CHAPTER IV

THE GEOCHEMICAL SIGNIFICANCE AND APPLICATION OF SOME BIOMARKERS

During sedimentation and maturation in the diagenesis of petroleum, biomarkers have been generated under many complex conversion processes. These biomarkers are derived from the original lipid fraction of biological system in varying degrees of thermodynamic stability. Because of their preservation with unchanged molecular structures, or minor changes, which are still linked to precursor molecules that are present in living organism systems, these molecules are capable of yielding very specific information regarding source, maturity, migration and biodegradation. The degree of information is affected by the chemical complexity of molecular structure, thus, the greater the complexity of the structure or transformation of stereochemical change, the greater specific information that can be derived. Therefore, the geochemical significance and application of biomarker can be classified into many classes depending upon molecular structures and, concurrently, occurrence and origin.

In recent years, development and application of the use of biomarkers in petroleum exploration have been widely accepted. Many specific classes of biomarkers

have been summarized (see PHILP, 1985). Characterization of biomarkers in source rocks and crude oils have been used for determining source materials, depositional environments, thermal maturity, biodegradation and relative migration distances (PHILP and LEWIS, 1985). Because of the relatively low concentration and complexity of source rock extracts and oils, identification techniques necessarily require highly sophisticated instrumentation. Currently, biomarkers are analyzed by computerized gas chromatography/mass spectrometry (C/GC/MS). The most extensive MS technique used for detecting trace amounts of a particular biomarker is selective ion monitoring (SIM) or multiple ion detection (MID). The concept of SIM or MID is based on the premise that certain compounds, or compound classes, will fragment in a particular manner to give rise to a characteristic fragment or ion (PHILP,1986). Library search facilities or compound classification programs have been established as the data system to provide a rapid method for assigning a structure of unknown compound by searching against a large number of standard spectra. Distribution of biomarkers can be revealed by looking at the specific mass fragmentogram, e.g. m/z 123 for sesquiterpanes and diterpanes, m/z 191 for pentacyclic triterpanes and m/z 217 for steranes. In this chapter the geochemical significance and applicability of some biomarkers will be described briefly.

NORMAL ALKANES

A n-alkane (I) is the simplest biomarker structure which can be easily detected by GC alone. Distribution of n-alkanes are used to infer the source of organic material, maturity and early stages of biodegradation. Differences in source material will give different n-alkane distributions even though differences in these chromatograms may be progressively obliterated with increasing burial and age. The distributions and the carbon preference index (CPI) are used for quantifying the interpretation. High molecular n-alkanes may reflect the contribution of terrigeneous organic input, whereas low molecular weight n-alkanes are derived from algae or other marine sources. Higher concentration of odd-number alkanes ($n\text{-}C_{27}$, $n\text{-}C_{29}$ and $n\text{-}C_{31}$) than even-number alkanes ($n\text{-}C_{28}$ and $n\text{-}C_{30}$) may indicate a high leaf wax input (EGLINTON et al., 1962, EGLINTON and HAMILTON, 1967; CALDICOTT and EGLINTON, 1973) whereas $n\text{-}C_{17}$ predominance is used for phytoplankton original source (ORO et al., 1967; BLUMER et al., 1971; GELPI et al., 1970). A predominance of n-alkanes in $n\text{-}C_{16}$ to $n\text{-}C_{24}$ region with no marked odd/even predominance indicates a bacterial contribution in environmental sedimentation (NISHIMURA and BAKER, 1986). Odd-carbon numbered alkanes of high molecular weight ($n\text{-}C_{25}$ to $n\text{-}C_{33}$) are derived from cuticular waxes of the continental higher plants through early diagenesis (defunctionalization) from the even-numbered acid alcohols or esters (TISSOT and WELTE,

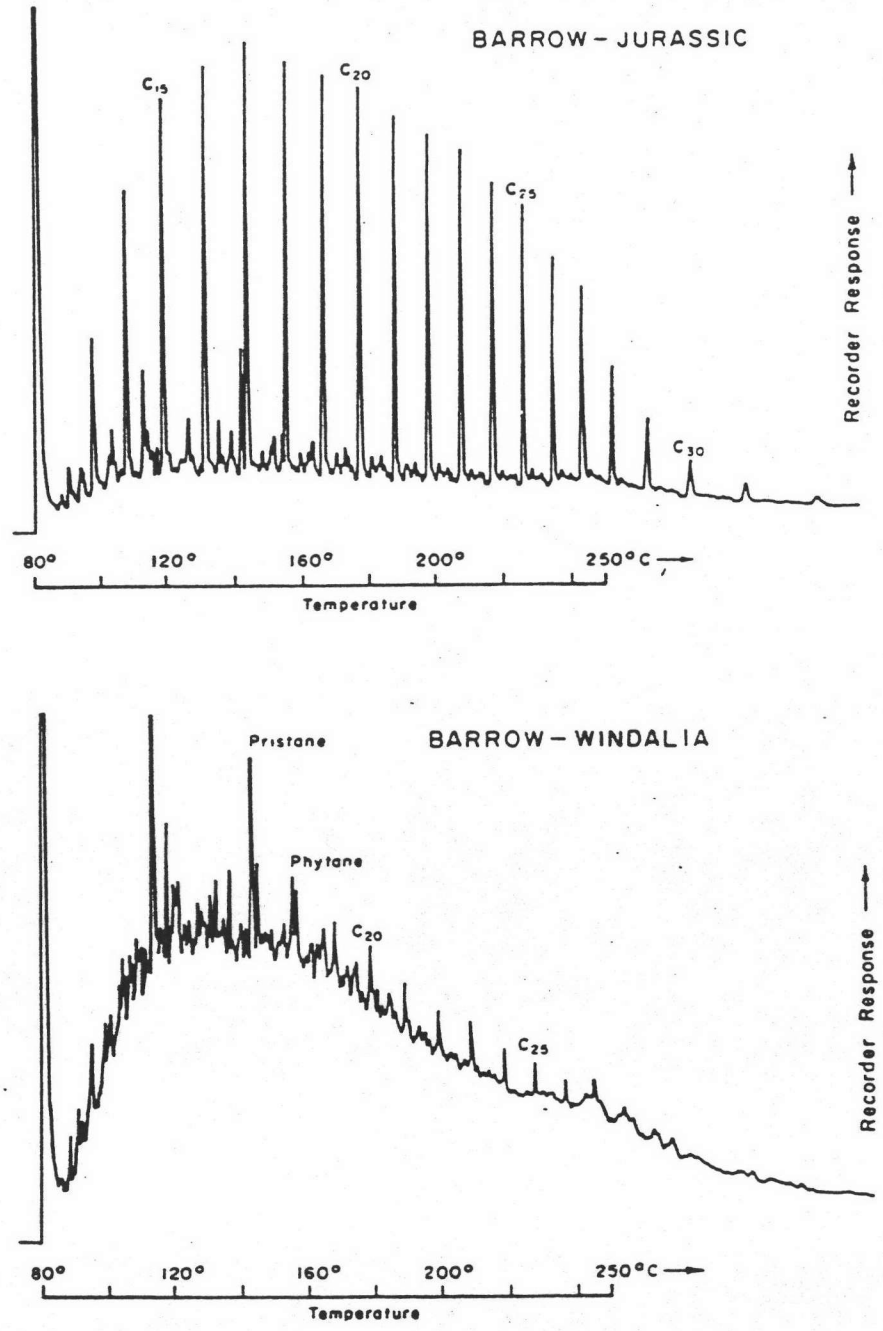

Figure 9. The effect of biodegradation on n-alkane distribution of crude oil (from PHILP, 1985).

Table 2. Effects of biodegradation on chemical and physical properties of crude oils (from WAPLES, 1985).

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1 - C_1-C_6 ↘

2 - GOR (GAS/OIL RATIO) ↘

3 - GASOLINE RANGE (C_6-C_{15}) ↘

4 - API GRAVITY ↘

5 - VISCOSITY ↗

6 - CHANGE IN GROSS COMPOSITION OF C_{15}^+ COMPOUNDS
alkanes ↘
aromatics ↘
NSO's compound ↗
asphaltenes ↗

7 - SULPHUR CONTENT ↗

8 - NITROGEN CONTENT ↗

9 - V AND Ni ↗

10 - OPTICAL ACTIVITY ↗

11 - POUR POINT ↘

12 - $\delta^{13}C$ whole oil
alkanes ↗
aromatic or ↘
asphaltenes ↘

13 - CHANGE IN OIL TYPES

paraffinic oil	→ naphthenic oils
paraffinic or paraffinic -napthenic oils	→ aromatic - naphthenic oils
paraffinic condensates	→ naphthenic - condensate
aromatic-intermediate oils	→ aromatic - asphaltic oils

Table 3. Classification of biodegraded crude oils according to the severity of biodegradation (from WAPLES, 1985).

Level of Biodagradation	Compounds Removed	Extent of Biodegradation
1	None	Undegraded
2	Short n-alkanes absent	Minor
3	> 90 % of n-alkanes removed	Moderate
4	Alkylcyclohexanes absent; isoprenoids reduced	Moderate
5	Isoprenoid absent	Moderate
6	Bicyclic alkanes absent	Extensive
7	> 50 % regular steranes removed	Very extensive
8	Steranes altered; demethylated hopanes abundant	Severe
9	Demethylated hopanes predominate; diasteranes formed; no steranes	Extreme

1978). Usually, the high molecular weight n-alkanes in crude oil inherited from terrestrial plants are diluted by hydrocarbons from kerogen degradation. This dilution will make the CPI value approach 1.0. Unusual even/odd predominance in n-C_{16} to n-C_{30} range in crude oil and sediment have also been reported by DOUGLAS and GRANTHAM (1974). Increasing maturity can effect the change in n-alkane distribution. No marked odd/even predominance can be seen in high maturity samples because of the trend to shift toward lower-carbon numbered n-alkane. This is the reason that n-alkane distributions are of little use as a high level maturity indicator.

