An Investigation into The Effect of Composition on Performance of Penetration Grade Road Bitumens Part 2: Biomarker Analysis

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Synopsis

In the first part of the investigation several grades of penetration grade bitumens from two sources were subjected to SARA analysis. Differences in response to ageing of the bitumen samples from the two sources were then investigated in terms of changes occurring to the proportions of saturates, aromatics, resins and asphaltenes. In this part of the investigation, it was decided to investigate, from a petroleum geochemical perspective at a more detailed level, the chemical differences in the make up of the bitumens. The bitumen samples were fractionated using column chromatography and the aliphatic fractions were subsequently subjected to Gas Chromatography (GC) and Gas Chromatography Mass Spectrometry (GCMS) analysis. GC analysis on its own was found not to be a suitable test for penetration grade bitumens. Using GCMS (ion m/z 85) it was possible to show significant differences between the n-alkane distribution of Middle Eastern and Kuwaiti bitumen samples, even though they both originate from the same source/depositional environment. Using GCMS (ions m/z 191 & 217) it was possible to analyse the hopane and sterane distributions respectively of the saturated hydrocarbon fractions of all bitumen samples. Fundamental differences were noted in the abundance and distribution of biomakers in the chromatograms between the Venezuelan and Middle Eastern bitumens. A range of biomarker diagnostic ratios was investigated allowing some analysis of thermal maturation and depositional environments of the organic rich source rocks (from which the oil was generated) to be carried out. Some potentially useful trends in diagnostic ratios were observed as one moves progressively across the various grades of bitumens for any one source, and between samples in the virgin state and those that had been subjected to age conditioning.

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Engineers involved with bituminous materials (asphalts) currently have at their disposal a wide range of dynamic and rheological characterisation tests with which to analyse, compare and theoretically model the performance of penetration grade bitumens and bituminous mixtures under laboratory and field conditions.

From a chemical point of view, at most, asphalt engineers will have access to SARA analysis (saturates, aromatics, resins and asphaltene contents). The test is typically carried out using an Iatroscan which in essence is a TLC-FID (i.e. separation of organic components using thin layer chromatography and detection by flame ionization system). Variations in the proportions of these four components, especially the asphaltene content, have been routinely linked to differences in viscosity as a result of changes in bitumen grade or as a consequence of bitumen ageing. In the first part of this investigation, there was ample evidence that in addition to the aforementioned, the use of colloidal instability coefficients was a very useful tool for analysing differences in the ageing behaviour of bitumens from different sources. In this part of the investigation it was decided to take a step further into the field of bitumen characterisation and attempt to characterise bitumens based on more sophisticated petroleum geochemical techniques.

Geochemical fossils (or biological markers) are molecules synthesized by plants or animals and incorporated in sediments with only minor changes. In particular, the carbon skeleton of hydrocarbons or other lipids is preserved. These molecules represent only a minor fraction of crude oils. However, they are of great interest to geologists and geochemists as they can be used for characterization, correlation, and /or reconstitution of the depositional environment, in the same manner as macro- or microfossils are commonly used by geologists.

Alkanes, fatty acids, terpenes, steroids, and porphyrins are the major groups of geochemical fossils. They can be traced from recent to ancient sediments where they progressively suffer thermal degradation and/or dilution by other hydrocarbons generated at greater depths (Tissot & Welte, 1984).

The primary objective of this investigation was to analyse and distinguish bitumens from different sources using biological markers (biomarkers). Biomarker distributions are best studied using chromatograms obtained from gas chromatography – mass spectrometry (GC-MS) analysis. The second objective was to assess the suitability of biomarker diagnostic ratios for identifying any consistent differences or trends between the various bitumen grades and/or bitumen conditioning state (e.g. virgin bitumen versus oven aged sample).

USE OF BIOMARKER INDICES

Influence of Weathering Processes on Bitumen from a Geochemical Perspective

Weathering processes including water-washing, biodegradation, photo-oxidation and oxidation do modify the gross composition of the bitumen, entailing a marked increase of polar fractions, namely resins, asphaltenes and insoluble residues. During this alteration process, C_{15+} aromatics tend to disappear and polar by-products are generated. Fortunately, C_{15+} alkanes are less affected by these weathering processes; consequently steranes and terpanes may be used to correlate bitumen from archaeological samples to their geological sources, namely natural asphalts (Connan, 1999).

Relationships Between Biomarker Indices, FTIR Indices and Bitumen Rheological Properties

Pieri *et al.* (1996) proposed an equation using selected biomarker indices to estimate the value of $G^*/\sin\delta$ determined by means of dynamic shear rheometer testing. The ratio $G^*/\sin\delta$ is widely accepted as indicative of the resistance to permanent deformation of a bituminous binder at high temperatures (Superpave SP-2).

Considering biomarker indices as distillation independent, it was thus argued that these indices can be estimated from crude oil data. However, different bitumen grades (i.e. corresponding to different rheological

properties) can be obtained from the same oil. Consequently, Pieri *et al.* (1996) added an extra FTIR index, the aromaticity index (A.I.) into the equation to take distillation into account, as this index is dependent on the distillation process. They found a good multivariate linear regression to predict G*/sinδ at 58°C (adjusted R^2 = 0.87, std error of estimate = 0.3) using the following equation:

$$Y_{G*/\sin\delta} = 0.60X_{Ts/(Ts+Tm)} + 0.92X_{NOR/Ts} + 0.75X_{V} + 0.28X_{AI} \quad (1)$$

The independent variables being;

NOR/Ts = [(29,30 bisnorhopane + 28,30 bisnorhopane + 17α(H),21β(H)-25-norhopane + 17α(H),21β(H)-30-norhopane)] ÷ Ts

Ts = 18α(H)-22,29,30-trisnorneohopane

Tm = 17α(H)-22,29,30-trisnorhopane

Aromaticity index (AI), AI = [$A_{1635\text{-}1538}$ ÷ ΣA], where A; area (area band limits are noted in subscript and unit is wavenumber, cm^{-1}), ΣA = sum of all measured FTIR bands

V = vanadium content

Consequently, it was suggested that G*/sinδ can be correctly estimated using only four indices from FTIR, biomarker and element analysis data. The more the bitumen or its parent crude oil shows a high maturity, a high aromatic structure content, a bacterial origin in an anoxic environment and a high vanadium content, the less suitable it will be to the mechanical ageing phenomena at high temperature. The vanadium content probably relates to the porphyrin content and would correspond to oils from marine carbonates or siliciclastics.

Sensitivity to thermal cracking of bitumens, is routinely assessed by measuring the creep stiffness at low temperatures in particular following ageing (short term and long term laboratory ageing). Pieri *et al.* (1996) proposed the following equation to reflect the stiffness at -10°C, S(-10°C) also obtained from a multivariate linear regression run with some geochemical data of crude oils and bitumen aromatic structure content that considers the distillation effect:

$$S_{(-10^{o}C)} = 0.38X_{R/S\ HOP} + 0.53X_{Ts/(Ts+Tm)} - 0.61X_{NM/C30} - 0.32X_{29,30BNH/C30H} + 0.41X_{A1} \quad (2)$$

where;

R/S HOP = [($C_{31\text{-}35}$) 17α(H),21β(H)-homohopanes 22R] ÷ [($C_{31\text{-}35}$) 17α(H),21β(H)-homohopanes 22S)],

NM/C_{30}H = [normoretane] ÷ [17α(H),21β(H)-hopane].

$X_{29,30BNH/C30H}$ = [29,30 bisnorhopane] ÷ [17α(H),21β(H)-hopane].

Equation 2 emphasises the relationship between sensitivity to thermal cracking at low temperatures and the following variables; the bitumen aromatic structure content (AI), the maturity level and the depositional environment of the parent crude oil (R/S HOP, Ts/(Ts+Tm), 29,30BNH/C_{30}H), and the biodegradation level of the parent crude oil (NM/C_{30}H).

Furthermore, by comparing Equations 1 and 2, it can be confirmed that a great deal of difficulty exists in selecting a bitumen with good rheological properties at both low and high temperatures because of the antagonist chemical characteristics which are required. Bitumens can be obtained from biodegraded oil because of the resulting good resistance to permanent deformation at high temperature. However, if the crude oil is biodegraded and immature such as those from California, the corresponding bitumen has a good chance of being too hard at low temperature, particularly following ageing.

MATERIALS AND METHODS

The same bitumen types and grades as used in Part 1 of the investigation (i.e. 35, 40/60, 70/100 and 160/220pen. Venezuelan and Middle Eastern bitumens, plus a Kuwaiti sample) were also used in Part 2. A Brent Crude Oil sample was also submitted for analysis to assist in subsequent data interpretation. As in Part 1, the bitumens were also tested in their virgin, short term oven aged and recovered from rubber/bitumen mixes states.

All bitumen samples were initially fractionated into their aliphatic and aromatic hydrocarbon fractions using column chromatography, as detailed below. The aliphatic hydrocarbon fractions were then analysed by gas chromatography (GC). The aliphatic and aromatic hydrocarbon fractions from all bitumen samples were also analysed by gas chromatography - mass spectormetry (GC-MS).

COLUMN CHROMATOGRAPHY

General Principles

In column chromatography, the stationary phase, a solid adsorbent, is packed in a vertical glass column. Silica gel (SiO_2) and alumina (Al_2O_3) are the two adsorbents most commonly used by the organic chemist for column chromatography. Adsorbent particle size affects how the solvent flows through the column.

The mixture to be analyzed by column chromatrography is applied to the top of the column. The mobile phase, a liquid solvent (the eluent), is passed through the column by gravity or by the application of air pressure. An equilibrium is established between the solute adsorbed on the adsorbent and the eluting solvent flowing down through the column. Because the different components in the mixture have different interactions with the stationary and mobile phases, they will be carried along with the mobile phase to varying degrees and a separation will be achieved. The individual components, or elutants, are collected as the solvent drips from the bottom of the column.

Compounds interact with the silica or alumina largely due to polar interactions. The polarity of the solvent which is passed through the column affects the relative rates at which compounds move through the column. Often a series of increasingly polar solvent systems are used to elute a column. A non-polar solvent is first used to elute a less-polar compound. Once the less-polar compound is off the column, a more-polar solvent is added to the column to elute the more-polar compound.

Procedure for Preparation of Column and Bitumen Samples

A pre-weighed sample of bitumen (up to 50mg dissolved in Dichloromethane (DCM)) was transferred into a vial containing dry alumina powder. The vial containing (alumina + bitumen sample + DCM) was then dried on a warm surface (until all the DCM has evaporated and the bitumen stained brown coloured alumina powder became free flowing).

In this investigation, a glass column containing Petroleum Ether (Pet. Ether, BP 40-60°C) was packed with enough silica granules (silica gel 60, pre-extracted and activated at 180°C) to form a compacted height of approximately 20cm. A layer of powdered alumina was then added into the glass column so that it formed a compacted plug on top of the silica, approximately 0.5-1.0cm in height.

The (alumina + bitumen) sample was then added to the top of the column. A layer of approximately 0.5cm of alumina was finally added to form another compacted plug on top of the specimen and to assist in confining the specimen.

In the first step, 70ml Pet. Ether eluent was gradually added into and allowed to pass through the column, collecting the elutant in a flask. The level of solvent was not allowed to drop below the packed sample level at any time. This step separated the aliphatic hydrocarbon fractions from the rest of the bitumen sample.

In the second step, 70ml of (Pet. Ether / DCM) mixture in the proportion of 60/40 was passed through the column in increments. A change in colour of the solvents in the column from colourless to light brown was observed as the Pet. Ether/DCM travelled through the column. At this stage, the aromatic hydrocarbon fractions were separated from the rest of the bitumen sample.

Drying Fractions and Determination of Masses

Using a rotary evaporator, the volumes of the dissolved fractions in each of the round bottom flasks were reduced in turn. The reduced fractions were transferred into clean marked vials. Using a fine stream of Nitrogen gas, the excess solvents from each of the vials were gently evaporated (until a constant mass of solvent free fraction was achieved). The amounts of each fraction in grams were weighed to an accuracy of 4 decimal places.

