School of Applied Chemistry

Molecular Sieving, Analysis and Geochemistry of Some Pentacyclic Triterpanes in Sedimentary Organic Matter

Carim Armanios

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

1995

Dedicated to

my wife, Marisa
and my mother, Muzayan

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

First and foremost, I wish to thank my principal supervisor Professor Robert Alexander for his untiring guidance, enthusiasm and encouragement thoroughout the past three and a half years. I wish to also acknowledge my co-supervisor Professor Robert Ian Kagi for his academic assistance and for raising funds to provide our research group with quality analytical instruments.

The following are also acknowledged: The Australian Government for providing me with financial assistance in the form of an Australian Postgraduate Research Award (APRA). Dr. Allan White and Dr. Lindsay Byrne, The University of Western Australia, for carrying out X-ray crystallography studies and for carrying out ^{13}C NMR analyses respectively. Mr. Steen Kristensen, CRA exploration Pty. Ltd., for providing the Heartbreak lignite samples used in the research. Mr. Geoff Chidlow and Mr. Steve Fisher, Curtin University of Technology, for their continuous and most generous technical assistance.

I wish to also thank my family members, in particular my mother, and friends who have encouraged me through the 'good' and 'not so good' times of research life. Finally, I wish to express my deepest thanks to my wife and best friend Marisa for her emotional support, encouragement and for just being there for me, while at the same time carrying out her own research work.

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

A liquid chromatographic technique using ultrastable-Y (US-Y) molecular sieve as the stationary phase and *n*-pentane as the mobile phase has been developed to fractionate and enrich pentacyclic triterpanes from petroleum. The sieve provides a shape-selective window which distinguishes between the various pentacyclic components, thus fractionating them on the basis of molecular shape differences. This sieving technique has been applied to isolate various pentacyclic triterpanes from sedimentary organic matter to enable better analysis of these biomarkers to be carried out.

Biodegraded crude oils from three Australian basins were analysed to assess the geochemistry of their rearranged hopanes. Enhanced abundances of 25-norhopanes, 18α-30-norneohopane and diahopanes relative to the regular hopanes were observed in the most severely biodegraded samples. Geochemical interpretation of these results suggests that the enhanced abundances are due to the greater resistance of rearranged hopanes to biodegradation compared to regular hopanes. These studies also indicate that enhanced relative abundances of 25-norhopanes in these samples is most likely due to selective bacterial demethylation of αβ-hopane precursors.

A branched and cyclic alkane fraction from a higher plant-derived crude oil was subjected to the US-Y chromatography procedure and the fractions eluted from the column were analysed using GC-MS. The compositions of the first two eluted fractions were markedly different from the initial branched and cyclic alkane mixture in that they were enriched in higher plant-derived triterpanes, such as bicadinanes, spirotriterpane and the oleananes and other, previously unreported, C_{29} and C_{30} triterpanes. A comparison of mass spectral data, GC retention, and molecular sieve sorption characteristics of these compounds with

those of known triterpanes of known molecular structure was used to suggest structures for the unknown compounds.

Isolation of crude oil fractions enriched in pentacyclic alkanes using the sieving procedure enabled lower concentrations of bicadinanes to be detected than was previously possible by applying selective ion detection GC-MS to branched and cyclic alkane fractions. Application of this technique to a higher-plant derived Jurassic crude oil and two Jurassic sediments from the Eromanga Basin, Australia has revealed the presence of bicadinanes. The occurrence of the *cis-cis-trans* and *trans-trans-trans* bicadinane biomarkers that have previously only been reported from angiosperms may indicate an early evolution of flowering plant-like species in this basin.

The molecular sieving technique has also been used to isolate three pentacyclic triterpanes from low rank coals in order to obtain unambiguous structural identification and to determine their geochemical significance. A major hopanoid component isolated from a Victorian brown coal was characterised by single crystal X-ray diffraction and ^{13}C NMR spectroscopy as *22R* 17α,21β(H)-homohopane. This compound was shown to correspond to the later eluting 17α,21β(H)-homohopane and hence, for the first time, confirmed the common practice of assigning the higher retention time peak in gas chromatograms of αβ-homohopanes as the *22R* diastereomer. Heating of the isolated *22R* αβ-homohopane on anthracite produced a mixture of the *22S* and *22R* diastereomers which implied a product-reactant relationship between the two epimers. Furthermore, a C_{29} and a C_{30} triterpane present in the hydrous pyrolysate of a Bremer Basin coal were also isolated using the molecular sieving procedure. 28-Nor-18α-oleanane was characterised by single crystal X-ray analysis while lupane was characterised by ^{13}C NMR spectroscopy and by co-chromatography with an authentic standard on four different GC phase columns. The unusual

occurrence of these triterpanes was attributed to the high sulphur content of the coal.

Finally, laboratory isomerisation and reduction of an isomeric mixture of oleanenes was carried out to investigate the origin of oleanane (18β-oleanane) and 18α-oleanane. Laboratory results indicated that oleanane was mainly derived from olean-18-ene, while 18α-oleanane was derived from 18α-olean-12-ene. Analysis of oleanene/oleanane abundances in a sedimentary sequence from Indonesia provided results consistent with laboratory evidence showing that 18α-olean-12-ene, rather than oleanane, is the main sedimentary precursor of 18α-oleanane.

CHAPTER 1

INTRODUCTION

1.1 ZEOLITE MOLECULAR SIEVES

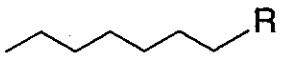
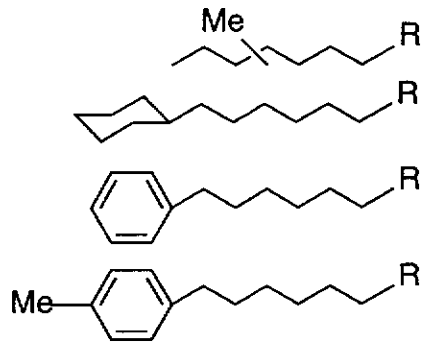
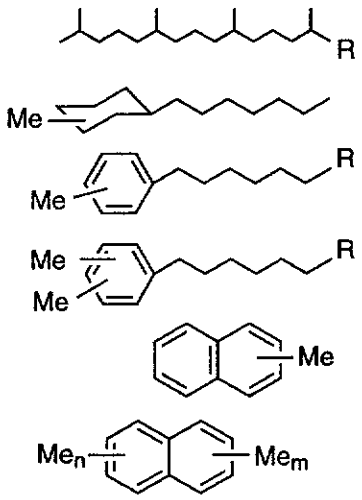
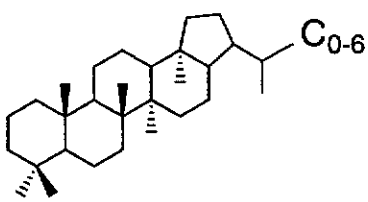
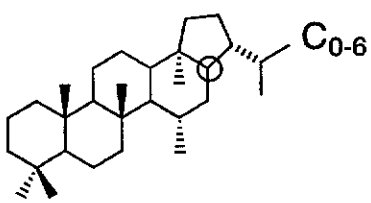
Zeolites are crystalline, hydrated aluminosilicates comprising an infinitely extending three-dimensional network of AlO_4 and SiO_4 tetrahedra linked to each other by the sharing of all oxygen atoms (*cf.* Breck, 1974; Flanigan *et al.*, 1978; Olson *et al.*, 1981). These structures contain non-framework elements, mainly group I or group II metal ions, which act as charge-compensating cations to balance the net negative charge of the aluminosilicate framework. Once these zeolites are dehydrated they reveal void spaces and interconnecting channels which are of molecular dimensions. In this form, zeolites can be used as molecular sieves to separate and trap molecules having dimensions which would enable them to enter the sieve channels (Ruthven, 1988).

1.2 ROLE OF MOLECULAR SIEVES IN THE SEPARATION OF PETROLEUM HYDROCARBONS

By far the major commercial application of molecular sieves has been in the area of catalysis (e.g., Heinemann, 1981; Pines, 1981; Maxwell, 1986; Chen and Degnan, 1988). The development of synthetic routes for manufacturing molecular sieves, together with their greater stability than conventional amorphous silica-alumina has led to their rapid adoption as hydrocarbon cracking catalysts. More pertinent to this thesis however, is the use of molecular sieves in the separation of petroleum hydrocarbons for geochemical analysis. Table 1.1 summarises the reported uses of a range of molecular sieves to separate petroleum components.

Molecular sieve sorption of simple alkanes has become a routine analytical procedure in organic geochemistry. 5A Molecular sieves have been used for the sorption and removal of straight chained alkanes from other branched and cyclic

Table 1.1 Application of molecular sieves for sorption of petroleum hydrocarbons.

Molecular Sieve	Pore Aperture (nm)	Hydrocarbons sorbed
5A	0.43 (0.49*)	 n-alkanes
ZSM-5 SILICALITE	0.51 x 0.56	 methylalkanes alkylcyclohexanes alkylbenzenes *p*-alkyltoluenes
MORDENITE	0.67 x 0.70	 isoprenoids methyl alkylcyclohexanes *o,m*-alkyltoluenes some alkylxylenes methylnaphthalenes some dimethylnaphthalenes some trimethylnaphthalenes some tetramethylnaphthalenes
10X 13X	0.80	 all hopanoids  17α(H)-diahopanes

* channel aperture when heated.

alkanes for more than two decades (e.g., Murphy, 1969; Jasra and Bhat, 1987; Kerr, 1989). More recently however, work has been directed to finding other molecular sieves which may be useful in separating more complex hydrocarbon components in petroleum. For example, a procedure using a silicalite molecular sieve, the all-silica end-member of the ZSM-5 series, was developed for the rapid and convenient removal of *n*-alkanes as well as methylalkanes, alkylcyclohexanes, alkylbenzenes and the *p*-substituted alkyltoluene components from a petroleum alkane fraction, leaving an enriched branched and cyclic fraction suitable for biomarker analysis by GC-MS (West *et al.*, 1990). Hoering and Freeman (1984) reported the use of 5A and silicalite molecular sieves for the complete isolation of monomethylalkanes from petroleum. Silicalite inclusion, combined with exclusion by 5A molecular sieves provided a narrow 10 nm-wide window for the isolation of monomethylalkanes, which have kinetic diameters (Hoering and Freeman, 1984) between 0.50 nm and 0.60 nm. Furthermore, these workers used column chromatography with silicalite to obtain a near complete separation of the 2-, 3- and 4-methylalkane isomers.

A ZSM-5 molecular sieve, which has channel dimensions similar to those of silicalite, has been used in a gas chromatographic mode by Weitkamp *et al.* (1991) to separate alkane diastereomers. These authors reported the fractionation of 3,4-dimethylhexane diastereomers using two capillary columns arranged in series which had been coated with the sodium form of ZSM-5 (NaZSM-5). Although, these authors did not suggest an explanation for the ability of this sieve to distinguish one diastereoisomeric form over another, they did point out that a similar result could not be achieved using either a smaller pore sieve such as ZSM-23 or a larger pore sieve such as 13X. This may indicate that ZSM-5 has channel dimensions which cause the preferential sorption of one alkane isomer resulting in it having a slightly longer retention time on the column and hence producing the observed separation of the two compounds.

A 0.70 nm mordenite molecular sieve was used to simplify the complex mixture of alkanes in a Green River Shale extract (Curran *et al.*, 1968). This sieve, having a pore size intermediate between that of the 5A and the 10X type sieves, included the branched alkanes such as pristane and phytane, but excluded the more bulky cyclic compounds, such as the steranes and the tri- tetra- and pentacyclic triterpanes. More recently, a dealuminated mordenite sieve was reported to selectively sorb *n*-alkylbenzenes, *n*-alkyltoluenes and some *n*-alkylxylenes and alkylnaphthalenes from mixtures containing bulkier aromatic compounds in mixtures of petroleum hydrocarbons (Ellis *et al.*, 1992). This technique enabled these authors to recognise compounds which were previously masked in the gas chromatogram by peaks from other components of complex mixtures.

A 0.8 nm 10X molecular sieve has been used to enrich polycyclic terpenoids from petroleum. Whitehead (1974) exhaustively extracted a branched and cyclic alkane fraction from a Nigerian distillate with activated 10X molecular sieve in order to enrich the pentacyclanes. The hopanoids were reported to be enriched by their selective sorption by the sieve, however not without significant fractionation of the extended αβ-hopane diastereomers. The *22S* diastereomers appeared to be more strongly sorbed by the sieve compared with the *22R* diastereomers. Further, Whitehead (1974) suggested that certain bulkier triterpanes of the lupane-oleanane-taraxastane types could be excluded from the sieve.

Dimmler and Strausz (1983) used a NaX (or 13X) molecular sieve, which has channel dimensions of approximately 0.8 nm, similar to those of 10X, to enrich polycyclic components from a branched and cyclic alkane fraction of an Athabascan tar sand. Hopanes and tricyclanes were selectively sorbed leaving a complex mixture in the excluded fraction that appeared as an unresolved 'hump' in the gas chromatogram. Complete desorption of the hydrocarbons included in NaX sieves was achieved by extraction with iso-octane for 36 hours.

1.3 BACTERIALLY-DERIVED PENTACYCLIC TRITERPANES

The hopanoids (Appendix, I-VI) are pentacyclic triterpanes found in crude oils and sediments. They occur as homologous series commonly containing 27 to 35 carbon atoms in a naphthenic structure composed of four six-membered rings and one five-membered ring (van Dorsselear *et al.*, 1977b). The C_{28} member is rarely observed since the formation of this compound from higher carbon number hopanes requires two bond cleavages at the same carbon atom, which is energetically unfavourable (Kimble *et al.*, 1974). The higher molecular weight homologues (C_{31}-C_{35}) are termed extended hopanes and contain a chiral centre at C-22 and so may exist as both the *22R* and *22S* diastereomers.

1.3.1 Regular Hopanes

The sedimentary hopanes, shown in Figure 1.1, have been reported to exist in three isomeric forms: 17β,21β(H), 17β,21α(H) and 17α,21β(H) (Appendix, I, II and III, respectively). The 17β,21β(H)-hopanes, which were first identified in Messel Oil Shale (Ensminger *et al.*, 1972), are reported to be derived from bacteriohopanetetrol and other polyfunctional C_{35} hopanoids common in prokaryotic microorganisms (Ourisson *et al.*, 1979; 1984; Rohmer, 1987). Mild maturation causes a decrease in the relative concentration of the ββ-hopanes which is postulated to be due to their conversion to the more thermodynamically stable moretanes (βα-hopanes) and αβ-hopanes (e.g. Ensminger *et al.*, 1974; 1977; Seifert and Moldowan, 1980). Upon further maturation, the extended *22R* 17α,21β(H)-hopanes are proposed to be gradually converted into a 3:2 mixture of *22S* and *22R* diastereomers which is typically observed in mature crude oils (e.g. Mackenzie *et al.*, 1980, Peters and Moldowan, 1991).

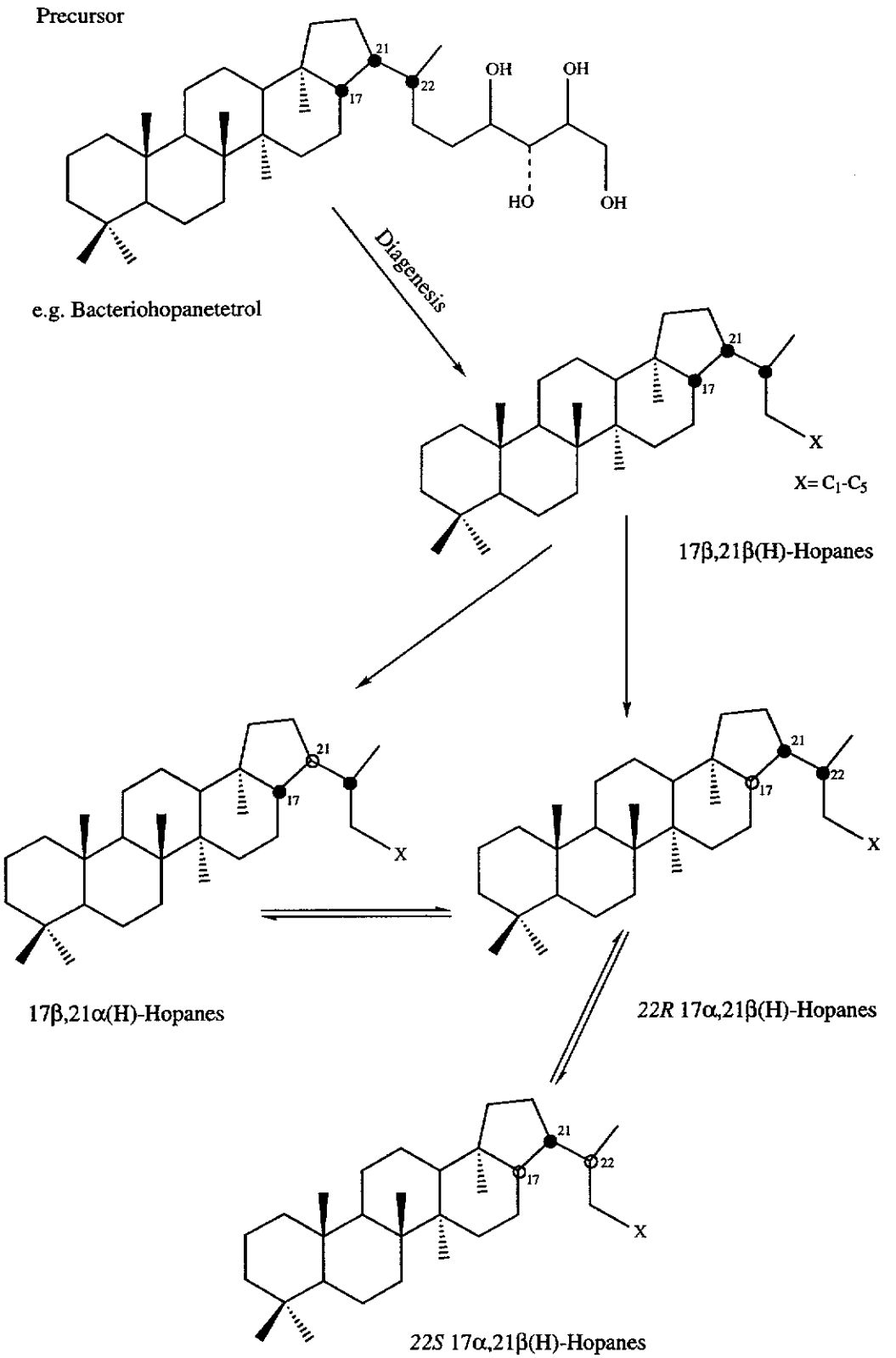


Figure 1.1 Proposed formation pathway of sedimentary hopanes from bacteriohopanetetrol found in the membranes of prokaryotic organisms (after Peters and Moldowan, 1991).

1.3.2 Rearranged Hopanes

Diahopanes

The diahopanes (Appendix, IV) occur as a homologous series (C_{27}, C_{29}-C_{34}) in a range of crude oils. The first unambiguous identification of this series was reported by Moldowan *et al.* (1991) who isolated the C_{30} member and characterised it using X-ray crystallography. This compound had been previously recognised in crude oils from several Australian basins and was proposed to be a possible terrestrial marker (Volkman *et al.*, 1983a; Philp and Gilbert, 1986).

Based on the rearranged structure of diahopane (Appendix, IVd), where the C-27 methyl is located at C-15, Moldowan *et al.* (1991) suggested that it may be formed by rearrangement of a hopanoid carrying a functionality on the D-ring. This is consistent with the reported formation of diahopane from dehydroxylation of 15α-hydroxyhopane (Corbett and Smith, 1969). It was further proposed by Moldowan *et al.* (1991) that diahopanes are derived from bacterial input to sediments containing clays and that deposition under oxic or sub-oxic conditions favours their formation.

18α-30-Norneohopane

18α-30-Norneohopane (C_{29}Ts; Appendix, Vc) was isolated from a Prudhoe Bay crude oil and characterised by advanced NMR methods (Moldowan *et al.*, 1991). Unlike diahopane which occurs as a member of a homologous series, C_{29}Ts is only the third member identified in the series along with 18α-22,29,30-trisnorneohopane (Ts; Appendix, Va) and a tentatively identified 18α-neohopane (Petrov *et al.*, 1985). Enhanced thermodynamic stability of C_{29}Ts compared with its hopane counterpart (Moldowan *et al.*, 1991) suggests that its formation may be via thermally induced isomerisation of the corresponding αβ-hopane analog. This is consistent with several reports which suggest that the abundance of C_{29}Ts

relative to that of 30-norhopane is related to thermal maturity (Hughes *et al.*, 1985; Sofer *et al.*, 1986; Sofer, 1988; Cornford *et al.*, 1988; Riediger *et al.*, 1990).

25-Norhopanes

The 25-norhopanes (Appendix, VI) are a homologous series of compounds (C_{26}-C_{34}) typical of many extremely biodegraded crude oils (Reed, 1977; Seifert and Moldowan, 1979; Rullkötter and Wendisch, 1982; Goodwin *et al.*, 1983; Seifert *et al.*, 1984; Trendel *et al.*, 1990; Cassani and Eglinton, 1991) and some sediments (Noble *et al.*, 1985; Chosson *et al.*, 1992). The origin of 25-norhopanes in crude oil has therefore been attributed to the bacterial removal of the methyl group at C-10 from the regular αβ-hopanes (e.g. Volkman *et al.*, 1983b; Peters and Moldowan, 1991). Furthermore, the occurrence of 25-norhopanes in apparently non-biodegraded crude oils has been explained by mixing of fresh crude oil with highly biodegraded residues from an older crude oil (e.g. Alexander *et al.*, 1983; Philp, 1983; Volkman *et al.*, 1983b, Talukdar *et al.*, 1986, 1988; Sofer *et al.*, 1986).Furthermore, Volkman *et al.* (1983a) and Peters and Moldowan (1991) completed mass balance calculations to show that 25-norhopanes must be formed *de novo* rather than just concentrated.

Alternatively, it has been proposed that minor amounts of 25-norhopanes are present in some crude oils which remain undetected until the more abundant αβ-hopanes are removed by extreme biodegradation (e.g. Blanc and Connan, 1992). In some cases 25-norhopanes have been reported to occur in source rock extracts (Chosson *et al.*, 1992). Noble *et al.* (1985) observed 25-norhopanes in some Australian shales but these compounds were absent in the hydrous pyrolysate. They concluded that these compounds only occurred in the free form and so would be diluted by the gradual increase of αβ-hopanes from the maturing kerogen.

1.4 HIGHER PLANT-DERIVED PENTACYCLIC TRITERPANES

Pentacyclic triterpanes based on the 17α,21β(H)-hopane skeleton are ubiquitous in sedimentary organic matter (e.g. van Dorsselaer *et al.*, 1974; Ensminger *et al.*, 1974). Higher plant-derived triterpanes having 'non-hopanoid' skeletons, on the other hand, show a more restricted occurrence in the geosphere. Higher plant-derived triterpanes discussed in this thesis have mostly been observed in terrestrially derived organic matter younger than Cretaceous, thus making them useful as age-specific biomarkers.

1.4.1 Bicadinanes

Grantham *et al.* (1983) first reported three bicadinane hydrocarbons which they named W, T and R. Cox *et al.* (1986) used X-ray crystallography and NMR spectroscopy to establish the structure of compound 'T' as *trans-trans-trans* bicadinane (Appendix, XI), and van Aarssen *et al.* (1990b) used NMR spectroscopy to characterise compound 'W' as *cis-cis-trans* bicadinane (Appendix, X).

Bicadinanes occur in Tertiary sedimentary organic matter from South East Asia (Grantham *et al.*, 1983; van Aarssen *et al.*, 1990a; Alam and Pearson, 1990; Sosrowidjojo *et al.*, 1994a). These and related compounds have been associated with dammar resins (Grantham *et al.*, 1983) of the extant angiosperms *Dipterocarpaceae* (van Aarssen *et al.*, 1990a). These plants produce copious resin containing a polycadinene which is reported to depolymerise upon thermal stress to form bicadinanes along with a range of cadinane moieties, namely cadinanes and polycadinanes (van Aarssen *et al.*, 1991). Because *Dipterocarpaceae* only occur in sediments of Oligocene age or younger (Muller, 1981; Bande and Prakash, 1986;

Lakhanpal and Guleria, 1986) bicadinanes have been used as age-specific biomarkers.

More recent evidence has shown that *Dipterocarpaceae* are not the only angiosperms from which sedimentary bicadinanes can be derived. A polysesquiterpene, which was reported to occur in the fruits of ancient representatives of *mastixioid Cornaceae* (Crelling *et al.*, 1991; Meuzelaar *et al.*, 1991), has been shown to also contain a similar polycadinene precursor (van Aarssen *et al.*, 1994). The occurrence of these fossil fruits in the Eocene Messel oil shale and the Eocene Dorset sediments (Chandler, 1962; Collinson, 1983), which were deposited in a region of cooler climates than those which existed in Southeast Asia, suggests that bicadinanes were not restricted to tropical conditions. Although dammar resins appear to be the major precursors of sedimentary bicadinanes so far reported, other angiosperms have produced bicadinanes, thus extending the age range of their precursors back to at least Eocene and possibly to Early Cretaceous times when angiosperms apparently first evolved (Stewart and Rothwell, 1993).

1.4.2 Oleanane and 18α-Oleanane

The well-characterised hydrocarbons oleanane and 18α-oleanane (Smith *et al.*, 1970) (Appendix, XIV and XVa respectively) are reported to be derived from geological transformation of pentacyclic triterpenoids typical of higher plants such as the amyrins and betulins (e.g. Whitehead, 1974; Grantham *et al.*, 1983; ten Haven and Rullkötter, 1988). In addition to these two compounds, 24,27-bisnor-18α-oleanane and 24,28-bisnor-18α-oleanane (Appendix, XVd and XVc respectively) have been isolated from an Egyptian crude oil and unequivocally identified using NMR spectrocopy (Trendel *et al.*, 1991a; 1991b, respectively).

Triterpenoids with oleanane skeletons are probably the most widespread of all the higher-plant derived biomarkers, having been reported to occur in a range of

Tertiary crude oils and sediments (e.g. Grantham *et al.*, 1983; Hoffman *et al.*, 1984; Riva *et al.*, 1988; Zumberge, 1987; Czochanska *et al.*, 1988; Ekweozor and Udo, 1988; Zeng *et al.*, 1988; Fu Jiamo and Sheng Guoying, 1989). Numerous functionalised C_{28}-C_{30} oleanenes have been reported to occur in immature sediments (e.g. ten Haven *et al.*, 1992a; 1992b). In more mature sediments and crude oils however, the 18α-oleanane and oleanane come into prominence as a result of diagenetic defunctionalisation and double bond reduction of their precursors, while in mature crude oils the more thermodynamically stable 18α-isomer assumes dominance (*cf.* Moldowan *et al.*, 1994).

1.4.3 Spirotriterpane

Hills and Whitehead (1966) were the first to report the mass spectrum of an unusual C_{30} triterpane in a Nigerian crude oil which was later characterised by Smith (1970) using X-ray crystallography as 1(10 -> 5) *abeo*-3β-methyl-24β-nor-5α-18α(H)-10β(H)-oleanane and named spirotriterpane (Appendix, XII). Since then, there have been several reports of its occurrence in Tertiary crude oils (e.g. Whitehead, 1974; Ekweozor and Udo, 1988; Woolhouse *et al.*, 1992).
Since spirotriterpenoids have not been reported as natural products, it has been suggested that the sedimentary spirotriterpane had been formed from naturally occurring triterpenoids similar in structure to 18α-oleanane by rearrangement reactions in sediments (Hills *et al.*, 1968). This was supported by the work of Halsall *et al.* (1966) who showed that A-ring spiro-formation can indeed take place under mildly acidic conditions. Hence, Hills *et al.* (1968) proposed a pathway for spiro A-ring formation, shown in Figure 1.2, based on a carbocation rearrangement mechanism. This would involve a 1:2 shift of the β-(axial) methyl at C-4 to the β-(equatorial) position at C-3, followed by a migration of the C-1,C-10 bond to C-5 to form a spiro junction.

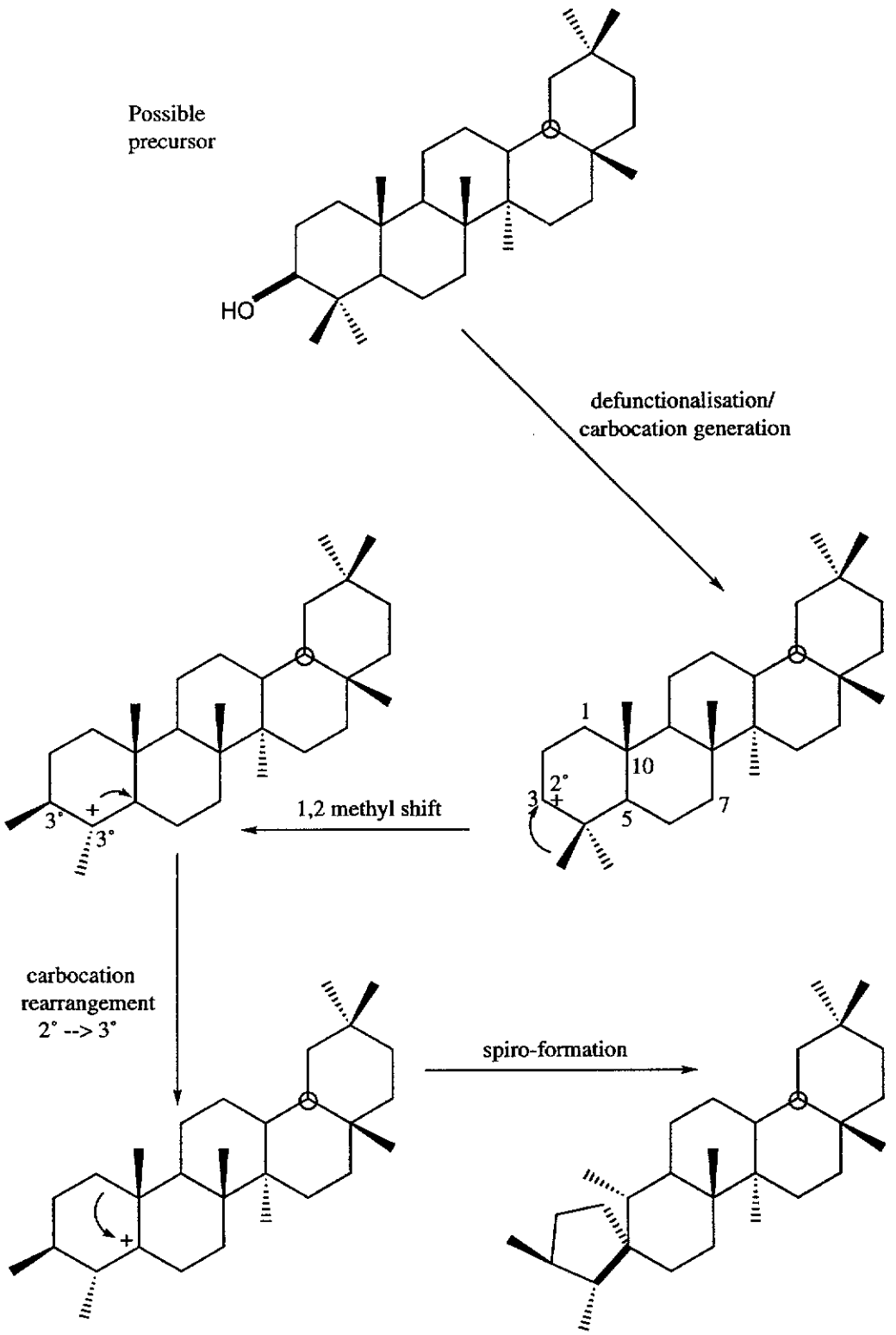


Figure 1.2 Proposed formation pathway for the spirotriterpane (Hills *et al.*, 1968).

1.4.4 Lupane

Functionalised triterpenoids of the lupane skeletal type occur widely in higher plants (*cf.* Dev *et al.*, 1989). Following diagenesis where defunctionalisation of these natural products takes place there have been several derivatives of lupane found in sedimentary organic matter. Rullkötter *et al.* (1982) first reported the widespread occurrence of two isomeric 24,28-bisnorlupanes (Appendix, XIIIc) in Tertiary sediments, and Trendel *et al.* (1991a) identified 24-norlupane (Appendix, XIIIb) in an Egyptian crude oil.

Interestingly, the presence of lupane (Appendix, XIIIa) itself has never been confirmed in sedimentary organic matter. It was tentatively identified in a Nigerian crude oil by Hills and Whitehead (1966), however co-chromatography with an authentic standard carried out later showed this assignment to be incorrect (Whitehead, 1974). Peters and Moldowan (1993) suggested that lupane is a common constituent of coal and gave reference to the work of Wang and Simoneit (1990) who had in fact only tentatively identified lup-20(29)-ene in a Tertiary brown coal.

The apparent lack of lupane in crude oils and sediments can be explained in two ways. First, the terminal $\Delta^{20,29}$ double bond of lupene is reported to undergo acid-catalysed isomerisation and ring expansion to form a six carbon membered E-ring. Lupeol has been shown to isomerise in acid to germanicol (olean-18-ene-3-ol) and taraxasterol (Ames *et al.*, 1952, 1954; Hasall *et al.*, 1952, 1954), while other lupane derivatives have been shown to readily rearrange in the presence of dilute acid to oxyallobetul-2-ene (Barton *et al.*, 1956). Defunctionalisation and double bond reduction of these isomerisation products would give oleananes (Appendix, XIV and XVa) and taraxastanes (*cf.* Rullkötter *et al.*, 1994) (Appendix, XVI) which occur widely in higher plant derived crude oils. An alternative explanation for the lack of reported occurrences of lupane in geological samples is due to

difficulty in its analysis using GC-MS techniques (ten Haven *et al.*, 1992b). Rullkötter *et al.* (1994) showed that lupane has almost identical GC retention behaviour to those of the more widely occurring oleanane and 18α-oleanane. Even under gas chromatographic conditions which separate oleanane and 18α-oleanane (Riva *et al.*, 1988), lupane remains unresolved from 18α-oleanane and so may go undetected in m/z 191 mass chromatograms of higher-plant derived crude oils and sediments.

1.5 ULTRASTABLE-Y MOLECULAR SIEVE

Ultrastable-Y molecular sieve is a decationised form of zeolite NaY which exhibits improved thermal and hydrothermal stability (McDaniel and Maher, 1968). The decationation process is detailed elsewhere (*cf.* Breck, 1974), in brief however, it consists of (i) exchange of the sodium ions in zeolite Y with ammonium ions, (ii) heating of the zeolite at temperatures of 500-600°C to effectively remove the ammonium ions as ammonia and (iii) further heating at temperatures of around 800°C to remove resulting hydroxyl groups as water. The resulting US-Y molecular sieve is reported to be thermally stable to 1000°C compared to the usual decomposition temperature of NaY zeolite of 600°C (Scherzer, 1978). This important feature makes US-Y molecular sieve very useful as a high temperature hydrocarbon cracking catalyst (e.g. Barrie *et al.*, 1991; Corma *et al.*, 1992).

US-Y molecular sieve contains channels which are marginally smaller than those of the NaY zeolite. The removal of the ammonium ion by thermal decomposition and dehydroxylation results in a significant decrease in the unit cell dimensions of the ultrastable product. For example, a unit cell dimension of 2.34 nm is reported for US-Y molecular sieve compared to a typical value of 2.47 nm for an ammonium

exchanged zeolite Y (Breck, 1974). As a consequence, ultrastable-Y has a channel aperture of approximately 0.74 nm, which is smaller than that for zeolite X or Y of approximately 0.80 nm (Breck, 1974). Zeolite X (13X) (*cf.* Table 1.1) has been previously reported to sorb a range of pentacyclic triterpanes (Dimmler and Strausz, 1983). The channels in US-Y are smaller than those in zeolite X and span the dimensions of various pentacyclic triterpane components, making it an ideal sieve for shape-selective fractionation of this class of hydrocarbons.

1.6 SCOPE AND SIGNIFICANCE OF PRESENT WORK

The scope of the present work is to analyse the pentacyclic triterpane components in various bacterially derived and higher-plant derived crude oils and sediments to obtain structural information and to assess their geochemical significance. Previously, these biomarker compounds were typically analysed using GC-MS. However, even with very time consuming temperature program conditions (*cf.* Subroto *et al.*, 1991), it was difficult to achieve adequate resolution of all pentacyclic triterpanes in the complex petroleum alkane mixtures. In this work, a molecular sieving technique using the shape-selective features of ultrastable-Y molecular sieve has been developed and applied to enrich and isolate the minor pentacyclic triterpanes from samples to a point where they may be better analysed.

Branched and cyclic alkane fractions from higher-plant derived geological samples were chromatographed on a US-Y molecular sieve column to fractionate and enrich the complex mixtures of non-hopanoid pentacyclic components. Analysis of the eluted fractions revealed the presence of previously unreported C_{29} and C_{30} triterpanes which have been tentatively identified based on comparison of their mass spectra, GC retention behaviours and molecular sieve sorption

characteristics with triterpanes of known structures. Furthermore, application of the sieving technique to some Jurassic samples revealed the first reported occurrence of angiosperm-derived pentacyclic biomarkers in pre-Cretaceous sedimentary organic matter.

The molecular sieving technique has also been used to isolate three pentacyclic triterpanes from low rank coals which were unambiguously identified for the first time. The characterised *22R* 17α,21β(H)-homohopane was shown to undergo laboratory epimerisation at C-22 position, which supports its proposed fate in mature sediments and crude oils. The other two triterpanes isolated from a high sulphur coal are reported for the first time in sedimentary organic matter. The geochemical implications of their unusual occurrence in this sample have been discussed.

The geochemistry of diahopanes in some biodegraded crude oils is investigated. Furthermore, data presented in this work sheds new light on the disputed origin of 25-norhopanes in biodegraded crude oils. In other work, the diagenetic origin of 18α-oleanane has been established by comparing the results of laboratory hydrogenation experiments with oleanane/oleanene distributions in a sedimentary sequence.

CHAPTER 2

EXPERIMENTAL

2.1 MATERIALS AND REAGENTS

Solvents

All analytical grade solvents were purified by fractional distillation. Laboratory grade methanol and cyclohexane were purified by refluxing with concentrated sulphuric acid prior to fractional distillation.

Silicic acid

Silicic acid (Sigma A.R. grade, 100-200 mesh) was used for column chromatography. It was activated at 250°C for at least 24 h before use.

Silica gel

Silica gel (Merck, 70-230 mesh) was used for column chromatography. It was activated at 250°C for at least 24 h before use.

Alumina

Neutral alumina (Fluka, 100-125 mesh) was used for preparative column chromatography.

Copper (precipitated)

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

Bis (trimethylsilyl) trifluoroacetamide

BSTFA (Supelco, Lot No. RA2932) was used as a silating agent to deactivate glass ampoules used for heating experiments.

Anthracite

A high rank coal (R_o = 2.0 - 3.0%) from Pennsylvania, U.S.A. was supplied by K. Sappal, Curtin University and was used in isomerisation reactions of triterpanes.

Platinum (IV) oxide hydrate

Platinum (IV) oxide hydrate (Aldrich) was used as a catalyst in the hydrogenation of germanicene (olean-18-ene) and ursa-9(11),12-diene.

Germanicene (olean-18-ene)

A sample of germanicene was provided by Associate Professor E. L. Ghisalberti (University of Western Australia).

Ursa-9(11),12-diene

A sample of ursa-9(11),12-diene was provided by Professor R. Alexander (Curtin University of Technology).

Aluminium smectite

The smectite fraction from a Wyoming bentonite (Standard Chemical Company, Melbourne, Australia) was isolated by dispersing the clay in water and collecting the fraction with a particle size of less than 2 μm. The surface area of the bentonite was found to be 33.0 $m^2 g^{-1}$ by the B.E.T. method and its cation exchange capacity, determined titrimetrically, was found to be 67.7 meq / 100 g (Fripiat and van Olphen, 1979). Aluminium smectite was prepared by treating the isolated smectite with aluminium chloride solution (1 N). The clay was washed free of excess ions by repeatedly centrifuging with deionised water, then dried, crushed and the particles less than 340 μm were collected using a sieve. It was finally activated at 120°C for 48 h before use.

2.2 MOLECULAR SIEVES

All molecular sieves were activated before use by heating under vacuum (1 mm Hg) at 160°C (except 5A sieve at 90°C) for 24 h in a Gallenkamp drying pistol.

5A molecular sieve

5A zeolite (BDH, 1/8" pellets) Lot No. 5664810B5009 was used to sorb *n*-alkanes from crude oil aliphatic fractions. A total alkane fraction (30 mg) was dissolved in a mixture of cyclohexane (40 mL) and sieve (5 g). The mixture was refluxed for 3 h, filtered and the sieve powder quickly washed with cold cyclohexane (2 x 50 mL).

Silicalite and ZSM-5 molecular sieves

Silicalite (Union Carbide, pellets) Trisiv batch No. 910187020003 or ZSM-5 (CBV 2802, The PQ Corporation) Lot No.9107766K were used to sorb *n*-alkanes, methylalkane and some alkycyclohexanes from crude oil alkane fractions.

Ultrastable-Y (US-Y) molecular sieve

US-Y zeolite (CBV 400, The PQ Corporation) Lot No. 4000N0002912 was used to sorb and fractionate pentacyclic triterpanes from crude oil and sediment branched and cyclic alkane fractions.

13X molecular sieve

13X zeolite (UOP, beads) Lot No. 945090060157-5 was used in a preliminary step to sorb and enrich tricyclic and pentacyclic terpanes from branched and cyclic alkane fractions. The branched and cyclic alkane fraction (30 mg) was dissolved in a mixture of *n*-pentane (50 mL) and sieve (5 g) and heated to reflux for 3 h. The triterpanes were recovered by dissolving the sieves in hydrofluoric acid.

2.3 GEOCHEMICAL AND ANALYTICAL TECHNIQUES

Sample preparation

Lignite samples were obtained as cores sealed in PVC tubes which were opened and allowed to dry in open air for 10 days and then ground to a fine powder using a mortar and pestle. Core sections that could not be ground in this manner were crushed to smaller chips in a van Gelder jaw crusher, then crushed to a powder using a Tema grinder.

Extraction of soluble organic matter (SOM) from sediments

Ground sediment (approximately 10 g) was extracted with dichloromethane (200 mL) using a continuous soxhlet extraction apparatus for 8 h. The solvent was removed from the filtrate by fractional distillation to obtain the soluble organic matter (SOM).

Fractionation of crude oil and SOM by column chromatography

Crude oils and rock extracts (50-90 mg) were separated into aliphatic, aromatic and polar fractions by liquid chromatography using either silica gel or silicic acid. Activated stationary phase was introduced into a glass column (40 cm x 1.0 cm i.d.) as a slurry in *n*-pentane (20 mL). The saturated hydrocarbons were eluted with *n*-pentane (20 mL), aromatic hydrocarbons with 10% dichloromethane in *n*-pentane (40 mL), and the polar compounds with a 1:1 mixture of dichloromethane/methanol (30 mL). The aliphatic fraction was routinely passed through a small column of activated copper (100 mg) to remove elemental sulphur.

Isolation of branched and cyclic alkane fractions (B/C)

Activated ZSM-5 or silicalite molecular sieve was dry packed into a Pasteur pipette plugged at the base with cotton wool. The aliphatic fraction (30 mg) was

introduced onto the top of the column in the minimum of *n*-pentane (200 µl). The column was then eluted with three bed volumes of *n*-pentane to yield a branched and cyclic alkane fraction.

Ultrastable-Y molecular sieve chromatography

Activated US-Y sieves (1 g) was tightly dry packed into a Pasteur pipette plugged at the base with cotton wool and then washed with two bed volumes of *n*-pentane. The branched and cyclic alkane fraction (30 mg) was introduced to the column in *n*-pentane (50 µL) and allowed to stand for two min, after which time the column was eluted with *n*-pentane at the rate of 1 drop every 10 s using compressed nitrogen. Fractions (500 µL) were collected and analysed by GC-MS techniques.

On a preparative scale, activated US-Y sieve (30 g) was dry packed into a glass column 40 cm x 1.0 cm i.d. The branched and cyclic alkane fraction was introduced to the column and chromatographed using *n*-pentane as described above.

Hydrous pyrolysis of sediment samples

Hydrous pyrolysis experiments were carried out in closed stainless steel vessels (50 mL) that were leak tested with water blank trials. Solvent-extracted sediment sample (1.5 g) was placed in the pyrolysis chamber with deionised water (6 mL). The reaction vessel was purged with nitrogen for 3-5 min, then sealed and placed in an oven at 330°C for 72 h (MoS_2 was used to coat the vessel thread to prevent seizing at high temperatures). After heating, the vessel was cooled in liquid nitrogen and then opened. The pyrolysed sediment was filtered, dried overnight and re-extracted with dichloromethane. The solvent was distilled off and the pyrolysate was chromatographed on silica gel to obtain aliphatic, aromatic and polar fractions. The alkane fraction was treated with either 5A or ZSM-5 molecular

sieves to obtain a branched and cyclic alkane fraction which was analysed by GC-MS techniques.

Preparative scale hydrous pyrolysis used in the isolation of 28-nor-18α-oleanane and lupane was based on a procedure reported by Lewan (1985). A one-litre stainless steel vessel was used to carry out hydrous pyrolysis on solvent extracted lignite (1.5 kg). Ground coal (200 g) was placed in the reactor vessel along with an excess of deionised water (150 mL). The remaining volume was purged with nitrogen for 10 min and the vessel was sealed and placed in an oven at 330°C for 72 h. The reactor was equipped with a pressure release safety valve which was set at approximately 27 x 10^3 kPa. After heating, the vessel was allowed to cool overnight and then opened. The hydrocarbon-rich water in the reactor was decanted into a separatory funnel where it was extracted with dichloromethane (3 x 30 mL). The dried pyrolysed coal was also extracted with dichloromethane (200 mL). The dichloromethane extracts were combined and the solvent was removed by distillation. The pyrolysates from seven such experiments were combined and a branched and cyclic alkane fraction was obtained by chromatography on alumina, silica gel and finally ZSM-5 molecular sieve.

Deactivation of glass reaction ampoules

Deactivation of glass ampoules was based on a modification of a previously published procedure by Alexander *et al.* (1988a). The glass capillaries were leached with hot concentrated hydrochloric acid until the wash solution remained colourless. They were then thoroughly washed with deionised water and sealed at one end. The ampoules were again leached in concentrated hydrochloric acid overnight, washed with deionised water and dried in an oven at 110°C for 3-5 h. Upon cooling, the ampoules were each treated with a silylation reagent BSTFA (2 drops) and placed in a glass vessel which was subsequently evacuated, sealed and heated to 140°C (for 10 min) at 10°C min^{-1}, then to 160°C (for 10 min) at 0.5°C

min^{-1}, and finally to 300°C (for 30 min) at 10°C min^{-1}. After cooling and opening the vessel the ampoules were washed well with dichloromethane and dried.

Heating reactions using aluminium smectite/anthracite

A solution of *22R* αβ-homohopane (0.15 mg) or branched and cyclic alkane fraction containing 28-nor-18α-oleanane (10 mg) in dichloromethane (20 μL) was transferred into a deactivated glass ampoule containing ground anthracite or aluminium smectite (10 mg), respectively. The reaction mixture was thoroughly stirred with a fine glass rod and the dichloromethane evaporated. This was repeated a second time after the side of the ampoule had been washed with *n*-pentane (10-20 μL). The glass ampoule containing the residue was then evacuated, sealed and heated at various temperatures for different periods of time, cooled with liquid nitrogen and opened. The anthracite/smectite was extracted with dichloromethane and then chloro-octadecane added as a normalisation standard.

Hydrogenation of germanicene and ursa-9(11),12-diene

Germanicene (olean-18-ene) was hydrogenated based on a procedure by Zürcher *et al.*, (1954). Germanicene (0.3 mg) was hydrogenated for 3 h over platinum (IV) oxide hydrate (0.1 mg) in glacial acetic acid (200 μl) at ambient temperature and slightly elevated pressure (approximately 200 kPa). GC-MS analysis of reaction mixture showed it to contain olean-13(18)-ene, olean-18-ene, 18α-olean-12-ene and oleanane in a ratio of 4:12:1:3, respectively.

Ursa-9(11),12-diene (1.0 mg) was hydrogenated over platinum (IV) oxide hydrate (0.3 mg) in glacial acetic acid (300 μL) for 12 h under the same conditions described above. The resulting reaction mixture contained ursa-9(11),12-diene, urs-12-ene, a tentatively identified urs-9(11)-ene and ursane in a ratio of 8:5:1:1, respectively.

2.4 INSTRUMENTATION

Medium performance liquid chromatography (MPLC)

The removal of residual monoaromatics from the branched and cyclic alkane fractions was carried out using a Waters Associates programmable solvent delivery module 590, equipped with a 22 cm x 1.5 cm i.d. silica glass column. A branched and cyclic alkane sample (20 mg) in hexane (60 μL) was injected into the injection port and eluted through the column with hexane at a flow rate of 2 mL min^{-1}. A Waters differential refractometer R40 was used to detect the eluting alkane fraction. The system was then backflushed to recover the retained aromatic residue which was detected by a Waters UV absorbance detector 440 operating at 254 nm.

Capillary gas chromatography (GC-FID)

Alkane fractions were analysed using a Hewlett-Packard 5880A gas chromatograph equipped with a 50 m x 0.22 mm i.d. fused silica capillary column coated with 5% diphenyldimethyl siloxane (BP-5, SGE) and an OCI-3 on-column injector (SGE). Hydrogen was used as the carrier gas at a linear flow velocity of 30 cm s^{-1}. The oven was programmed from 70°C to 300°C (20 min) at 4°C min^{-1}. The FID (flame ionization detector) was maintained at 300°C.

Preparative-gas chromatography

Pure (>99.5%) αβ-homohopane was obtained from a mixture of homohopane and isopimarane by preparative gas chromatography. This was carried out using a Hewlett Packard 5890 gas chromatograph fitted with a 30 m x 0.53 mm i.d. HP series 530 μm fused silica column, an OCI-3 on-column injector (SGE) and a thermal conductivity detector (HP). Hydrogen at 5 mL/min was used as the carrier gas. The oven was programmed from 70° to 240°C (25 min) at 10°C min^{-1} to

collect the isopimarane, and then programmed to 280°C (20 min) at 5°C min^{-1} to collect the homohopane.

Gas chromatography-mass spectromentry (GC-MS)

All branched and cyclic alkane fractions were analysed for their biomarker content using GC-MS techniques. Three different GC-MS systems were used over the course of this study.

I Branched and cyclic alkane fractions were routinely analysed using a Hewlett Packard 5890 series II gas chromatograph interfaced to a HP 5971 mass selective detector operating at 70 eV. The gas chromatograph was fitted with a 60 m x 0.25 mm i.d. fused silica capillary column coated with dimethyl siloxane phase (DB-1, J&W). Helium was used as the carrier gas at a linear flow velocity of 22 cm s^{-1}. The oven was programmed from 70°C to 300°C (20 min) at 4°C min^{-1}. The samples were injected on-column using a Hewlett Packard 7673 automatic sampler and analysed using the full data acquisition (SCAN) and/or selected ion monitoring (SIM) mode. Typical mass spectrometer operating conditions were: Electron multiplier voltage 2000 V; emission current 220 μA; electron energy 70 eV; source temperature 250°C.

II Branched and cyclic alkane fractions were regularly reanalysed on a different column phase to obtain better resolution of certain alkane components. These analyses were carried out using a Hewlett Packard 5970 MSD equipped with the RTE/A data system. The gas chromatograph was fitted with a 40 m x 0.2 mm i.d. fused silica capillary column coated with diphenyldimethyl siloxane phase (DB-5, J&W). Helium was used as the carrier gas at a linear flow velocity of 30 cm s^{-1}. The oven temperature was programmed from 50 to 274°C at 8°C min^{-1}, then to

300°C (20 min) at 1°C min^{-1}. The samples were injected on-column using a HP7673A automatic sampler (HP) and analysed using the SCAN and SIM modes.

III Branched and cyclic alkane fractions of higher plant origin, which contained triterpanes which were unresolved on DB-1 and DB-5 columns, were subsequently analysed using more polar columns. These analyses were carried out using a Hewlett Packard 5985 GC/MS system equipped with the RTE/A data system. The gas chromatograph was fitted with a 60 m x 0.25 mm i.d. fused silica capillary column coated with cyanopropylphenyl siloxane phase (DB-1701, J&W). Hydrogen was used as the carrier gas at a linear flow velocity of 40 cm s^{-1}. The oven temperature was programmed from 50 to 270°C (30 min) at 5°C min^{-1}. Alternatively the gas chromatograph was fitted with a 50 m x 0.22 mm i.d. fused silica capillary column coated with polyethylene glycol phase (BP-20, SGE). Hydrogen was used as the carrier gas at a linear flow velocity of 40 cm s^{-1}. The oven temperature was programmed from 50-250°C (50 min) at 5°C min^{-1}.