Biodegradation is another of many effects which can also influence n-alkane distributions. At the first stage of bacterial degration, the low-molecular weight alkane in crude oil will be removed followed by the n-C_{16} to n-C_{25} range and finally by those above n-C_{25}. The effect of biodegradation on chemical and physical properties of crude oils, and the severity classifition of biodegraded crude oils are shown in Table 2 and 3. An example chromatogram of biodegraded oil is also shown in Figure 9. However, biodegradation is sometimes accompanied by water washing which produces similar changes in the n-alkane distributions (MILLER et al., 1977).

The fourth factor which can effect n-alkane distributions in crude oils is migration. The higher-molecular weight n-alkanes migrate more slowly than lower

members of the series during migration. The n-C_{15} to n-C_{19} alkanes tend to be expelled in the thin shale sequences faster than thick shales as a result of compaction and expulsion (MACKENZIE et al.,1983).

ISOPRENOIDS

Isoprenoids (II) are the second class of biomarkers in which only the regular C_{13}-C_{20} isoprenoids can be detected by GC alone. Other isoprenoids, including those with higher molecular weights must be detected by using GC\MS and SIM techniques. Isoprenoids are formed from various combinations of C_5 isoprene units (III). These functionalyzed C_5 isoprene precursors occur in living plant and to a smaller extent in animals and can even occur as hydrocarbon, alcohol or ether derivatives and are important feature of most biochemical cycles. There are three main families of isoprenoids which are based on the linkage of isoprene units. There are the head-to-tail linked isoprenoids, the tail-to-tail linked isoprenoids and the head-to-head linked isoprenoids. Pristane (IV) and phytane (V) are the most common head-to-tail linkage isoprenoids. Members of this series can range up to at least C_{40} to C_{45}. The regular isoprenoids have branching points at the 2,6,10,14 carbon atoms, whereas the anteisoprenoid has branching points at 3,7,11,15 carbon atoms. The regular C_{13}-C_{20} isoprenoids are generally present in relatively high concentrations compared to the other isoprenoids. The most common

geochemical application for of this type isoprenoid is that they are derived from diagenesis of the phytol side chain of chlorophyll (VI) (TREIBS, 1934). The abundance of pristane and phytane or pristane/phytane ratios can refer to diagenetic pathways of organic source material or depositional environment (DIDYK et al., 1978). It has been suggested that the formation of pristane prefers an oxidizing depositional environment coal-swamp whereas phytane formation is preferentially formed from chlorophyll in a more reducing deltaic or marine-type environment (BROOKS et al., 1969; POWELL and MCKIRDY, 1973; DIDYK et al., 1978). Pristane/phytane ratios and also pristane/C_{17} and phytane/C_{18} ratios can be used as correlation parameters to study the depositional environment, maturity and second step of biodegradation (RACHID, 1979; VOGLER et al., 1981; ALEXANDER et al., 1981). The degree of oxidation and reduction in any type of depositional environment, maturity and biodegradation will also effect these ratios. Pristane originates from the phytol side chain of chlorophyll cleavage followed by decarboxylation in oxidation pathway, while phytane reduction requires reducing condition. As the level of maturity increases, n-alkanes are generated faster than the isoprenoids, contrary to the effect of biodegradation, where n-alkanes will be removed by bacteria preferentially over the isoprenoids. The schematic diagenetic pathways of pristane and phytane under depositional condition is shown in Figure 10.

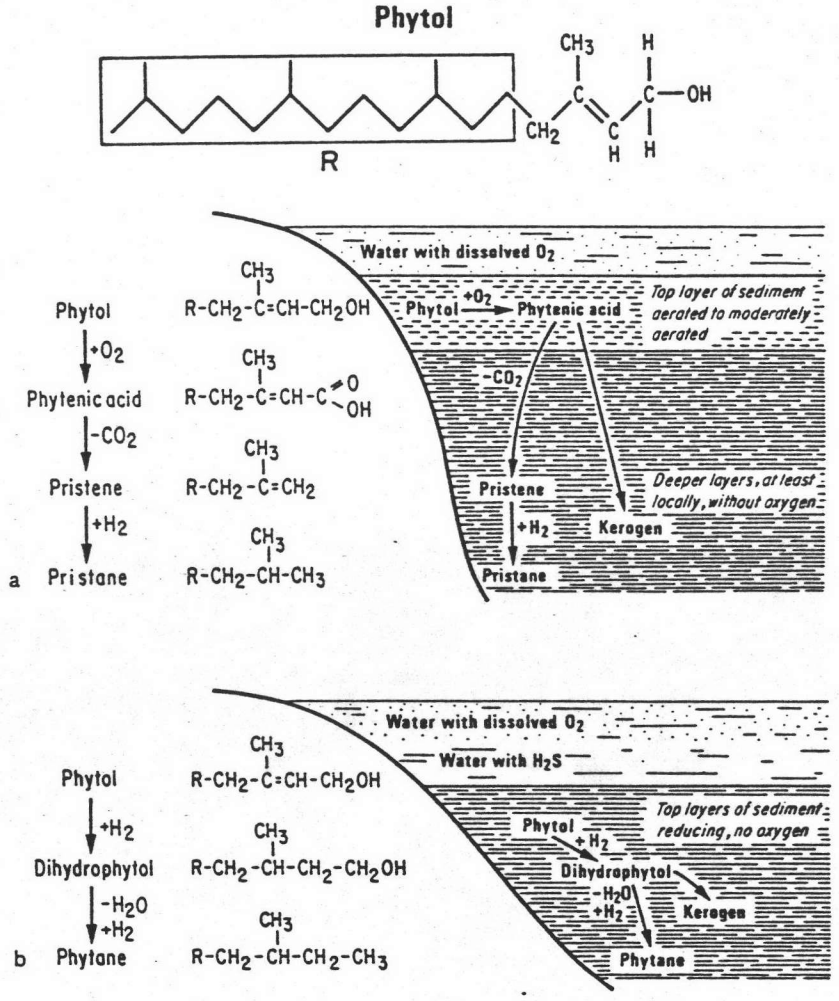

Figure 10. Diagenetic pathways from phytol to pristane (C_{19}) and phytane (C_{20}) a) in the presence of oxygen and b) in the absence of oxygen (from TISSOT and WELTE, 1978).

Isoprenoids with carbon number range above C20 have provided much geochemical information following their detection by GC/MS and MID. The C_{25} regular isoprenoid was proposed to be a biomarker for lagoonal-type saline environment (WAPLES et al., 1974). Some regular head-to-tail isoprenoids up to C_{40} were investigated in Spanish crude oil (ALBAIGES and TORRADOS, 1977; ALBAIGES et al., 1978) and have also be proposed to originate from long-chain oligoterpenyl alcohols present in many naturally occurring materials (HAN and CALVIN, 1969).

Head-to-head linked isoprenoids have been found in crude oils (ALBAIGES, 1979; MOLDOWAN and SEIFERT, 1979) and are presently used to investigate the occurrence of many oils and source rocks. Their possible origin is from cell wall membranes of the thermoacidophilic bacteria of Calderiella species (de ROSA et al., 1977). In addition, head-to-head linked isoprenoids are thought to have been alternatively generated from the methanogenic members of Archaebacteria (MICHAELIS and ALBRECHT, 1979).

Tail-to-tail linked isoprenoids are the other class of isoprenoids derived from naturally occurring unsaturated precursors. The common ones are squalane (VII), perhydro-β-carotane (VIII) and lycopane (IX). This type of isoprenoid is limited to a much smaller number of compounds than those with head-to-head or head-to-tail linkages. Therefore, they have not been widely

used directly as a biomarker although their unsaturated counterparts occur as natural products. The distribution chromatogram of head-to-tail and head-to-head isoprenoid mixtures which have been detected by GC\MS and MID of the ion at 183 are shown in Figure 11.

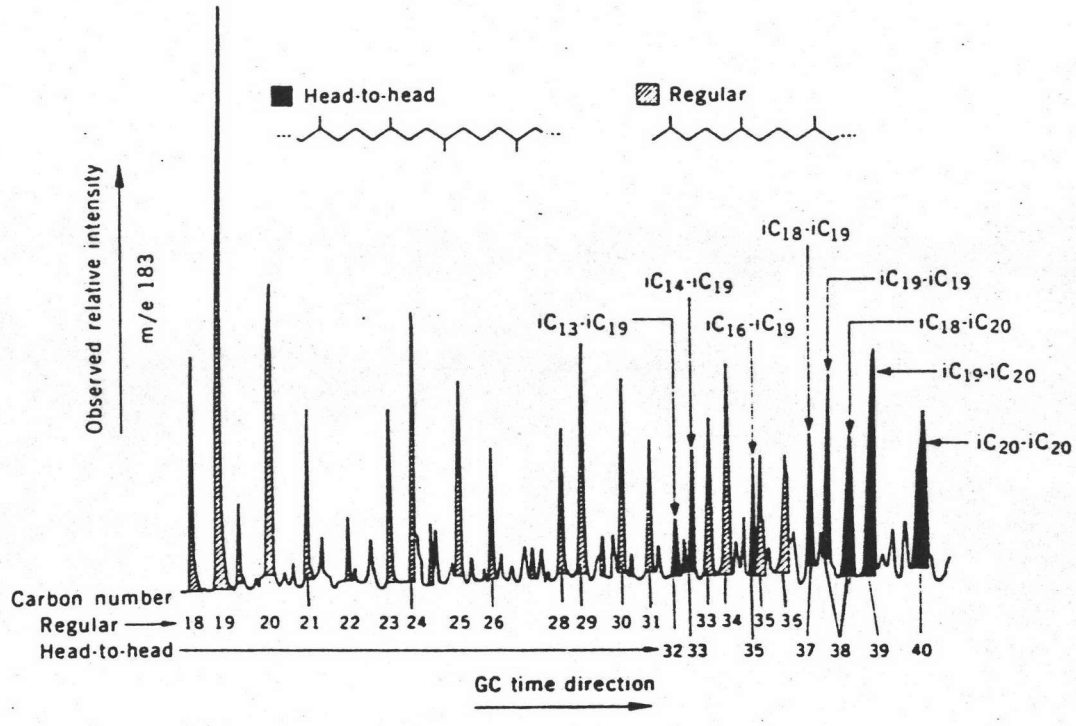

Figure 11. Distribution chromatogram of head-to-tail and head-to-head isoprenoids in crude oil (from PHILP, 1985).