The aliphatic hydrocarbon fractions were submitted for further analysis by gas chromatography (GC) and GC-MS. The concentration of the sample required for GC analysis was pre-set initially at 100 μl of DCM per mg of sample. Only a small quantity, approximately 1ml of aliphatic hydrocarbon fractions in DCM was required for GC testing.

GAS CHROMATOGRAPHY (GC)

Background Information on GC

Gas chromatography is a chromatographic technique that can be used to separate volatile organic compounds. The GC system consists of a liquid with a high boiling point impregnated on an inert solid support as the stationary phase and typically helium gas as the mobile phase. The stationary phase is packed into a thin metal column and helium gas is allowed to flow through it. The column is attached to an injection port, and the entire system is heated in an oven. A solution of the mixture is injected into the column through the injection port by means of a syringe and is immediately volatilized. The injection port is maintained at a higher temperature than the boiling point of the least volatile component in the sample mixture. The helium gas then sweeps the components out of the column and past a detector. The organic compounds are separated due to differences in their partitioning behaviour between the mobile gas phase and the stationary phase in the column. The polarity of the compounds and their volatility determines how long they are retained by the column. When each component passes the detector, a peak is registered on a recorder. The relative quantities of the components can be determined from the relative areas under the peaks.

Since the partitioning behaviour is dependant on temperature, the separation column is usually contained in a thermostat-controlled oven. Separating components with a wide range of boiling points is accomplished by starting at a low oven temperature and increasing the temperature over time to elute the high-boiling point components.

GC Test Conditions

In this investigation, gas chromatographic analysis was performed on a Hewlett Packard 5890 II instrument equipped with a split/splitless injector and flame ionization detector. The samples, dissolved in DCM were injected using a HP 7673 auto-sampler and separation was performed on a Hewlett Packard HP-5MS fused silica capillary column (30m × 0.25mm i.d.; film thickness = 0.25µm) using hydrogen as carrier gas. The oven temperature was held at 50°C for 2 minutes and then ramped at 4°C/minute to 300°C where it was held for 20 minutes.

DISTRIBUTION OF N-ALKANES IN THE SATURATED HYDROCARBON BITUMEN FRACTIONS

Gas Chromatography results

GC analysis was carried out on the aliphatic hydrocarbon fraction of selected bitumen samples to analyse the distribution of n-alkanes. The original aliphatic hydrocarbon samples were dissolved in DCM at the normal concentration of 1mg/300µL for injection into the GC column. In addition, the samples contained squalane (0.5% by mass of bitumen), which was added to all the bitumen samples prior to open column liquid chromatography on silica-gel. Squalane elutes between n-C_{26} and n-C_{27} and can thus help to identify the rest of the n-alkane peaks.

Unfortunately, the n-alkane peaks from the aliphatic hydrocarbon fractions of the bitumen samples were of such low intensity and abundance that it was not possible to analyse these samples (very low signal to noise ratio). Each GC trace was totally dominated by the squalane peak and the complex unresolved hump.

The samples were reanalysed by GC after they were concentrated to approximately 1mg/150µL (by blowing down the aliphatic hydrocarbons in DCM samples in a stream of nitrogen to about half volume). Figures 1 and 2 below show the saturated hydrocarbon gas chromatograms of two bitumen samples. Once again it was very clear that the peak heights of the n-alkanes were too small (units on the y axis too small) to allow good quantitative analysis, since the peak heights/areas must be determined from an integration baseline which follows the top of the unresolved complex mixture (UCM). Furthermore the squalane peak, which elutes at around 54 seconds (not shown in Figures 1 and 2), was still highly dominant and would not allow the peak heights or areas under the n-alkane peaks to be accurately quantified.

In all the gas chromatograms, a large hump (UCM) was also very dominant. Heavily degraded oils are characterized by a large UCM that rises above the baseline on gas chromatograms (Peters & Moldowan, 1993). It was therefore decided not to proceed with any further GC analysis, but to concentrate on GC-MS as an alternative.

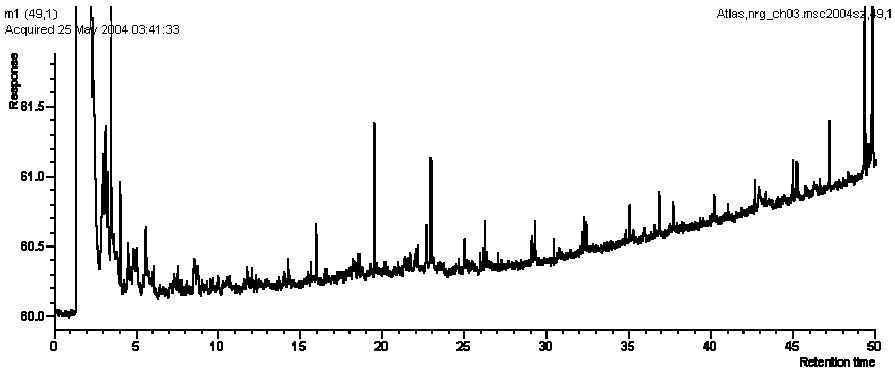

Figure 1: GC trace of aliphatic hydrocarbon fraction of 200 penetration grade Middle Eastern Virgin bitumen.

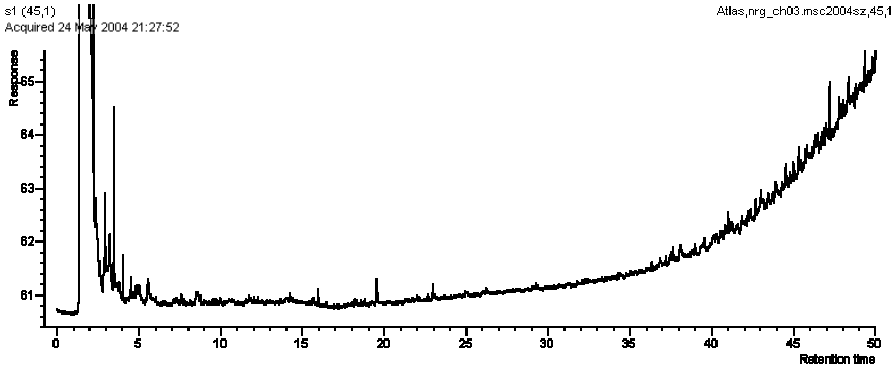

Figure 2: GC trace of aliphatic hydrocarbon fraction of 200 penetration grade Venezuelan Virgin bitumen.

MASS SPECTROMETRY

Mass spectrometry is used to determine the molecular weight of a compound. It also provides information about isotopic abundances, and the ways in which molecular ions decompose. The sample to be analysed is first vaporized by heating and is then ionized. Ionization takes place with a beam of high energy electrons. The ions pass through a magnetic field where their paths are deflected. The amount of deflection is mass dependent. The output from the detector is called the mass spectrum, and is a plot of signal intensity against mass:charge (m/z) ratio.

In a mass spectrum, peaks arising from ions with mass lower than the parent ion are often observed. These arise from bond cleavage which leads to fragmentation of the molecule. By assigning identities to fragment peaks in a mass spectrum, it is possible to 'reconstruct' a molecule and hence use fragmentation patterns to aid the identification of unknown compounds.

GAS CHROMATOGRAPHY - MASS SPECTROMETRY (GCMS)

GC-MS Test Setup

Mass spectral characterisation of compounds in the hydrocarbon fractions was carried out using combined gas chromatography/mass spectrometry (GC/MS). In this investigation, GCMS analysis of aliphatic and aromatic hydrocarbon fractions were performed on a Hewlett-packard 5890 II GC split/splitless injector (280°C) linked to a Hewlett-Packard 5972MSD (electron voltage 70eV, filament current 220μA, source temperature 160°C, multiplier voltage 2000V, interface temperature 300°C). The acquisition was controlled by a HP Vectra 486 pc chemstation computer, initially in full scan mode (50-550 amu/sec) or in selected ion mode (30ions 0.7cps 35ms dwell) for greater sensitivity. The sample (1μl) in DCM was injected by an HP7673 auto sampler and the split opened after 1 minute. After the solvent peak had passed the GC temperature programme and data acquisition commenced. Separation was performed on a fused silica capillary column (30m × 0.25mm i.d) coated with 0.25μm 5% phenyl methyl silicone (HP-5). The GC was temperature programmed from 40°C to 300°C at 4°C/min. and held at peak temperature for 20 minutes with Helium as the carrier gas (flow 1ml/min., pressure of 50kPa, split at 30 mls/min.). The acquired data was stored on DVD tape for later data processing, integration and printing.

GC-MS (ion m/z 85)

The n-alkanes give more intense m/z 85 fragments than most biomarkers and the n-alkanes become more distinct from the co-eluting biomarkers. Figure 3 shows a typical oil sample n-alkane homologous series.

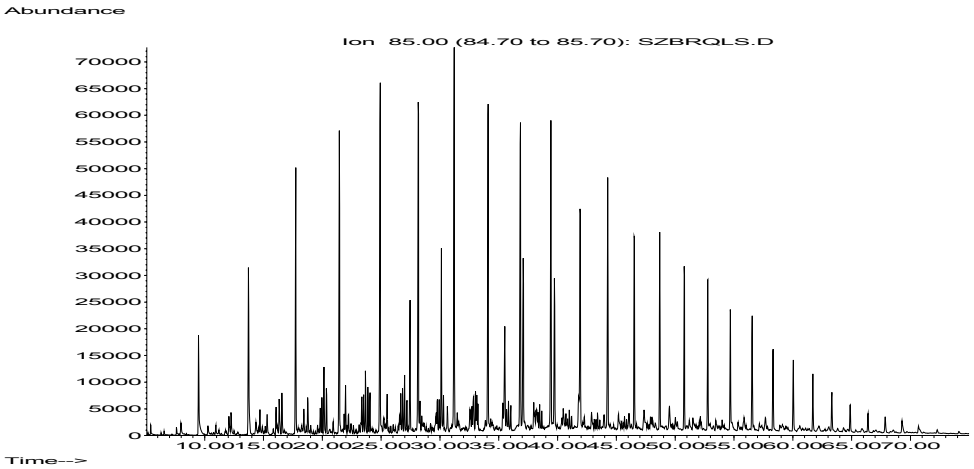

Figure 3: Mass chromatogram m/z 85 of the saturated hydrocarbon fraction from Brent Crude oil

For a bitumen sample viewed under normal magnification, the squalane peak (eluting at around 55.5 minutes) was very dominant and the abundance of the n-alkanes was very low. When the y axis scale is magnified, typical chromatograms m/z 85 of the saturated hydrocarbon fractions from various bitumen samples are shown in Figures 4 and 5. A visual comparison of the GC-MS Chromatograms of 200 pen. bitumens from the two sources show that it was difficult to make specific identification of the various samples purely based on their n-alkane distributions.

