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# 17,21-Secohopanoic acids, 25-norhopanoic acids, and 28-norhopanoic acids in source rocks and crude oils

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**Abstract**

The presence of three families of hopanoic acids, 17,21-secohopanoic acids, 25-norhopanoic acids, and 28-norhopanoic acids, is discussed. Oils from West Siberia and tar balls from the Seychelles Islands were found to contain relatively high proportions of 17,21-secohopanoic acids. These acids have not been previously reported in any oils or source rocks. A heavily biodegraded West Siberian oil, was found to contain an homologous series of 25-norhopanoic acids co-occurring with the 25-norhopanes as previously reported in only a small number of oils from Campos Basin, Brazil. 28-Norhopanoic acids have been reported in various sediments and extracts of the Monterey Shale, but in this study their occurrence has been extended to oils, degraded oils, and tar balls sourced from the Monterey Shale. The primary purpose herein is to report the occurrence of these acids and possible relationships between the acids and corresponding hydrocarbons.


## 1. Introduction

Hydrocarbon biomarkers, particularly steranes and terpanes, have played a major role in many aspects of crude oil exploration and exploitation but relatively little effort has been directed towards functionalized biomarkers such as carboxylic acids. Previous work on cyclic carboxylic acids in crude oils has focused primarily on hopanoic acids with molecular structures equivalent to the ubiquitous hopanes in crude oils and source rocks (Peters and Moldowan, 1993). The presence of hopanoic acids in crude oils may be related to source, or to oxidation products of the corresponding hopanes, with oxidation occurring either during early diagenesis or in-reservoir degradation (Watson et al., 2002). Alternatively, the acids may originate from remnants of the microbial community responsible for degradation of the oil (Meredith et al., 2000). The hopanoic acids have the potential to be used in a manner analogous to the hydrocarbons but relatively few systematic studies have been undertaken on potential applications, or utilization, of the acids as source, maturity, depositional environment indicators or correlation parameters. The first comprehensive review of carboxylic acids in crude oils was published by Seifert (1975). The identities of a number of hopanoic acids were established by Schmitter et al. (1978) and this information was used in many papers that followed, including a sequence of papers focusing on the effects of maturation on the hopanoic acid distributions and their utilization as migration indicators (Jaffé et al.,

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1988a,b, 1992; Jaffé and Gardinali, 1990; Jaffé and Gallardo, 1993). Quirk et al. (1984) discussed the abundance of hopanoic acids, particularly the relatively high abundance of the $C_{32}$-bishomohopanoic acid in recent peat samples and Behar and Albrecht (1984) made a number of observations related to the biodegradation of hopanoic acids. Carboxylic acids in crude oils from the Starogroznensk field, West Siberia, were described by Vanyukova et al. (1991) who also noted the acids may be source related or formed through in-reservoir alteration processes. The presence of hopanoic acids in crude oils resulting from biodegradation of the oils or the biomass of the degrading bacteria was addressed in a study of 33 oils from various fields, degraded to varying degrees, where it was observed that the $C_{30}$–$C_{32}$ hopanoic acids increased in concentration for all but the most heavily biodegraded oils (Watson et al., 1999; Meredith et al., 2000). Concomitant with this trend was a significant increase in the concentration of the ββ-hopanoic acids except for the most heavily degraded oils. The presence of the ββ-hopanoic acids led to the suggestion that these acids may have been derived from the bacteria responsible for the degradation of the oil (Meredith et al., 2000). An increase in the ββ-hopanoic acids being incorporated into oils generated at higher maturity levels had been noted previously and it was suggested that these may represent secondary generation of ββ-hopanoic acids tightly bound into geopolymers (Jaffé and Gardinali, 1990). Such a secondary generation pulse would only represent a small percentage of acids and unlikely would be the source of the relatively high amounts of ββ-hopanoic acids present in the degraded oils examined by Meredith et al. (2000). Formation of hopanoic acids was also observed to occur during the aerobic degradation of a crude oil in a laboratory study and it was proposed that under the experimental conditions 1–3.5% of the individual hopanes were converted to the corresponding $C_{29}$, $C_{31}$, and $C_{32}$ hopanoic acids (Watson et al., 2002). The absence of the $C_{30}$ hopanoic acid was explained on the basis of molecular mechanics calculations showing that formation of the $C_{30}$ acid was less thermodynamically favorable than the other three acids. $C_{31}$ Hopanes and the $C_{30}$–$C_{32}$ hopanoic acids from a Jurassic silty horizon in the Isle of Skye, Scotland have been quantified and molecular maturity parameters determined as a function of the distance from an igneous intrusion. Results suggested that the free hopanoic acids could be one potential source of the hopanes (Bennett and Abbott, 1999). Hopanoic acids bound into the macromolecular fraction, and released at higher levels of maturity, could represent another source for the hopanes. Bennett and Abbott (1999) also noted an increase in the ββ-hopanoic acids generated at higher maturity levels possibly representing secondary generation of ββ-hopanoic acids tightly bound into geopolymers as previously observed (Jaffé and Gardinali, 1990). The distributions of acid biomarkers in Albacora oils from the Campos Basin in Brazil were discussed by Nascimento et al. (1999) and it was proposed that since the carboxylic acid biomarkers were more resistant to biodegradation than the hydrocarbons, they could provide information on past reservoir history and degradation of the neutral biomarkers. This situation may be complicated by the production of carboxylic acids during biodegradation (Meredith et al., 2000; Watson et al., 2002). In another study by the Brazilian group, characterization of the acids and hydrocarbons from oils of the Sergipe-Alagoas Basin, Brazil suggested that decarboxylation was an important mechanism for the formation of the alkyl-steranes and monoaromatic alkyl-steroids from carboxylic acid precursors (Rodrigues et al., 2000). A similar observation was made following the analyses of the marine evaporitic oils from Fazenda Belem, Potiguar Basin (Lopes et al., 1997, 1999). The distribution of hopanoic acids, their origin, and relationship to the hopanes in a suite of 30 Mesozoic sedimentary rocks of varying maturity were studied and it was concluded the acids were not products of weathering of outcrop samples but represented early diagenetic products of bacteriohopanepolyols (Farrimond et al., 2002). Characterization of the free and bound acids from the Messel oil shale suggested that hopanoic acids released from kerogen were not exclusively converted to hopanes via decarboxylation or reduction (Sugden and Abbott, 2002).