X-ray crystallography

An Enraf-Nonius machine was used to establish the molecular structures and stereochemisty of *22R* 17α,21β(H)-homohopane and 28-nor-18α-oleanane by XRD examination of single crystals.

Nuclear Magnetic Resonance Spectroscopy (NMR)

A Bruker ARX-500 nuclear magnetic resonance spectrometer operating at 125.77 MHz was used to obtain ^{13}C spectra for the *22R* 17α,21β(H)-homohopane, lupane and 28-nor-18α-oleanane which were isolated from Australian lignites. The spectra were recorded in deuterochloroform solution and TMS was used as an internal standard. Chemical shifts were reported in ppm.

Computerised molecular modeling

A CSC Chem3D Plus molecular modeling computer package version 3.1 was used to estimate steric energies and effective molecular diameters for the various petroleum pentacyclic components discussed in this thesis.

Melting point apparatus

Melting points for the three isolated triterpanes were carried out in triplicate using an Electrothermal IA9000 series digital melting point apparatus. The oven temperature was driven to a predetermined temperature at a maximum rate, then allowed to increase linearly at 1°C/min until melt analysis takes place.

2.5 ISOLATION OF TRITERPANES FROM LIGNITES

22R 17α,21β(H)-Homohopane

The homohopane was isolated from Loy Yang coal (1.5 kg) which was ground using a mortar and pestle and extracted with dichloromethane (2 L) using a Soxhlet apparatus for 48 h to ensure complete extraction. The dichloromethane was distilled, leaving an extract concentrate (24 g) which was then partially dissolved in *n*-pentane. At this stage the insoluble asphaltenes (8 g) were removed by filtration. The *n*-pentane soluble fraction (16 g) was then chromatographed on an alumina column 40 cm x 2 cm which retained most of the highly polar components. The eluted mixture which contained the alkane and aromatic components (4 g) was then chromatographed on a silicic acid column 30 cm x 1 cm using *n*-pentane to obtain an alkane fraction. The alkane fraction (1.0 g) was treated with ZSM-5 molecular sieve to remove the *n*-alkanes and activated copper (to remove elemental sulphur) leaving a fraction containing only branched and cyclic alkanes. The branched and

cyclic alkane fraction (0.6 g) was chromatographed on a US-Y molecular sieve column 40 cm x 1 cm i.d. using

n-pentane and three eluted fractions were collected (15 mL, 15 mL, 10 mL respectively). Gas chromatography of the third eluted fraction (15 mg) showed it to be a mixture of homohopane and isopimarane (85:15 mixture, 96% by GC). Preparative gas chromatography (refer to page 24) was repeatedly performed on the mixture to obtain approximately 8 mg of the homohopane solid (>99.5% by GC, melting point of 134-135°C).

The homohopane was crystallised from hexane by very slow evaporation of the solvent. The homohopane (8 mg) was dissolved in hexane (400 μL) which had previously been filtered through a 0.45 μ millipore filter and placed in a glass vial which was free of particulate matter. The vial was placed in a small desiccator next to a similar vial containing a hexane solution super-saturated in 1-chloro-octadecane (20 mg in 100 μL). The desiccator was evacuated, tightly sealed and stored away from any vibrations for 10 days. Apart from an initial drop in the hexane level in the homohopane solution due to equilibration of the atmosphere in the desiccator, further reduction in the hexane level was attributed to the very slow transfer of hexane vapour into the concentrated chloro-octadecane solution. This process facilitated the very slow but continuous evaporation of hexane from the vial containing the homohopane. After 10 days when nearly all the hexane had evaporated, the homohopane began to crystallise into long needle-like crystals which were suitable for a single crystal X-ray crystallography study.

28-nor-18α-Oleanane and lupane

28-Nor-18α-oleanane and lupane were isolated from a low rank coal from the Bremer Basin, southwest of Australia (Heartbreak HCH 32 24.3-24.8 m). The coal (1 kg) was ground using a Tema grinder and extracted with dichloromethane (2 L) using a Soxhlet apparatus for 48 h. The extracted coal was placed in a

stainless steel vessel (1 L) which was then sealed and pyrolysed at 330°C for 72 h. After cooling to ambient temperature the vessel was opened and the water and coal were separately extracted with dichloromethane (2 x 200 mL). The extracts were combined and the dichloromethane removed by distillation to leave a black concentrate (125 g). This extract was dissolved in *n*-pentane (300 mL) and filtered to remove the insoluble asphaltenes (60 g).

The *n*-pentane soluble fraction was concentrated (150 mL) and mixed with activated alumina (80 g) and left to stand for 3 h. The mixture was then filtered and washed well with cold *n*-pentane (200 mL). The reduced filtrate (30 mL) containing a mixture of alkane and aromatic components (30 g) was chromatographed on a silica gel column 50 cm x 2 cm i.d. using *n*-pentane to obtain an alkane fraction which was then passed through a short column of activated copper to remove elemental sulphur. The alkane fraction (10 g) was treated with ZSM-5 molecular sieve to obtain a branched and cyclic alkane fraction (4.5 g). This mixture was distilled at reduced pressure (0.2 mm of Hg) to obtain a high molecular weight cut which was enriched in the two triterpanes of interest.

The viscous alkane fraction (0.7 g) was chromatographed on a US-Y molecular sieve column 40 cm x 1 cm i.d. using *n*-pentane and three eluted fractions were collected (5 mL, 25mL, 15 mL respectively). The first eluted fraction (9 mg) showed that it was enriched in the two triterpanes (85% by GC, 90:10 mixture). 5.8 mg of 28-nor-18α-oleanane was fractionally crystallised in cold ethylmethylketone (200 μL) and the fine crystals filtered (98% pure by GC, melting point of 185-186°C). 28-Nor-18α-oleanane was then recrystallised from ethylacetate (150 μL) to obtain long needle-like crystals suitable for X-ray analysis. The remaining solution was reduced (50 μL) and stored for a few days before 0.4 mg of lupane was crystallised and filtered (95% pure by GC, melting point of 194-195°C). The lupane was positively identified using ^{13}C NMR analysis (Ammann

et al., 1982) and confirmed by co-chromatography with an authentic lupane standard on four different GC columns (DB-1, DB-5, DB-1701 and BP-20).

CHAPTER 3

SHAPE-SELECTIVE FRACTIONATION OF PENTACYCLIC TRITERPANES USING AN ULTRASTABLE-Y SIEVE

Summary

A liquid chromatographic procedure using ultrastable-Y (US-Y) molecular sieve as the stationary phase and *n*-pentane as the mobile phase has been developed to fractionate petroleum hopanoid classes based on molecular size and shape. US-Y molecular sieve has a reported channel aperture of approximately 0.74 nm (Breck, 1974) which is intermediate between the molecular cross-sections of the various hopanoids. Compounds which are too large to enter the sieve channels and are eluted with the solvent front, while smaller compounds able to enter the sieve channels are retained to varying degrees. Pentacyclic triterpanes can therefore be fractionated on the sieve column based on their relative molecular dimensions.

3.1 ANALYTICAL PROCEDURE

3.1.1 Batchwise Versus Counter-Current Sorption Systems

Sorption of a branched and cyclic alkane fraction onto ultra stable-Y molecular sieves was carried out in order to compare the separation efficiencies of batchwise and counter current (liquid chromatography) sorption systems (refer to section 2.2 and 2.3, respectively). Results demonstrate that the batchwise sorption technique does not appear to adequately fractionate the hopanoids and that the chromatographic method offers a vastly improved means for enriching diahopanes. Figure 3.1 shows partial m/z 191 mass chromatograms for an alkane fraction before (a) and after batchwise sorption (b) onto US-Y sieves and the first three eluted sieve fractions from sieve chromatography (c-e). Comparison of the first two chromatograms shows that there is a marked increase in the abundance of the diahopanes relative to the 17α-hopanes in the excluded alkane fraction from batchwise sorption suggesting that diahopanes are less strongly sorbed by the sieves. However, using the same sieve in a chromatographic mode (Figure 3.1 c-e), diahopanes are not only enriched in the first eluted fraction, but have been separated from the 17α-hopanes which are the major components in the chromatograms for Fraction 2 and 3.

The inadequate enrichment of the diahopanes using batchwise sorption may be due to a number of factors. This method offers only one equilibrium stage of separation and it appears that the complete separation of diahopanes from hopanes for example, based on their subtle cross-sectional differences requires many equilibrations for satisfactory separation. Furthermore, batchwise sorption involves heating of the sieves may cause thermal vibration of the framework atoms in the sieve pores (Ruthven, 1988) thereby increasing in the channel aperture and decreasing size-selectivity.

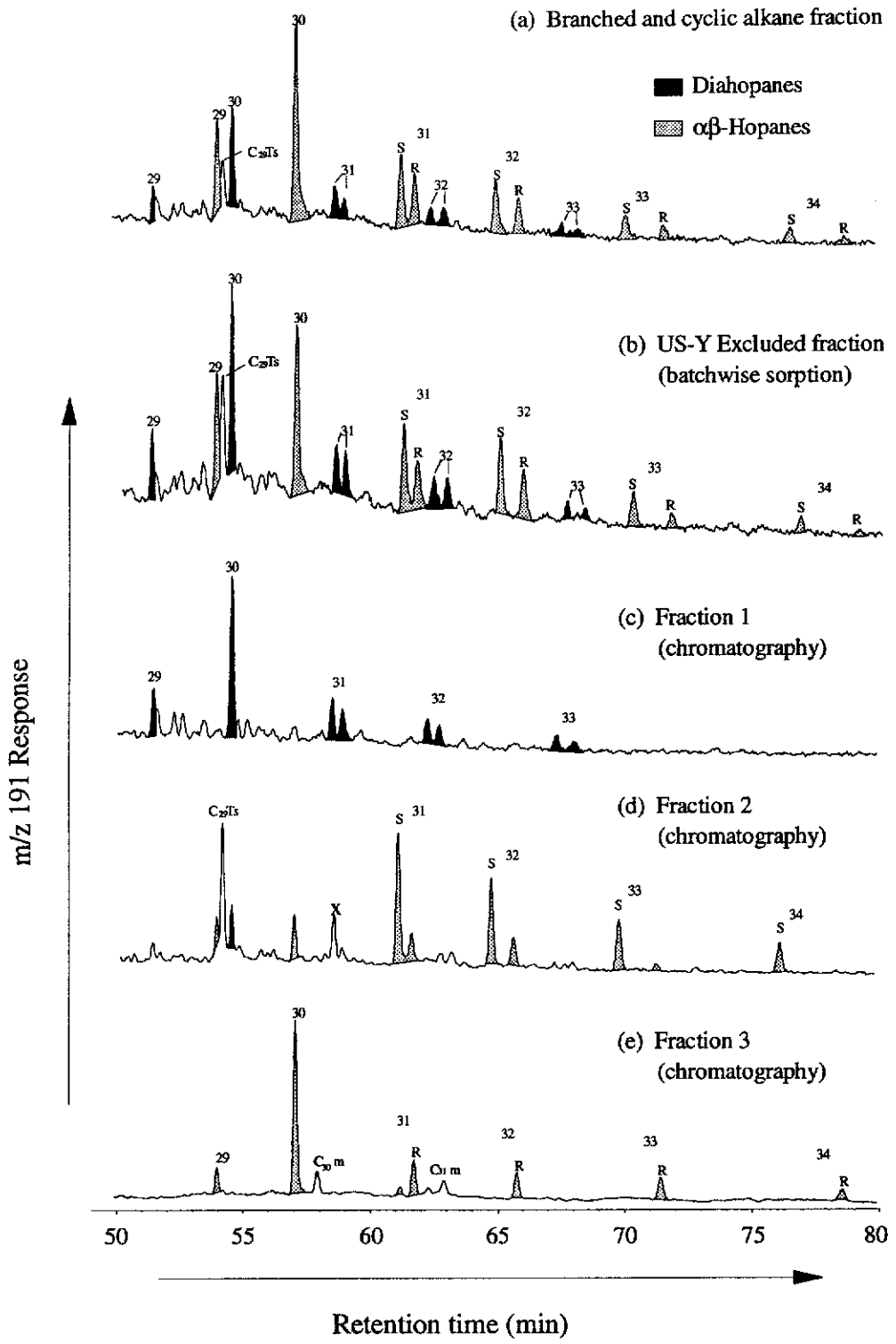


Figure 3.1 Partial m/z 191 mass chromatograms for Lambert crude oil branched and cyclic alkane fraction before (a) and after treatment with US-Y molecular sieves in batchwise mode (b) and counter current (chromatography) mode (c-e). Numbers denote the total number of carbons in each homologue.

Liquid chromatography techniques using molecular sieves, on the other hand, have been previously used to enhance inadequate separation of components achieved by batchwise sieving techniques (Zinnen *et al.*, 1985; Hoering and Freeman, 1984). A major advantage of chromatography over batchwise separation is that many theoretical plates are possible with even a modest system such as the one used. As a result, the relative selectivities for adsorption of the different triterpane components from the mixture need not be high for a satisfactory separation to be achieved.

3.1.2 Size-Shape Selectivity Using Liquid Chromatography

The fractionation obtained using the sieve liquid chromatographic procedure is based predominantly on size and shape of the pentacyclic triterpanes. The sorption behaviour of triterpanes on a US-Y sieve chromatography column was investigated by subjecting a range of branched and cyclic alkane fractions from selected crude oils and sediments to the US-Y liquid chromatography procedure. In each experiment, the mixture eluting from the sieve column was collected in three fractions and each fraction was then analysed for the known triterpanes. The behaviour of the various triterpanes is summarised in Table 3.1. These compounds have been grouped into two categories; hopanoids and non-hopanoids (or higher plant triterpanes). Compounds having a molecular diameter greater than approximately 0.81 nm are not sorbed, while compounds having molecular diameters ranging from 0.70 nm to 0.73 nm are sorbed within the sieve channels (as estimated using CSC Chem-3D Plus 3.1). Compounds having molecular diameters of approximately 0.75 nm also appear to be sorbed by the sieve, but to a lesser degree, and so will be described as 'weakly sorbed'. Results presented here show a close correlation between sorption behaviour of compounds and their effective molecular diameters relative to that of the sieve channels.

Table 3.1 Cross-sectional diameters of some hopanoid and non-hopanoid components in petroleum and their sorption behaviour on US-Y molecular sieves. The effective cross-sectional diameters were estimated using a CSC Chem-3D Plus computer package version 3.1.

Compound Type	Compound	Structure Number*	Estimated Diameter (nm)	Sorption Behaviour
HOPANOIDS	8,14-Secohopane	VII	0.88	not sorbed
	Diahopane	IVd	0.82	not sorbed
	17β,21β(H)-Hopane	Id	0.76	weakly sorbed
	30-Norneohopane	Vc	0.75	weakly sorbed
	17α,21β(H)-Hopane	IIId	0.73	sorbed
	25-Norhopane	VIc	0.72	sorbed
	30-Norhopane	IIIc	0.72	sorbed
	Moretane	IId	0.70	sorbed
NON HOPANOIDS	c-c-t-Bicadinane (W)	X	1.14	not sorbed
	t-t-t-Bicadinane (T)	XI	0.93	not sorbed
	Cadinane	XVII	0.89	not sorbed
	Lupane	XIIIa	0.87	not sorbed
	18α-Oleanane	XVa	0.85	not sorbed
	28-Nor-18α-oleanane	XVb	0.84	not sorbed
	Spirotriterpane	XII	0.84	not sorbed
	Taraxastane	XVI	0.81	not sorbed
	Oleanane	XIV	0.82	not sorbed
	Ursane	XVIII	0.83	not sorbed

* Structures shown in Appendix.

Although the fractionation of triterpanes using US-Y sieve chromatography is mainly controlled by to size-selectivity, adsorption/desorption effects may also contribute to the separation. If adsorption was a dominant process in the observed triterpane fractionation, then similar fractionation results would be obtained using stationary phases with similar chemical properties but having different channel apertures. In preliminary experiments, a number of sieves having similar SiO_2/Al_2O_3 compositions to that of the US-Y sieve and a variety of channel apertures were tested as adsorbents. These were found to have little or no capacity to discriminate between pentacyclic triterpanes. Whereas the effective channel apertures of sieves such as ZSM-5 and mordenite proved to be too small for the fractionation of pentacyclic triterpanes, the channel aperture of 13X sieve was found to be a little too large. In contrast, an obvious and reproducible fractionation effect was observed using various forms of US-Y sieves (US-Y 400 to US-Y 760) which differ only in their SiO_2/Al_2O_3 compositions.

The remainder of this chapter presents, a selection of results obtained from the fractionation of pentacyclic triterpanes and related compounds on US-Y sieve (*cf.* Table 3.1) and a discussion of the sorption mechanism for individual components.

3.2 FRACTIONATION OF HOPANOIDS

The branched and cyclic alkane fraction isolated from a Lambert crude oil from the Barrow Sub-Basin, Western Australia, which contains a full range of hopanoid components, was subjected to liquid chromatography using the US-Y molecular sieve. Figure 3.1 shows m/z 191 mass chromatograms of the branched and cyclic alkane fraction of the crude oil (a) together with the three fractions (c-e) collected from the column. These results are discussed in detail below, but briefly: Fraction 1 contains abundant diahopanes; the hopanoids in Fraction 2 consist mainly of the

22S diastereomers of the extended αβ-hopanes together with 30-norneohopane (C_{29}Ts); Fraction 3 contains 30-norhopane (29), αβ-hopane (30) and the *22R* diastereomers of the extended αβ-hopanes (31-34) as well as moretane (C_{30}m) and homomoretane (C_{31}m).

3.2.1 Analysis of Rearranged Hopanes

Diahopanes

The high abundance of diahopanes in Fraction 1 indicates that they were excluded from the channels of the molecular sieve and so were not retained on the column. Examination of computer models of hopane and diahopane (Figure 3.2, f and h) indicates that they both have effective molecular diameters (o.73 and 0.82 nm, respectively) similar to that of the molecular sieve channel aperture (0.74 nm). In the case of the diahopane however, the equatorial methyl substituent located at C-15 results in a slightly greater effective diameter of 0.82 nm compared to 0.73 nm for αβ-hopane, where the methyl substituent is in an axial position and is located at C-14. The orientation of the methyl substituent on C-15 in diahopanes appears to hinder them from entering into the sieve channels, therefore causing their observed enrichment in Fraction 1.

A more accurate analysis of the relative amounts of some diahopane components is possible using the fractionated branched and cyclic alkane fraction (Fraction 1). For example, the mass chromatogram of the branched and cyclic alkane fraction, shown in Figure 3.1a, shows the C_{32} diahopanes with a relative diastereomer abundance of approximately 1:1. However the mass chromatogram from Fraction 1 indicates that this ratio is nearer to 3:2. This discrepancy is due to coelution of the later-eluting C_{32} diahopane diastereomer with homomoretane (C_{31}m). After fractionation, the homomoretane is in Fraction 3 and therefore no longer interferes with the C_{32} diahopane. Similarly, a comparison of the pair of C_{31} diahopane

diastereomers in the mass chromatograms for the alkane fraction and Fraction 1 shows that these compounds are better resolved in the sieved fraction which is attributed to removal of co-eluting component(s) after liquid chromatography. One interferring C_{30} component has eluted in Fraction 2 (peak labelled "x" in the mass chromatogram) which has a retention time intermediate between the C_{31} diahopane diastereomers.

30-Norneohopane

30-Norneohopane (C_{29}Ts) eluted in Fraction 2 and hence, unlike the diahopanes, was slightly retained by the sieve column. Its intermediate retention on the column suggests that C_{29}Ts was not strongly sorbed by the sieves, which may be explained based on relative steric energies of its rotational conformations. Figure 3.2 shows two conformations of C_{29}Ts (d and e) which have significantly different steric energies (Table 3.2). The less sterically hindered conformation (d, 320 kJ/mole) is where the ethyl side chain is protruding away from the methyl on C-17, which is oriented toward the side chain. In such a conformation the side chain extends beyond the molecular cross-sectional diameter described by the rest of the molecule and so would hinder its sorption into the sieve channels. For C_{29}Ts to be completely sorbed into the sieve channel it must assume a significantly higher energy conformation (e, 340 kJ/mole) where the side chain ethyl group is in close proximity to the methyl on C-17. It is apparent from the sieving results that relatively few molecules assume this higher energy conformation. Hence C_{29}Ts is not strongly sorbed within the sieve channels and elutes in Fraction 2.

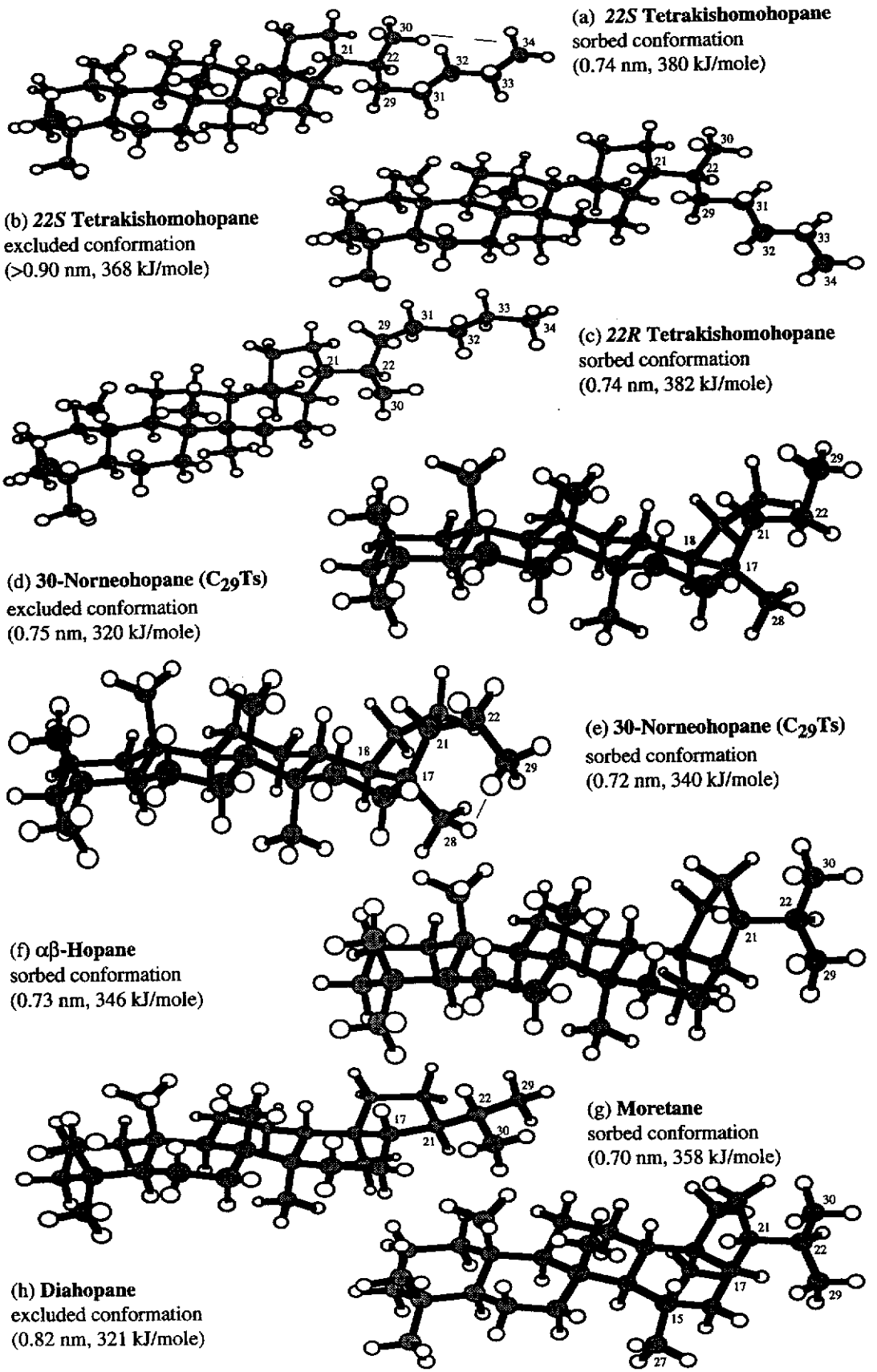


Figure 3.2 Computer generated molecular models of some petroleum triterpanes showing their estimated cross-sectional diameters (nm) and their calculated relative steric energies (kJ/mole).

In the case of 30-norhopane (29) however, which elutes in Fraction 3 (Figure 3.1e), there is little difference between the calculated steric energies of the conformations which would be sorbed or not sorbed into the sieve channels (Table 3.2, 340 and 335 kJ/mole respectively) since the methyl group on C-17, which promotes steric crowding in C_{29}Ts, is now located on C-18, away from the ethyl side chain. Hence, a greater proportion of 30-norhopane molecules are likely to assume the conformation which can be sorbed into the sieve channels and so results in its longer retention on the sieve column compared to C_{29}Ts.

3.2.2 Analysis of Hopanes

αβ-Hopanes

The presence of αβ-hopanes and moretanes only in Fractions 2 and 3 (Figure 3.1d and e) indicates that they are sorbed into the sieve channels. The molecular skeletons of hopane and moretane (Figure 3.2 f and g, respectively) are of small enough dimensions to permit their sorption into the sieve channels. Hence, 30-norhopane (29), αβ-hopane (30) and the moretanes (C_{30}m and C_{31}m) are strongly retained by the sieve and so elute predominantly in Fraction 3 from the sieve column.

Differences in the sorption behaviour of the *22R* and *22S* epimers of the αβ-hopanes are related to conformational effects of the C-22 side chain. The presence of *22S* and *22R* extended αβ-hopane diastereomers in different fractions indicates differences in sorption energies of the diastereomers. In order to examine likely conformational effects on these compounds when sorbed into the molecular sieves, computer models and steric energies of *22S* and *22R* tetrakishomohopanes were examined. Figure 3.2 shows models for a high energy sorbed conformation of the *22S* epimer (a), a low energy conformation of the same epimer (b) (which would hinder the molecule from entering the sieve pores), and a relatively low energy

conformation of the *22R* epimer (c) (which is able to be sorbed into the sieve channels). The model for the most stable rotational conformation of the *22S* epimer (b, 368 kJ/mole), where the C-29 to C-34 chain is orientated away from C-30 methyl, clearly exceeds the molecular dimensions described by the remainder of the molecule and so would be hindered from entering the sieve channels. For this molecule to be sorbed it must adopt a rotational conformation (a, 380 kJ/mole) where the side chain comes into closer proximity to C-30 methyl. The significant difference in the steric energies for the two conformers suggests that a greater proportion of the molecules would adopt the more stable conformation which is less strongly sorbed by sieve and hence *22S* tetrakishomohopane elutes in Fraction 2. In the case of the *22R* tetrakishomohopane epimer however, a comparison of the steric energies of the rotational conformations which may and may not be strongly sorbed by the sieves (Table 3.2, 382 kJ/mole and 379 kJ/mole, respectively) suggests that a greater proportion of molecules are likely to have the more strongly sorbed conformation. As a consequence, *22R* tetrakishomohopane is more strongly sorbed by the sieves than its *22S* epimer and so has a longer retention on the column.

Additional support for the differences in sorption behaviour of *22R* and *22S* epimers being related to conformational effects on C-22 side chain is provided by the increasing selectivity of this separation process with increase in carbon number of the extended αβ-hopanes. The mass chromatogram of Fraction 2 (Figure 3.1) shows an increase in abundance of the *22S* diastereomer relative to the *22R* isomer when the C_{33} or C_{34} hopanes (33-34) are compared with the C_{31} or C_{32} components (31-32). Comparison of steric energies for rotational conformations of *22S* homohopane (C_{31} homologue Table 3.2, 368 and 364 kJ/mole) with those for the C_{34} homologue (382 and 379 kJ/mole) indicates that a greater proportion of the *22S* homohopane molecules are likely to adopt the 'sorbed' conformation compared with *22S* tetrakishomohopane. Hence, increase in bulk of the alkyl group in the

side chain of extended *22S* hopanes results in higher energies of interaction and energetically less favourable confined conformations within the channels of the sieve. Such effects therefore result in the observed increase in selectivity with increasing carbon number.

Discrimination between αβ-hopane diastereomers on the basis of sorption behaviours on molecular sieves has been observed previously. Close inspection of a published chromatogram of a pentacyclic concentrate from 10X sieves (Whitehead, 1974) showed preferential sorption of the *22S* diastereomers compared to the *22R* isomers. This is contrary to the observations with the US-Y sieves. However, Dimmler and Strausz (1983) observed that the desorption of a polycyclic terpenoid fraction from 13X sieves using iso-octane gave incomplete recovery of the C_{29}, C_{30} and the *22R* C_{31}-C_{35} hopanes, thus suggesting that these hopanes, also present in Fraction 3 (Figure 3.1), are sorbed more strongly within the sieve channels.

Table 3.2 Steric energies for different rotational conformations of some hopanoids which will allow them to be sorbed or not sorbed within the sieve channels.

COMPOUND	Energy of most stable sorbed conformation kJ/mole	Energy of most stable conformation kJ/mole
30-Norhopane	340	335
30-Norneohopane	340	320
22R αβ-Homohopane	371	371
22S αβ-Homohopane	368	364
22R αβ-Tetrakishomohopane	382	379
22S αβ-Tetrakishomohopane	380	368

ββ-Hopanes

A branched and cyclic alkane fraction from a low rank coal was chromatographed on US-Y molecular sieves in order to examine the sorption behaviour of ββ-hopanes. Figure 3.3 shows m/z 191 mass chromatograms for the original alkane fraction and three fractions obtained from the sieve column. Fraction 1 contains mainly C_{29}-C_{32} triterpanes of unknown structure which were initially present in the sample in minor amounts. Nearly all the ββ-hopanes in the sample eluted in Fraction 2 demonstating that they are sorbed by the sieve. The cross-sectional diameter estimated for the ββ-hopane is 0.76 nm compared with that for the US-Y sieve of 0.74 nm. Evidently the channels are large enough to permit sorption of ββ-hopanes, but the fit is tight and the sorption is not as strong as that of the αβ-hopanes in the sample.

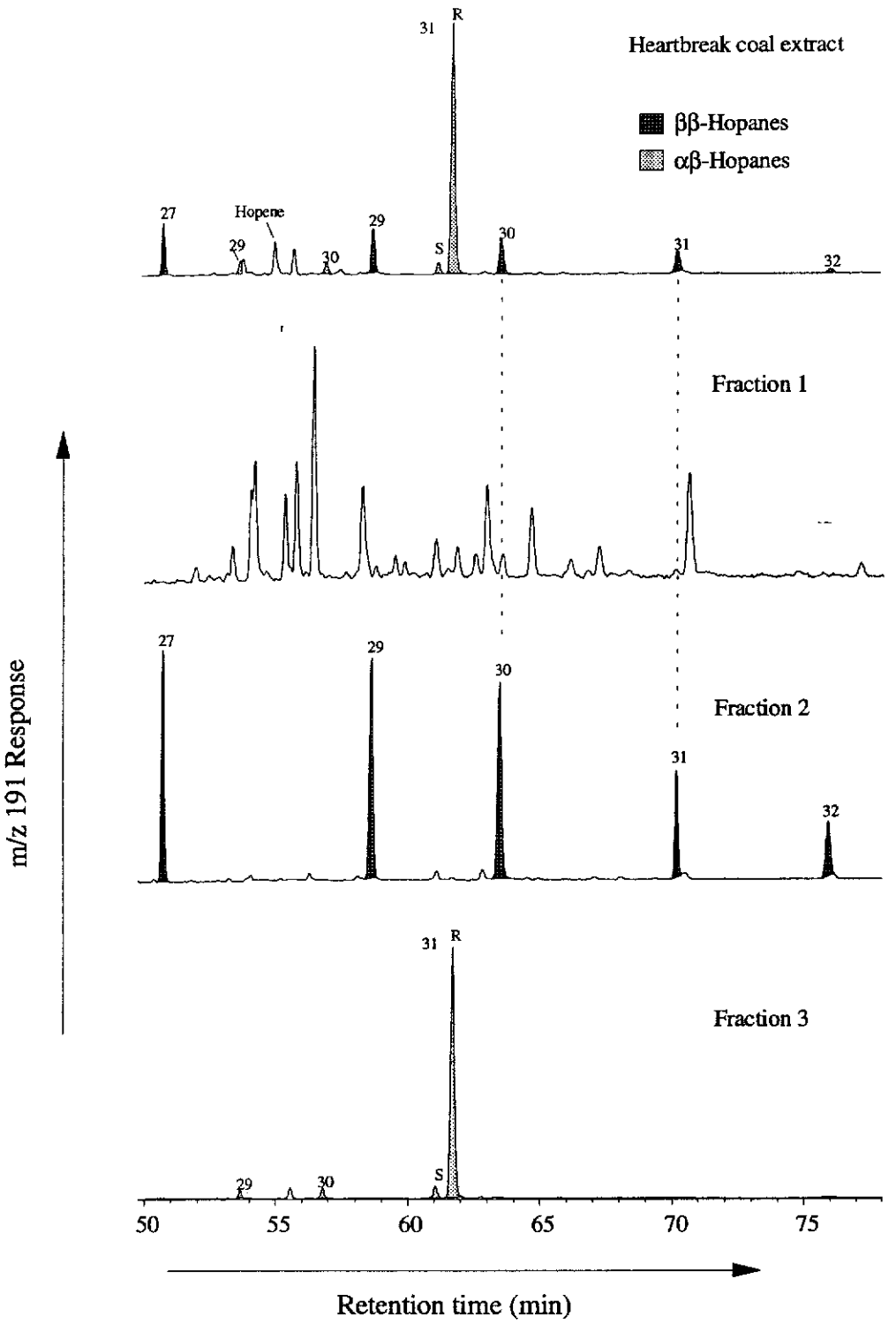


Figure 3.3 Partial m/z 191 mass chromatograms of the branched and cyclic alkane fraction from Heartbreak coal extract and three eluted fractions from the US-Y sieve column. Numbers denote the total number of carbons in each homologue.

3.3 FRACTIONATION OF CADINANE-TYPE COMPOUNDS

3.3.1 Samples

Indonesian crude oils from the Central Sumatra Basin (CSB) and South Sumatra Basin (SSB) (Figure 3.4), containing markedly different higher-plant biomarker distributions were chosen to demonstrate the potential use of US-Y molecular sieve chromatography to separate the non-hopanoid pentacyclic components in crude oil. Capillary gas chromatograms of the branched and cyclic alkanes in the two Indonesian crude oils are shown in Figure 3.5. CSB crude oil contains high amounts of botryococcane which indicates a predominantly algal source (Maxwell *et al.*, 1968; Cox *et al.*, 1973; Moldowan and Seifert, 1980; Seifert and Moldowan, 1981; Metzger *et al.*, 1991); however the sample was selected because it contains barely detectable amounts of bicadinanes and was therefore ideal to demonstrate the effectiveness of the molecular sieve liquid chromatography procedure for concentrating these compounds. SSB crude oil was selected because of abundant bicadinanes and high concentrations of other pentacyclic triterpanes of higher plant origin which will be discussed in Chapter 5.

3.3.2 Enrichment of Cadinane and Bicadinane Isomers

The branched and cyclic alkane fraction from SSB crude oil was chromatographed on a column of US-Y molecular sieve in order to show the effects of molecular sieve chromatography on cadinanes and homocadinanes. The branched and cyclic alkane fraction of this crude oil contained very high concentrations of bicadinanes and high concentrations of the associated cadinane and homocadinane components.

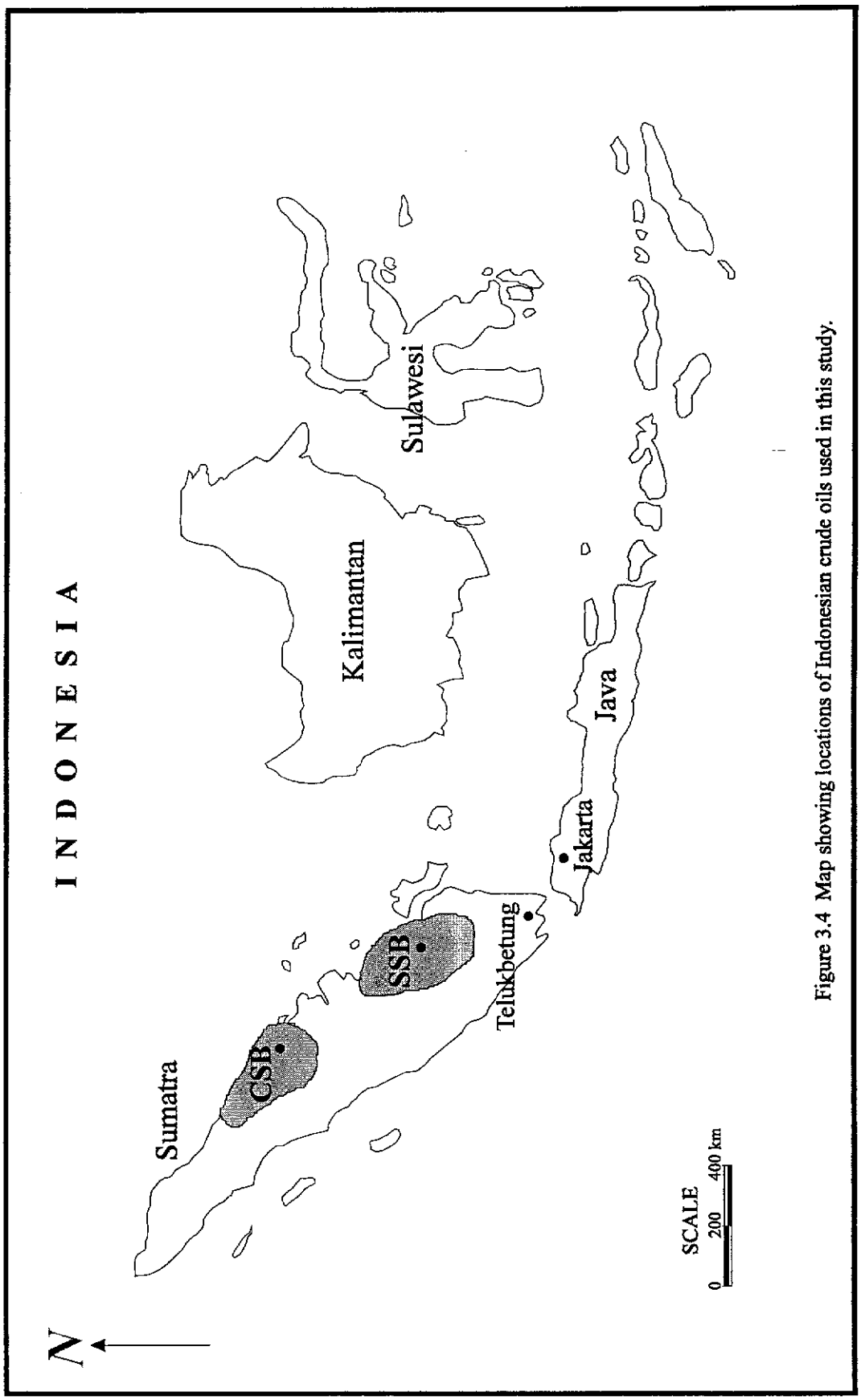


Figure 3.4 Map showing locations of Indonesian crude oils used in this study.

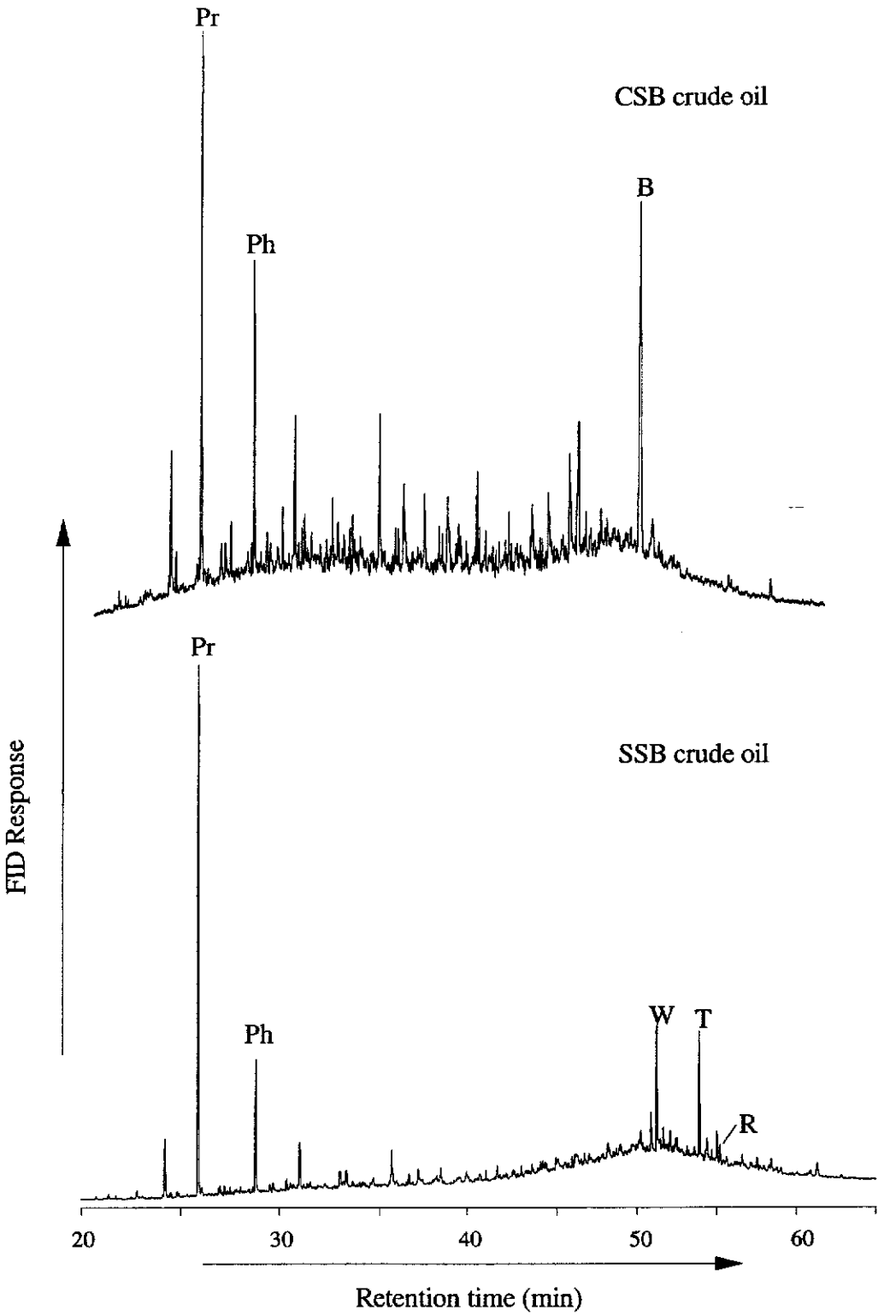


Figure 3.5 Partial capillary gas chromatograms of the branched and cyclic alkane fractions from two Indonesian crude oils. Pr=pristane; Ph=phytane; B=botryococcane; W=*cis-cis-trans* bicadinane; T=*trans-trans-trans* bicadinane; R=C_{30} bicadinane.

Figure 3.6 shows combined m/z 208 & 165 mass chromatograms indicating the cadinanes and m/z 222 & 179 mass chromatograms indicating the homocadinanes in the unsieved branched and cyclic alkane fraction and in Fraction 1 from the sieve chromatography. These results show that the cadinanes and the homocadinanes elute in the first chromatography fraction, consistent with calculated molecular cross-sections of approximately 0.89 nm (Table 3.1), indicating that they are too large to be sorbed into the sieve channels.

The sieving procedure not only enriches cadinanes but also appears to remove small amounts of interfering components evident in the chromatogram of the unsieved material. Figure 3.6 shows that there are less peaks in Fraction 1 than in the unsieved branched and cyclic alkane fraction. Computer models of various isomeric cadinanes show that they have effective diameters ranging from 0.85 nm to 0.89 nm and therefore are all too large to be sorbed into the sieve channels. It is therefore likely that the peaks which are solely present in the chromatogram of the unsieved sample represent compounds which are not based on the cadinane skeleton and were sorbed by the sieves.

The high concentrations of homobicadinanes and secobicadinanes in SSB crude oil also enabled the molecular sieve chromatography behaviour of these compound types to be studied. The mass chromatograms in Figure 3.7 show signals from these compound types along with the bicadinanes. In each case, combined mass chromatograms of the major fragment ion and the parent ion have been used to enhance the responses of the particular component type. The peaks were tentatively assigned on the basis of mass spectral data and their published relative retention times (van Aarssen *et al.*, 1992). The presence of these components in Fraction 1 shows that they too were not sorbed into the sieve channels.

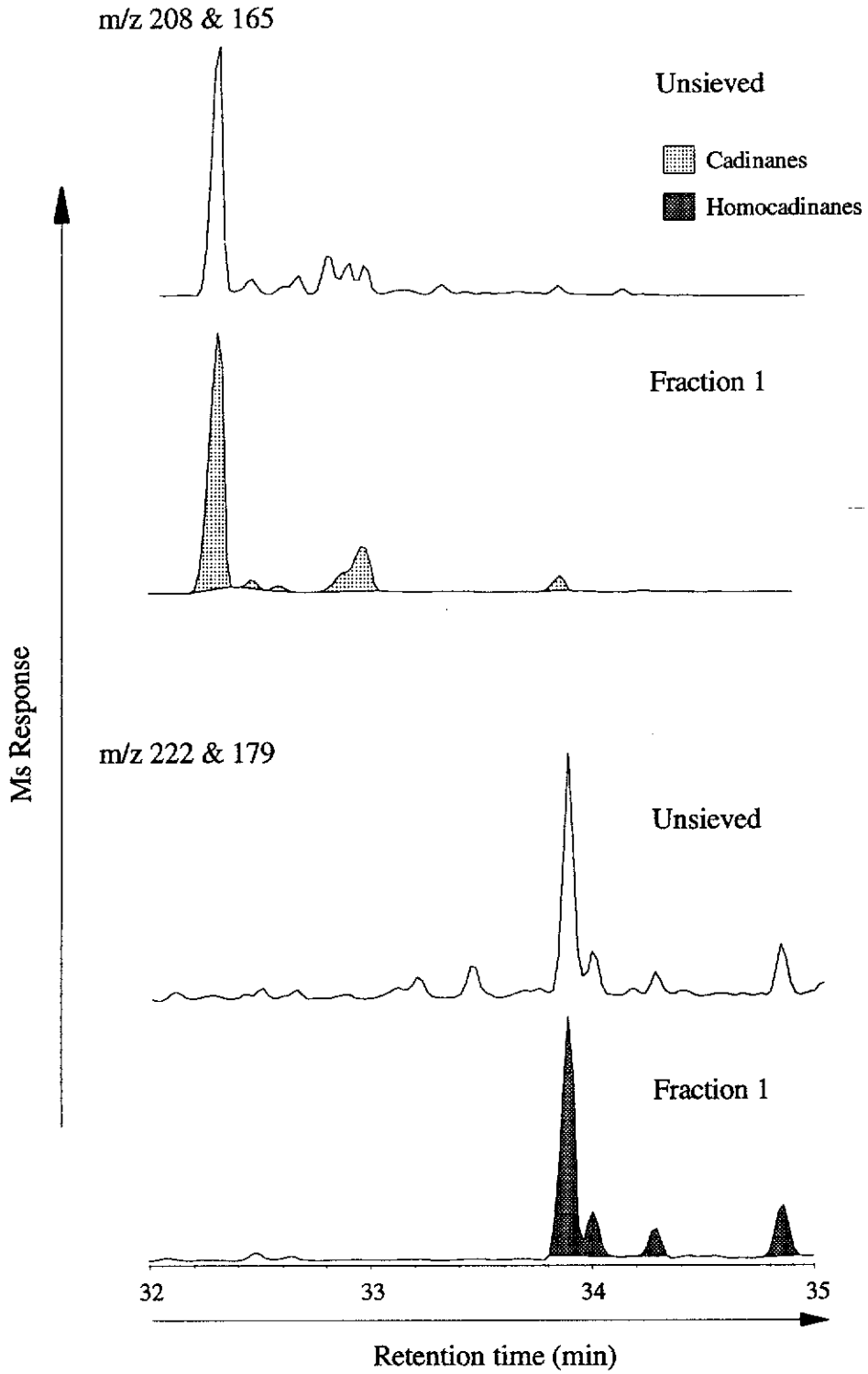


Figure 3.6 Combined partial mass chromatograms of the branched and cyclic alkane fraction from SSB crude oil compared with those of a fraction obtained by chromatography on US-Y molecular sieves illustrating the selective enrichment of cadinanes and homocadinanes after sieving.

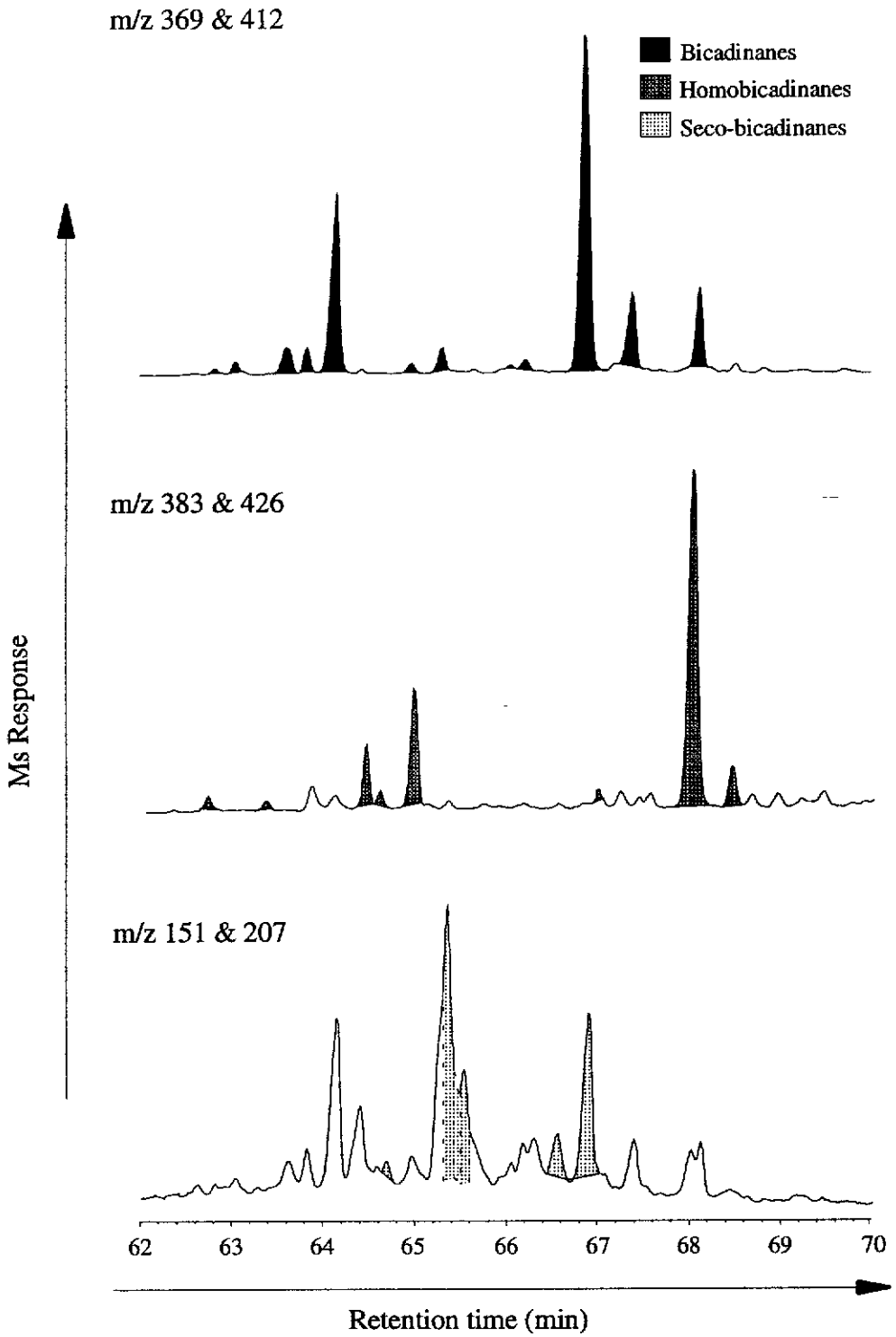


Figure 3.7 Combined partial mass chromatograms of the first eluted branched and cyclic alkane fraction from SSB crude oil showing the bicadinanes (m/z 369 + 412), homobicadinanes (m/z 383 + 426), and secobicadinanes (m/z 151 + 207).