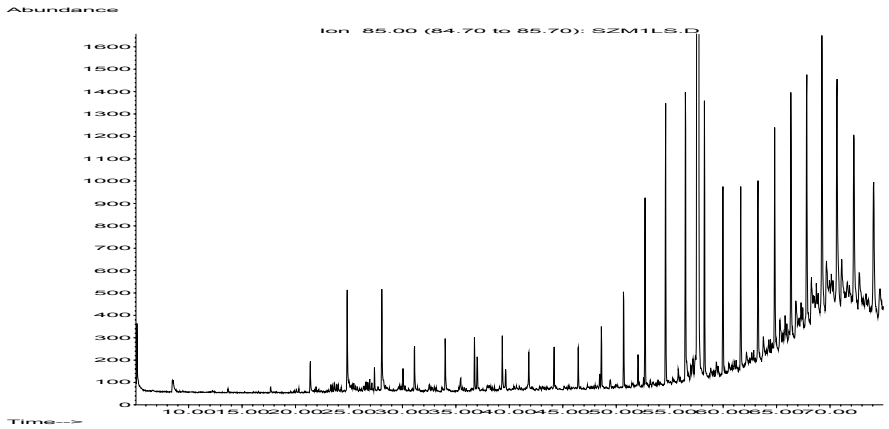

Figure 4: Mass chromatogram m/z 85 of the saturated hydrocarbon fraction from a 200 penetration grade Middle Eastern virgin bitumen sample

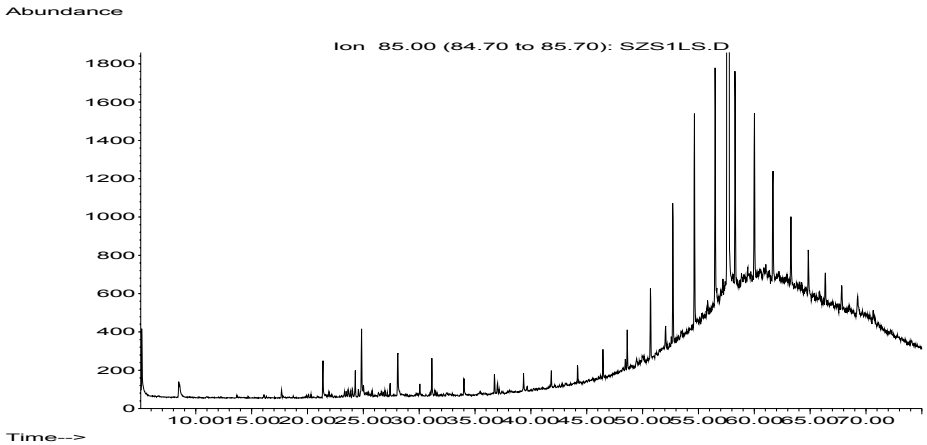

Figure 5: Mass chromatogram m/z 85 of the saturated hydrocarbon fraction from a 200 penetration grade Venezuelan virgin bitumen sample

General Distribution of n-paraffins

n-paraffins are typically the major peaks in gas chromatograms of mature oils and bitumens. The distribution of n-paraffins depends on organic source type, thermal maturity, expulsion, migration and biodegradation. Biodegradation results in depletion of n-paraffins prior to significant alteration of any other compound class, including the acyclic isoprenoids pristane and phytane. Mildly biodegraded oils will show higher pristane/nC_{17} and phytane/nC_{18} ratios than related, non-biodegraded oils. Decreased abundances of volatile paraffins (<C_{15}) can be related to low thermal maturity, migrational effects (phase separation), evaporative loss (weathering), or water washing (Peters & Moldowan, 1993).

DIAGNOSTIC RATIOS USED FOR DATA INTERPRETATION

To enable a more detailed interpretation of the GC-MS data, the following diagnostic ratios were used to analyse the GC-MS m/z 85 results.

Carbon Preference Index (CPI)

The carbon preference index (CPI) is a numerical means of representing odd-over-even predominance in n-alkanes in a particular carbon number range (Tissot & Welte, 1984). It can be used as a maturity measurement when there is an obvious odd to even preference in C_{25}-C_{33} n-alkanes resulting from higher plant waxes. Values of CPI will initially be >1.0 but will tend towards a final value of 1.0 with increasing maturity, as the n-alkanes in this carbon number range become diluted by the generation of large amounts of additional n-alkanes in the same range without any preference for odd or even carbon numbers.

In crude oils, the high molecular weight n-alkanes inherited from terrestrial plants are normally diluted by hydrocarbons from kerogen degradation, and the CPI is around 1.0 (Tissot & Welte, 1984).

$$\mathrm{CPI} = \frac{1}{2}\left[\frac{C_{25}+C_{27}+C_{29}+C_{31}+C_{33}}{C_{24}+C_{26}+C_{28}+C_{30}+C_{32}} + \frac{C_{25}+C_{27}+C_{29}+C_{31}+C_{33}}{C_{26}+C_{28}+C_{30}+C_{32}+C_{34}}\right] \quad (3)$$

additionally, the following equation was used for comparison:

$$\left(\frac{2C_{29}}{C_{28}+C_{30}}\right) \quad (4)$$

Acyclic Isoprenoids

Generally in geochemistry the term “regular isoprenoid” is restricted to an acyclic, branched, saturated molecule with a methyl group on every fourth carbon atom, irrespective of the total number of carbon atoms: such arrangement implies a “head to tail” linkage of the isoprene groups. The best known and most common isoprenoids are pristane and phytane (Tissot & Welte, 1984).

These compounds are normally associated with the ubiquitous pristane (Pr) and phytane (Ph), (2,6,10,14-tetramethylpentadecane and 2,6,10,14-tetramethylhexadecane respectively). These C_{19} and C_{20} alkanes tend to dominate the branched and cyclic alkane fractions of petroleums and sedimentary rock extracts. Pristane is thought to arise via oxidation and decarboxylation of phytol, whilst phytane could result from dehydration and reduction. Thus pristane/phytane ratios have been proposed as measures of redox potential of sediments (Mackenzie, 1984).

Some commonly accepted indications for the Pr/Ph ratio are as follows; (Pr/Ph) > 3 indicates higher terrestrial organic matter in oxic conditions, (Pr/Ph) < 0.6 indicates anoxic marine / lacustrine and often a hypersaline environment, whilst the range 0.6 < (Pr/Ph) < 2.5 is difficult to interpret without additional support (Tissot & Welte, 1984; Killops & Killops, 1993; Farrimond, 2003).

Nonetheless, there are several problems that must be kept in mind when using the Pr/Ph ratio. The first one is that the ratio increases with maturity. Secondly, a problem exists with highly branched isoprenoids co-eluting with pristane. Finally, phytol is not the only precursor of pristane and phytane.

Additional Parameters

Amongst the Norwegian Industry standard analytical procedure requirements and reporting guidelines to organic geochemical analysis (Weiss, 2000), the following ratios are also used: Pr/n-C_{17}, n-C_{15}/n-C_{20}, n-C_{30}/n-C_{20}, and n-C_{17}/(n-C_{17} + n-C_{27}). Finally, the ratio [$\Sigma(C_{12\text{-}23}) \div \Sigma(C_{24\text{-}35})$] was also introduced based on private consultations.

Results

Results from the various diagnostic ratios are shown below in Tables 1a to 1d.

Table 1a: Results from various diagnostic indices

	35pen. Middle East Virgin	50pen. Middle East Virgin	100pen. Middle East Virgin	200pen. Middle East Virgin	60/70pen. Middle East Virgin
CPI	1.03	1.01	1.09	1.07	1.22
$2C_{29}/(C_{28}+C_{30})$	0.99	0.95	0.90	0.99	0.93
Pr/Ph	1.50	1.00	1.50	1.50	0.40
Pr/C_{17}	0.21	0.18	0.25	0.63	0.42
C_{15}/C_{20}	1.09	0.96	0.89	1.00	0.59
C_{30}/C_{20}	1.36	2.70	2.89	4.15	1.26
$C_{17}/(C_{17}+C_{27})$	0.37	0.27	0.05	0.16	0.19
$\Sigma(C_{12-23})/\Sigma(C_{24-35})$	0.68	0.37	0.18	0.26	0.53

Table 1b: Results from various diagnostic indices

	35pen. Middle East Aged	50pen. Middle East Aged	100pen. Middle East Aged	200pen. Middle East Aged
CPI	1.01	1.01	1.00	0.66
$2C_{29}/(C_{28}+C_{30})$	0.94	0.99	0.96	0.24
Pr/Ph	1.67	1.67	1.67	1.00
Pr/C_{17}	0.38	0.38	0.36	0.50
C_{15}/C_{20}	0.75	1.28	0.82	1.33
C_{30}/C_{20}	3.30	4.06	4.59	41.67
$C_{17}/(C_{17}+C_{27})$	0.09	0.17	0.22	0.10
$\Sigma(C_{12-23})/\Sigma(C_{24-35})$	0.27	0.28	0.27	0.09

Table 1c: Results from various diagnostic indices

	35pen. Venezuelan Virgin	50pen. Venezuelan Virgin	100pen. Venezuelan Virgin	200pen. Venezuelan Virgin
CPI	1.04	1.05	1.03	1.05
$2C_{29}/(C_{28}+C_{30})$	0.87	1.17	1.01	0.93
Pr/Ph	1.89	1.50	2.20	2.50
Pr/C_{17}	0.65	0.33	0.79	0.63
C_{15}/C_{20}	2.10	1.50	2.50	2.00
C_{30}/C_{20}	1.50	9.40	2.80	3.88
$C_{17}/(C_{17}+C_{27})$	0.19	0.07	0.22	0.08
$\Sigma(C_{12-23})/\Sigma(C_{24-35})$	0.76	0.17	1.04	0.32

Table 1d: Results from various diagnostic indices

	35pen. Venezuelan Aged	50pen. Venezuelan Aged	100pen. Venezuelan Aged	200pen. Venezuelan Aged
CPI	1.08	1.01	1.02	1.08
$2C_{29}/(C_{28}+C_{30})$	0.91	1.03	1.11	1.09
Pr/Ph	1.50	1.13	3.00	2.50
Pr/C_{17}	0.38	2.00	0.75	0.56
C_{15}/C_{20}	0.89	0.80	1.36	0.89
C_{30}/C_{20}	2.44	11.10	2.27	4.67
$C_{17}/(C_{17}+C_{27})$	0.07	0.07	0.21	0.11
$\Sigma(C_{12-23})/\Sigma(C_{24-35})$	0.27	0.17	0.89	0.31

Analysis of GCMS m/z 85 Results

The data presented in Tables 1a-d reveals a large amount of scatter in results. In particular, the diagnostic ratios reported for the 200 pen Middle Eastern aged sample in Table 1b appear to be particularly abnormal, though the reasons for this are unclear. None of the diagnostic ratio values changed in a consistent manner, either as a consequence of systematically changing the bitumen grade or as a consequence of changing the

bitumen source. The only possible exception was the Pr/C_{17} ratio, which had on average higher values for the Venezuelan than the Middle Eastern bitumens.

On the other hand, some interesting comparisons of the diagnostic ratios obtained from the Kuwaiti bitumen sample and the rest of the Middle Eastern bitumens were observed (see Table 1a).

The first interesting observation was the CPI values. All the Middle Eastern and Venezuelan samples (virgin and aged results shown, results of recovered samples not shown) had CPI values that ranged from 1.01 to 1.09 with an average value of 1.04, whilst the Kuwaiti bitumen sample had a higher CPI value of 1.22.

The second observation was that the Pr/Ph value of all the Middle Eastern and Venezuelan samples ranged from 1.0 to 3.0 with an average value of 1.76. The Pr/Ph value of the Kuwaiti sample was much lower at 0.4.

The third observation was that the C_{15}/C_{20} value of all the Middle Eastern and Venezuelan samples ranged from 0.75 to 2.5 with an average value of 1.24. The C_{15}/C_{20} value of the Kuwaiti sample was lower at 0.59. This ratio is an indication of proportion of light, liquid n-alkanes to the heavy, waxy n-alkanes.

The last observation was that the C_{30}/C_{20} value of all the Middle Eastern and Venezuelan samples ranged from 1.36 to 11.1 with an average value of 4.19. The C_{30}/C_{20} value of the Kuwaiti sample was lower at 1.26.

In theory there should be no fundamental differences between the Kuwaiti and Middle Eastern bitumens since they all originate from a similar source. This point is proven in the work presented in subsequent sections of this report by analysis of hopane and sterane distributions and biomarker diagnostic ratios.

The only logical argument for the differences in diagnostic ratios can explained by differences in the fractional distillation conditions that the crude oil had endured during bitumen production. The Kuwaiti bitumen is known to have been produced at a Kuwaiti refinery and the rest of the Middle Eastern and Venezuelan samples are known to have been vacuum distilled at a UK based oil refinery. It would have been an ideal situation if based on the n-paraffins and isoprenoids diagnostic ratios, one is able to determine which of the two refining techniques is more severe, though further analysis is required to test this.

GC-MS ANALYSIS OF TERPANES (HOPANES) FROM THE SATURATED HYDROCARBON FRACTIONS

Following fractionation of the bitumen samples using gravity column chromatography, the saturated hydrocarbon fractions from selected samples were dissolved in DCM (1mg/100µl) and subsequently submitted for GC-MS analysis (in Full Scan mode). The resultant mass chromatograms (fragmentograms), in particular m/z 191, were difficult to interpret due to the very low peak intensities. The samples were therefore re-concentrated to 1mg/20µl by evaporating some of the DCM solvent (using a stream of nitrogen gas) and reanalysed by GC-MS in selected ion mode (SIM).

