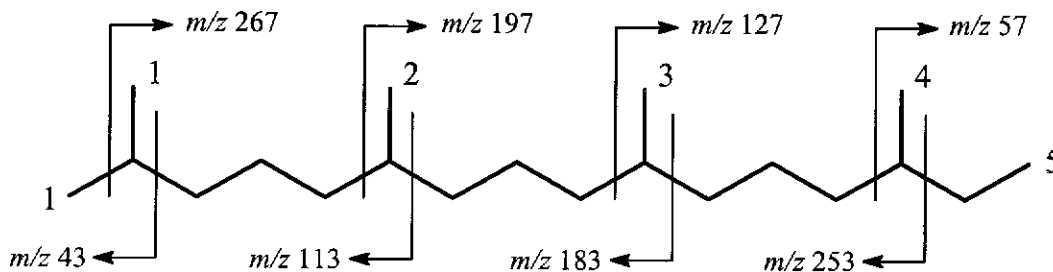


Figure 7.15. Structure of phytane showing the possible positions of deuterium attachment (numbered 1 to 5) and the diagnostic mass fragments arising through cleavage at the tertiary centres.

Compounds differing only in the position of a single deuterium atom would exhibit virtually identical GC retention behaviour, and the mass spectrum would therefore be a composite of the co-eluting isomers. In order to determine whether more than one co-eluting deuterium-position isomer of phytane was present in the sample, the mass spectrum of the methyl ester of the carboxylic acid from which the deuterated pristane was derived was analysed (Figure 7.14c). The diagnostic m/z 74 and 101 ions are produced by McLafferty rearrangement of an alkanoic acid methyl ester with a methyl branch at C-3, confirming that the ester, and hence the deuterium, is at the position marked 5 on Fig. 7.15. There was no indication of the presence in the sample of any of the compounds with the ester functionality at any of the other position. This type of analysis was applied to all of the other isoprenoids identified in the sample. In all cases, it was determined that the deuterium was attached to the main-chain carbon atom with the highest number according to the IUPAC system of nomenclature.

*Origins of isoprenoids and their aromatic precursors*

Isoprenoidal carboxylic acids have been identified in the ruthenium tetroxide oxidation products of asphaltenes (Trifilieff *et al.*, 1992; Richnow *et al.*, 1992, 1993) and kerogen (Standen *et al.*, 1991a). However, this is the first report of their occurrence in the ruthenium tetroxide oxidation products of crude oil aromatic fractions. The presence of these isoprenoid alkanes indicates that compounds with isoprenoid side chains attached directly to aromatic rings were present in the initial aromatic fractions, and that the branch methyls on the side chain were in the ω-2, ω-6, etc. positions.

Sinninghe Damsté *et al.* (1988) identified a series of methylated phytanylbenzenes (Fig. 7.16a) in crude oils and sediment extracts ranging in age from Miocene to Permian from various geographical locations. It was proposed that these compounds were formed either from isoprenoid quinones (Henninger, *et al.*, 1963; Barr and Arntzen, 1969; Threlfall and Whistance, 1971; Hughes and Tove, 1982; Janiszowska and Rygier, 1985) during diagenesis, or by direct biosynthesis (Langworthy *et al.*, 1982). The presence of such compounds in the initial
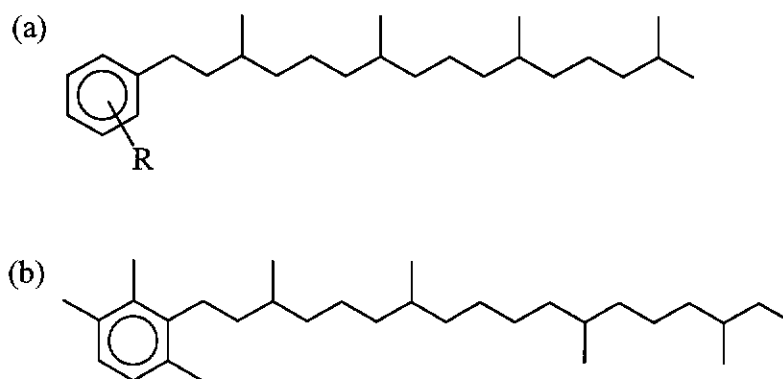
Figure 7.16. (a) Phytanylbenzene compounds (R = -H, -CH₃, -(CH₃)₂ and -(CH₃)₃) (Sinninghe Damsté *et al.*, 1988), and; (b) example of an irregular monoaromatic isoprenoid hydrocarbon reported in sedimentary organic matter (Summons and Powell, 1987).

monoaromatic fraction would account for the occurrence of the isoprenoids in the monodeuterated hydrocarbon sample.

There have been numerous other reports of monoaromatic hydrocarbons with an isoprenoidal side chain in sedimentary organic matter (e.g. Ostroukhov *et al.*, 1982; Powell *et al.*, 1984; Summons and Powell, 1987; Requejo *et al.*, 1992). However, the isoprenoidal side chain of these compounds is irregular, containing a head-to-head connection (Figure 7.16*b*), and thus these compounds could not be the source of the monodeuterated isoprenoids identified in this sample, all of which contained only head-to-tail connections.

The occurrence in crude oil of naphthalenes bearing phytane substituents has been reported (Gontcharov, 1993). As with the deuterated *n*-alkanes from the crude oil diaromatic fraction, the isoprenoids could be derived from precursors in which a monoaromatic system has attached to an aryl isoprenoid. Such compounds have not been reported to date in crude oils.

Isoprenoidal monoaromatic compounds may also be formed geosynthetically, by alkylation of aromatic rings with functionalised isoprenoids in a manner similar to that proposed for the $n$-alkylaromatic precursors to the monodeuterated $n$-alkanes. The $C_{20}$ isoprenoid alcohol, phytol, is ubiquitous in nature (Sinninghe Damsté et al., 1988), and it is proposed that the saturated isoprenoidal hydrocarbons to $C_{20}$ commonly found in sediments are derived from this (Killops and Killops, 1993). This is the same carbon number range for the isoprenoids observed in unbiodegraded Gippsland Basin oils. Substitution of phytol onto an aromatic ring followed by ruthenium tetroxide oxidation would result in the formation of a $C_{21}$ isoprenoid carboxylic acid with the methyl groups in the $\omega$-2, $\omega$-6, etc. positions. This compound, when reduced using the procedure described in this study, is identical to the $C_{21}$ monodeuterated hydrocarbon identified in the current sample. The monodeuterated hydrocarbons extend from $C_{10}$ to $C_{21}$, which corresponds to side chains to $C_{20}$, which is the same carbon number range as the saturated isoprenoids. Thus the monoaromatic isoprenoids may be derived from alkylation of aromatic rings with functionalised isoprenoidal compounds. The distribution of the monodeuterated isoprenoids, however, is different to that of the isoprenoids in the saturate fraction of an unbiodegraded Gippsland Basin crude oil, indicating that the isoprenoidal aromatics have at least one other source, such as the specific biological precursors mentioned.

The technique used here of determining the position of the deuterium atom on the hydrocarbon molecule was also applied to several other groups of compounds identified in the sample. Details of these compound classes, and the postiton of deuterium substitution, are described below.

### 7.2.3.3 Methylalkanes

*Identification of monodeuterated methylalkanes*

A suite of methylalkanes has been identified in all three monodeuterated samples, identified on the basis of gas chromatographic retention behaviour and mass spectra. In the sample from the monoaromatic fraction, all methylalkanes from

$C_{10}$ to $C_{31}$ were identified. As an example, the *m/z* 225, 211, 197, 183, 169, 155 and 141 mass chromatograms showing the $C_{18}$ methylalkane isomers from this sample is given in Fig 7.17*a* (together with the corresponding portion of the TIC). The mass spectrum of 5-methylheptadecane, shown in Fig. 7.17*b* as a typical example of the mass spectrum of a monodeuterated methylalkane. All methylalkanes from $C_{10}$ to $C_{23}$ were identified in the sample from the diaromatic fraction. Higher carbon number homologues may also be present, but the abundance of the methylalkanes relative to the *n*-alkanes is much lower than in the deuterated hydrocarbons from the monoaromatic fraction. In the sample from the triaromatic fraction, monodeuterated methylalkanes from $C_{10}$ to $C_{28}$ were identified. The abundance of these compounds relative to the *n*-alkane of the same carbon number is similar to that observed in the diaromatic sample. The relative distributions of methylalkane isomers in the samples from the diaromatic and triaromatic fractions are very similar to those from the monoaromatic fraction, and for this reason are not shown. In all three samples, the relative abundances of all of the methylalkanes at a given carbon number were approximately equal, within a factor of two.

As with the isoprenoids, an analysis of the ratios of the ions generated by cleavage at the tertiary centre was performed in order to determine the position of the deuterium atom. In the case of the methylalkanes, there are three primary carbon atoms, to which the deuterium atom could be attached (with the exception of 2-methylalkanes, which have only two different primary carbon atoms). The values of the ratio (m + 1)/m, where m is the mass of the odd-mass ion generated *via* cleavage at the tertiary centre, were observed to increase in the case of all three fragments for each methylalkane, with the ratios of the fragments containing C-1 (according to the IUPAC system of nomenclature) showing the most marked increase. These observations suggest that while the deuterium atom is often at C-1, isomers in which the deuterium atom is attached to either of the remaining two primary carbon atoms may also be present in the sample. Two homologous series of compounds with fragments of *m/z* 88 and 101, and 74 and 101, corresponding to 2- and 3-methylalkanoic acid methyl esters respectively
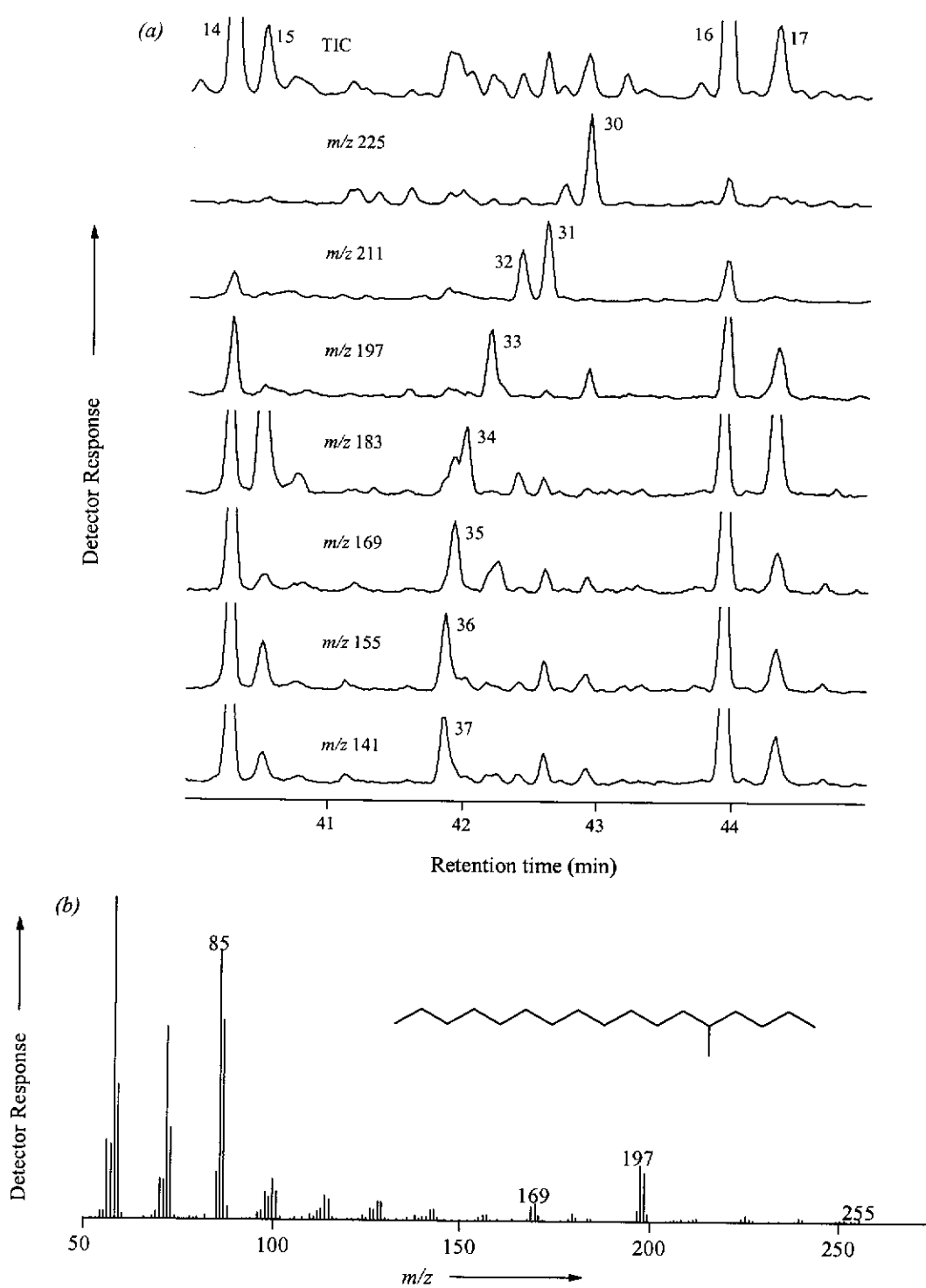
Figure 7.17. (a) TIC and $m/z$ 225, 211, 197, 183, 169, 155 and 141 RICs showing monodeuterated $C_{18}$ methylalkanes (for peak identification refer to Table 7.4), and; (b) the mass spectrum of monodeuterated 5-methylheptadecane.

were observed in the methyl ester sample of the carboxylic acid oxidation product (Figure 7.18*a*). Example mass spectra are given in Fig. 7.18*b* and 7.18*c*. Similar series of compounds were also observed in the carboxylic acid methyl esters obtained from the diaromatic and triaromatic fractions. These results indicate that aromatic species with alkyl side chains containing methyl branches at all positions were present in the crude oil aromatic fractions. Several of the deuterated methylalkane isomers appeared to have the deuterium atom situated on the methyl branch, with the position of the deuterium unable to be determined with confidence in several other isomers. As discussed with the deuterated isoprenoids, this may be the result of the presence of co-eluting isomers with the deuterium in different positions.

The occurrence of 2- and 3-methyl-branched alkanoic acids, which in the current study have been reduced to deuterated methylalkanes (alkyl-*d*), has been reported in the ruthenium tetroxide oxidation products of monoaromatic refinery fractions and a biodegraded crude oil aromatic fraction (Thomas, 1995). However, this is the first report of the occurrence of 2-alkylalkanoic acids (side chain > methyl) in such oxidation products (present in the current study as methyl-*d* methylalkanes). This is also the first report of the occurrence of compounds with a methylalkane carbon skeleton from the ruthenium tetroxide oxidation of crude oil diaromatic and triaromatic fractions.

*Origins of methylalkanes and their aromatic precursors*

Precursor compounds for these methylalkanes have two different types of alkyl side chains attached to the aromatic ring. For methylalkanes with the deuterium at the end of the long carbon chain, the precursor must have been a compound with a monomethyl-branched side chain attached to the aromatic ring. The occurrence in sedimentary organic matter of compounds containing such a structural moiety has not been reported in the literature, and this is the first evidence for the existence of such compounds. Methylalkanes with the deuterium attached to the methyl group are derived from precursors with a phenylalkane structural moiety. The apparent occurrence of phenylalkanes in crude oils has

*(a)*

Detector Response

38
*

*

39
*

*

*

*

*

40
*

*

*

*

41
*

*

*

*

*

42
*

20    30    40    50    60    70    80

Retention time (min)

*(b)*

88

Detector Response

57

74

101

C–OCH₃
O

129    143    155    186

50    100    150    200

*m/z*

*(c)*

74

Detector Response

57

87

101

C–OCH₃
O

111

129    143    155    186

50    100    150    200

*m/z*
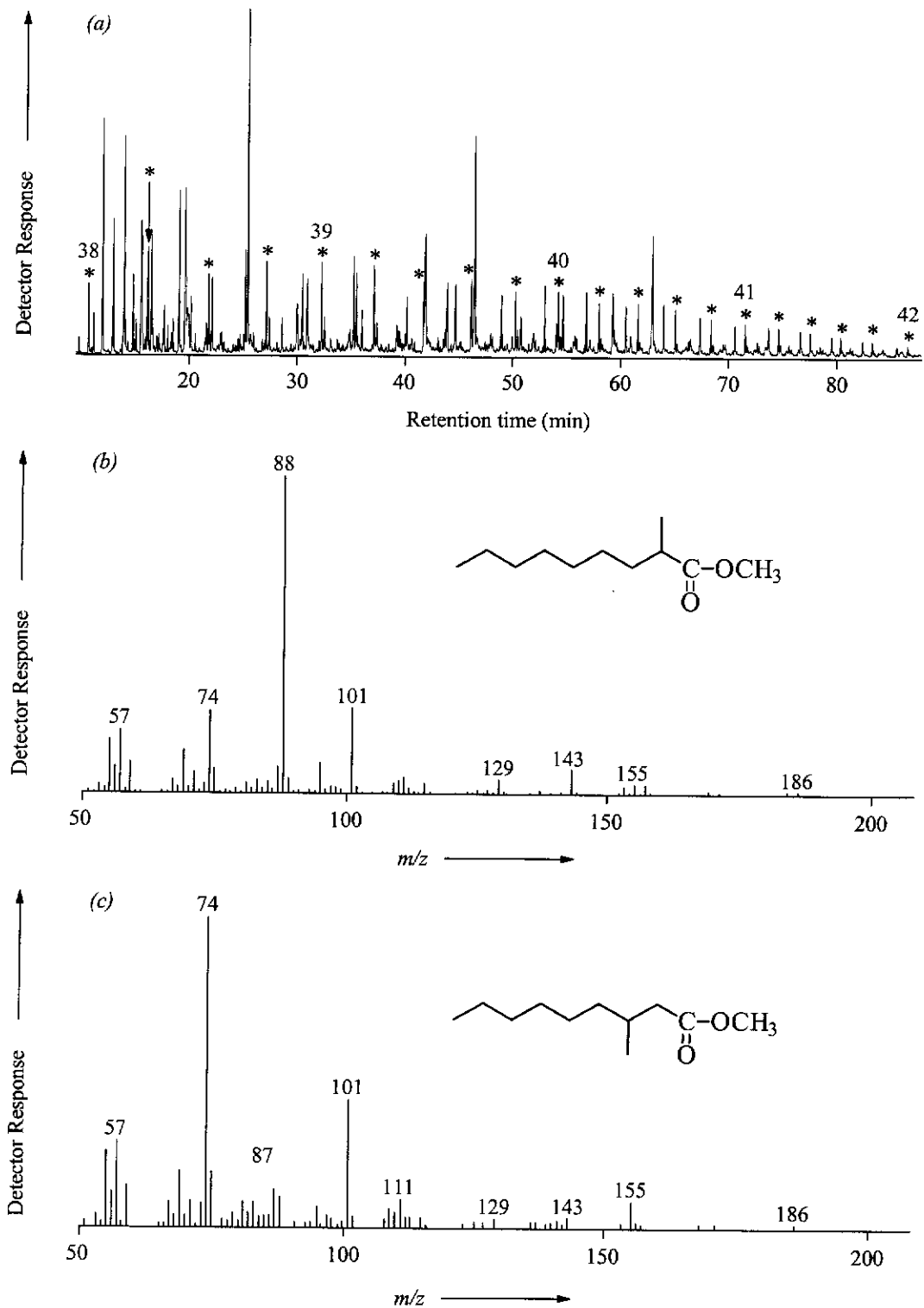
Figure 7.18. (a) m/z 101 Reconstructed ion chromatogram showing 2- and 3-methyl alkanoic acid methyl esters derived from the crude oil monoaromatic fraction (* - 2-methyl alkanoic acid, with the corresponding 3-methyl isomer eluting marginally later; for peak identification refer to Table 7.4); (b) mass spectrum of methyl 2-methylnonanoate, and; (c) mass spectrum of methyl 3-methylnonanoate.

long been considered the result of contamination with surfactants, however Ellis *et al.* (1996) present new evidence to suggest that these compounds are in fact indigenous to petroleum. Diaromatic and triaromatic compounds containing either a monomethyl branched alkyl group attached to an aromatic ring, or a phenylalkane or naphthylalkane structural moiety have not been reported to occur in crude oils. These results therefore constitute the first evidence for the presence of such compounds in crude oils.