There are other isoprenoids and branched hydrocarbon which have been found in sediments and crude oils. Botryoccocane (X) which derived from botryococcene and present only in the fresh or brackish water algae has been discovered in crude oil from Indonesia (MOLDOWAN and SEIFERT, 1980). The limited occurrence in geological samples of this compound is useful for identifying the organic input even though the exact origin still remains to be determined.

SESQUITERPANES

Sesquiterpanes make up the largest terprenoid classes, consisting of approximately 30 main structural types and 70 less common skeletons. These biomarkers occur in resins, essential oils and some marine organisms, e.g. algae. The most abundant sesquiterpanes present in crude oil are bicyclic sesquiterpanes (XI), especially C_{14} and C_{15} bicyclo-paraffin, namely drimane (XII) and eudesmane (XIII). These compounds were partially identified by GC/MS and MID based on the base peaks at m/z 109 and 123. They have been suggested to form from degradation of onoceranes (BENDORAITIS, 1974) and 8,14-secohopanes (XIV) (SCHMITTER et al., 1982). An other possible origin of these compounds has been proposed by BENDORAITIS (1975) from the degradation of pentacyclic triterpanes such as β-amyrin (XV) during the maturation process. Two bicyclic sesquiterpanes, drimane and eudesmane, have been identified in crude oils from

Australia and have been suggested that eudesmane derived from higher plant materials while drimane probably has a microbial source origin (ALEXANDER et al., 1983). The distribution of C_{14}-C_{16} bicyclic sesquiterpanes can be used to confirm the unambiguous evidence for terrestrial contribution to crude oils (PHILP et al., 1981; RICHARDSON and MILLER, 1982). The present isomer mixture of these compounds suggests to the possibility that ratios of certain bicyclic sesquiterpane isomers may be useful as maturity parameter (PHILP, 1985). The biodegradation can not affect to these compounds (PHILP et al., 1981). An example of bicyclic sesquiterpane distribution detected by MID at M/Z 123 is shown in Figure 12.

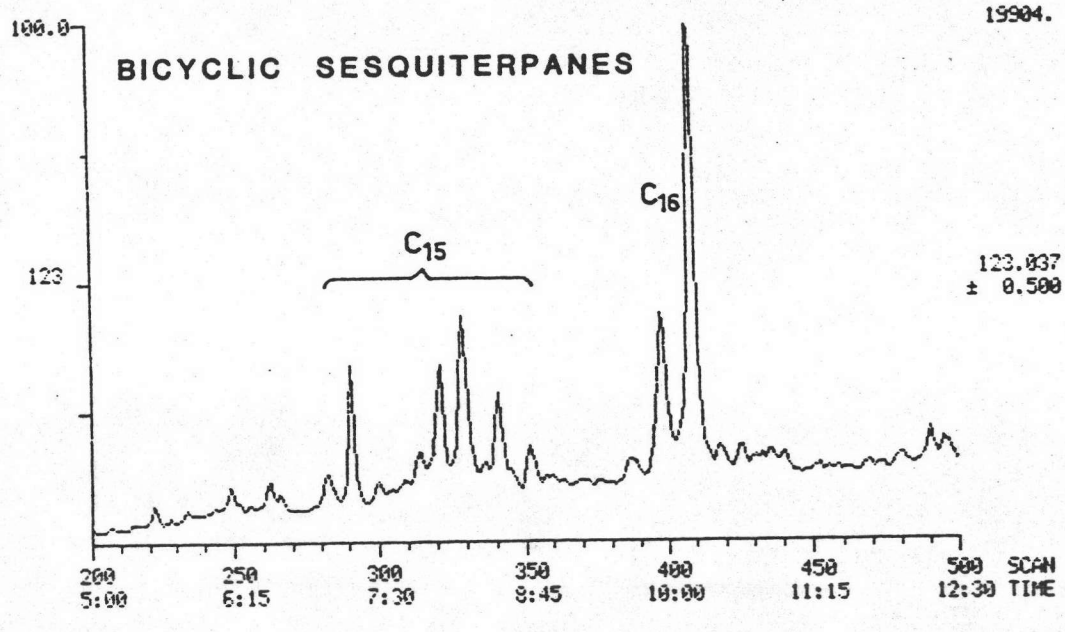

Figure 12. Distribution of bicyclic sesquiterpanes in terrestrial crude oil from New Zealand, detecting by MID at m/z 123 (from PHILP, 1985).

DITERPANES

Diterpanes in fossil fuels have been used widely as an indicator of terrestrial source material, particularly resins, to oils, source rocks, and coals. Their concentrations usually present a relatively low in most oils besides oil derived from higher plant source. They can be detected by GC/MS and MID using the ions at m/z 109, or 123, plus 191. The precursors of diterpanes are generally accepted to be diterprenoid acids based on the abietane (XVI), pimarane (XVII) and labdane (XVIII) skeletons which occur in plant resins as complex mixtures of di- and tri-enoic acids. The natural occurrence of diterprenoids has been reported more as a coal biomarker than related purely to oil or source rock. A number of diterpanes namely phyllocladane (XIX), kaurane (XX), norabietane (XXI) and norpimarane (XXII) were investigated in brown coal from Rhenisch area, West Germany (HAGEMANN and HOLLERBACH, 1981). The first two compounds are common in recent phyllocladus and kauriconifers (STRIEBL and HEROUT, 1969) and the remaining compounds have been identified as common constituents of fossil resins (MAXWELL et al., 1971) and lignites (DOUGLAS and GRANTHAM, 1975). Mixture of nine C_{19} and C_{20} diterprenoids in many oils from offshore Gippland basin, Australia have been examined by PHILP et al. (1981) by using GC/MS and MID of the characteristic ion at M/Z 123. In addition, crude oils which contained high concentration of diterpanes could have resinite as a

major source contributor to the oils (SNOWDON and POWELL, 1982).

At the early stages of coalification, defunctionalization will occur to form the saturated hydrocarbon diterpanes whereas at higher stages of coalification, aromatization occurs and the diterprenoid structures are subsequently converted into methyl-substituted phenanthrenes and other aromatic components (PHILP, 1985). Therefore, change in the distribution of various classes of biomarkers, including diterpanes, could be used as additional organic geochemical coalification parameters besides the petrologically determined values such as vitrinite reflectance (HOLLERBACH and HAGEMANN, 1981). PHILP et al.(1981) proposed that a mixture of diterprenoids was formed at low temperatures and would be progressively aromatized as the hydrogenation temperature was increased. The diterprenoids were not related to the abietane or pimarane skeletons and probably represent a series of diterprenoids associated with algal-type material. An example distribution of diterprenoids in four Australian crude oils is shown in Figure 13.

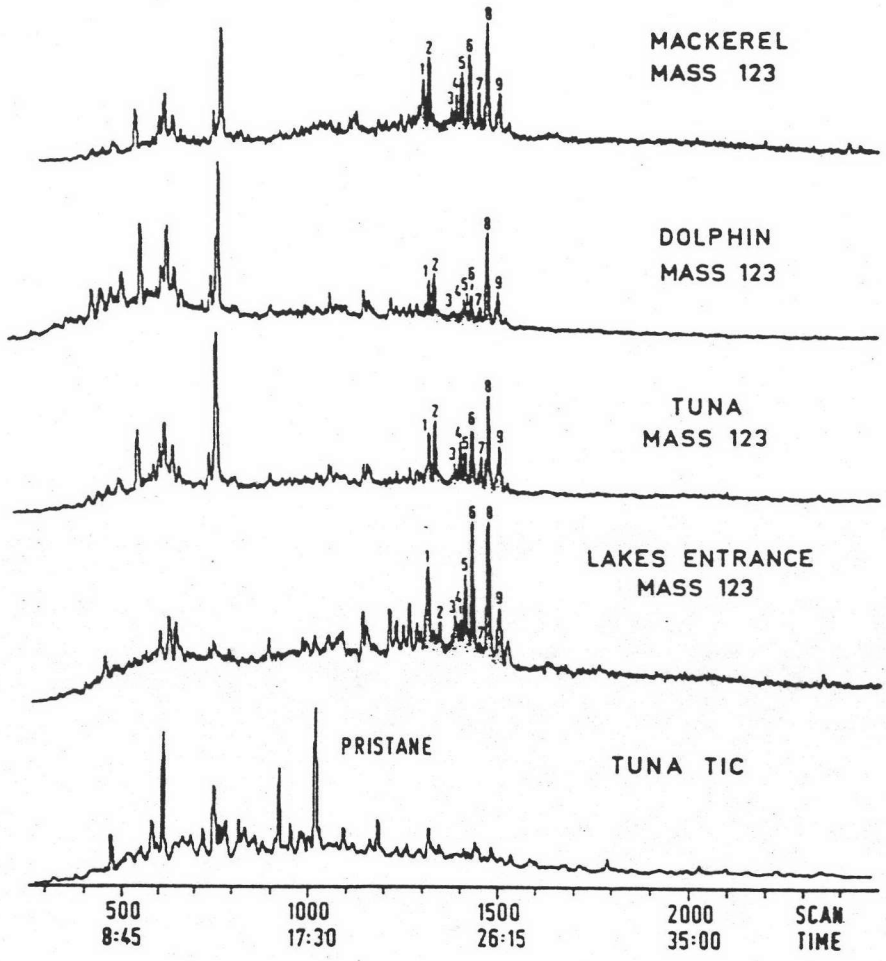

Figure 13. Distribution of diterprenoids in crude oil from Gippsland Basin, Australia. Peaks 1 and 2 are C_{19} diterprenoids and peak 3-9 are C_{20} diterprenoids (from PHILP, 1985)