3.3.3 Unmasking of Bicadinanes

The branched and cyclic alkane fraction from CSB crude oil was chromatographed using US-Y molecular sieve in an attempt to concentrate the bicadinanes to facilitate detection. Figure 3.8 shows partial m/z 191 and m/z 412 mass chromatograms of the branched and cyclic alkanes from CSB crude oil and from Fraction 1 after molecular sieve chromatography of this fraction. It is apparent from a comparison of the m/z 191 mass chromatograms that *cis-cis-trans* bicadinane (W), *trans-trans-trans* bicadinane (T) and bicadinane R are all present in higher relative concentrations in Fraction 1 than in the unsieved sample. Examination of the mass spectra obtained at a retention time corresponding to the peak labelled R in the two m/z 191 mass chromatograms showed that R in the unsieved sample consisted of more than one component and that the bicadinane was a minor contributor. In contrast, the peak labelled R in Fraction 1 was mainly bicadinane. Furthermore, this enrichment procedure does not change the relative proportions of the bicadinanes. This is evident from the m/z 412 mass chromatograms (Figure 3.8) which show less interference in analysis of the bicadinanes than the m/z 191 mass chromatograms and show similar relative amounts of the three bicadinanes in Fraction 1 and in the unsieved sample.

In order to quantify the improved detection limits for bicadinanes that result from analysis of the fraction recovered from molecular sieve chromatography, experiments were carried out to determine the detection limits for the *cis-cis-trans* bicadinane (W) in CSB crude oil branched and cyclic alkane fraction before and after molecular sieve chromatography. The branched and cyclic alkane fraction and Fraction 1 obtained from sieve chromatography were successively diluted with a solution of an internal standard lupane in *n*-pentane. In a separate experiment it had been established that lupane, having a molecular diameter of 0.87 nm (Table 3.1), was not sorbed in the separation process.

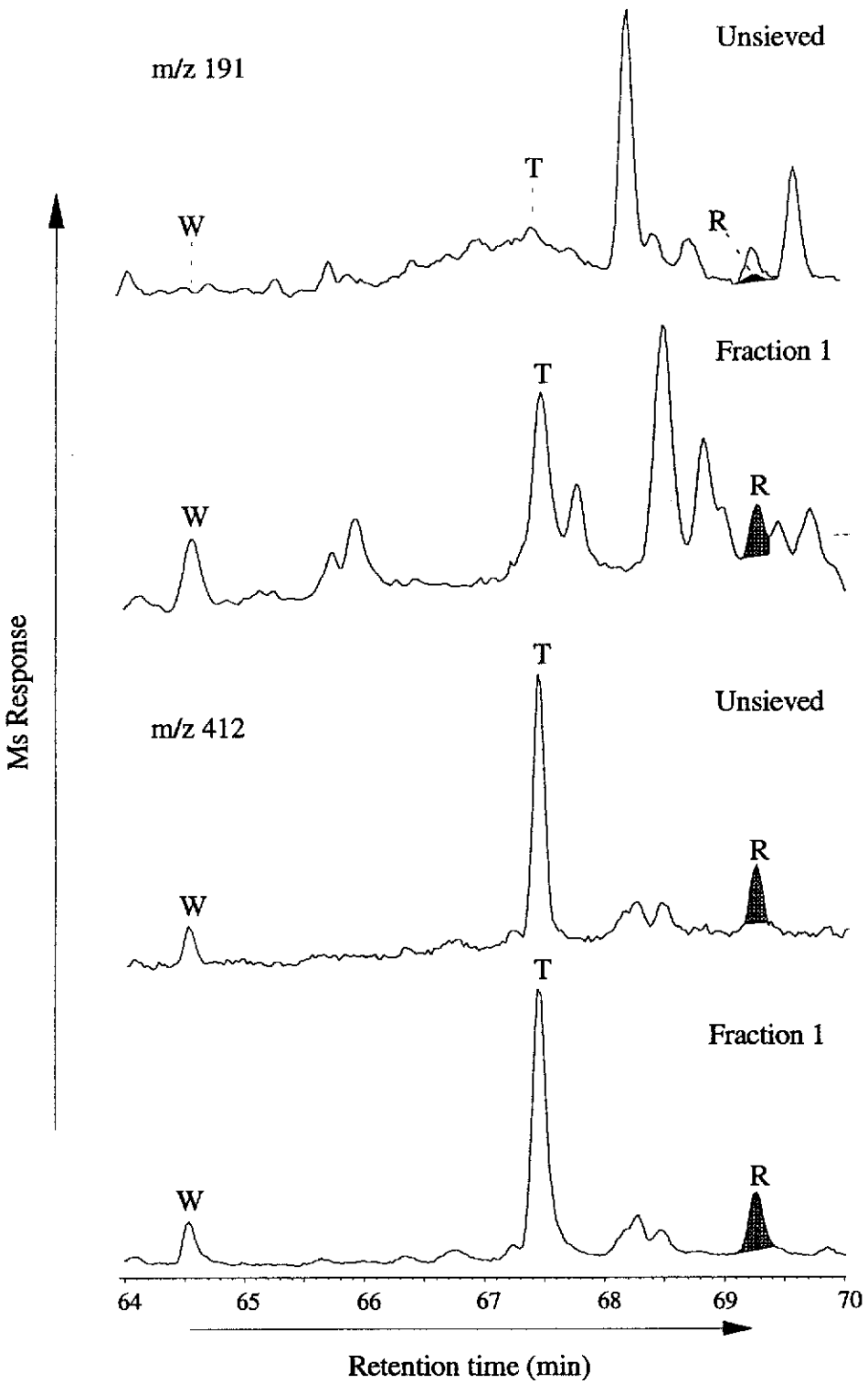


Figure 3.8 Partial m/z 191 and m/z 412 mass chromatograms showing the relative abundances of *cis-cis-trans* bicadinane (W), *trans-trans-trans* bicadinane (T) and bicadinane R in the branched and cyclic alkane fraction from CSB crude oil before sieving compared with that in Fraction 1 obtained from molecular sieve chromatography.

Figure 3.9 shows results from GC-MS analysis of the unsieved and sieved samples after successive dilution with internal standard. The enhanced response for the bicadinanes resulting from sieving is immediately apparent from the first set of chromatograms (dilution 1:17). Comparison of these chromatograms shows the complete removal of αβ-hopane in the sieved fraction and the removal of most of the unresolved complex hydrocarbon mixture (hump) which elutes in the same region as the bicadinanes. This unresolved hydrocarbon mixture has been suggested to be made up of fairly simple alkanes comprising substituted alkyl chains (Killops and Al-Juboori, 1990; Gough and Rowland, 1990) which may be sorbed into the sieve channels and so, unlike the bicadinanes, would be removed from the eluent.

The detection limits for bicadinanes where their signal/noise ratio equals 2 was determined in both the sieved and unsieved fractions. The response from the *cis-cis-trans* bicadinane (W) in the m/z 412 mass chromatograms was used as an example to determine the signal/noise ratio. These data were then used along with the area ratios obtained from peaks representing lupane and bicadinane (W) to obtain the plot shown in Figure 3.10. It is apparent from this plot that the signal/noise ratio at each relative concentration is greater for the sample that had been treated with molecular sieves. Linear extrapolation of the data showed that when the signal/noise value is two, a lower relative concentration of *cis-cis-trans* bicadinane can be measured after sieving. Clearly, the molecular sieve chromatography process improves the detection limit for the bicadinanes when they are analysed using selective ion monitoring techniques. This effect is attributed to the removal of compounds that have retention times similar to the bicadinanes, thereby reducing the chemical background noise in the m/z 412 mass chromatograms. A geochemical application of this technique, where these bicadinanes have been unmasked for the first time in samples older than Late Cretaceous, is described in Chapter 6.

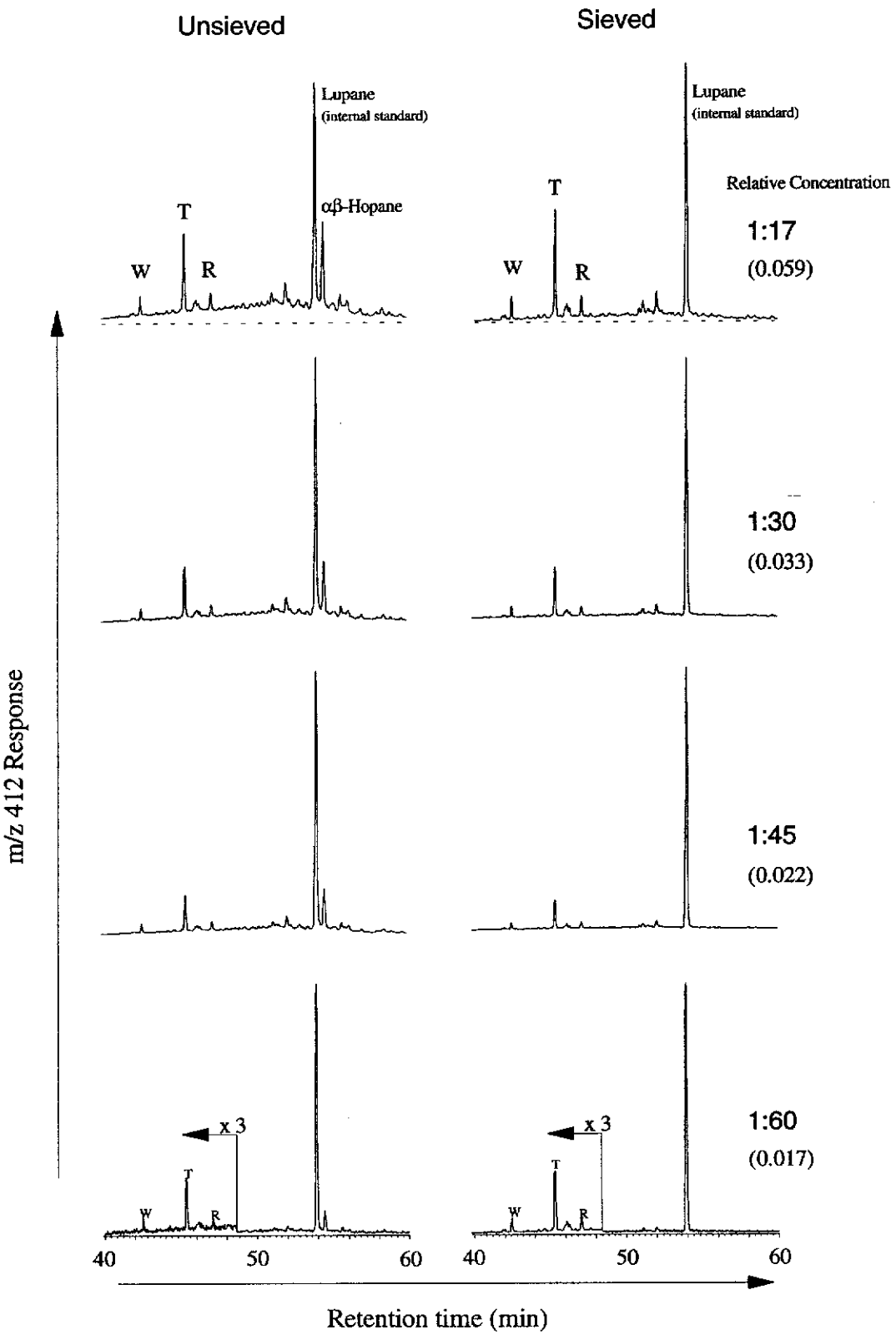


Figure 3.9 Partial m/z 412 mass chromatograms of a branched and cyclic alkane fraction of CSB crude oil before and after sieving and their successive dilution with internal standard lupane. The ratios denote the amount of bicadinane W relative to that of the internal standard.

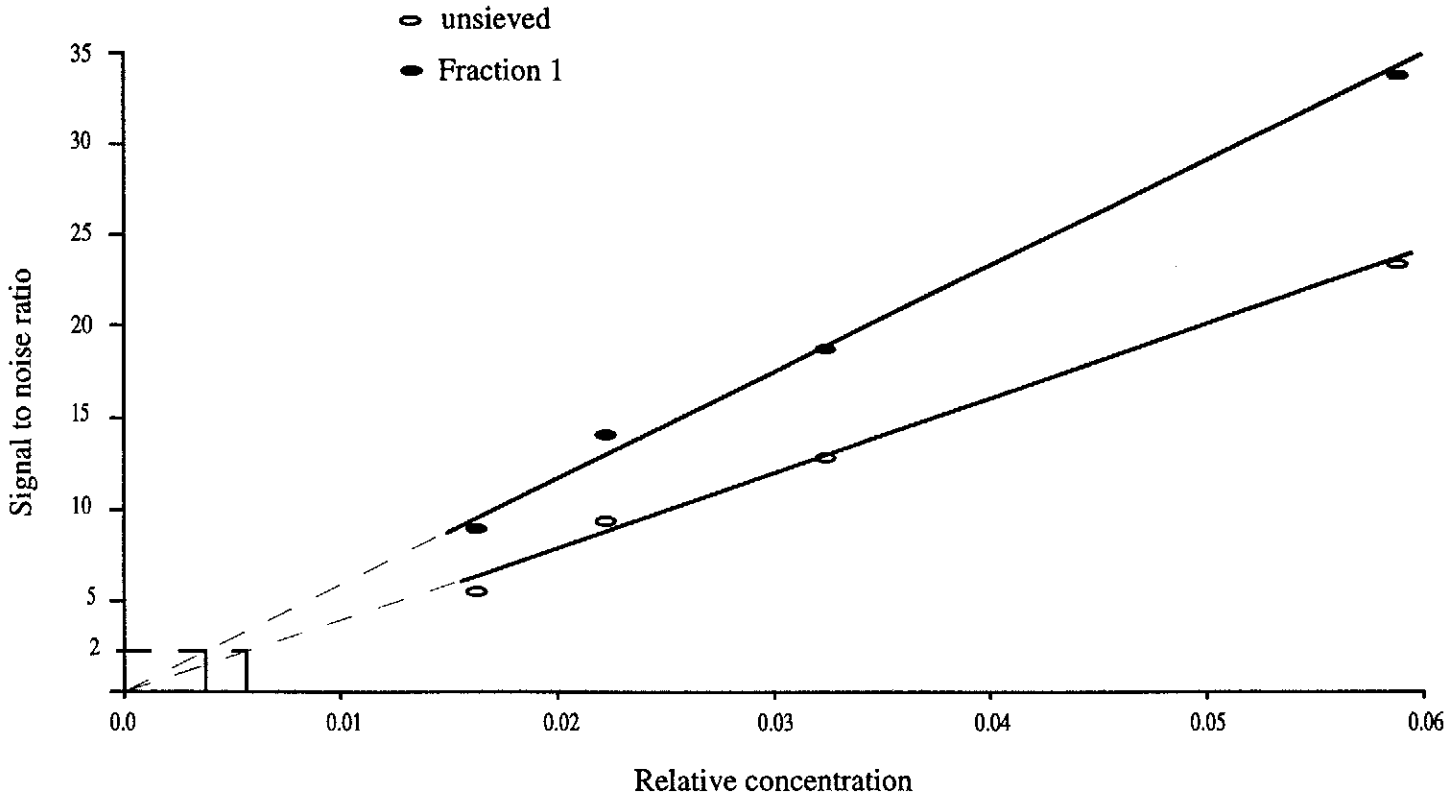


Figure 3.10 A plot of signal to noise ratio for *cis-cis-trans* bicadinane versus its concentration relative to that of the internal standard lupane in an unseieved and first eluted branched and cyclic alkane fraction from CSB crude oil. This figure illustrates the improved detection limits of *cis-cis-trans* bicadinane obtained after sieving.

3.4 CONCLUSIONS

A liquid chromatographic procedure using ultra stable-Y (US-Y) molecular sieve as the stationary phase and *n*-pentane as the mobile phase has been developed to fractionate petroleum hopanoid classes based on molecular shape and size. Compounds having molecular diameters greater than approximately 0.81 nm are excluded from the sieve and so elute in the first fraction from the sieve column. Compounds having molecular diameters of approximately 0.75 nm are weakly sorbed by the sieve and elute in Fraction 2. Finally, compounds having molecular diameters ranging from 0.70 nm to 0.73 nm are strongly sorbed by the sieve and elute in Fraction 3.

Throughout the remainder of the thesis, this liquid chromatographic technique using US-Y molecular sieve will be applied to geochemical studies of selected petroleum triterpanes which would have otherwise been more difficult using conventional separation methodology.

CHAPTER 4

ISOLATION, CHARACTERISATION AND ISOMERISATION

REACTIONS OF *22R* 17α,21β(H)-HOMOHOPANE

Summary

22R 17α,21β(H)-Homohopane was isolated from a lignite and characterised using X-ray crystallography and ^{13}C NMR spectroscopy. Co-chromatography of this hopanoid with a branched and cyclic alkane fraction of a crude oil showed that it corresponds to the later eluting 17α,21β(H)-homohopane diastereomer, thus rigorously confirming for the first time the common practice of assigning the higher retention time peak of each αβ-homohopane pair as the *22R* diastereomer.

22R αβ-homohopane was heated in the presence of anthracite for various periods of time and was observed to partially isomerise to give the *22S* epimer. The reaction is believed to proceed via a free radical mechanism.

4.1 BACKGROUND

The gas chromatographic elution order of the *22R* and *22S* diastereomers of the ββ-hopane series was established by Ensminger *et al.* (1974) who synthesised the pair of C_{31} ββ-homohopanes from diploptene and found that only the second eluting epimer *(22R)* was present in an immature sediment. In the case of the extended αβ-hopanes however, the assignment of the C-22 epimers was probably based upon the fact that in immature sedimentary rocks the second-eluting epimer is in greater concentration and thus, by analogy with the ββ-hopanes, was assumed to be the *22R* epimer (e.g. Mackenzie *et al.*, 1980). In the following work the assignment of αβ-homohopane diastereomers is rigorously established by co-chromatography with an authentic standard of *22R* αβ-homohopane.

4.2 ISOLATION OF A HOMOHOPANE FROM A LIGNITE

The procedure employed to isolate the homohopane from a low rank coal is schematically illustrated in Figure 4.1 (refer to section 2.5 for full experimental procedure). Loy Yang coal was chosen for the preparative work because it contained the homohopane as the dominant component in the branched and cyclic alkane fraction (Figure 4.2a). Briefly, Loy Yang coal was ground and extracted with dichloromethane using a Soxhlet apparatus. The branched and cyclic alkane fraction was isolated from the extract using conventional liquid chromatographic procedures. This alkane fraction was then subjected to further chromatography with US-Y molecular sieves to obtain a fraction enriched in homohopane and isopimarane (Figure 4.2b). Preparative gas chromatography was finally used to isolate the homohopane (>99.5 % by GC) which was further purified by recrystallisation from hexane.

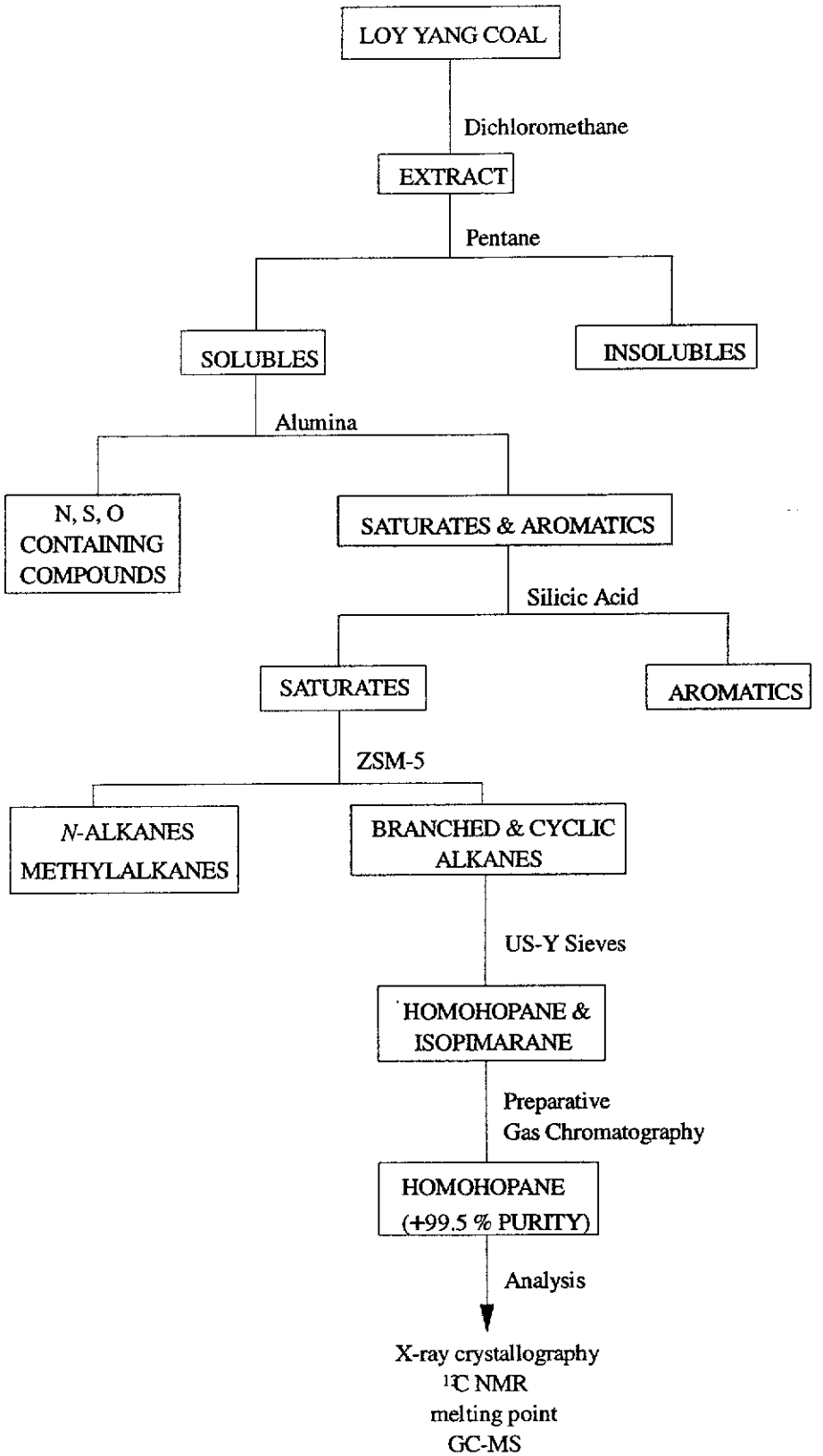


Figure 4.1 Techniques used to isolate and analyse the sedimentary homohopane from a lignite.

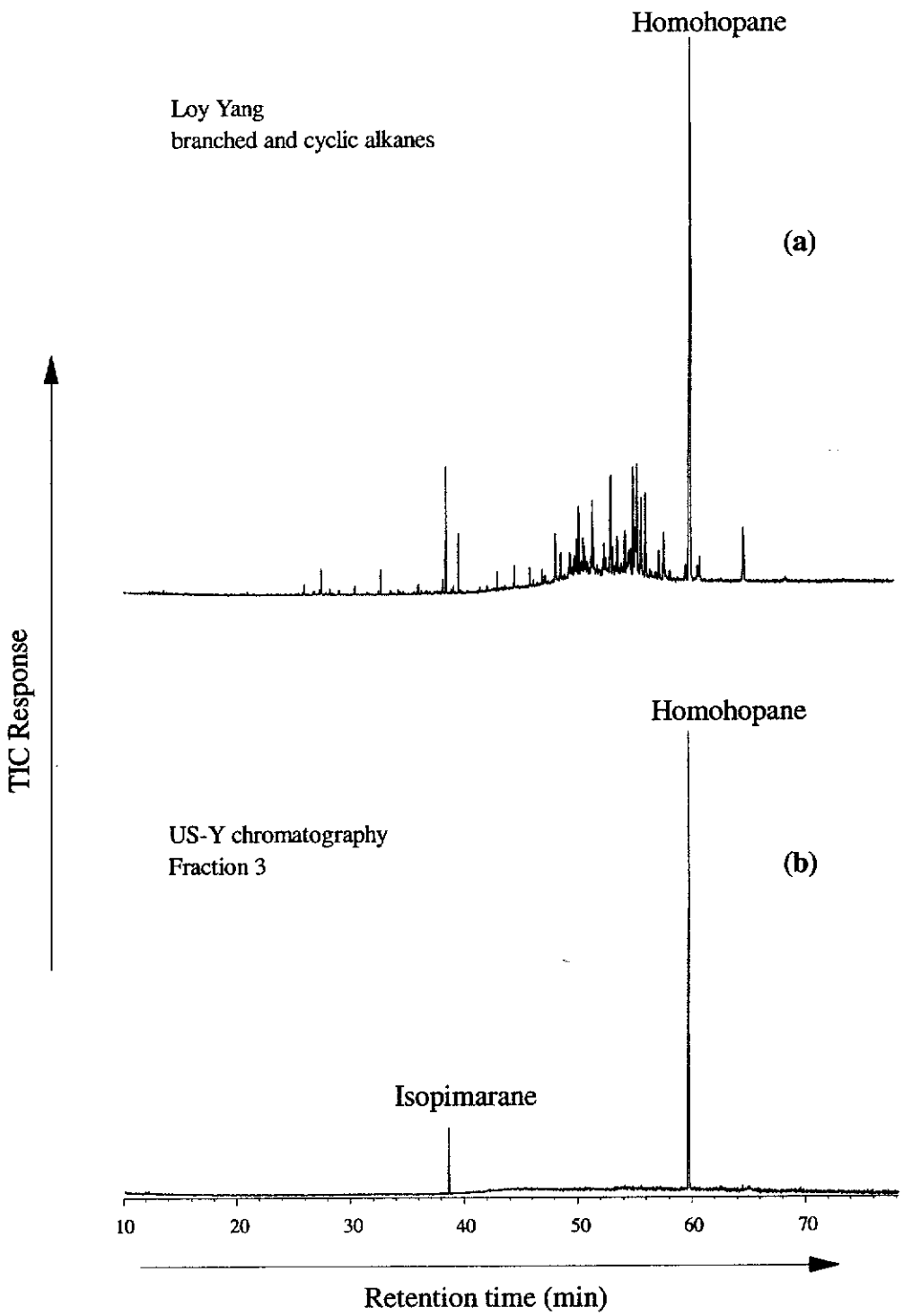


Figure 4.2 Total ion chromatograms for (a) the branched and cyclic alkane fraction from Loy Yang coal and (b) the homohopane enriched Fraction 3 which was eluted from the US-Y molecular sieve column.

4.3 CHARACTERISATION OF *22R* 17α,21β(H)-HOMOHOPANE

4.3.1 X-ray Crystallography

The homohopane was crystallised from hexane by very slow evaporation of the solvent to give long needle-like crystals which were suitable for X-ray crystallography. The molecular structure and stereochemistry of this triterpane was then established by a single crystal X-ray study to be *22R* 17α,21β(H)-homohopane. A unique room-temperature diffractometer data set (T~295 K; Enraf-Nonius machine θ/2θ scan mode, $2\theta_{max}$ 45°; monochromatic Mo Kα radiation, λ = 0.7107 Å) was measured on a small, weakly diffracting fibre mounted needle, 0.10 x 0.15 x 0.75 mm, yielding 2056 independent reflections, 1544 with $I > \sigma(I)$ being considered "observed" and used in the full matrix least squares refinement without absorption correction. Despite the weakness of the data, anisotropic thermal parameters refined meaningfully for the carbon atoms; $(x, y, z, U_{iso})_H$ were included at estimated values. Conventional residuals R, R_w on $|F|$ at convergence were 0.098, 0.093; neutral atom complex scattering factors were employed (chirality being assigned from the chemistry), computation using the XTAL 3.2 program system (Hall *et al.*, 1992). A three-dimensional structure consistent with the connectivity and stereochemistry is shown in Figure 4.3 (a). Pertinent results are given in Table 4.1; full molecular non-hydrogen geometries and thermal and hydrogen parameters have been deposited with the Cambridge Crystallographic Data Centre.

Crystal data - $C_{31}H_{54}$, $M_r = 426.7$. Orthorhombic, space group $P2_12_12_1$ (D_2^4, No. 19), $a = 7.720(5)$, $b = 15.655(5)$, $c = 21.895(5)$ Å, $V = 2646$ Å^3. D_c ($Z = 4$) = 1.07 g.cm.$^{-3}$; $F(000) = 960$. $\mu_{Mo} = 0.4$ cm^{-1}.

Table 4.1 Atomic positional and isotropic displacement parameters obtained from a single crystal X-ray study of *22R* 17α,21β(H)-homohopane.

	x	y	z	U
C(1)	0.596(1)	0.8774(7)	0.1725(5)	0.051(4)
C(2)	0.626(1)	0.9652(8)	0.2013(6)	0.059(4)
C(3)	0.482(2)	1.0277(8)	0.1825(5)	0.055(4)
C(4)	0.292(1)	0.9972(7)	0.1965(5)	0.049(4)
C(5)	0.275(1)	0.9069(7)	0.1686(5)	0.042(4)
C(6)	0.098(1)	0.8680(8)	0.1747(5)	0.056(4)
C(7)	0.069(1)	0.7963(7)	0.1309(5)	0.054(4)
C(8)	0.199(1)	0.7226(7)	0.1377(5)	0.037(3)
C(9)	0.387(1)	0.7609(7)	0.1425(4)	0.037(3)
C(10)	0.411(1)	0.8382(7)	0.1856(5)	0.045(4)
C(11)	0.516(1)	0.6872(7)	0.1494(5)	0.051(4)
C(12)	0.504(1)	0.6217(7)	0.0991(5)	0.049(4)
C(13)	0.318(1)	0.5857(6)	0.0913(4)	0.031(3)
C(14)	0.195(1)	0.6615(6)	0.0804(4)	0.035(3)
C(15)	0.008(1)	0.6204(7)	0.0730(5)	0.048(4)
C(16)	-0.001(1)	0.5547(7)	0.0217(5)	0.051(4)
C(17)	0.123(1)	0.4816(7)	0.0322(4)	0.042(3)
C(18)	0.312(1)	0.5083(7)	0.0472(5)	0.046(4)
C(19)	0.372(2)	0.4289(8)	0.0785(6)	0.063(4)
C(20)	0.231(1)	0.3980(7)	0.1190(5)	0.056(4)
C(21)	0.064(1)	0.4192(7)	0.0823(5)	0.045(4)
C(22)	-0.031(2)	0.3393(7)	0.0596(5)	0.051(4)
C(23)	0.257(2)	1.0042(8)	0.2637(6)	0.065(4)
C(24)	0.179(1)	1.0594(8)	0.1625(6)	0.063(4)
C(25)	0.412(2)	0.8130(7)	0.2533(5)	0.057(4)
C(26)	0.148(2)	0.6747(8)	0.1973(6)	0.062(5)
C(27)	0.233(1)	0.7083(7)	0.0209(5)	0.050(4)
C(28)	0.408(1)	0.5222(8)	-0.0136(6)	0.063(4)
C(29)	-0.117(2)	0.2898(8)	0.1131(6)	0.070(5)
C(30)	-0.164(1)	0.3535(9)	0.0100(7)	0.081(5)
C(31)	-0.188(2)	0.204(1)	0.0997(7)	0.094(6)

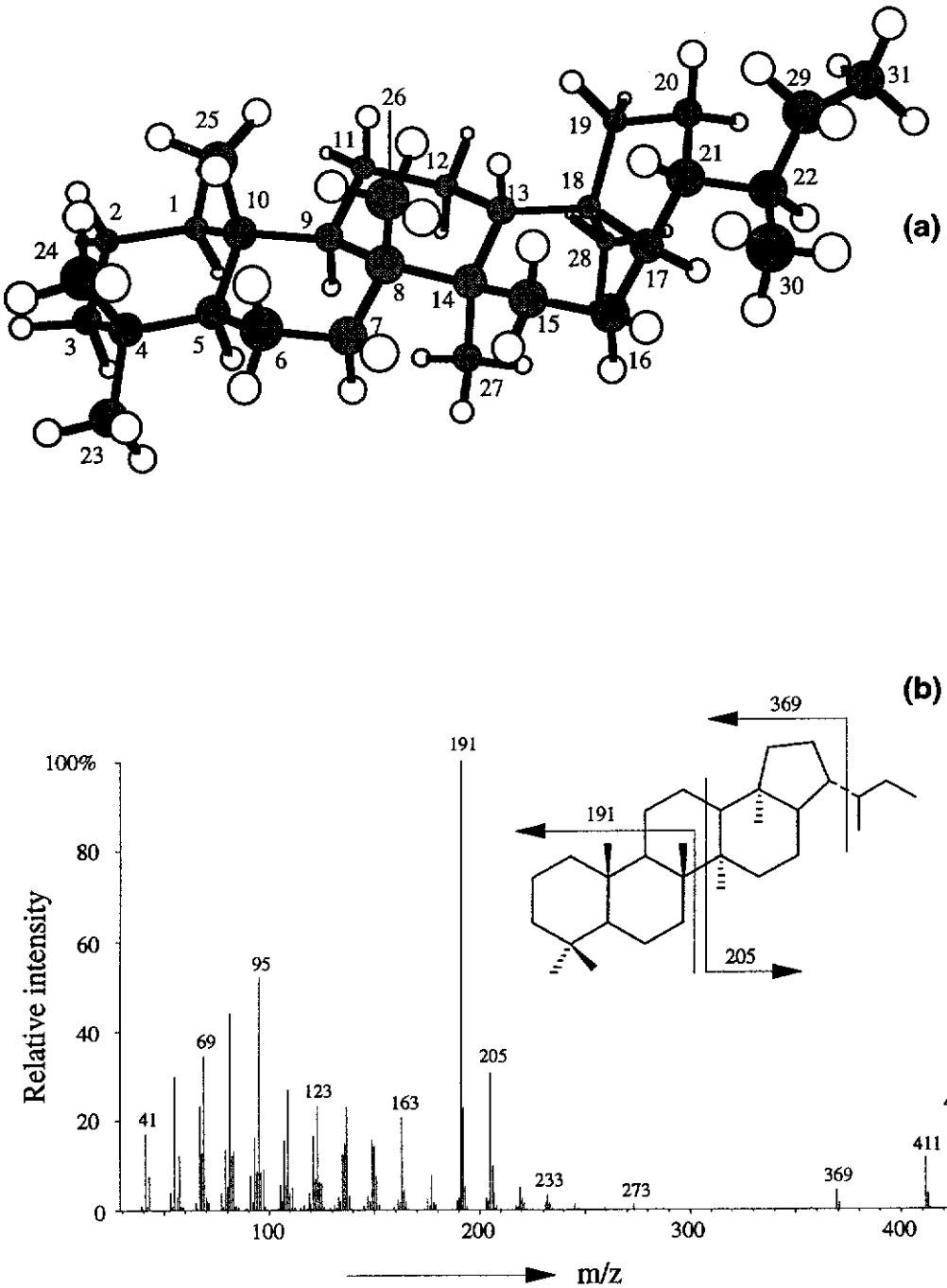


Figure 4.3 Three-dimensional perspective view (a) and mass spectrum (b) of *22R* αβ-homohopane isolated from Loy Yang lignite.

4.3.2 NMR Spectroscopy

The ^{13}C NMR spectrum of *22R* αβ-homohopane showed 31 resonances which were resolved with the aid of DEPT experiments into eight methyl, twelve methylene, six methine and five quaternary carbon signals. The ^{13}C NMR chemical shifts for the homohopane were assigned based on those previously published for the C_{30} homologue. Table 4.2 shows chemical shift assignments for the ^{13}C spectrum of *22R* αβ-homohopane along with those reported for the spectrum of αβ-hopane. Balogh *et al.* (1973) first reported the ^{13}C chemical shift assignments for αβ-hopane isolated from an oil shale, but these were later revised by Wilkins *et al.* (1987). More recently, since 2D NMR data became available, it was apparent that further re-assignments were required (A. Wilkins, personal communication). Comparison of the ^{13}C NMR shifts for the two homologues shows that for the homohopane the chemical shifts of E-ring carbons closest to the side chain (ie. C-17, C-20 and C-21) and of course the isopropyl group carbons (ie. C-22, C-29 and C-30) are those most affected by the introduction of the C-31 carbon. Interestingly, the C-16 signals in the two samples differ significantly by 0.5 ppm showing that, even though it is five carbons removed from C-31, C-16 is also mildly affected by the introduction of the C-31.

4.4 ANALYSIS OF *22R* αβ-HOMOHOPANE

4.4.1 GC-MS Analysis

The mass spectrum of *22R* αβ-homohopane in Figure 4.3 (b) shows a strong molecular ion at m/z 426 and a base peak at m/z 191 for the AB ring fragment. A fragment ion at m/z 369 [M-57] indicates loss of a C_4 group while a

Table 4.2 ^{13}C chemical shift assignments (ppm relative to TMS using $CDCl_3$ as internal reference) for αβ-hopane (Wilkins *et al.*, 1987) and *22R* αβ-homohopane.

Carbon	αβ-Hopane	*22R* αβ-Homohopane
1	40.4	40.3
2	18.8	18.67*
3	42.2	42.1
4	33.3	33.3
5	56.5	56.4
6	18.8	18.71*
7	33.2	33.1
8	42.2	42.1
9	51.1	50.9
10	37.6	37.5
11	21.7	21.6
12	23.8	23.7
13	39.1	39.0
14	41.5	41.3
15	26.8	26.9
16	22.2	22.5
17	47.7#	46.9
18	44.2	44.2
19	41.7	41.6
20	25.0	25.6
21	49.4#	48.7
22	31.0	38.6
23	33.5	33.4
24	21.7	21.6
25	16.1	16.1
26	16.4	16.3
27	16.7	16.8
28	24.1#	24.0
29	18.3	26.1
30	22.1#	17.9
31	-	12.2

* ^{13}C resonance assignments are interchangeable

revised assignments as elucidated in a combination of 2D ^{13}C-^{1}H (^{1}J and long range coupled data sets), DQFCOSY and NOESY NMR experiments (A. L. Wilkins, personal communication).

relatively strong ion at m/z 205 is due to a dominant DE ring fragment.
The GC retention behaviour of the isolated *22R* αβ-homohopane was compared with the pair of *22R* and *22S* epimers commonly found in crude oil. Figure 4.4 shows results from the co-chromatography of the authenticated *22R* αβ-homohopane and a typical crude oil branched and cyclic alkane fraction. This figure shows that *22R* αβ-homohopane has the same retention time as the later eluting αβ-homohopane diastereomer in crude oil. This therefore rigorously validates the common practice of assigning the higher retention time peak in gas chromatograms of αβ-homohopanes in crude oil as the *22R* diastereomer. Furthermore, the unequivocal identification of high amounts of an αβ-homohopane having a *22R* configuration in a low rank coal is consistent with reports of a reactant-product relationship between the biologically produced *22R* hopane polyols and sedimentary αβ-hopanes (Ensminger *et al.*, 1974, 1977; Seifert and Moldowan, 1980; Peters and Moldowan, 1991).

4.4.2 Melting Point

A relatively low melting point of 134-135°C was recorded for *22R* αβ homohopane compared with a melting point of 156-158°C for αβ-hopane (Tsuda *et al.*, 1967) and approximately 180°C for 30-norhopane (Smith, 1975). The decrease in the melting point of these homologues with increasing carbon number can be attributed to the increase in the bulk of the side chain producing notable differences in their lattice energies. The homohopane molecules, which have an extended side chain, are hindered from being as efficiently packed into the crystal lattice compared to the C_{29} and C_{30} homologues. The homohopane crystal lattice is therefore less ordered and less stable than those of the C_{29} and C_{30} homologues.

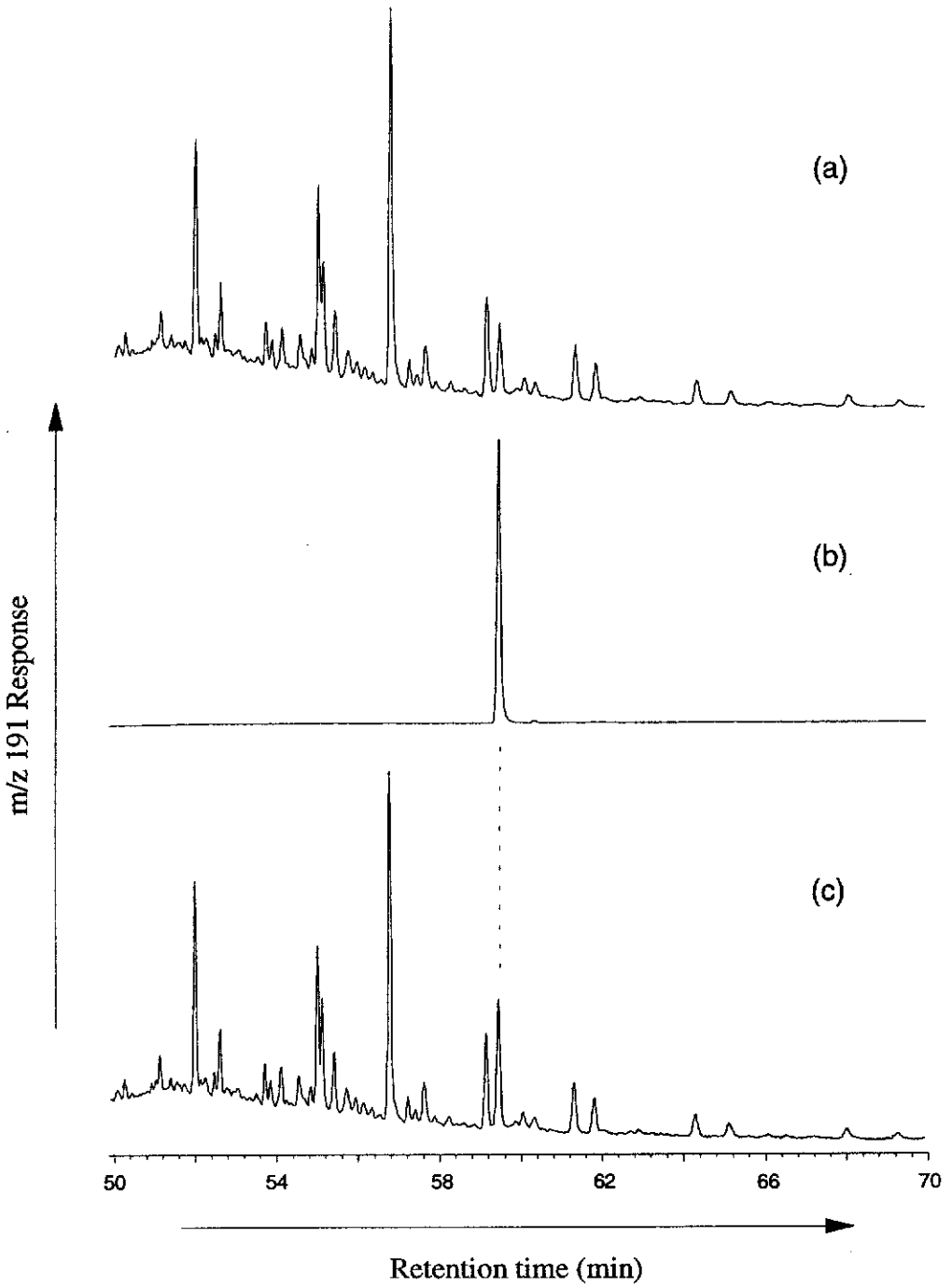


Figure 4.4 Partial m/z 191 mass chromatograms for a) a branched and cyclic alkane fraction from a typical crude oil, b) *22R* αβ-homohopane and c) co-chromatography of the homohopane and the crude oil branched and cyclic alkane fraction.

4.5 ISOMERISATION OF *22R* αβ-HOMOHOPANE

Previous laboratory attempts to replicate the sedimentary isomerisation of *22R* αβ-homohopanes to their *22S* epimers have met with limited success. Van Dorsselaer *et al.* (1977a) and Connan (1974) heated Yallourn lignite at 300°C for various periods of time and observed a gradual increase in the abundance of *22S* relative to *22R* αβ-homohopane. However, since only relative abundances were obtained, their results were inconclusive in showing isomerisation of *22R* to *22S* as opposed to simple selective destruction of the *22R* epimer. In contrast, Peters *et al.* (1990) observed a decrease in *22S/22R* ratios at high pyrolysis temperatures. More recently, Bisseret and Rohmer (1990) heated single hopanoid compounds with bromine, *n*-bromosuccinimide (NBS), or sulphur, all of which had been reported to be capable of inducing free radical isomerisation of saturated hydrocarbons (Kohen and Stevenson, 1965; Gorodetsky *et al.*, 1970, Abbott *et al.*, 1984; 1985). Sulphur, for example exists largely in the molecular form of an eight-membered ring which at elevated temperatures undergoes thermal scission to give linear sulphenyl diradicals (Pryor, 1962). In particular, Bisseret and Rohmer (1990) reported that heating of the acetate (at C-31) of *22R* 17β,21β(H)-homohopane with sulphur gave a product mixture dominated by αβ-hopane in a *22S/22R* ratio of 15:85. Hence, these authors were able to unambiguously demonstrate for the first time the interconversion of *22R* to *22S* diastereomers. However, this experimental result does not adequately replicate sedimentary conversion processes for the following reasons : (i) A *22S/22R* ratio of 15:85 was obtained compared to an apparent equilibrium ratio of 60:40 observed in mature sediments and crude oils. (ii) Only a 20% recovery of the reaction mixture was obtained and so the experiment does not exclude the possibility that the *22S* epimer may have formed directly from the *22R* ββ-hopane reactant instead of via the *22R* αβ-hopane

product. (iii) Finally, heating with sulphur may not be representative of diagenetic conditions, a point also noted by the authors.

In the following section, evidence is presented which extends the work of Bisseret and Rohmer (1990) on the isomerisation of homohopane diastereomers. Intimate mixtures of *22R* αβ-homohopane and powdered anthracite were heated at high temperatures in deactivated Pyrex glass ampoules sealed under vacuum. Anthracite, a high rank coal, was chosen instead of sulphur because it offered both a convenient source of free radicals (Marchard *et al.*, 1969; Pusey, 1973; Tissot and Welte, 1984) and an organic matrix on which the isomerisation can take place. Furthermore, it may be more representative of carbonaceous material in sediments than the molten sulphur used in previous laboratory experiments.

Laboratory reaction of 22R αβ-homohopane with anthracite

A mixture of *22R* αβ-homohopane and ground anthracite was heated in deactivated glass ampoules at 330°C for various periods of time. Figure 4.5 shows total ion mass chromatograms for the reaction mixtures along with calculated recoveries and relative abundances of *22R* and *22S* epimers. A chromatogram for a mixture heated at 180°C was included in this figure to show that even at a relatively low reaction temperature the reactant was not completely recovered. A comparison of the remaining chromatograms shows that with increasing reaction time there was a corresponding increase in the abundance of *22S* αβ-homohopane relative to its *22R* epimer in the reaction mixture. These results therefore show that isomerisation of *22R* αβ-homohopane can be performed under laboratory conditions, thus demonstrating a reactant-product relationship of *22R* αβ-homohopane to its thermodynamically more stable *22S* epimer.

A *22S/22R* ratio of 40:60 was observed in the 6 day reaction mixture. Although the relative abundance of the formed *22S* epimer is far greater than that reported by Bisseret and Rohmer (1990), it is nevertheless low compared to the steady state

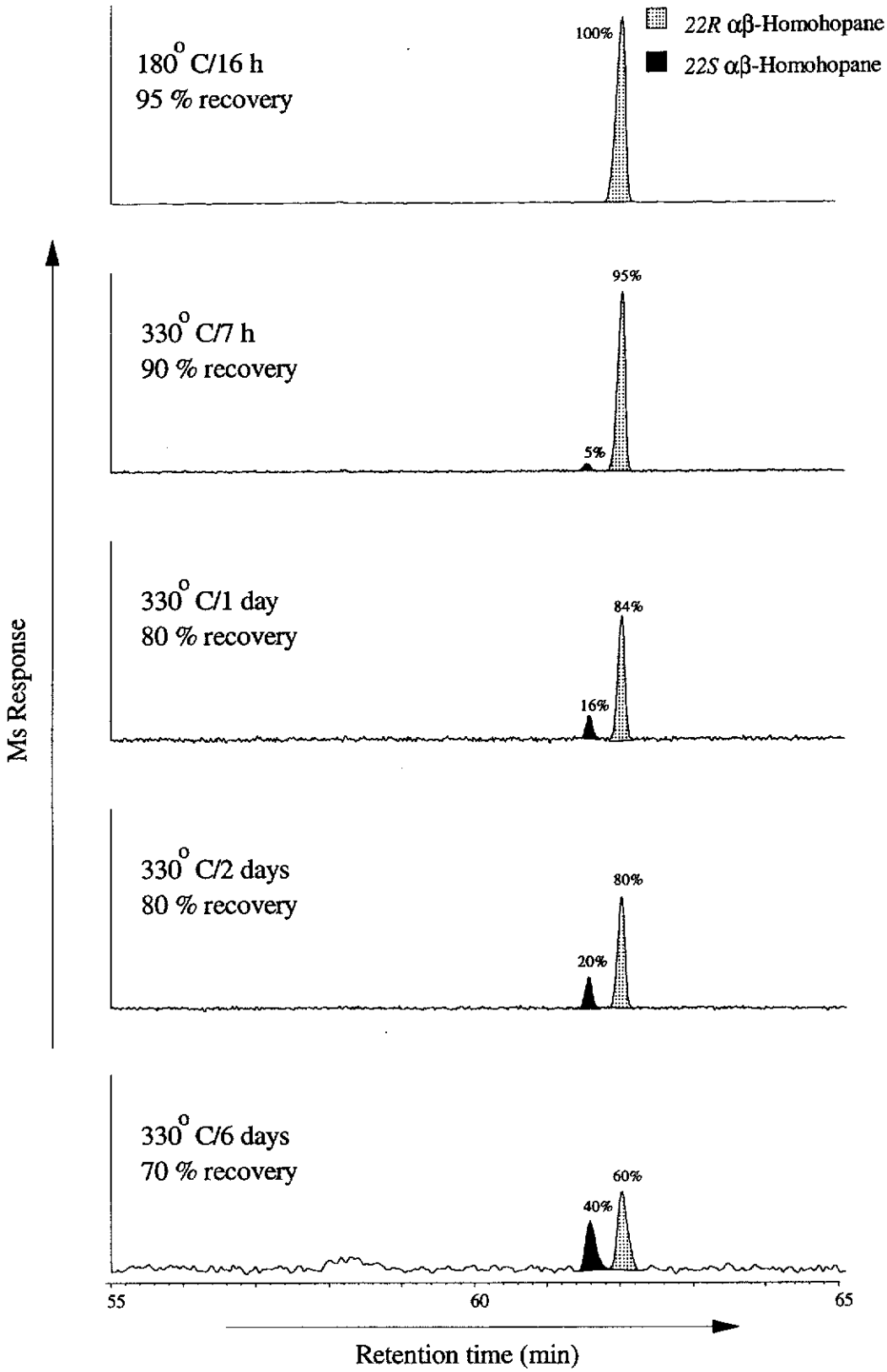


Figure 4.5 Total ion mass chromatograms for the reaction mixtures obtained from heating *22R* αβ-homohopane on anthracite. Percentage recoveries were calculated from the combined abundance of *22S* and *22R* epimers compared to the normalisation standard.

mixture of 60:40 (*22S/22R*) commonly observed in mature crude oil. Heating of *22R* αβ-homohopane at 330°C beyond 6 days resulted in a dramatic decrease in compound recovery with little difference in the diastereomer relative abundance. Failure to obtain an equilibrium mixture of the homohopane epimers in the laboratory may be due to experimental parameters which differ from those occurring in nature. Temperatures used to induce the epimer conversion in the laboratory, for example, are much higher than those prevailing in sediments over geological time. Hence, under relatively extreme laboratory conditions, reactions involving thermal and/or catalytic destruction of compounds are likely to dominate over the more delicate equilibration reactions which are responsible for producing the steady state epimer distributions found in mature sediments and crude oils.

4.6 CONCLUSIONS

The abundant hopanoid in an Australian lignite was isolated and characterised as *22R* αβ-homohopane. GC-MS analysis of this hopane enabled the first confirmation of the common practice of assigning the later eluting αβ-homohopanes in mass chromatogram of crude oils as the *22R* diastereomers. A product-reactant relationship has been observed in the laboratory between *22S* and *22R* αβ-homohopane based on free radical isomerisation.

CHAPTER 5

GEOCHEMISTRY OF REARRANGED HOPANES

IN SOME AUSTRALIAN CRUDE OILS

Summary

Biodegraded crude oils from three Australian basins were analysed for their hopanoid components in order to assess the effect of biodegradation and maturation on the rearranged hopanes. Rearranged hopanes were observed in greatest abundance relative to the regular hopanes in two extremely biodegraded crude oils. The co-occurrence of elevated levels of diahopanes, 30-norneohopane and 25-norhopanes in these samples could not be explained as source or maturation effects and so were attributed predominantly to their greater resistance to biodegradation compared to the regular hopanes. Furthermore, evidence is presented in this chapter which sheds new light on the disputed origin of 25-norhopanes in biodegraded crude oils.