A possible mechanism of formation of phenylalkane structural moieties involves electrophilic substitution of an aromatic system with an *n*-alkyl chain containing a secondary carbocation. Protonation of *n*-alkenes or primary alcohols on clay surfaces forms a secondary carbocation (Olah and Molnár, 1995), which *via* a series of 1,2-hydride shifts, is free to move along the alkyl chain. Alexander *et al.* (1991a) have reported the formation of *n*-alkenes from the thermal decomposition of carboxylic esters in sediments, and alkenes are also reported to form from cracking reactions which occur as sediments are subjected to high levels of thermal stress (van de Meent *et al.*, 1980).

The monodeuterated methylalkanes in the sample from the monoaromatic fraction extends from $C_{10}$ to $C_{31}$ ($C_{10}$ to $C_{23}$ for the diaromatic and $C_{10}$ to $C_{28}$ for the triaromatic fractions), indicating monomethylated alkyl side chains to $C_{30}$ in the initial crude oil aromatic fraction. The methylalkanes in unbiodegraded Gippsland Basin oils also extend to around $C_{30}$, and it was also observed that the monodeuterated methylalkanes and the methylalkanes present in the saturate fractions of Gippsland Basin crude oils had similar, smooth distributions of isomers. The similarities in the carbon number ranges, and isomer distributions within and between carbon numbers suggests that in an analogous manner to the *n*-alkanes, the methylalkanes and the methylalkane side chains attached to aromatic carbons have common precursors. This suggests that, in a manner similar to that described for the *n*-alkanes, the methylalkyl aromatics are the product of the alkylation of aromatic rings by these precursor compounds.

### 7.2.3.4 Monoalkylalkanes

*Identification of monodeuterated monoalkylalkanes*

A range of monoalkylalkanes has been identified in the monodeuterated hydrocarbon samples from the crude oil monoaromatic, diaromatic and triaromatic fractions. The majority of all possible isomers from $C_{10}$ to $C_{20}$ were identified in the sample from all three aromatic fractions according to the procedure detailed in Chapter Four. The $m/z$ 225, 211, 197 and 183 RICs, showing the $C_{18}$ ethylalkanes, propylalkanes, butylalkanes and pentylalkanes respectively, in the sample from the monoaromatic fraction are given in Figure 7.19, together with a typical mass spectrum, that of 5-ethylhexadecane. Some isomers were unable to be identified due to co-elution with compounds giving a strong signal for the diagnostic ion used for the analysis. Also, monoethylalkanes were identified in the monodeuterated hydrocarbon samples derived from the monoaromatic ($C_{21}$ to $C_{27}$), diaromatic ($C_{21}$ to $C_{24}$) and triaromatic ($C_{21}$ to $C_{25}$) fractions. The relative distributions of the monoalkylalkanes in the corresponding mass chromatograms from the diaromatic and triaromatic fractions were very similar in appearance to those given in Fig. 7.19.

Analysis of the ratios of the ions generated by cleavage at the tertiary centre was performed in order to determine the position of deuterium attachment for those isomers for which a good quality mass spectrum could be obtained. As was the case with the monomethylalkanes, the deuterium appeared to be attached predominantly at C-1, with lesser amounts of the isomers with the deuterium attached to either of the remaining two primary carbon atoms.

This is the first report of the occurrence of monoalkyl-branched alkanoic acids with branch lengths greater than methyl (identified by reduction to monodeuterated monoalkylalkanes) as products of the ruthenium tetroxide oxidation of crude oil aromatics. The occurrrence of suitable precursors in sedimentary organic matter has not been reported to date, and this therefore constitutes the first evidence for the presence of such compounds.
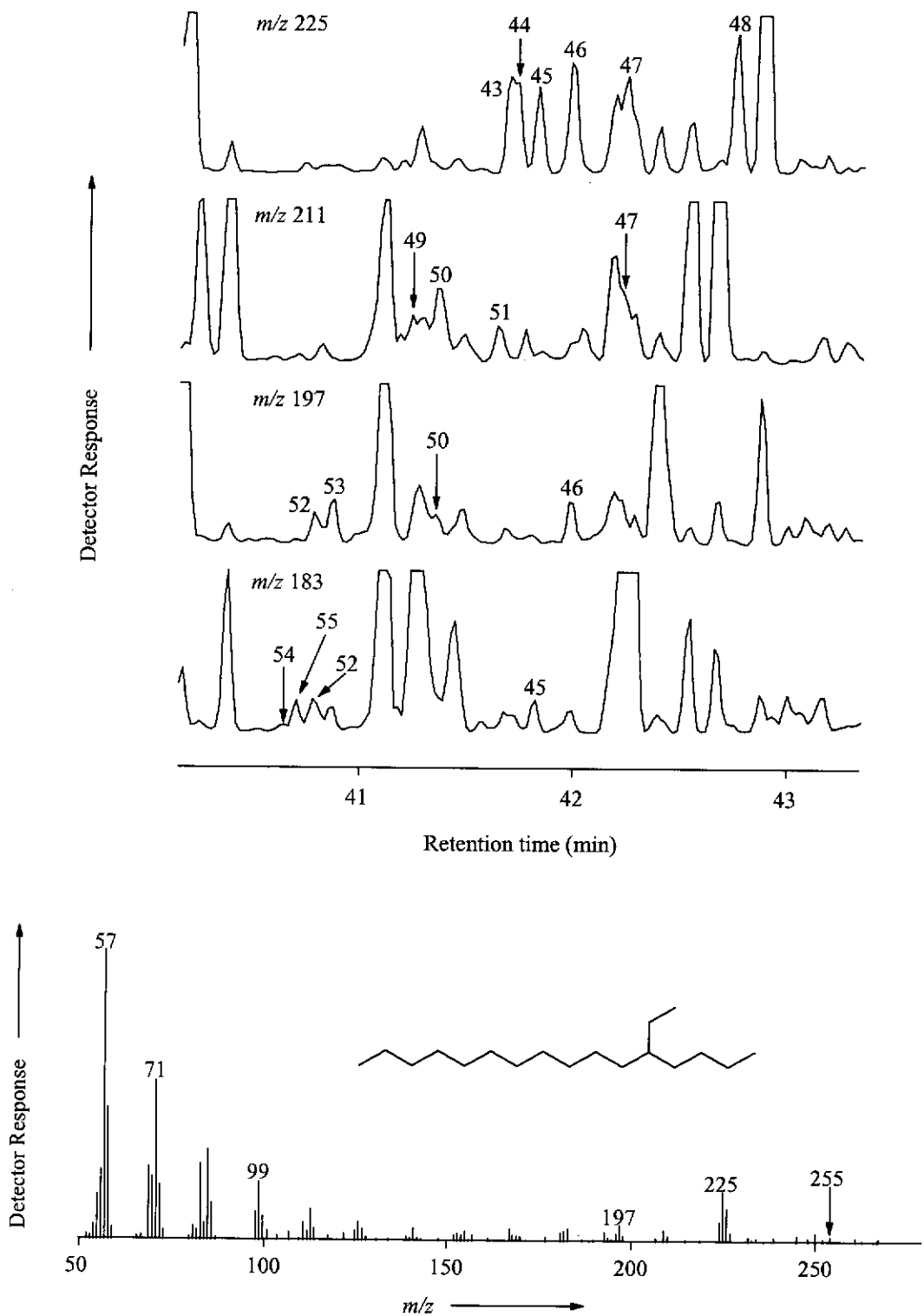
Figure 7.19. m/z 225, 211, 197 And 183 reconstructed ion chromatograms showing: (a) the monodeuterated C$_{18}$ ethylalkanes, propylalkanes, butylalkanes and pentylalkanes respectively (for peak identification refer to Table 7.4), and; (b) the mass spectrum of 5-ethylhexadecane.

These compounds are derived from precursors containing an alkyl group with a branching carbon chain, attached to an aromatic ring. The structural similarity between these compounds and the monoalkylalkanes discussed in Chapters Four and Five suggests that the two types of compounds may have a similar origin. In Chapter Five, evidence was presented that certain monoalkylalkane isomers are derived from naturally-occurring functionalised precursors with the same carbon skeleton, while a mixture of all isomers was formed via acid-catalysed carbon skeleton rearrangement of $n$-alkenes (cf. Fig. 5.7). Two possible mechanisms of formation of the branched-chain alkylaromatic compounds are: 1) alkylation of an aromatic system by a branched alkyl species containing an electrophile such as a carbocation; and, 2) rearrangement of the alkyl chain of a straight-chain alkylaromatic compound. As the carbon $\alpha$ to the aromatic ring (and hence the carbon which would bear the carbocation prior to alkylation) is secondary in these branched-chain alkylaromatic compounds, formation via the first possibility is unlikely as primary carbocations are highly unstable and would very likely rapidly undergo an intermolecular 1,2-hydride shift to form the more stable secondary carbocation. Alkenylbenzenes, $n$-alkenes with a terminal benzene ring, have been reported in the pyrolysates of kerogens (Ellis et al., 1999), and rearrangement of these compounds via a process similar to that given in Fig. 5.7 could yield monoalkylalkanes with a terminal aromatic ring.

## 7.2.3.5 Alkylcyclohexanes and methylalkylcyclohexanes

Two homologous series of monocyclic compounds, the monodeuterated alkylcyclohexanes from $C_{10}$ to $C_{33}$ and the monodeuterated methylalkyl-cyclohexanes from $C_{10}$ to $C_{29}$ (four isomers at each carbon number), have been identified in the monodeuterated hydrocarbon sample derived from the crude oil monoaromatic fraction. For the sample from the diaromatic fraction, the carbon number ranges are $C_{10}$ to $C_{25}$ and $C_{10}$ to $C_{13}$ respectively, while for the triaromatic fraction they are $C_{10}$ to $C_{28}$ and $C_{10}$ to $C_{13}$ respectively. Figure 7.20 shows the $m/z$ 83 (alkylcyclohexanes) and Figure 7.21 the $m/z$ 97 (methyl alkylcyclohexanes) reconstructed ion chromatograms for the sample from the monoaromatic fraction.

Examples of the mass spectra of compounds of each of these types are shown with the relevant chromatograms. Investigation of the monocarboxylic acid methyl ester samples from all three aromatic fractions revealed that the alkylcyclohexane series continues down to $C_8$, the smallest members in each series. A series of $C_1$ to $C_3$ alkyl substituted cyclohexanecarboxylic acids were also identified in the ester samples.

In the case of the alkylcyclohexanes, as there is only one primary carbon atom present in the molecule, there is only one possible position for the deuterium atom. This is at the end of the alkyl chain furthest from the cyclohexane ring. Methyl-$n$-alkylcyclohexanes on the other hand have a second primary carbon – the methyl carbon attached to the cyclohexane ring. The diagnostic ion for methylalkylcyclohexanes is the $m/z$ 97 ion corresponding to cleavage of the alkyl side chain at the ring carbon with charge retention on the fragment containing the ring. The presence of a deuterium on the methyl substituent would result in the formation of a $m/z$ 98 fragment. The size of the $m/z$ 98 fragment relative to the $m/z$ 97 fragment indicates that isomers with the deuterium attached to both the alkyl chain and the methyl group are present in the sample.

Cyclohexanecarboxylic acid has been reported in the products of the ruthenium tetroxide oxidation of monoaromatic and diaromatic refinery fractions, and a biodegraded crude oil. Its presence in the monoaromatic and total aromatic fractions was attributed to the occurrence of cyclohexylbenzene in the original aromatic fractions (Thomas, 1995); however, its presence in the oxidation product from the diaromatic refinery fraction was unexplained. The occurrence of a homologous series of ω-cyclohexylalkanoic acids (identified by conversion into deuterated hydrocarbons) has not previously been reported. This is also the first report of compounds with a methylalkylcyclohexane carbon skeleton as oxidation products of crude oil aromatics.
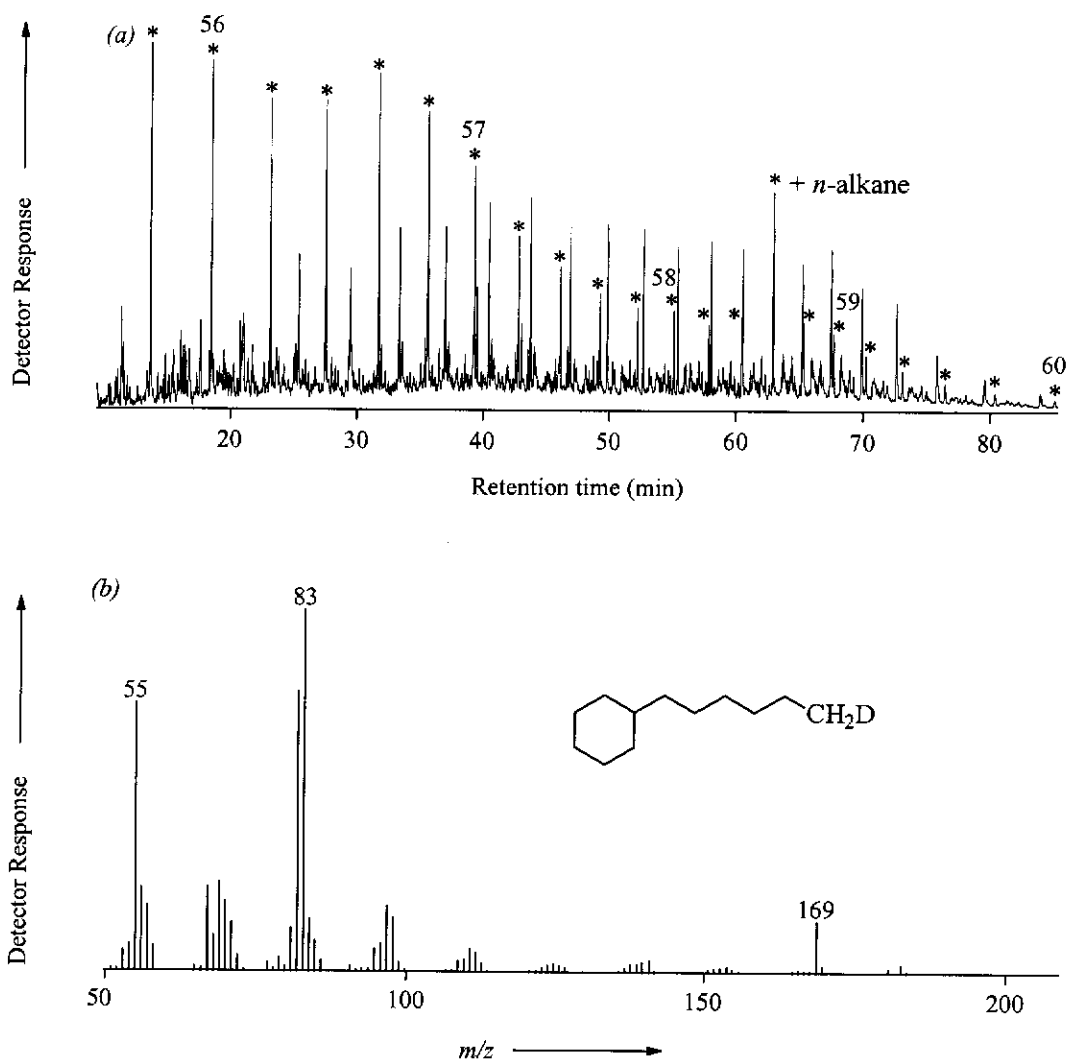
Figure 7.20. (a) GC-MS *m/z* 83 reconstructed ion chromatogram showing monodeuterated alkylcyclohexanes (indicated by *; for peak identification refer to Table 7.4), and; (b) mass spectrum of monodeuterated *n*-hexylcyclohexane.
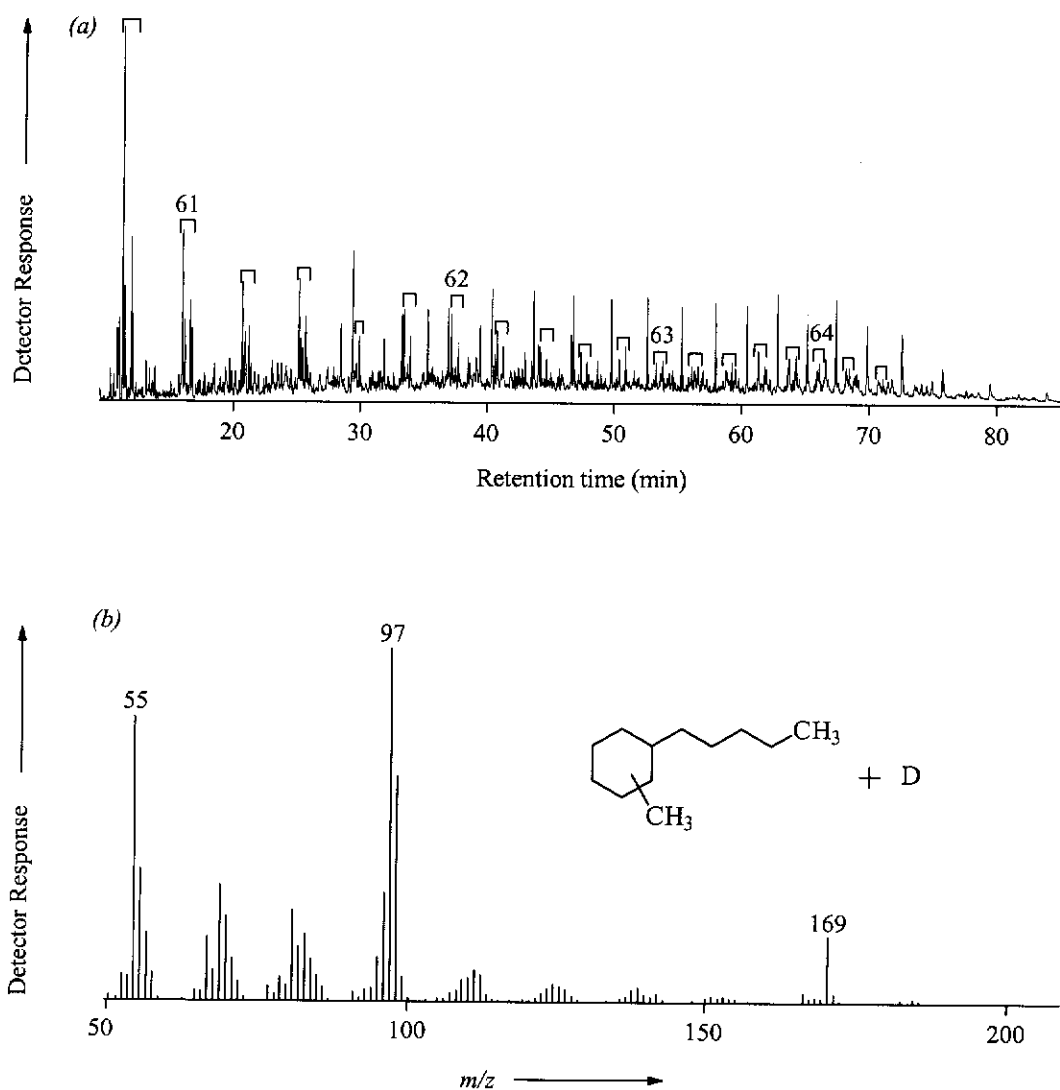
Figure 7.21. (a) GC-MS *m/z* 97 reconstructed ion chromatogram showing monodeuterated methylalkylcyclohexanes (indicated by square bracket; for peak identification refer to Table 7.4), and; (b) mass spectrum of monodeuterated methyl-*n*-pentylcyclohexane.