Hopane distributions were revealed primarily by m/z 191 mass chromatograms. The mass chromatograms of the bitumen samples were typically complex and the peaks not easy to identify. To assist in peak identification, a Brent crude oil sample, with a very well understood m/z 191 chromatogram hopane distribution, was analysed by GC-MS (unfractionated oil sample dissolved in DCM) in the same batch as the bitumen saturated hydrocarbon fractions. This ensured that the oil and bitumen samples were subjected to the same test conditions in the column and hence the retention times of the various eluting components (i.e. hopanes, tricyclic and tetracyclic terpanes) from all samples were almost identical. Hence knowing the retention time of any particular peak in a bitumen chromatogram, one is able to refer back to the Brent oil sample for identification of that compound.

To further assist in peak identification the following ion fragments (not shown in this paper) were used to enhance specific hopane isomers; m/z 177 was used to enhance C_{29} hopanes, m/z 205 was used to enhance the C_{31} hopanes, m/z 219 was used to enhance the C_{32} hopanes ($\alpha\beta$ elutes before $\beta\alpha$) and m/z 233 enhances C_{33} hopanes ($\alpha\beta$ elutes before $\beta\alpha$ and S elutes before R for the $\alpha\beta$). Unfortunately in each case demethylated hopanes are also enhanced, thus complicating the overall picture.

Hopanoids (Bacterial in Origin)

The preferred “biological” 17β(H),21β(H) configuration inherited by the alkanes of immature sediments is lost with increasing maturity in favour of the more stable 17α(H),21β(H) and 17β(H),21α(H) configurations, but mainly the former, see Figure 6. At higher levels of maturity, the 17β(H),21α(H) hopanes also convert to the 17α(H),21β(H) form (Farrimond, 2003).

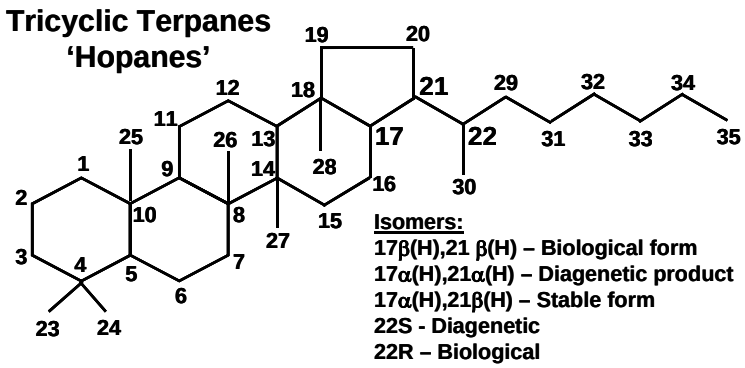

Figure 6: Illustration of the molecular structure of Tricyclic Terpanes (Hopanes)

For C_{31} and higher, a chiral centre exists at C-22. Only the 22R configuration has been seen in hopanoid natural products, and dominates in immature sediments. With increasing maturity, this preference is lost in favour of a 60:40 mixture of the S and R isomers (Peters & Moldowan, 1993).

Tricyclic and Tetracyclic Triterpenoid Derivatives

Tricyclic and tetracyclic terpanes also give an m/z 191 ion in their mass spectra, and thus can be seen in the m/z 191 mass chromatogram, although most of them elute before the hopane region. A series of tricyclic terpanes ranging from C_{19} to C_{30} is found in most oils and bitumens. All four isomers at C-13 and C-14 ($\beta\alpha$, $\alpha\alpha$, $\alpha\beta$, $\beta\beta$) are present in immature rocks with $\beta\alpha$ and $\alpha\alpha$ predominating, but with increasing maturity, the $\beta\alpha$ isomer becomes dominant (Peters & Moldowan, 1993).

For tricyclic terpanes containing 25 or more carbon atoms, C-22 is an asymmetric centre resulting in two stereoisomers or a doublet on m/z 191 mass chromatograms. For tricyclic terpanes containing 30 or more carbon atoms, C-27 is also an asymmetric centre and four stereoisomers are expected for each compound (Peters & Moldowan, 1993).

The effect of maturity on the triterpane distributions of crude oils is not so well known. Most of the isomerizations are complete before the oil generation zone. This creates problems in the interpretation of oil/source rock correlations. High amounts of non-hopanoid triterpanes can help distinguish an oil or a sedimentary rock extract (Mackenzie, 1984).

Oils and bitumens from carbonate rocks appear to show low concentrations of tricyclic terpanes above C_{26} compared to those from other depositional environments where the C_{26}-C_{30} and C_{19}-C_{25} homologs show similar concentrations. Because of their extreme resistance to biodegradation, tricyclic terpanes permit correlation of intensely biodegraded oils. They also appear resistant to thermal maturation compared to homohopanes, although the lower carbon number homologs are favoured at high thermal maturity (Peters & Moldowan, 1993).

The ratios of tetracyclic terpanes/hopanes increases in more mature source rocks and oils, indicating greater stability of the tetracyclic terpanes. Tetracyclic terpanes also appear more resistant to biodegradation than the hopanes. For these reasons, they are occasionally used in correlations of altered petroleums. The C_{24} tetracyclic terpane shows the most widespread occurrence, followed by C_{25} to C_{27} homologs. An abundant C_{24} tetracyclic terpane in petroleum appears to be a marker for carbonate and evaporite depositional environments (Peters & Moldowan, 1993).

It has also been suggested that within the triterpanes the tricyclic components migrate more easily than the pentacyclic hopanes, and so are relatively rich in highly migrated oils (Mackenzie, 1984).

Ion Chromatograms m/z 191

Mass fragmentograms or ion chromatograms of the saturated hydrocarbon fractions from a Brent Crude Oil sample, a 50 penetration grade Venezuelan virgin bitumen and a 50 penetration grade Middle Eastern virgin bitumen are shown below in Figures 7 to 9. The list of Diterpane and Triterpane (m/z 191) compounds used in Figures 7 to 9 is shown in Table 2.

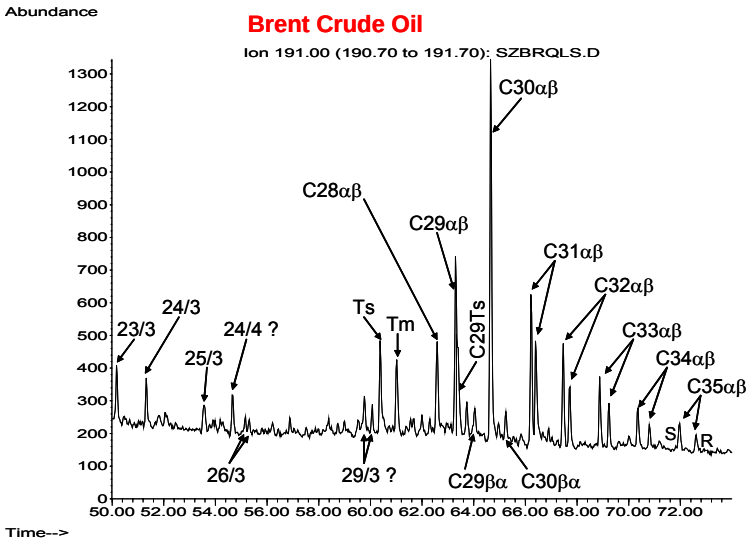

Figure 7: Mass chromatogram m/z 191 of the saturated hydrocarbon fractions from Brent Crude oil sample with annotated peaks.

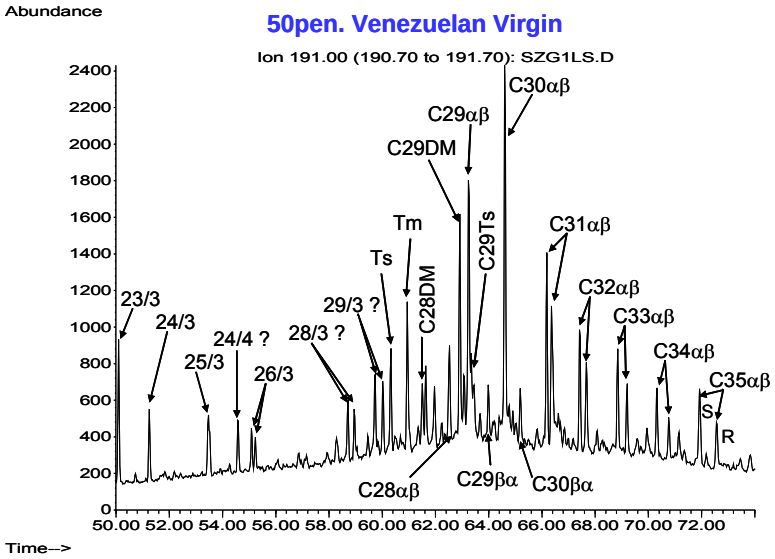

Figure 8: Mass chromatogram m/z 191 of the saturated hydrocarbon fractions from 50 penetration grade Venezuelan virgin bitumen sample with annotated peaks.

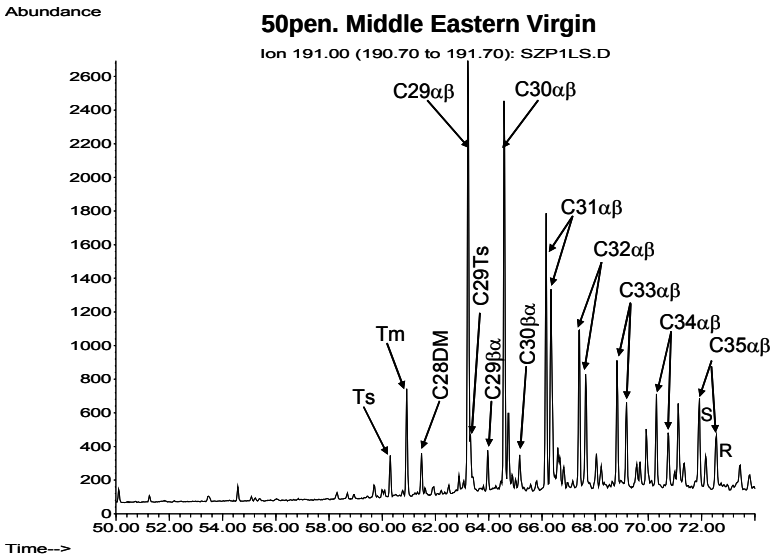

Figure 9: Mass chromatogram m/z 191 of the saturated hydrocarbon fractions from 50 penetration grade Middle Eastern virgin bitumen sample with annotated peaks.

Table 2: Compound List of Diterpanes and Triterpanes (m/z 191)

Abbreviated name	Full name
23/3	Tricyclic Terpane ($C_{23}H_{42}$)
24/3	Tricyclic Terpane ($C_{24}H_{44}$)
25/3	Tricyclic Terpane ($C_{25}H_{46}$)
24/4	Tetracyclic Terpane ($C_{24}H_{42}$)
26/3	Tricyclic Terpane ($C_{26}H_{48}$)
28/3	Tricyclic Terpane ($C_{28}H_{52}$)
29/3	C_{29} Tricyclic Terpane ($C_{29}H_{54}$)
Ts	(C_{27}) 18α(H)-22,29,30-trisnorneohopane
Tm	(C_{27}) 17α(H)-22,29,30-trisnorhopanes
C_{28}DM	C_{28} Demethylated Hopane (25-Norhopane)
$C_{28}\alpha\beta$	17α(H),21β(H)-28,30-Bisnorhopane, (C_{28} $\alpha\beta$ Demethylated Hopane)
C_{29}DM	C_{29} Demethylated Hopane (17α(H),21β(H)-25-Norhopane)
$C_{29}\alpha\beta$	C_{29} 17α(H),21β(H)-30-Norhopane
C_{29}Ts	C_{29} 18α(H)-30-Norneohopane
$C_{29}\beta\alpha$	C_{29} 17β(H),21α(H)-30-norhopane (Normoretane)
$C_{30}\alpha\beta$	C_{30} 17α(H),21β(H) Regular Hopane
$C_{30}\beta\alpha$	C_{30} 17β(H),21α(H) Hopane (Moretane)
$C_{31}\alpha\beta$	C_{31} 17α(H),21β(H) Homohopane, 22S elutes before 22R
$C_{32}\alpha\beta$	C_{32} 17α(H),21β(H) Bishomohopane, 22S elutes before 22R
$C_{33}\alpha\beta$	C_{33} 17α(H),21β(H) Trishomohopane, 22S elutes before 22R
$C_{34}\alpha\beta$	C_{34} 17α(H),21β(H) Tetrakishomohopane, 22S elutes before 22R
$C_{35}\alpha\beta$	C_{35} 17α(H),21β(H) Pentakishomohopane, 22S elutes before 22R

When comparing the general characteristics of GC-MS m/z 191 chromatograms from the saturate fractions of Middle Eastern v.s. Venezuelan bitumens, several distinctive features were noted.