EXTENDED TRICYCLIC TERPANES

Extended tricyclic terpanes (XXIII) have been proposed to be derived from marine organic source materials, unlike C_{19} and C_{20} diterprenoids which are derived only from terrestrial materials. The series of these extended tricyclic terpanes appear from C_{19} to C_{30} carbon atoms and can be detected by using GC/MS and MID, particularly the abundant characteristic fragment ion at m/z 191. The major tricyclic series in sediments and petroleum are C_{19} and C_{20} members. The proposed precursor for this series could be C_{30}-tricyclohexaprenol formed anaerobically from a universal cell constituent, hexaprenol. The saturated counterpart of hexaprenol has been reported in petroleum (ALBAIGES et al., 1978) and in the lipids of Archaebacteria (HOLZER et al., 1979). C_{23} tricyclic terpanes (XXIV) of the series have been proposed to be derived from a C_{30} hexaisoprenoid precursor synthesized with either a head-to-tail coupling of six isoprene units or head-to-tail dimerization of two C_{15} units (EKWEOZER and STRAUSZ, 1983; 1982) Recently, the series of tricyclic hydrocarbons has been extended from the range C_{19}-C_{30} to C_{19}-C_{45} (MOLDOWAN et al., 1983). It has been proposed that this series supports the prediction of the biogenic origin of the tricyclic terpanes from cyclization of regular polyprenol in bacterial membranes. The precursor of the C_{45} tricyclic terpane is probably a C_{45} or larger unsaturated isoprenoid alcohol known to occur in plants (HAN and

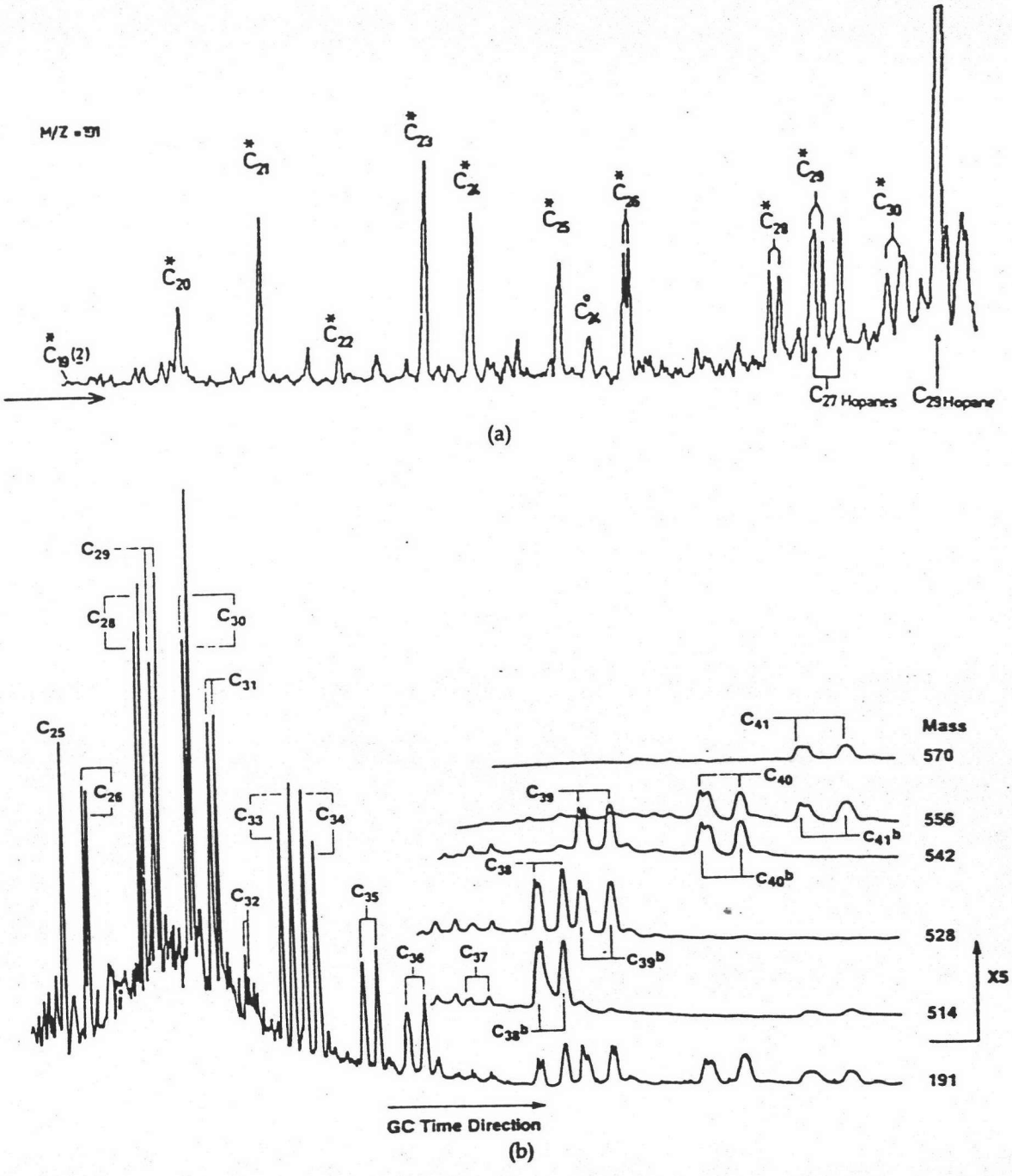

Figure 14. (a) Mass fragmentogram (m/z 191) of the tricyclic (*) and tetracyclic (o) terpanes in crude oil from Congo (from AQUINO et al., 1983) and (b) extended series of tri cyclic terpanes past 40 in California crude oil (from MOLDOWAN et al., 1983).

CALVIN, 1969). The variations in the distribution of individual members of this extended tricyclic homologous series relative to the ubiquitous hopanes then makes the tricyclic terpanes a potentially valuable source correlation parameter for product/precursor relationship. Many puplished papers have been reported (CYR and STRAUSZ, 1983; CHICARELLI et al., 1988; AQUINO NETO et al., 1986). The examples of mass fragmentation (m/z 191) of distributed tricyclic terpanes in crude oil and the extended series past C_{40} tricyclic terpanes are shown in Figure 14.

TETRACYCLIC TERPANES

Tetracyclic terpanes (XXV), which are usually present in a series from C_{24} to C_{27} in crude oil and source rock, can be detected by using GC/MS and MID of the characteristic ion at m/z 191. They show a probable relationship to pentacyclic hopanoids from the 17(21) bond cleavage by thermal or microbial degradation during early diagenesis or maturation (TRENDEL et al., 1982; AQINO et al., 1983). The widespread occurrence of 17,21-secohopanes in sediment and petroleum has been proposed to be due to the thermocatalytic degradation of hopanes precursor, by microbial opening of ring E, or cyclization of the precursor squalene stopping at ring D to produce tetracyclic precursor which can be further reduced by geochemical process (TRENDEL et al., 1982). Hopanoids, precursors of hopanes, are found widely distributed among

bacteria and blue-green algae (OURISSON et al., 1979). Other possible origins have been suggested to be a diagenetic product of terpenes contained in sponges living contemporaneously with the deposition of clay sediments (SCHOLEFIELD and WHITEHURST, 1980). The C_{24} member of this series is always predominant in general crude oils except those derived from terrestrial sources which only show trace amounts. Differences in probable precursors of tetracyclic and tricyclic terpanes make the ratio of C_{24} tetracyclic terpane over C_{26} tricyclic terpane a useful source parameter (JONES, 1986). Even though the tetracyclic terpanes have not been widely reported in crude oils or source rocks, their proposed origin by thermal evolution from pentacyclic triterpanes would suggest a role as indicators in petroleum exploration studies.

PENTACYCLIC TRITERPANES

The pentacyclic triterpanes (XXVI) can be divided into two groups; the major one being the hopane-type triterpanes and the remaining triterpanes making up the other group. They are far more diversified in their application than many of the other biomarkers and can be used as source maturity, migration and biodegradation indicators.

Hopane-type triterpanes

Hopane-type triterpanes (XXVII) are ubiquitous

biomarker in fossil fuels. Their commonly accepted precursors, diloptene (XXVIII) and C_{35} tetrahydroxyhopane (XXIX), have been found in various microorganisms, recent sediments and are widely distributed in bacteria and cyanobacteria (blue green-algae) (OURISSON et al., 1979), tropical trees, some grasses, lichens, and several ferns. The naturally occurring precursor compounds have the 17β(H),21β(H) stereochemistry. Diagenesis and maturation of the organic material containing the precursors lead to defunctionalization and formation of the saturated hopanes with the thermodynamically more stable 17α(H),21β(H) configuration pocessed by mature hopanes in crude oils and source rocks. At the same time, formation of another series of hopane analogs, known as moretanes, with the 17β(H),21α(H)configuration occurs. Therefore, increasing maturity will change the distribution of the hopanes and related compounds. The less mature sample contains relatively high concentration of the 17β(H),21β(H) hopanes (i.e., naturally occurring stereochemistry) and, as the maturity level increases, the 17α(H),21β(H) isomers predominate and the 17β(H),21β(H) moretanes appear. The C_{31} and higher homologue can occur as either 22S or 22R epimers due to the chiral central at the position C_{22}. The naturally occurring precursor compounds have the 22R configuration but an increase in maturation leads to a mixture of 22S and 22R epimers whose equilibrium ratio is approximately 60 to 40 (PHILP, 1986).