The presence of these alkylcyclohexanes in the deuterated samples indicates that a series of compounds consisting of a cyclohexane ring and a monoaromatic system at opposite ends of an $n$-alkyl chain of variable length from $C_0$ to at least $C_{26}$ ($C_{21}$ if the cyclohexane ring contains a methyl substituent) were present in the crude oil monoaromatic fraction. The maximum side chain carbon numbers for the diaromatic and triaromatic fractions are $C_{19}$ ($C_6$ with methyl) and $C_{22}$ ($C_6$ with methyl) respectively. Alkyl substituted cyclohexanecarboxylic acids were present in the ester sample, indicating the presence of compounds containing a (saturated ring-substituted) alkylcyclohexylbenzene structural moiety in the crude oil.

Cyclohexylbenzenes with an unsubstituted or monomethylated aliphatic ring have been reported as constituents of crude oils (Alexander *et al.*, 1993) and are potential sources of the substituted cyclohexanecarboxylic acids observed in the oxidation products. The more highly substituted cyclohexanecarboxylic acids are formed from analogous as-yet unidentified precursors. Compounds containing a cyclohexane ring and an aromatic ring connected by a $C_2$ alkyl chain have been reported in the Green River Shale (Gallegos, 1973). For these compounds, an origin from aromatisation and ring opening of tricyclic or higher terpenoids was proposed. Suitable aromatic precursors for the deuterated long-chain alkylcyclohexanes have not been reported in the literature, and this constitutes the first evidence for the existence of such compounds in petroleum.

The carbon number ranges and isomer distributions of the monodeuterated alkylcyclohexanes is similar to those of the saturated analogues in unbiodegraded Gippsland Basin crude oils. These observations indicate that the alkylcyclohexanes found in the saturate fractions of crude oils and the alkylcyclohexyl substituents may have similar precursor compounds, and that the $\omega$–cyclohexylmonoaromatics are formed by alkylation of aromatic rings by such precursors. The formation of alkylaromatic compounds from alkylation of aromatic systems with acylium carbocations (from carboxylic acids) has been reported (Wilkes *et al.*, 1998), and the occurrence of suitable precursor

compounds, the ω–cyclohexylalkanoic acids, in bacteria has been reported (de Rosa *et al.*, 1974; Suzuki *et al.*, 1981).

### 7.2.3.6     Bicyclic compounds

A series of monodeuterated bicyclic compounds from $C_{11}$ to $C_{18}$ were identified in the samples from the monoaromatic, diaromatic and triaromatic fractions, with those from the monoaromatic fraction shown in Figure 7.22. The chromatograms for the diaromatic and triaromatic fractions are very similar to those for the monoaromatic fraction, and are not shown. The most abundant component in all three samples (peak 65), the mass spectrum of which is shown in Figure 7.23, is a $C_{17}$ compound with a base peak of *m/z* 123. This compound is tentatively assigned the structure shown in Fig. 7.23. What appears to be a homologous series of bicyclic compounds from $C_{11}$ to $C_{14}$ is also apparent in the *m/z* 137 mass chromatogram shown in Fig. 7.22. Mass spectra of the compounds suggests that they are monosubstituted decalins with an alkyl side chain from $C_1$ to $C_4$, with the deuterium atom situated at the end of the alkyl chain. Several other bicyclic compounds are also present, the molecular structures of which have not been determined. This is the first report of the occurrence of compounds with a bicyclic carbon skeleton as products of the ruthenium tetroxide oxidation of crude oil aromatics, although the occurrence of this class of compound was proposed by Revill (1992).

It appears likely that, for the sample from the monoaromatic fraction, the $C_{17}$ monodeuterated compound shown in Fig. 7.23 is derived from D-ring monoaromatic 8,14-secohopanoids (Hussler *et al.*, 1984) as shown. Similar structures have also been proposed by Bendoraitis (1974). In the case of the diaromatic fraction, the prominent $C_{17}$ isomer may be derived from D-ring aromatic benzo-8,14-secohopanoid compounds, whilst no suitable triaromatic precursor has been reported to date. It is probable that the other bicyclic compounds are derived from similar aromatic species formed by ring-opening of partially aromatised polycyclic precursor compounds. Revill (1992) postulated
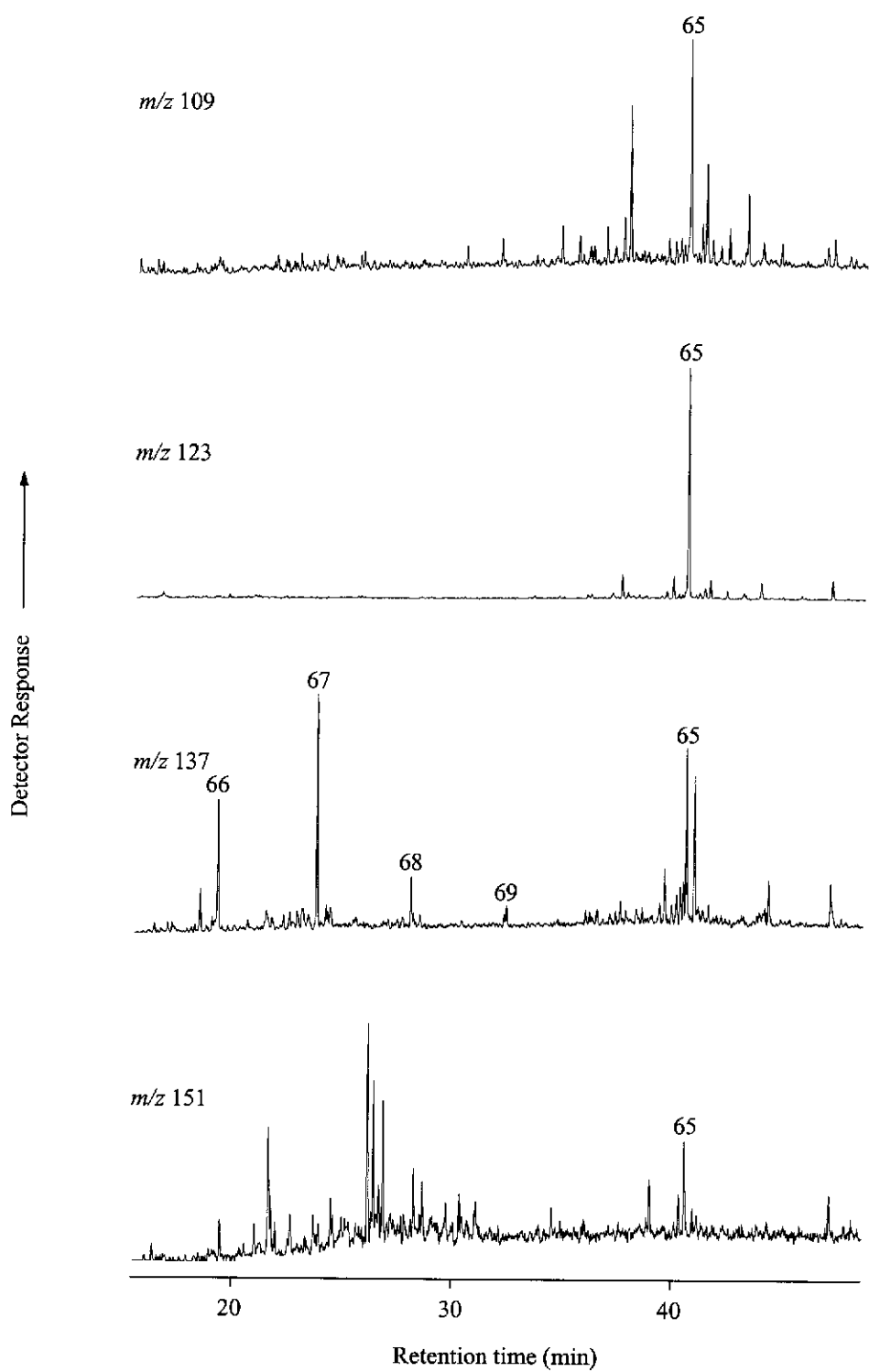
Figure 7.22. RICs at *m/z* 109, 123, 137 and 151 showing monodeuterated bicyclic compounds (all major peaks correspond to bicyclic compounds; for peak identification refer to Table 7.4).
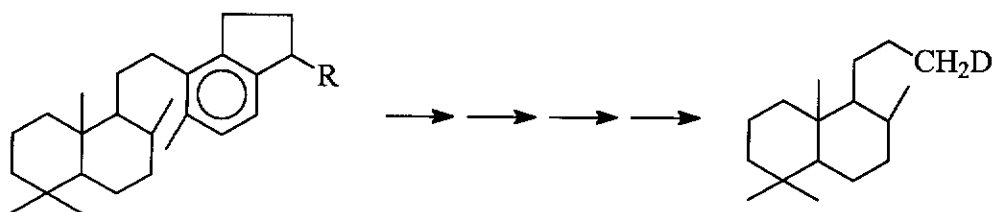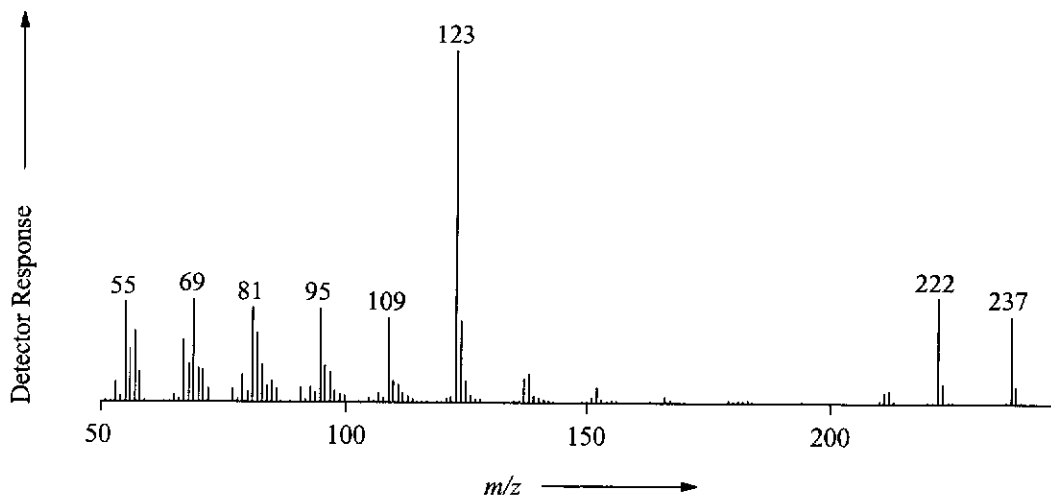
Figure 7.23. Mass spectrum of $C_{17}$ monodeuterated bicyclic compound and the proposed structure of the compound and its proposed D-ring monoaromatic 8,14-secohopane precursor.

the presence in UCMs of compounds with the general naphthenoaromatic structures shown in Figure 7.2. Similar species, with the alkyl chain attached to the aromatic ring at one end, and the decalin moiety at the other, are likely precursors for the homologous series of monodeuterated alkyldecalins observed.

### 7.2.3.7 Aromatic components

Numerous aromatic compounds were identified in the monocarboxylic acid methyl ester and monodeuterated hydrocarbon samples derived from the crude oil diaromatic and triaromatic fractions. Aromatic compounds were not observed as oxidation products of the crude oil monoaromatic fraction, as expected.

The major components of the monocarboxylic acid methyl ester fraction from oxidation of the crude oil diaromatic and triaromatic fractions included various aromatic compounds. The type and relative distribution of the aromatic compounds generated by oxidation of the crude oil diaromatic fraction is similar to that of the triaromatic fraction, with the main difference being that the overall abundance of the aromatic compounds relative to the aliphatic components is slightly lower in the case of the triaromatic sample. A series of alkyl-substituted benzoic acids have been identified in the samples, including benzoic acid, all three toluic acids, and a series of $C_2$ and $C_3$ alkyl-substituted benzoic acids, as shown in Figure 7.24. Also present in both samples were a series of $\omega$-phenyl alkanoic acids and $\omega$-(methylphenyl) alkanoic acids with carbon chains to $C_4$.

Investigation of the deuterated hydrocarbon samples revealed that the series of alkylbenzenes extended to a chain length of $C_{12}$, while the $m$-alkyltoluenes to a chain length of $C_{11}$ and $p$-alkyltoluenes to $C_8$ were also present ($C_{12}$, $C_6$ and $C_6$ respectively for the triaromatic fraction). The deuterated alkyltoluenes are derived from both the alkyltoluic acids and the $\omega$-(methylphenyl) alkanoic acids, with the only difference being the position of the deuterium atom. The relative abundances of the $m/z$ 105, 106 and 107 ions for the peaks corresponding to the alkyltoluenes suggest that coeluting compounds with the deuterium attached to the methyl group and the alkyl group are present in the sample.
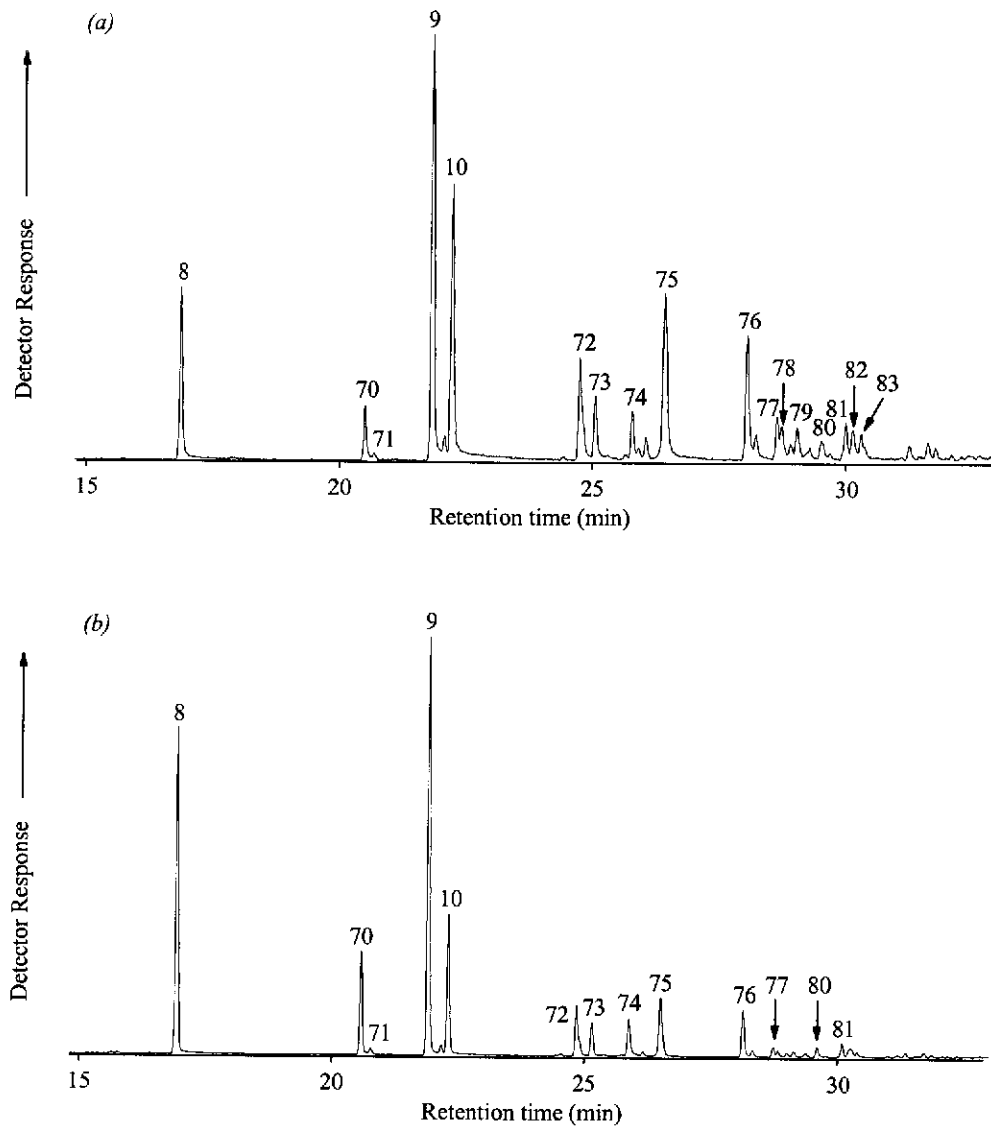
Figure 7.24. Summed m/z 91, 105, 119, 133 and 147 mass chromatograms showing the methyl esters of $C_0$ to $C_3$ alkyl substituted benzoic acids and $C_0$ to $C_2$ alkyl substituted benzeneacetic acids from the ruthenium tetroxide oxidation of Leatherjacket-1 788.5m crude oil (a) diaromatic; and, (b) triaromatic fractions (for peak identification refer to Table 7.4).