The Venezuelan bitumen chromatograms in general appear to have a greater abundance of unresolved complex mixtures (humps) compared to the Middle Eastern bitumens.

Almost all the tricyclic and tetracyclic terpanes eluting before the Ts peak were absent (or of very low abundance) from the Middle Eastern bitumens. This included the following compounds: 23/3, 24/3, 25/3, 24/4, 26/3, 28/3 and 29/3. Other missing peaks present in the Venezuelan bitumens but not in the Middle Eastern samples include the $C_{28}\alpha\beta$ peaks and the relatively intense C_{29}DM peaks.

The peak intensity (height) of the $C_{29}\alpha\beta$ (17α(H),21β(H)-30-Norhopane) was consistently greater than the $C_{30}\alpha\beta$ (17α(H),21β(H) regular hopane), for the Middle Eastern bitumens only. This is a characteristic feature of Middle Eastern oils. The reverse was true for the Venezuelan bitumens. This can be verified by analysing the norhopane ratios in Tables 3a-e.

It was not possible to distinguish the Kuwaiti bitumen sample from amongst the Middle Eastern samples based purely on the general appearance of the m/z 191 chromatograms. This prompted the author to conduct a more detailed investigation of the distribution of peaks from the Middle Eastern and Kuwaiti bitumen samples (m/z 191 chromatograms) and a comparison was carried out with hopane distributions of a range of Kuwaiti oils available in the literature (Bufarsan et al., 2002). Following this analysis, there was no doubt that all the Middle Eastern and Venezuelan bitumens originate from the same Kuwaiti oil, furthermore, it was possible to identify the source as being the giant Burgan oil field South of Kuwait city.

Thermal Maturation

An assessment of the extent of thermal maturation experienced by an organic-rich sedimentary rock in the temperature range ca. 50-150°C can be determined using so-called organic molecular parameters. Thermal maturation encompasses the temperature-dependent chemical and physicochemical reactions which are experienced in the diagenesis and catagenesis zones (Mackenzie, 1984). The best examples of this approach involve the monitoring of the extent of certain reactions by measuring the relative concentrations of the reactants (A) and products (B):

$$[A] \underset{k2}{\overset{k1}{\Longleftrightarrow}} [B] \qquad (5)$$

The best measure of the reaction extent is:

$$\frac{[B]}{[A]+[B]} \qquad (6)$$

The relative concentrations of A and B are normally determined by GC-MS.

Diagnostic Indices

For Hopanes, the following diagnostic indices (ratios) of biomarkers were used in this part of the investigation:

Ts/Tm; The ratio of the more stable 18α(H)-22,29,30-trisnorneohopane (Ts) (a rearranged hopane) to the less stable 17α(H)-22,29,30-trisnorhopanes (Tm) (a normal hopane) is also used to define level of maturity. The ratio covers a wide maturity range; from 0% at immaturity to about 50% in the oil window, and tends to increase to 80-90% at late maturity. The ratio is influenced by source type (Faksness *et al*., 2002).

$$\left(\frac{C_{27}\text{Ts}(191)}{C_{27}\text{Ts}(191)+C_{27}\text{Tm}(191)}\right)\times 100 \qquad (7)$$

Norhopane; This ratio is related to both source type and thermal maturity. It is of limited value for the North Sea crudes, but useful for other oils (Faksness *et al*., 2002).

$$\left(\frac{C_{29}\alpha\beta(191)}{C_{29}\alpha\beta(191)+C_{30}\alpha\beta(191)}\right) \qquad (8)$$

C-22 in 17α(H),21β(H) hopanes; This isomerization can be followed in the C_{31}-C_{35} hopanes, as revealed by m/z 191 fragmentograms for the GC-MS analysis of a separated alkane fraction. The 22S isomer elutes before the 22R isomer in each case. Either an average of the isomer ratios for all five carbon numbers can be measured or one carbon number consistently chosen. The results for C_{31} are prone to error, because often the two isomers are not resolved to baseline, and gammacerane, another pentacyclic triterpane, coelutes with the 22R isomer under certain conditions. The C_{32} hopane (Bishomohopane) is probably the best to use for making the measurement (Mackenzie, 1984; Farrimond, 2003).

$$\frac{22S}{(22R+22S)} \quad \text{for } 17\alpha\text{(H) hopanes.} \qquad (9)$$

Typical values varies from 0 to 60% with increasing maturity. Samples showing 22S/(22S+22R) ratios in the range 0.5 to 0.54 have barely entered oil generation, while ratios in the range 0.57 to 0.62 indicate that the main phase of oil generation has been reached or surpassed. Some oils exposed to very light thermal stress show 22S/(22S+22R) ratios as low as about 0.55 (Peters & Moldowan, 1993).

$\beta\alpha$-Moretanes/$\alpha\beta$-hopanes; The ratio of 17β(H),21α(H)-moretanes to their corresponding 17α(H),21β(H)-hopanes decreases with increasing thermal maturity from about 0.8 in immature bitumens to values of less than 0.15 in mature source rocks and oils to a minimum of 0.05. Oils from Tertiary source rocks show higher moretane/hopane ratios (0.1-0.3 with many values between 0.15 and 0.2) than those from older rocks (generally 0.1 or less). The C_{30} compounds and the C_{29} compounds are most often used for moretane/hopane ratio (Peters & Moldowan, 1993).

C-17 and C-21 in hopanes ≥ C_{29}; The stability of hopanes increases in the order 17β(H),21β(H) < 17β(H),21α(H) < 17α(H),21β(H). 17β(H),21β(H) is usually the dominant stereochemistry of very immature sediments and it disappears fairly rapidly with increasing maturity. Thus the following expression can be used in the early stages of oil generation (Mackenzie, 1984):

$$\frac{[17\alpha(H),21\beta(H)]}{[17\beta(H),21\alpha(H)]+[17\alpha(H),21\beta(H)]} \qquad (10)$$

The values of Equation 10 rise from around 50% up to approximately (90-100%). Use of the previous equation is best confined to the C_{30} hopanes (i.e. C_{30} homologue), because it tends to be the largest peak area and therefore produces the lowest errors.

Homohopane Index; Records preferential preservation of intact C_{35} hopanoid skeleton in marine anoxic sediments. Homohopane Index records preferential preservation of intact C_{35} hopanoid skeleton in marine anoxic sediments and therefore the index is generally higher in reducing sulphidic conditions. C_{35}>C_{34} can be an indicator of anoxia. Furthermore, Homohopane Index falls with maturity due to generation of shorter hopanes from kerogen. The Homohopane Index is often higher in carbonate environments and evaporates (Farrimond, 2003).

$$\frac{C_{35}\alpha\beta(S+R)}{\sum C_{31-35}\alpha\beta(S+R)}\% \qquad (11)$$

C_{35}/C_{34} homohopane; In oils generated from normal marine sediments the homohopanes typically decrease in abundance from C_{31} to C_{35}. As mentioned in the previous section, high C_{35}/C_{34} homohopane ratios (>50%) have been interpreted as a general indicator of highly reducing marine conditions during deposition of the source rock, but are also influenced by thermal maturity, the most mature samples showing the lowest ratios (Faksness *et al*., 2002).

$$\left(\frac{C_{35}\alpha\beta(S+R)}{C_{34}\alpha\beta(S+R)+C_{35}\alpha\beta(S+R)}\right) \quad (12)$$

Tricyclic v.s. pentacyclic terpanes; Ratios between the abundances of tricyclic and pentacyclic terpanes are useful correlation ratios. Comparability between laboratories is, however, poor due to discrimination problems (wide retention time interval). The ratio generally tends to increase at high maturities, but is strongly influenced by organic matter type and/or depositional environment of the source rock. This ratio requires good control of peak height (or area) discrimination, as a wide retention time interval is involved (Faksness *et al*., 2002).

$$\left(\frac{[24/3(191)]}{[24/3(191)]+[C_{30}\alpha\beta(191)]}\right)\times 100 \quad (13)$$

The ratio of tricyclics/17α(H)-hopanes is primarily a source parameter that compares a group of bacterial or algal lipids (tricyclics) with markers that arise from different prokaryotic species (hopanes). Peters & Moldowan (1993) recommended measurement of the sum of the four tricyclic terpane peaks, 22R and 22S doublets, representing the C_{28} and C_{29} pseudo-homologs of tricyclohexaprenane (29,30-bisnortricyclohexaprenane and 30-nortricyclohexaprenane, respectively), for the numerator of the ratio. The sum of the C_{29}-C_{33} 17α(H)-hopanes comprises the denominator of the ratio. The tricyclics/17α(H)-hopanes ratio increases systematically for related oils of increasing thermal maturity. The ratio increases because proportionally more tricyclic terpanes than hopanes are released from kerogen at higher levels of maturity.

(Tricyclics) ÷ (17α(H)-hopanes) (14)

Tetracyclic terpanes; As mentioned earlier, the ratios of tetracyclic terpanes/hopanes increases in more mature source rocks and oils, indicating greater stability of the tetracyclic terpanes and tetracyclic terpanes also appear more resistant to biodegradation than the hopanes. The following ratio was proposed in this investigation to test this behaviour:

(24/4)/(30$\alpha\beta$) in m/z 191 (15)

Additional ratios; Amongst the Norwegian Industry standard analytical procedure requirements and reporting guidelines to organic geochemical analysis (Weiss, 2000), the following ratios are also used: [(23/3)/30$\alpha\beta$] in m/z 191 and [35$\alpha\beta$R/30$\alpha\beta$] in m/z 191.

Results

Tables 3a to 3e list the values obtained from the various diagnostic ratios used in this investigation.