Changes in the hopane distributions as a result of increasing maturity have been used to indicate whether a source rock is mature enough to have generated crude oil. Virtually all crude oils contain only the 17α(H) hopane series plus minor concentrations of the moretane [17β(H),21α(H)] series and the 17β(H)-C_{27} isomer, and the 22S and 22R epimer for C_{31} higher homologues in a 60 to 40 ratio.

Tm, 17α(H)-22,29,30-trisnorhopane (XXX) and Ts, 18α(H)-22,29,30-trisnorhopane (XXXI), are of particular use in evaluating oil and source rock maturity. Tm display similar characteristics to other 17α(H)-hopanes; degrading at roughly the same rate with increasing maturity (SEIFERT and MOLDOWAN, 1978). Ts is ubiquitous in oil and source rocks (SMITH and WHITEHEAD, 1973),and more resistant to thermal maturation than Tm. Therefore, the ratio of Tm over Ts (Tm/Ts), is used as a maturity parameter for oil and source rock samples of similar source materials (SEIFERT and MOLDOWAN, 1978). The larger the value of Tm/Ts, the less mature a particular sample is in relation to other samples of a similar source.

The hopane distribution can be determined by using GC/MS and MID of m/z 191 and has been widely used for the correlation of oils with suspected source rocks or families of oils thought to be derived from the similar sources (e.g. SEIFERT and MOLDOWAN, 1978 and 1981; VOLKMANN et al., 1983). In the majority of oils

and source rock studied, the regular hopanes observed range from C_{27} to C_{35}, with the absence of the C_{28} member. The formation of the C_{28} hopanes (XXVI; R=CH) from the higher homologue in the series requires cleavage of two carbon-carbon bonds in the side chain rather than one as required for the other members of this series. However, in a few notable exceptions, a C_{28} hopane has been reported as the predominant triterpane. SEIFERT et al. (1978) isolated this compound from the Monterey shale extract and proved its structure to be 17α(H),18α(H), 21β(H)-28,30-bisnorhopane (XXXII). The origin of this compound has provoked much discussion. SEIFERT et al. (1978) initially suggested a possible origin from certain constituents of ferns, but GRANTHAM et al. (1980) suggested that the presence of the C_{28} bisnorhopane might reflect a specific environment of deposition, for there was a correlation between C_{28} bisnorhopane concentrations and sulfur content in several of the sample examined.

There have been many studies reported on whether biological markers are biodegradable. RUBINSTEIN et al. (1977) and CONNAN et al. (1980) reported that triterpanes and steranes were unaffected in either reservoirs or laboratory experiments, while REED (1977) and SEIFERT and MOLDOWAN (1979) concluded from their studies of oil in reservoirs that these compounds were degradable. SEIFERT and MOLDOWAN (1979) proposed that extensive biodegradation would affect the hopanes and lead to the formation of a series of demethylated hopanes. GOODWIN

et al. (1983) performed some laboratory experiments design to enhance the naturally occurring biological activity and observed some specific changes, including reduction in relative concentration of the C_{27} regular and rearranged steranes plus a reduction of the C_{35} hopanes. Evidence was also obtained to suggest that 22R epimers were degraded at a slightly faster rate than the related 22S epimers.

In the past few years, many source, maturity and migration parameters of crude oils have been proposed by SEIFERT and MOLDOWAN. The example of hopane distribution in crude oil is shown in Figure 15 and the most significant parameters involving the hopanes are summarized in Table 6 (PHILP, 1985).

Pentacyclic triterpanes other than hopanes

Many pentacyclic triterpanes other than hopanes, e.g. lupanes (XXXIII), oleananes (XXXIV), fernanes (XXXV) and ursanes (XXXVI) have been reported (SNOWDON and PEAKE, 1978; RICHARDSON and MIILLER, 1982; RICHARDSON and MIILER, 1983; HOERING, 1977). In most reports on these compounds, their use as source indicators has been emphasized. These compounds are found most frequently in coals or in oils derived from terrestrial source material, because their precursors are wildly distributed in higher plants.

In this study, oleanane and gammacerane are detected in some crude oils, therefore they are briefly described.

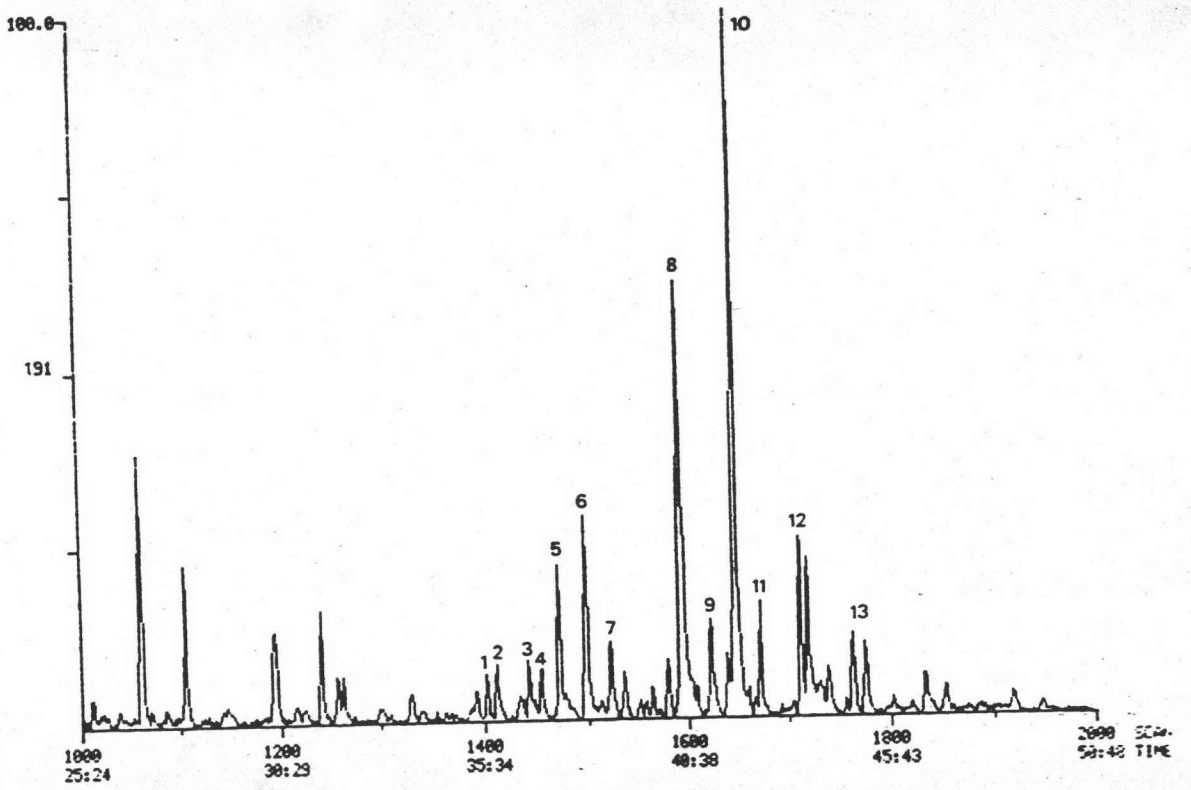

Figure 15. Distribution of hopane-type triterpanes in Australia crude oil. Peak identities are listed in Table 4 (from PHILP, 1985).

Table 4. Identification of triterpanes of m/z 191 shown in Figure 15 (from PHILP, 1985).

Peak number	Compound
1 2 3 4	Tricyclic terpanes
5	18α(H)-22,29,30-trisnorhopane
6	17α(H)-22,29,30-trisnorhopane
7	17β(H)-22,29,30-trisnorhopane
8	17α(H),21β(H)-30-norhopane
9	17β(H),21α(H)-30-normoretane
10	17α(H),21β(H)-hopane
11	17β(H),21α(H)-moretane
12	22S and R-17α(H),21β(H)-30-homohopanes
13	22S and R-17α(H),21β(H)-30,31-bishomohopanes

Oleananes. The precursors of the oleanane are presumed to be the oleanene triterprenoids which are associated with highly specialized terrestrial plants. Therefore, the oils containing oleanane are generally supposed to have a terrestrial or higher plant source.

Gammaceranes. Gammacerane (XXXVII) was reported to occur in extracts of the green river shale by HENDERSON et al. (1969). Though they proposed gammacerane was derived from tetrahymamol (which is the only pentacyclic triterpane found in animals), the precise origin of gammacerane is still unclear. However, it does appear to be indicative of specific depositional environments. For example, high gammacerane concentration may signal hypersaline episodes of source rock deposition occurring in alkaline lakes as well as in lagoonal carbonate evaporite environments.

STERANES

Steranes (XXXVIII) are derived from sterols that are widely dispersed in plants and microorganisms, with the C_{27} and C_{28} sterol most abundant in marine organisms and the C_{29} sterol in higher plants. The major use of steranes in fossil fuel studies is mainly based on the stereochemical complexity of their basic skeleton. Sterol generally contain a 5,6 double bond and occur naturally as the 20R epimer with the 14α(H),17α(H) configuration. As diagenesis begins, the double is hydrogenated and a new epimeric center with a mixture of

5∝(H) and 5β(H) stereochemistries is formed with a predominance of the 5∝(H) epimer due to its greater thermal stability (SEIFERT, 1980). As the level of maturity increases the 14β(H),17β(H) isomers, which predominate at high maturity levels in petroleum (SEIFERT and MOLDOWAN, 1981), are formed as a mixture of the 20R and 20S epimers. The complex sterane distribution is used for source correlations, maturity determinations and observing the effects of biodegradation on crude oils. Migration also affects sterane distributions (SEIFERT and MOLDOWAN, 1981; CAlSON and CHAMBERLAIN, 1986), and it has been proposed that isomers migrating faster than isomers and diasteranes faster than regular steranes.