5.1 GEOLOGICAL SETTING AND SAMPLE DESCRIPTION

5.1.1 Barrow Sub-basin

The Barrow Sub-basin is an elongate crustal depression within the Carnarvon Basin in Western Australia. It is Mesozoic in age and is located near the central Western Australian coastline (Figure 5.1). Detailed accounts of the petroleum geology of the Barrow Sub-basin can be found in papers by Crank (1973), Thomas and Smith (1974) and Thomas (1978). The three crude oils from this basin were sourced from the Jurassic Dingo Claystone (Volkman *et al.*, 1983b) and accumulated at different times in the continually subsiding reservoir sands under varying biodegradation conditions (Volkman *et al.*, 1983b). Detailed studies on the geochemistry of the crude oils have been reported (Volkman *et al.*, 1983a; 1983b). In brief, the Barrow Upper Jurassic (UJ) sample is a paraffinic light crude oil which shows no evidence of biodegradation. The Flinders Shoal and Mardie samples are naphthenic crude oils with compositions consistent with their having been subjected to severe biodegradation and water washing.

5.1.2 Bonaparte Basin

The Bonaparte Basin (Figure 5.1) is located mostly offshore in northwest Australia (Lavering and Ozimic, 1988). Lee and Gunn (1988) provide a geological review of the area which contains sediments dating from Devonian to Tertiary. Gas accumulations at Petrel and Tern and are numerous oil shows in the Permian sandstones of the Kulshill Formation evidence for the presence of mature source rocks in this basin (Gunn and Ly, 1989). The samples from the Turtle and Barnett exploration wells studied here are two such oil shows which are located just offshore in the southern Bonaparte Basin. Jefferies (1988) used available

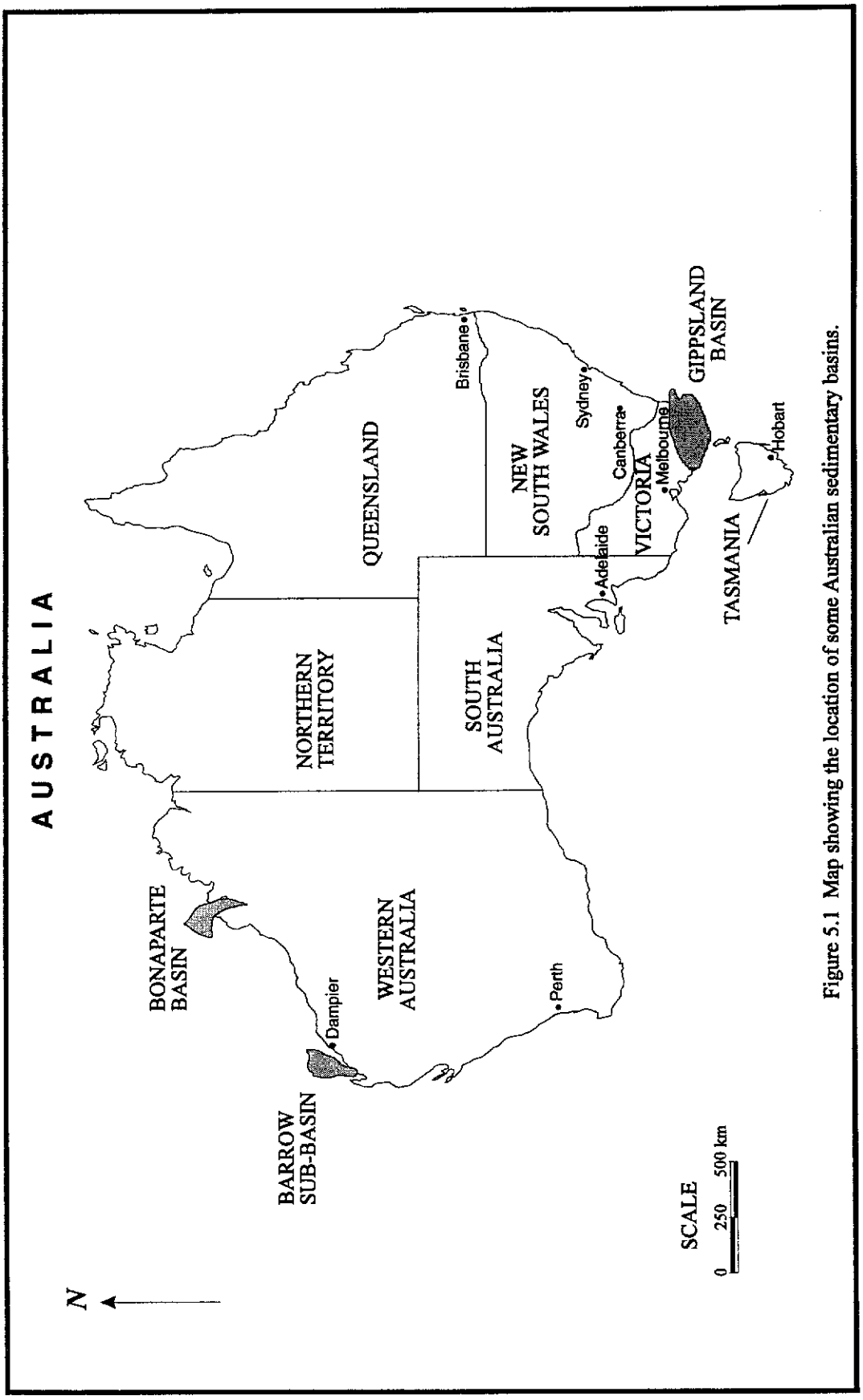


Figure 5.1 Map showing the location of some Australian sedimentary basins.

geochemical and geological data to infer that these crude oils are both sourced from the thick marine shales of the Late Devonian-Early Carboniferous Milligans Formation.

The Barnett 1 crude oil contains abundant of *n*-alkanes with no detectable 25-norhopanes and therefore can be classified as a non-degraded crude oil. Turtle 2 crude oil, on the other hand, contains some high molecular weight *n*-alkanes along with abundant 25-norhopanes. This unusual composition is reported (Jefferies, 1988) to have arisen from the mixing of two phases of oil generation. An initial oil was severely biodegraded in the reservoir prior to a decline in bacterial activity and a second pulse of oil generation. The latter oil was subjected to very mild degradation which removed the lower molecular weight *n*-alkanes.

5.1.3 Gippsland Basin

The Gippsland Basin is located mostly offshore near the Victorian Ranges in the southeast of mainland Australia (Figure 5.1) and contains the largest known oil deposits in Australia. Stainforth (1984) and Davidson *et al.* (1984) give detailed reviews of the geology and hydrocarbon generation of the basin which contains thick Lower Cretaceous to Recent sediments. Crude oils from this basin are typically reservoired in the Upper Cretaceous to Eocene Latrobe Group (Davidson *et al.*, 1984) with the former being their proposed source (Saxby, 1980). The four crude oils analysed from this basin have been subjected to varying degrees of biodegradation however, unlike the other samples discussed above, the Gippsland Basin samples are not as biodegraded as Mardie or Turtle 2 oil. None of the four samples have been sufficiently biodegraded to alter their hopanoid components.

5.2 COMPOSITIONS OF REARRANGED HOPANES

5.2.1 GC-MS Analysis of Rearranged Hopanes in Crude Oil

Diahopanes and neohopanes were identified in m/z 191 mass chromatograms based on their mass spectral and GC retention characteristics, as shown in Figure 5.2. Diahopane (30) and 30-norneohopane (C_{29}Ts) were assigned by their published retention times relative to the αβ-hopanes (Moldowan *et al.*, 1991). Under standard GC conditions C_{29}Ts has a slightly longer retention time relative to 30-norhopane (29) but shorter than diahopane (30). Nordiahopane (29) and the extended diahopanes (31-33) were also assigned based on their published retention times relative to the extended αβ-hopanes. Moldowan *et al.* (1991) used GC-MS-MS techniques to identify the parent ions of m/z 191 daughter ions in a crude oil alkane fraction and showed a probable pseudohomologous series of C_{29}-C_{33} diahopanes. Each member pair of this series eluted before the corresponding pair of αβ-hopanes with the same carbon number. In Figure 5.2 and throughout this thesis, the assignment of the extended diahopanes in m/z 191 mass chromatograms was based on GC-MS techniques alone and was supported by the mass spectra in so far as all compounds assigned as diahopanes displayed prominent m/z 191 and parent ion responses.

Various hopane and sterane biomarker ratios together with sample information are shown in Table 5.1. These ratios were calculated from peak areas of m/z 191 mass chromatograms (hopanes) and m/z 217 mass chromatograms (steranes).

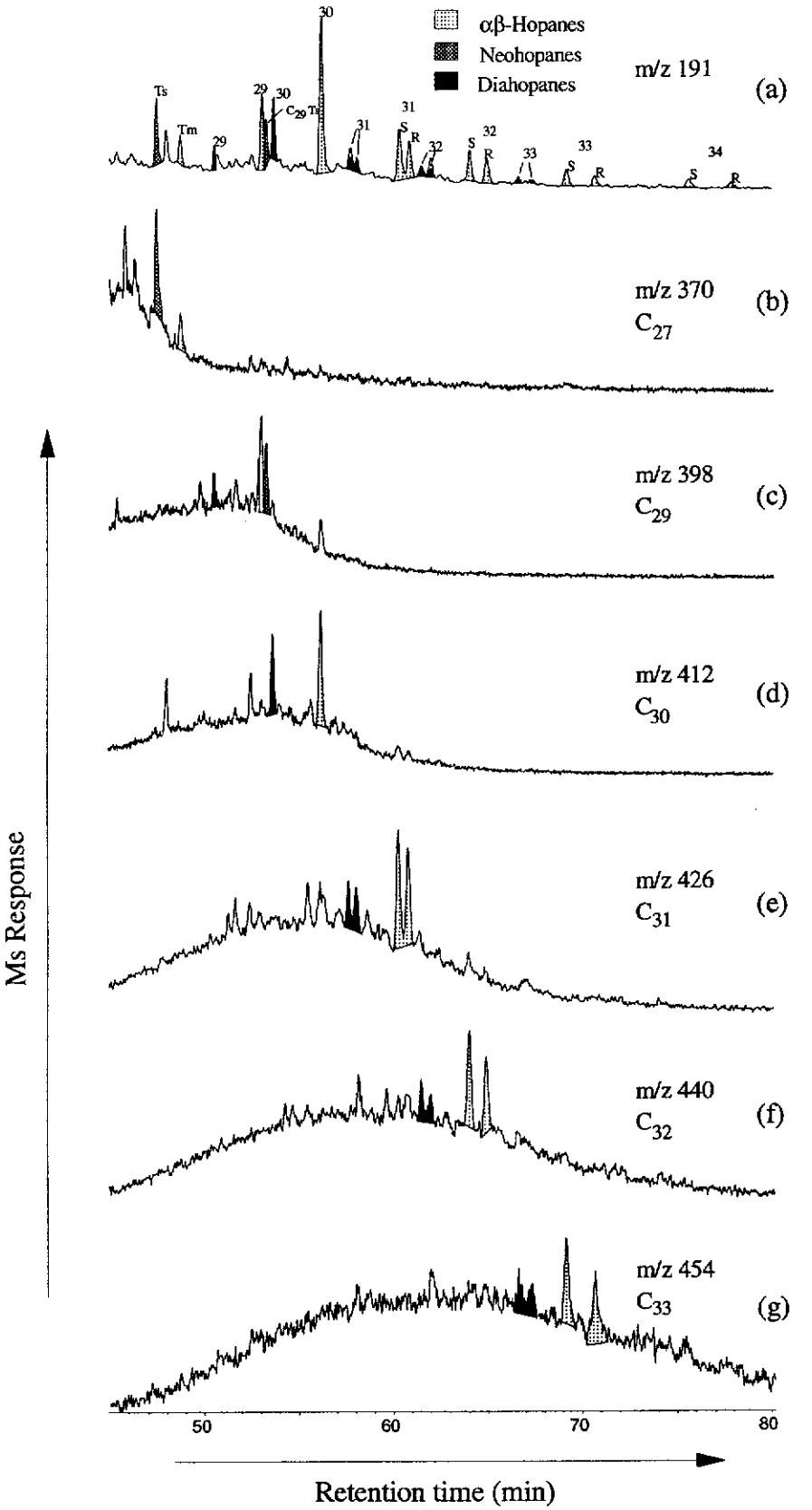


Figure 5.2 Partial m/z 191 (a) and C_{27}, C_{29} to C_{33} molecular ion (b-g) mass chromatograms for a branched and cyclic alkane fraction of a crude oil. Number labels on peaks represent carbon numbers within each compound class.

5.2.2 Results

Suites of crude oils from three Australian basins were analysed for hopanoid biomarkers using GC-MS techniques. Table 5.1 shows geological and geochemical data for each of the crude oils used in this study. The sample suites from each basin contain a range of non-degraded to heavily biodegraded crude oils so that compositional differences in the samples should largely reflect biodegradation effects.

A comparison of the biomarker ratios (Table 5.1) for the various crude oils shows that these values are similar for basin-related samples with the exception of the heavily biodegraded Mardie (Barrow Sub-basin) and Turtle 2 (Bonaparte Basin) crude oils, which contain significantly higher relative amounts of rearranged hopanes. This observation suggests that rearranged hopanes are more resistant to microbial attack than are the hopanes and hence are concentrated in Mardie and Turtle 2 crude oils. However, this simple interpretation of the data in Table 5.1 depends on the assumption that the basin related crude oils were derived from similar source rocks containing organic matter of similar maturity. In fact, this assumption appears to be incorrect when comparing source and maturity parameters of basin related crude oils. Significant differences in the Ts/Tm ratio for the Barrow Sub-basin crude oils (Table 5.1), for example, suggest differences in their source rocks. Since biodegradation is known to dramatically alter the crude oil hopanoids (e.g., Volkman *et al.*, 1983a; Peters and Moldowan, 1993), it is very difficult to predict the original hopanoid composition of a biodegraded crude oil, hence making an assessment of its source and maturity nearly impossible.

In the following sections an attempt has been made to provide a thorough geochemical interpretation of the occurrence of rearranged hopanes in these Australian biodegraded crude oils. Abundant rearranged hopanes in the most

Table 5.1 Sample information and some biomarker ratios for three sets of Australian crude oils.

Location	Crude Oil	Proposed Source	Biodegradation Level [4]	Ts/Tm	C_{29}Ts/ C_{29}H	C_{29}DIA/ C_{29}H	C_{30}DIA/ C_{30}H	C_{31}DIA/ C_{31}H	C_{32}DIA/ C_{32}H	C_{30}DIA/ C_{29}DST	C_{29}D/ C_{30}DIA
Barrow Sub-basin	Barrow UJ	Dingo Claystone [1]	Non-degraded (1)	3.0	0.6	0.2	0.2	0.1	0.4	0.3	0.4
	Flinders Shoal		Severe (8)	1.7	0.5	0.2	0.2	0.1	0.4	0.3	1.7
	Mardie		Extreme (9)	1.0	5.6	3.5	1.3	3.0	2.5	0.3	5.0
Bonaparte Basin	Barnett 1 RFT4 2033m	Milligans Formation [2]	Non-degraded (1)	1.5	0.5	0.3	0.1	0.2	0.2	0.2	<0.1
	Turtle 2 RFT2 1673m		Extreme (9)	1.3	1.3	0.7	0.4	0.5	0.4	0.2	6.0
Gippsland Basin	Tuna	Latrobe Group [3]	Non-degraded (1)	0.6	0.2	0.1	0.1	0.1	0.4	0.4	<<0.1
	West Seahorse		Minor (2)	0.5	0.2	0.1	0.1	0.1	0.4	0.4	<<0.1
	Lakes Entrance		Severe (8)	0.7	0.3	0.1	0.2	0.2	0.6	0.4	0.1
	LeatherJacket		Severe (8)	0.7	0.3	0.1	0.2	0.2	0.4	0.4	0.1

Abbreviations used in this table:

Ts - 22,29,30-Trisnorneohopane
Tm - 22,29,30-Trisnorhopane
C_{29}Ts - 30-Norneohopane
C_{29}H - 30-Norhopane
C_{29}DIA - 30-Nordiahopane
C_{29}DST - *20S+20R* C_{29} diasteranes
C_{29}D - 25-Norhopane
C_{30}DIA - Diahopane
C_{30}H - Hopane
C_{31}DIA - *22S+22R* Homodiahopanes
C_{31}H - *22S+22R* Homohopanes
C_{32}DIA - *22S+22R* Homodiahopanes
C_{32}H - *22S+22R* Homohopanes

[1] Volkman *et al.*, 1983b
[2] Jefferies, 1988
[3] Stainforth, 1984
[4] Numerical values are based on those established by Volkman *et al.* (1983a).

heavily biodegraded samples is interpreted as a function of the organic matter type and maturity of the sediments from which the crude oils were expelled, and the possibility of biodegradation effects on the observed hopanoid distributions is considered.

5.3 EFFECTS OF SOURCE AND MATURITY ON REARRANGED HOPANES IN CRUDE OILS

This section investigates the possibility that abundant rearranged hopanes in Mardie and Turtle 2 crude oils predominantly reflect the composition of their respective source rocks at time of hydrocarbon expulsion, rather than the effects of biodegradation.

5.3.1 Source Effects

An origin from clay-rich, highly mature source rocks appears to be necessary to explain the observed high relative abundances of diahopanes in Mardie and Turtle 2 crude oils. Relative to the regular hopanes with the same carbon number (Table 5.1, $C_{29}H$ to $C_{32}H$) Mardie and Turtle 2 crude oils both contain abundant diahopanes ($C_{29}DIA$ to $C_{32}DIA$), 30-norneohopane ($C_{29}Ts$) and 25-norhopane ($C_{29}NH$). Elevated levels of diahopanes in nondegraded crude oils has been previously associated with clay-rich source rocks containing high amounts of terrestrial organic material deposited under oxic to suboxic conditions (Peters and Moldowan, 1993). However, the unusually high relative abundances of diahopanes in these two crude oils, in particular Mardie, indicates that their respective source rocks were also highly mature, since diahopanes have been reported to be more thermally resistant than the regular hopanes (Moldowan *et al.*,

1991) and so would be selectively preserved during maturation. This is also consistent with the work of Seifert and Moldowan (1978) who observed that the concentration of αβ-hopanes in sediments decreased with increasing maturity, thus suggesting that they may be thermally depleted.

However, high levels of diahopanes and 25-norhopanes in Turtle 2 and especially Mardie crude cannot be adequately explained as simply originating from mature, terrestrially derived source rocks. A proposed origin of Mardie and Turtle 2 crude oils from mature, terrestrially derived source rocks is inconsistent with the co-occurrence of high amounts of 25-norhopanes in these samples. Based on pyrolysis of source rock extracts and shales, Noble *et al.* (1985) concluded that 25-norhopanes did not appear to be formed from kerogen cracking and occurred only as free hydrocarbons in sediments. This suggests that as bound αβ-hopanes are released with increasing sediment maturation, any free 25-norhopanes initially present would be diluted by the regular hopanes in more mature sediments. Based on this reasoning, 25-norhopanes are likely to be in their highest concentration in immature source rocks. Further, Blanc and Connan (1992) have shown that 25-norhopanes are usually associated with marine sediments. High relative concentrations of 25-norhopanes in Mardie and Turtle 2 crude oils therefore may contradict their terrestrial origin.

5.3.2 Maturation Effects

Because source effects cannot be used to explain the elevated relative abundances of 25-norhopanes, norneohopane, and diahopanes in Mardie and Turtle 2 crude oils, an alternative explanation based on differences in thermal stability of hopanoids was investigated. Table 5.2 shows steric energies for various hopanoids calculated using the CSC molecular modeling package along with values previously reported (Moldowan *et al.*, 1991; Kolaczkowska *et al.*, 1990). This table shows lower steric energies for the rearranged hopanes compared to the hopanes. Figure 5.3 shows three-dimensional molecular models which illustrate steric energy differences between some regular and rearranged hopanes. In the case of the hopane skeleton, the methyls at C-14 (C-27) and at C-18 (C-28) are in a high energy 1,3 diaxial conformation. However, in the case of the rearranged hopane skeletons this unfavourable conformation of the two methyls no longer exists. For diahopanes the methyl group at C-14 is relocated to C-15. For neohopanes the methyl group at C-18 is relocated to C-17 (neohopanes). This relief in steric strain results in the enhanced stability of diahopanes and 30-norneohopane compared to αβ-hopanes. Similarly, the removal of the methyl group at C-10 greatly enhances the stability of the 25-norhopanes compared to the αβ-hopanes (Figure 5.3). In the αβ-hopane, C-25 is in an unfavourable 1,3,5 triaxial methyl conformation with C-24 and C-26. The absence of this methyl in the 25-norhopane means that the overall steric crowding of the molecule is lower, as is evident from the calculated steric energies of 335 kJ/mol in 30-norhopane and 292 kJ/mol in 25-norhopane (note: comparisons of steric energies are generally valid only for among isomers). Hence, if a crude oil was subjected to extreme thermal conditions it could become relatively depleted in the regular hopanes and enriched in both 25-norhopanes and rearranged hopanoids if these compounds were present initially.

Table 5.2 Steric energies of some petroleum hopanoids (values were obtained using CSC Chem-3D Plus computer package version 3.1). Note, only steric energies from isomeric compounds can be used to assess relative stabilities.

CARBON NUMBER	COMPOUND	STERIC ENERGY (kJ/mole)
27	22,29,30-Trisnorhopane	(326[a]) 328
27	22,29,30-Trisnorneohopane	(308[b]) 308
29	30-Norhopane	(334[a]) 335
29	30-Norneohopane	(319[b]) 320
29	25-Norhopane	292
30	ββ-Hopane	(373[a]) 373
30	αα-Hopane	(366[a]) 367
30	βα-Hopane (moretane)	(355[a]) 358
30	αβ-Hopane	(344[a]) 346
30	Diahopane	(318[b]) 321
30	8,14-Secohopane	306
31	*22R* αβ-Homohopane	(351[a]) 371*
31	*22S* αβ-Homohopane	(349[a]) 362*

Values reported by [a]Kolaczkowska *et al.* (1990) and [b]Moldowan *et al.* (1991).

*large discrepancies between these values compared to those reported in the literature may be due to the inability of the CSC Chem-3D package to correctly calculate energies associated with extended side chains.

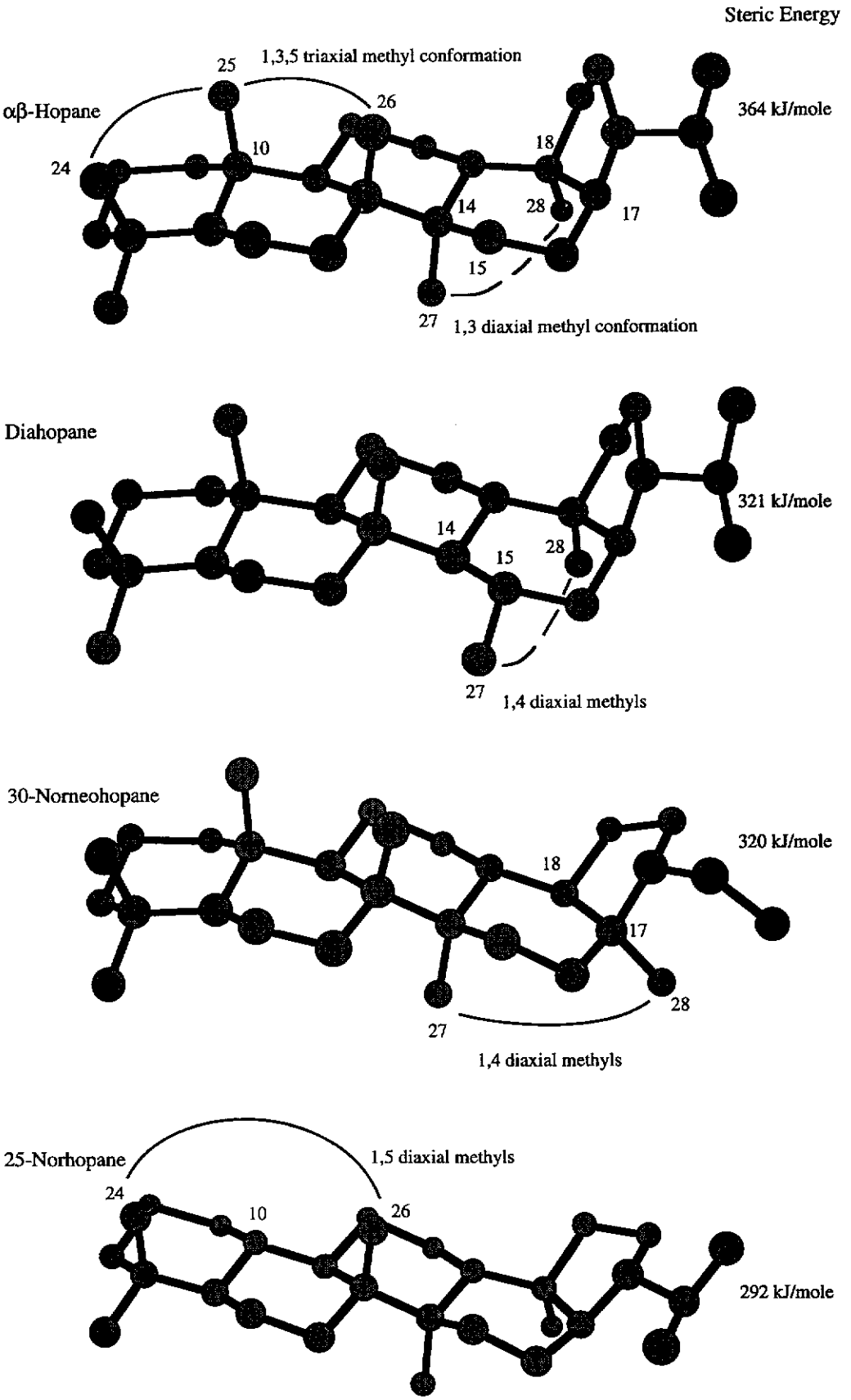


Figure 5.3 Three-dimensional molecular models of some hopanoids in crude oil.

Using this reasoning, enhanced diahopanes and 25-norhopanes and depleted regular hopanes in Mardie and Turtle 2 crude oils may reflect extreme thermal maturation. Maturity assessment of extremely biodegraded crude oils such as Mardie crude oil is however difficult because many of the compounds typically used as maturity indicators are either absent or severely affected by biodegradation. For example, alkylnaphthalenes and alkylphenanthrenes in Mardie are either in very low abundance or have been significantly altered by biodegradation rendering them unreliable for maturity ratios. Volkman *et al.* (1984) however, reported that the relative proportions of short chain (C_{21},C_{22}) and long chain (C_{29}) monoaromatic steranes were similar in Barrow UJ, Flinders Shoal and Mardie crude oils and were typical of mature source rocks. Therefore, if all three crude oils had similar sources, it is unlikely that Mardie oil has been subjected to unusually severe thermal conditions, relative to the Barrow UJ and Flinders Shoal crude oils.

5.4 BIODEGRADATION EFFECTS ON REARRANGED HOPANES

Barrow Sub-basin

The alkane compositions of the three Barrow Sub-basin crude oils were examined to assess their biodegradation history. Figure 5.4 shows gas chromatograms for Barrow UJ, Flinders Shoal and Mardie crude oils which are similar to those previously reported by Volkman *et al.* (1983a; 1983b) who attributed the marked differences in these chromatograms to different biodegradation histories for each crude oil. The gas chromatogram of the Barrow UJ sample shows a typical alkane distribution which is dominated by the *n*-alkane homologous series. The presence of these readily biodegraded alkanes (Volkman *et al.*, 1983a) in the Barrow UJ crude oil indicates that it contains material which has not been subjected to bacterial degradation. The gas chromatograms of

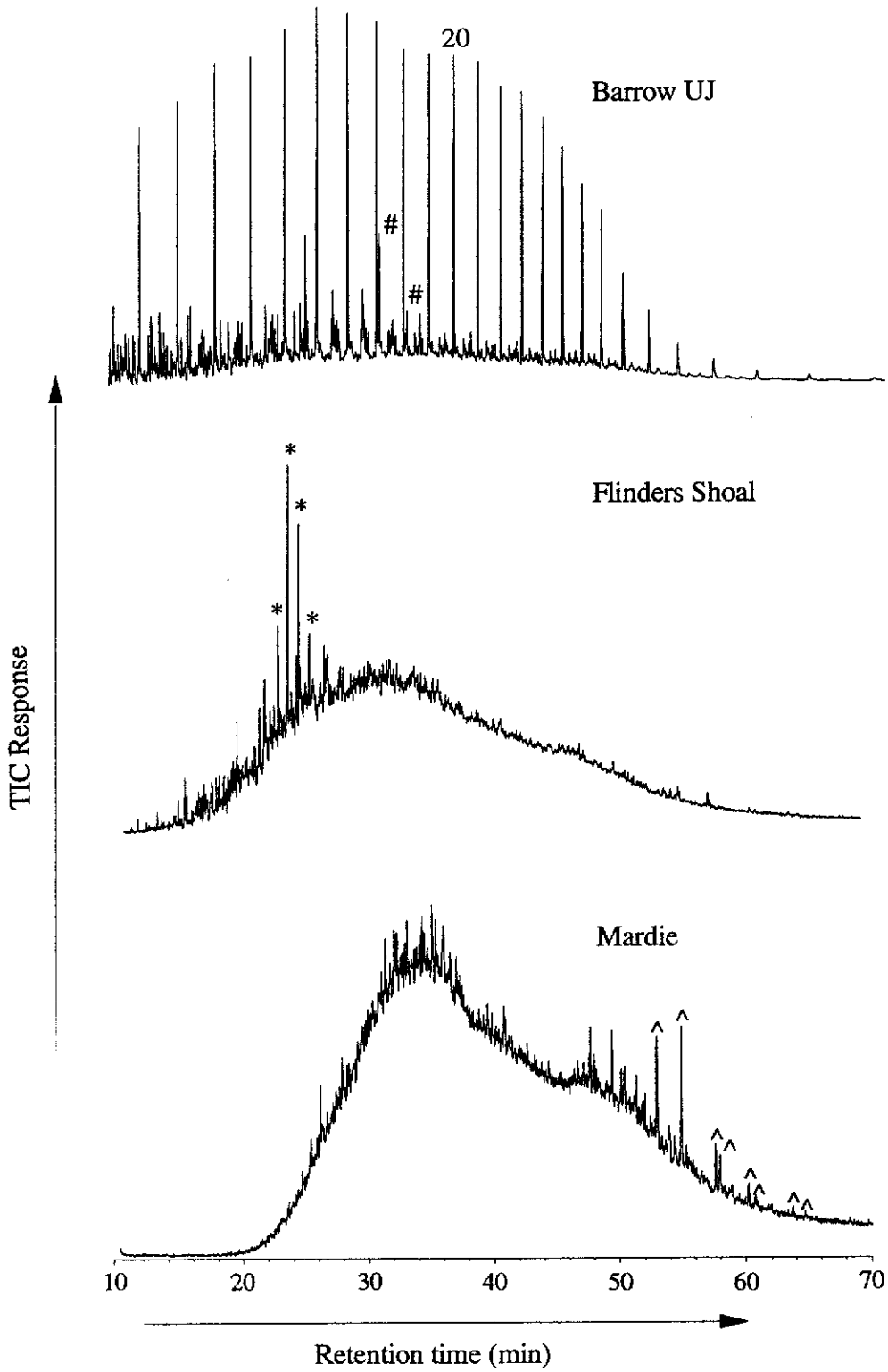


Figure 5.4 Total ion chromatograms for the total alkane fractions from the three crude oils from the Barrow Sub-basin, Western Australia which are similar to those published by Volkman *et al.* (1983a; 1983b). C_{20} *n*-alkane is indicated by (20), the isoprenoid alkanes pristane and phytane by (#), the C_{14} to C_{16} bicyclanes by (*), and the 25-norhopanes by (^).

Flinders Shoal and Mardie crude oils, on the other hand, show no *n*-alkanes and comprise unresolved complex mixtures of branched and cyclic alkanes consistent with the crude oils having been biodegraded. Bicyclic alkanes are absent in Mardie oil but are present in Flinder Shoal crude oil, which indicates that biodegradation has proceeded farther in Mardie (Volkman *et al.*, 1983a).

Figure 5.5 shows partial m/z 191 mass chromatograms for the branched and cyclic alkane fractions from the three crude oils from the Barrow Sub-basin. The mass chromatogram of the Flinders Shoal sample is similar to that of the Barrow sample, except that it contains a higher relative concentration of the highly resistant 25-norhopane represented by the peak labelled $C_{29}D$. The presence of this compound in Flinders Shoal crude oil has previously been interpreted as resulting from mixing of a heavily biodegraded crude oil containing some 25-norhopanes with a less biodegraded crude oil (Alexander *et al.*, 1983; Volkman *et al.*, 1983a). The mass chromatogram of Mardie, on the other hand, shows a dominant peak for 25-norhopane ($C_{29}D$) and relatively minor peaks for the αβ-hopanes. Interestingly, 30-nordiahopane (29), diahopane (30) and 30-norneohopane ($C_{29}Ts$) are present in high relative amounts in Mardie crude oil and hence appear to be more resistant to biodegradation than αβ-hopanes.

Bonaparte Basin

Figure 5.6 shows partial m/z 191 mass chromatograms which reveal the hopane distributions in Barnett 1 and Turtle 2 crude oils. The very similar sterane distributions of the two crude oils, shown in the insets of this figure, support the suggestion made by Jefferies (1988) that these crude oils were derived from similar sources. The greater relative abundances of rearranged hopanes in the extremely biodegraded Turtle 2 crude oil is apparent from comparison of the m/z 191 mass chromatograms in Figure 5.6 and the hopane ratios in Table 5.1.Therefore,

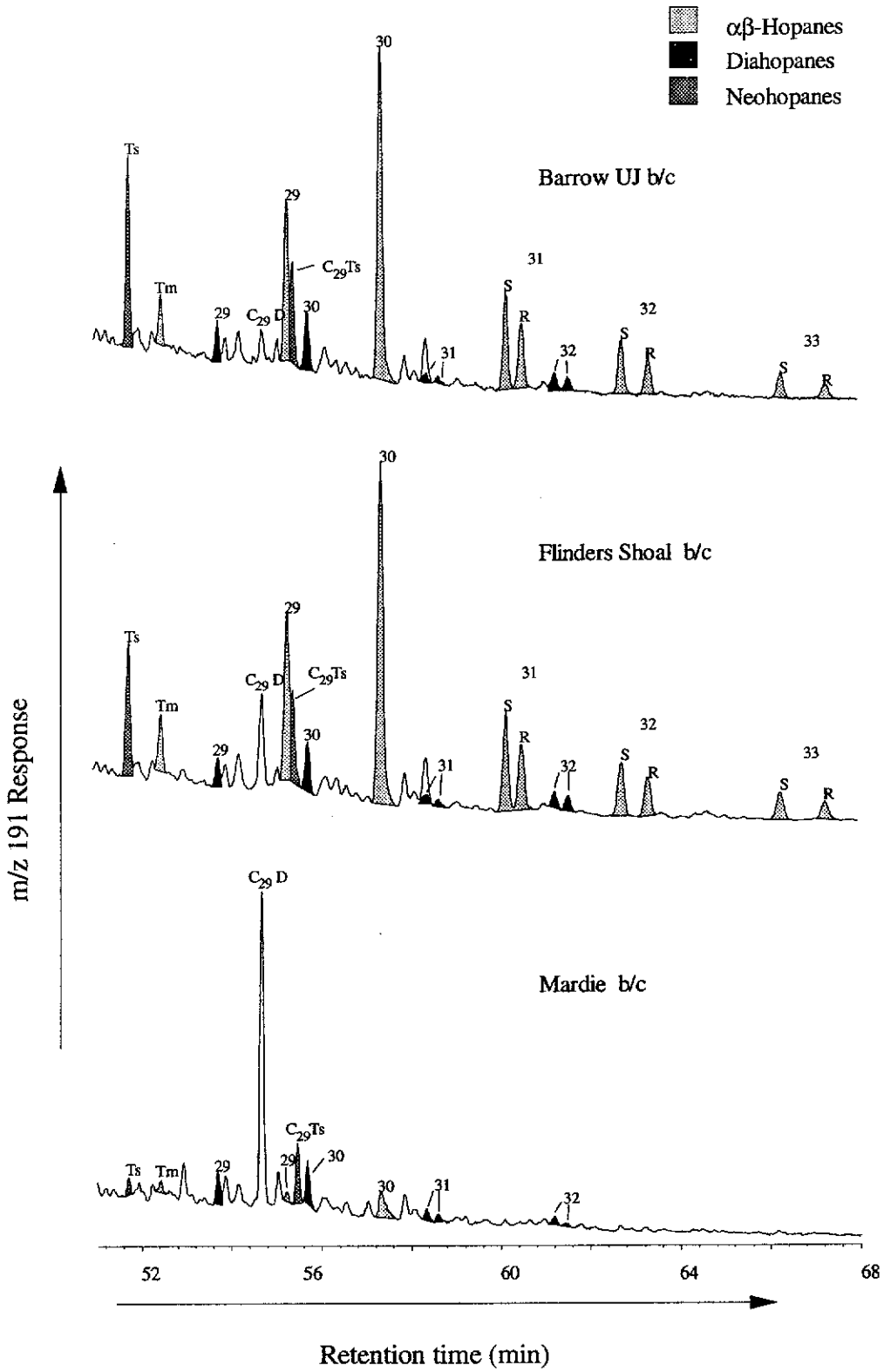


Figure 5.5 Partial m/z 191 mass chromatograms for the branched and cyclic alkane fractions of three crude oils from the Barrow Sub-basin. Numbers 29-32 denote the total number of carbons in each homologue; Ts=22,29,30-trisnorneohopane, Tm=22,29,30-trisnorhopane, $C_{29}D$=25-norhopane, $C_{29}Ts$=30-norneohopane.

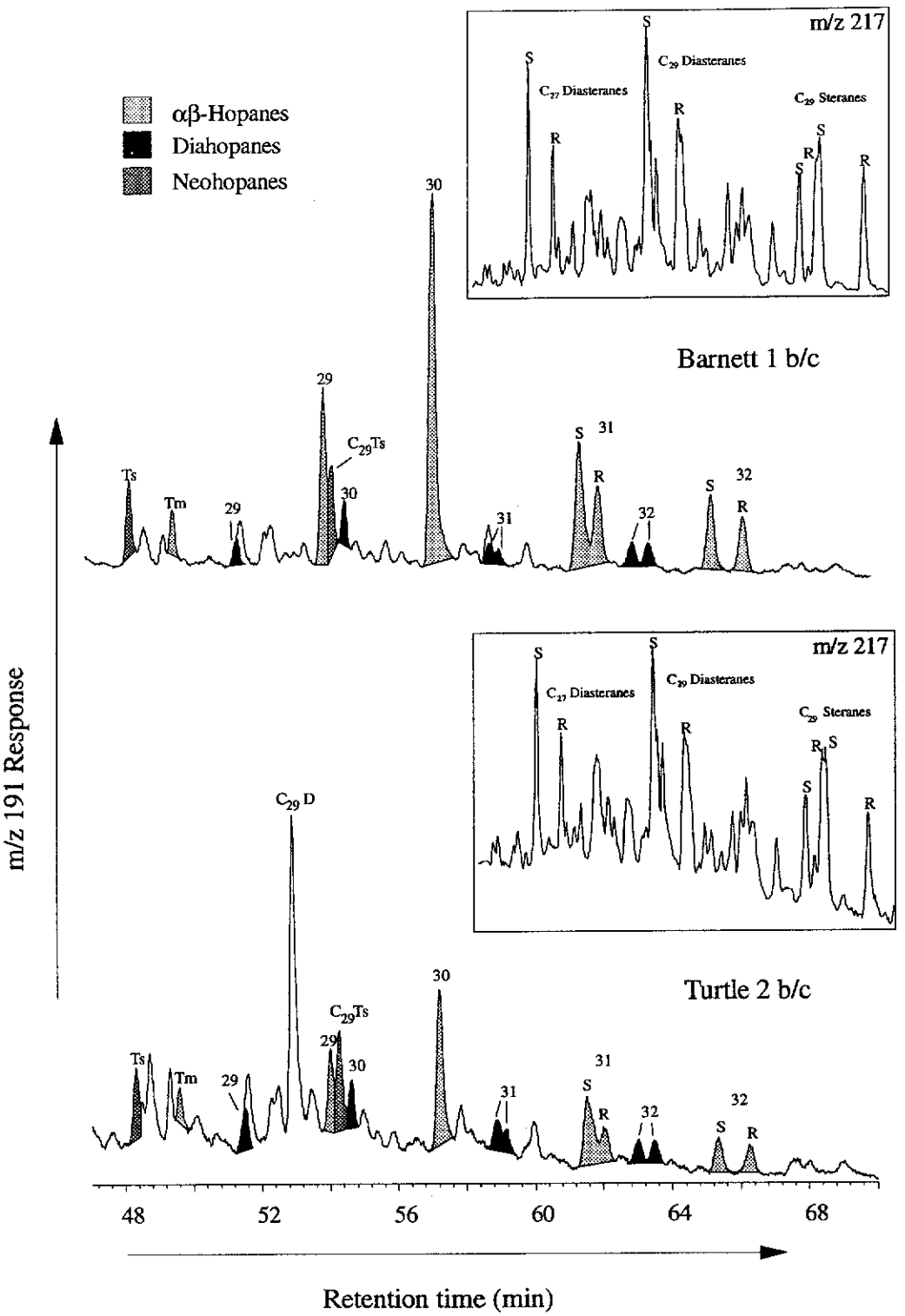


Figure 5.6 Partial m/z 191 mass chromatograms for the branched and cyclic alkane fraction of two crude oils from the Bonaparte basin. The m/z 217 mass chromatograms for these alkane fractions are shown in the insets. Numbers 29-32 denote the total number of carbons in each homologue; Ts=22,29,30-trisnorneohopane, Tm=22,29,30-trisnorhopane, $C_{29}D$= 25-norhopane, $C_{29}Ts$=30-norneohopane.

enhanced abundances of rearranged hopanes are again attributed to their greater resistance to thermal degradation relative to the hopanes.

Gippsland Basin

Crude oils from the Gippsland Basin (Table 5.1) show similar values for a given rearranged hopanes to hopane ratio. Since none of these crude oils shows regular hopanes altered by biodegradation, this observation suggests that enhanced abundances of rearranged hopanes in the Mardie and Turtle 2 crude oils are in fact caused by preferential degradation of the hopanes.

Biodegradation of rearranged hopanes

The extent of biodegradation of rearranged hopanes in Mardie oil was assessed by comparing the relative abundances of hopane (30) and 30-norneohopane (C_{29}Ts) in the nondegraded Barrow UJ sample with those of 25-norhopane (C_{29}D) and 30-norneohopane (C_{29}Ts) in the Mardie sample (Figure 5.5). Assuming that hopane in Barrow UJ had been demethylated to produce 25-norhopane in Mardie without alteration of 30-norneohopane (C_{29}Ts), then the relative response of peak labelled C_{29}D (i.e. relative to that labelled C_{29}Ts) in the mass chromatogram from Mardie should be only about one half the relative response of peak labelled 30 in the mass chromatogram of the Barrow crude oil since the 191 fragment is derived only from the D/E ring moiety of C_{29}D while the peak labelled 30 (hopane) has a 191 contribution from both the A/B and D/E ring moieties. Clearly the relative response from C_{29}D, shown in Figure 5.5, is much higher in Mardie than anticipated suggesting that 30-norneohopane and the diahopanes are indeed degraded but at a slower rate than the αβ-hopanes.

Furthermore, the increased abundance of 30-nordiahopane (29) and diahopane (30) relative to those of the extended homologs (31+32) in the Mardie sample compared to the Barrow UJ sample suggests that the C_{29} and C_{30} compounds are

depleted at a slower rate than their extended chain homologues. This may be attributed to preferential bacterial attack upon the saturated side chain in the extended diahopanes which parallels the reported degradation of extended αβ-hopanes prior to their lower carbon number homologues (Rullkötter and Wendisch, 1982).

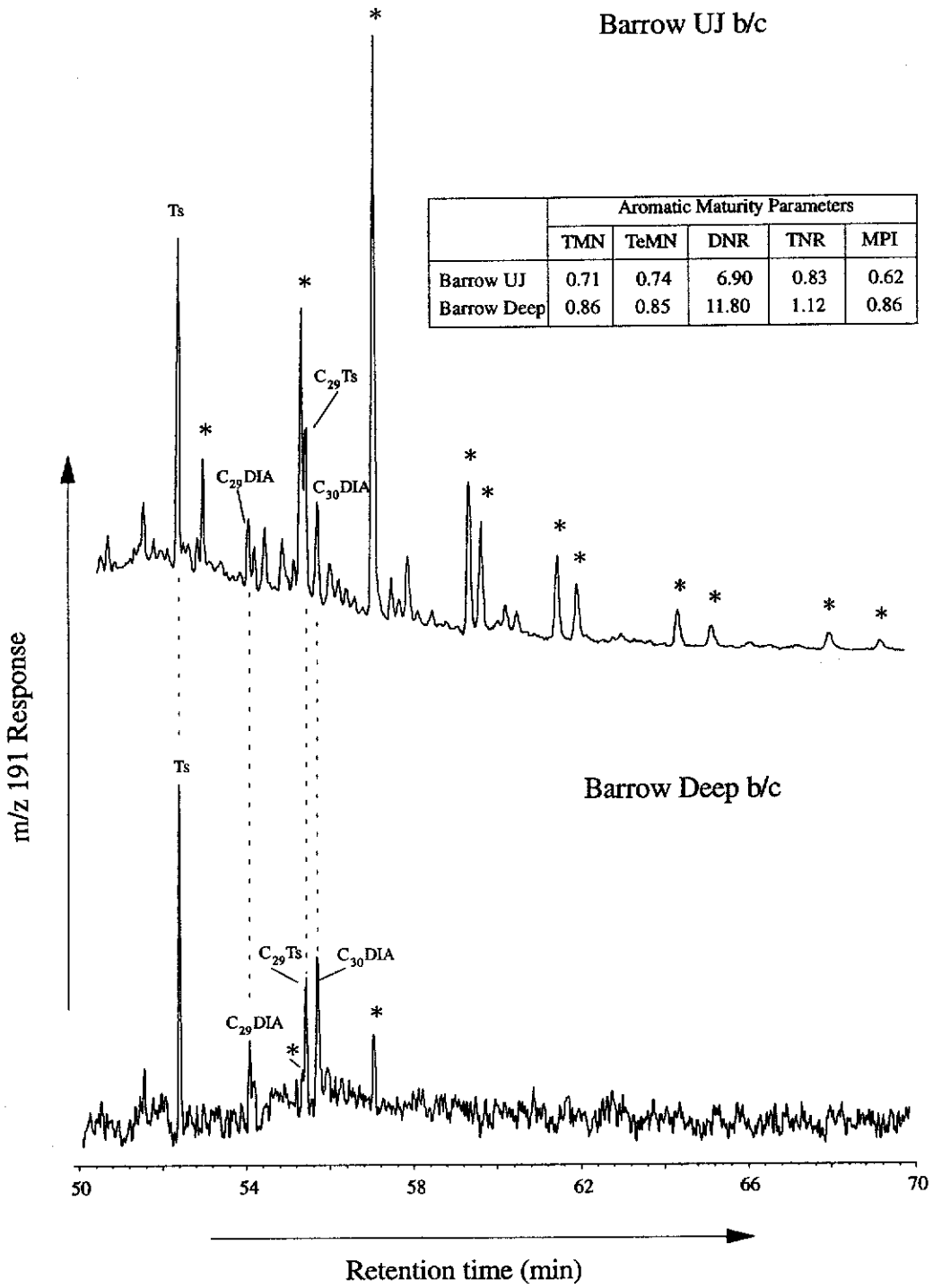
The branched and cyclic alkane fraction from Mardie crude oil was analysed for possible hydrocarbon degradation products of 30-norneohopane and the diahopanes. The reported occurrence of 25,30-bisnorneohopane in a biodegraded asphalt (Trendel *et al.*, 1990) suggests that 30-norneohopane may undergo demethylation processes similar to those proposed for the αβ-hopanes (Volkman *et al.*, 1983a). However, a detailed analysis of a Mardie alkane fraction enriched in diahopanes using US-Y molecular sieves provided no evidence for the presence of the 25,30-bisnorneohopane or 25-nordiahopanes. However, it is possible that diahopanes are degraded to non-hydrocarbon products that were not detected by the analytical procedure.

Finally, the relative abundances of diahopanes and diasteranes within sample suites were compared to assess their relative resistances to biodegradation. Table 5.1 shows values for the relative abundances of these compounds in the crude oils. Within each sample suite the ratio C_{30}DIA/C_{29}DST was the same for all crude oils regardless of their biodegradation history suggesting that diahopanes and diasteranes have similar resistance to biodegradation. Since diasteranes are presumed to form during catagenesis, the similarity in their abundance with diahopanes supports the suggestion made by Moldowan *et al.* (1991) that diahopanes too have a catagenetic origin rather than being formed via bacterial alteration of the αβ-hopanes.

5.5 ORIGIN OF 25-NORHOPANES IN CRUDE OIL

Data in Table 5.1 provide additional evidence on the disputed origin of 25-norhopanes in biodegraded crude oil. Previously, 25-norhopanes have been reported to originate from either bacterial demethylation of the hopanes (e.g. Volkman *et al.*, 1983a; Peters and Moldowan 1991) or, alternatively, from early diagenetic processes and are only observed in biodegraded crude oils due to the selective removal of the hopanes (e.g. Blanc and Connan, 1992). Since diahopanes are presumed to have a diagenetic origin (Moldowan *et al.*, 1991) and have a high resistance to biodegradation, their abundances in biodegraded crude oils can be used to infer the origin of the 25-norhopanes. A comparison of the relative abundance ratio $C_{29}NH/C_{30}DIA$ (Table 5.1) shows that it increases with increasing biodegradation. This suggests that 25-norhopane is being formed in the crude oils.

A very mature crude oil from the Barrow Deep well in the Barrow Sub-basin was analysed for its hopane distribution to assess the effect of extreme thermal maturation on the relative abundances of rearranged hopanes. Figure 5.7 shows m/z 191 mass chromatograms for the Barrow Deep crude oil and an adjacent, possibly source-related, Barrow UJ crude oil along with some calculated aromatic maturity parameters. The Barrow Deep crude oil has an unusual hopanoid distribution dominated by neohopanes (Ts, $C_{29}Ts$) and diahopanes ($C_{29}DIA$, $C_{30}DIA$) with only minor amounts of the regular hopanes. Comparison of steric energies of these hopanoids (Table 5.2) and the aromatic maturity parameters (Figure 5.7) indicates that the low levels of hopanes in this sample are caused by their lower stability and preferential destruction under extreme thermal conditions. However, if 25-norhopanes are indigenous to source material, they too should be enriched in the Barrow Deep crude oil along with the rearranged hopanes. Analysis



	Aromatic Maturity Parameters				
	TMN	TeMN	DNR	TNR	MPI
Barrow UJ	0.71	0.74	6.90	0.83	0.62
Barrow Deep	0.86	0.85	11.80	1.12	0.86

Figure 5.7 Partial m/z 191 mass chromatograms for the branched and cyclic alkanes from two crude oils from the Barrow Sub-Basin. The hopanes are denoted by (*) while the rearranged hopanes are abbreviated Ts and C_{29}Ts (neohopanes) and C_{29} DIA and C_{30}DIA (diahopanes). TMN (trimethylnaphthalene) = 2,3,6/(2,3,6+1,2,5); TeMN (tetramethylnaphthalene) = 1,3,6,7/(1,3,6,7+1,2,5,6); DNR (dimethylnaphthalene ratio) = (2,6+2,7)/1,5; TNR (trimethylnaphthalene ratio) = 2,3,6/(1,4,6+1,3,5); MPI (methylphenanthrene index) = 1.5[(2+3)/(P+1+9)].

of an m/z 177 mass chromatogram for this sample showed no evidence for 25-norhopanes suggesting that, in this case at least, these compounds were not formed in the source rocks. Therefore, assuming that the sources of Barrow Deep and the nearby Barrow Sub-basin crude oils are similar, 25-norhopanes in Flinders Shoal and Mardie were most likely derived from bacterial demethylation of the hopanes as previously suggested by Volkman *et al.* (1983a), and not simply enriched by maturation.

5.6 CONCLUSIONS

Abundant diahopanes, 30-norneohopane, and 25-norhopanes relative to αβ-hopanes were observed in two extremely biodegraded crude oils from Australia. A comprehensive geochemical interpretation of these results suggests that the prominence of these three compound types in these samples is most likely due to selective bacterial degradation of the αβ-hopanes to give 25-norhopanes, which in turn results in enhanced abundances of diahopanes and 30-norneohopane.

Abundant diahopanes and 30-norneohopane were also observed in a crude oil of a very high maturity. This was attributed to the greater resistance of these compounds to thermal maturation compared with the αβ-hopanes. Absence of thermodynamically stable 25-norhopanes in this sample provided evidence supporting their reported origin from demethylation of αβ-hopanes in crude oil, rather than from diagenetic processes in sediments.