Table 3a: Results from various diagnostic indices

	Brent Crude	35 pen. Mid. East Virgin	50 pen. Mid. East Virgin	100 pen. Mid. East Virgin	200 pen. Mid. East Virgin
Ts/Tm	53.93	23.12	26.76	26.12	27.27
$C_{29}\alpha\beta/(C_{29}\alpha\beta+C_{30}\alpha\beta)$	34.61	51.34	51.85	50.98	51.04
C_{31} 22S/(S+R) 17α(H)	56.66	48.78	50.17	49.38	53.18
C_{32} 22S/(S+R) 17α(H)	58.80	56.85	58.01	60.6	56.84
C_{30} $\beta\alpha/\alpha\beta$	0.08	0.09	0.10	0.08	0.08
$C_{30}\alpha\beta/(\alpha\beta+\beta\alpha)$	93.00	92.15	91.25	92.42	92.89
Homohopane Index	8.08	14.52	13.40	13.34	14.31
$C_{35}/(C_{35}+C_{34})$ $\alpha\beta$(S+R)	43.38	54.31	54.18	54.59	54.72
[24/3]/([24/3]+[$C_{30}\alpha\beta$])	12.60	N.A.	N.A.	N.A.	N.A.
Tricyclics/17α(H)hopanes	N.A.	N.A.	N.A.	N.A.	N.A.
[24/4]/$C_{30}\alpha\beta$	0.13	N.A.	N.A.	N.A.	N.A.
[23/3]/$C_{30}\alpha\beta$	0.17	N.A.	N.A.	N.A.	N.A.
$C_{35}\alpha\beta R/C_{30}\alpha\beta$	0.06	0.23	0.19	0.19	0.22

Table 3b: Results from various diagnostic indices

	60/70 pen. Kuwait	35 pen. Ven. Virgin	50 pen. Ven. Virgin	100 pen. Ven. Virgin	200 pen. Ven. Virgin
Ts/Tm	26.04	38.36	38.62	38.94	36.41
$C_{29}\alpha\beta/(C_{29}\alpha\beta+C_{30}\alpha\beta)$	55.45	49.73	35.46	46.95	46.84
C_{31} 22S/(S+R) 17α(H)	50.67	42.92	49.71	44.97	47.22
C_{32} 22S/(S+R) 17α(H)	56.31	52.58	54.01	52.38	56.47
C_{30} $\beta\alpha/\alpha\beta$	0.07	0.19	0.14	0.20	0.16
$C_{30}\alpha\beta/(\alpha\beta+\beta\alpha)$	93.35	84.09	87.38	83.10	86.08
Homohopane Index	12.80	16.07	15.61	14.59	13.37
$C_{35}/(C_{35}+C_{34})$ $\alpha\beta$(S+R)	54.24	56.95	59.89	60.90	57.71
[24/3]/([24/3]+[$C_{30}\alpha\beta$])	N.A.	12.21	13.42	21.39	33.59
Tricyclics/17α(H)hopanes	N.A.	0.17	0.20	0.35	0.41
[24/4]/$C_{30}\alpha\beta$	0.09	0.16	0.13	0.28	0.34
[23/3]/$C_{30}\alpha\beta$	N.A.	0.23	0.28	0.51	0.99
$C_{35}\alpha\beta R/C_{30}\alpha\beta$	0.16	0.24	0.16	0.17	0.14

Table 3c: Results from various diagnostic indices

	35 pen. Mid. East Aged	50 pen. Mid. East Aged	100 pen. Mid. East Aged	200 pen. Mid. East Aged
Ts/Tm	23.02	26.90	26.03	27.13
$C_{29}\alpha\beta/(C_{29}\alpha\beta+C_{30}\alpha\beta)$	51.83	51.66	52.47	50.06
C_{31} 22S/(S+R) 17α(H)	49.46	51.51	49.54	49.69
C_{32} 22S/(S+R) 17α(H)	56.62	57.52	55.89	57.00
C_{30} $\beta\alpha/\alpha\beta$	0.08	0.09	0.12	0.08
$C_{30}\alpha\beta/(\alpha\beta+\beta\alpha)$	92.37	91.53	89.47	92.42
Homohopane Index	14.15	12.97	13.40	14.40
$C_{35}/(C_{35}+C_{34})$ $\alpha\beta$(S+R)	53.27	52.09	53.09	54.54
[24/3]/([24/3]+[$C_{30}\alpha\beta$])	N.A.	N.A.	N.A.	N.A.
Tricyclics/17α(H)hopanes	N.A.	N.A.	N.A.	N.A.
[24/4]/$C_{30}\alpha\beta$	N.A.	N.A.	N.A.	N.A.
[23/3]/$C_{30}\alpha\beta$	N.A.	N.A.	N.A.	N.A.
$C_{35}\alpha\beta R/C_{30}\alpha\beta$	0.23	0.17	0.20	0.24

Table 3d: Results from various diagnostic indices

	35 pen. Ven. Aged	50 pen. Ven. Aged	100 pen. Ven. Aged	200 pen. Ven. Aged
Ts/Tm	36.69	38.14	35.63	36.74
$C_{29}\alpha\beta/(C_{29}\alpha\beta+C_{30}\alpha\beta)$	47.64	37.83	39.05	46.88
C_{31} 22S/(S+R) 17α(H)	43.77	48.17	45.35	46.65
C_{32} 22S/(S+R) 17α(H)	56.04	58.17	53.88	56.03
C_{30} $\beta\alpha/\alpha\beta$	0.18	0.14	0.13	0.14
$C_{30}\alpha\beta/(\alpha\beta+\beta\alpha)$	84.57	87.75	88.37	87.82
Homohopane Index	16.06	12.97	15.16	15.12
$C_{35}/(C_{35}+C_{34})$ $\alpha\beta$(S+R)	55.58	55.73	57.70	57.65
[24/3]/([24/3]+[$C_{30}\alpha\beta$])	10.06	12.42	19.13	26.69
Tricyclics/17α(H)hopanes	0.16	0.20	0.36	0.39
[24/4]/$C_{30}\alpha\beta$	0.16	0.13	0.26	0.27
[23/3]/$C_{30}\alpha\beta$	0.22	0.26	0.43	0.74
$C_{35}\alpha\beta R/C_{30}\alpha\beta$	0.24	0.12	0.17	0.14

Table 3e: Results from various diagnostic indices

	35 pen. Mid. East Recovered	50 pen. Mid. East Recovered	35 pen. Ven. Recovered	50 pen. Ven. Recovered
Ts/Tm	23.01	28.11	35.06	39.65
$C_{29}\alpha\beta/(C_{29}\alpha\beta+C_{30}\alpha\beta)$	53.55	51.45	47.72	46.61
C_{31} 22S/(S+R) 17α(H)	48.81	49.52	44.30	46.89
C_{32} 22S/(S+R) 17α(H)	57.06	57.57	53.23	52.50
C_{30} $\beta\alpha/\alpha\beta$	0.08	0.09	0.27	0.14
$C_{30}\alpha\beta/(\alpha\beta+\beta\alpha)$	92.21	91.80	78.73	87.49
Homohopane Index	14.09	12.22	15.96	12.88
$C_{35}/(C_{35}+C_{34})$ $\alpha\beta$(S+R)	56.05	50.83	55.75	53.61
[24/3]/([24/3]+[$C_{30}\alpha\beta$])	N.A.	N.A.	7.04	12.80
Tricyclics/17α(H)hopanes	N.A.	N.A.	0.15	0.19
[24/4]/$C_{30}\alpha\beta$	N.A.	N.A.	0.11	0.15
[23/3]/$C_{30}\alpha\beta$	N.A.	N.A.	0.14	0.27
$C_{35}\alpha\beta R/C_{30}\alpha\beta$	0.20	0.16	0.22	0.15

General Comments on Results of Diagnostic Ratios

The ratio Ts/Tm of all the Venezuelan bitumens were at least 10 units higher than the Middle Eastern bitumens. Since the numerical values of the ratios shown in Tables 3a-e are all below what is expected from materials in the oil window or late maturity, it was not possible to speculate about the thermal maturity of the samples.

The ratio (22S)/(22R+22S) for the 17α(H) C_{32} hopanes was always lower for the Venezuelan bitumens compared to the Middle Eastern samples. As discussed earlier, some oils exposed to very light thermal stress show 22S/(22S+22R) ratios as low as about 0.55. It is possible that the petroleum from which the Venezuelan bitumens were produced were exposed to very light thermal stress.

As discussed earlier, the ratio of C_{30} 17β(H),21α(H)-moretanes to their corresponding 17α(H),21β(H)-hopanes decreases with increasing thermal maturity. Oils from Tertiary source rocks show higher moretane/hopane ratios (0.1-0.3 with many values between 0.15 and 0.2) than those from older rocks (generally 0.1 or less). Since all the Middle Eastern bitumen samples had moretane/hopane ratios lower than the Venezuelan samples, one can assume that the Middle Eastern oil originates from older rocks. The ratio [$C_{30}\alpha\beta/(\alpha\beta+\beta\alpha)$] being consistently higher in the Middle Eastern samples also confirms that the Middle Eastern oil originates from more mature sediments.

Tables 3a-e show that the Homohopane Indices of Middle Eastern bitumens were predominantly lower than the Venezuelan samples. As discussed earlier, the Homohopane Index falls with maturity due to generation of shorter hopanes from kerogen. This indicates that the Middle Eastern bitumens are likely to originate from more mature sources.

In earlier sections, it was also argued that the Homohopane Index is often higher in reducing sulphidic conditions, carbonate environments and evaporates, and that abundant C_{24} tetracyclic terpane (Compare Figures 8 & 9 for the presence of peak 24/4) in petroleum appears to be a marker for carbonate and evaporite depositional environment. These two observations support the hypothesis that the Venezuelan bitumens originate from carbonate-rich and/or evaporite depositional environments.

Comparison Based on Bitumen Grade

It would have been useful if for any one bitumen source (e.g. Middle Eastern), changes in the values of the diagnostic ratios could be related to changes in the bitumen grades (i.e. penetrations), but unfortunately finding such a trend was not possible.

By observing the variations in the diagnostic ratios across the rows in Tables 3a and 3c, it became clear that for the Middle Eastern bitumens, none of the ratios did change in a consistent manner across any of the rows as the bitumens became softer.

On the other hand, for the Venezuelan bitumens in Tables 3b and 3d, there was consistent increase in the ratios of the tricyclic v.s. pentacyclic terpanes as the bitumens became softer, more specifically, the following three ratios: [24/3]/([24/3]+[$C_{30}\alpha\beta$]), Tricyclics/17α(H)hopanes, and [23/3]/$C_{30}\alpha\beta$.

In Table 3b, there was also the possibility of the Homohopane Index showing a trend of decreasing values as the Venezuelan bitumens grades became softer. Unfortunately, this trend was not duplicated for the aged samples in Table 3d.

Comparison Based on Short Term Bitumen Ageing

The results were also examined for consistent changes in diagnostic ratios between bitumens in the virgin state (Tables 3a,b) and bitumens that had been subjected to short term oven ageing (Tables 3c,d) as described in Part 1 of the paper. A particular ratio was considered a reliable indicator of ageing only when the same direction of change (increase or decrease in value) occurred across all the bitumen grades. For example, for the Venezuelan bitumens, if a diagnostic ratio increased with ageing for the 35, 40/60 and 70/100 penetration grades but decreased for the 160/220 penetration grade, the ratio was considered unreliable.

For the Middle Eastern bitumen the C_{35}/C_{34} homohopane ratio, i.e. $C_{35}/(C_{35}+C_{34})$ $\alpha\beta$(S+R), was the only possible diagnostic ratio that showed a consistent decrease in value for all grades as a result of oven ageing.

On the other hand, for the Venezuelan bitumen, in addition to the C_{35}/C_{34} homohopane ratio which also showed a reduction in value across all grades with short term oven ageing, the $C_{30}\alpha\beta/(\alpha\beta+\beta\alpha)$ values also showed consistent change (increase) with short term ageing.

More importantly for the Venezuelan bitumens, the ratios of [24/3]/([24/3]+[$C_{30}\alpha\beta$]) and [23/3]/$C_{30}\alpha\beta$ also demonstrated a trend of decrease in value with short term oven ageing. These ratios are more important because they also displayed a trend with change in bitumen grade as explained in the previous section.

Comparison Based on Individual Bitumen Types

Mixing bitumen with rubber at elevated temperatures is known to remove some of the lighter fractions from the bitumen by absorption into the rubber thus causing an increase in the viscosity of the recovered bitumen beyond what is normally expected following short term oven ageing (Rahman, 2004)

By ignoring consistent trends in diagnostic ratios across all the bitumen grades, and concentrating instead on one bitumen grade/type at a time as the bitumen is converted from the virgin state to short term oven aged and finally to a recovered condition, it is possible to identify a few possibly useful ageing diagnostic ratios.

When analysing the Middle Eastern bitumens, it was noticeable that the Homohopane Index values decreased as the bitumen was short term oven aged and decreased even further as the bitumens were additionally hardened by rubber interaction. This applied to both the 35 and 50 penetration grade Middle Eastern bitumens. There was also an indication that the $C_{35}\alpha\beta R/C_{30}\alpha\beta$ diagnostic ratio might display similar properties with ageing.

In the case of the Venezuelan bitumens, the Homohopane Index values did also decrease with increasing severity of laboratory induced ageing. This was evident for both the 35 and 50 penetration grades. Furthermore, there was an indication that the Tricyclics/17α(H)hopanes diagnostic ratio also decreased as a result of increased laboratory ageing of Venezuelan bitumens.