In the case of the toluic acids, it can be seen in Fig. 7.24 that the 3- and 4-methyl isomers are present in much higher abundance than the 2-methyl isomer in both samples. This is in agreement with literature reports that biphenyls with *ortho* substituents are in much lower abundance than those with substituents in the *meta* and *para* positions in mature sedimentary organic matter (Alexander *et al.*, 1986; Cumbers *et al.*, 1987).

Thomas (1995) reported the occurrence of only one aromatic monocarboxylic acid, phenylacetic acid, from the ruthenium tetroxide oxidation of diaromatic refinery fractions. Also identified in the same study, in the oxidation of a total crude oil aromatic fraction were benzoic acid, 4-methylbenzoic acid and 3,5-dimethylbenzoic acid. None of the other aromatic monocarboxylic acids identified in the present study have previously been reported in the oxidation of crude oil aromatics.

The only possible diaromatic precursors for the deuterated alkyltoluenes (methyl-*d*) are alkylated biphenyls. Alkylbiphenyls have been reported to occur in crude oils (Mair and Mayer, 1964; Yew and Mair, 1966; Adams and Richardson, 1953), coal tar (Mostecky *et al.*, 1970) and shale oil (Rovere *et al.*, 1983). More detailed studies have resulted in all isomers of methylbiphenyl, ethylbiphenyl and dimethylbiphenyl, and 17 of the 24 trimethylbiphenyl isomers, being identified in crude oils and sediments (Cumbers *et al.*, 1986, 1987; Alexander *et al.*, 1986, 1991b). There have been no reports in the literature of the occurrence of long chain alkylbiphenyls in crude oils and sediments.

Deuterated alkylbenzenes and alkyltoluenes (alkyl-*d*) are derived from diaromatic compounds in which the two aromatic rings are linked by an alkyl chain, such as alkyldiphenylmethanes. Diphenylmethane and the four methyldiphenylmethanes have recently been reported as constituents of crude oils (Trolio *et al.*, 1996). Compounds in which the linking carbon chain is greater than $C_1$ have not been reported in crude oils and sediments.

Possible triaromatic precursors of the deuterated alkyltoluenes (methyl-$d$) include phenylnaphthalenes and other compounds in which an aromatic ring is attached by a single carbon-carbon bond to another aromatic ring, such as terphenyls. Deuterated alkyltoluenes (alkyl-$d$) and alkylbenzenes are derived from analogous triaromatic compounds in which the carbon-carbon bond joining the aromatic ring systems is replaced with an alkyl chain. Such compounds have thus far not been reported to occur in sedimentary organic matter.

## 7.2.4 Analysis of the dicarboxylic acids generated from the alkyl substituents of crude oil aromatic components

A large number of the methyl esters of the dicarboxylic acids derived from the alkyl substituents of the crude oil aromatic fractions have been characterised using GC-MS techniques. The TICs of the methyl esters from the monoaromatic, diaromatic and triaromatic fractions are given in Figure 7.25. In the chromatogram for the sample from the monoaromatic fraction, 76 % of the material is resolved. The proportion of resolved material in the samples from the diaromatic and triaromatic fractions are 65 % and 61 % respectively. Compound classes identified in the samples include the dimethyl esters of $\alpha,\omega$-alkanedioic acids, alkylcyclopentane-dicarboxylic acids and alkylcyclohexane-dicarboxylic acids, and aromatic dicarboxylic acids

### 7.2.4.1 Aliphatic components

*$\alpha,\omega$-Alkanedioic acid dimethyl esters*

The dimethyl esters of 1,5-pentanedioic acid and several alkyl-substituted isomers have been identified in the dicarboxylic acid dimethyl ester samples from the crude oil aromatic fractions on the basis of expected mass fragmentation patterns. Diagnostic ions for the dimethyl esters include $M^+$-31 (corresponding to the loss of -$OCH_3$) and $M^+$-59/60 (corresponding to the loss of -$COOCH_3$). A series of alkyl 1,6-hexanedioic acid dimethyl esters has also been identified in the sample on the basis of expected mass spectrum fragmentation patterns. Figure 7.26 shows
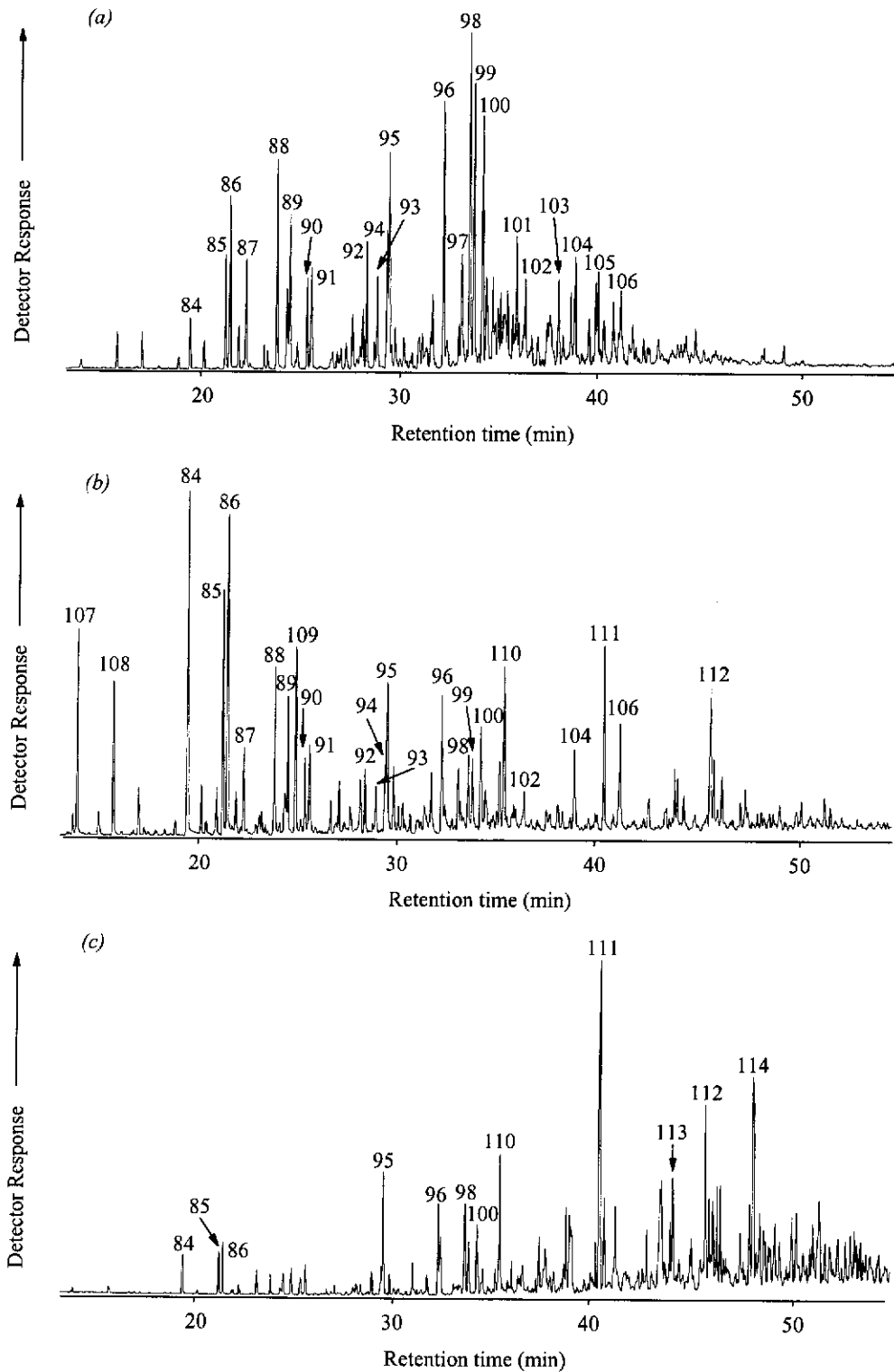
Figure 7.25. GC-MS total ion chromatograms showing dimethyl esters of water soluble dicarboxylic acids from ruthenium tetroxide oxidation of Leatherjacket-1 788.5m (a) monoaromatic; (b) diaromatic; and, (c) triaromatic fractions (for peak identification refer to Table 7.4).

peaks corresponding to the dimethyl esters of both the 1,5-pentanedioic acid and 1,6-hexanedioic acid isomers in the sample from the monoaromatic fraction, with the identified isomers given in Table 7.4. The same compounds were also identified in the samples from the diaromatic and triaromatic fractions.

Dimethyl pentanedioate and the two monomethyl isomers have been identified in the sample, together with three of the six $C_2$-substituted isomers and a single $C_3$-substituted isomer. Dimethyl hexanedioate and the two monomethyl isomers have also been identified in the sample. Peaks corresponding to pentanedioic and hexanedioic acids from $C_7$ to $C_{10}$ are present in the relevant M-31 RICs (loss of -OCH$_3$). Due to the similarities in the mass spectra of the alkyl substituted pentanedioic and hexanedioic acids, the exact structures of the higher molecular weight isomers were unable to be determined.

$\alpha,\omega$-Alkanedioic acids can have two possible sources in the ruthenium tetroxide oxidation product of aromatic systems. They can be produced from the bridging alkyl chains which link two aromatic systems together, or from cyclic systems in which both ends of the alkyl chain are attached to the same aromatic ring. For the sample derived from the crude oil monoaromatic fraction, the latter of these two explanations proposed must be correct. Therefore, the presence of 1,5-pentanedioic acids in the oxidation product indicates that compounds with alkylindane structural moieties were present in the initial monoaromatic fraction, and many of these compounds contained alkyl substituents on the saturated part of the alkylindane portion of the molecule. The presence of alkylated 1,6-hexanedioic acids in the water soluble dicarboxylic acid fraction of the ruthenium tetroxide oxidation products indicates that compounds with tetralin structures, in which alkyl groups may be attached to the saturated ring, were present in the original monoaromatic source material.

In the dimethyl ester sample from the crude oil diaromatic fraction, a series of $\alpha,\omega$-alkanedioic acid methyl esters from $C_4$ to $C_{10}$ have been identified (Figure
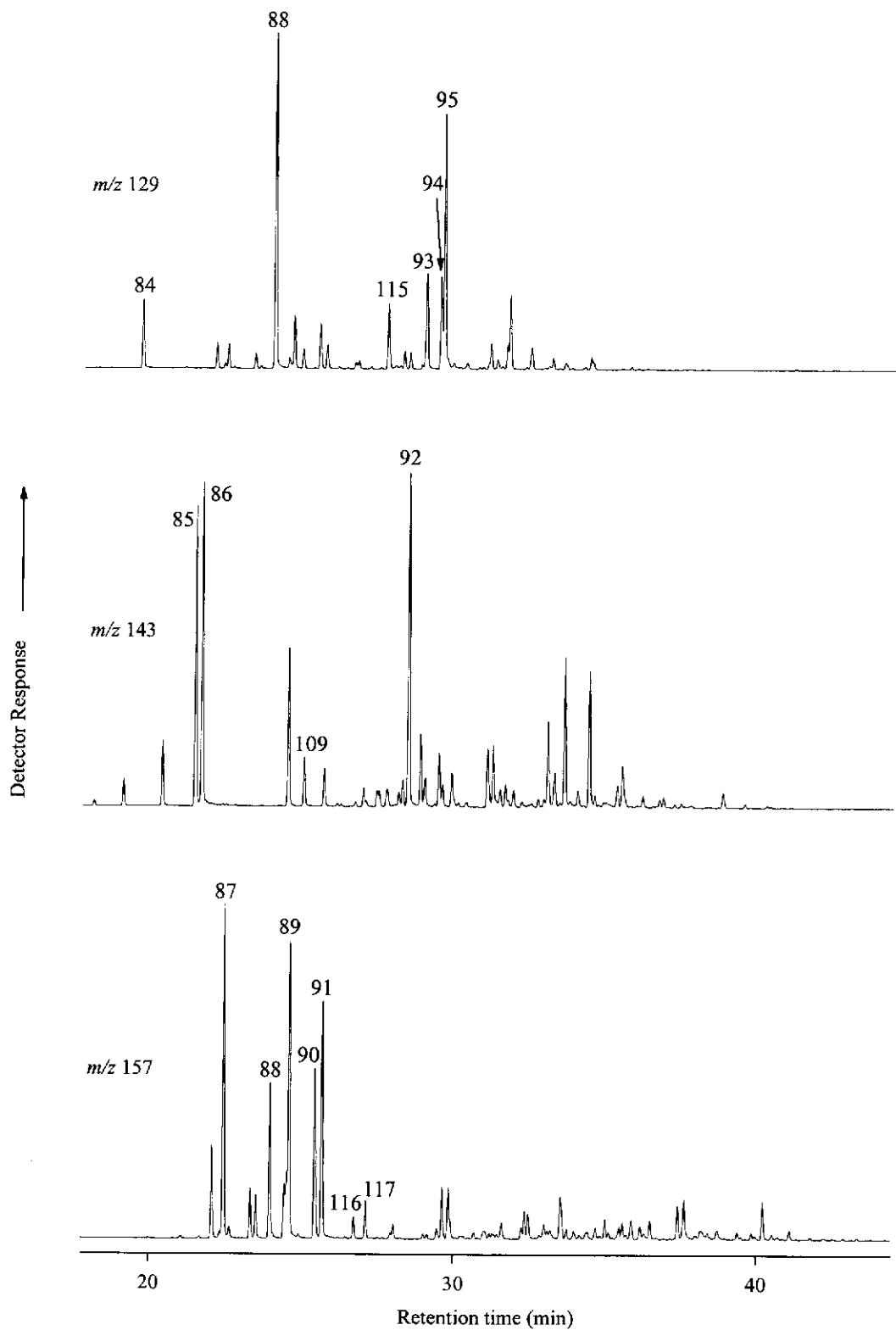
Figure 7.26. RICs at m/z 129, 143 and 157 showing various alkylated 1,5-pentanedioic acid and 1,6-hexanedioic acid dimethyl esters from the ruthenium tetroxide oxidation of Leatherjacket-1 788.5m monoaromatic fraction (for peak identification refer to Table 7.4).

7.27), with the $C_4$ to $C_6$ isomers present in considerably higher abundance relative to the other isomers. A similar distribution was observed for the $\alpha,\omega$-alkanedioic acid methyl esters from the triaromatic fraction, with the series extending to $C_{12}$. These results indicate that, as well as indane and tetralin structures, aromatic systems linked by alkyl chains from $C_2$ to $C_{10}$ were present in the original diaromatic and triaromatic fractions.

Thomas (1995) reported the occurrence of $C_2$ and $C_4$ to $C_6$ straight chain $\alpha,\omega$-alkanedioic acids, as well as unspecified $C_5$ to $C_7$ branched $\alpha,\omega$-alkanedioic acids, in the oxidation products of refinery fractions and a biodegraded crude oil. In the current study, several of the branched $\alpha,\omega$-alkanedioic acids have been identified, as shown in Table 7.4.

While the occurrence in crude oils of alkylated indanes has been proposed (e.g. Hussler et al., 1984; Rinaldi et al., 1988), there are very few reports of the positive identification of specific alkylindane isomers. One such study reports on the occurrence of indane and 1-, 2-, 4- and 5-methylindane in several crude oils (Sokolov et al., 1972). Another potential source of the 1,5-pentanedioic acids is from the D ring of the steranes with an aromatic C-ring (e.g. Schaefle et al., 1978; Mackenzie et al., 1981).

Alkyltetralins containing methyl substituents have been reported to occur in crude oils (e.g. Gallegos, 1973; Sokolov et al., 1972; Forster et al., 1989a; Alexander et al., 1992b) and shales (Anders et al., 1973; Koopmans et al., 1997). Williams et al. (1988) also reported anomolous concentrations of long chain alkyltetralins in West Texas and Michigan crude oils.