CHAPTER 6

ANALYSIS OF HIGHER PLANT-DERIVED TRITERPANES IN SEDIMENTARY ORGANIC MATTER

Summary

Molecular sieve chromatography of a higher-plant derived crude oil improves better analysis of unusual pentacyclic triterpane components. The branched and cyclic alkane components were chromatographed using a US-Y sieve column and the eluted fractions were analysed using GC-MS techniques. The compositions of the first two fractions differ markedly from the unchromatographed mixture in that they were enriched in the various higher-plant components, some of which had previously not been reported. A comparison of mass spectral data, GC retention, and molecular sieve sorption characteristics of these compounds with known triterpanes used to suggest structures for the unknown compounds.

The US-Y chromatography procedure has also been applied to investigate the occurrence of bicadinanes in sedimentary organic matter older than Cretaceous. Rocks from the Jurassic of the Eromanga Basin were subjected to the sieving technique in order to identify trace amounts of bicadinanes. These angiosperm-derived compounds were indeed present in some of the samples, thus extending the Eocene age-range of sedimentary organic matter known to contain bicadinanes, as well as suggesting a possible pre-Cretaceous evolution of angiosperm-like plants.

Using a molecular modeling package, steric energies for a number of higher-plant derived triterpanes such as the bicadinanes, spirotriterpane, oleananes and taraxastanes have been reported for the first time. Relative thermodynamic stabilities of these non-hopanoids have been used to explain their relative abundances in sedimentary organic matter.

6.1 ANALYSIS OF HIGHER-PLANT DERIVED TRITERPANES IN A CRUDE OIL FROM INDONESIA

6.1.1 Sample

SSB crude oil used in this study was recovered from the South Sumatra Basin, Indonesia (see Figure 3.4 for location). The geology of this basin has been detailed elsewhere (e.g. Davies, 1984; de Coster, 1975; Daly *et al.*, 1987). Crude oils from this basin are believed to be sourced from fluvio-deltaic Tertiary sediments of the Talang Akar Formation (de Coster, 1975; Robinson, 1987; Suseno *et al.*, 1992) and are characterised by abundant of higher-plant derived biomarkers, including bicadinanes, oleanane (18β-oleanane), 18α-oleanane and other triterpenoids of unknown structure (Sosrowidjojo *et al.*, 1994a).

6.1.2 Analysis

SSB crude oil was subjected to liquid chromatography using silicic acid and *n*-pentane as the eluent to obtain a total alkane fraction. Subsequent treatment with ZSM-5 removed the *n*-alkanes and methylalkanes leaving a branched and cyclic alkane fraction. This fraction was chromatographed on a column of ultrastable-Y molecular sieves using *n*-pentane. Fractions of eluent were collected and analysed using GC-MS techniques. Fractions 1 and 2 from molecular sieve chromatography of the branched and cyclic alkanes from SSB crude oil were subjected to detailed GC-MS analysis using two columns in order to resolve the complex mixture of pentacyclic terpanes.

6.1.3 Fractionation of C_{29} and C_{30} triterpanes

The pentacyclic triterpane region of m/z 191 mass chromatograms obtained for higher plant-derived crude oils commonly shows a complex distribution of peaks (*cf.* van Aarssen *et al.*, 1992; Rullkötter *et al.*, 1994). In such samples, the ubiquitous αβ-hopanes series (Appendix, III) may be less easily identified due to an abundance of higher-plant derived non-hopanoids, making the analysis of individual components more difficult. Furthermore, the relatively low amounts of minor non-hopanoid components means that little structural information is available on these compounds.

US-Y molecular sieve chromatography of such a complex mixture of compounds overcomes the problems described above by separating hopanoid from non-hopanoid components as well as enriching minor components to enable further analysis. The resulting eluted fractions from the US-Y column contain less complex triterpane distributions which enable a more detailed analysis of individual components. Figure 6.1 shows partial m/z 191 mass chromatograms of the initial branched and cyclic alkane fraction together with those of Fraction 1 and Fraction 2 from molecular sieve chromatography of the SSB crude oil. As discussed in Chapter 3, triterpanes which elute in Fraction 1 are too large to enter the sieve channels and thus are not retained on the sieve column, while components in Fraction 2 are small enough to enter the sieve and hence be retained on the column. The αβ-hopanes which eluted in Fraction 3 are not shown in this figure. Compound assignments are listed in Table 6.1 and comprise hopanoids, non-hopanoids, and a number of previously unreported triterpanes.

Figure 6.1 shows that Fraction 1 contains several higher-plant derived triterpanes and no αβ-hopanes. 30-Norhopane (7), which coelutes with the spirotriterpane (8) (Appendix, XII; Hills and Whitehead, 1966), is not present in Fraction 1 or 2 and has therefore been sorbed by the sieve. The 30-normoretane

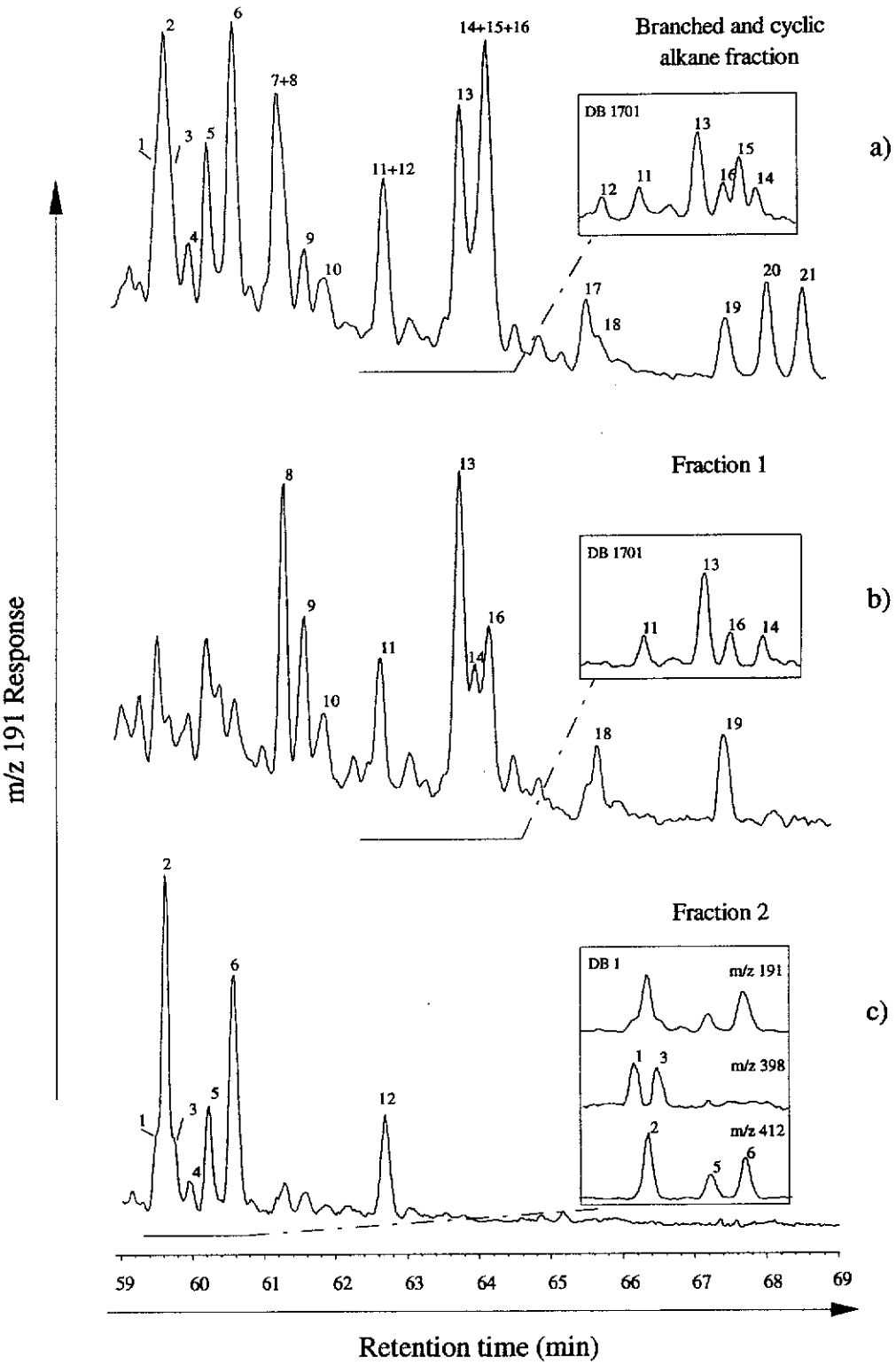


Figure 6.1 Partial m/z 191 mass chromatograms of the branched and cyclic alkane fraction of SSB crude oil (a) and the first two eluted fractions from the sieve column (b-c). Insets show the elution order of some triterpenoid components using a DB 1701 column (a-b) and mass chromatograms showing molecular ion responses (DB 1 column) (c) Compound identifications are in Table 6.1.

Table 6.1 Triterpanes present in the branched and cyclic alkane fraction from SSB crude oil from Indonesia (a - Ekweozor *et al.*, 1979; b - Moldowan *et al.*, 1984; c - Ensminger *et al.*, 1977 and van Dorsselaer *et al.*, 1977b; d - Hills *et al.*, 1968; e - Whitehead, 1974; f - Kimble *et al.*, 1974).

Peak No.	Triterpane	Structure	Reference
1*	C_{29} Triterpane	A	-
2	C_{30} Triterpane	-	a
3*	C_{29} Triterpane	B	-
4	28,30-Bisnorhopane	IIIb	b
5	C_{30} Triterpane	-	a
6	C_{30} Triterpane	-	a
7	30-Norhopane	IIIc	c
8	Spirotriterpane	XII	d
9*	C_{29} Ttriterpane	C	-
10*	C_{29} Triterpane	D	-
11	30-Normoretane	IIc	c
12*	C_{30} Triterpane	E	-
13	18β + 18α-Oleananes	XIV, XVa	e
14*	C_{29} Triterpane	F	-
15	αβ-Hopane	IIId	c
16*	C_{30} Triterpane	G	-
17	Moretane	IId	c
18*	C_{30} Triterpane	G	-
19	Taraxastanes	XVI	f
20	*22S* αβ-Homohopane	IIIe	c
21	*22R* αβ-Homohopane	IIIe	c

* Denotes tritepanes reported for the first time which have been tentatively assigned structures A-G; Roman numerals refer to rigorously determined structures shown in Appendix.

(11) and a coeluting C_{30} triterpane (12), which are completely resolved on a DB 1701 column (inset Figure 6.1a), have also been separated by the sieving procedure and elute in Fractions 1 and 2, respectively (Figure 6.1b,c). A C_{29} (14) and a C_{30} (16) triterpane, which are also resolved on a DB 1701 column (inset Figure 6.1a) are easily detected in Figure 6.1b due to the selective removal of αβ-hopane (15). Further, a C_{30} triterpane (18) which elutes closely following moretane (17) is enriched in the first fraction. Finally, the coeluting oleanane and 18α-oleanane (13) (Appendix, XIV and XV respectively) and the coeluting taraxastane isomers (19), which have larger diameters than those of the sieve channels due to their 6-membered E-ring (see Table 3.1), are present in Fraction 1.

The chromatogram for Fraction 2 (Figure 6.1c) shows two C_{29} (1,3) and three C_{30} (2,5,6) triterpanes. The inset in this figure shows their molecular ion responses. The two C_{29} triterpanes (1,3) observed in this crude oil appear not to have been reported previously in the chemical literature. However, close inspection of published m/z 191 mass chromatograms of crude oils as well as published mass spectra reveals that the C_{30} triterpanes in this sample have the same GC retention behaviour as those previously reported (Ekweozor *et al.*, 1979; Ekweozor and Udo, 1988; Czochanska *et al.*, 1988; van Aarssen *et al.*, 1992; Woolhouse *et al.*, 1992, Rullkötter *et al.*, 1994) as higher plant-derived triterpanes of unknown structure. As well as showing a molecular ion at m/z 412, the previously published mass spectra of triterpanes 2, 5 and 6 (Ekweozor *et al.*, 1979) all show an ion at m/z 397 [M-15] and a base peak at m/z 191. The mass spectra for triterpanes 2 and 5 also contain an ion at m/z 369 indicating the loss of an isopropyl group. Based largely upon these mass spectral data, Ekweozor *et al.* (1979) tentatively assigned triterpanes 2 and 5 as neohopane isomers and triterpane 6 as being an oleanane isomer. However, in light of the recent identification of 30-norneohopane (C_{29}Ts, Moldowan *et al.*, 1991) in crude oil, which has a significantly longer retention time than triterpanes 2 and 5 eluting after 30-norhopane (7), it is unlikely that these

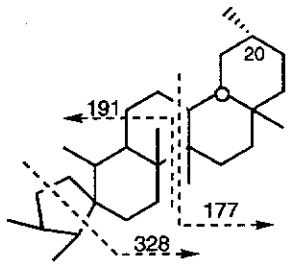
triterpanes have neohopane skeletons. Since triterpanes 2, 5 and 6 were sorbed by the sieve and eluted in Fraction 2 (Figure 6.1c), these compounds may have structures related to those tentatively proposed in the following section for triterpanes 1, 3 and 12, which elute in the same fraction (Figure 6.1c).

6.1.4 Suggested Structures for Triterpanes

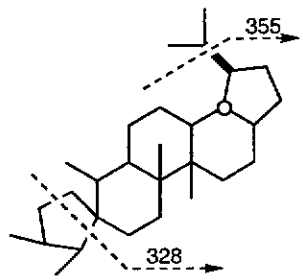
The unknown triterpanes listed in Table 6.1 have been tentatively assigned structures based on mass spectral features as well as GC and US-Y molecular sieve retention behaviour. The numbers given to each mass spectrum in Figure 6.3 correspond to peaks assigned in Figure 6.1.

The mass spectra of triterpanes 1, 3 and 9 all show molecular ions at m/z 398 indicating C_{29} pentacyclic compounds. They all also show an unusual fragment ion at m/z 328 [M-70]. An analogous fragment ion at m/z 342 [M-70] for a C_{30} component has been reported in the spectrum of the C_{30} spirotriterpane (Hills and Whitehead, 1966). This M-70 ion has been suggested by Woolhouse *et al.* (1992) to be due to cleavage of a C-5 fragment from the spiro-A ring, as shown in Appendix (XII). In the present context, this suggests that triterpanes 1, 3 and 9 may have structures incorporating a five-membered spiro A-ring system.

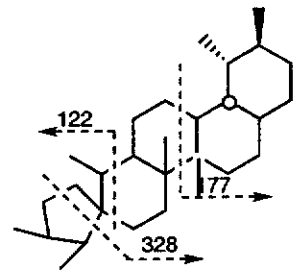
The mass spectrum of triterpane 1 also shows a fragment ion at m/z 383 [M-15] and a base peak at m/z 177. A structure which is consistent with this information appears to be a C_{29} pentacyclic compound with a spiro-A ring and a six-membered E-ring. Triterpane 1 eluted in Fraction 2 indicating that it is weakly sorbed by the sieve, unlike the C_{30} spirotriterpane (8) which eluted in Fraction 1. Hence, it is speculated that the absence of a methyl in the C_{29} compound gives a lower cross-sectional diameter of the molecule. Examination of a model of the C_{30} spirotriterpane indicates that the β (equatorial) methyl on C-20 is the structural element which controls the effective diameter of the molecule. Hence, absence of



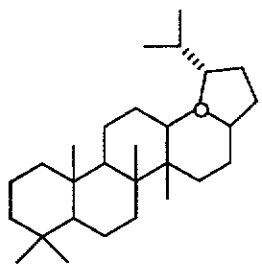
A - Triterpane 1



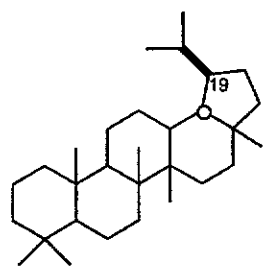
B - Triterpane 3



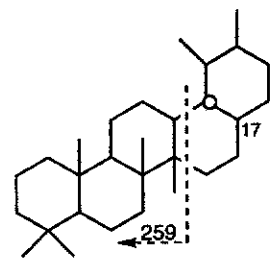
C - Triterpane 9



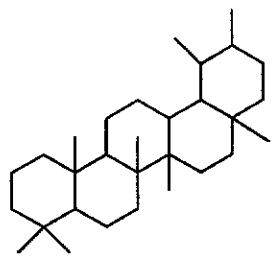
D - Triterpane 10



E - Triterpane 12



F - Triterpane 14



G - Triterpanes 16 & 18

Figure 6.2 Suggested structures for some triterpanes in SSB crude oil.

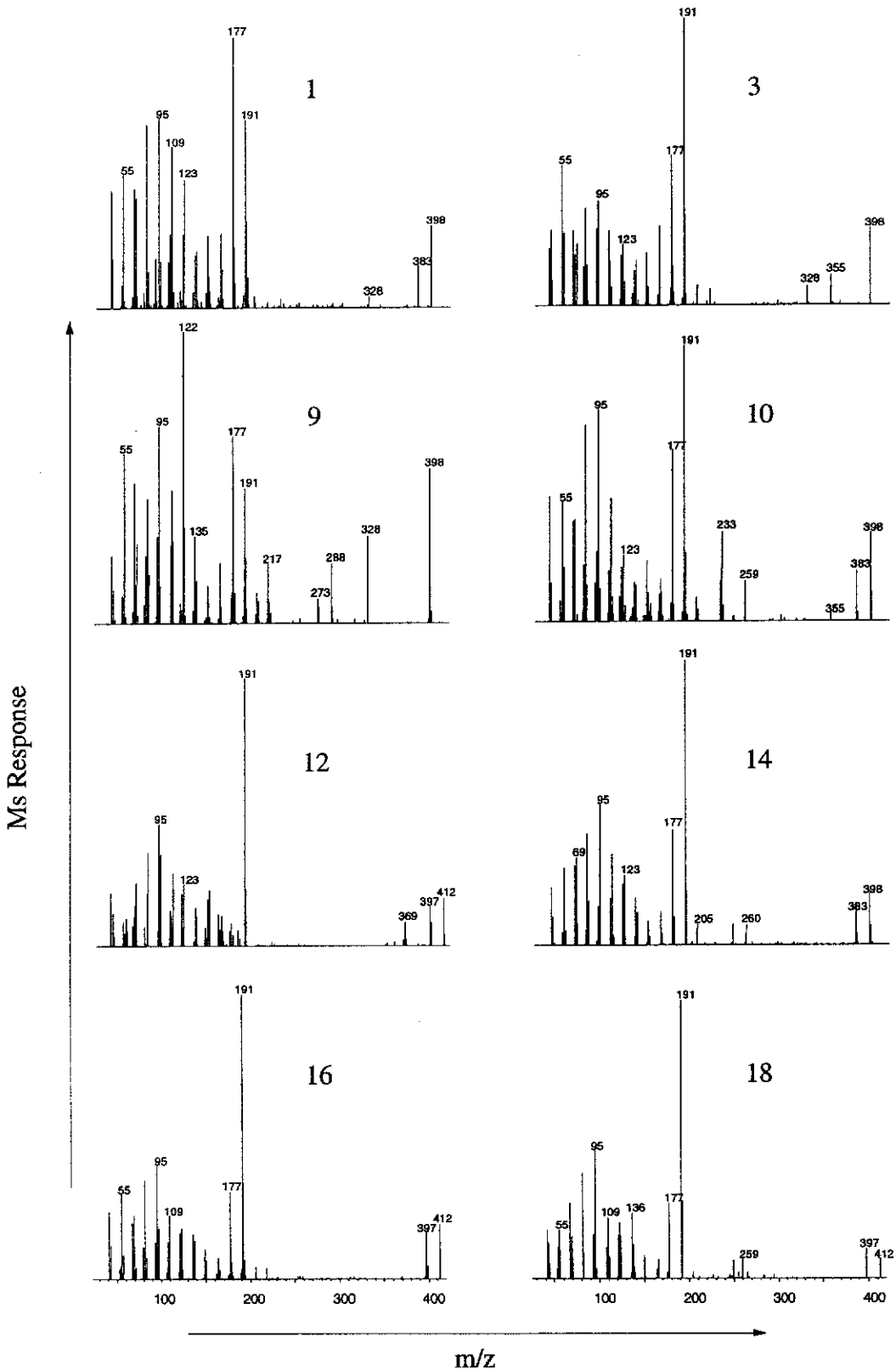


Figure 6.3 Mass spectra (corrected for background) of structurally unknown C_{29} and C_{30} triterpanes in SSB crude oil. The numbers given to each mass spectrum correspond to the peaks assigned in Figure 6.1.

this substituent could account for the observed sorption behaviour. Triterpane 1 has therefore been tentatively assigned 30-norspirotriterpane (Figure 6.2, A).

The mass spectrum of triterpane 3 shows a C_{29} molecular ion at m/z 398 and fragment ions at m/z 355 [M-43] and m/z 328 [M-70]. These features suggest a spiro A-ring system and an isopropyl substituent. The two most common ring systems for potential precursors of such a spiro compound are the lupane and hopane types. A lupane precursor is more likely because natural products based on this skeletal type, unlike those of the hopane-type, are commonly functionalised at C-3. They can therefore facilitate formation of a carbocation at this position and initiate the process that results in spiro ring formation. A computer model of a spiro A-ring lupane shows that the presence of an α-isopropyl substituent on the E-ring would cause the molecule to be completely excluded from the sieve. This is consistent with the observation from a separate experiment where lupane itself, having an α-isopropyl substituent at C-19, was excluded from the US-Y sieve. Models also show that a β-isopropyl substituent at C-19 position does not extend the overall dimensions of the molecule and hence would less hinder its sorption by the sieves. Since triterpane 3 eluted in Fraction 2 and so was slightly retained by the sieve column, a 19α(H) spiro-norlupane structure (Figure 6.2, B) is suggested for this compound. The absence of a methyl substituent at C-17 in structure B is suggested as this is a common position for labile oxygen-containing functional groups which could be removed during diagenesis.

The mass spectrum of triterpane 9 shows a very strong C_{29} molecular ion at m/z 398 and fragment ions at m/z 383 [M-15] and m/z 328 [M-70] and a base peak at m/z 122. The mass spectrum of this component shows no evidence for loss of methyl or isopropyl substituents from the molecule and has a low intensity m/z 191. Based on a consideration of GC retention behaviour, a spiro-taraxastane rather than a spiro-oleanane skeleton is indicated: Triterpane 9 cannot be a C_{29} spiro-oleanane because it elutes after the well-characterised C_{30} spiro-oleanane,

spirotriterpane (8). Since the taraxastanes (19) have longer retention times than the oleananes (13) it is likely that the corresponding C_{29} spiro-taraxastane would elute after a C_{29} spiro-oleanane. Hence, this C_{29} compound with a relatively long GC retention time may be a spiro-taraxastane (Figure 6.2, C). As mentioned above for triterpane 3, the absence of a methyl substituent at C-17 is suggested since this is a common position for functional groups in natural products.

Triterpane 10 has a mass spectrum showing a strong C_{29} molecular ion at m/z 398 along with fragment ions at m/z 383 [M-15], m/z 355 [M-43] and m/z 259. These features, together with its exclusion from the sieve channels (contained in Fraction 1) are all consistent with a lupane carbon skeleton having lost a methyl group. The most likely positions where a methyl substituent may be missing are at C-4 (24-nor) or C-17 (28-nor), since lupane natural products often contain functional groups at these positions (*cf.*, Connolly and Overton, 1972; Dev *et al.*, 1989). Based on GC retention behaviour of this compound, it cannot be 24-norlupane which is reported to elute just before 30-norhopane (Curiale, 1991). Hence a 28-norlupane structure (Figure 6.2, D) is tentatively assigned to triterpane 10.

The mass spectrum of triterpane 12 shows a C_{30} molecular ion at m/z 412 along with fragment ions at m/z 397 [M-15], and m/z 369 [M-43]. These features are consistent with ring-A contracted triterpanes (*cf.*, ten Haven *et al.*, 1992a) as well as triterpanes with lupane-type or hopane-type carbon skeletons. A ring-A contracted structure was discounted because examination of computer models indicates that the A-ring isopropyl in such compounds has an unfavourable configuration for sorption within the molecular sieve and hence would not have the mild sorption characteristics of triterpane 12 (Figure 6.2c). It is further suggested that it is more likely to have a lupane-type rather than a hopane-type skeleton because this compound co-occurs with other compounds of higher plant origin and is not a hopane of known structure. As described earlier, lupane is excluded from

the sieve channels and is not sorbed, while triterpane 12 is weakly sorbed by the sieve. The GC retention time is also less than that of lupane (which coelutes with oleanane) by approximately one and a half minutes. Both the sorption and GC retention behaviour of this compound are consistent with a lupane-type skeleton but one containing a β-isopropyl group on the E-ring. The effect of a β-isopropyl group on the US-Y sorption behaviour has already been discussed with reference to triterpane 3. The effect of a change from an α to a β-isopropyl on the GC retention behaviour however, can be predicted by comparing the retention times of 17β,21β(H)-hopane (α-isopropyl) and 17β,21α(H)-moretane (β-isopropyl). In this case, the β-substituted compound has a retention time much less than the α-substituted compound and supports the assignment of a β-isopropyl group for triterpane 12 on the basis of its reduced retention time relative to lupane. Based on this analogy, a 19α(H)-lupane (Figure 6.2, E) is proposed for triterpane 12.

The mass spectrum of triterpane 14 shows a C_{29} molecular ion and fragment ions at m/z 383 [M-15] and m/z 259. It was also completely excluded from the sieve. These features suggest either an oleanane or taraxastane type skeleton for this component. The GC retention behaviour of this compound on columns of different polarity appears to be consistent with that for a taraxastane rather than an oleanane. The retention times of the oleananes (13), taraxastanes (19) and triterpane 14 relative to that of hopane (15) were examined using DB-1 and DB-1701 columns. It is evident from Figure 6.1a that the relative retention time of the oleananes changed little when chromatographed using the two columns. However the relative retention times of triterpane 14 and the taraxastanes (19) increased by approximately a half of a minute when a DB 1701 column was used. Assuming that C_{29} homologues behave in an analogous manner to the C_{30} compounds, it is likely that triterpane 14 is a C_{29} taraxastane (Figure 6.2, F). A 28-nor-skeleton is suggested for this compound since many extant structurally related natural products have oxygenated functional groups at C-17 (*cf.*, Dev *et al.*, 1989).

Triterpanes 16 and 18 both show a C_{30} molecular ion at m/z 412, an ion at m/z 397 [M-15] and a dominant base peak at m/z 191 in their mass spectra. Their mass spectral features together with their exclusion from the sieve pores and their GC elution order between oleananes and taraxastanes suggest that triterpanes 16 and 18 have structures related to those of taraxastane or ursane (Figure 6.2, G).

Formation of spiro-compounds

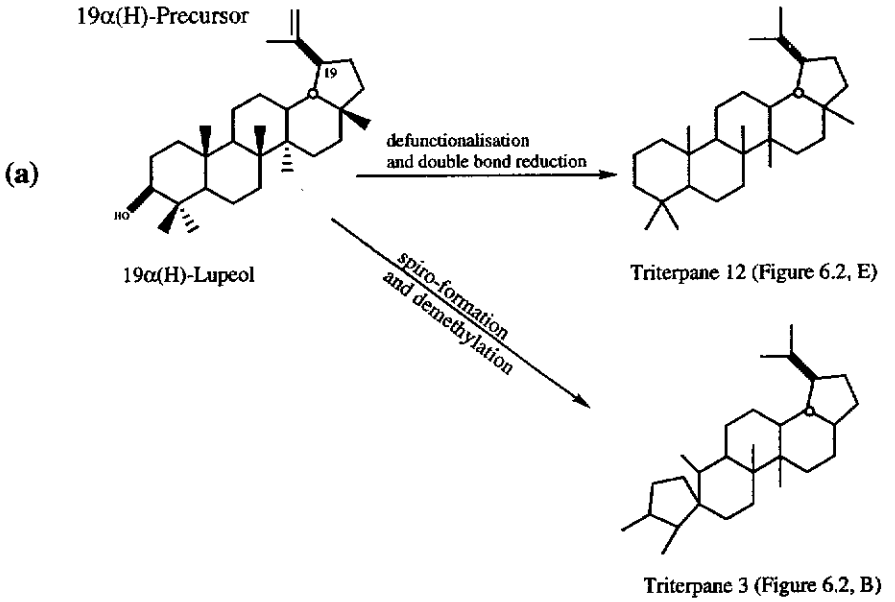
The co-occurrence of the spirotriterpanes 1, 3, 8 and 9 in SSB crude oil is consistent with the same spiro-formation mechanism proposed by Hills *et al.* (1968) in so far as the proposed structures A, B and C (Figure 6.2) are based on the formation of A-ring spiro-compounds from precusors with oleanane, lupane and taraxastane-type skeletons, respectively. Both lupane and taraxastane have AB-ring systems identical with 18α-oleanane, the proposed precursor of the well-characterised spirotriterpane (Appendix, XII). Furthermore, their possible precursors such as lupan-3β-ol and lupan-3-one, which have been identified in sediments (ten Haven *et al.*, 1992a), are nearly always functionalised at C-3 which is necessary for carbocation formation. It is therefore not surprising that spiro formation has apparently occurred from precursor natural products other than oleananes.

Origin of 19α(H)-lupanes

19α(H)-Lupanes have two possible sources from either (1) a natural product precursor having the same β-isopropyl stereochemistry or (2) isopropyl isomerisation of regular lupane precursors during diagenesis and maturation to give compounds with the β-isopropyl stereochemistry. Figure 6.4 shows possible formation schemes for triterpanes 3 and 12. Natural products of the 19α(H)-lupane skeletal type such as 19α(H)-lupeol (Figure 6.4a) and neolupeol have been reported to occur in plants (*cf.* Dev *et al.*, 1989) and may undergo diagenetic

defunctionalisation and hydrogenation to give a saturated triterpane similar in structure to that proposed for the two triterpanes 3 and 12 (Figure 6.4a). However, due to the lack of reported natural products of this type functionalised at C-17 which would give a C_{29} hydrocarbon, triterpane 3 may alternatively be derived from regular lupane [19β(H)-] natural products via acid-catalysed isomerisation of the isopropyl substituent in lupene intermediates (Figure 6.4b), as shown from laboratory experiments by Baddeley *et al.* (1976). This proposed pathway would begin with the defunctionalisation of a suitable natural product precursor such as betulic acid. This has been reported to give a mixture of Δ^{17}, Δ^{16} and $\Delta^{17(22)}$ lupenes, which after acid-catalysed isomerisation affords a Δ^{17} component where the stereochemistry of the isopropyl group at C-19 has changed from an α to a β-orientation (Baddeley *et al.*, 1976). Meanwhile dehydroxylation at C-3 may lead to spiro A-ring formation (Hills *et al.*, 1968). Finally migration of the Δ^{17} tetra-subtituted double bond followed by its reduction may then give a C_{29} triterpane which is similar in structure to triterpane 3. It is important to note however that, based on steric energy calculations (Table 6.3), isomerisation of the isopropyl substituent in lupane is not a thermodynamically favoured process. So even though it has been observed to occur under laboratory conditions, it is questionable whether such a process would take place in sediments.

The co-occurrence of spiro-compounds and β-isopropyl lupanes in this Indonesian crude oil is consistent with it having been subjected to acidic conditions during petroleum formation. Since spiro-formation is acid-catalysed (Hills *et al.*, 1968) it occurs under similar conditions to the isopropyl isomerisation of lup-17-ene (Baddeley *et al.*, 1976) and it is not surprising that a triterpane may undergo both spiro-formation and isopropyl isomerisation (Figure 6.4b) in the same sediment to give a product such as a spiro-19α(H)-norlupane (triterpane 3).



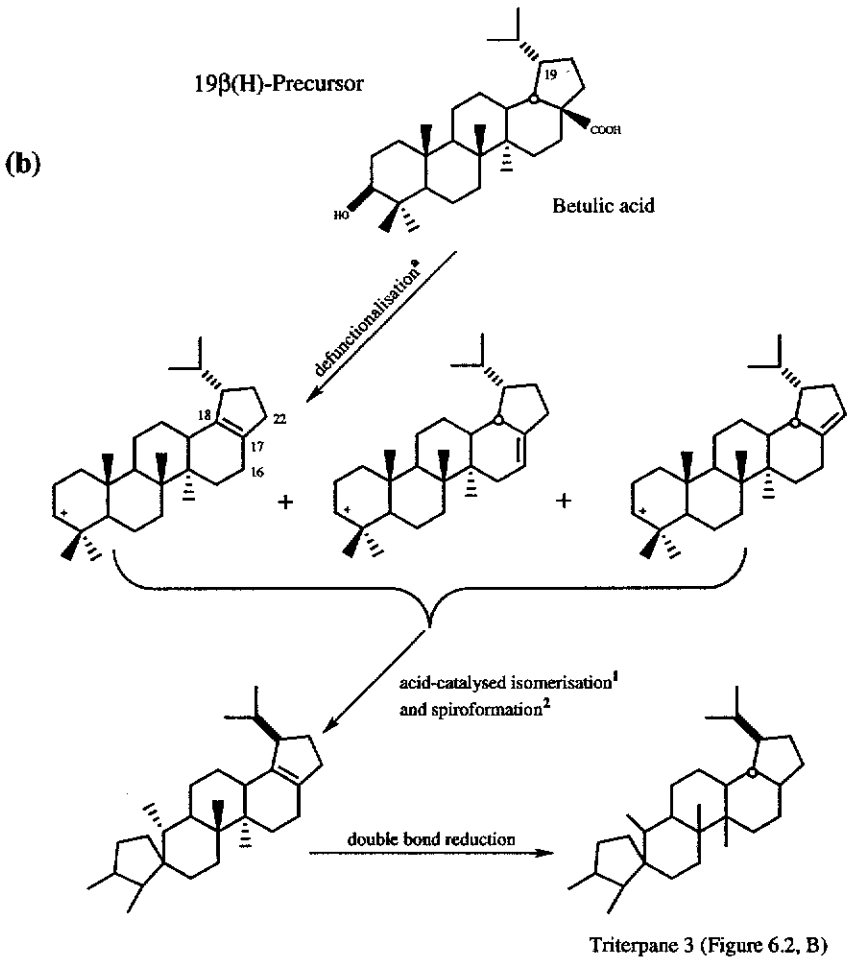


Figure 6.4 Proposed formation pathways of the rearranged 19α(H)-lupanes ([1] - Baddeley *et al.*, 1976; [2] - Hills *et al.*, 1968).

6.2 IDENTIFICATION OF BICADINANES IN JURASSIC SEDIMENTARY ORGANIC MATTER FROM THE EROMANGA BASIN, AUSTRALIA

6.2.1 Background

Recent evidence suggests a pre-Cretaceous evolution of angiosperms. Based on studies of extant primitive angiosperms and of Jurassic fossils with angiosperm-like leaf and wood characteristics, several unorthodox theories have been proposed for the time and place of origin of flowering plants. Large concentrations of extant primitive angiosperms found in the southeastern Asia, northern Australia and the southwestern Pacific have led to the suggestion that angiosperms originated in these areas during the Jurassic or earlier times (e.g., Bailey, 1949; Axelrod, 1952; 1960; Takhtajan, 1969). More recently however, the likelihood of a pre-Cretaceous origin for the angiosperms has been rigorously established (*cf.*, Palmer, 1994). Based on calculations of 'molecular clocks' in the DNA of living plants (Martin *et al.*, 1989; Crane *et al.*, 1989) and on cladistic hypotheses that link angiosperms with extinct Triassic plants such as *Bennettitales* and *Gnetales* (*cf.*, Crane, 1993) it was concluded that the lineage that led to the flowering plants must have diverged from these Triassic plants more than 230 million years ago. Further support has come from Cornet (1993; *cited in* Palmer, 1994 and Crane, 1993) who reported the presence of a single small leaf and two 'flowers' of *Pannanlika triassica* in a Triassic shale in North Carolina.

It has been shown in Chapter 3 that isolation of crude oil fractions enriched in pentacyclic alkanes using US-Y molecular sieves has enabled lower concentrations of bicadinanes to be analysed than was previously possible by applying selective ion detection GC-MS techniques. In the following section, the molecular sieve

chromatography procedure has been applied to investigate the occurrence of very low levels of bicadinanes in Jurassic samples from the Eromanga Basin, Australia.

6.2.2 Geological Setting and Sample Description

The three samples used in this study were from the Jurassic Eromanga Basin which overlies the Permian-Triassic Cooper Basin in eastern Australia (Figure 6.5). The regional geology and lithostratigraphy of the Eromanga Basin, shown in Figure 6.6, have been well documented (e.g., Bowering and Harrison, 1982; Price *et al.*, 1985). The Corona-1 DST 1 crude oil was recovered from the Hutton Formation (Figure 6.6) of Middle Jurassic age (Green *et al.*, 1989) and is therefore presumed to be from a source of similar age or older. It is reported to contain biomarkers typical of the *Araucariaceae* Jurassic flora of the region (Alexander *et al.*, 1988b). Thargomindah #2 1240.53 m and 1242.51 m sediment samples described as a dark grey mudstone and a dark grey siltstone respectively were recovered from the Westbourne Formation (Figure 6.6) which is Late Jurassic in age (Green *et al.*, 1989). Table 6.2 lists some geochemical parameters obtained for these samples.

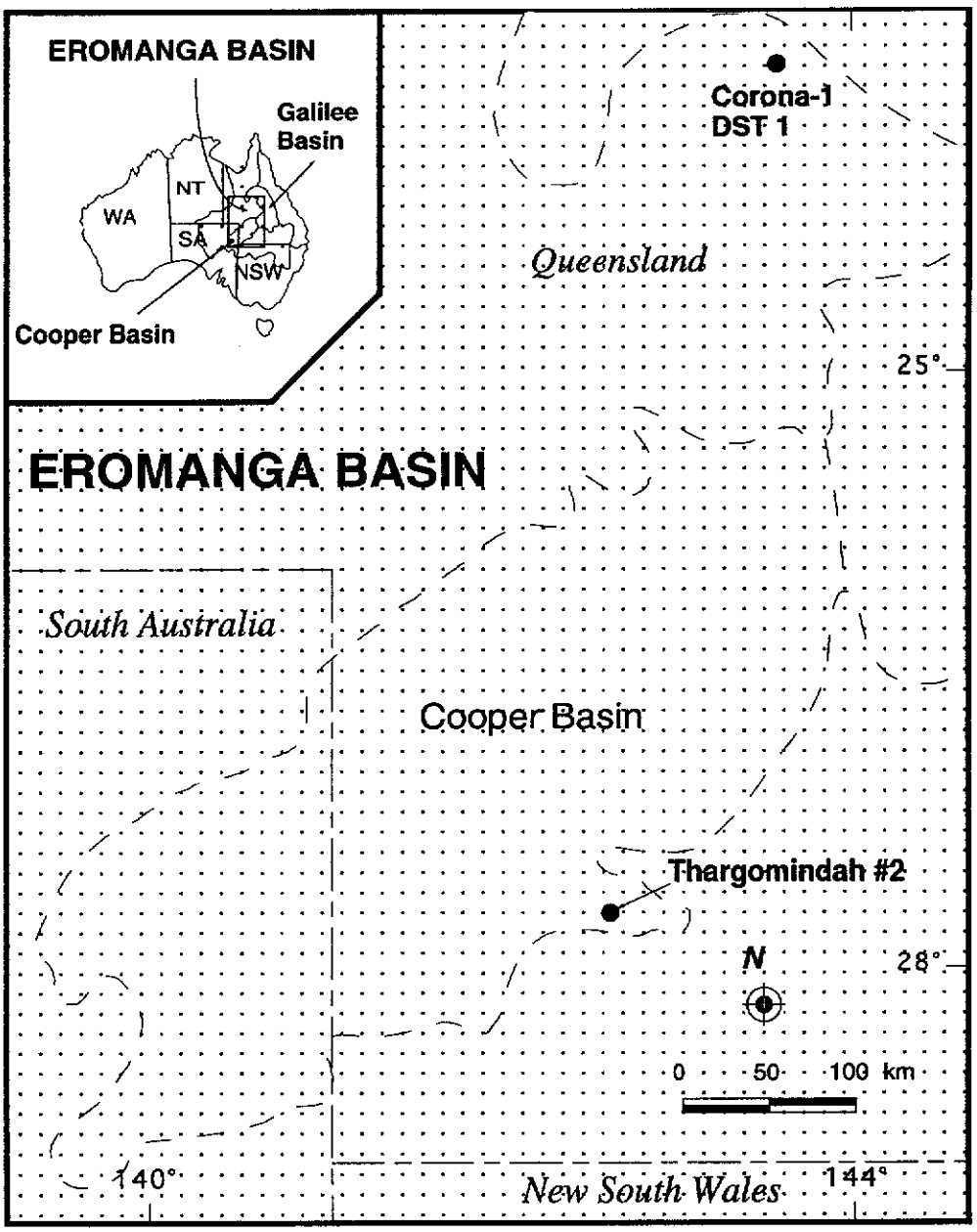


Figure 6.5 Map showing the locations of Jurassic samples from the Eromanga Basin.

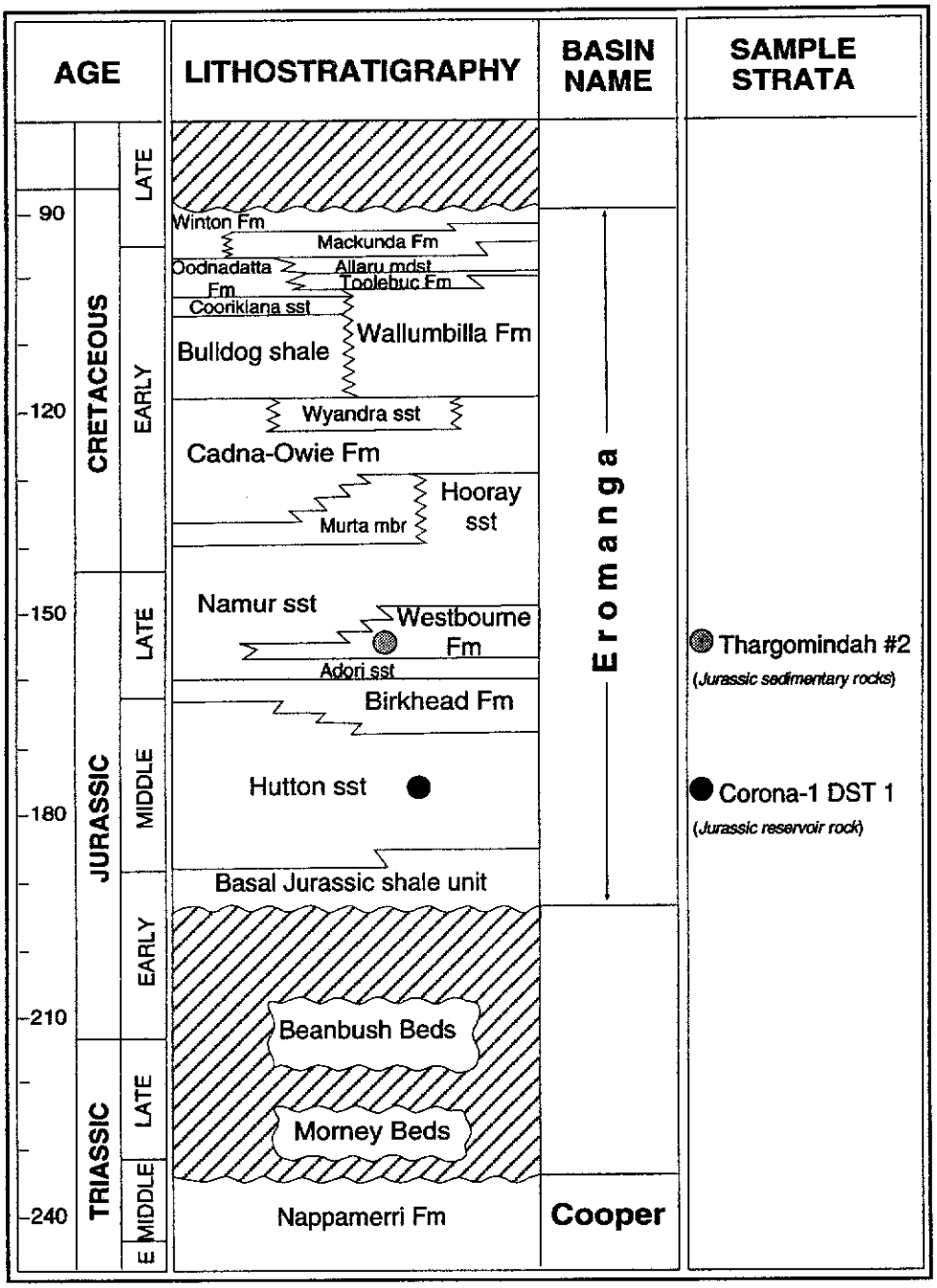


Figure 6.6 Stratigraphy of the Eromanga Basin (after Price *et al.*, 1985).

Table 6.2 Selected geochemical parameters for Eromanga samples used in this study.

Sample	TOC (%)	Tmax	SOM (%)	S1	S2	PI	R_O (%)
Thargomindah #2 1240.53 m	0.75	445	0.0330	0.04	0.40	0.09	0.62
Thargomindah #2 1242.51 m	0.69	439	0.0344	0.04	0.55	0.07	0.62

TOC - total organic carbon (%)
Tmax - temperature corresponding to the S_2 maxima Rock-Eval pyrolysis (°C)
SOM - soluble organic matter (%)
R_o - vitrinite reflectance (%)
PI - production index

Sample	Pr/Ph	Ts/Tm	$C_{30}H/C_{30}M$	*S/R* $C_{31}H$	$C_{27}/C_{29}S$	*S/R* $C_{29}S$
Thargomindah #2 1240.53 m	3.35	0.20	2.80	1.46	0.13	1.27
Thargomindah #2 1242.51 m	3.67	0.15	3.62	1.43	0.13	1.21
Corona-1 DST 1	6.91	0.18	7.50	1.40	<0.1	0.82

Pr/Ph - Pristane/Phytane
Ts/Tm - 22,29,20-Trisnorneohopane/22,29,30-Trisnorhopane
$C_{30}H/C_{30}M$ - αβ-Hopane/Moretane
S/R $C_{29}S$ - C_{29} *20S* ααα-Sterane/C_{29} *20R* ααα-Sterane
C_{27}/C_{29} S- C_{27} ααα-Steranes/C_{29} ααα-Steranes
S/R $C_{31}H$ - *22S* αβ-Homohopane/*22R* αβ-Homohopane

6.2.3 GC-MS Analysis

The branched and cyclic alkane fractions from the crude oil and the two sediments were chromatographed using US-Y molecular sieves to enrich the bicadinanes. Figure 6.7 shows a comparison of m/z 412 mass chromatograms for the branched and cyclic alkanes from the crude oil and sediments before sieving and in Fraction 1 from the sieve column. The bicadinanes were identified based on their m/z 412 parent ion and m/z 369 ion fragment responses as well as their relative retention times compared with those for a set of bicadinane isomers in the

branched and cyclic alkane fraction from an Indonesian crude oil. Close inspection of the mass chromatograms for the unsieved alkane fractions shows that the presence of bicadinanes is equivocal, even though a weak response for *trans-trans-trans*-bicadinane (T) may be apparent. The mass chromatograms of Fraction 1 however, show clear evidence for the presence of bicadinanes in these Jurassic samples. The bicadinanes, which are not sorbed by the sieve, were selectively enriched and separated from other coeluting components in Fraction 1, thus improving their limits of detection.

6.2.4 Origin of Bicadinanes in the Eromanga Basin

The occurrence of very low concentrations of bicadinanes in these Jurassic samples whose sources of organic matter predate the widespread occurrence of angiosperms can be interpreted in two ways. First, older plants than the well-known *Dipterocarpaceae* (van Aarssen *et al.*, 1990a) and the fruiting plant mastixioid *Cornaceae* (Crelling *et al.*, 1991; Meuzelaar *et al.*, 1991; van Aarssen *et al.*, 1994) may have contributed bicadinane precursors to sediments. Hence, although polycadinenes originating from angiosperm resins are the main precursors of the bicadinanes in sediments of Tertiary age and younger, other earlier plant types may also have been capable of producing these compounds. Alternatively, the bicadinanes in the Eromanga Basin may have originated from angiosperms similar to the primitive extant angiosperms in the nearby north-east Queensland rainforests. The large concentration of primitive extant species of flowering plants in this region has led to suggestions that angiosperms originated in this region during pre-Cretaceous times (e.g., Takhtajan, 1969). Combined with other evidence that angiosperms diverged from more primitive plants as early as the Triassic (Martin *et al.*, 1989; Crane *et al.*, 1989; Cornet, 1993), a Jurassic angiosperm is a possible source for these bicadinanes.

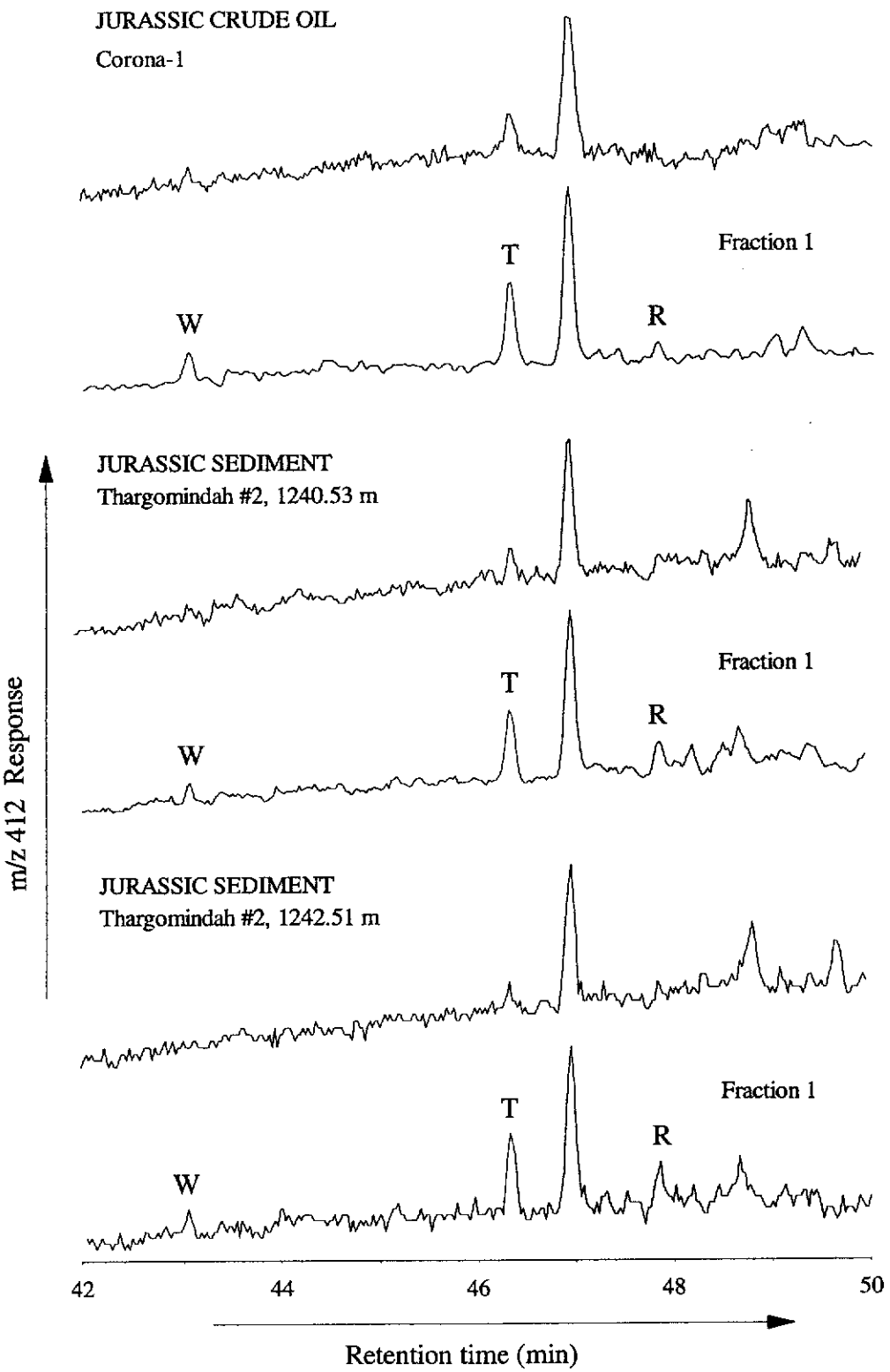


Figure 6.7 Partial m/z 412 mass chromatograms of the branched and cyclic alkane fractions from a Jurassic crude oil and two Jurassic sediments showing the relative abundances of the bicadinanes before sieving compared to that in Fraction 1 from the sieve column.

6.3 STERIC ENERGY CALCULATIONS FOR SOME HIGHER PLANT-DERIVED TRITERPANES

Steric energy calculations for the various petroleum hopanoids have been previously used to explain their occurrence and relative abundance in geological samples (Kolaczkowska *et al.*, 1990; Moldowan *et al.*, 1991). In this section, steric energies for a number of higher-plant derived triterpanes are reported for the first time and are used to account for their relative abundances in sedimentary organic matter.