GC-MS ANALYSIS OF STERANES FROM THE SATURATED HYDROCARBON FRACTIONS

Sterane Identification

In addition to analysing the hopane distributions in all bitumen samples, the m/z 217 mass chromatograms were also analysed to identify steranes & diasteranes (Farrimond, 2003). An illustration of the molecular structure of a regular sterane is shown in Figure 10. Even more so than hopanes, the m/z 217 chromatograms of the bitumen samples were complex and the peaks not easy to identify. To assist in peak identification, a Brent Crude oil sample, with a well documented m/z 217 chromatogram sterane distribution, was submitted for GC-MS analysis at the same time as the saturated hydrocarbon fractions of the bitumen. This ensured that the oil and bitumen samples were subjected to the same test conditions in the column and hence the retention times of the various eluting components from all samples were almost identical. As for the hopanes, knowing the retention time of any particular peak in a bitumen chromatogram, one is able to refer back to the Brent oil sample sterane distribution for identification of the compound.

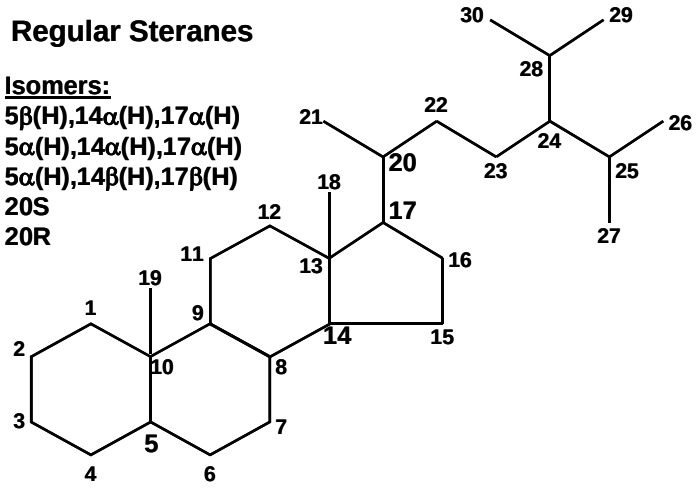

Figure 10: Illustration of the molecular structure of a regular sterane

Figures 11 and 12 compare the sterane distributions of a 50 penetration grade Venezuelan virgin bitumen and a 50 penetration grade Middle Eastern bitumen. Table 4 lists all the peaks identified in numerical order.

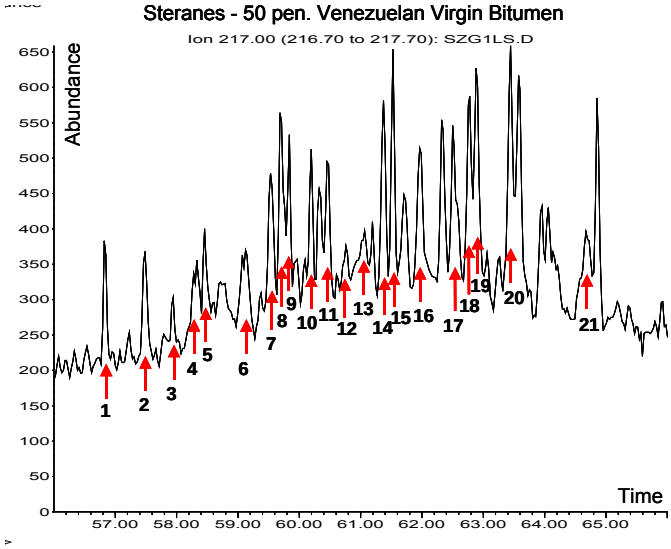

Figure 11: Mass chromatogram m/z 217 of the saturated hydrocarbon fraction from a 50 pen. grade Venezuelan virgin bitumen sample with the identifiable peaks marked.

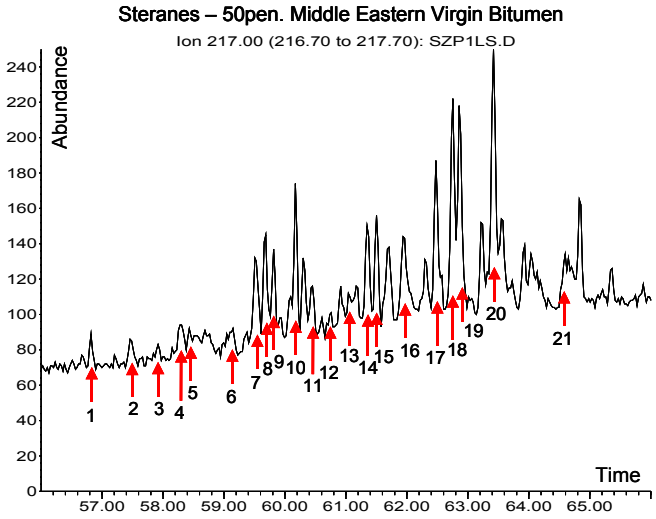

Figure 12: Mass chromatogram m/z 217 of the saturated hydrocarbon fraction from a 50 pen. grade Middle Eastern virgin bitumen sample with the identifiable peaks marked

Comparing the sterane distributions of the Venezuelan and Middle Eastern bitumens (Figures 11 v.s. 12), some very striking differences can be observed. The first obvious difference in the overall appearance of the chromatograms is the low intensity of the m/z 217 peaks in the Middle Eastern bitumen samples (typically less than half the intensities of the Venezuelan samples. The second main difference is the lack of diasterane peaks (or very low intensities) in the Middle Eastern samples, in particular the following early eluting compounds; 27dS, 27dbR, 27daS, 27daR, 28dbS and 28dbR. These peaks were very distinct and relatively easy to identify in the Venezuelan samples.

Table 4: Compound list of steranes and diasteranes (m/z 217)

Peak no.	Abbreviated name	Full name
1	27dbS	13β(H),17α(H),20(S)-cholestane (diasterane)
2	27dbR	13β(H),17α(H),20(R)-cholestane (diasterane)
3	27daS	13α(H),17β(H),20(S)-cholestane (diasterane)
4	27daR	13α(H),17β(H),20(R)-cholestane (diasterane)
5 & 6	28dbS	24-methyl-13β(H),17α(H),20(S)-cholestane (diasterane)
7	28dbR	24-methyl-13β(H),17α(H),20(R)-cholestane (diasterane)
8	27aaS	5α(H),14α(H),17α(H),20(S)-cholestane
9	27bbR + 29dbS	5α(H),14β(H),17β(H),20(R)-cholestane 24-ethyl-13β(H),17α(H),20(S)-cholestane (diasterane)
10	27bbS	5α(H),14β(H),17β(H),20(S)-cholestane
11	27aaR	5α(H),14α(H),17α(H),20(R)-cholestane
12	29dbR	24-ethyl-13β(H),17α(H),20(R)-cholestane (diasterane)
13	29daS	24-ethyl-13α(H),17β(H),20(S)-cholestane (diasterane)
14	28aaS	24-methyl-5α(H),14α(H),17α(H),20(S)-cholestane
15	28bbR	24-methyl-5α(H),14β(H),17β(H),20(R)-cholestane
16	28bbS	24-methyl-5α(H),14β(H),17β(H),20(S)-cholestane
17	28aaR	24-methyl-5α(H),14α(H),17α(H),20(R)-cholestane
18	29aaS	24-ethyl-5α(H),14α(H),17α(H),20(S)-cholestane
19	29bbR	24-ethyl-5α(H),14β(H),17β(H),20(R)-cholestane
20	29bbS	24-ethyl-5α(H),14β(H),17β(H),20(S)-cholestane
21	29aaR	24-ethyl-5α(H),14α(H),17α(H),20(R)-cholestane
22	30aaR	24-propyl-5α(H),14α(H),17α(H),20(R)-cholestane

GENERAL CRITERIA USED FOR STERANES

Early immature sediments are normally dominated by the biological configurations, i.e. the 5β(H),14α(H),17α(H),20R for each carbon number, plus the 5α(H),14α(H),17α(H),20R steranes. The latter are normally two to three times more abundant than the former. Absence of diasteranes is also expected. Increasing maturity sees the appearance of the 20S isomers and the 5α(H),14β(H),17β(H) species. For late mature samples; one would expect to see Diasteranes C_{29}-dia. One should also start to see ααα20S rising in relative amounts to the total distribution (Farrimond, 2003).

Comparison of Diagnostic Indices Based on Bitumen Source

Diasteranes/Regular Steranes; The abundance of diasteranes relative to regular steranes is mainly a source type indicator, but influenced by thermal maturity (increases with increasing maturity). The ratio is typically high in oils derived from marine siliciclastic (i.e. shale-type / containing abundant clays) sources. However, high diasteranes/steranes ratios have also been observed in bitumens from organic-lean carbonate rocks, probably deposited in an acidic (low pH), oxic (high Eh) environment. High diasteranes/steranes ratios in some petroleums appear to result from high thermal maturity and/or heavy biodegradation (Peters & Moldowan, 1993).

On the other hand, the ratio is low in oils from marine carbonate (anoxic, clay-poor) source rocks. During diagenesis of these carbonate sediments, bicarbonate and ammonium ions are provided by bacterial activity resulting in increased water alkalinity. Under these conditions of low Eh and high pH, calcite tends to precipitate and organic matter preservation is improved (Peters & Moldowan, 1993).

Heavy biodegradation can result in selective destruction of steranes relative to diasteranes. This interpretation can be supported by other evidence of heavy biodegradation, including depletion of n-paraffins and isoprenoids, or the presence of 25-norhopanes. However, it is possible that a non-biodegraded oil might mix with a heavily biodegraded oil showing a much higher diasteranes/steranes ratio. In such cases, only careful quantitative assessment of each biodegradation-sensitive parameter can lead to a correct interpretation (Peters & Moldowan, 1993).

$$\left(\frac{27\text{dbS}(217)+27\text{dbR}(217)}{27\text{dbS}(217)+27\text{dbR}(217)+27\text{bbR}(217)+27\text{bbS}(217)}\right)\times 100 \qquad (16)$$

In this investigation, when the magnitude of the diagnostic index values of any particular Venezuelan bitumen grade were compared to a Middle Eastern bitumen of the same grade (both virgin and oven aged), the following trends were observed:

The values of the diasteranes/steranes ratios were consistently higher in the Venezuelan samples. Taking an average value of the diasteranes/steranes ratios across the virgin, aged and recovered samples, the average value calculated for the Venezuelan bitumens was 39.35 versus an average value of 23.11 for the Middle Eastern bitumens.

The information in the previous few paragraphs shows that interpreting the diasteranes/steranes ratio is definitely not as straight forward as some of the other ratios described earlier, especially when making a selection between a siliciclastic or carbonate source rocks. But since there is plenty of evidence in the previous sections from diagnostic ratios of m/z 191 tricyclic terpanes that the Middle Eastern samples originate from a more mature source, the higher diasterane/sterane ratios obtained in this section for the Venezuelan samples cannot therefore be attributed to increased thermal maturity. Nonetheless, a combination of the presence of 25-norhopanes in the Venezuelan bitumen (Figure 8), the higher average Pr/C17 ratio for the Venezuelan bitumens (Table 1a-d), and a higher diasteranes/steranes ratio all seem to indicate either a degree of biodegradation or at least some mixing with heavily biodegraded oils (Head *et al.*, 2003)

Sterane Ternary Plots; It has been suggested that the distribution of the C_{27}-, C_{28}-, and C_{29}- homologous sterols in sediments when plotted on a ternary diagram (Figure 13) can be used to differentiate ecosystems. Sterane ternary plots can be used to identify algal v.s. land plant input. One complication is that C_{29} sterols are also produced by some algae (Farrimond, 2003). Similarly, the relative abundance of C_{27}-, C_{28}-, and C_{29}- sterane homologs in oils reflect the carbon number distribution of the sterols in the organic matter of the source rocks for these oils (Peters & Moldowan, 1993).