The parent and alkylated 1,5-pentanedioic acids and 1,6-hexanedioic acids identified in the dicarboxylic acid dimethyl ester sample from the diaromatic fraction may be derived from naphthenoaromatic systems containing two aromatic rings. One specific example of such a compound is simonellite, a biomarker which indicates input of higher plant material into sediments (Simoneit, 1977),
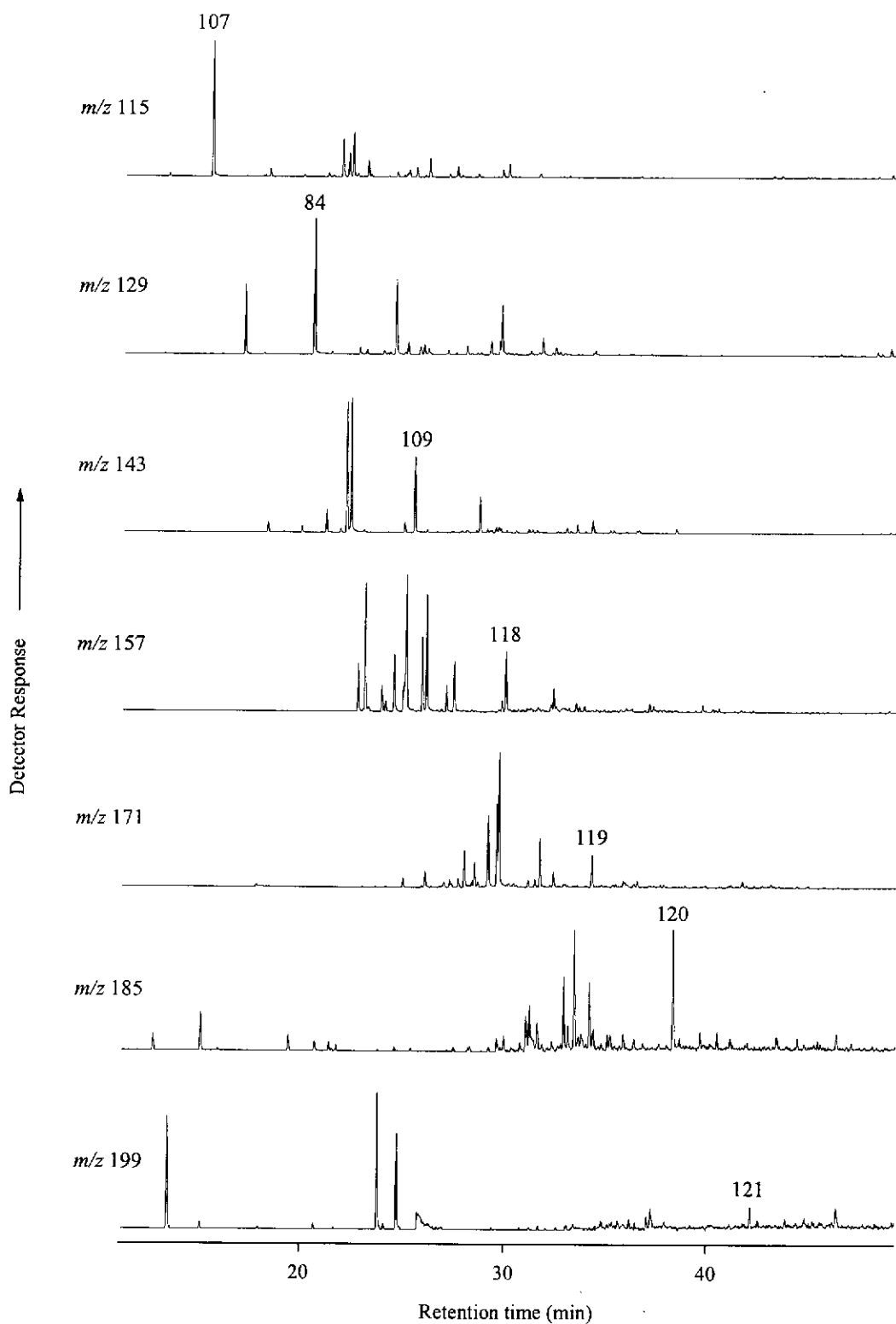
Figure 7.27. Mass chromatograms (*m/z* 115, 129, 143, 157, 171, 185 and 199) showing dimethyl esters of the dicarboxylic acid fraction from ruthenium tetroxide oxidation of Leatherjacket-1 788.5m crude oil diaromatic fraction (for peak identification refer to Table 7.4).

and other examples are known (e.g. Gallegos, 1973; Simoneit *et al.*, 1986; Murray *et al.*, 1997 and references therein). Examples of triaromatic compounds which may give rise to 1,5-pentanedioic acids and 1,6-hexanedioic acids on oxidation with ruthenium tetroxide include triaromatic steranes (Mackenzie *et al.*, 1981; Ludwig *et al.*, 1981) and other partially aromatised polycyclic compounds (e.g. Chaffee *et al.*, 1984; Simoneit, 1986; Murray *et al.*, 1997). Suitable precursor compounds for the $\alpha,\omega$-alkanedioic acids of different chain lengths have not to date been reported, and this constitutes the first evidence for the existence of such compounds.

*Cyclopentane- and cyclohexane-dicarboxylic acids*

Several compounds which have been tentatively identified as alkylcyclopentane-dicarboxylic acids and alkylcyclohexane-dicarboxylic acids have also been detected in the dicarboxylic acid dimethyl ester samples from the three crude oil aromatic fractions studied. Figure 7.28 shows the *m/z* 67, 81 and 95 reconstructed ion chromatograms corresponding to diagnostic fragment ions for some of these compounds, from the sample derived from the crude oil monoaromatic fraction. The samples derived from the crude oil diaromatic and triaromatic fractions contain essentially the same components, with their relative abundances varying slightly between the three samples. As is the case with the $\alpha,\omega$-alkanedioic acids, the mass spectra of isomeric compounds are very similar, and therefore positive identification of specific structures was unable to be made without reference compounds. This is the first report of the occurrence of cyclic dicarboxylic acids in the ruthenium tetroxide oxidation of crude oil aromatics.

The presence of cyclopentane-dicarboxylic acids and cyclohexane-dicarboxylic acids in the oxidation products indicates that tricyclic (at least) material was present in the initial monoaromatic fraction of this oil. Fused ring polycyclic monoaromatic compounds are well known constituents of crude oils, the most common of which are the monoaromatic steranes (e.g. Shaefle *et al.*, 1978; Hussler *et al.*, 1981; Mackenzie *et al.*, 1981). Other tricyclic and tetracyclic monoaromatic terpenes have also been reported in sedimentary organic matter
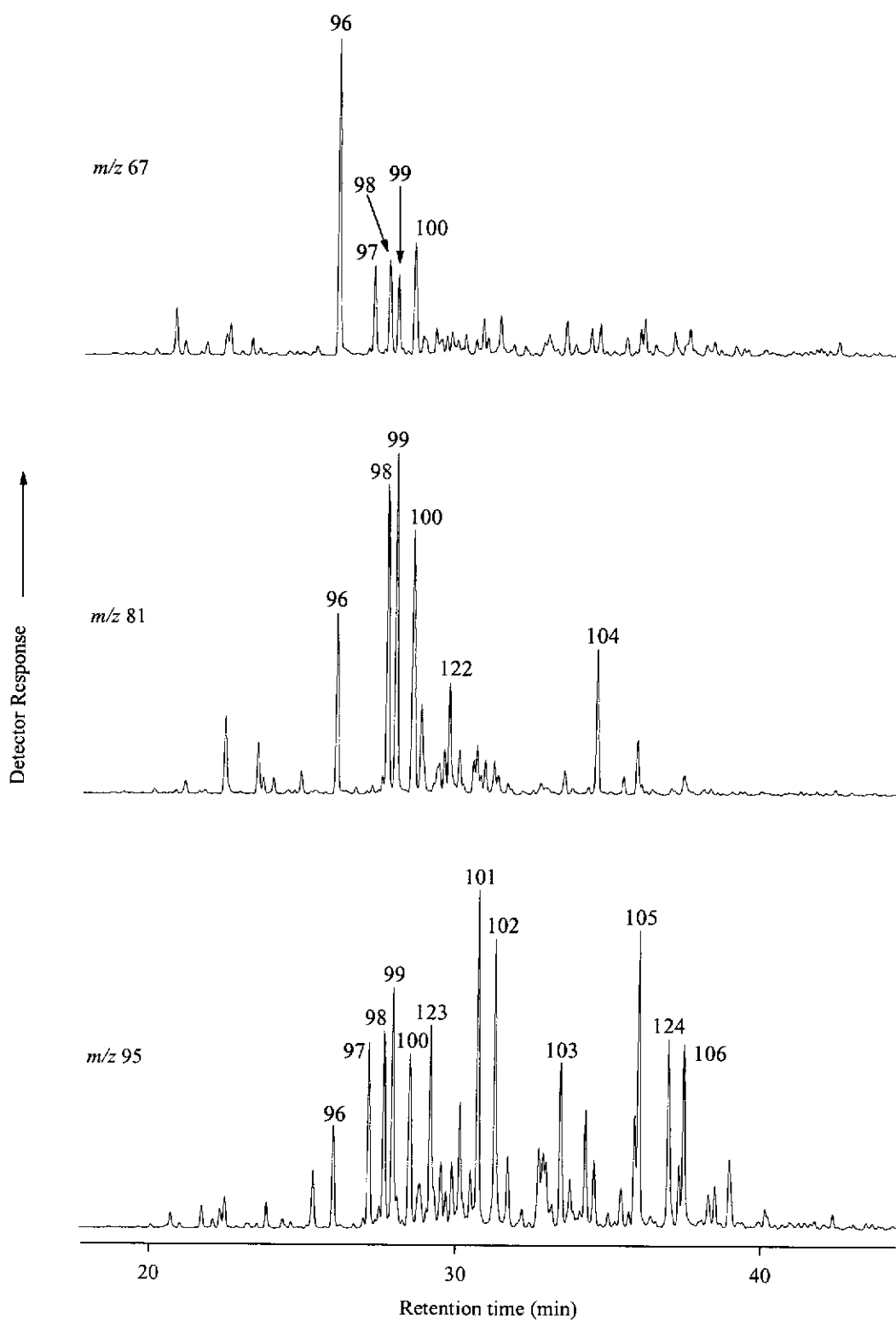
Figure 7.28.  GC-MS m/z 67, 81 and 95 reconstructed ion chromatograms showing various alkylated cyclopentane-dicarboxylic acid dimethyl esters and cyclohexane-dicarboxylic acid dimethyl esters in the sample derived from the Leatherjacket-1 788.5m monoaromatic fraction (for peak identification refer to Table 7.4).

(e.g. Anders *et al.*, 1973; Simoneit *et al.*, 1986; Boreham and Wilkins, 1995; Murray *et al.*, 1997 and references therein).

## 7.2.4.2 Aromatic components

*Aromatic dicarboxylic acids*

A series of aromatic dicarboxylic acids have been identified in the dicarboxylic acid dimethyl ester sample derived from the crude oil diaromatic and triaromatic fractions (Figure 7.29). The peak in the *m/z* 163 mass chromatogram (Peak 110, Fig. 7.29) has been attributed to phthalic acid on the basis of co-chromaography with an authentic standard. This is the product expected from the ruthenium tetroxide oxidation of fused polycyclic aromatic systems such as naphthalene or phenanthrene, or their alkylated homologues, with the alkyl substitution on the part of the ring system which is destroyed (cf. Spitzer and Lee, 1974). Isophthalic acid and terephthalic acid were not detected in the reaction mixture. A single $C_9$ aromatic dicarboxylic acid was also detected, and its mass spectrum indicates that it is most likely to be 3- or 4-methylphthalic acid. Several other isomers ranging from $C_{10}$ to $C_{12}$ have also been identified in the sample. As expected, dimethyl esters of aromatic dicarboxylic acids were not detected in the sample from the crude oil monoaromatic fraction, due to the absence of fused ring polycyclic aromatic structures.

Phthalic acid was identified in the ruthenium tetroxide oxidation product of a crude oil aromatic fraction (Thomas, 1995). It was uncertain, however, whether this compound was derived from compounds present in the initial aromatic fraction, or was the result of contamination, as it was also present in a procedural blank. The current results therefore constitute the first report of the occurrence of aromatic dicarboxylic acids from the oxidation of a crude oil aromatic fraction.

Phthalic acids are produced by ruthenium tetroxide oxidation of fused ring aromatic systems, such as naphthalenes in the case of diaromatic compounds and phenanthrenes in the case of triaromatic compounds (Spitzer and Lee, 1974; Stock
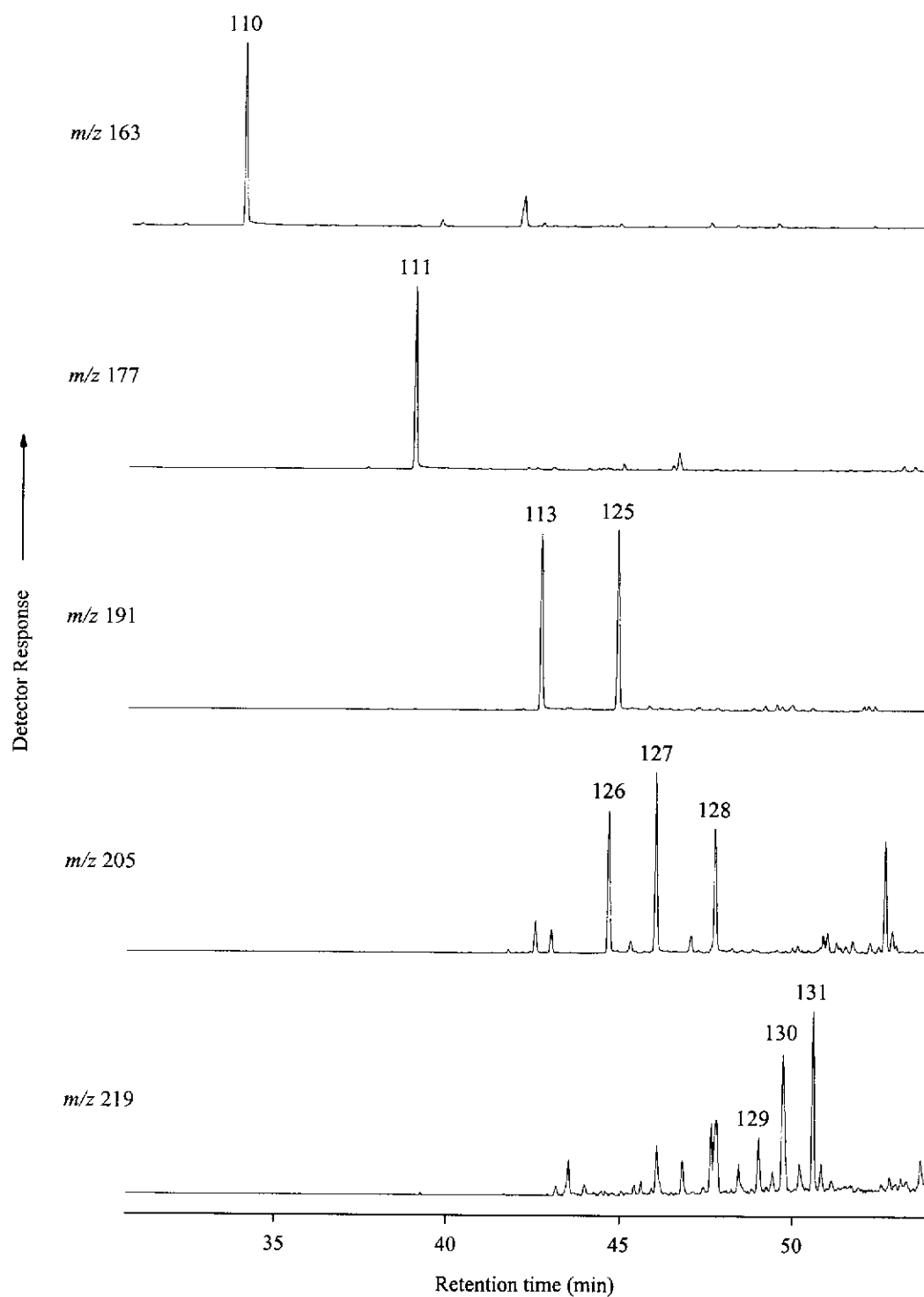
Figure 7.29. *m/z* 163, 177, 191, 205 And 219 mass chromatograms showing dimethyl esters of $C_8$ to $C_{12}$ aromatic dicarboxylic acids respectively from the ruthenium tetroxide oxidation of Leatherjacket-1 788.5m diaromatic fraction (for peak identification refer to Table 7.4).

and Tse, 1983). Alkylnaphthalenes are among the most well-known and widely studied aromatic constituents of sedimentary organic matter (e.g. Mair and Mayer, 1964; Yew and Mair, 1966; Radke *et al.,* 1982a, b, 1984, 1986, 1990a, b; Alexander *et al.,* 1983, 1984c, 1985; Rowland *et al.,* 1984; Puttman and Villar, 1987; Strachan *et al.,* 1988; Forster *et al.,* 1989b; Heppenheimer *et al.,* 1992; Yawanarajah and Kruge, 1994; Audino *et al.,* 1996; Borrego *et al.,* 1997; Bastow *et al.,* 1998). The occurrence of alkylphenanthrenes in sedimentary organic matter has also been extensively reported (e.g. Mair and Mayer, 1964; Yew and Mair, 1966; Radke *et al.,* 1982a, b, 1984a, b, 1986, 1990a, b, 1993; Garrigues and Ewald, 1983; Melikadze *et al.,* 1985; Garrigues *et al.,* 1987; Boreham *et al.,* 1988; Killops, 1991; Budzinski *et al.,* 1992, 1993, 1995; Fisher *et al.,* 1996).

A single aromatic tricarboxylic acid, benzene-1,2,4-tricarboxylic acid (trimethyl ester), was also identified in the sample from the crude oil triaromatic fraction. This compound would be produced from the oxidation of 2-phenylnaphthalene species present in the initial triaromatic fraction. It is likely that compounds containing a 1-phenylnaphthalene structural moiety are also present, but the corresponding oxidation product, benzene-1,2,3-tricarboxylic acid has a large degree of steric hindrance, which may impede its formation and/or esterification.

### 7.2.5 Summary of oxidation products identified and their possible aromatic precursors

Compound classes identified in the deuterated hydrocarbon fraction derived from reduction of the monocarboxylic acids generated by ruthenium tetroxide oxidation of a crude oil monoaromatic fraction include *n*-alkanes, methylalkanes, monoalkylalkanes, isoprenoids, alkylcyclohexanes and bicyclic compounds. Alkyl-1,5-pentanedioic acids, alkyl-1,6-hexanedioic acids, alkylcyclopentane-dicarboxylic acids and alkylcyclohexane-dicarboxylic acids were present in the methyl esters derived from the dicarboxylic fraction of the same oxidation product. Figure 7.30 summarises the oxidation products identified

in this study, and relates them to the aromatic moieties from which they are most likely derived.

The deuterated hydrocarbon fraction derived from reduction of the monocarboxylic acids generated by ruthenium tetroxide oxidation of a crude oil diaromatic fraction was similar to those of the monoaromatic fraction. Compound classes identified included $n$-alkanes, methylalkanes, monoalkylalkanes, isoprenoids, alkylcyclohexanes and bicyclic compounds. The monocarboxylic acid compounds identified were alkyl-substituted benzoic and benzeneacetic acids. The aliphatic components of the methylated dicarboxylic acid sample were also similar to those of the monoaromatic fraction, and included alkyl-1,5-pentanedioic acids, alkyl-1,6-hexanedioic acids, alkylcyclopentane-dicarboxylic acids and alkylcyclohexane-dicarboxylic acids. A series of $\alpha,\omega$-alkanedioic acids from $C_4$ to $C_{10}$ were also identified in the sample. Aromatic components of the dicarboxylic acid sample were identified as $C_0$ to $C_5$ alkyl-substituted phthalic acids.

The oxidation products which are similar to those produced from the monoaromatic fraction derive from precursors similar to those shown in Figure 7.30, but containing an extra aromatic ring. The aromatic rings may be fused (e.g. naphthalenes), or attached by either a carbon-carbon bond (e.g. biphenyls) or an alkyl chain (e.g. diphenylmethanes). A summary of the oxidation products which are different to those produced from the monoaromatic fraction, and the diaromatic moieties from which they are most likely derived, is given in Figure 7.31.