Disteranes (XXXIX) are also found in crude oils and source rocks (SEIFERT and MOLDOWAN, 1978; ENSMINGER et al., 1978). Since these compounds are formed by acid-clay-catalyzed backbone rearrangement of regular steranes (RUBINSTEIN et al., 1975) they can be used for depositional environment determinations.

In some studies, biodegradation has been shown to have an effect on the sterane distribution. The first epimer to be removed is the C_{29}-20R (VOLKMAN et al., 1983). As the extent of biodegradation increases, the regular steranes are removed, leaving only the rearranged steranes in the extensively biodegraded oils (SEIFERT and MOLDOWAN, 1979).

The example distribution of the C_{27} to C_{29} steranes in crude oil and peak identification are shown

in Figure 16 and Table 5. In addition, many important parameters involving steranes for source maturation, migration and biodegradation indicators are summarized in Table 6.

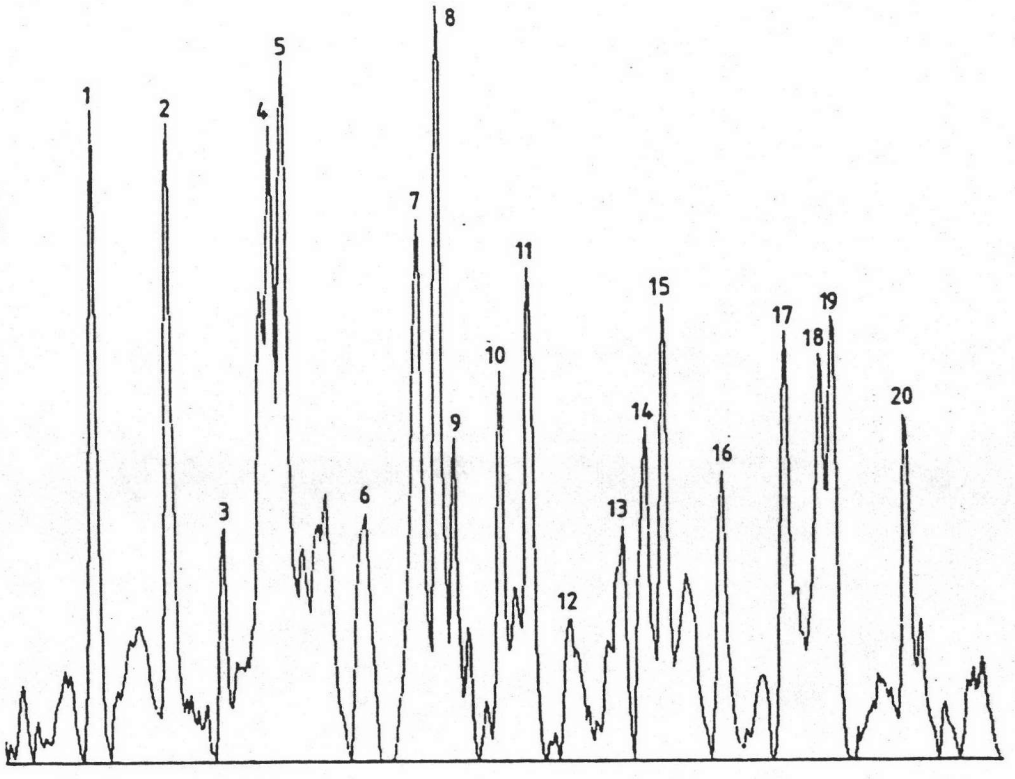

Figure 16. Distribution of steranes in an Alaska crude oil at mass ion m/z 217. Peak identifications are given in Table 5. (from PHILP, 1985).

Table 5. Identification of steranes of m/z 217 shown in Figure 16 (from PHILP, 1985).

Peak number	Compound
1	13β,17α-Diacholestane (20S)
2	13β,17α-Diacholestane (20R)
3	13α,17β-Diacholestane (20S)
4	13α,17β-Diacholestane (20R)
5	24-Methyl-13β,17α-diacholestane (20R)
6	24-Methyl-13β,17α-diacholestane (20R)
7	24-Methyl-13α,17β-diacholestane (20S) + 14α-cholestane (20S)
8	24-Methyl-13β,17α-diacholestane (20S) + 14β,17β-cholestane (20R)
9	14β,17β-Cholestane (20S) + 24-methyl-13α,17β-diacholestane (20R)
10	14α,17α-Cholestane (20R)
11	24-Ethyl-13β,17α-diacholestane (20R)
12	24-Ethyl-13α,17β-diacholestane (20S)
13	24-Methyl-14α,17α-cholestane (20S)
14	24-Ethyl-13α,17β-diacholestane (20R) + 24-methyl-14β,17β-cholestane (20R)
15	24-Methyl-14β,17β-cholestane (20S)
16	24-Methyl-14α,17α-cholestane (20R)
17	24-Ethyl-14α,17α-cholestane (20S)
18	24-Ethyl-14β,17β-cholestane (20R)
19	24-Ethyl-14β,17β-cholestane (20S)
20	24-Ethyl-14α,17α-cholestane (20R)

Table 6. Summary of biomarker parameters based on steranes and triterpanes in petroleum studies (from PHILP, 1985).

Biomarker parameter	Application
Tricyclic terpanes (%)	Source
Change in paraffin concentration plus increase in sterane and triterpane concentration	Migration and/or maturation specific if common source established
$\frac{C_{29} + C_{30} \text{ primary terpanes}}{C_{27} + C_{28} \text{ secondary terpanes}}$	Source and maturation
$\frac{17\alpha(H)\text{-}22,29,30\text{-trisnorhopane } (T_m)}{18\alpha(H)\text{-}22,29,30\text{-trisnorhopane } (T_s)}$	Source and maturation
5β(H) steranes/17α(H) hopanes	Migration if common source established
$\frac{17\beta(H),21\beta(H)}{17\beta(H),21\beta(H) + 17\beta(H),21\alpha(H) + 17\alpha(H),21\beta(H)}$ hopanes	Maturation
$\frac{22R}{22R + 22S}$ 17α(H),21β(H)-homohopanes	Maturation
$\frac{20S}{20S + 20R}$ 13β(H),17α(H)-diacholestane	Maturation
$\frac{24S}{24S + 24R}$ (20R)-24-methyl-ααα-cholestane[a]	Maturation
$\frac{20R}{20R + 20S}$ -24-ethyl-ααα-cholestane[a]	Maturation
$\frac{\alpha\beta\beta}{\alpha\beta\beta + \alpha\alpha\alpha}$ (20R + 20S) 24-ethylcholestane[a]	Maturation
$\frac{\alpha\alpha\alpha\text{-}C_{28} \text{ steranes}^a}{\alpha\alpha\alpha\text{-}C_{29} \text{ steranes}}$	Source
$\frac{\alpha\alpha\alpha\text{-}C_{27} \text{ steranes}^a}{\alpha\alpha\alpha\text{-}C_{29} \text{ steranes}}$	Source
$\frac{\beta\alpha\alpha \text{ (20R)} + \alpha\beta\beta \text{ (20R)}}{\alpha\alpha\alpha \text{ (20R)}}$ C_{29} steranes	Migration
$\frac{13\beta(H),17\alpha(H)(20S)}{\alpha\alpha\alpha \text{ (20R)}}$ $C_{27} + C_{28} + C_{29}$ steranes	Migration
$\frac{\beta\alpha\alpha \text{ (20R)-}C_{28} + \alpha\beta\beta \text{ (20R + 20S)-}C_{28} + \beta\beta\beta \text{ (20R + 20S)-}C_{29}}{\alpha\alpha\alpha \text{ (20R)-}C_{28}}$ steranes	Source
$\frac{C_{27} \text{ triaromatic steranes}}{C_{27} \text{ triaromatic} + C_{28} \text{ monoaromatic steranes}}$	Maturation and aromatization
$\frac{C_{21} \text{ monoaromatic steranes}}{C_{21} \text{ triaromatic} + C_{28} \text{ monoaromatic steranes}}$	Maturation and bond breaking
$\frac{C_{26} \text{ triaromatic steranes}}{C_{20} \text{ triaromatic} + C_{27} \text{ triaromatic steranes}}$	Maturation and bond breaking
Preferential removal of C_{27}-20S diasterane over 20R epimer	Biodegradation
Preferential removal of regular steranes over diasteranes	Biodegradation
$\frac{17\alpha(H)\text{-}C_{30} \text{ hopane}}{17\beta(H)\text{-}(C_{29} + C_{30}) \text{ moretanes}}$	Differentiation of source rock stratigraphies by pyrolysis
17α(H)/17β(H)—trisnorhopanes	Differentiation of source rock stratigraphies by pyrolysis

METHYLSTERANES

Methylsteranes (XL) found in sediments and crude oils have been reported to be derived from dinoflagellates (WITHER, 1983). They can be presented in samples starting from C_{28} to C_{31} methylsteranes. The general molecular structure of these compound is 4-methylsterane. The C_{28}-C_{30} methylsteranes can be found in both marine and nonmarine crude oils (MOLDOWAN et al., 1985). The mass ion studied in the MID mode of GC/MS is based on m/z 231. An important and appearantly characteristic feature of methylsteranes in natural presumed precursor of many dinoflagellates has been reported is the 4β-methyl stereoisomer (WITHER, 1983). This isomer is a relatively less thermodynamically stable isomer compareed to 4αconfigulation. The presence of 4α-methylsteroids (4α-methylsterols, 4α-methylstanones, etc) in marine and lacustrine dinoflagellates in recent sediments associated with methylsteranes in crude oils (BOON et al., 1979; GAGOSIAN et al., 1980; BRASSEL and EGLINTON, 1983; DE LEEUW et al., 1983) has been used to infer the contribution of dinoflagellates in such a depositional environment. This may be suggested that the widespread occurrence of methylsteranes in crude oils may reflect the participation of dinoflagellates in ecosystem that eventually contribute to petroleum genesis (MOLDOWAN et al., 1985). Because of a difference thermodynamically stable between these two isomers and a significant abundance of 4α-methyl counterparts that are

affected by increasing maturity (WITHER ,1983), the ratio of 4β /4∝ methylsterane has been suggested therefor to be a possible maturity parameter (RUBINSTEIN and ALBRECHT, 1975; MACKENZIE et al., 1980). An example chromatogram of 4-methylsterane distribution in crude oil is shown in Figure 17.