Three-dimensional molecular models were built and steric energies of all bonds minimized in order to approximate the steric energy of each compound. This was carried out by sequentially applying three types of minimizations on each model. Briefly, the first minimization reduces the error in ring closures; the second minimizes structural error by adjusting atom positions to optimize bond lengths and bond angles; and the third minimizes the overall steric energy of the model. Each steric energy value presented in this thesis is a summation of seven individual energies which are detailed in the CSC Chem-3D Plus (3.1) instruction manual:

1) Stretch - represents the energy associated with stretching and compressing bonds from their optimal length.

2) Bend - represents the energy associated with deforming bond angles from their optimal values.

3) Stretch/Bend - the energy required to stretch the two bonds involved in a bond angle when that bond angle is severely compressed.

4) Torsion - represents the energy associated with deforming torsional angles in the molecule.

5) Non 1,4 Van der Waals - represents the energy for the interaction of atoms bonded to non-adjacent atoms.

6) 1,4 Van der Waals - represents the energy for the interaction of atoms bonded to adjacent atoms.

7) Dipole/Dipole - represents the energy associated with the interaction of bond dipoles.

Table 6.3 lists the various higher plant-derived triterpanes along with their calculated steric energies which have been used to compare the stabilities of the various triterpanes. For isomeric molecules, differences in the heats of formation are related to differences in intramolecular steric crowding (Moldowan *et al.*, 1991).

Table 6.3 Steric energies of some higher plant-derived triterpanes (values were obtained using CSC Chem-3D Plus computer package version 3.1).

COMPOUND	STERIC ENERGY (kJ/mole)
Gammacerane	363
Ursane	356
19α(H)-Lupane	358
20β(H)-Taraxastane	354
20α(H)-Taraxastane	347
Lupane	342
Oleanane	332
8αH,14βH-Onocerane (ax,ax methyls)	320
8αH,14αH-Onocerane (ax,eq methyls)	311
8βH,14βH-Onocerane (eq,ax methyls)	311
18α-Oleanane	311
28-Nor-18α-oleanane	308
8βH,14αH-Onocerane (eq,eq methyls)	299
Spirotriterpane	295
trans-trans-trans Bicadinane (T)	262
cis-cis-trans Bicadinane (W)	237

Bicadinanes

Of the compounds examined, the two bicadinanes having the *cis-cis-trans* (W) and *trans-trans-trans* (T) configuration had the lowest steric energies and hence are the most thermodynamically stable. The steric energy of 237 kJ/mole calculated for *cis-cis-trans* bicadinane compared with 262 kJ/mole for the *trans-trans-trans* bicadinane suggests that a measure of W/T in samples may be useful in assessing the extent of maturation. This is consistent with work by Murray *et al.* (1994) who noted that the abundance of *cis-cis-trans* bicadinane relative to *trans-trans-trans* bicadinane in source-related samples increased with maturity. Further, the relative abundances of the two bicadinanes (W/T) have been measured in samples from two sedimentary sequences (BJB-124 1798-3030 m; GNK-67 1112-2330 m) from the South Sumatra Basin, Indonesia (Sosrowidjojo, PhD thesis). The results show that with increasing depth there was a steady increase in the ratio W/T.

Oleananes

Table 6.3 shows that 18α-oleanane has a significantly lower steric energy than oleanane (311 versus 332 kJ/mole, respectively). These energy values appear to be reflected in the relative abundances of these oleananes in sedimentary organic matter. In sediments and immature crude oils oleanane dominates, while 18α-oleanane becomes more important in more mature crude oils. This observation has previously been attributed to the thermally induced isomerisation of oleanane to 18α-oleanane (ten Haven and Rullkötter, 1988; Ekweozor and Telnaes, 1990). Interestingly, examination of the relative abundances of these isomers in some geological samples does not always support this idea. If isomerisation was the only process taking place, then it would not adequately explain the presence of minor amounts of 18α-oleanane (isomerisation product) in immature sediment (e.g., Riva *et al.*, 1988) or high amounts of oleanane in crude oil (e.g. Ekweozor and Udo, 1988). With this in mind, one cannot rule out the possibility that the

formation of 18α-oleanane proceeds independently to that of oleanane and that the change in relative amounts of the two isomers with increasing maturation may predominantly be due to the selective destruction of the less stable isomer. This proposal is discussed in detail in Chapter 8.

Spirotriterpane

Based on steric energy calculations (Table 6.3), spiro-formation is a thermodynamically favoured process since spirotriterpane (295 kJ/mole) is more stable than its proposed precursor 18α-oleanane (311 kJ/mole). Examination of three-dimensional molecular models of these two triterpanes (Figure 6.8a) illustrates that the enhanced thermodynamic stability of spirotriterpane is due to the relief of strain caused by the 1,3,5 triaxial methyl conformation (C-24, C-25 and C-26) in the A/B ring system of 18α-oleanane. Prior to spiro-formation the C-24 methyl migrates away from the unfavourable triaxial conformation leaving a diaxial conformation. During spiro-formation, the C-25 migrates from a β- (axial) to an α- (equatorial) position. Similar energy differences are also observed for the proposed spirolupane (Figure 6.2, B) and spirotaraxastane (Figure 6.2, C) compared with their 6-membered A-ring analogs. The greater thermodynamic stabilities of spiro-compounds relative to their 6-membered A-ring analogs suggests that they may be more prevelant in mature crude oils than previously anticipated.

19α(H)-Lupanes

Although spiro-formation affords greater thermal stability, the isomerisation of the α to β-isopropyl substituent in lupane does the reverse by increasing the overall steric energy of the molecule. Based on inspection of Drieding models, Baddeley *et al.* (1976) reported that the conversion from an α- to a β-isopropyl orientation greatly reduces the unfavourable steric interactions between the side chain and C-ring. However, a comparison of molecular models of lupane and 19α(H)-lupane

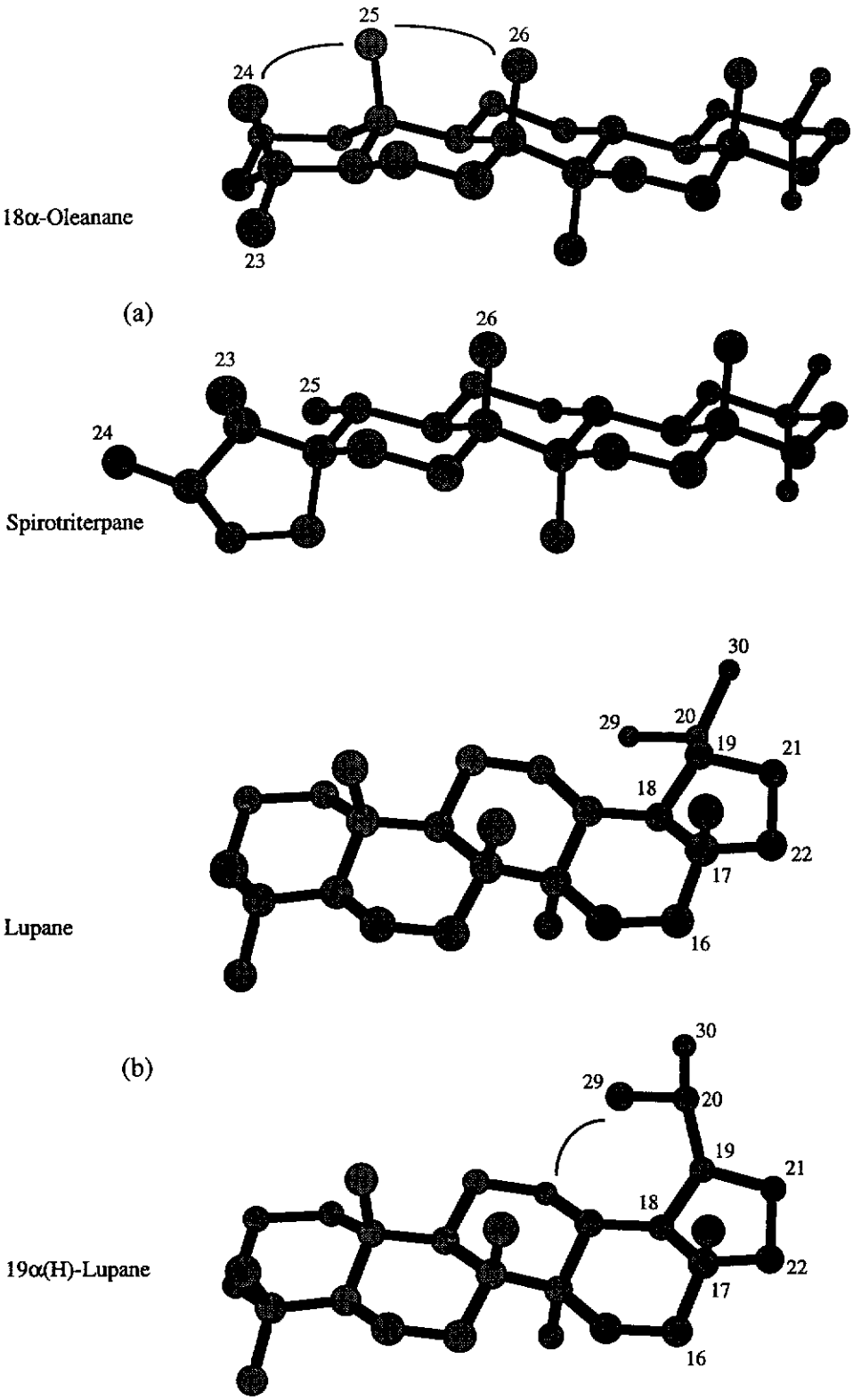


Figure 6.8 Three-dimensional molecular models of (a) 18α-oleanane and spirotriterpane (b) lupane [19β(H)] and 19α(H)-lupane.

(Figure 6.8b) using the more sophisticated CSC Chem-3D Plus (3.1) computer package showed that it is the β-isopropyl group, rather than the α-isopropyl, which comes in closer proximity to the rest of the molecule. Thus, the β-isopropyl lupane isomer actually has a calculated steric energy which is higher than that of the corresponding α-isopropyl isomer by 16 kJ/mole (Table 6.3).

Onoceranes

Of the four possible onocerane isomers (Table 6.3) only three (8αH, 14αH; 8βH, 14βH; and 8βH, 14αH) have been reported to occur in geological samples (e.g., Ourisson *et al.*, 1964; Curiale, 1988). This can be attributed to the relative thermodynamic stabilities of these isomers (steric energies from 229 to 311 kJ/mole) compared to that of the fourth isomer which has both methyls at C-8 and C-14 in an unfavourable diaxial configuration (320 kJ/mole).

Taraxastanes

Steric energies for various isomeric taraxastanes can provide some insight into the most likely structures of the taraxastanes in higher-plant derived crude oils. To date only two isomeric taraxastanes (Figure 6.1a; Table 6.1) have been tentatively identified in geological samples. Although the basic taraxastane skeleton (Appendix, XVI) is known, the precise configuration of the E-ring C-30 methyl substituent in each isomer has not been rigorously proven since all likely precursor compounds have an unsaturation at either Δ^{20} or $\Delta^{20(30)}$ (*cf.* Dev *et al.*, 1989). Hydrogenation of these taraxastenes would most likely proceed from the least hindered face; however, molecular models show that the α and β faces are equally accessible. Hence, assuming that hydrogenation of Δ^{20} or $\Delta^{20(30)}$ leads to approximately equal amounts of the 20α(H)- and 20β(H)- saturated isomers, it is appropriate to consider thermodynamic stabilities in order to assess the relative abundance of these isomers in mature crude oils. Steric energy values of 347

kJ/mole and 354 kJ/mole calculated for the 20α(H)- and 20β(H)- isomers respectively show that thermodynamically there is little difference between the two isomers. The isomer abundance data from analysis of various crude oil alkane fractions supports the steric energy considerations, in that the pair of isomeric taraxastanes typically occur in equal amounts, with a slightly enhanced relative abundance of the earlier eluting isomer. Based on steric energy calculations alone, it is suggested that the more abundant earlier eluting isomer is probably the more thermodynamically stable 20α(H)-taraxastane.

Ursane and lupane

Although thermodynamic stablity is an important factor in the preservation and occurrence of specific biomarker compounds in sedimentary organic matter, other factors sometimes prevail. For example, based on comparison of steric energy values for the various triterpanes, both lupane and ursane appear to be sufficiently stable to exist in crude oil yet their occurrence has never been reported. In the case of ursane, the Δ^{12} double bond in the precursor urs-12-ene has been reported to be chemically inert and does not undergo acid-catalysed isomerisation (Corey and Ursprung, 1956) and hence may explain the apparent absence of the saturated analog in crude oil. In the case of lupane, various structural rearrangements of the lup-20(29)-ene precursor to oleanane and taraxastane structures are known in the chemical literature (Ames *et al.*, 1952, 1954; Halsall *et al.*, 1952, 1954) which may explain the lack of preservation of the C_{30} compound (the fate of sedimentary lupane is discussed further in Chapter 7). These rearrangement processes are most likely to occur during early diagenesis of sediments alongside natural product precursor defunctionalisation processes. This would explain why the lupane skeleton has only been reported in sediments when it contains oxygen functionalities (e.g., Wintersteiner *et al.*, 1965; ten Haven *et al.*, 1992b).

6.4 CONCLUSIONS

Eight previously unreported triterpanes have been observed in an Indonesian crude oil of higher-plant origin. Based on their mass spectral data, GC retention and molecular sieve sorption characteristics, tentative structures such as 19α(H)-lupanes and a spiro-nortaraxastane and a spiro-lupane have been proposed for these compounds.

Using the US-Y liquid chromatographic procedure described in Chapter 3, low levels of bicadinanes have been detected in Jurassic samples from the Eromanga Basin, Australia. Interpretation of these results suggests either that plants older than angiosperms are capable of producing bicadinanes or that angiosperms evolved prior to Cretaceous times.

Steric energies have been calculated for a number of higher-plant derived triterpanes. These relative steric energy values provide a generally adequate explanation for the observed relative abundances of these compounds in sedimentary organic matter.

CHAPTER 7

ISOLATION, CHARACTERISATION AND GEOCHEMISTRY OF TWO HIGHER PLANT-DERIVED TRITERPANES IN A LIGNITE

Summary

A lignite of Eocene age from Heartbreak Ridge, Bremer Basin, Western Australia was subjected to hydrous pyrolysis. From the pyrolysate, 28-nor-18α-oleanane was isolated and characterised by single crystal X-ray analysis. Lupane was also isolated and characterised by ^{13}C NMR spectroscopy and by co-chromatography with an authentic standard on four different GC phases.

28-Nor-18α-oleanane has a likely origin from C-28 functionalised higher-plant natural products. The absence of the 18β-isomer (28-noroleanane) in this sample suggests a formation pathway for 28-nor-18α-oleanane different to that proposed for the formation of 18α-oleanane from oleanane. 28-Nor-18α-oleanane was also identified in the hydrous pyrolysate of another lignite sample, thus indicating that it may be a common constituent in Tertiary lignites, while its selective destruction when heated in the presence of acidic clay may explain its absence in mature sediments and crude oils.

The unusually high abundance of lupane in the sulphur-rich Heartbreak lignite has been attributed to its diagenetic sulphur incorporation into the kerogen, thus preserving its otherwise reactive five carbon membered E-ring from conversion to one having six carbon atoms. Using gas chromatographic conditions which enable the complete resolution of lupane from the oleananes, it was observed that lupane was either absent or present in negligible amounts in a wide range of higher-plant derived sediments and crude oils analysed.

7.1 GEOLOGICAL SETTING AND SAMPLE DESCRIPTION

The Bremer Basin is situated in the southeast of Western Australia and comprises an area of Tertiary sediments extending along the south coast (Figure 7.1). The Tertiary sediments of the onshore part of the basin were deposited over a highly irregular surface of Precambrian rocks. The resulting scattered exposures of marine and continental Tertiary rocks occur in a belt up to 64 km wide over an area of about 12,400 km^2. These sediments have been geologically classified as belonging to either the Plantagenet or Eundynie Groups. The Eocene Plantagenet Group consists of the Werillup Formation and the Pallinup Siltstone. The Werillup Formation is dated as Late Eocene and contains the Heartbreak lignite deposit.

Information on the geology of the Upper Eocene Heartbreak lignite deposit was provided by CRA Exploration Pty. Ltd. (unpublished data). The lignite deposit was developed autochthonously in backswamp regimes of a fluvial system. Sedimentation was mainly controlled by eustatic changes in sea level, producing a northerly advancing marine transgression with more open water conditions prevailing to the south. On a regional basis the lignites are thicker and have higher mineral matter content in the north and east suggesting that the rate of organic matter accumulation has remained constant whilst clastic input has varied regionally. The beds are not tectonically deformed and have not undergone significant burial based on the low rank of the coal (R_o=0.2%).

Gamma logs provided for a number of sediment cores show that within each sequence there are notable changes in the coal quality. These sediments range from carbonaceous shales (coals containing >40% by wt. mineral matter content will be referred to as carbonaceous shales) to humic coals which have little mineral matter (<1%). The change in mineral content has been suggested to reflect a gradation from a fluvial to an upper deltaic depositional environment for the preserved organic material.

The Heartbreak lignite is characterised by its high chloride and sulphur content, typically 7% and 5% respectively (CRA Exploration Pty. Ltd., unpublished data), which is probably due to marine influences at the time of deposition. Most primary sulfur originated from early diagenetic reactions between the deposited organic matter and aqueous sulfide species, such as hydrogen sulfide or polysulfides (e.g., Francois, 1987). Sulfides are produced by sulfate-reducing bacteria, such as *Desulfovibrio*, which thrived in the highly reducing backswamp environment in which the lignite developed.

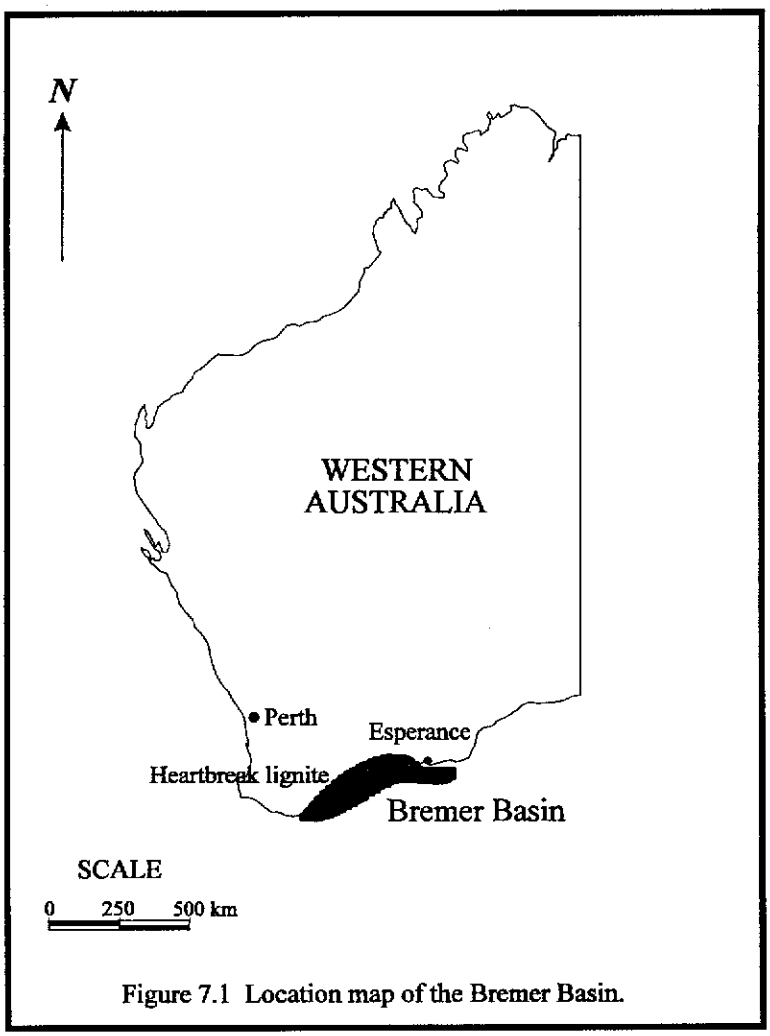


Figure 7.1 Location map of the Bremer Basin.

7.2 GC-MS ANALYSIS OF TRITERPANE COMPONENTS

The lignite samples analysed were provided by CRA Exploration as core material sealed in PVC tubes to prevent moisture loss and exposure to air during storage. Samples from different locations of the lignite deposit were analysed. Within each sediment core, coal sections containing various amounts of mineral matter were analysed to assess differences in their pentacyclic triterpane distributions. The triterpane components in the soluble organic matter of most samples were analysed as well as the kerogen-bound triterpanes generated by hydrous pyrolysis.

The triterpane distributions in samples having different mineral contents were examined and compared. Figure 7.2 shows m/z 191 mass chromatograms for the soluble organic matter and hydrous pyrolysate from a carbonaceous shale and a coal sample which occurred close together in the same borehole. Comparison of the chromatograms of the extracts (Figure 7.2a,c) shows that, although there are minor differences in the relative abundances of individual components, the same triterpanes are present in both samples. Both extract triterpane distributions are dominated by *22R* αβ-homohopane with lesser amounts of ββ-hopanes (27-32), 30-normoretane (29) and possible trace amounts of αβ-hopanes (29,30). The presence of the ββ-hopanes and significant amounts of hopene in these samples is consistent with their low maturity. Comparison of the chromatograms for the triterpanes in the two hydrous pyrolysates (Figure 7.2b,d), on the other hand, shows striking differences between the two samples. Both chromatograms show a more mature distribution of triterpanes which is dominated by the αβ-hopane and moretane series of compounds. In addition to the well-characterised hopanoids, four compounds represented by peaks labelled 1-4, were major components in the coal hydrous pyrolysate (Figure 7.2b), but were absent in the shale pyrolysate.

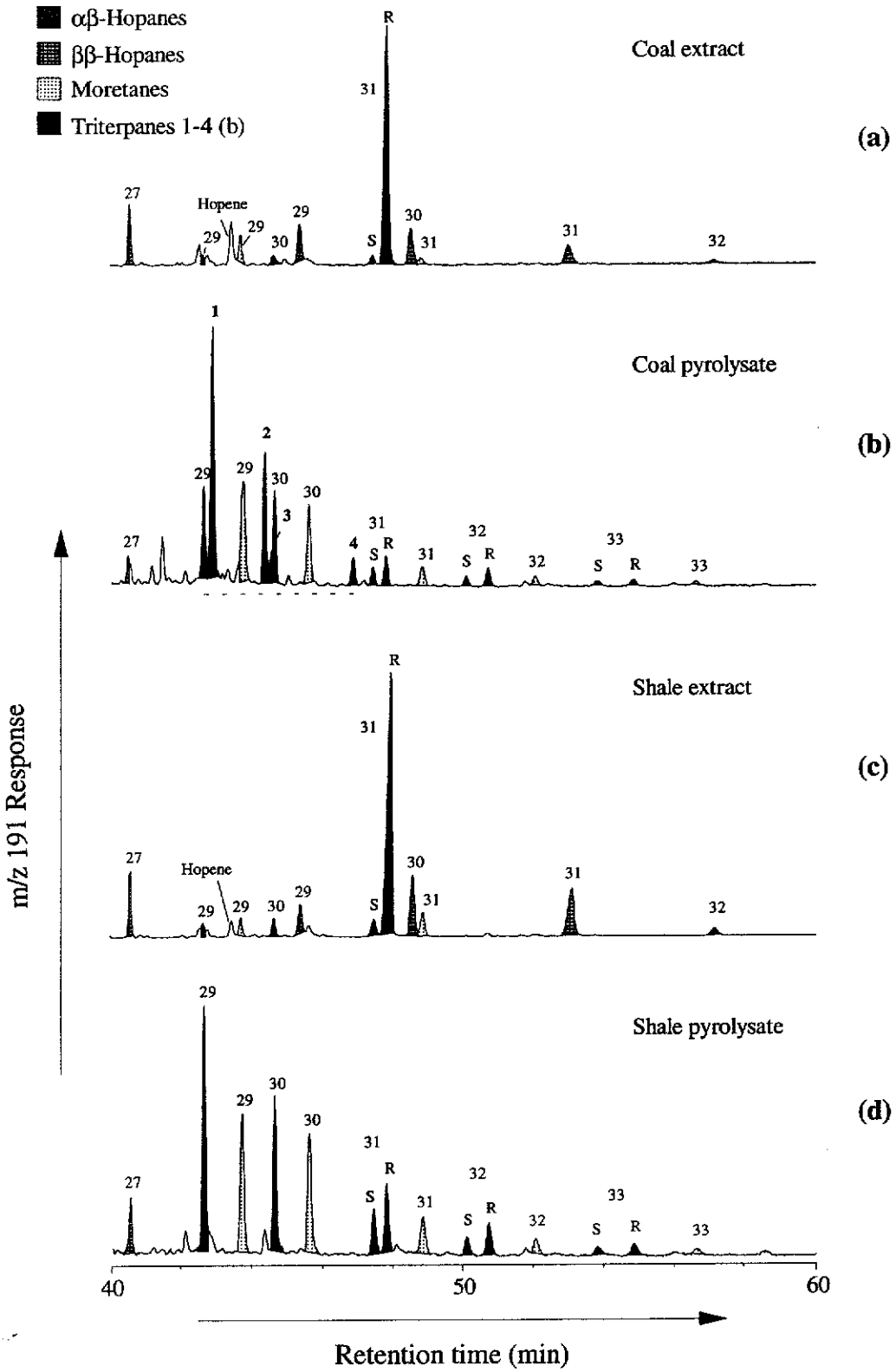


Figure 7.2 Partial m/z 191 mass chromatograms of the branched and cyclic alkanes obtained from a coal and adjacent carbonaceous shale. Peaks 1-4 represent higher-plant derived triterpanes of previously unknown structure. Numbers 27-33 denote the total number of carbons in each homologue. 1=28-nor-18α-oleanane and 2=lupane.

Compounds represented by peaks 1-4 (Figure 7.2b) were assigned structures based on published relative retention times and mass spectral features. Two coeluting C_{30} triterpanes (4) were tentatively identified as isomeric taraxastanes (Kimble *et al.*, 1974; Rullkötter *et al.*, 1994). A C_{29} triterpane (3) which co-elutes with αβ-hopane (30) was also present in an Indonesian crude oil (see Figure 6.1, peak 14) and was tentatively identified as 28-nortaraxastane. The C_{29} and C_{30} triterpanes (1 and 3) however, which show GC retention times similar to 30-norneohopane and oleanane respectively, do not appear to be common constituents in sedimentary organic matter of higher-plant origin. The remainder of this chapter describes the work carried out to isolate, characterise and investigate the geochemistry of these two triterpanes.

7.3 ISOLATION AND CHARACTERISATION OF TRITERPANES

7.3.1 Isolation Procedure

Figure 7.3 is a schematic of the procedure used to isolate the two triterpanes from the Heartbreak lignite. The coal was dried, crushed and extracted with dichloromethane for 48 h using a Soxhlet apparatus. The extracted coal was pyrolysed in the presence of water in a stainless steel vessel at 330°C for 72 h. The aqueous phase was extracted with dichloromethane and the insoluble material was air dried and extracted with dichloromethane. The two dichloromethane extracts were combined and the solvent was removed by distillation. The resulting bitumen extract was subjected to a series of liquid chromatography procedures. First, the extract was chromatographed on alumina to collect the less polar components eluted with *n*-pentane. This fraction was chromatographed on silica gel to obtain a total

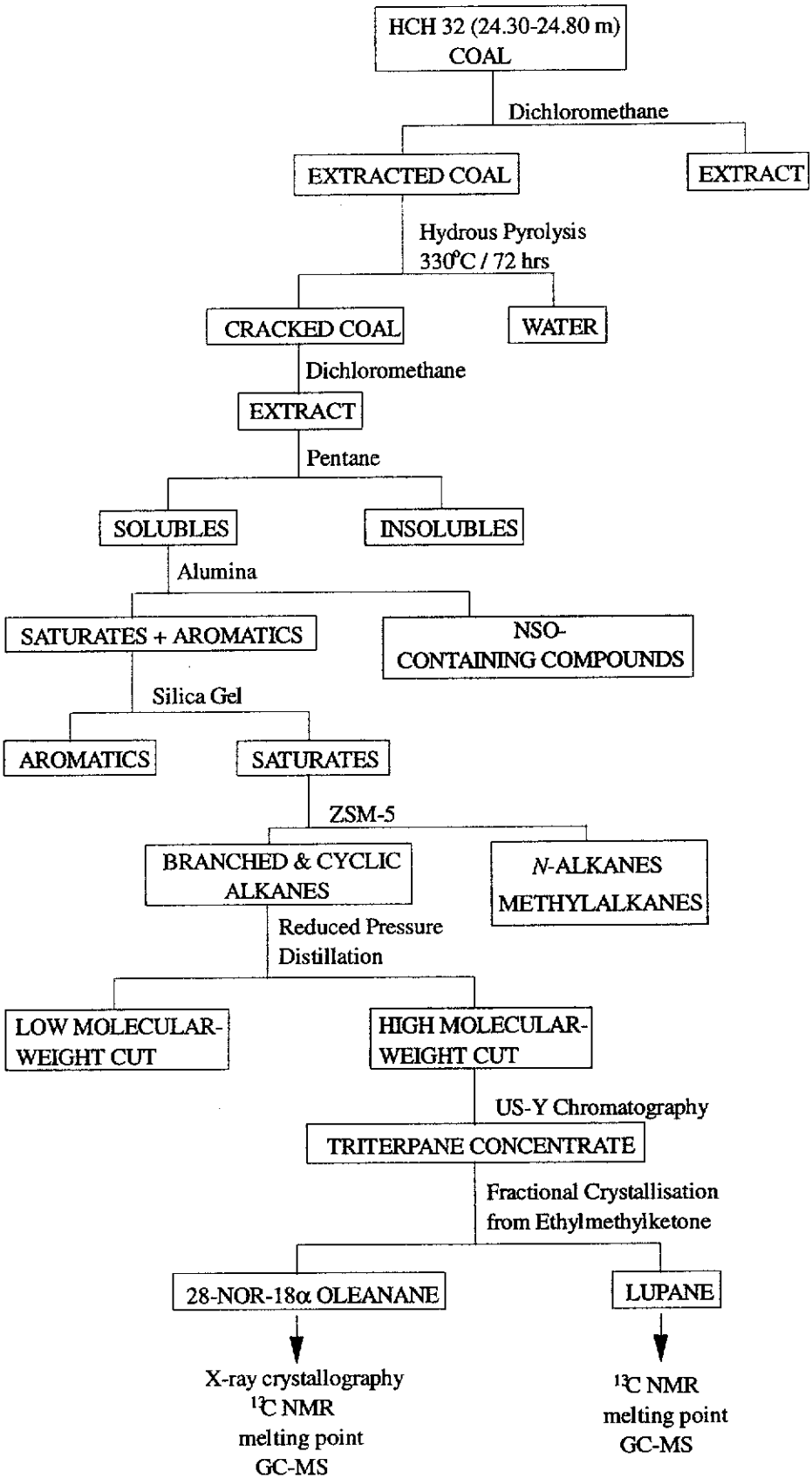


Figure 7.3 Techniques used to isolate and analyse 28-nor-18α-oleanane and lupane from a lignite.

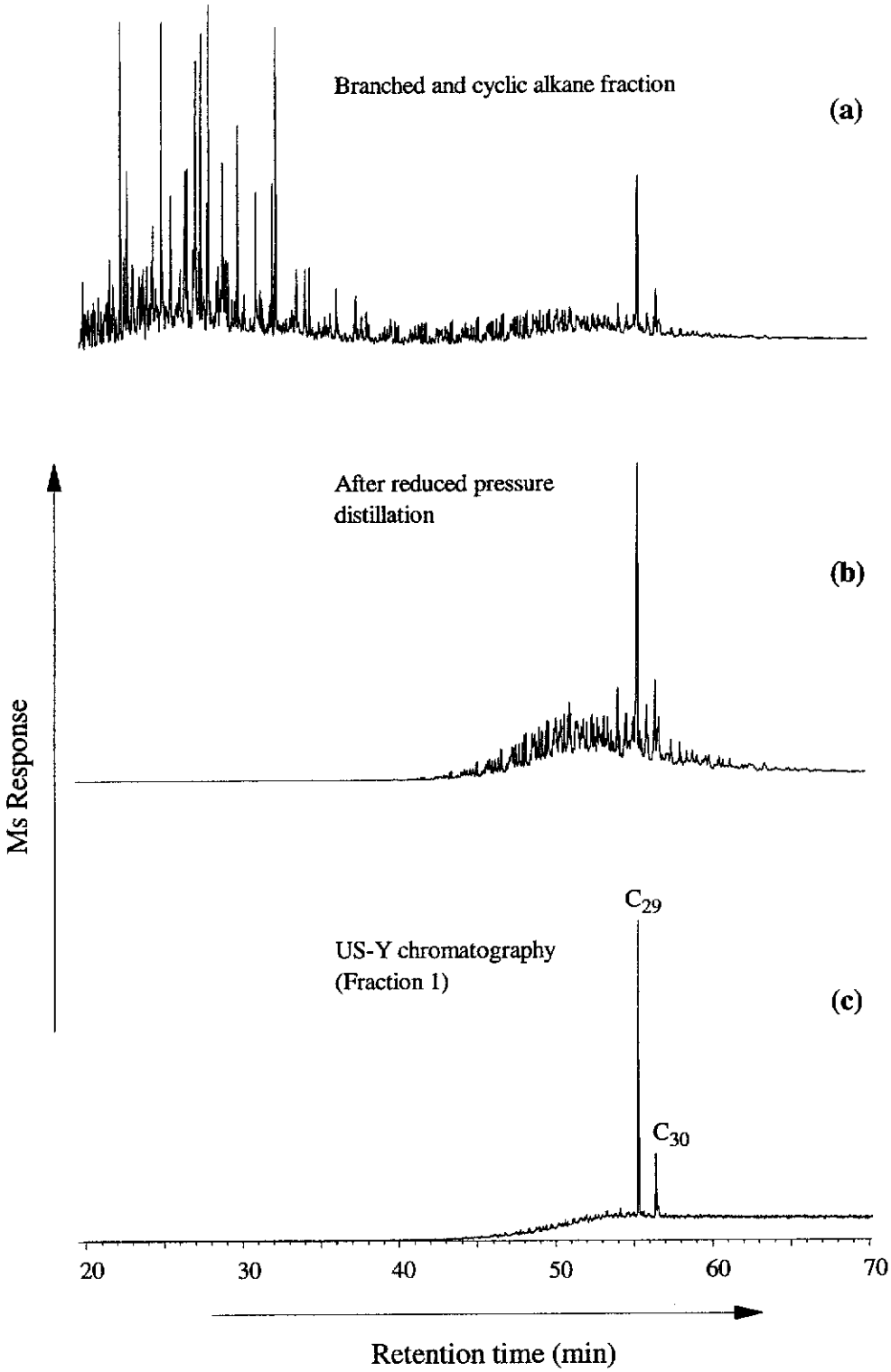


Figure 7.4 Total ion chromatograms of the branched and cyclic alkane fraction from the hydrous pyrolysate of the Heartbreak lignite showing the progressive enrichment of the two unknown triterpanes.

aliphatic fraction which was then chromatographed on ZSM-5 molecular sieve to give a branched and cyclic alkane fraction.

The branched and cyclic alkane fraction was subjected to further separation procedures in order to isolate the two triterpane components of interest. Total ion mass chromatograms showing progessive enrichment of the two triterpanes are given in Figure 7.4. The branched and cyclic alkane fraction (Figure 7.4a) was distilled under reduced pressure to obtain a pot residue (Figure 7.4b) which was enriched in the high molecular-weight alkanes. This fraction was then chromatographed on a US-Y molecular sieve column using *n*-pentane and fractions were collected. The first eluted fraction (Figure 7.4c), which contained compounds too large to be sorbed by the sieves, contained the two unidentified C_{29} and C_{30} triterpanes which accounted for 75% of the total GC response. The two compounds were separated and isolated by fractional crystallisation from ethylmethylketone.

7.3.2 Analysis and Structural Determination of 28-Nor-18α-oleanane

X-ray structure determination

The major triterpane component of the branched and cyclic alkane mixture (Figure 7.2, peak 1) was fractionally crystallised from ethylmethylketone (melting point of 185-186°C) and then recrystallised from ethylacetate into crystals used for X-ray analysis. The molecular structure and stereochemistry of the isolated compound was established by the X-ray study to be 28-nor-18α-oleanane. A unique room-temperature diffractometer data set (T~295 K; Enraf-Nonius machine, monochromatic Mo*K*α radiation, λ 0.7107_3 Å; ω scan mode, $2\theta_{max}$ 45°) was measured, yielding 2693 independent reflections, 877 with $I > 3\sigma(I)$ being considered 'observed' and used in the large block least squares refinement without absorption correction. The weak and limited data available from a marginally small

specimen would not support meaningful anisotropic thermal parameter refinement and the isotropic form was refined throughout for C,O; $(x, y, z, U_{iso})_H$ were included constrained at estimated values. The assignment of the methyl substituent disposition throughout, nevertheless, appears unambiguous. Conventional residuals R, R_w, on $|F|$ at convergence were 0.074, 0.060 (statistical weights, derivative of $\sigma^2(I) = \sigma^2(I_{diff} + 0.0004\ \sigma^4(I_{diff}))$. Neutral atom complex scattering factors were used, chirality being assigned from the expectations of the associated chemistry; computation used the XTAL 3.2 program system (Hall *et al.*, 1992). Pertinent results obtained from two independant molecules are given in Figure 7.5(a) and Table 7.1. Full molecular non-hydrogen geometries and thermal and hydrogen parameters have been deposited with the Cambridge Crystallographic Data Centre.

Crystal data - $C_{29}H_{50}$, M 398.7. Orthorhombic, space group $P2_12_12_1$ (D_2^4, No. 19), a 43.27(3), b 15.91(1), c 7.217(5) Å. V 4970 Å^3. D_c (Z = 8) 1.07 g.cm.$^{-3}$; $F(000)$ 1792. μ_{Mo} 0.6 cm^{-1}; specimen: 0.04 x 0.05 x 0.62 mm.

^{13}C NMR spectroscopy

The ^{13}C NMR spectrum of the 28-nor-18α-oleanane showed 29 resonances which were resolved with the aid of DEPT experiments into seven methyl, twelve methylene, five methine and five quaternary carbon signals. The ^{13}C NMR chemical shifts (Table 7.2) were assigned based on those reported for 24,28-bisnor-18α-oleanane (Trendel *et al.*, 1991b) for the DE-ring carbons, and those for lupane (Ammann *et al.*, 1982) for the ABC-ring carbons. A comparison of the AB-ring carbon chemical shifts of the two 18α-oleanane homologs shows a significant difference between the two sets of data, owing to the absence of C-24 in the A-ring of the C_{28} homolog.

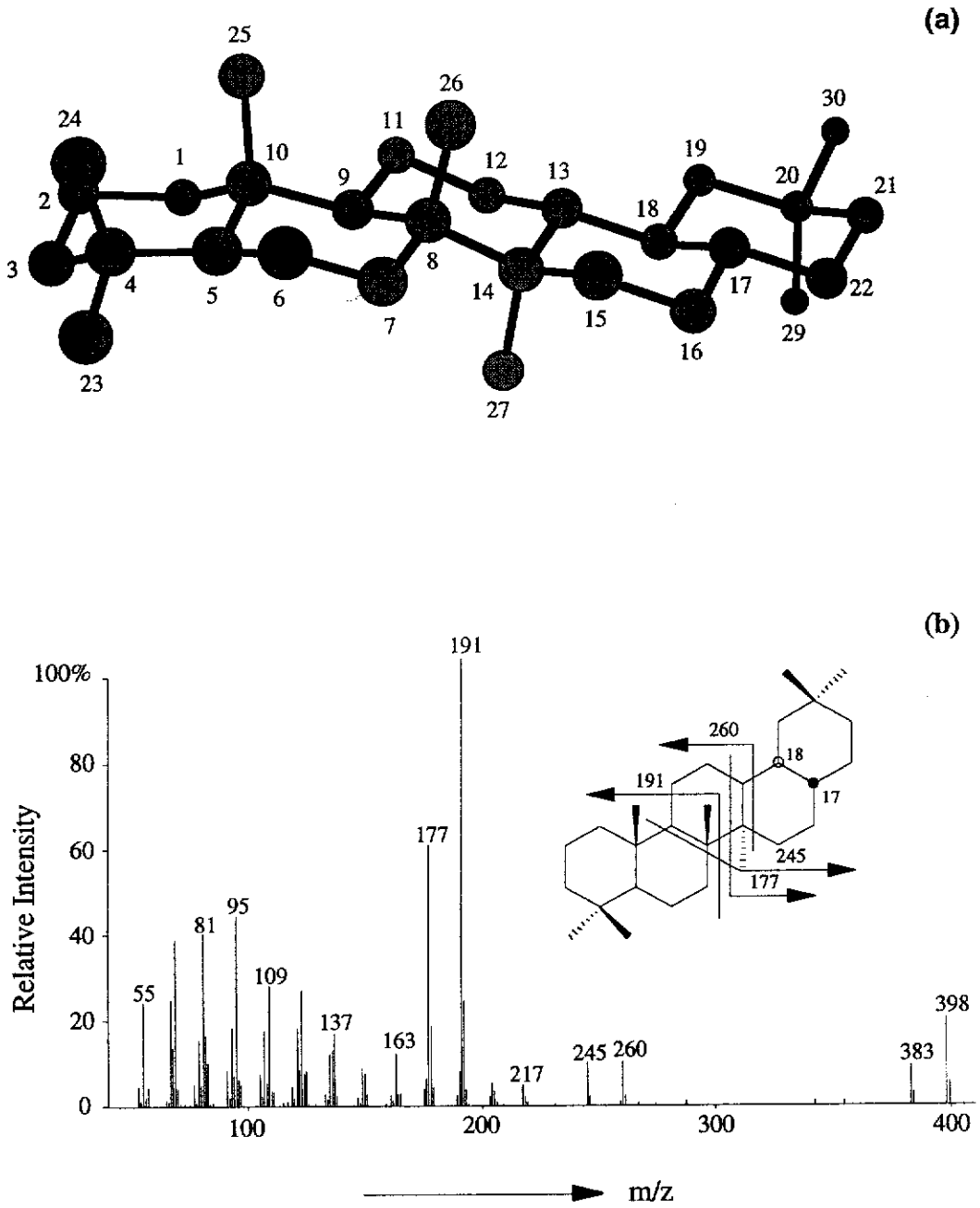


Figure 7.5 Three-dimensional perspective view (a) and mass spectrum (b) of 28-nor-18α-oleanane isolated from the hydrous pyrolysate of a lignite.

Table 7.1 Non-hydrogen positional and isotropic displacement parameters obtained from a single crystal X-ray study of 28-nor-18α-oleanane.

	x	y	z	U
C(1)	0.5890(5)	0.538(1)	0.793(4)	0.047(8)
C(2)	0.5916(6)	0.638(1)	0.819(4)	0.060(9)
C(3)	0.5907(6)	0.679(1)	0.632(5)	0.065(9)
C(4)	0.5627(6)	0.659(2)	0.511(5)	0.053(9)
C(5)	0.5594(6)	0.560(2)	0.499(4)	0.05(1)
C(6)	0.5317(5)	0.528(1)	0.378(4)	0.037(8)
C(7)	0.5387(5)	0.434(2)	0.330(5)	0.062(9)
C(8)	0.5424(6)	0.376(2)	0.487(4)	0.039(8)
C(9)	0.5657(5)	0.414(1)	0.637(4)	0.039(8)
C(10)	0.5608(6)	0.513(2)	0.676(5)	0.054(9)
C(11)	0.5690(6)	0.358(2)	0.813(5)	0.08(1)
C(12)	0.5789(5)	0.269(1)	0.744(4)	0.054(9)
C(13)	0.5590(5)	0.230(2)	0.606(4)	0.032(8)
C(14)	0.5564(5)	0.284(2)	0.428(4)	0.031(9)
C(15)	0.5356(6)	0.245(2)	0.287(5)	0.07(1)
C(16)	0.5428(6)	0.153(2)	0.236(5)	0.061(9)
C(17)	0.5457(5)	0.099(2)	0.413(4)	0.047(9)
C(18)	0.5690(6)	0.137(1)	0.548(4)	0.035(8)
C(19)	0.5711(5)	0.083(2)	0.719(4)	0.050(9)
C(20)	0.5798(6)	-0.012(2)	0.667(4)	0.051(9)
C(21)	0.5576(6)	-0.046(2)	0.535(5)	0.07(1)
C(22)	0.5542(5)	0.009(2)	0.367(5)	0.06(1)
C(23)	0.5324(6)	0.701(2)	0.589(5)	0.08(1)
C(24)	0.5648(6)	0.693(2)	0.321(5)	0.08(1)
C(25)	0.5328(5)	0.523(2)	0.817(4)	0.059(8)
C(26)	0.5106(5)	0.364(1)	0.571(4)	0.051(8)
C(27)	0.5875(5)	0.298(1)	0.338(4)	0.048(8)
C(29)	0.6126(6)	-0.018(2)	0.592(5)	0.08(1)
C(30)	0.5789(5)	-0.062(1)	0.858(4)	0.059(9)

Table 7.2 ^{13}C chemical shift assignments (ppm relative to TMS using $CDCl_3$ as internal reference) for lupane (Ammann *et al.*, 1982), the isolated C_{30} triterpane, 24,28-bisnor-18α-oleanane (Trendel *et al.*, 1991b) and the isolated and characterised 28-nor-18α-oleanane.

Carbon	Lupane	C_{30} Triterpane	24,28-Bisnor-18α-oleanane	28-Nor-18α-oleanane
1	40.36	40.26	39.81	40.37
2	18.69+	18.69	21.51	18.69#
3	42.16	42.10	36.63	42.11
4	33.20	33.25	30.58	33.26
5	56.36	56.28	54.42	56.46
6	18.72+	18.63	20.97	18.60#
7	34.42	34.30	33.06	33.88
8	41.12	41.04	40.49*	40.77
9	50.25	50.13	48.55	50.58
10	37.47	37.40	37.07	37.44
11	21.88	21.90	21.21	20.89
12	26.94	26.89	25.03	24.90
13	37.88	37.75	43.04	42.98
14	43.08	43.04	41.61*	41.50
15	27.36	27.26	31.06	31.11
16	35.61	35.54	29.66	29.57
17	43.12	43.16	43.75	43.69
18	47.69	47.60	37.88	37.82
19	44.75	44.67	43.94	43.86
20	29.36	29.36	31.09	31.05
21	21.93	21.90	39.19	39.11
22	40.43	40.41	30.41	30.33
23	33.34	33.36	20.54	33.35
24	21.57	21.57	-	21.54
25	16.00	16.01	14.13	16.25
26	16.08	16.07	15.78	15.70
27	14.49	14.42	14.91	15.02
28	18.08	18.05	-	-
29	15.13	15.14	25.39	25.35
30	22.93	23.00	33.74	33.69

+,#,* ^{13}C resonance assignments are interchangeable

Mass spectrometry

The mass spectrum of 28-nor-18α-oleanane (Figure 7.5b) shows a strong molecular ion at m/z 398 and a base peak at m/z 191. Fragment ions at m/z 260 and m/z 245 are due to ABC-ring and CDE-ring fragmentation, respectively. A strong ion at m/z 260/259 is a common feature in the mass spectra of other higher-plant derived triterpanes like oleanane, lupane and taraxastane. The absence of this fragment ion in the mass spectra of the regular hopanes suggests that cleavage at the DE-ring junction in the molecule is due to the lack of a methyl substituent at C-18, a common structural feature in higher-plant derived triterpanes, but not in hopanoids. Further support to this suggestion comes from inspection of a published mass spectrum of 30-norneohopane (C_{29}Ts) (Moldowan *et al.*, 1991), a rearranged hopane lacking a C-18 methyl substituent, which shows fragment ions at m/z 260 and 245 similar to those in the mass spectrum of 28-nor-18α-oleanane (Figure 7.5b). The m/z 260 fragment ion is therefore characteristic of a pentacyclic triterpane lacking methyl substituents at the C-18 position.

GC-MS analysis

GC-MS analysis was carried out to distinguish between the GC retention behaviour of 28-nor-18α-oleanane and the closely eluting 30-norneohopane. Figure 7.6 shows partial m/z 191 mass chromatograms obtained from the co-injection of the isolated 28-nor-18α-oleanane standard with a typical branched and cyclic alkane fraction from a crude oil containing 30-norneohopane (C_{29}Ts). The figure shows that 28-nor-18α-oleanane is not well resolved from 30-norneohopane. However, 28-nor-18α-oleanane has a slightly longer retention time than 30-norneohopane and hence may be distinguished from the latter compound in m/z 191 mass chromatograms of crude oils.

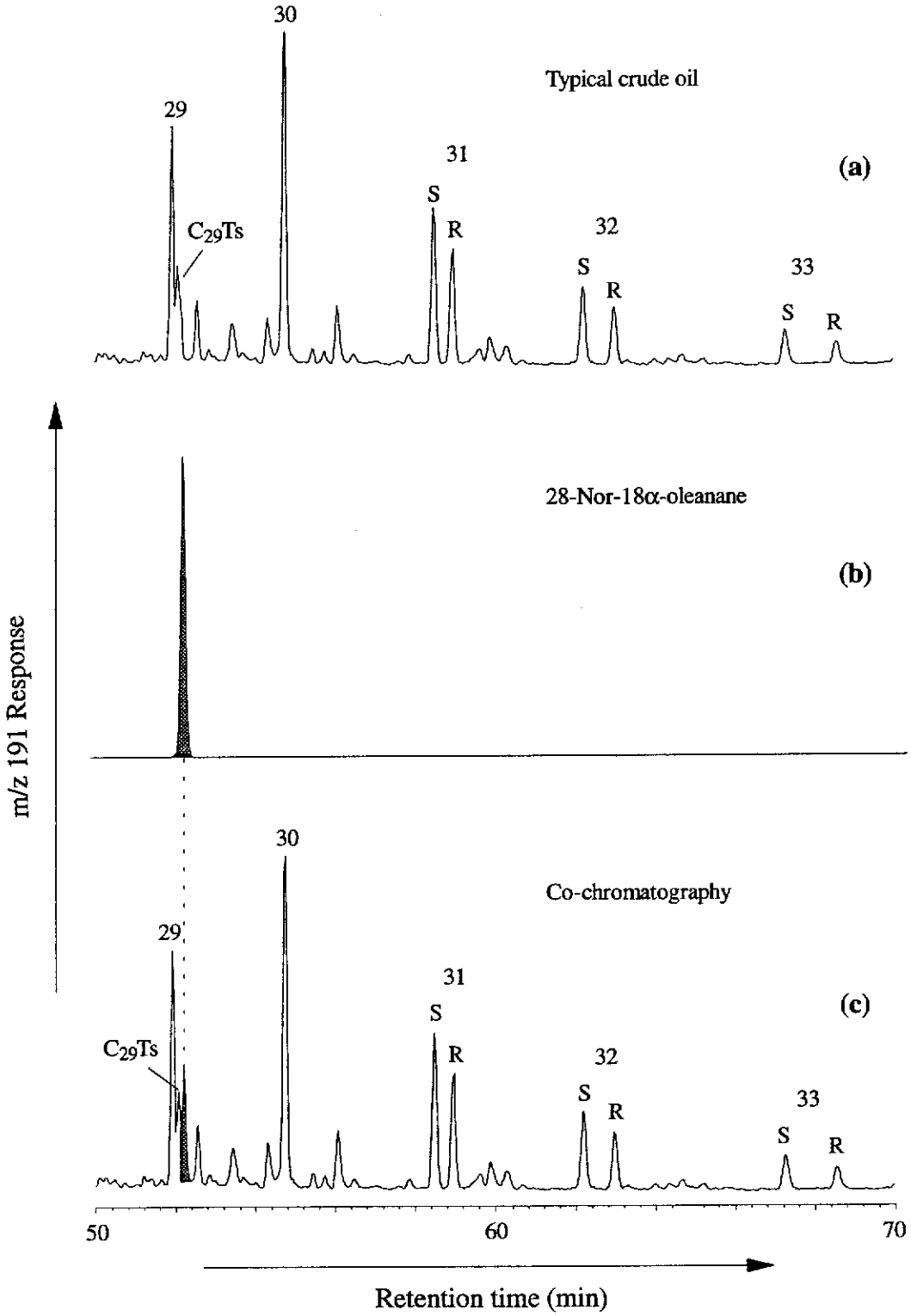


Figure 7.6 Partial m/z 191 mass chromatograms for a typical crude oil branched and cyclic alkane fraction before and after co-chromatography with 28-nor-18α-oleanane standard. Peaks labelled 29-33 represent the series of αβ-hopanes.

7.3.3 Analysis and Identification of Lupane

The C_{30} triterpane was crystallised (87% pure by GC, melting point of 194-195°C) from a minimum quantity of ethylmethylketone into very fine needle-like crystals which were not suitable for X-ray analysis. The structure of this triterpane was therefore based on ^{13}C NMR data and supported by comparison of its GC retention behaviour with that of an authentic reference compound.