Deuterated hydrocarbons formed from the reduction of aliphatic monocarboxylic acids derived from the oxidation of the crude oil triaromatic fraction include $n$-alkanes, methylalkanes, monoalkylalkanes, isoprenoids, alkylcyclohexanes and bicyclic compounds. Also identified in the monocarboxylic acid methyl ester sample were series of alkyl-substituted benzoic acids, $\omega$-phenyl alkanoic acids and $\omega$-(methylphenyl) alkanoic acids, the higher molecular weight isomers of which

**Monodeuterated Product** / **Precursor \*** / **Number of carbon atoms in product**

7-34

10-21

10-31

7-33

11-18

**Methylated Dicarboxylic** / **Precursor**

3-8
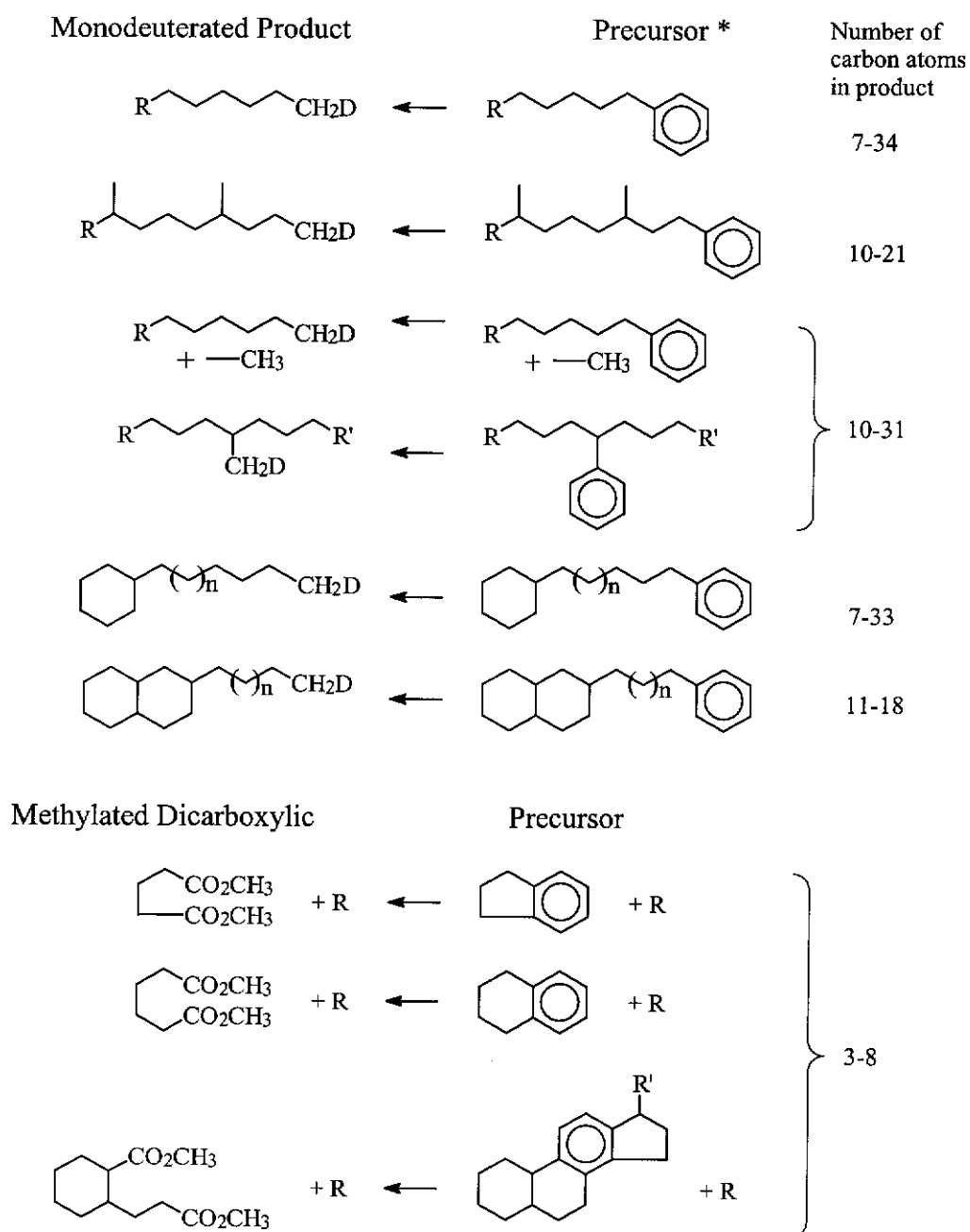
Figure 7.30. A summary of the monodeuterated hydrocarbon products obtained from the ruthenium tetroxide oxidation of crude oil monoaromatic fraction, and the general structures of aromatic moieties from which these oxidation products are most likely derived (\* aromatic rings may contain other substituents, both acyclic and alicyclic; R = alkyl).

were present in the deuterated hydrocarbon sample as their deuterated analogues. The aliphatic components of the dicarboxylic acid sample included alkyl-1,5-pentanedioic acids, alkyl-1,6-hexanedioic acids, alkylcyclopentane-dicarboxylic acids, alkylcyclohexane-dicarboxylic acids and $\alpha,\omega$-alkanedioic acids. Aromatic components of this sample included phthalic acid and a series of $C_9$ to $C_{12}$ aromatic dicarboxylic acids, as well as benzene-1,2,4-tricarboxylic acid. Most of the oxidation products are similar to those produced from the monoaromatic and diaromatic fractions, the aromatic precursors of which are shown in Figs. 7.30 and 7.31 respectively. In this case, the precursors are triaromatic, and the extra ring(s) may be fused, attached by a carbon-carbon bond or an alkyl chain. A summary of the oxidation products which are different to those produced from the monoaromatic and diaromatic fractions, and the triaromatic moieties from which they are most likely derived, is given in Figure 7.32.

Product                          Precursor *
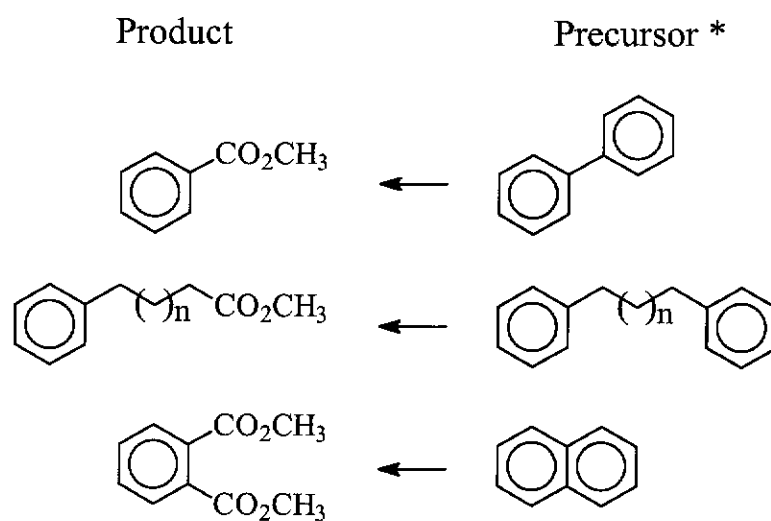


Figure 7.31.  A summary of some of the products obtained from the oxidation of a crude oil diaromatic fraction, and the aromatic moieties from which these compounds are most likely derived (* aromatic rings may contain other substituents, both acyclic and alicyclic).

Product                          Precursor *
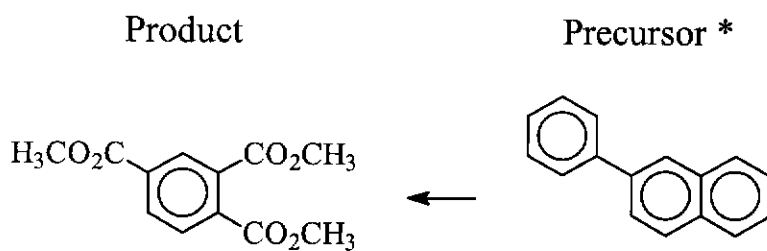


Figure 7.32.  A summary of some of the products obtained from the oxidation of a crude oil triaromatic fraction, and the aromatic moieties from which these compounds are most likely derived (* aromatic rings may contain other substituents, both acyclic and alicyclic).

## 7.3 CONCLUSIONS

Monoaromatic, diaromatic and triaromatic fractions from a biodegraded crude oil were oxidised with ruthenium tetroxide to cleave the side-chains off the aromatic systems at the *ipso* carbon to form carboxylic acids, in order to facilitate the characterisation of the side chains of components of the aromatic UCM by GC-MS.

The GC-MS total ion chromatogram of the deuterated hydrocarbon sample obtained by reduction of the monocarboxylic acids present in the monoaromatic fraction contained many deuterated analogues of compounds present in the saturate fraction of an unbiodegraded crude oil. Compound classes identified in the sample included monodeuterated *n*-alkanes, methylalkanes, alkylalkanes, alkylcyclohexanes, methylalkylcyclohexanes, isoprenoids and bicyclic compounds. Many of the compounds identified have not previously been reported in the products of the oxidation of crude oils. These observations indicate that compounds present in the original monoaromatic fraction contain straight, branched and isoprenoidal side chains, *n*-alkyl chains with ω-cyclohexyl groups and bicyclic components, attached to monoaromatic ring compounds. The occurrence of many of these precursor compounds has not previously been reported in crude oils

The dicarboxylic acids from the monoaromatic fraction contained alkyl-1,5-pentanedioic and alkyl-1,6-hexanedioic acids, as well as alkylcyclopentane- and alkylcyclohexane-dicarboxylic acids. Therefore, it can be concluded that compounds such as alkylindanes and alkyltetralins, as well as components with three or more fused rings, of which one is aromatic, are present in the original monoaromatic fraction.

The deuterated hydrocarbon samples derived from the diaromatic and triaromatic fractions are broadly similar in composition to those from the monoaromatic fraction. One significant difference is the presence of aromatic compounds such

as alkylbenzenes and alkyltoluenes (alkyl-*d*), indicating the presence of diaromatic and triaromatic compounds in which the aromatic rings are linked by an alkyl chain. Alkyltoluenes (methyl-*d*) were also identified, and indicate the presence of compounds such as biphenyls in the original diaromatic fraction, and phenylnaphthalenes or triaromatic compounds with a biphenyl structural moiety in the original triaromatic fraction.

The presence of phthalic acids in the dicarboxylic acid samples from the diaromatic and triaromatic fractions indicates the presence of polyaromatic fused ring systems in the original aromatic fractions, such as naphthalenes and phenanthrenes.

While some of these compounds have previously been reported in the ruthenium tetroxide oxidation products of crude oils, the results presented here constitute the first report of the occurrence of many others. MS analysis of the original aromatic fractions showed that sulfur-containing compounds were insignificant components. Thus it is clear that the oxidation products reported in this chapter were the moieties which were attached to aromatic rings in the original crude oil aromatic fractions. A summary of the previously reported oxidation products, and those identified in this study, is presented in Table 7.7. The structural information summarised in this table was unable to be obtained using currently available GC-MS techniques and instrumentation, as reported in Section 7.2.1

The similarities in the compound types, carbon number ranges and isomer distributions between the monodeuterated hydrocarbons derived from oxidation of alkylaromatics, and the saturate hydrocarbons present in unbiodegraded crude oils from the same geographic location suggest that the alkyl side chains and the saturate hydrocarbons in many cases have common precursors. Evidence was presented to support the proposal that the alkylaromatics are in part derived from alkylation of aromatic rings by functionalised acylium carbocations. The similarities in the composition of the monodeuterated hydrocarbon samples formed from the monoaromatic, diaromatic and triaromatic fractions, indicating

that the side chains are similar in all three fractions, independent of the nature of the aromatic systems to which they are attached, provides further evidence for this proposal. Sedimentary alkylation of aromatics may also provide an explanation for the existence of unresolved complex mixtures. Alkylation at different positions on aromatic systems may lead to a complex mixture comprising vast numbers of structurally similar isomeric groups of compounds, which would therefore not be resolved by GC.

Table 7.7. A summary of the compounds identified in this study as products of the ruthenium tetroxide oxidation of crude oil aromatics, together with previous literature reports of their occurrence.

| Compound class * | Identification in this study | Previous reports |
|---|---|---|
| $n$-alkanes | monoaromatic fraction: $C_7$-$C_{34}$ <br> diaromatic fraction: $C_7$-$C_{31}$ <br> triaromatic fraction: $C_7$-$C_{33}$ | $C_3$-$C_{22}$ (monoaromatics), $C_5$-$C_{10}$ (diaromatics), $C_6$-$C_{23}$ (aromatics)[a] <br> $C_8$-$C_{22}$ (aromatics)[b] |
| isoprenoid alkanes | monoaromatic fraction: $C_{10}$-$C_{21}$ <br> diaromatic fraction: $C_{10}$-$C_{21}$ <br> triaromatic fraction: $C_{10}$-$C_{21}$ | none |
| methylalkanes | monoaromatic fraction: $C_{10}$-$C_{31}$ (all isomers) <br> diaromatic fraction: $C_{10}$-$C_{23}$ (all isomers) <br> triaromatic fraction: $C_{10}$-$C_{28}$ (all isomers) | $C_8$-$C_{18}$ 2- and 3-methylalkanes (monoaromatics), $C_6$-$C_{14}$ 2- and 3-methylalkanes (aromatics)[a] |
| alkylalkanes | monoaromatic fraction: $C_{10}$-$C_{20}$ (most isomers); $C_{21}$-$C_{27}$ (ethylalkanes) <br> diaromatic fraction: $C_{10}$-$C_{20}$ (most isomers); $C_{21}$-$C_{24}$ (ethylalkanes) <br> triaromatic fraction: $C_{10}$-$C_{20}$ (most isomers); $C_{21}$-$C_{25}$ (ethylalkanes) | none |
| alkylcyclohexanes | monoaromatic fraction: $C_7$-$C_{33}$ <br> diaromatic fraction: $C_7$-$C_{25}$ <br> triaromatic fraction: $C_7$-$C_{28}$ | $C_7$ (monoaromatics, diaromatics, aromatics)[a] |
| methylalkylcyclohexanes | monoaromatic fraction: $C_8$-$C_{29}$ <br> diaromatic fraction: $C_8$-$C_{13}$ <br> triaromatic fraction: $C_8$-$C_{13}$ | none |

Table 7.7 (cont'd). A summary of the compounds identified in this study as products of the ruthenium tetroxide oxidation of crude oil aromatics, together with previous literature reports of their occurrence.

| bicyclic compounds | monoaromatic fraction: $C_{11}$-$C_{18}$ (various isomers, unconfirmed structures)<br>diaromatic fraction: $C_{11}$-$C_{18}$ (various isomers, unconfirmed structures)<br>triaromatic fraction: $C_{11}$-$C_{18}$ (various isomers, unconfirmed structures) | none |
|---|---|---|
| aromatic monocarboxylic acids | monoaromatic fraction: n/a<br>diaromatic fraction: benzoic acid, toluic acids (all three), $C_2$ and $C_3$ benzoic acids (several isomers), $C_7$-$C_{18}$ alkylbenzenes, $C_8$-$C_{18}$ $m$-alkyltoluenes (both methyl-$d$ and alkyl-$d$), $C_8$-$C_{15}$ $p$-alkyltoluenes (both methyl-$d$ and alkyl-$d$).<br>triaromatic fraction: benzoic acid, toluic acids (all three), $C_2$ and $C_3$ benzoic acids (several isomers), $C_7$-$C_{12}$ alkylbenzenes, $C_8$-$C_{14}$ $m$- and $p$-alkyltoluenes (both methyl-$d$ and alkyl-$d$). | phenylacetic acid (diaromatics), benzoic acid, $m$- and $p$-toluic acid, 3,5-dimethylbenzoic acid, phenylacetic acid (aromatics) [a] |
| $\alpha,\omega$-alkanedioic acids | monoaromatic fraction: pentanedioic acid, methylpentanedioic acids (both isomers), $C_2$ (three isomers) and $C_3$ (one isomer) pentanedioic acids, hexanedioic acid, methylhexanedioic acids (both isomers), $C_7$-$C_{10}$ $\alpha,\omega$-alkanedioic acids (various isomers, unconfirmed structures)<br>diaromatic fraction: $C_4$-$C_{10}$ straight chain $\alpha,\omega$-alkanedioic acids, others as for monoaromatic fraction<br>triaromatic fraction: $C_4$-$C_{12}$ straight chain $\alpha,\omega$-alkanedioic acids, others as for monoaromatic fraction | $C_2$-$C_6$ straight chain $\alpha,\omega$-alkanedioic acids, unspecified branched dicarboxylic acids (monoaromatics), $C_2$, $C_6$ straight chain $\alpha,\omega$-alkanedioic acids [a] |

Table 7.7 (cont'd). A summary of the compounds identified in this study as products of the ruthenium tetroxide oxidation of crude oil aromatics, together with previous literature reports of their occurrence.

| cycloalkanedicarboxylic acids | monoaromatic fraction: <br> diaromatic fraction: <br> triaromatic fraction: | none |
|---|---|---|
| aromatic dicarboxylic acids | monoaromatic fraction: n/a <br> diaromatic fraction: phthalic acid, $C_1$-$C_5$ substituted phthalic acids (various isomers, unconfirmed structures) <br> triaromatic fraction: as for diaromatic fraction | phthalic acid (aromatics) [a] |
| aromatic tricarboxylic acids | monoaromatic fraction: n/a <br> diaromatic fraction: n/a <br> triaromatic fraction: benzene-1,2,4-tricarboxylic acid | none |

* : Hydrocarbons identified in this study are all monodeuterated and are derived from the reduction of monocarboxylic acids, and both of these compound types are considered equivalent. Thus for example, n-alkanes refers to both monodeuterated n-alkanes and the corresponding n-alkanoic acids. In a similar manner, a direct comparison is made between the monodeuterated hydrocarbons identified here and the carboxylic acids identified in earlier studies.

a: Thomas, 1995; b: Revill, 1992.

# REFERENCES

Adams, N. G. and Richardson, D. M. (1953) Isolation and identification of biphenyls from West Edmond crude oil. *Analytical Chemistry* **25**, 1073-1074.

Albaigés, J., Algaba, J., Clavell, E. and Grimalt, J. (1986) Petroleum geochemistry of the Taragona Basin (Spanish Mediterranean off-shore). In *Advances in Organic Geochemistry 1985* (Eds. D. Leythaeuser and J. Rullkötter), Pergamon Press, Oxford.

Alexander, R., Kagi, R. I. and Sheppard, P. N. (1983) Relative abundance of dimethylnaphthalene isomers in crude oils. *Journal of Chromatography* **267**, 367-372.

Alexander, R., Cumbers, M. and Kagi, R. I. (1984a) Geochemistry of some Canning Basin crude oils. In *The Canning Basin W.A.: Proceedings of the Geological Society of Australia/ Petroleum Exploration Society of Australia Symposium* (Ed. P. G. Purcell), Perth.

Alexander, R., Kagi, R. I. and Larcher, A. V. (1984b) Clay catalysis of alkyl hydrogen exchange reactions - reaction mechanisms. *Organic Geochemistry* **6**, 755-760.