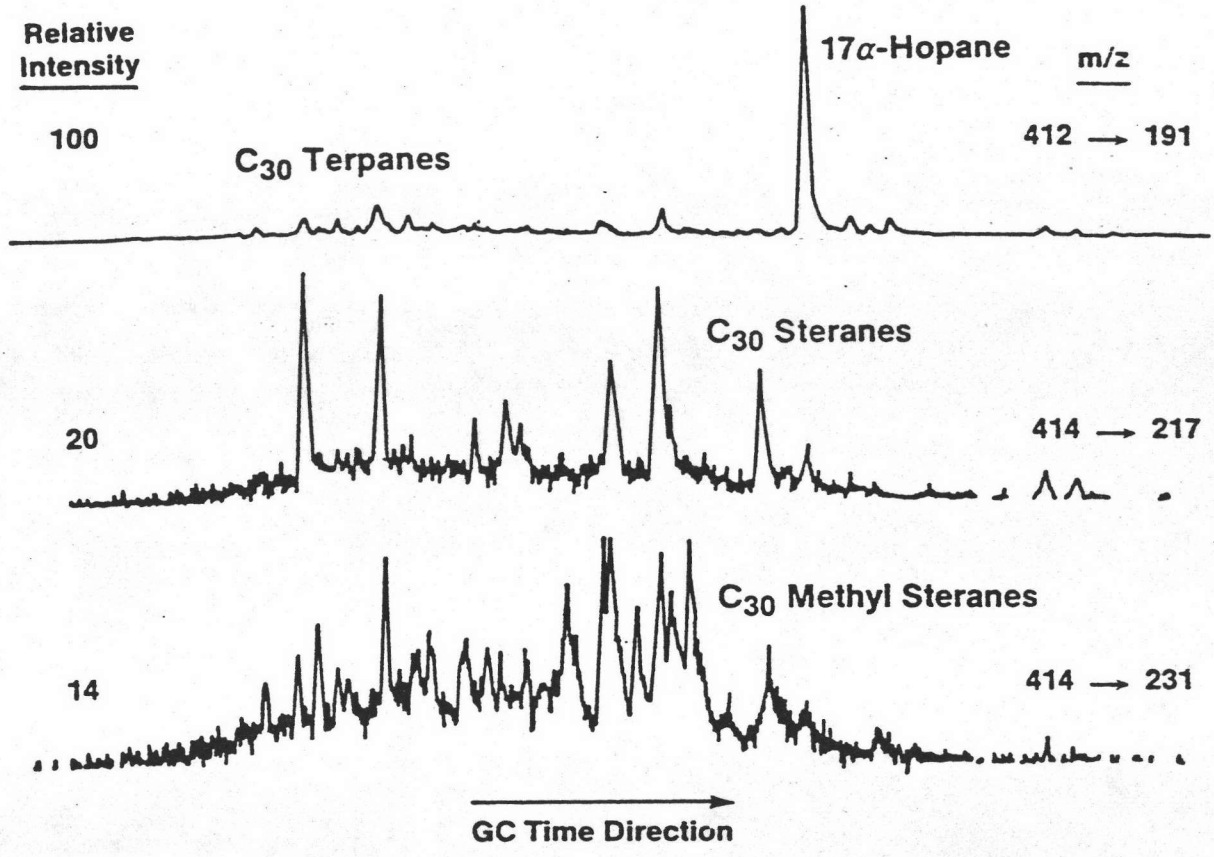

Figure 17. Distribution of C_{30} methylsteranes in Sadlerochit, Alaska (marine) crude (from MOLDOWAN et al., 1985).

AROMATIC COMPOUNDS

Aromatic hydrocarbons studied in source rock and sediment, unlike saturated hydrocarbon, are related to gross change rather than individual compound variations. Several aromatic hydrocarbon ratios have been used as maturity parameters and tend toward to both oil- and gas-prone source rock assessment (HALL et al., 1985). Polycyclic aromatic compounds found in recent and ancient sediments have been reported to be derived from non-aromatic biogenic precursors (LAFLAMME and HITES, 1978; WAKEHAM et al., 1980) and by some processes during diagenesis (YOUNGBLOOD and BLUMER, 1975; LAFLAMME and HITES, 1978; WAKEHAM et al., 1980). The precursor compounds were suggested including unsaturated fatty acids, carotenoids, polyhydroxyquinones and terprenoids (HALL et al., 1985 and reference therein). However, the variation of aromatic compounds in sediments mostly related to variations in source and maturity rather than depositional environment (HALL et al., 1985). The main groups of aromatic compounds found in most samples are naphthalene and alkylnaphthalene group (XLI), phenanthrene and alkylphenanthrene group (XLII) and aromatic steranes (XLIII), aromatic triterpanes (XLIV) plus other types of polycyclic compounds. The variations in the relative amounts of these three difference groups related to a combination of maturity and type of kerogen was concluded by HALL et al. (1985). Many aromatic parameters applied to maturity studies of source rock

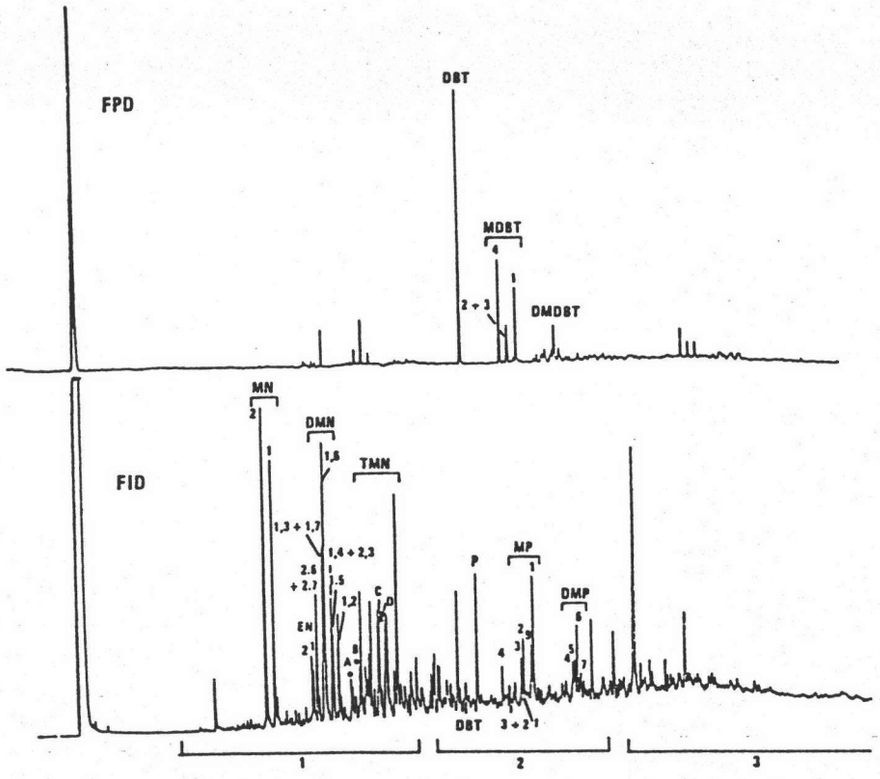

Figure 18. Distribution of aromatic hydrocarbons in organic source rock (from HALL et al., 1985).

Table 7. Maturity parameters from aromatic compounds.

$$*\text{MNR} = \frac{\text{2-methylnaphthalene}}{\text{1-methylnaphthalene}}$$

$$*\text{BPHR} = \frac{\text{biphenyl}}{\text{1,6 dimethyl naphthalene}}$$

$$*\text{ENR} = \frac{\text{2-ethyl naphthalene}}{\text{1-ethyl naphthalene}}$$

$$*\text{DMNR} = \frac{\text{2,6 + 2,7-dimethyl naphthalene}}{\text{1,5-dimethyl naphthalene}}$$

$$\frac{\text{4MDBT}}{\text{DBT}} = \frac{\text{4-methyl dibenzothiophene}}{\text{dibenzothiophene}}$$

$$\frac{*\text{4MDBT}}{\text{1MDBT}} = \frac{\text{4-Methyl dibenzothiophene}}{\text{1-methyl dibenzothiophene}}$$

$$\frac{\text{3/2MDBT}}{\text{1MDBT}} = \frac{\text{3 + 2-methyl dibenzothiophene}}{\text{1-methyl dibenzothiophene}}$$

$$*\text{2/1 MP} = \frac{\text{2-methyl phenanthrene}}{\text{1-methyl phenanthrene}}$$

$$*\text{MPI 1} = \frac{1.5 \times (3\text{MP} + 2\text{MP})}{\text{P} + 9\text{MP} + 1\text{MP}}$$

where P = phenanthrene and MP = methylphenanthrene

and sediment have been reported (HALL et al., 1985; RADKE et al., 1981, 1986). The example chromatogram of polycyclic aromatic distribution is shown in Figure 18. Parameters which usually used to indicate maturity are also shown in Table 7.