^{13}C NMR spectroscopy

The ^{13}C NMR spectrum of the isolated C_{30} triterpane showed 30 resonances which were resolved with the aid of DEPT experiments into eight methyl, eleven methylene, six methine and five quaternary carbon signals. The ^{13}C NMR chemical shifts, listed in Table 7.2, were assigned based on those previously reported for lupane (Wenkert *et al.*, 1978; Ammann *et al.*, 1982). Inspection of the two sets of data showed that the carbon chemical shifts for the isolated triterpane compared favourably (within 0.3 ppm range) with published data for lupane and it was therefore concluded that the isolated triterpane was lupane (Figure 7.7a).

Mass spectrometry

The mass spectrum of the isolated lupane (Figure 7.7b) shows a C_{30} molecular ion at m/z 412 and a base peak at m/z 191. The presence of an ion m/z 259 response is due to ABC-ring and CDE-ring ion fragments and, as previously mentioned, is characteristic of a triterpane skeleton lacking a methyl substituent at C-18. The most important feature of the spectrum however, which distinguishes it from those of the coeluting oleananes (e.g., ten Haven *et al.*, 1992b; Rullkötter *et al.*, 1994), is the presence of a fragment ion at m/z 369 [M-43] which indicates the loss of an isopropyl substituent.

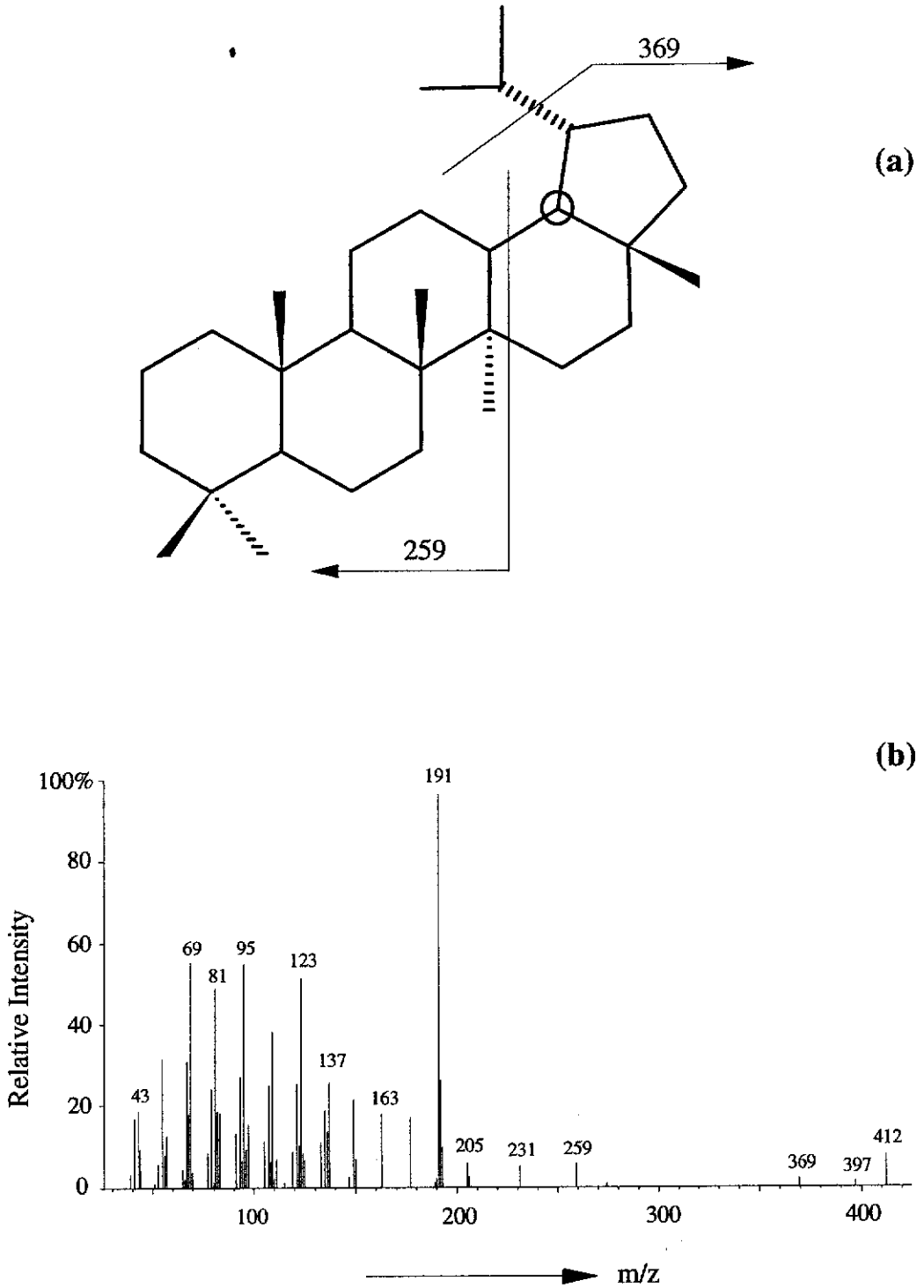


Figure 7.7 Structure (a) and mass spectrum (b) of lupane isolated from the hydrous pyrolysate of a lignite.

GC-MS analysis

To confirm the presence of lupane in the hydrous pyrolysate of the Heartbreak lignite, co-chromatography of the hydrous pyrolysate alkane fraction with an authentic standard of lupane was carried out using four different GC columns. Under standard GC conditions (see experimental section, part I, p.25) lupane and the isomeric oleananes were found to coelute when analysed using either a BP-1 or BP-5 columns. However, using a more polar BP-10 and in particular a BP-20 column it was found that these triterpanes were adequately resolved. Figure 7.8 shows partial m/z 191 mass chromatograms from two co-chromatography experiments using a BP-20 column. Co-injection of an alkane fraction from a typical higher-plant derived crude oil with a lupane standard (Figure 7.8a) shows that lupane is absent from the former. However, co-injection of lupane standard with the alkane fraction of the hydrous pyrolysate of the Heartbreak lignite (Figure 7.8b) confirms the presence of lupane.

7.4 GEOCHEMISTRY OF 28-NOR-18α-OLEANANE

7.4.1 Origin of 28-Nor-18α-oleanane

28-Nor-18α-oleanane most likely originates from C-28 functionalised natural products which occur widely in extant plants (Pant and Rastogi, 1979; Das and Mahato, 1983). During diagenesis, these natural product precursors may undergo defunctionalisation and hydrogenation to give 28-nor-triterpenoids (ten Haven *et al.*, 1992b). Acid catalysed interconversion of triterpanes of different skeletal types may occur (e.g., Ames *et al.*, 1954; Halsall *et al.*, 1952; Coates, 1967) and such processes have been reported to occur in sediments (e.g., ten Haven and Rullkötter, 1988; Ekweozor and Telnaes, 1990). Thus, 28-nor-18α-oleanane may

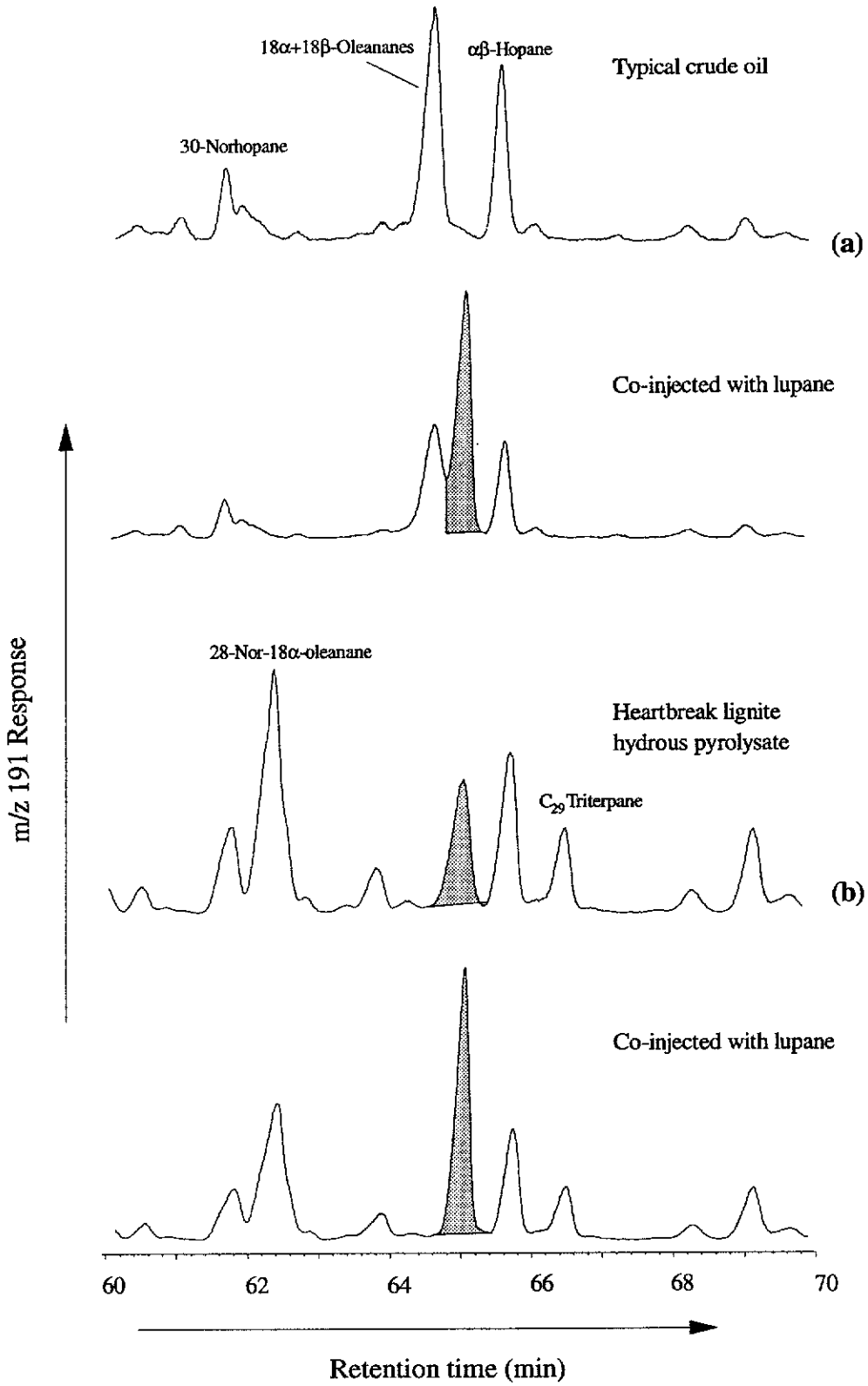


Figure 7.8 Partial m/z 191 mass chromatograms for the branched and cyclic alkane fractions of a typical higher-plant derived crude oil and the Heartbreak lignite hydrous pyrolysate before and after co-chromatography with lupane standard using a BP-20 capillary column.

be derived from a range of other 28-nor-triterpenoids, provided that the absence of a substituent at C-28 does not inhibit the rearrangement processes.

The presence of 28-nor-18α-oleanane in the hydrous pyrolysate of the Heartbreak lignite, but not in the unheated soluble organic matter indicates that it (or its precursor) is bound to the kerogen. The apparent absence of 28-noroleanane from this low maturity sample is unusual because it is not consistent with the proposed formation of 18α-oleanane from oleanane. This apparent inconsistency could be related to the high sulphur content of the lignite. Noroleanene species present during diagenesis may have been reduced and incorporated into the kerogen by polysulphide linkages (Figure 7.9).

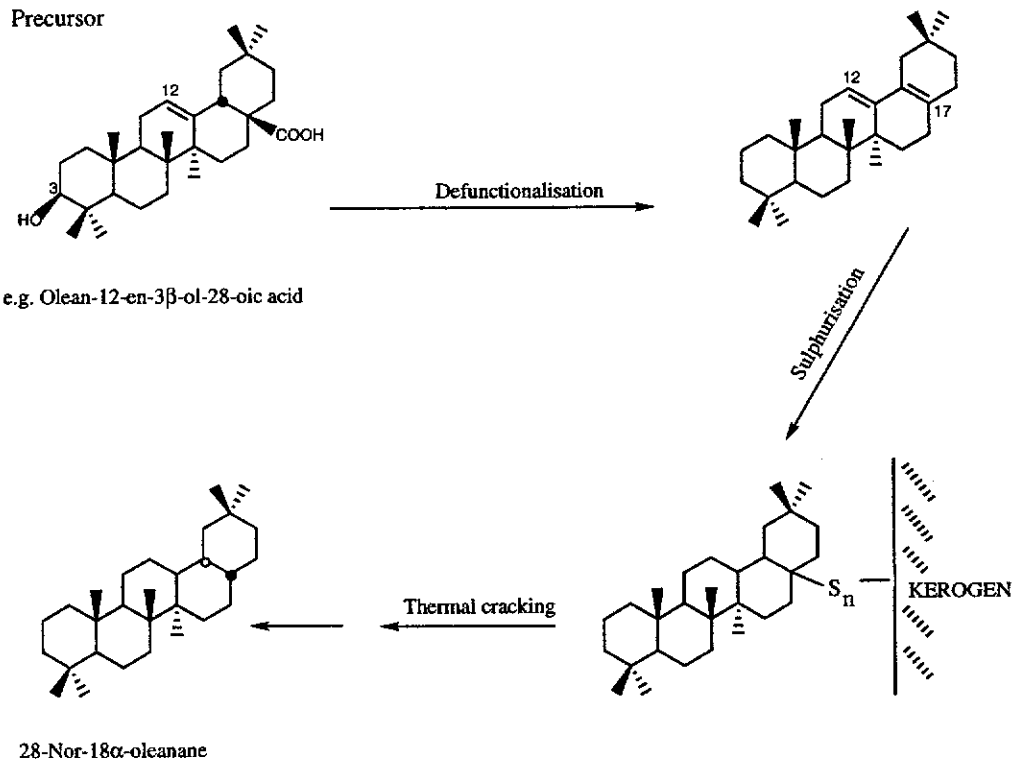


Figure 7.9 Proposed formation of 28-nor-18α-oleanane isolated from a sulphur-rich lignite.

This suggestion is supported by the fact that no noroleanenes were detected in the lignite either in the free or bound forms. Hence, if sulphur incorporation of noroleanene species occurred prior to conditions which favour their sedimentary hydrogenation then it is conceivable that their diagenetic pathway to form alkane species would be altered.

7.4.3 Occurrence 28-Nor-18α-oleanane in Sedimentary Organic Matter

The occurrence of 28-nor-18α-oleanane in kerogen from the Heartbreak lignite suggests that 28-noroleananes might be more widespread in sedimentary organic matter of Tertiary age than has previously been recognised. The Heartbreak lignite was deposited in a temperate climate. The compound 28-norolean-12-ene-3-one is a probable precursor for 28-nor-18α-oleanane and has been reported to occur in sediments from the Mahakam Delta (Corbet et al., 1980) deposited under humid, tropical conditions. Inspection of previously published m/z 191 mass chromatograms suggests that 28-nor-18α-oleanane occurred in the dry pyrolysate of a Tertiary lignite but was not recognised (Strachan *et al.*, 1989). Furthermore, data presented here confirms doubts expressed by ten Haven *et al.* (1992b) regarding the authenticity of a previous assignment of 28-noroleanane in a Jurassic coal by Wang *et al.* (1990). Their compound had a significantly lower GC retention time (relative to 30-norhopane) compared to that of our authentic standard. One explanation for the apparent absence of 28-nor-18α-oleanane in sediments and crude oils is that it is poorly resolved from C_{29}Ts (Moldowan *et al.*, 1991) (*cf.*, Figure 7.6) and the spirotriterpane (Hills *et al.*, 1968) using common stationary phases and may have gone undetected (e.g., Ekweozor and Udo, 1988).

A study was carried out to assess the occurrence of 28-nor-18α-oleanane in sedimentary organic matter. Samples analysed included higher-plant derived crude

oils rich in 18α-oleanane as well as Tertiary sediment extracts and hydrous pyrolysates. Interestingly, 28-nor-18α-oleanane was either absent or present in negligible amounts in all but one other sample, the hydrous pyrolysate of a Tertiary Loy Yang lignite (Figure 7.10a). The peak shown in this figure was assigned as 28-nor-18α-oleanane rather than 28-noroleanane based on its retention time and mass spectrum being identical to that for the isolated compound (*cf.* Figure 7.5b). The sole occurrence of 28-nor-18α-oleanane in this lignite and the Heartbreak lignite from which it was isolated suggests that it may be confined to Tertiary lignites.

7.4.4 Geochemical Fate of 28-Nor-18α-oleanane

The absence of 28-nor-18α-oleanane in more mature sediments and crude oils prompted an investigation of the fate of this compound following its release from the kerogen. It was observed that coals of higher rank than lignites did not contain this triterpane in either the free or bound form. Its absence may be due to either the lack of suitable natural product precursors at time of deposition or its selective destruction or alteration upon being released from the kerogen. It is unlikely that such an alteration process would be temperature dependent because 28-nor-18α-oleanane has a high thermodynamic stability comparable to that for 18α-oleanane.

28-Nor-18α-oleanane may occur in the bound form in low rank coals and upon its thermally induced release from the kerogen it is destroyed in the presence of acid clays. Laboratory dry pyrolysis of a Loy Yang lignite alone and in the presence of intimately mixed aluminium smectite was carried out to observe whether the proportion of 28-nor-18α-oleanane in the pyrolysate was affected by the presence of clay. Comparison of the m/z 191 mass chromatograms for the two pyrolysates, shown in Figure 7.10, shows the absence of 28-nor-18α-oleanane in the chromatogram for the sample from the clay experiment, along with an enhanced

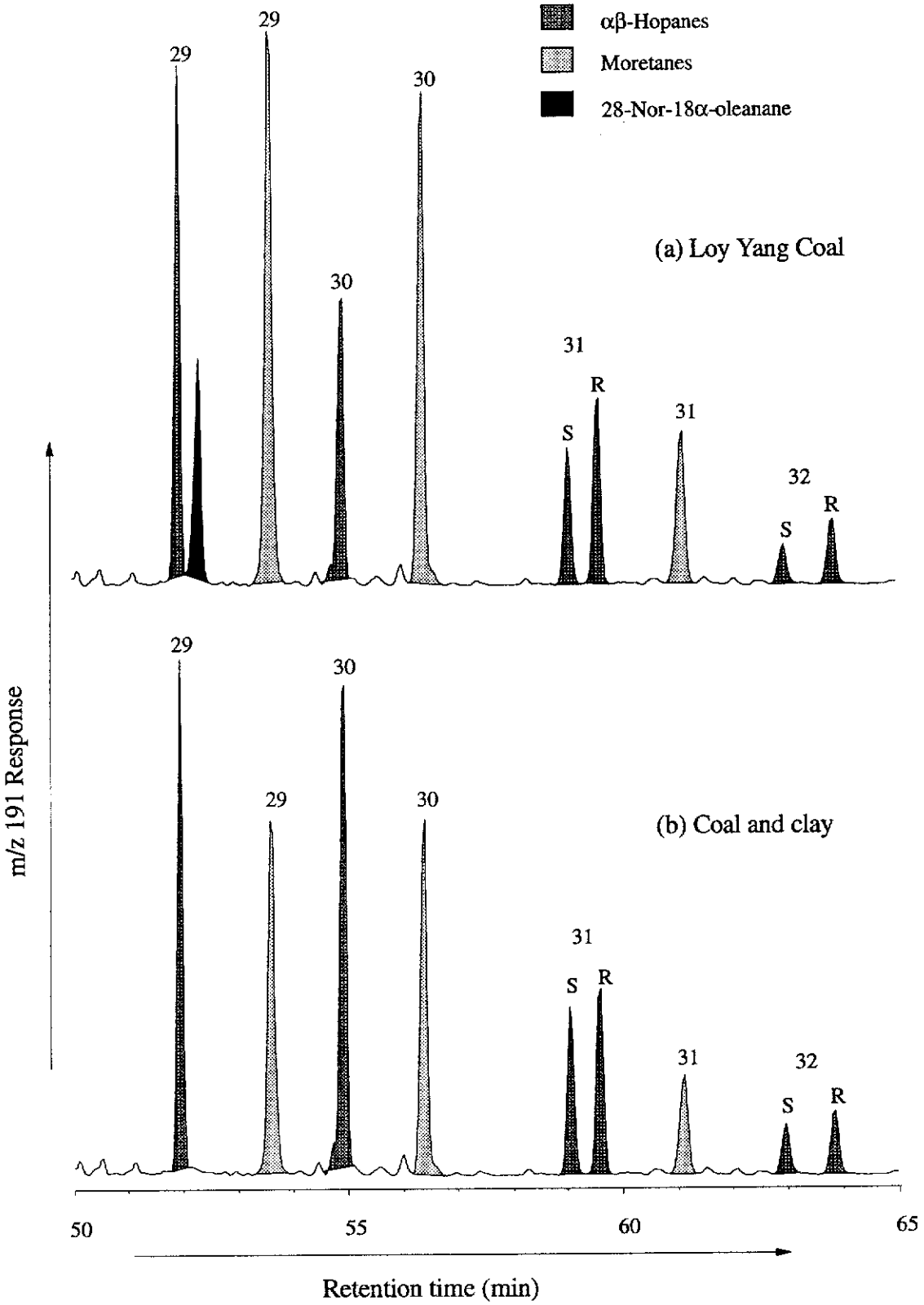


Figure 7.10 Partial m/z 191 mass chromatograms of the branched and cyclic alkane fraction from dry pyrolysate of (a) Loy Yang coal and (b) the same coal in a 50/50 mixture with an acidic clay. Numbers 29-32 denote the total number of carbons in each homologue.

relative abundance of the more stable αβ-hopanes. This result provides one explanation for the absence of 28-nor-18α-oleanane in mature sediments and crude oils.

7.5 OCCURRENCE OF LUPANE

The unusually high abundance of lupane in the sulphur-rich Heartbreak lignite (*cf.*, Figure 7.2b, peak 2) suggests that sulphur may have played a crucial role in the early bonding and subsequent preservation of the reactive lupane precursors. It has been shown that sulphurisation or sulphur incorporation into organic matter can occur very early in diagenesis (e.g., Sinninghe Damsté *et al.*, 1989; Kohnen *et al.*, 1989; Rowland *et al.*, 1993). Schouten *et al.* (1994) described laboratory sulphurisation experiments on various compounds which contained either a double bond, ketone or aldehyde functionality. By analogy, sulphurisation and preservation of lupane in this sulphur-rich lignite may have occurred prior to diagenetic conditions which favoured isomerisation of lupanoid precursors. Most of the numerous lupane-related natural products reported to date (*cf.*, Dev *et al.*, 1989) have either an oxygen containing functionality in the E-ring or, more importantly, a double bond on the isomerisation-prone isopropyl group. Bonding of the lupane precursors at the isopropyl group via sulphur bonds to the kerogen during early diagenesis might therefore preserve the lupane skeleton. Upon thermal cracking, the sulphur bonds would cleave giving lupane.

7.6 CONCLUSIONS

28-Nor-18α-oleanane and lupane have been unambiguously identified in the hydrous pyrolysate of a Heartbreak lignite. Their occurrence in high abundance in this sample has been attributed to incorporation via sulphur linkages of their precursors into the kerogen at early stages of diagenesis.

CHAPTER 8

FORMATION OF 18α-OLEANANE IN SEDIMENTS

Summary

Double bond isomerisation of olean-18-ene followed by catalytic hydrogenation of the resulting oleanene mixture was carried out to investigate the origin of 18α-oleanane. Laboratory results showed that 18α-olean-12-ene, a major product from acid-catalysed rearrangement of olean-18-ene, when hydrogenated gives predominantly 18α-oleanane. Analysis of the relative abundances of oleanenes, oleanane and 18α-oleanane in a low maturity sedimentary sequence from Indonesia supported laboratory findings indicating that 18α-olean-12-ene, rather than oleanane, is the main precursor of 18α-oleanane in sedimentary organic matter. These results are in agreement with those recently published by Rullkötter *et al.* (1994).

8.1 BACKGROUND

Both oleanane and 18α-oleanane are believed to originate in sediments from the hydrogenation of oleanene intermediates. ten Haven and Rullkötter (1988) suggested that Δ^{18} oleanene is hydrogenated, while Ekweozor and Telnaes (1990) prefer the $\Delta^{13(18)}$ species as the more probable candidate for hydrogenation. More recently, unpublished work by Peakman and co-workers (cited in ten Haven *et al.*, 1992b) indicates that the Δ^{12} and Δ^{18} species are the most probable candidates for hydrogenation. Regardless of which alkene species is preferentially hydrogenated in sediment, hydrogenation would preferentially give oleanane because the β-face in each molecule is the least hindered (e.g., ten Haven and Rullkötter, 1988). Therefore, it was inferred that the formation pathway for the sedimentary 18α-oleanane can only be via thermally induced isomerisation of the less stable 18β-isomer (e.g., Ekweozor and Telnaes, 1990). This conclusion was supported by various reports, such as one by Riva *et al.* (1988), who identified the 18α- and 18β-isomers in sediments in proportions suggesting that oleanane forms first and then isomerises to 18α-oleanane.

By analogy with the route proposed for the formation of 28-nor-18α-oleanane (Chapter 7) from precursor intermediates other than 28-noroleanane, the possibility of formation of 18α-oleanane from precursors other than oleanane has been explored. In the following section the diagenetic formation of 18α-oleanane from oleanene intermediates is reported. This work assumes that catalytic hydrogenation in the laboratory is a valid model for reduction of alkenes in sediments.

8.2 IDENTIFICATION OF OLEANENE ISOMERS IN LABORATORY AND GEOLOGICAL SAMPLES

Olean-13-(18)-ene, olean-18-ene and olean-12-ene were identified from their relative retention times (ten Haven *et al.*, 1992a; 1992b) and comparison of their mass spectra (Figure 8.1) with those published (Budzikiewicz *et al.*, 1963; Djerassi *et al.*, 1962; Karliner and Djerassi, 1966; respectively).

18α-Olean-12-ene, which is reported to coelute with urs-12-ene (ten Haven *et al.*, 1992a), was identified based on characteristic features of its mass spectrum compared with that of urs-12-ene. Figure 8.2 shows total ion chromatograms of mixtures obtained from the hydrogenation of ursa-9(11),12-diene and olean-18-ene. Urs-12-ene was observed to have a slightly shorter retention time than the tentatively identified 18α-olean-12-ene in the latter chromatogram. More importantly, comparison of the mass spectra for the two peaks shown in the insets of this figure showed characteristic differences as previously reported (*cf.*, Karliner and Djerassi, 1966). The mass spectrum of urs-12-ene shows less intense ions at m/z 395 and m/z 203 relative to m/z 410 and m/z 191, while the mass spectrum of 18α-olean-12-ene shows more intense ions at m/z 395 and m/z 203.

8.3 LABORATORY ISOMERISATION AND HYDROGENATION OF OLEAN-18-ENE

Acid-catalysed rearrangement of olean-18-ene followed by catalytic hydrogenation of the resulting mixture was carried out to investigate the formation pathway of oleanane and 18α-oleanane from oleanene precursors.

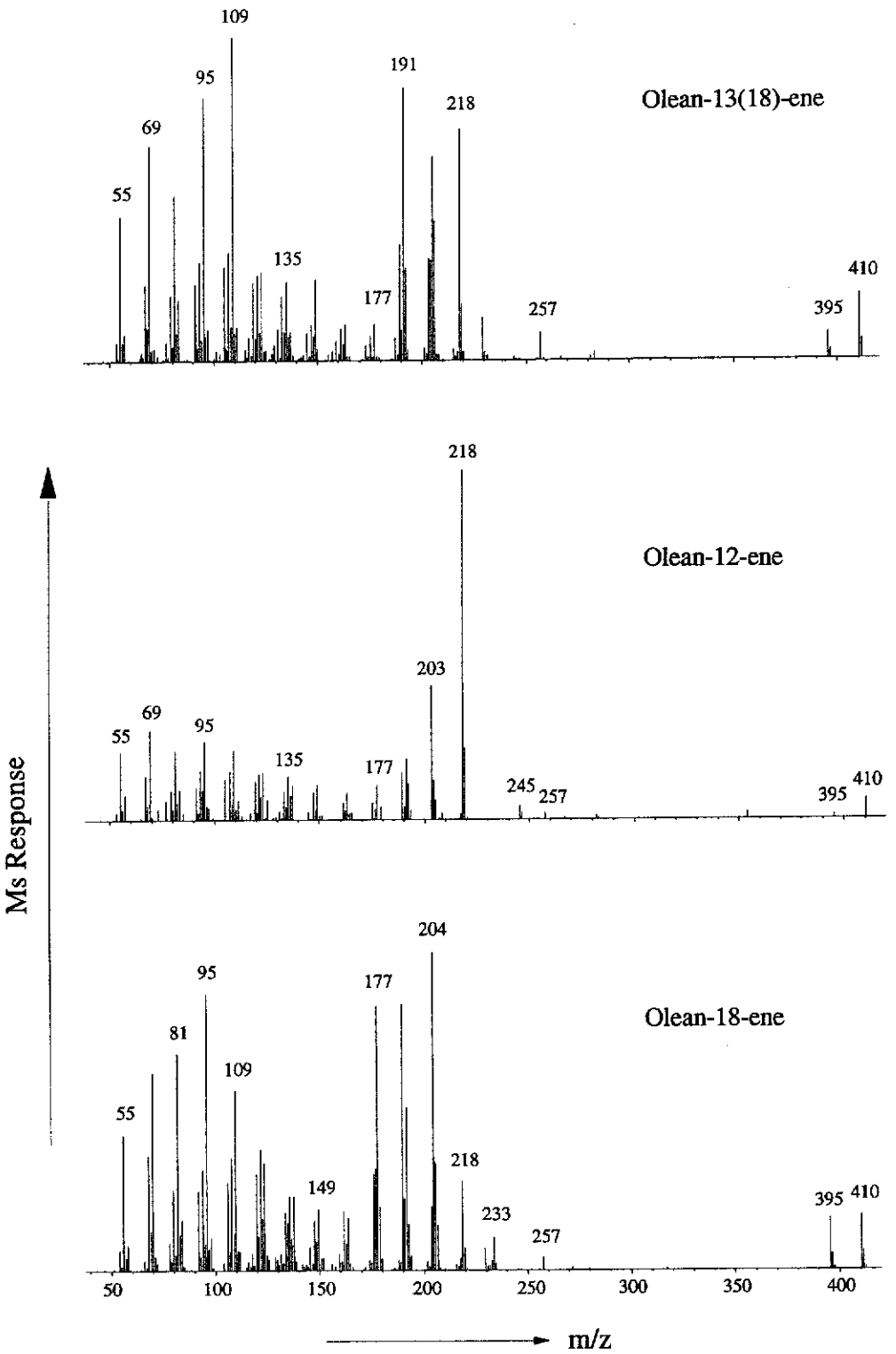


Figure 8.1 Mass spectra of oleanene isomers identified in this study.

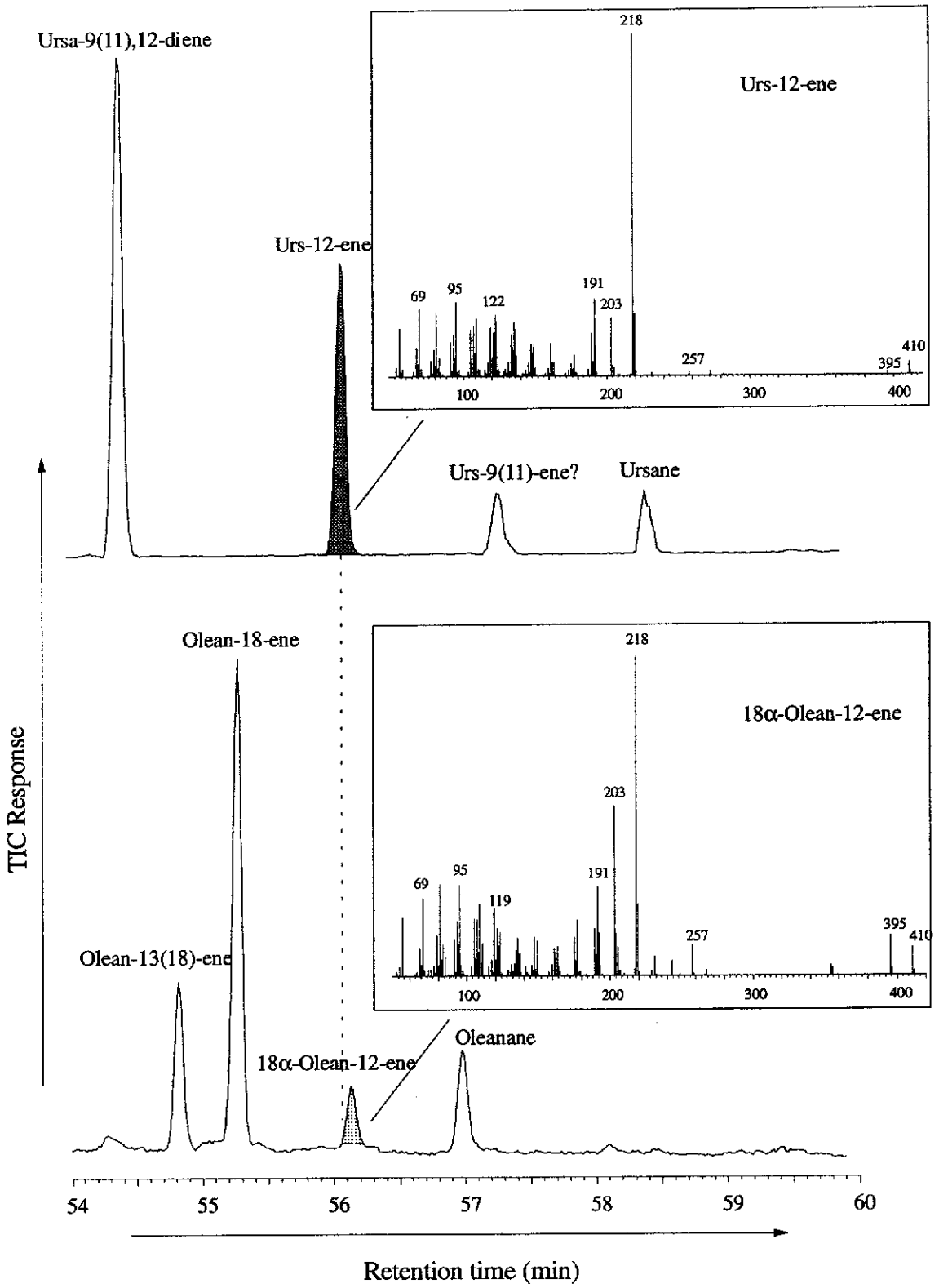


Figure 8.2 Total ion chromatograms for compound mixtures obtained from the hydrogenation of ursa-9(11),12-diene and olean-18-ene. The insets show mass spectra of urs-12-ene and 18α-olean-12-ene which have similar gas chromatographic retention times.

Figure 8.3b-d shows total ion chromatograms obtained from heating olean-18-ene (Figure 8.3a) in the presence of aluminium smectite at various temperatures. Comparison of the reaction mixture compositions shows that with increasing temperature olean-18-ene isomerises to the more thermodynamically stable olean-13(18)-ene and 18α-olean-12-ene. This is consistent with work of Brownlie *et al.* (1956) and Ageta *et al.* (1987) who reported olean-13(18)-ene and 18α-olean-12-ene to be the major products from acid-catalysed rearrangement of olean-12-ene. Above a reaction temperature of 120°C, little change occurred in the isomer distribution, but degradation products tentatively identified as alkylindenes and alkylnaphthalenes began to form. It was therefore suggested that the isomer mixture d (120°C/24h), which comprised olean-13(18)-ene, olean-18-ene and 18α-olean-12-ene in a ratio of approximately 6:1:3, was probably nearing equilibrium for the solid state process under these experimental conditions. This is broadly consistent with the data of Brownlie *et al.* (1956) who reported the equilibrium mixture, obtained from a homogeneous system by refluxing olean-18-ene in aqueous acid solution, to be made up of olean-13(18)-ene and 18α-olean-12-ene in a ratio of 4:3.

Hydrogenation of oleanene mixture d over platinum (IV) oxide hydrate catalyst in glacial acetic acid produced a mixture (Figure 8.3f) which, in addition to the alkenes, contained an unidentified C_{30} triterpane (?) and approximately equal amounts of 18α and 18β oleananes. The notable absence of olean-18-ene in the hydrogenation product mixture indicates that this alkene is most susceptible to reduction. The fact that this alkene predominantly forms oleanane is demonstrated by the hydrogenation of olean-18-ene carried out in a separate experiment (Figure 8.3e). Hence, the presence of 18α-oleanane in mixture f, and its absence in the product mixture from hydrogenation olean-18-ene, shows that it was derived from reduction of either or both olean-13(18)-ene or 18α-olean-12-ene. Reduction of 18α-olean-12-ene is suggested to be the more likely scenario

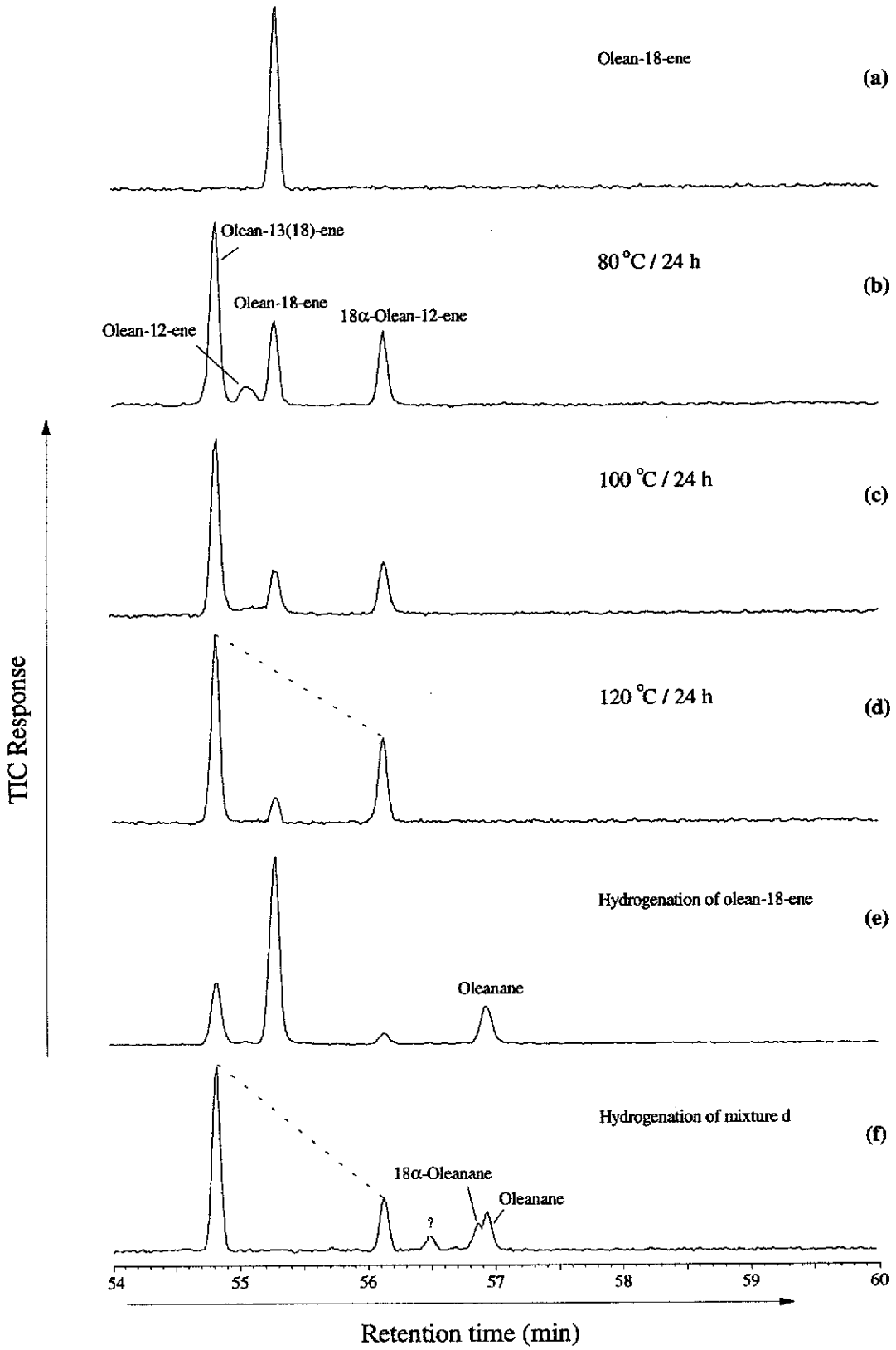


Figure 8.3 Total ion chromatograms obtained from heating olean-18-ene (a) in the presence of aluminium smectite at various temperatures (b-d), and from the hydrogenation of olean-18-ene (e) and mixture d (f).

for the formation of 18α-oleanane since, based on accepted hypotheses of hydrogenation (*cf.* Augustine, 1965), the tetrasubstituted double bond in olean-13(18)-ene should be more resistant to hydrogenation than the trisubstituted double bond in 18α-olean-12-ene.

Comparison of component abundances in mixtures before and after hydrogenation (Figure 8.3d and f, respectively) also reveals a precursor-product relationship between the oleanenes and their saturated analogs. The amount of oleanane produced after hydrogenation corresponds to that of olean-18-ene before hydrogenation thus confirming the reduction of olean-18-ene to oleanane. Further, a decrease in the amount of 18α-olean-12-ene relative to olean-13(18)-ene after hydrogenation shows that it was reduced at a faster rate. Since most of the oleanane in mixture f has been accounted for, it is deduced that 18α-olean-12-ene was the major source of 18α-oleanane in the product mixture.

8.4 HYDROGENATION OF OLEANENES IN SEDIMENTS

The relative abundances of oleanenes and oleananes in an immature sedimentary sequence from the South Sumatra Basin (see Figure 3.4) were analysed in an effort to find evidence for the formation of 18α-oleanane from 18α-olean-12-ene in sediments. These shales from the Miocene Gumai Formation had been deposited in a marine environment (*cf.*, Sosrowidjojo *et al.*, 1994b). The shallowest, least mature samples (380-1352m) were chosen for this study so that formation of 18α-oleanane by thermal maturation is not an issue. Figure 8.4 shows m/z 191 mass chromatograms for the branched and cyclic alkane components extracted from five sediment samples from the sequence (see Table 8.1 for peak identification). Inspection of the mass chromatograms of the four shallowest samples (a-d) shows that, apart from having high amounts of

oleanenes (insets a-d), these sediments contain high abundances of 30-normoretane, moretane (5 and 10) and *22R* αβ-homohopane (11) which is a feature of their low maturity. In contrast, the deepest sample (Figure 8.4e) appears to be significantly more mature based on its equilibrated homohopane distribution (11,12) and the lack of remaining oleanenes (inset e).

Table 8.1 Triterpanes identified in the branched and cyclic alkane fractions from a sedimentary sequence from Indonesia (peak numbers refer to Figure 8.4).

Peak	Compound
1	Olean-13(18)-ene
2	30-Norhopane
3	Olean-12-ene
4	Olean-18-ene
5	30-Normoretane
6	18α-Olean-12-ene
7	18α-Oleanane
8	Oleanane
9	αβ-Hopane
10	Moretane
11	*22R* αβ-homohopane
12	*22S* αβ-homohopane

The occurrence of 18α-oleanane in these immature samples supports its diagenetic origin from 18α-olean-12-ene. The second shallowest sample (Figure 8.4b) appears to have reached a stage marked by the commencement of alkene

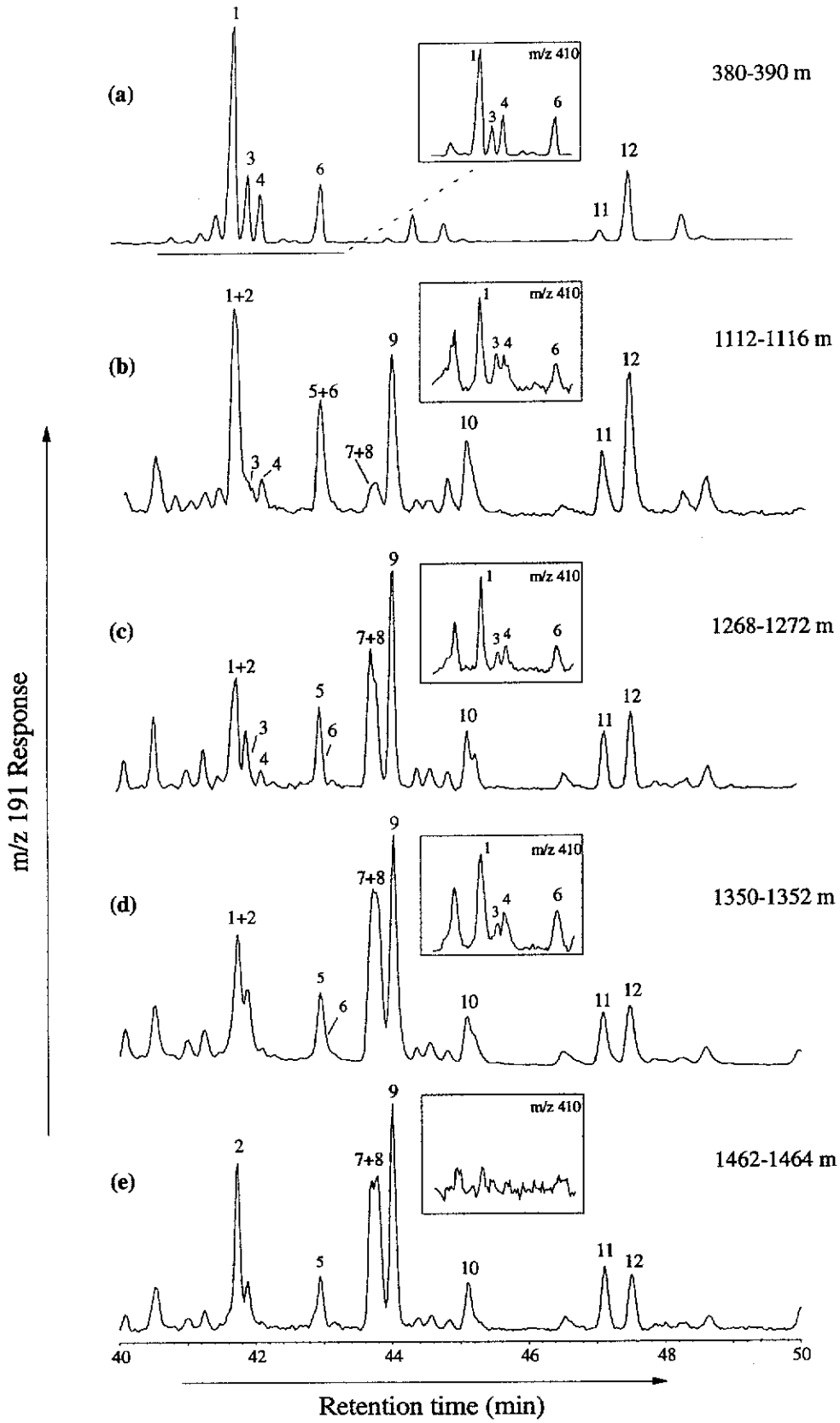


Figure 8.4 Partial m/z 191 mass chromatograms showing the pentacyclic triterpane distributions in a sedimentary sequence from Indonesia (see Table 8.1 for peak identification). Insets show m/z 410 mass chromatograms for detecting oleanenes.

reduction, as evident from the minor amounts of oleanane and 18α-oleanane (7+8). With increasing depth, hydrogenation proceeds by producing the alkane species until all precursors are depleted, as observed in the chromatogram of the deepest sample (inset Figure 8.4e). Due to the low maturity of the intermediate samples, it is highly unlikely that oleanane would have isomerised to form 18α-oleanane. Combined with laboratory evidence for the hydrogenation of 18α-olean-12-ene, it is evident that this alkene is the major precursor of 18α-oleanane in this sedimentary sequence.

Figure 8.5 summarises the proposed scheme for oleanene-oleanane conversion in sediments. Natural products of various skeletal-types undergo early diagenetic processes to give olean-12-ene (i) which then rearranges under acid catalysed conditions to give an equilibrated mixture of isomers, including olean-13(18)-ene (ii) olean-18-ene (iii) and18α-olean-12-ene (iv). With increased sediment maturation, these isomers are reduced at different rates. Olean-18-ene appears to be reduced at the fastest rate to form oleanane, while 18α-olean-12-ene is reduced at a slower rate to give 18α-oleanane. Upon further sediment maturation, it appears that oleanane may either be preferentially destroyed or isomerised to the more thermodynamically stable 18α-oleanane.

The diagenetic origin of 18α-oleanane from 18α-olean-12-ene may have important geochemical implications regarding its occurrence in immature samples. Since 18α-olean-12-ene appears to be derived from isomerisation of naturally occurring oleanenes, it can be inferred that high abundances of 18α-oleanane in sediments reflects acidic diagenetic conditions. Alternatively, elevated amounts of 18α-olean-12-ene in samples may reflect the type of natural product precursors present at the time of depostion. In other words, 18α-oleanene species in sediments may, in part, be derived from natural products which have an 18α-configuration, rather than solely being derived from the acid-catalysed

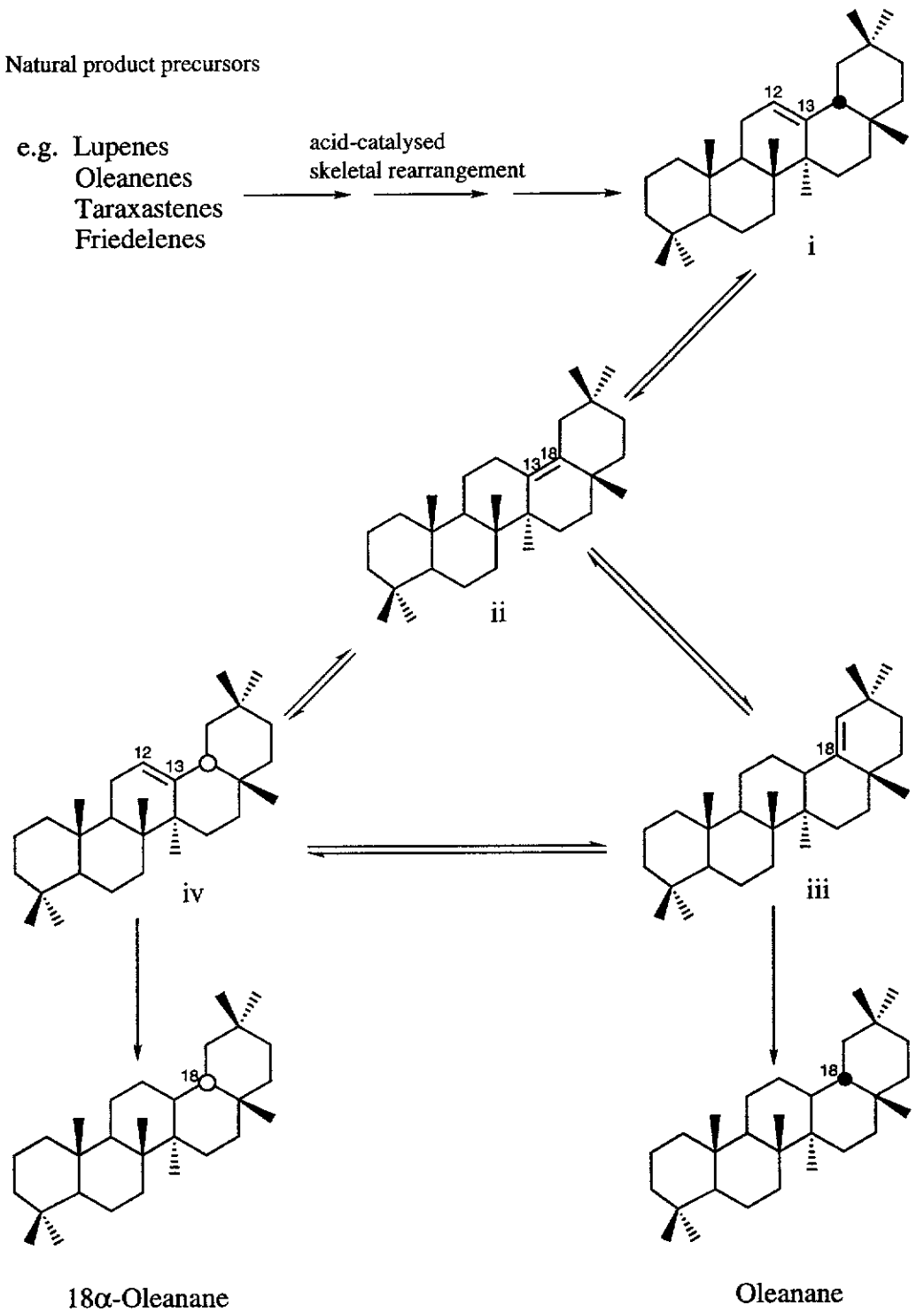


Figure 8.5 Proposed diagenetic formation pathway of 18α-oleanane and oleanane via 18α-olean-12-ene and olean-18-ene precursors, respectively.

rearrangement of oleanenes. Structurally similar natural products such as 18α-olean-19α-ol-3-one, which was identified in a tree *Samadera indica* (Wintersteiner *et al.*, 1965), may undergo defunctionalisation to give 18α-oleanene species. Such extant natural products are, however, not common and so clearly would not account for the widespread occurrence of 18α-oleanane in sedimentary organic matter of higher-plant origin. Another possible group of precursors which are widespread in extant plants are the lupene-type natural products. Lupenes have been shown to readily undergo acid catalysed rearrangement to give 18α-oleanane (Halsall *et al.*, 1952; Corbett and Ding, 1971). This would involve the reduction of the $\Delta^{20(30)}$ double bond in lupeol to form a tertiary carbocation which promotes the acid catalysed rearrangement from a five to a six-membered E-ring, while retaining its 18α-configuration.