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 distribution of dimethylnaphthalenes and trimethylnaphthalenes in some Ancient sediments and petroleums. *Geochimica et Cosmochimica Acta* **49**, 385-395.

Alexander, R., Cumbers, M. and Kagi, R. I. (1986) Alkylbiphenyls in ancient sediments and petroleums. In *Advances in Organic Geochemistry 1985* (Eds. D. Leythaeuser and J. Rullkötter), Pergamon Press, Oxford. *Organic Geochemistry* **10**, 841-845.

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), Pergamon Press, Oxford.

Alexander, R., Kralert, P. G., Marzi, R., Kagi, R. I. and Evans, E. J. (1991a) 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., Ngo, S. H. and Kagi, R. I. (1991b) Identification and analysis of trimethybiphenyls in petroleum. *Journal of Chromatography* **538**, 424-430.

Alexander, R., Kralert, P. G. and Kagi, R. I. (1992a) Kinetics and mechanism of the thermal decomposition of esters in sediments. In *Advances in Organic Geochemistry 1991* (Eds. C. B. Eckardt, J. R. Maxwell, S. R. Larter and D. A. C. Manning), Pergamon Press, Oxford. *Organic Geochemistry.* **19**, 133-140.

Alexander, R., Bastow, T. P., Kagi, R. I. and Singh, R. K. (1992b) Identification of 1,2,2,5-tetramethyltetralin and 1,2,2,5,6-pentamethyltetralin as racemates in petroleum. *Journal of the Chemical Society Chemical Communications* **23**, 1712-1714.

Alexander, R., Baker, R. W., Kagi, R. I. and Warton, B. (1993) Cyclohexylbenzenes in crude oils. *Chemical Geology* **113**, 103-115.

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.

Audino, M., Alexander R. and Kagi R. I. (1996) Ethylmethylnaphthalenes in crude oils. *Polycyclic Aromatic Compounds* **8**, 93-103.

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

Barr, R. and Arntzen, C. J. (1969) The occurrence of δ-tocopherylquinone in higher plants and its relationship to senescence. *Plant Physiology* **33**, 591-598.

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), Technip, Paris.

Biemann, K. (1962) *Mass Spectrometry Organic Chemical Applications*, pp. 78-85. McGraw-Hill, New York.

Birch, E. J., Knight, T. W. and Shaw, G. J. (1989) Separation of male goat pheromones responsible for stimulating ovulatory activity in ewes. *New Zealand Journal of Agricultural Research* **32**, 337-341.

Blanc, P. and Albrecht, P. (1990) Molecular markers in bitumen and macromolecular matrix of coals. Their evaluation as rank parameters. In *Advanced Methodologies in Coal Characterisation* (Ed. H. Charcosset), Elsevier, Amsterdam.

Blumer, M., Ehrhardt, M. and Jones, J. H. (1973) The environmental fate of stranded crude oil. *Deep-Sea Research* **20**, 239-259.

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.

Boreham, C. J. and Wilkins, A. L. (1995) Structure and origin of the two major monoaromatic hydrocarbons in a tasmanite oil shale from Tasmania, Australia. *Organic Geochemistry* **23,** 461-466.

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.

Budzinski, H., Radke, M., Garrigues, P., Wise, S. A., Bellocq, J. and Willsch, H. (1992) Gas chromatographic retention behaviour of alkylated phenanthrenens on a smectic liquid crystalline phase- Application to organic geochemistry. *Journal of Chromatography* **627,** 227-239.

Budzinski, H., Garrigues, P., Radke, M., Connan, J. and Oudin, J. L. (1993) Thermodynamic calculations on alkylated phenanthrenes - Geochemical applications to maturity and origin of hydrocarbons. *Organic Geochemistry* **20,** 917-926.

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.

Cainelli, G. and Cardillo, G. (1984) *Chromium Oxidations in Organic Chemistry.* Springer-Verlag, Berlin.

Carlsen, P. H. J., Katsuki, T., Martin, V. S. and Sharpless, K. B. (1981) A greatly improved procedure for ruthenium tetraoxide catalysed oxidations of organic compounds. *Journal of Organic Chemistry* **46,** 3936-3938.

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.

Clerc, R. J., Hood, A. and O'Neal, M. J. (1955) Mass spectrometric analysis of high molecular weight, saturated hydrocarbons. *Analytical Chemistry* **27**, 868-875.

Condon, F. E. (1951) Synthesis and mass spectra of some hexa-, hepta- and octadeuterated derivatives of propane. *Journal of the American Chemical Society* **73**, 4675-4678.

Condon, F. E., McMurry, H. L. and Thornton V. (1951) Infrared and mass spectra of some deuterium-containing derivatives of propane. *The Journal of Chemical Physics* **19**, 1010-1013.

Connan, J. (1984) Biodegradation of crude oils in reservoirs. In *Advances in Organic Geochemistry*, (Eds. J. Brooks and D. H. Welte), 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.

Courtney, J. L. (1986) Ruthenium tetroxide oxidations. In *Organic Syntheses by Oxidation with Metal Compounds* (Eds. W. J. Mijs and C. R. H. de Jonge), Plenium, New York.

Cumbers, K. M., Alexander, R. and Kagi, R. I. (1986) Analysis of methylbiphenyls, ethylbiphenyls and dimethylbiphenyls in crude oils. *Journal of Chromatography* **361**, 385-390.

Cumbers, K. M., Alexander, R. and Kagi, R. I. (1987) Methylbiphenyl, ethylbiphenyl and dimethylbiphenyl isomer distributions in some sediments and crude oils. *Geochimica et Cosmochimica Acta* **51**, 3105-3111.

Dachs, J., Bayona, J. M., Fowler, S. W., Miquel, J. C. and Albaiges, J. (1998) Evidence For Cyanobacterial Inputs and Heterotrophic Alteration of Lipids in Sinking Particles in the Alboran Sea (SW Mediterranean). *Marine Chemistry* **60**, 189-201.

de Rosa, M., Gambacorta, A., Minale, L. and Bu'lock, J. D. (1972) The formation of ω-cyclo-hexyl fatty acids from shikimate in an acidophilic thermophilic bacillus. *Biochemical Journal* **128**, 751-754.

Deming, J. W. and Baross, J. A. (1993) The early diagenesis of organic matter: bacterial activity. In *Organic Geochemistry Principles and Applications,* (Eds. M. H. Engel and S. A. Macko). Plenum Press, New York.

Djerassi, C. and Engle, R. R. (1953) Oxidations with ruthenium tetroxide. *Journal of the American Chemical Society* **75**, 3838-3840.

Dong, J.-Z., 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.

Dowling, N. J. E., Widdel, F. and White, D. C. (1986) Phospholipid ester-linked fatty acid biomarkers of acetate-oxidising sulphate reducers and other sulphide-forming bacteria. *Journal of General Microbiology* **132**, 1815-1825.

Downing, D. T. (1976) Mammalian waxes. In *Chemistry and Biochemistry of Natural Waxes* (Ed. P. E. Kolattukudy), Elsevier Scientific Publishing Company, Amsterdam.

Eglinton, G. and Hamilton, R. J. (1967) Leaf epicuticular waxes. *Science* **156,** 1322-1325.

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

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., Fisher, S. J., Singh, R. K., Alexander, R. and Kagi, R. I. (1999) Identification of alkenylbenzenes in pyrolyzates using GC-MS and GC-FTIR techniques: evidence for kerogen aromatic moieties with various binding sites. *Organic Geochemistry* (in press).

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

Ellis, L., Langworthy, T.A. and Winans, R. (1996) Occurrence of phenylalkanes in some Australian crude oils and sediments. *Organic Geochemistry* **24,** 57-69.

Farcasiu, M. and Rubin, B. R. (1987) Aliphatic elements of structure in petroleum residues. *Energy and Fuels* **1,** 381-386.

Fisher, S. J., Alexander, R., Ellis, L. and Kagi, R. I. (1996) The analysis of alkylphenanthrenes by direct deposition gas chromatography-fourier transform infrared spectroscopy (GC-FTIR). *Polycyclic Aromatic Compounds* **9,** 257-264.

Forster, P., Alexander, R. and Kagi, R. I. (1989a) Identification of 2,2,7,8-tetramethyl-1,2,3,4-tetratetrahydronaphthalene 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.

Fowler, M. G. and Douglas, A. G. (1987) Saturated hydrocarbon biomarkers in oils of Late Precambrian age from Eastern Siberia. *Organic Geochemistry* **11**, 201-213.

Friedman, L., Fishel, D. L. and Shechter, H. (1965) Oxidation of alkylarenes with aqueos sodium dichromate. A useful method for preparing mono- and polyaromatic carboxylic acids. *Journal of Organic Chemistry* **30**, 1453-1457.

Furniss, B. S., Hannaford, A. J., Smith, P. W. G. and Tatchell, A. R. (1989) *Vogel's Textbook of Practical Organic Chemistry,* 5th edn. Longman Scientific & Technical, Harlow, England.

Gallegos, E. J. (1973) Identification of new steranes, terpanes and branched paraffins in Green River shale by combined capillary gas chromatography and mass spectrometry. *Analytical Chemistry* **45**, 1151-1160.

Garrigues, P. and Ewald, M. (1983b) Natural occurrence of 4-methylphenanthrene in petroleums and recent marine sediments. *Organic Geochemistry* **5**, 54-56.

Garrigues, P., Parlanti, E., Radke, M., Bellocq, J., Willsch, H. and Ewald, M. (1987) Identification of alkylphenathrenes in shale oil and coal by liquid and capillary gas chromatography and high-resolution spectrofluorimetry (Shpol'skii effect). *Journal of Chromatography* **395**, 217-228.

Gates, B. C., Katzer, J. R. and Schuit, G. C. A. (1979) *Chemistry of Catalytic Processes,* pp. 12-22. McGraw Hill, New York.

Gelpi, E., Schneider, H., Mann, J. and Oro, J. (1970) Hydrocarbons of geochemical significance in microscopic algae. *Phytochemistry* **9,** 603-612.

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* (Ed. 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 (London)* **344,** 648-650.

Gough, M. A. and Rowland, S. J. (1991) Characterisation of unresolved complex mixtures of hydrocarbons from lubricating oil feedstocks. *Energy and Fuels* **5,** 869-874.

Gough, M. A., Rhead, M. M. and Rowland, S. J. (1992) Biodegradation studies of unresolved complex mixtures of hydrocarbons: model UCM hydrocarbons and the aliphatic UCM. *Organic Geochemistry.* **18,** 17-22.

Greensfelder, B. S., Voge, H. H. and Good, G. M. (1949) Catalytic and thermal cracking of pure hydrocarbons. Mechanisms of reaction. *Industrial and Engineering Chemistry* **41,** 2573-2584.

Gunstone, F. D. (1996) *Fatty Acid and Lipid Chemistry.* Chapman and Hall, Glasgow.

Gurgey, K. (1998) Geochemical effects of asphaltene separation procedures: changes in sterane, terpane, and methylalkane distributions in maltenes and asphaltene co-precipitates. *Organic Geochemistry* **29,** 1139-1147.

Han, J., McCarthy, E. D. and Calvin, M. (1968) Hydrocarbon constituents of the blue-green algae *Nostoc muscorum, Anacystis nidulans, Phormidium luridum and Chlorogloea fritschii*. *Journal of the Chemical Society* (C), 2785-2791.

Henninger, M. D., Dilley, R. A. and Crane, F. L. (1963) Restoration of ferricyanide reduction in acetone-extracted chloroplasts by $\beta$- and $\gamma$-tocopherol quinones. *Biochemical and Biophysical Research Communications* 10, 237-242.

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.

Hillbrick, G. C., Tucker, D. J. and Smith, G. C. (1995) The lipid composition of cashmere goat fleece. *Australian Journal of Agricultural Research* 46, 1259-1271.

Hoering, T. C. (1980) Monomethyl, acyclic hydrocarbons in petroleum and rock extracts. *Carnegie Institute of Washington Yearbook* 80, 389-394.

Hoering, T. C. and Freeman, D. H. (1984) Shape-selective sorption of monomethylalkanes by silicalite, a zeolitic form of silica. *Journal of Chromatography* 316, 333-341.

Hoffmann, C. F., Foster, C. B., Powell, T. G. and Summons, R. E. (1987) Hydrocarbon biomarkers from Ordovician sediments and the fossil alga *Gloeocapsomorpha prisca* Zalessky 1917. *Geochimica et Cosmochimica Acta* 51, 2681-2697.

Holzer, G., Oro, J. and Tornabene, T. G. (1979) Gas chromatographic-mass spectrometric analysis of neutral lipids from methanogenic and thermoacidophilic bacteria. *Journal of Chromatography* 186, 795-809.

Hood, A. and O'Neal, M. J. (1959) Status of application of mass spectrometry to heavy oil analysis. In *Advances in Mass Spectrometry* (Ed. J. D. Waldron), Academic Press, London.

Hood, A., Clerc, R. J. and O'Neal, M. J. (1959) The molecular structure of heavy petroleum compounds. *Journal of the Institute of Petroleum* **45**, 168-173.

Hudlický, M. (1990) *Oxidations in Organic Chemistry.* American Chemical Society, Washington D.C.

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

Hussler, G., Connan, J. and Albrecht, P. (1984) Novel families of tetra- and hexacyclic aromatic hopanoids predominant in carbonate rocks and crude oils. *Organic Geochemistry* **6**, 39-49.

Ilsley, W. H., Zingaro, R. A. and Zoeller Jr., J. H. (1986) The reactivity of ruthenium tetroxide towards aromatic and etheric functionalities in simple organic compounds. *Fuel* **65**, 1216-1220.

Ioppopo-Armanios, M. (1995) *The occurrence and origins of some alkylphenols in crude oils.* Ph.D. Thesis, Curtin University of Technology, Perth, Australia.

Jackson, M. J., Powell, T. G., Summons, R. E. and Sweet, I. P. (1986) Hydrocarbon shows and petroleum source rocks in sediments as old as $1.7 \times 10^9$ years. *Nature* **322**, 727-729.

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

Kabalka, G. W., Varma, M., Varma, R. S., Srivastava, P. C. and Knapp Jr., F. F. (1986) Tosylation of alcohols. *Journal of Organic Chemistry* **51**, 2386-2388.

Kaneda, T. (1977) Fatty acids of the genus *Bacillus*: an example of branched-chain preference. *Bacteriological Reviews* **41**, 391-418.

Killops, S. D. (1991) Novel aromatic hydrocarbons of probable bacterial origin in a Jurassic lacustrine sequence. *Organic Geochemistry* **17**, 25-36.

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. and Killops, V. J. (1993) *An Introduction to Organic Chemistry.* Longman Scientific and Technical, Harlow, England.

Kissin, Y. V. (1987) Catagenesis and composition of petroleum: Origin of *n*-alkanes and isoalkanes in petroleum crudes. *Geochimica et Cosmochimica Acta* **51**, 2445-2457.

Kissin, Y. V. and Feulmer, G. P. (1986) Gas chromatographic analysis of alkyl-substituted paraffins. *Journal of Chromatographic Science* **24**, 53-59.

Klomp, U. C. (1986) The chemical structure of a pronounced series of iso-alkanes in South Oman crudes. *Organic Geochemistry* **10**, 807-814.

Kolattukudy, P. E. (1976) Introduction to natural waxes. In *Chemistry and Biochemistry of Natural Waxes* (Ed. P. E. Kolattukudy), Elsevier Scientific Publishing Company, Amsterdam.

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.

Kralert, P. G. (1995) *Pyrolytic elimination reactions of alkyl esters and their application to the reconstruction of thermal histories of sediments.* Ph.D. Thesis, Curtin University of Technology, Perth, Australia.

Krasavchenko, M. I., Zemskova, Z. K., Mikhnovskaya, A. A., Pustilnikova, S. D. and Petrov, A. A. (1971) Monosubstituted alkanes $C_{10}$ - $C_{16}$ in paraffinic oils. *Neftekhimiya* **11**, 803-809.

Kurashova, E. K., Musayev, I. A., Smirnov, M. B., Simanyuk, R. N., Mikaya, A. I., Ivanov, A. V. and Sanin, P. I. (1989) Hydrocarbons of Khar'yag crude oil. *Petroleum Chemistry U.S.S.R.* **29** (3), 206-220.

Kursanov, D. N., Parnes, Z. N., Bassova, G. I., Loim, N. M. and Zdanovich, V. I. (1967) Ionic hydrogenation of the ethylene bond and the double bond of the carbonyl group. *Tetrahedron* **23**, 2235-2242.

Langworthy, T. A., Tornabene, T. G. and Holzer, G. (1982) Lipids of Archaebacteria. *Zentralblatt fur Bakteriologie, Mikrobiologie und Hygiene 1. Abt. Originale C* **3**, 228-244.

Lee, D. G. (1980) *The Oxidation of Organic Compounds by Permanganate Ion and Hexavalent Chromium,* pp. 43-55. Open Court Publishing Company, La Salle, Illinois.

Lee, D. G. and Spitzer, U. A. (1969) The oxidation of ethylbenzene with aqueous sodium dichromate. *Journal of Organic Chemistry* **34**, 1493-1495.

Ludwig, B., Hussler, G., Wehrung, P. and Albrecht, P. (1981) $C_{26}$-$C_{29}$ triaromatic steroid derivatives in sediments and petroleums. *Tetrahedron Letters* **22**, 3313-3316.

Mackenzie, A. S., Hoffmann, C. F. and Maxwell, J. R. (1981) Molecular parameters of maturation in the Toarcian shales, Paris Basin, France-III. Changes in aromatic steroid hydrocarbons. *Geochimica et Cosmochimica Acta* **45**, 1345-1355.

Mair, B. J. and Mayer, T. J. (1964) Composition of the dinuclear aromatics, $C_{12}$ to $C_{14}$, in the light gas oil fraction of petroleum. *Analytical Chemistry* **36**, 351-362.

Makushina, V. M., Aref'yev, O. A., Zabrodina, M. N. and Petrov, A. A. (1978) New relict paraffins of crude oils. *Neftekhimiya* **18**, 847-854.

Mallaya, N. and Zingaro, R. A. (1984) Ruthenium tetroxide - a reagent with the potential for the study of oxygen functionalities in coal. *Fuel* **63**, 423-425.

March, J. (1992) *Advanced Organic Chemistry. Reactions, Mechanisms and Structure,* 4th edn. John Wiley and Sons, New York.

McCarthy, E. D., Han, J. and Calvin, M. (1968) Hydrogen atom transfer in mass spectrometric fragmentation patterns of saturated aliphatic hydrocarbons. *Analytical Chemistry* **40**, 1475-1480.