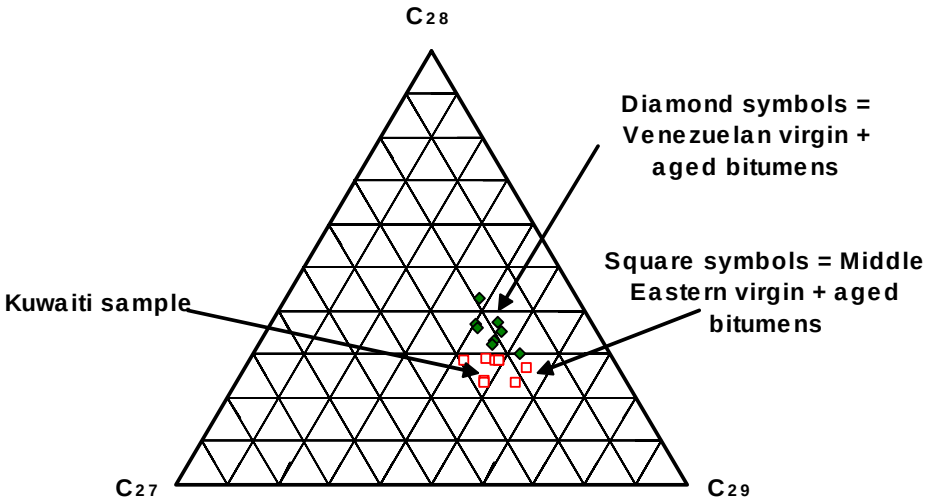

Figure 13: Ternary diagram showing the relative abundances of C_{27}-C_{28}-C_{29} regular steranes (ααα20R isomers) in the saturate hydrocarbon fractions of bitumens determined by GC-MS (m/z 217).

The ternary plot shows two very clear clusters of data points, one for the Venezuelan the other for the Middle Eastern samples. In terms of source, the ternary plot only confirms that both bitumen types are marine.

C_{28}/C_{29} steranes: A general increase in the relative content of C_{28} steranes and a decrease in C_{29} steranes in marine petroleum is expected through geologic time. The increase in the C_{28} steranes may be related to increased diversification of phytoplankton assemblages including diatoms, coccolithophores and dinoflagellates in the Jurassic and Cretaceous (Peters & Moldowan, 1993).

C_{28} and C_{29} steranes are age diagnostic markers, and in oil samples a ratio of $C_{28}/C_{29} \geq 0.7$ indicates an Upper Jurassic and younger oils, (Farrimond, 2003; Peters & Moldowan, 1993). In this investigation, to calculate the ratio of C_{28} to C_{29} steranes, it was decided to sum the ααα**S**, ααα**R**, αββ**S** and αββ**R** isomers for each sterane.

The values of the [C_{28}/C_{29}] ratios were much higher in the Venezuelan bitumens. Even though the results (not shown in this paper) show some scatter, taking an average value of the [C_{28}/C_{29}] ratio across all the Middle Eastern samples a value of 0.44 is obtained, whilst for the Venezuelan bitumens, the average value is 0.70. Therefore, the Middle Eastern bitumens most likely originate from an oil older than 144 Ma and the Venezuelan bitumen samples originate from an oil younger than 144 Ma.

When comparing the Kuwaiti bitumen sample to other Middle Eastern bitumen samples, it was not possible to distinguish any specific features that would allow one to single out the Kuwaiti sample. The similarities applied to the general appearance of the m/z 217 chromatograms (not shown in this report), and to the values of the diagnostic indices.

Comparison of diagnostic indices based on grade of bitumen

Compared with the results reported in the section describing hopane distributions, there was less overall confidence in the accuracy of the values of diagnostic ratios. This was caused on the one hand by the difficulties encountered during peak identification and on the other hand by the low peak intensities of many sterane compounds, in particular the diasteranes.

Hence it was decided to apply stricter rules when analysing trends in sterane and diasterane diagnostic index values with change in bitumen penetration (viscosity). Only the ratios which showed consistent change (consistent rise or fall in value) as the penetration values of the bitumens increased from 35 pen to 160/220 pen for both the virgin and the aged bitumens were allowed to be reported as useful diagnostic indicators.

Using such a strict criteria, it was found that the only possibly useful diagnostic indicator were the C-14 and C-17 chiral centres in the C_{29} steranes, which were analysed as follows:

$$\frac{[\alpha\beta\beta(20R)]}{[\alpha\beta\beta(20R)]+[\alpha\alpha\alpha(20R)]} \qquad (17)$$

typical values range from anything between (0% to 50%) to as high as 80%. Caution must be exercised when the value of equation 17 is less than 50%, which was the case for all the bitumen samples analysed in this investigation and hence using this parameter specifically as a maturity indicator is not advisable. (Farrimond, 2003; Peters & Moldowan, 1993).

The values of this ratio were found to decrease gradually as the penetration value of the Middle Eastern bitumens increased. This was applicable to the virgin, oven aged and recovered bitumens.

Surprisingly, the values of the same ratio were found to increase as the penetration values of the Venezuelan virgin and recovered bitumens increased, although some scatter of results was observed for the oven aged samples.

GC-MS ANALYSIS OF AROMATIC HYDROCARBON FRACTIONS

Following fractionation of the bitumen samples using gravity column chromatography (silica adsorbent), the aromatic hydrocarbon elutant fractions from representative samples were submitted for GC-MS analysis (in Full Scan). The mass chromatograms of a large number of characteristic fragment ions were then investigated (Lee *et al*., 1981). Unfortunately, none of the peaks in the reconstructed ion chromatograms were of adequate intensity to allow analysis or correlations to be formed from the results with any confidence. GC-MS analysis was repeated in SIM (selected ion mode) and the improvement in peak intensities was minimal. Such an outcome was unexpected and it was therefore decided to proceed with pyrolysis-GC-MS.

The molecular mass of a given polycyclic aromatic hydrocarbon (PAH) peak can generally be determined by GC-MS. The potential for this method as an identification tool is however limited, because it does not normally allow for a differentiation between isomeric PAH (Radke, 1987). The exact details of the pyrolysis test conditions adopted in this investigation are beyond the scope of this paper. Nonetheless, it is worth reporting that the following PAH compounds and their alkylated derivatives do appear promising and require further investigations; naphthalene, fluorene, phenanthrene, dibenzothiophene, biphenyl and methylphenanthrene.

CONCLUSIONS

Bitumen Types Used in This Investigation

The same bitumen types and grades as used in part 1 of the investigation (i.e. 35, 40/60, 70/100 and 160/220pen. Venezuelan and Middle Eastern bitumens, plus a Kuwaiti sample) were also used in Part 2. A Brent Crude Oil sample was also submitted for analysis to assist in subsequent data interpretation. As in part 1, the bitumens were also tested in their virgin, short term oven aged and recovered from rubber/bitumen mixes states.

All bitumen samples were initially fractionated into their aliphatic and aromatic hydrocarbon fractions using column chromatography. The aliphatic hydrocarbon fractions were then analysed by gas chromatography (GC). The aliphatic and aromatic hydrocarbon fractions from all bitumen samples were also analysed by gas chromatography - mass spectormetry (GC-MS).

Gas Chromatography Analysis of Aliphatic Hydrocarbon Fractions

Unfortunately, even after concentrating the samples to 1mg in 150µL DCM, the peak intensities of the n-alkanes in the chromatograms were far too small to allow proper analysis of the distribution of n-alkanes. A distinctive dominant feature of all GC chromatograms was the large (UCM) hump.

GC-MS of Saturated Hydrocarbon Fractions, Analysis of Ion m/z 85

It was difficult to make specific identification of the various samples based purely on their n-alkane distributions in m/z 85 chromatograms. None of the diagnostic ratio values investigated did change in a consistent manner either as a consequence of systematically changing the bitumen grade or source. The only possible exception was the Pr/C_{17} ratio, which had on average higher values for the Venezuelan bitumens than the Middle Eastern ones.

Some interesting results were observed when a comparison was carried out on the diagnostic ratios obtained from the Kuwaiti bitumen sample and the rest of the bitumens. The Kuwaiti bitumen sample had a higher CPI value, a lower Pr/Ph ratio, a lower C_{15}/C_{20} ratio and a lower C_{30}/C_{20} ratio than all the other Middle Eastern and Venezuelan samples. This was hypothesised to be caused by differences in the fractional distillation conditions that the crude oil had endured during bitumen production.

GC-MS of Saturated Hydrocarbon Fractions, Analysis of Hopanes (m/z 191)

Almost all the tricyclic and tetracyclic terpanes eluting before the Ts peak were absent from the Middle Eastern bitumens. This included the following compounds: 23/3, 24/3, 25/3, 24/4, 26/3, 28/3 and 29/3. Other missing peaks present in the Venezuelan bitumens but not in the Middle Eastern samples include the $C_{28}\alpha\beta$ peaks and the relatively intense C_{29}DM peaks.

The peak intensity of the $C_{29}\alpha\beta$ was consistently greater than the $C_{30}\alpha\beta$, for the Middle Eastern bitumens only, which is very characteristic of Middle Eastern oils. The reverse was true for the Venezuelan bitumens.

The ratio Ts/Tm of all the Venezuelan bitumens were at least 10 units higher than the Middle Eastern bitumens. The ratio 22S/(22S+22R) for the 17α(H) C_{32} hopanes was always lower for the Venezuelan bitumens compared to the Middle Eastern samples. On the other hand, the ratio of C_{30} $\beta\alpha$ to their corresponding $\alpha\beta$-hopanes of the Middle Eastern bitumen samples ratios were lower than the Venezuelan samples. Similarly, the Homohopane indices of Middle Eastern bitumens were predominantly lower than the Venezuelan samples.

There was a lot of biomarker evidence indicating that the Middle Eastern oil, from which the bitumen was fractionated, originates from more mature source rock sediments than the Venezuelan samples. There is also some evidence to indicate that the Venezuelan oil was exposed to light thermal stress and that this oil originates from a carbonate rich and/or evaporitic depositional environment.

It was not possible to relate changes in bitumen grade to changes in the values of diagnostic ratios for the Middle Eastern samples. For the Venezuelan bitumens, a few possibilities existed as there was consistent increase in the ratios of the tricyclic v.s. pentacyclic terpanes as the bitumen penetration increased.

For both bitumen sources the C_{35}/C_{34} homohopane ratio was the only diagnostic ratio showing a consistent decrease in value for all grades as a result of oven ageing.

GC-MS of Saturated Hydrocarbon Fractions, Analysis of Steranes (m/z 217)

Even more so than hopanes, the m/z 217 mass chromatograms of the bitumen samples were complex and the peaks not easy to identify. The first obvious difference in the overall appearance of the chromatograms was the lower intensity of the m/z 217 peaks in the Middle Eastern bitumen samples. The second main difference was the lack of diasterane peaks in the Middle Eastern samples, in particular the relative absence of the following early eluting compounds; 27dS, 27dbR, 27daS, 27daR, 28dbS and 28dbR. These peaks were very distinct and relatively easy to identify in the Venezuelan samples.

The values of the [diasteranes/steranes], [C_{28}/C_{29}], [$C_{30}/(C_{27}$ to $C_{30})$], [$(C_{27}\beta\beta$ S+R)/(C_{27}+C_{28}+C_{29})] and the [$(C_{28}\beta\beta$ S+R)/(C_{27}+C_{28}+C_{29})] diagnostic ratios were all consistently higher in the Venezuelan samples.

The values of the [C_{28}/C_{29}] ratios were much higher in the Venezuelan bitumens. Even though the results show some scatter, taking an average value of the [C_{28}/C_{29}] ratio across all the Middle Eastern samples a value of 0.44 is obtained, whilst for the Venezuelan bitumens, the average value is 0.70. This supports the earlier observations of the diagnostic ratios from the hopanes, i.e. the Middle Eastern bitumens are very likely to have originated from an oil older than 144 Ma and that the Venezuelan bitumen samples originated from an oil younger than 144 Ma (Upper Jurassic or younger).

The combination of the presence of 25-norhopanes in the Venezuelan bitumen, the higher average Pr/C17 ratio for the Venezuelan bitumens, and a higher diasteranes/steranes ratio all seem to indicate either a degree of biodegradation or at least some mixing with heavily biodegraded oils.

Overall, GC-MS analysis of the saturated hydrocarbon fractions (steranes and/or hopanes) is a very useful tool for distinguishing between bitumens from different sources regardless of the bitumen grade or the fractional distillation process.

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