8.5 CONCLUSIONS

18α-Olean-12-ene has been shown in laboratory hydrogenation experiments to be reduced to give predominantly 18α-oleanane. Results obtained from the relative abundances of oleanenes, oleanane and 18α-oleanane in a low maturity sedimentary sequence are consistent with laboratory observations and hence indicate that 18α-olean-12-ene, rather than oleanane, is indeed the main diagenetic precursor of 18α-oleanane in sediments.

REFERENCES

van Aarssen B. G. K., Cox H. C., Hoogendoorn P. and De Leeuw J. W. (1990a) A cadinene biopolymer present in fossil and extant dammar resins as a source for cadinanes and bicadinanes in crude oils from South East Asia. *Geochim. Cosmochim. Acta* **54**, 3021-3031.

van Aarssen B. G. K., Kruk C., Hessels J. K. C. and de Leeuw J. W. (1990b) Cis-cis-trans-bicadinane, a novel member of an uncommon triterpane family isolated from crude oils. *Tetrahedron Lett.* **31**, 4645-4648.

van Aarssen B. G. K., Horsfield B. and de Leeuw J. W. (1991) A comparative study of three different pyrolysis methods used to characterise a biopolymer isolated from fossil and extant dammar resins. *J. Anal. Appl. Pyrol.* **20**, 125-139.

van Aarssen B. G. K., Hessels J. K. C., Abbink O. A. and De Leeuw J. W. (1992) The occurrence of polycyclic sesqui-, tri-, and oligoterpenoids derived from a resinous polymeric cadinene in crude oils from southeast Asia. *Geochim. Cosmochim. Acta* **56**, 1231-1246.

van Aarssen B. G. K., de Leeuw J. W., Collinson M., Boon J. J. and Goth K. (1994) Occurrence of polycadinene in fossil and recent resins. *Geochim. Cosmochim. Acta* **58**, 223-229.

Abbott G. D., Lewis C. A. and Maxwell J. R. (1984) Laboratory simulation studies of steroid aromatisation and alkane isomerisation. In *Advances in Organic Geochemistry 1983* (Edited by Schenck P. A., de Leeuw J. W. and Lijmbach G. W. M.), *Org. Geochem.* **6**, pp. 31-38. Pergamon Press, Oxford.

Abbott G. D., Lewis C. A. and Maxwell J. R. (1985) Laboratory models for aromatization and isomerization of hydrocabons in sedimentary basins. *Nature* **318**, 651-653.

Ageta H., Shiojima K. and Arai Y. (1987) Acid-induced rearrangement of triterpenoid hydrocarbons belonging to the hopane and migrated hopane series. *Chem. Pharm. Bull.* **35**, 2705-2716.

Alam M. and Pearson M. J. (1990) Bicadinanes in oils from the Surma Basin, Bangladesh. *Org. Geochem.* **15**, 461-464.

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

Alexander R., Fisher S. J., Kagi R. I. (1988a) 2,3-Dimethylbiphenyl: Kinetics of its cyclisation reaction and effects of maturation upon its relative concentration in sediments. In *Advances in Organic Geochemistry 1987* (Edited by Mattavelli L. and Novelli L.), pp. 833-837. Pergamon Press, Oxford.

Alexander R., Larcher A. V., Kagi R. I. and Price P. L. (1988b) The use of plant-derived biomarkers for correlation of oils with source rocks in the Cooper/Eromanga Basin system, Australia. *APEA J.* **28**, 310-324.

Ames T. R., Davy G. S., Halsall T. G. and Jones E. R. H. (1952) The chemistry of triterpenes and related compounds. Part XII. The action of formic acid on lupeol. *J. Chem. Soc.*, 2826-2872.

Ames T. R., Beton J. L., Bowers A., Halsall T. G. and Jones E. R. H. (1954) The chemistry of the triterpenes and related compounds. Part XXIII. The structure of taraxasterol, ψ-taraxasterol (heterolupeol), and lupenol-I. *J. Chem. Soc.*, 1905-1919.

Ammann W., Richarz R., Wirthlin T. and Wendisch D. (1982) ^{1}H and ^{13}C chemical shifts and coupling constants of lupane. Application of two-dimensional NMR techniques. *Org. Magn. Res.* **20**, 260-265.

Augustine R. L. (1965) *Catalytic Hydrogenation.* Edward Arnold Publishers Ltd., London.

Axelrod D. I. (1952) A theory of angiosperm evolution. *Evolution* **6**, 29-60.

Axelrod D. I. (1960) The evolution of flowering plants. *Evolution After Darwin* **1**, 277-305.

Baddeley G. V., Fookes C. J. R. and Nigam S. K. (1976) The acid-catalysed rearrangement of 28-norlupenes. *Australian J. Chem.* **29**, 2707-2712.

Bailey I. W. (1949) Origin of the angiosperms: Need for a broadened outlook. *J. Arnold Arboretum* **30**, 64-70.

Balogh B., Wilson D. M., Christiansen P. and Burlingame A. L. (1973) 17α(H)-Hopane identified in oil shale of the Green River Formation (Eocene) by Carbon-13 NMR. *Nature* **242**, 603-605.

Bande M. B. and Prakash U. (1986) The Tertiary flora of South East Asia with remarks in its palaeoenvironment and phytogeography of the Indo-Malayan region. *Rev. Palaeobot. Palynol.* **49**, 203-233.

Barrie P. J., Gladden L. F. and Klinowski J. (1991) Neutron diffraction studies of realuminated zeolite Y. *J. Chem. Soc., Chem. Commun.*, 592-594.

Barton D. H. R., Carruthers W. and Overton K. H. (1956) Triterpenoids. Part XXI. A triterpenoid lactone from petroleum. *J. Chem. Soc.*, 788.

Bisseret P. and Rohmer M. (1990) Bromine, *n*-bromosuccinimide and sulphur induced isomerizations in the hopane series. *Tetrahedron Lett.* **31**, 7445-7448.

Blanc P. and Connan J. (1992) Origin and occurrence of 25-norhopanes: a statistical study. *Org. Geochem.* **18**, 813-828.

Bowering O. J. W. and Harrison D. M. (1982) The Merrimelia oil and gas field. In *Eromanga Basin Symposium*, Summary Paper (Compiled by Moore P. S. and Mount T. J.), pp. 344-345. Geological Society of Australia & Petroleum Exploration Society of Australia, Adelaide.

Breck D. W. (1974) *Zeolite Molecular Sieves.* John Wiley, New York.

Brownlie G., Fayez M. B. E., Spring F. S., Stevenson R. and Strachan W. S. (1956) Triterpenoids. Part XLVIII. Olean-13(18)-ene: Isomerisation of olean-12-ene and related hydrocarbons with mineral acid. *J. Chem. Soc.*, 1377-1381.

Budzikiewicz H., Wilson J. M. and Djerassi C. (1963) Mass spectrometry in structural and stereochemical problems. XXXII. Pentacyclic triterpenes. *J. Amer. Chem. Soc.* **85**, 3688-3699.

Cassani F. and Eglinton G. (1991) Organic geochemistry of Venezuelan extra-heavy crude oils 2. Molecular assessment of biodegradation. *Chem. Geol.* **91**, 315-333.

Chandler M. E. J. (1962) *The Lower Tertiary Floras of Southern England. II. Flora of the Pipe-Clay Series of Dorset (Lower Bagshot).* British Museum (Natural History), London.

Chen N. Y. and Degnan T. F. (1988) Industrial catalysis applications of zeolites. *Chem. Eng. Prog.* **16**, 32-41.

Chosson P., Connan J., Dessort D. and Lanau C. (1992) In vitro biodegradation of steranes and terpanes: A clue to understanding geological situations. In *Biological Markers in Sediments and Petroleum* (Edited by Moldowan J. M., Albrecht P. and Philp R. P.), pp. 320-349. Prentice Hall, Englewood Cliffs.

Coates R. M. (1967) On the friedelene-oleanene rearrangement. *Terahedron Lett.* **13**, 4143-4146.

Collinson M. E. (1983) Fossil plants of the London Clay. *Palaeontological Association Field Guides to Fossils No 1.* Palaeontological Association, London.

Connan J. (1974) Diagenese neaturelle et diagenese artificielle de la matiere organique a éléments végétaux prédominants. In *Advances in Organic Geochemistry 1973* (Edited by Tissot B. and Bienner F.), pp. 73-95. Ed. Technip, Paris.

Connolly J. D. and Overton K. H. (1972) The triterpenoids. In *Chemistry of Terpenes and Terpenoids* (Edited by Newman A. A.), pp. 207-287. Academic Press, New York.

Corbet B., Albrecht P. and Ourisson G. (1980) Photochemical or photomimetric fossil triterpenoids in sediments and petroleum. *J. Amer. Chem. Soc.* **102**, 1171-1173.

Corbett R. E. and Smith R. A. (1969) Lichens and fungi. Part VI. Dehydration rearrangements of 15-hydroxyhopanes. *J. Chem. Soc.* (C), 44-47.

Corbett R. E. and Ding H. L. (1971) Lichens and Fungi. Part VIII. 18α-Oleanane. *J. Chem. Soc. (C)*, 1884-1885.

Corey E. J. and Ursprung J. J. (1956) The stereochemistry of the α-amyrins. *J. Amer. Chem. Soc.* **78**, 183-188.

Corma A., Miguel P. J., Orchilles A. V. and Koermer G. S. (1992) Cracking of long-chain alkyl aromatics on USY zeolite catalysts. *J. Catal.* **135**, 45-59.

Cornet B. (1993) (?) *Modern Geology* **19**, 81-99.

Cornford C., Christie O., Endresen U., Jensen P. and Myhr M. -B. (1988) Source rock and seep oil maturity in Dorset, Southern England. *Org. Geochem.* **13**, 399-409.

de Coster G. L. (1975) The geology of the Central and South Sumatra Basins. In *Proceedings of the Indonesian Petroleum Association. Third Annual Convention*, pp. 77-110. Indonesian Petroleum Association, Jakarta.

Crane P. R. (1993) Time for the angiosperms. *Nature* **366**, 631-632.

Crane P. R., Donoghue M. J., Doyle J. A. and Friis E. M. (1989) Angiosperm origins. *Nature* **342**, 131-132.

Crank K. (1973) Geology of Barrow Island oil field. *APEA J.* **13**, 49-57.

Crelling J. C., Pugmire R. J., Meuzelaar H. L. C., McClennen W. H., Huai H. and Karas J. (1991) The chemical structure and petrology of resinite from the Hiawatha "B" coal seam. *Energy and Fuels* **5**, 688-694.

Cox R. E., Burlingame A. L., Wilson D. M., Eglinton G. and Maxwell J. R. (1973) Botryococcane - a tetramethylated acyclic triterpenoid of algal origin. *J. Chem. Soc. Chem. Commun.* 284-285.

Cox H. C., de Leeuw J. W., Schenck P. A., van Koningsveld H., Jansen J. C., van de Graaf B., van Geerestein V. J., Kanters J. A., Kruk C. and Jans A. W.

H. (1986) Bicadinane, a C_{30} pentacyclic isoprenoid hydrocarbon found in crude oil. *Nature* **319**, 316-318.

Curiale J. A. (1988) Molecular genetic markers and maturity indices in intermontane lacustrine facies: Kishenehn Formation, Montana. In *Advances in Organic Geochemistry 1987* (Edited by Mattavelli L. and Novelli L.), pp. 633-638. Pergamon Press, Oxford.

Curiale J. A. (1991) The petroleum geochemistry of Canadian Beaufort Tertiary "non-marine" oils. *Chem. Geol.* **93**, 21-45.

Curran R., Eglinton G., Maclean I., Douglas A. G. and Dungworth G. (1968) Simplification of complex mixtures of alkanes using 7A molecular sieve. *Tetrahedron Lett.* **14**, 1669-1673.

Czochanska Z., Gilbert T. D., Philp R. P., Sheppard C. M. Weston R. J., Wood T. A. and Woolhouse A. D. (1988) Geochemical application of sterane and triterpane biomarkers to a description of oils from the Taranaki Basin in New Zealand. *Org. Geochem.* **2**, 123-135.

Daly M. C., Hooper B. G. D. and Smith D. G. (1987) Tertiary plate tectonics and basin evolution in Indonesia. In *Proceedings of the Indonesian Petroleum Association. Sixteenth Annual Convention*, pp. 399-428. Indonesian Petroleum Association, Jakarta.

Das M. C. and Mahato S. B. (1983) Triterpenoids. *Phytochem.* **22**, 1071-1095.

Davidson J. K., Blackburn G. J. and Morrison K. C. (1984) Bass and Gippsland Basins: A comparison. *APEA J.* **24**, 101-109.

Davies P. R. (1984) Tertiary structural evolution and related hydrocarbon occurrences, North Sumatra Basin. In *Proceedings of the Indonesian Petroleum Association. Thirteenth Annual Convention*, pp. 19-47. Indonesian Petroleum Association, Jakarta.

Dev S., Gupta A. S. and Patwardhan S.A. (1989) *CRC Handbook of Terpenoids - Triterpenoids Volume II.* CRC Press, Boca Raton.

Dimmler A. and Strausz O. P. (1983) Enrichment of polycyclic terpenoid, saturated hydrocarbons from petroleum by adsorption on zeolite NaX. *J. Chromatogr.* **270**, 219-225.

Djerassi C., Budzikiewicz H. and Wilson J. M. (1962) Mass spectrometry in structural and stereochemical problems. VIII. Unsaturated pentacyclic triterpenoids. *Tetrahedron Lett.* **7**, 263-270.

van Dorsselaer A., Ensminger A., Spyckerelle C., Dastillung M., Sieskind O., Arpino P., Albrecht P., Ourisson G., Brooks P. W., Gaskell S. J., Kimble B. J., Philp R. P., Maxwell J. R. and Eglinton G. (1974) Degraded and extended hopane derivatives (C_{27} to C_{35}) as ubiquitous geochemical markers. *Tetrahedron Lett.* 1349-1352.

van Dorsselaer A., Albrecht P. and Connan J. (1977a) Changes in composition of polycyclic alkanes by thermal maturation (Yallourn lignite, Australia). In *Advances in Organic Geochemistry 1975* (Edited by Campos R. and Goni J.), pp. 53-59. Enadimsa, Madrid.

van Dorsselaer A., Albrecht P. and Ourisson G. (1977b) Identification of novel (17αH) hopanes in sediments and crude oils. *Bull. Soc. Chim. France*, 165-170.

Ekweozor C. M., Okogun J. I., Ekong D. E. U. and Maxwell J. R. (1979) Preliminary organic geochemical studies of samples from the Niger Delta (Nigeria). I. Analysis of crude oils for triterpanes. *Chem. Geol.* **27**, 11-28.

Ekweozor C. M. and Udo O. T. (1988) The oleananes: Origin, maturation and limits of occurrence in Southern Nigeria sedimentary basins. In *Advances in Organic Geochemistry 1987* (Edited by Mattavelli L. and Novelli L.), *Org. Geochem.* **13**, pp. 131-140. Pergamon Press, Oxford.

Ekweozor C. M. and Telnaes N. (1990) Oleanane parameter: Verification by quantitative study of the biomarker occurrence in sediments of the Niger delta.

In *Advances in Organic Geochemistry 1989* (Edited by Durand B. and Behar F.), pp. 401-413. Pergamon Press, Oxford.

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

Ensminger A., Albrecht P., Ourisson G., Kimble B. J., Maxwell J. R. and Eglinton G. (1972) Homohopane in Messel Oil Shale: First identification of a C_{31} pentacyclic triterpane in nature. Bacterial origin of some triterpanes in ancient sediments? *Tetrahedron Lett.* **36**, 3861-3864.

Ensminger A., van Dorsselaer A., Spyckerelle C., Albrecht P. and Ourisson G. (1974) Pentacyclic triterpenes of the hopane type as ubiquitous geochemical markers: origin and significance. In *Advances in Organic Geochemistry 1973* (Edited by Tissot B. and Bienner F.), pp. 245-260. Editions Technip, Paris.

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

Flanigen E. M., Bennett J. M., Grose R. W., Cohen J. P., Patton R. L., Kirchner R. M. and Smith J. V. (1978) Silicalite, a new hydrophobic crystalline silica molecular sieve. *Nature* **271**, 512-516.

Francois R. (1987) A study of sulphur enrichment in the humic fraction of marine sediments during early diagenesis. *Geochim. Cosmochim. Acta* **51**, 17-27.

Fripiat J. J. and van Olphen V. (1979) *Data Handbook for Clay Matrials and other Non-Metallic Minerals.* Pergamon Press, Oxford.

Fu Jiamo and Sheng Guoying (1989) Biological marker composition of typical source rocks and related oils of terrestrial origin in the People's Republic of China; a review. *Appl. Geochem.* **4**, 13-22.

Goodwin N. S., Mann A. L. and Patience R. L. (1983) Crude oil biodegradation. In *Advances in Organic Geochemistry 1981* (Edited by Bjorøy *et al.*), pp. 650-658. J. Wiley and Sons, New York.

Gorodetsky M., Kogan D. and Mazur Y. (1970) Free radical epimerization at unactivated tertiary carbon atoms in cyclohexane derivatives. *J. Am. Chem Soc.* **92**, 1094-1096.

Gough M. A. and Rowland S.J. (1990) Characterisation of unresolved complex mixtures of hydrocarbons in petroleum. *Nature* **344**, 648-650.

Grantham P. J., Posthuma J. and Baak A. (1983) Triterpanes in a number of Far Eastern crude oils. In *Advances in Organic Geochemistry 1981* (Edited by Bjorøy M. *et al.*), pp. 675-683. John Wiley, Chichester.

Green P. M., Brain T. J. and John B. H. (1989) Possible stratigraphic controls on hydrocarbon distribution within the Jurassic-Early Cretaceous rocks, Eromanga Basin, southern Queensland. In *The Cooper & Eromanga Basins Australia.* Proceedings of Petroleum Exploration Society of Australia (Edited by O'Neil B. J.) pp. 252-264, Society of Petroleum Engineers, Australian Society of Exploration Geophysicists (SA Branches), Adelaide.

Gunn P. J. and Ly K. C. (1989) The petroleum prospectivity of the Joseph Bonaparte Gulf area, northwestern Australia. *APEA J.* **29**, 509-526.

Hall S. R., Flack H. D. and Stewart J. M. (Editors) (1992) The XTAL 3.2 reference manual. Universities of Western Australia, Geneva and Maryland.

Halsall T. G., Jones E. R. H. and Meakins G. D. (1952) The chemistry of triterpenes and related compounds. Part XI. The conversion of lupeol into germanicol (isolupeol). The structure of lupeol hydrochloride. *J. Chem. Soc.*, 2862-2867.

Halsall T. G., Jones E. R. H. and Swayne R. E. H. (1954) The chemistry of triterpenes and related compounds. Part XXII. The conversion of lupeol into ψ-taraxasterol (heterolupeol). *J. Chem. Soc.*, 1902-1905.

Halsall T. G., Jones E. R. H., Tan E. L. and Chaudhry G. R. (1966) The chemistry of triterpenes and related compounds. Part XLV. The acid-catalysed rearrangements of the 5α,6α-diols and 5,6-epoxides of some 4,4-dimethylsteroids. *J. Chem. Soc.*, 1374-1383.

ten Haven H. L. and Rullkötter J. (1988) The diagenetic fate of taraxer-14-ene and oleanene isomers. *Geochim. Cosmochim. Acta* **52**, 2543-2548.

ten Haven H. L., Peakman T. M. and Rullkötter J. (1992a) Δ^2-Triterpanes: Early intermediates in the diagenesis of terrigenous triterpenoids. *Geochim. Cosmochim. Acta* **56**, 1993-2000.

ten Haven H. L., Peakman T. M. and Rullkötter J. (1992b) Early diagenetic transformation of higher-plant triterpenoids in deep-sea sediments from Baffin Bay. *Geochim. Cosmochim. Acta* **56**, 2001-2024.

Heinemann H. (1981) Technological applications of zeolites in catalysis. *Cat. Rev.-Sci. Eng.* **23**, 315-322.

Hills I. R. and Whitehead E. V. (1966) Triterpanes in optically active petroleum distillates. *Nature* **209**, 977-979.

Hills I. R., Smith G. W. and Whitehead E. V. (1968) Optically active spirotriterpane in petroleum distillates. *Nature* **219**, 243-246.

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

Hoffman C. F., Mackenzie A. S., Lewis C. A., Maxwell J. R., Oudin J. L., Durand B. and Vandenbroucke M. (1984) A biological marker study of coals, shales, and oils from the Mahakam Delta, Kalimantan, Indonesia. *Chem. Geol.* **42**, 1-23.

Hughes W. B., Holba A. G., Miller D. E. and Richardson J. S. (1985) Geochemistry of greater Ekofisk crude oils. In *Geochemistry in Exploration of*

the Norwegian Shelf (Edited by Thomas B. M.), pp. 75-92. Graham and Trotman.

Jasra R. V. and Bhat S. G. (1987) Sorption kinetics of higher n-paraffins on zeolite molecular sieve 5A. *Ind. Eng. Chem. Res.* **26**, 2544-2546.

Jefferies P. J. (1988) Geochemistry of the Turtle oil accumulation, offshore southern Bonaparte Basin. In *The North West Shelf Australia.* Proceedings of Petroleum Exploration Society Australia Symposium, Perth. (Edited by Purcell P.G. and Purcell R.R.), pp. 563-569. Petroleum Exploration Society Australia, Perth.

Karliner J. and Djerassi C. (1966) Terpenoids LVII. Mass spectral and nuclear magnetic resonance studies of pentacyclic triterpene hydrocarbons. *J. Org. Chem.* **31**, 1945-1956.

Kerr G. T. (1989) Synthetic zeolites. *Scient. Am.* **261**, 82-87.

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. *Org. Geochem.* **15**, 147-160.

Kimble B. J., Maxwell, J. R., Philp, R. P. and Eglinton G. (1974) Identification of steranes and triterpanes in geolipid extracts by high-resolution gas chromatography and mass spectromentry. *Chem. Geol.* **14**, 173-198.

Kohen F. and Stevenson R. (1965) The bromine and *n*-bromosuccinimide oxidation of the saturated hydrocarbon, friedelane. *J. Org. Chem.* **30**, 2479-2480.

Kohnen M. E. L., Sinninghe Damste J. S., ten Haven H. L., and de Leeuw J. W. (1989) Early incorporation of polysulphides in sedimentary organic matter. *Nature* **341**, 640-641.

Kolaczknowska E., Slougui N. -E., Watt D. S., Maruca R. E. and Moldowan J. M. (1990) Thermodynamic stability of various alkylated, dealkylated and rearranged 17α- and 17β-hopane isomers using molecular mechanics

calculations. In *Advances in Organic Geochemistry 1989* (Edited by Durand B. and Behar F.), pp. 1033-1038. Pergamon Press, Oxford.

Lakhanpal R. N. and Guleria J. S. (1986) Fossil leaves of *Dipterocarpus* from the Lower Siwalik beds near Jawalamukhi, Himachal Pradesh. *The Palaeobotanist* **35**, 258-262.

Lavering I. H. and Ozimic S. (1988) Bonaparte Basin Petroleum Accumulations. In *The North West Shelf Australia.*. Proceedings of Petroleum Exploration Society Australia Symposium, Perth. (Edited by Purcell P.G. and Purcell R.R.), pp. 331-337. Petroleum Exploration Society Australia, Perth.

Lee R. J. and Gunn P. J. (1988) The Bonaparte Basin. In *Petroleum in Australia, the First Century*, pp. 252-269. Australian Petroleum Exploration Association.

Lewan M. D. (1985) Evaluation of petroleum generation by hydrous pyrolysis experimentation. *Phil. Trans. R. Soc. Lond.* **A315**, 123-134.

McDaniel C. V. and Maher P. K. (1968) *Molecular Sieves.* Society of Chemical Industry, London.

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

Marchand A., Libert P. and Combaz A. (1969) Essai de caractérisation physico-chimique de la diagénese de quelques roches organiques biologiquement homogenes. *Rev. Inst. Fr. Pét.* **24**, 3-20.

Martin W., Gierl A. and Saedler H. (1989) Molecular evidence for pre-Cretaceous angiosperm origins. *Nature* **339**, 46-48.

Maxwell I. E. (1986) Shape selective catalysis and process technology via molecular inclusion in zeolites. *J. Incl. Phenom.* **4**, 1-7.

Maxwell J. R., Douglas A. G., Eglinton G. and McCormick A. (1968) The botryococcenes-hydrocarbons of novel structure from the alga *Botryococcus braunii, Kutzing*. *Phytochemistry* **7**, 2157-2171.

Metzger P., Largeau C. and Casadevall E. (1991) Lipids and macromolecular lipids of the hydrocarbon-rich microalga Botryococcus *braunii*. Chemical structure and biosynthesis. Geochemical and biotechnological importance. *Progress in the Chemistry of Organic Natural Products* **57**, 1-70.

Meuzelaar H. L. C., Huai H., Lo R. and Dworzanski J. P. (1991) Chemical composition and origin of fossil resins from Utah Wasatch Plateau coal. *Fuels Proc. Technol.* **28**, 119-134.

Moldowan J. M. and Seifert W. K. (1980) First discovery of botryococcane in petroleum. *J. Chem. Soc., Chem. Commun.* 912-914.

Moldowan J. M., Seifert W. K., Arnold E. and Clardy J. (1984) Structure proof and significance of stereoisomeric 28,30-bisnorhopanes in petroleum and petroleum source rocks. *Geochim. Cosmochim. Acta* **48**, 1651-1661.

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

Moldowan J. M., Dahl J., Huizinga B. J., Fago F. J., Hickey L. J., Peakman T. M. and Taylor D. W. (1994) The molecular fossil record of oleanane record of oleanane and its relation to angiosperms. *Science* **265**, 768-771.

Muller J. (1981) Fossil pollen records of extant angiosperms. *The Botanical Review* **47**, 1-146.

Murphy M. T. K. (1969) Analytical methods. In *Organic Geochemistry* (Edited by Eglinton G. and Murphy M. T. J.), pp. 74-88. Springer, Berlin.

Murray A. P., Summons R. E., Boreham C. J. and Dowling L. M. (1994) Biomarker and *n*-alkane isotope profiles for Tertiary oils: An organofacies approach. In *Advances in Organic Geochemistry 1993*, in press.

Noble R., Alexander R. and Kagi R. I. (1985) The occurrence of bisnorhopane, trisnorhopane and 25-norhopanes as free hydrocarbons in some Australian shales. *Org. Geochem.* **8**, 171-176.

Olson D. H., Kokotailo G. T., Lawton S. L. and Meier W. M. (1981) Crystal structure and structure-related properties of ZSM-5. *J. Phys. Chem.* **85**, 2238-2243.

Ourisson G., Crabbé P. and Rodig O. R. (1964) Tetracyclic triterpenes. In *Chemistry of Natural Products* (Edited by Lederer E.), Hermann, Paris.

Ourisson G., Albrecht P. and Rohmer M. (1979) The hopanoids. Palaeochemistry and biochemistry of a group of natural products. *Pure Appl. Chem.* **51**, 709-729.

Ourisson G., Albrecht P. and Rohmer M. (1984) The microbial origin of fossil fuels. *Scien. Amer.* **251**, 44-51.

Palmer D. (1994) First flowers emerge from Triassic mud. *New Scientist* **1910**, 15.

Pant P. and Rastogi R. P. (1979) The triterpenoids. *Phytochem.* **18**, 1095-1108.

Peters K. E. and Moldowan J. M. (1991) Effects of source, thermal maturity, and biodegradation on the distribution and isomerisation of homohopanes in petroleum. *Org. Geochem.* **17**, 47-61.

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

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

Petrov A. A., Vorobieva N. S. and Zemskova Z. K. (1985) Sterenes and triterpenes in brown coals. *Org. Geochem.* **8**, 269-273.

Philp R. P. (1983) Correlation of crude oils from the San Jorges Basin, Argentina. *Geochim. Cosmochim. Acta* **47**, 267-275.

Philp R. P. and Gilbert T. D. (1986) Biomarker distributions in oils predominantly derived from terrigenous source material. In *Advances in Organic Geochemistry 1985* (Edited by Leythaeuser D. and Rullkötter J.), pp. 73-84. Pergamon Press, Oxford.

Pines H. (1981) *The Chemistry of Catalytic Hydrogenation Conversion.* Academic Press, New York.

Price P. L., Filatoff J., Williams A. J., Pickering S. A. and Wood G. R. (1985) Late Palaeozoic and Mesozoic palynostratigraphical units. In *CSR Oil & Gas Division Palynological Facility Report No. 274/25*, pp. 1-20, Queensland Department of Mines Open File Report No. 14012.

Pryor W. A. (1962) *Mechanisms of Sulphur Reactions.* McGraw-Hill, New York.

Pusey W. C. (1973) How to evaluate potential gas and oil source rock. *World Oil* **176**, 71-75.

Reed W. E. (1977) Molecular composition of weathered petroleum and comparison with its possible source. *Geochim. Cosmochim. Acta* **41**, 237-247.

Riediger C. L., Fowler M. G., Brooks P. W. and Snowdon L. R. (1990) Triassic oils and potential Mesozoic source rocks, Peace River Arch area, Western Canada Basin. *Org. Geochem.* **16**, 295-305.

Riva A., Caccialanza P. G. and Quagliaroli F. (1988) Recognition of 18β(H)-oleanane in several crudes and Tertiary-Upper Cretaceous sediments. Definition of a new maturity parameter. In *Advances in Organic Geochemistry 1987*

(Edited by Mattavelli L. and Novelli L.), pp. 671-675. Pergamon Press, Oxford.

Robinson K. M. (1987) An overview of source rocks and oils in Indonesia. In *Proceedings of the Indonesian Petroleum Association. Sixteenth Annual Convention*, pp. 97-122. Indonesian Petroleum Association, Jakarta.

Rohmer M. (1987) The hopanoids, prokaryotic triterpenoids and sterol surrogates. In: *Surface Structures of Microorganisms and Their Interactions with the Mammalian Host* (Edited by Schriner E. *et al.*) Proceedings of the Eighteenth Workshop Conference, pp. 227-242. Hocchst, Schloss Ringberg.

Rowland S., Rockey C., al-Lihaibi S.S. and Wolff G.A. (1993) Incorporation of sulphur into phytol derivatives during simulated early diagenesis. *Org. Geochem.* **20**, 1-5.

Rullkötter J. and Wendisch D. (1982) Microbial alteration of 17α(H)-hopanes in Madagascar asphalts: Removal of C-10 methyl group and ring opening. *Geochim. Cosmochim. Acta* **46**, 1545-1553.

Rullkötter J., Leythaeuser D. and Wendisch D. (1982) Novel 23,28-bisnorlupanes in Tertiary sediments. Widespread occurrence of nuclear demethylated triterpanes. *Geochim. Cosmochim. Acta* **46**, 2501-2509.

Rullkötter J., Peakman T. M., ten Haven H. L. (1994) Early diagenesis of terrigenous triterpenoids and its implications for petroleum geochemistry. *Org. Geochem.* **21**, 215-233.

Ruthven D. M. (1988) Zeolites as selective adsorbents. *Chem. Eng. Prog.* **143**, 42-50.

Saxby J. D. (1980) Source rock and maturation in the Gippsland Basin. In *South-Eastern Australia Oil Exploration Symposium, Technical Paper and Abstracts*, Petroleum Exploration Society of Australia, Victorian and Tasmanian Branch, Melbourne.

Scherzer J. (1978) Dealuminated faujasite-type structures with SiO_2/Al_2O_3 ratios over 100. *J. Catal.* **54**, 285-288.

Schouten S., van Driel G. B., Sinninghe Damsté J. S. and de Leeuw J. W. (1994) Natural sulphurization of ketones and aldehydes: A key reaction in the formation of organic sulphur compounds. *Geochim. Cosmochim Acta* **58**, 5111-5116.

Seifert W. K. and Moldowan J. M. (1978) Applications of steranes, terpanes, and monoaromatics to the maturation, migration, and source of crude oils. *Geochim. Cosmochim. Acta* **42**, 77-95.

Seifert W. K. and Moldowan J. M. (1979) The effects of biodegradation on steranes and terpanes in crude oils. *Geochim. Cosmochim. Acta* **43**, 111-126.

Seifert W. K. and Moldowan J. M. (1980) The effect of thermal stress on source-rock quality as measured by hopane stereochemistry. In *Advances in Organic Geochemistry, 1979* (Edited by Douglas A. G. and Maxwell J. R.), pp. 229-237. Pergamon Press, Oxford.

Seifert W. K. and Moldowan J. M. (1981) Paleoreconstruction by biological markers. *Geochim. Cosmochim. Acta* **45**, 783-794.

Seifert W. K., Moldowan J. M. and Demaison G. J. (1984) Source correlation of biodegraded oils. In *Advances in Organic Geochemistry 1983* (Edited by Schenck P. A., de Leeuw J. W. and Lijmbach G. W. M.), pp. 633-643. Pergamon Press, Oxford.

Sinninghe Damsté J. S., Rijpstra W. I. C., Kock-van Dalen A. C., de Leeuw J. W. and Schenk P. A. (1989) Quenching of labile functionalised lipids by inorganic sulphur species: Evidence for the formation of sedimentary organic sulphur compounds at the early stages of diagenesis. *Geochim. Cosmochim. Acta* **53**, 1443-1455.

Smith G. W. (1970) Crystal and molecular structure of a spirotriterpane extracted from crude petroleum. *Acta Crystallogr.* **B26**, 1746-1755.

Smith G. W., Fowell D. T. and Melsom B. G. (1970) Crystal structure of 18α(H)-oleanane. *Nature* **228**, 355-356.

Smith G. W. (1975) The crystal and molecular structure of 29-nor-17αH-hopane, $C_{29}H_{50}$. *Acta Crystallogr.* **B31**, 526-530.

Sofer Z. (1988) Biomarkers and carbon isotopes of oils in the Jurassic Smackover Trend of the Gulf Coast States, U.S.A. *Org. Geochem.* **12**, 412-432.

Sofer Z., Zumberge J. E. and Lay V. (1986) Stable carbon isotopes and biomarkers as tools in understanding genetic relationship, maturation, biodegradation, and migration in crude oils in the Northern Peruvian Oriente (Maranon) Basin. *Org. Geochem.* **10**, 377-389.

Sosrowidjojo I. B., Alexander R. and Kagi R. I. (1994a) The biomarker composition of some crude oils from Sumatra. *Org. Geochem.* **21**, 303-312.

Sosrowidjojo I. B., Setiardja B., Zakaria C., Kralert P. G., Alexander R. and Kagi R. I. (1994b) A geochemical method for assessing the maturity of petroleum: Application to the South Sumatra Basin. In *Proceedings of the Indonesian Petroleum Association. Twenty Third Annual Convention.* Indonesian Petroleum Association, Jakarta. (in press).

Stainforth J. G. (1984) Gippsland hydrocarbons - a perspective from the basin edge. *APEA J.* **24**, 91-100.

Stewart W. N. and Rothwell G. W. (1993) *Paleobotany and the evolution of plants*. Cambridge University Press, Cambridge.

Strachan M. G., Alexander R. and Kagi R. I. (1989) A comparison of selected biological marker compounds in some natural and synthetic liquid fuels. *Fuel* **68**, 641-647.

Subroto E. A., Alexander R. and Kagi R. I. (1991) 30-Norhopanes: their occurrence in sediments and crude oils. *Chem. Geol.* **93**, 179-192.

Suseno P. H., Zakaria M. N. and Subroto E. A. (1992) Contribution of Lahat Formation as hydrocarbon source rock in South Palembang area, South

Sumatra, Indonesia. In *Proceedings of the Indonesian Petroleum Association. Twenty first Annual Convention*, pp. 325-337. Indonesian Petroleum Association, Jakarta.

Takhtajan A. (1969) *Flowering Plants: Origin and Dispersal.* Oliver & Boyd, Edinburgh.

Talukdar S., Gallango O. and Chin-A-Lien M. (1986) Generation and migration of hydrocarbons in the Maracaibo Basin, Venezuela: An integrated basin study. *Org. Geochem.* **10**, 261-279.

Talukdar S., Gallango O. and Ruggiero A. (1988) Generation and migration of oil in the Maturin Subbasin, Eastern Venezuelan Basin. *Org. Geochem.* **13**, 537-547.

Thomas B. M. (1978) Robe River-an onshore shallow oil accumulation. *APEA J.* **18**, 3-12.

Thomas B. M. and Smith D. N. (1974) A summary of the petroleum geology of the Carnarvon Basin. *APEA J.* **14**, 66-76.

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

Trendel J.-M., Guilhem J., Crisp P., Repeta D., Connan J. and Albrecht P. (1990) Identification of two C-10 demethylated C_{28} hopanes in biodegraded petroleum. *J. Chem. Soc., Chem. Commun.* 424-425.

Trendel J. -M., Albrecht P., Riva A. and Ghiselli C. (1991a) Novel demethylated higher plant triterpanes in petroleum. In *Organic Geochemistry. Advances and Applications in Energy and the Natural Environment* (Edited by Manning D. A. C.), pp. 212-214. Manchester University Press, Manchester.

Trendel J.-M., Graff R., Albrecht P. and Riva A. (1991b) 24,28-*dinor*-18α-oleanane, a novel demethylated higher plant derived triterpene hydrocarbon in petroleum. *Tetraheron Lett.* **32**, 2959-2962.

Tsuda Y., Isobe K., Fukushima S. and Ageta H. (1967) Final clarification of the saturated hydrocarbons derived from hydroxyhopanone, diploptene, zeorin, and dustanin. *Tetrahedron Lett.* **1**, 23-28.

Volkman J. K., Alexander R., Kagi R. I. and Woodhouse G. W. (1983a) Demethylated hopanes in crude oils and their applications in petroleum geochemistry. *Geochim. Cosmochim. Acta* **47**, 785-794.

Volkman J. K., Alexander R., Kagi R. I., Noble R. A. and Woodhouse G. W. (1983b) A geochemical recontruction of oil generation in the Barrow Sub-basin of Western Australia. *Geochim. Cosmochim. Acta* **47**, 2091-2105.

Volkman J. K., Alexander R., Kagi R. I., Rowland S. J. and Sheppard P. N. (1984) Biodegradation of aromatic hydrocarbons in crude oils from the Barrow Sub-basin of Western Australia. *Org. Geochem.* **6**, 619-632.

Wang T.-G. and Simoneit B. R. T. (1990) Organic geochemistry and coal petrology of Tertiary brown coal in the Zhoujing mine, Baise Basin, South China. 2. Biomarker assemblage and significance. *Fuel* **69**, 12-20.

Wang T.-G., Simoneit B. R. T., Philp R. P. and Yu C. -P. (1990) Extended 8β(H)-drimane and 8,14-*seco*-hopane series in a Chinese boghead coal. *Ener. Fuels* **4**, 177-183.

Weitkamp J., Schafer K. and Ernst S. (1991) Selective adsorption of diastereomers in zeolites. *J. Chem. Soc., Chem. Commun.*, 1142-1143.

Wenkert E., Baddeley G. V., Burfitt I. R. and Moreno L. N. (1978) Carbon-13 nuclear magnetic resonance spectroscopy of naturally-occurring substances LVII. Triterpenes related to lupane and hopane. *Org. Magn. Res.* **11**, 337-343.

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

Whitehead E. V. (1974) The structure of petroleum pentacyclanes. In *Advances in Organic Geochemistry 1973* (Edited by Tissot B. and Bienner F.), pp. 225-243. Editions Technip, Paris.

Wilkins A. L., Bird P. W. and Jager P. M. (1987) Carbon-13 NMR study of some triterpene hydrocarbons of the hopane group. *Mag. Res. Chem.* **25**, 503-507.

Wintersteiner O., Krakower G. and Moore M. (1965) Lupenone and 18α-oleanan-19α-ol-3-one from *Samadera indica*. *J. Org. Chem.* **30**, 2847-2849.

Woolhouse A. D., Oung J. -N., Philp R. P. and Weston R. J. (1992) Triterpanes and ring-A degraded triterpanes as biomarkers characteristic of Tertiary oils derived from predominantly higher plant sources. *Org. Geochem.* **18**, 23-31.

Zeng X. and Liu S. and Ma S. (1988) Biomarkers as source input indicators in source rocks of several terrestrial basins of China. In *Geochemical Biomarkers* (Edited by Yen T. F. and Moldowan J. M.), pp. 25-49. Harwood Academic, Chur.

Zinnen H. A., Hobbs S. H. and Gembicki S. A. (1985) Separating aromatics. *ChemTech*, 762-766.

Zumberge J. E. (1987) Prediction of source rock characteristics based on terpane biomarkers in crude oils: A multivariate statistical approach. *Geochim. Cosmochim. Acta* **51**, 1625-1637.

Zürcher A., Jeger O. and Ruzicka L. (1954) Zur Kenntnis der triterpene. Überführung der chinovasaure in phyllanthol und uvaol. Über die konstitution der ursolsaure und des uvaols. *Helvet. Chim. Acta* **37**, 2145-2152.

LIST OF JOURNAL PUBLICATIONS

Armanios C., Alexander R. and Kagi R.I. (1992) Shape-selective sorption of petroleum hopanoids by ultrastable Y zeolite. *Org. Geochem.* **18(4)**, 399-406.

Armanios C., Alexander R. and Kagi R.I. (1992) High diahopane and neohopane abundances in a biodegraded crude oil from the Barrow Sub-basin of Western Australia. *Org. Geochem.* **18(5)**, 641-645.

Armanios C., Alexander R., Kagi R.I. and Sosrowidjojo I.B. (1994) Fractionation of sedimentary higher-plant derived pentacyclic triterpanes using molecular sieves. *Org. Geochem.* **21(5)**, 531-543.

Armanios C., Alexander R., Kagi R.I., Sobolev A. N. and White A. (1994) The molecular structure of a sedimentary 17α,21β(H)-homohopane from X-ray diffraction. *Geochim. Cosmochim. Acta* **58(13)**, 2941-2943.

Armanios C., Alexander R., Kagi R.I., Skelton B. W. and White A. (1994) Occurrence of 28-*nor*-18α-oleanane in the hydrous pyrolysis of a lignite. *Org. Geochem.* (in press).

Armanios C., Alexander R., Sosrowidjojo I.B. and Kagi R.I. (1994) Identification of bicadinanes in Jurassic sedimentary organic matter from the Eromanga Basin, Australia. *Org. Geochem.* (in press).

LIST OF CONFERENCE PRESENTATIONS

Armanios C., Alexander R. and Kagi R.I. High diahopane and neohopane abundances in a biodegraded crude oil from the Barrow Sub-basin of Western Australia. *Australian Organic Geochemistry*, Melbourne, Australia, May 1991. *(poster presentation).*

Armanios C., Alexander R. and Kagi R.I. Separation of diahopanes and the *22S* and *22R* diastereomers of the extended hopanes using large-pore, high-silica molecular sieves. *15th Meeting of the EAOG*, Manchester, England, September 1991. *(poster presentation).*

Armanios C., Alexander R., Kagi R.I. and Sosrowidjojo I.B. Molecular sieving of higher-plant derived pentacyclics from crude oil. *16th Meeting of the EAOG*, Stavanger, Norway, September 1993. *(poster presentation).*

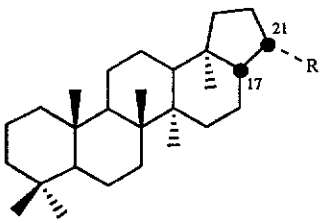
Fisher S.J., Armanios C., Ellis L. and Kagi R.I. The use of molecular sieves for separation of complex mixtures of hydrocarbons into fractions based upon molecular size. *RACI 12AC/3EC*, Perth, W. Australia, September 1993. *(oral presentation given by R. I. Kagi).*

Armanios C., Alexander R. and Kagi R.I. Application of molecular sieve separation in organic geochemistry. *Australian Organic Geochemistry*, Sydney, Australia, March 1994. *(oral presentation given by C. Armanios).*

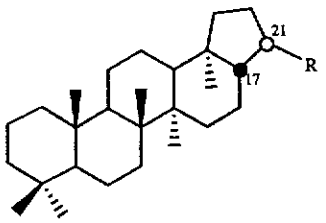
APPENDIX

STRUCTURE AND NOMENCLATURE OF PENTACYCLIC TRITERPANES DISCUSSED IN THE THESIS

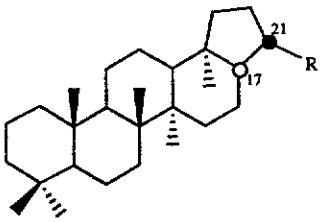
STRUCTURES OF BACTERIALLY-DERIVED PENTACYCLIC TRITERPANES REFERRED TO IN THE THESIS.



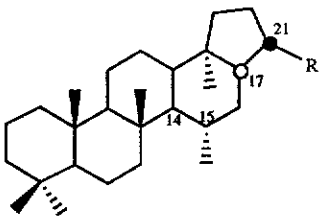
I- 17β,21β(H)-Hopanes



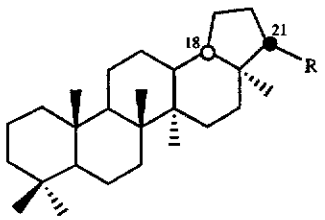
II- Moretanes



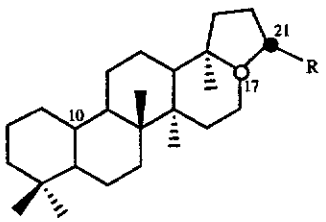
III- 17α,21β(H)-Hopanes



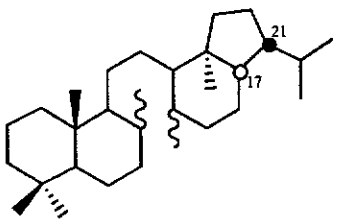
IV- 17α,21β(H)-Diahopanes



V- 18α,21β(H)-Neohopanes



VI- 17α,21β(H)-25-Norhopanes

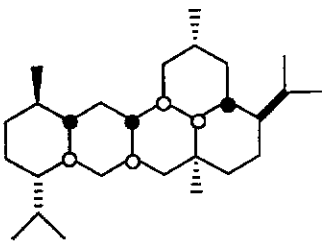


VII- 17α,21β(H)-8,14-Secohopane

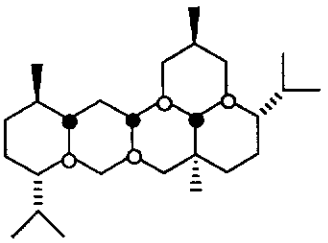
NOMENCLATURE OF PENTACYCLIC COMPONENTS REFERRED TO IN THIS THESIS

Structure No.	Compound
Ia (R=H)	17β-22,29,30-Trisnorhopane
Ic (R=C_2H_5)	17β,21β(H)-30-Norhopane
Id (R=C_3H_7)	17β,21β(H)-Hopane
Ie (R=C_4H_9)	17β,21β(H)-Homohopane (*22R*)
If (R=C_5H_{11})	17β,21β(H)-Bishomohopane (*22R*)
Ig (R=C_6H_{11})	17β,21β(H)-Trishomohopane (*22R*)
IIc (R=C_2H_5)	30-Normoretane
IId (R=C_3H_7)	Moretane
IIe (R=C_4H_9)	Homomoretane (*22R*)
IIf (R=C_5H_{11})	Bishomomoretane (*22R*)
IIg (R=C_6H_{11})	Trishomomoretane (*22R*)
IIIa (R=H)	17α-22,29,30-Trisnorhopane (Tm)
IIIc (R=C_2H_5)	17α,21β(H)-30-Norhopane
IIId (R=C_3H_7)	17α,21β(H)-Hopane
IIIe (R=C_4H_9)	17α,21β(H)-Homohopane (*22S&22R*)
IIIf (R=C_5H_{11})	17α,21β(H)-Bishomohopane (*22S&22R*)
IIIg (R=C_6H_{11})	17α,21β(H)-Trishomohopane (*22S&22R*)
IIIh (R=C_7H_{13})	17α,21β(H)-Tetrakishomohopane (*22S&22R*)
IIIi (R=C_8H_{15})	17α,21β(H)-Pentakishomohopane (*22S&22R*)
IVc (R=C_2H_5)	17α,21β(H)-30-Nordiahopane
IVd (R=C_3H_7)	17α,21β(H)-Diahopane
IVe (R=C_4H_9)	17α,21β(H)-Homodiahopane
IVf (R=C_5H_{11})	17α,21β(H)-Bishomodiahopane
IVg (R=C_6H_{11})	17α,21β(H)-Trishomodiahopane (*22S&22R*)
IVh (R=C_7H_{13})	17α,21β(H)-Tetrakisdiahomohopane (*22S&22R*)
Va (R=H)	18α-22,29,30-Trisnorneohopane (Ts)
Vc (R=C_2H_5)	18α,21β(H)-30-Norneohopane (C_{29}Ts)
VIc (R=C_2H_5)	17α,21β(H)-25,30-Bisnorhopane
VId (R=C_3H_7)	17α,21β(H)-25-Norhopane
VIe (R=C_4H_9)	17α,21β(H)-25-Norhomohopane (*22S&22R*)
VIf (R=C_5H_{11})	17α,21β(H)-25-Norbishomohopane (*22S&22R*)
VIg (R=C_6H_{11})	17α,21β(H)-25-Nortrishomohopane (*22S&22R*)
VII	17α,21β(H)-8,14-Secohopane

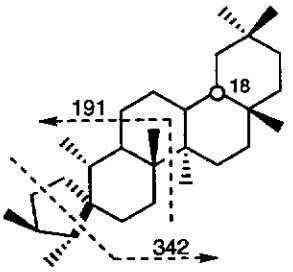
STRUCTURES OF HIGHER PLANT-DERIVED PENTACYCLIC TRITERPANES REFERRED TO IN THE THESIS.



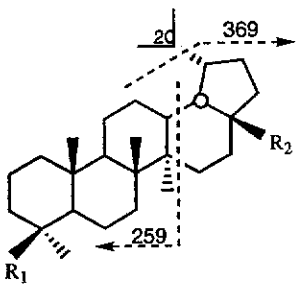
X- *cis-cis-trans* Bicadinane



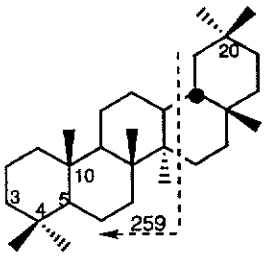
XI- *trans-trans-trans* Bicadinane



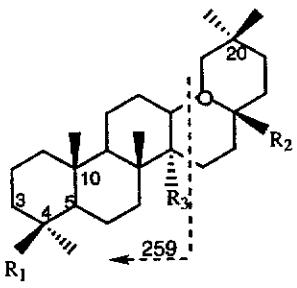
XII- Spirotriterpane



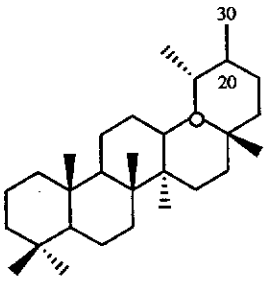
*XIII



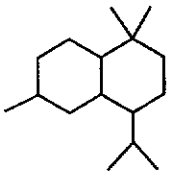
XIV-Oleanane



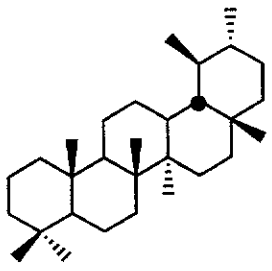
*XV



XVI-Taraxastane



XVII- Cadinane



XVIII- Ursane

*
XIIIa- R_1=CH_3, R_2=CH_3 -Lupane
XIIIb- R_1=H, R_2=CH_3 - 24-Norlupane
XIIIc- R_1=H, R_2=H - 24,28-Bisnorlupane

* **XVa-** R_1=CH_3, R_2=CH_3, R_3=CH_3 - 18α-Oleanane
XVb- R_1=CH_3, R_2=H, R_3=CH_3 - 28-Nor-18α-oleanane
XVc- R_1=H, R_2=H, R_3=CH_3 - 24,28-Bisnor-18α-oleanane
XVd- R_1=H, R_2=CH_3, R_3=H - 24,27-Bisnor-18α-oleanane