McLafferty, F. W., Turecek, F., 1993. *Interpretation of Mass Spectra, Fourth Edition,* pp. 226-229. University Science Books, Mill Valley, California.

Melikadze, L. D., Lekveishvili, E. G., Tevdorashvili, M. N. and Dznaparidze, K. D. (1985) Isolation and study of phenanthrene hydrocarbons from high boiling oil fractions of Mirzaani oil by photocondensation with maleic anhydride. *Soobschcheniya Akademii Nauk Gruzinskoi SSR* **119**, 309-312.

Monson, R. S. (1971) *Advanced Organic Synthesis. Methods and Techniques.* Academic Press, Inc. New York.

Mostecky, J., Popl, M. and Kriz, J. (1970) Determination of $C_{12}$ alkylnaphthalenes and methylbiphenyls in aromatic fractions by capillary gas chromatography. *Analytical Chemistry* **42**, 1132-1135.

Murray, A. P., Sosrowidjojo, I. B., Alexander, R., Kagi, R. I., Norgate, C. M. and Summons, R. E. (1997) Oleananes in oils and sediments: Evidence of marine influence during early diagenesis? *Geochimica et Cosmochimica Acta* **61**, 1261-1276.

Musayev, I. A., Kurashova, E. K., Simanyuk, R. N., Polyakova, A. A., Yermakova, L.S., Smirnov, M. B., and Sanin, P. I. (1985) Hydrocarbons from West Siberian crude oils. *Petroleum Chemistry U.S.S.R.* **25** (2), 76-82.

Nakanishi, K. and Solomon, P. I. (1977) *Infrared Absorption Spectroscopy,* 2nd edn. Holden-Day Inc., Oakland, California.

Nelson, D. R. (1978) Long-chain methyl-branched hydrocarbons: Occurrence, biosynthesis and function. *Advances in Insect Physiology* **13**, 1-33.

Newman, M. S. and Boden, H. (1961) Synthesis of the six carboxybenzo[c]-phenanthrenes. *Journal of Organic Chemistry* **26**, 1759-1761.

Olah, G. A. and Molnár, Á (1995) *Hydrocarbon Chemistry.* John Wiley & Sons, Inc., New York.

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) $C_{12}$-$C_{30}$ n-Alkylbenzenes in crude oils. *Petroleum Chemistry U.S.S.R.* **23**, 1-12.

Palmer, S. E. (1993) Effect of biodegradation and water washing on crude oil composition. In *Organic Geochemistry Principles and Applications,* (Eds M. H. Engel and S. A. Macko). Plenum Press, New York.

Perrin, D. D., Armarego, W. L. F. and Perrin, D. R. (1983) *Purification of Laboratory Chemicals,* 2nd edn. Pergamon Press, Oxford.

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.

Petrov, A. A. (1984) Petroleum Hydrocarbons. Springer-Verlag, Berlin.

Pond, J. L., Langworthy, T. A. and Holzer, G. (1986) Long-chain diols: a new class of membrane lipids from a thermophilic bacterium. *Science* **231**, 1134-1136.

Powell, T. G., Macqueen, R. W., Barker, J. F. and Bree, D. G. (1984) Geochemical character and origin of Ontario oils. *Bulletin of Canadian Petroleum Geology* **32**, 289-312.

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., 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). Academic Press, London.

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.

Reed, J. D., Illich, H. A. and Horsfield, B. (1986) Biochemical evolutionary significance of Ordovician oils and their sources. In *Advances in Organic Geochemistry 1985* (Eds. D. Leythaeuser and J. Rullkötter), Pergamon Press, Oxford. *Organic Geochemistry* **10**, 374-378.

Requejo, A. G., Allan, J., Creaney, S., Gray, N. R. and Cole, K. S. (1992) Aryl isoprenoids and diaromatic carotenoids in Paleozoic source rocks and oils from the Western Canada and Williston Basins. In *Advances in Organic Geochemistry 1991* (Eds. C. B. Eckardt, J. R. Maxwell, S. R. Larter and D. A. C. Manning), Pergamon Press, Oxford. *Organic Geochemistry* **19**, 245-264.

Revill, A. T. (1992) *Characterisation of unresolved complex mixtures of hydrocarbons by degradative methods.* Ph.D. Thesis, Polytechnic South West, Plymouth, U.K.

Richnow, H. H., Jenisch, A. and Michaelis W. (1992) Structural investigations of sulphur-rich macromolecular oil fractions and a kerogen by sequential chemical degradation. In *Advances in Organic Geochemistry 1991* (Eds. C. B. Eckardt, J. R. Maxwell, S. R. Larter and D. A. C. Manning), Pergamon Press, Oxford. *Organic Geochemistry* **19**, 351-370.

Richnow, H. H., Jenisch, A. and Michaelis, W. (1993) The chemical structure of macromolecular fractions of a sulfur-rich oil. *Geochimica et Cosmochimica Acta* **57**, 2767-2780.

Rieley, G. Collier, R. J., Jones, D. M., Eglinton, G., Eakin, P. A. and Fallick A. E. (1991) Sources of sedimentary lipids deduced from stable carbon-isotope analyses of individual compounds. *Nature (London)* **352,** 425-427.

Rigby, D., Gilbert, T. D. and Smith, J. W. (1986) The synthesis of alkyl aromatic hydrocarbons and its geochemical implications. *Organic Geochemistry* **9,** 225-264.

Rinaldi, G. G. L., Leopold, V. M and Koons, C. B. (1988) Presence of benzohopanes, monoaromatic secohopanes and saturate hexacyclic hydrocarbons in petroleums from carbonate environments. In *Geochemical Biomarkers* (Eds. T. F. Yen and J. M. Moldowan), pp. 331-353. Harwood Academic Publishers, Chur.

Robinson, N. and Eglinton, G. (1990) Lipid chemistry of Icelandic hot spring microbial mats. *Organic Geochemistry* **15,** 291-298.

Rossini, F. D., Mair, B. J. and Streiff, A. J. (1953) *Hydrocarbons From Petroleum* (API Research Project 6). ACS Monograph Series, Reinhold, New York.

Rovere, C. E., Crisp, P. T., Ellis, J. and Bolton, P. D. (1983) Chemical characterisation of shale oil from Condor, Australia. *Fuel* **62,** 1274-1282.

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., Revill, A. T., Thomas, K. V., Baas, M., Largeau, C., de Leeuw, J. W., Hodges, M., Lyne, P. and Smith, C. (1995) Unresolved complex mixtures of hydrocarbons in oils: Origins and characterization. In *Organic Geochemistry: Developments and Applications to Energy, Climate, Environment and Human*

*History* (Eds. J. O. Grimalt and C. Dorronsoro), A.I.G.O.A., Donostia-San Sebastián, Spain.

Rullkötter, J. (1993) The thermal alteration of kerogen and the formation of oil. In *Organic Geochemistry Principles and Applications,* (Eds M. H. Engel and S. A. Macko), Plenum Press, New York.

Rullkötter, J., Meyers, P. A., Schaefer, R. G. and Dunham, K. W. (1986) Oil generation in the Michigan Basin: a biological marker and carbon isotopic approach. *Organic Geochemistry* **10,** 359-375.

Sam, D. J., and Simmons, H. E. (1972) Crown polyether chemistry. Potassium permanganate oxidations in benzene. *Journal of the American Chemical Society* **94,** 4024-4025.

Schaefle, J., Ludwig, B., Albrecht, P. and Ourisson, G. (1978) Aromatic hydrocarbons from geological sources, VI. New aromatic steroid derivatives in sediments and crude oils. *Tetrahedron Letters* **43,** 4163-4166.

Schissler, D. O., Thompson, S. O. and Turkevich, J. (1951) Behaviour of paraffin hydrocarbons on electron impact. *Discussions of the Faraday Society* **10,** 46-53.

Shiea, J., Brassell, S. C. and Ward, D. M. (1990) Mid-chain branched mono- and dimethyl alkanes in hot spring cyanobacterial mats: A direct biogenic source for branched alkanes in ancient sediments? *Organic Geochemistry* **15,** 223-231.

Shiea J., Brassell, S. C. and Ward, D. M. (1991) Comparative analysis of extractable lipids in hot spring microbial mats and their component photosynthetic bacteria. *Organic Geochemistry* **17,** 309-319.

Silverstein, R. M., Bassler, G. C. and Morrill, T. C. (1981) *Spectrometric Identification of Organic Compounds,* 4th edn. John Wiley and Sons, New York.

Simoneit, B. R. T. (1986) Cyclic terpenoids of the geosphere. In *Biological Markers in the Sedimentary Record* (Ed. R. B. Johns), pp. 43-99. Elsevier, Amsterdam.

Simoneit, B. R. T. (1977) Diterpenoid compounds and other lipids in deep-sea sediments and their geochemical significance. *Geochimica et Cosmochimica Acta* **41,** 463-476.

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.

Sinninghe Damsté, J. S., Kock-van Dalen, A. C. and de Leeuw, J. W. (1988) Identification of long-chain isoprenoid alkylbenzenes in sediments and crude oils. *Geochimica et Cosmochimica Acta* **52,** 2671-2677.

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*-alkylbenzenesin Amposta crude oil from the Taragona Basin (Spanish Mediterranean): implications for the origin and fate of alkylbenzenes. *Geochimica et Cosmochimica Acta* **55,** 3677-3683.

Sinninghe Damsté, J. S., Keely, B. J., Betts, S. E., Baas, M., Maxwell, J. R. and de Leeuw, J. W. (1993) Variations in abundances and distributions of isoprenoid chromans and long-chain alkylbenzenes in sediments of the Mulhouse Basin: a molecular sedimentary record of paleosalinity. *Organic Geochemistry* **20,** 1201-1215.

Smirnov, M. B. (1990) Saturated petroleum hydrocarbons of T-shaped structure. *Petroleum Chemistry U.S.S.R.* **30** (1), 46-54.

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.

Solli H., Larter S. R. and Douglas A. G. (1980) Analysis of kerogens by pyrolysis-gas chromatography-mass spectrometry using selective ion monitoring. III. Long-chain alkylbenzenes. In *Advances in Organic Geochemistry 1979* (Eds. A. G. Douglas and J. R. Maxwell), Pergamon Press, Engelwood Cliffs, New Jersey.

Spitzer, U. A. and Lee, D. G. (1974) Oxidation of hydrocarbons. V. Oxidation of naphthalenes by ruthenium tetroxide. *Journal of Organic Chemistry* **39**, 2468-2469.

Standen, G., Boucher, R. J., Rafalska-Bloch, J. and Eglinton, G. (1991a) Ruthenium tetroxide oxidation of natural organic macromolecules: Messel kerogen. *Chemical Geology* **91**, 297-313.

Standen, G., Patience, R. L., Boucher, R. J. and Eglinton, G. (1991b) Ruthenium tetroxide oxidation: the assessment of putative petroleum source rocks. In *Organic Geochemistry – Advances and Applications in the Natural Environment.* (Ed. D. A. C. Manning), Manchester University Press, Manchester, England.

Stevenson, D. P. and Wagner, C. D. (1951) The mass spectra of $C_1$-$C_4$ monodeutero paraffins. *The Journal of Chemical Physics* **19**, 11-16.

Stock, L. M. and Tse, K. (1983) Ruthenium tetroxide catalysed oxidation of Illinois No. 6 coal and some representative hydrocarbons. *Fuel* **62**, 974-976.

Stock, L. M., Tse, K. and Wang, S.-H. (1985) The ruthenium (VIII) catalysed oxidation of Texas lignite and Illinois No. 6 bituminous coal. The aliphatic and

aromatic carboxylic acids. *American Chemical Society. Division of Fuel Chemistry* **30**, 493-502.

Stock, L. M. and Wang, S.-H. (1985) Ruthenium tetroxide catalysed oxidation of Illinois No. 6 coal. The formation of volatile hydrocarbons. *Fuel* **64**, 1713-1717.

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.

Strausz, O. P. and Lown, E. M. (1991) Structural features of Athabasca bitumen related to upgrading performance. *Fuel Science Technology International* **9**, 269-281.

Sugiyama, T., Matsuura, H., Sasada, H., Masaki, J. and Yamashita, K. (1986) Characterization of fatty acids in the sebum of goats according to sex and age. *Agricultural and Biological Chemistry* **50**, 3049-3052.

Sugiyama, T., Sasada, H., Masaki, J. and Yamashita, K. (1981) Unusual fatty acids with specific odor from mature male goat. *Agricultural and Biological Chemistry* **45**, 2655-2658.

Summons, R. E. (1987) Branched alkanes from ancient and modern sediments: Isomer discrimination by GC/MS with multiple reaction monitoring. *Organic Geochemistry* **11**, 281-289.

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.

Summons, R. E., Brassell, S. C., Eglinton, G., Evans, E., Horodyski, R. J., Robinson, N. and Ward, D. M. (1988a) Distinctive hydrocarbon biomarkers from fossiliferous

sediment of the Late Proterozoic Walcott Member, Chuar Group, Grand Canyon, Arizona. *Geochimica et Cosmochimica Acta* **52**, 2625-2637.

Summons, R. E., Powell, T. G. and Boreham, C. J. (1988b) Petroleum geology and geochemistry of the Middle Proterozoic McArthur Basin, Northern Australia: III. Composition of extractable hydrocarbons. *Geochimica et Cosmochimica Acta* **52**, 1747-1763.

Suzuki, K. I., Saito, K., Kawaguchi, A., Okuda, S. and Komogata, K. (1981) Occurrences of ω-cyclohexyl fatty acids in curtobacterium pussilum strains. *Journal of General Applied Microbiology* **27**, 261-266.

Thiel, V., Jenisch, A., Worheide, G., Lowenberg, A., Reitner, J. and Michaelis, W. (1999) Mid-chain branched alkanoic acids from "living fossil" demosponges: a link to ancient sedimentary lipids? *Organic Geochemistry* **30**, 1-14.

Thomas, K. V. (1995) *Characterisation and environmental fate of unresolved complex mixtures of hydrocarbons.* Ph.D. Thesis, University of Plymouth, Plymouth, U.K.

Threlfall, D. R. and Whistance, G. R. (1971) Biosynthesis of isoprenoid quinones and chromanols. In *Aspects of Terpenoid Chemistry and Biochemistry* (Ed. T. W. Goodwin), pp. 357-404. Academic Press, London.

Tietze, L.-F. and Eicher, T. (1989) *Reactions and Syntheses in the Organic Chemical Laboratory.* University Science Books, Mill Valley, California.

Tissot, B. P. and Welte, D. H. (1984) *Petroleum Formation and Occurrence.* Springer Verlag, Berlin.

Tornabene, T. G., Morrison, S. J. and Kloos, W. E. (1970) Aliphatic hydrocarbon contents of various members of the family *Micrococcaceae. Lipids* **5**, 929-937.

Trifilieff, S., Sieskind, O. and Albrecht, P. (1992) Biological markers in petroleum asphaltenes: Possible mode of incorporation. In *Biological Markers in Sediments and Petroleum* (Eds. J. M. Moldowan, P. Albrecht and R. P. Philp), Prentice Hall, Inc., Englewood Cliffs, New Jersey.

Trolio, R., Alexander, R. and Kagi, R. I. (1996) Alkyldiphenylmethanes in crude oils. *Polycyclic Aromatic Compounds* **9,** 137-142.

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

Vitorovic, D. (1980) Structure elucidation of kerogen by chemical methods. In *Kerogen. Insoluble Organic Matter from Sedimentary Rocks* (Ed. B. Durand), pp. 301-338. Éditions Technip, Paris.

Watson, D. G. (1993) Chemical derivatization in gas chromatography. In *Gas Chromatography – A Practical Approach* (Ed. P. J. Baugh), pp. 146-149. Oxford University Press, Oxford.

West, N., Alexander, R. and Kagi, R. I. (1990) The use of silicalite for rapid isolation of branched and cyclic alkane fractions of petroleum. *Organic Geochemistry* **15,** 499-501.

Wilkes, H., Disko U. and Horsfield B. (1998) Aromatic aldehydes and ketones in the Posidonia shale, Hils syncline, Germany. *Organic Geochemistry* **29,** 107-117.

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. In *Advances in Organic Geochemistry 1987* (Eds. L. Mattavelli and L. Novelli), Pergamon Press, Oxford. *Organic Geochemistry* **13**, 47-59.

Yawanarajah, S. R. and Kruge, 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. and Mair, B. J. (1966) Isolation and identification of $C_{13}$ to $C_{17}$ alkylnaphthalenes, alkylbiphenyls and alkyldibenzofurans from the 275° to 305°C dinuclear aromatic fraction of petroleum. *Analytical Chemistry* **38**, 231-237.

**APPENDIX**

## JOURNAL PAPERS

**Warton B.,** Alexander R. and Kagi R. I. (1997) Identification of some single branched alkanes in crude oils. *Organic Geochemistry* **27**, 465-476.

**Warton B.,** Alexander R. and Kagi R. I. (1998) The effect of maturation on the distribution of monoethylalkanes in Late Cretaceous sedimentary rocks and crude oils from the Gippsland Basin, Australia. In *Advances in Organic Geochemistry 1997* (Eds. B. Horsfield, M. Radke, R. G. Schaefer and H. Wilkes); *Organic Geochemistry* **29**, 593-604.

**Warton B.,** Alexander R. and Kagi R. I. (1999) Characterisation of the ruthenium tetroxide oxidation products from the aromatic unresolved complex mixture of a biodegraded crude oil. *Organic Geochemistry* (in press).

## CONFERENCE PRESENTATIONS

**Warton B.,** Alexander R. and Kagi R. I. Identification and occurrence of monoalkylalkanes (T-branched alkanes) in crude oils and sedimentary rocks. *Australian Organic Geochemistry Conference,* Perth, Australia, October 1996. *(Oral presentation - prize awarded for the best student oral presentation.)*

**Warton B.,** Alexander R. and Kagi R. I. The effect of maturation on the distribution of T-branched alkanes in a Late Cretaceous sedimentary sequence from the Gippsland Basin, Australia. *18ᵗʰ Meeting of the European Association of Organic Geochemists,* Maastricht, Netherlands, September, 1997. *(Poster presentation.)*

**Warton B.,** Alexander R. and Kagi R. I. Composition of the aromatic unresolved complex in a biodegraded crude oil. *Australian Organic Geochemistry Conference,* Canberra, Australia, September 1998. *(Oral presentation given by R. I. Kagi)*

**Warton B.,** Alexander R. and Kagi R. I. The effect of maturation on the distribution of monoethylalkanes in Late Cretaceous sedimentary rocks and crude oils from the Gippsland Basin, Australia. *Australian Organic Geochemistry Conference,* Canberra, Australia, September 1998. *(Poster presentation)*