STABLE CARBON ISOTOPES

Stable carbon isotope ratio is one of the parameters has been used widely in geochemical study of petroleum. They have been applied to many correlation studies (STAHL, 1978; SCHOELL et al., 1983; PETERS et al., 1986; CHUNG et al., 1981) and the determination of the deposition of an oil source (SOFER, 1984; PETERS et al., 1986). The measurement of carbon isotope ratios can also determine the extent of the biodegradation (STAHL, 1980), maturity (PETERS et al., 1981; LEWAN, 1983) and migration (BONILLA and ENGEL, 1986) of oils and bitumens. It can be applied to the whole oil, a whole extracted, asphaltene, NSO, aromatic and saturate fraction of the samples. The difference in the isotopic composition of the oils is explained by the difference in the from of CO_2 used by photosynthesizing organism that contribute to the different groups of organic source material, marine and terrigeneous (DEGEN, 1969). On the basic of the carbon isotope analysis, isotopic variations of marine and non-marine (terrigeneous) organic material is a function of distance from the coast and the influence of rivers (SILVERMAN and EPSTEIN, 1958). Crude oils derived

from terrigeneous organic source are usually isotopically more negative than oil from marine source (HUNT, 1970; TISSOT and WELTE, 1978; ROGERS, 1980). Some of marine and non-marine Tertiary crude oils from different environments had been analysed and the the C-isotope values differed by at least 3 ‰ (SILVERMAN and EPSTEIN, 1958). The $\delta^{13}C$ values of oils derived from marine sources was reported to rangs from -23 to -27 whereas oils derived from non-marine source were ranged from -30 to -32 ‰. The aromatic hydrocarbon fraction is generally more positive than saturate fraction (STAHL, 1977). The general trend of enrichment of light isotope ^{12}C with increasing age was caused by the intensity of photosynthesis which would change the isotopic composition of the atmopheric CO_2 (STAHL, 1976; WELTE, 1970). Therefore, this means the change in average carbon isotope composition of crude oils depends on various geologic ages (DEGENS, 1969). In addition, it can change owing to maturation, specific migration and the difference original organic source material.

Sofer (1984) used the isotopic relationship of the saturate and aromatic hydrocarbon fraction to differentiate oils derived from terrigeneous and oils derived from marine organic matters. Because both oils do not manifest themselves in the range of absolute values of only one fration, the isotopic relationship between the saturate and aromatic fraction then was created following the equations (SOFER, 1984);

For oils derived from terrigeneous source,

$$\delta^{13}C_{aro} = 1.12\ \delta^{13}C_{sat} + 5.45$$

For oils derived from marine source,

$$\delta^{13}C_{aro} = 1.10\ \delta^{13}C_{sat} + 3.75$$

The variations in these relationships also be suggested was determined by (a) source of the oils, (b) the absolute isotopic values and (c) the maturity of the oils. A statistical parameter used to evaluate the difference between these two equations is called Canonical Variable (CV), definded as the perpendicular distance of a given sample from the best separating line on the $\delta^{13}C_{aro}$ versus $\delta^{13}C_{sat}$ plane (SOFER, 1984). The relationship between CV value and isotopic composition was illustrated by the following equation;

$$CV = -2.53\ \delta^{13}C_{sat} + 2.22\ \delta^{13}C_{aro} - 11.65$$

The CV value which is larger than 0.47 was mentioned indicate predominantly a terrigeneous source of the oil whereas the value smaller than 0.47 indicate mostly oil derived from marine organic source (Figure 19).

An example of isotopic compositions ($\delta^{13}C_{aro}$ versus $\delta^{13}C_{sat}$) and gas chromatograph of different oil samples due to Sofer type plot is shown in Figure 20.

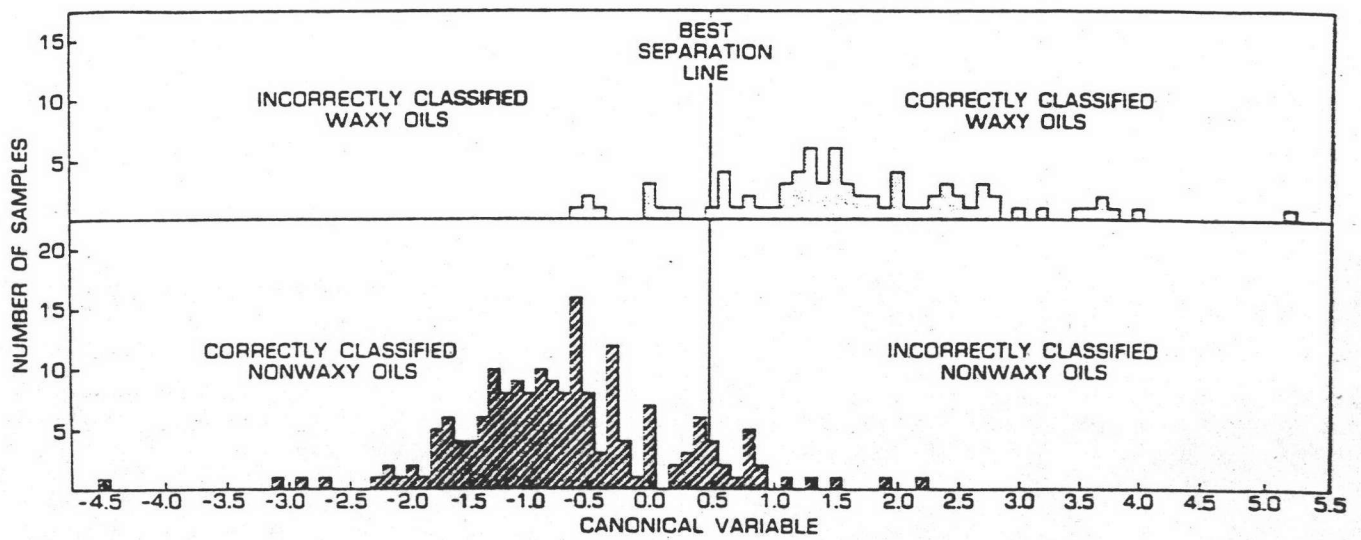

Figure 19. Histograms of canonical variable (CV) for $\delta^{13}C_{aro}$ versus $\delta^{13}C_{sat}$ (from SOFER, 1984).

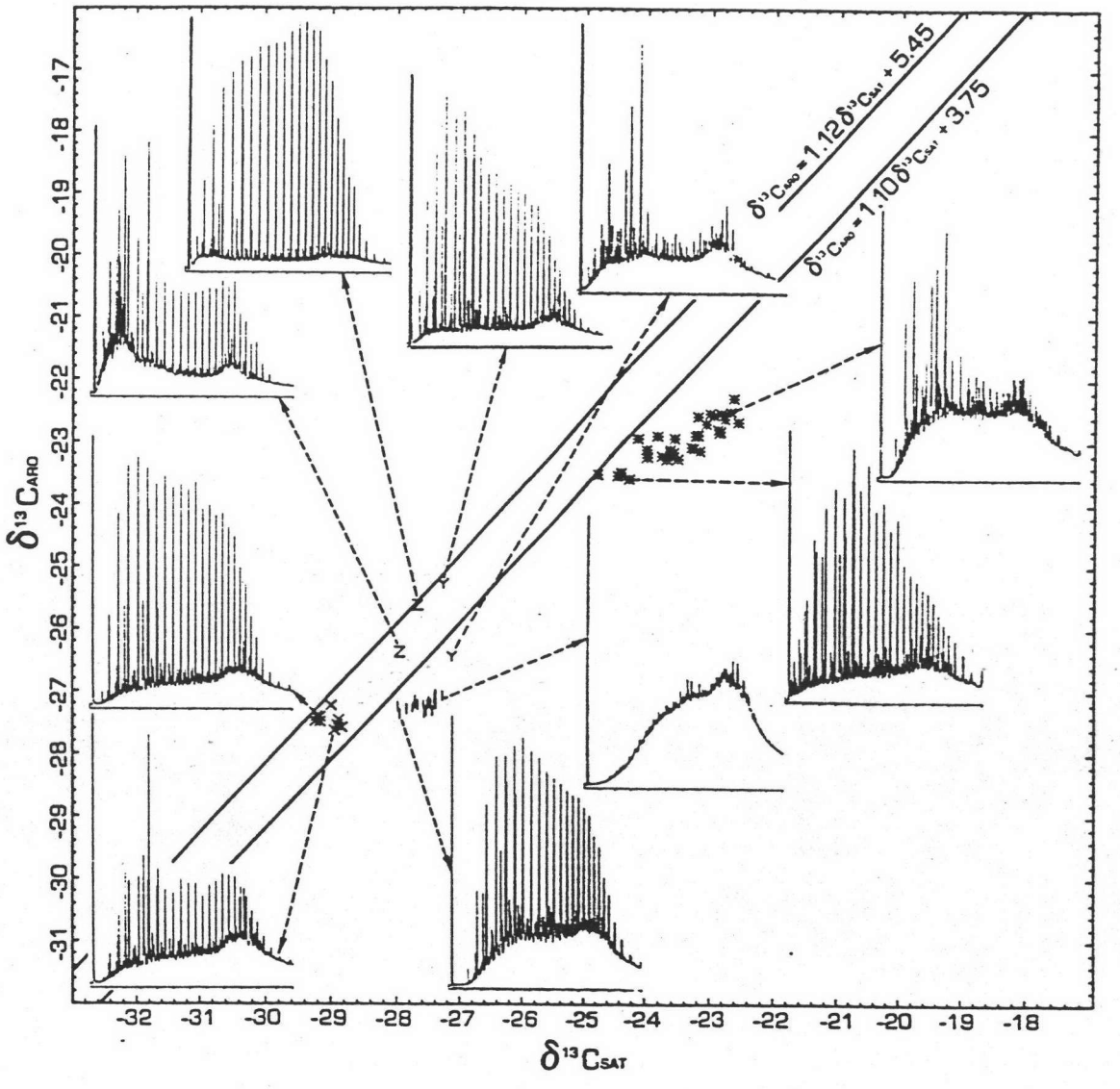

Figure 20. Isotopic compositions ($\delta^{13}C_{aro}$ versus $\delta^{13}C_{sat}$) and gas chromatograph of biodegraded and nondegraded marine and nonmarine oils. Marine oils: * = California; ¦ = Colombia. Nonmarine oils: x = location B; z = Java Sea; Y = undisclosed location (from SOFER, 1984).