28-Norhopanoic acids have been observed in a number of Neogene sediments from Japan and the Monterey Formation in California (Yamamoto et al., 2005). These acids were found to exist primarily as the free acids but were partly bound to the kerogen and the polar fraction via ester linkages. Isotopic studies of individual acids suggested the regular acids and demethylated acids were derived from different precursor organisms.

The major purpose of this paper is to report the novel occurrence of the 17,21-secohopanoic acids in certain crude oils and extend the reported occurrence

of the 25-norhopanoic and 28-norhopanoic acids. Unlike the hopanes and the regular hopanoic acids, 17,21-secohopanoic acids, 25-norhopanoic acids and 28-norhopanoic acids, are not ubiquitous in oils or source rocks and may reflect specific environmental conditions or source inputs. The Novolabitovskaya oil from Novolabitovskoe oil field, W. Siberia, contained relatively high concentrations of the 17,21-secohopanoic acids but did not contain the analogous secohopanes. The heavily biodegraded van Egan oil, from West Siberia, contained 25-norhopanoic acids and had a similar distribution of the 25-norhopanes suggesting a possible relationship between the acids and hydrocarbons. Tar balls from the beaches of California, thought to be derived from the Monterey shale-sourced oils, contained high concentrations of the 28-norhopanoic acids with the hydrocarbon fractions being dominated by the 28,30-bisnorhopanes. The concept of fatty acids being precursors of certain hydrocarbons in crude oils was originally discussed many years ago (Cooper and Bray, 1963). Quantitative data from more recent studies on the hopanoic acids in certain rock extracts have supported the idea that in certain cases the hydrocarbons can be derived from the acids (Bennett and Abbott, 1999). To the contrary, data have also been published from crude oil studies which suggest that under certain conditions the acids can be formed from the hydrocarbons via an oxidative mechanism (Watson et al., 2002). In our opinion, and from the results reported herein, the relationship between the acids and the corresponding hydrocarbons may to some extent be determined by the nature of the matrix in which they occur, i.e., oil or rock extract.

## 2. Experimental

### 2.1. Samples

Samples utilized in this study are listed in Table 1 and are derived from a number of different locations. The degraded and non-degraded oils from the West Siberian Basin have been described previously by Peters et al. (1991); the Californian tar balls by Hostettler et al. (2004); the Monterey Shale has been described in a wide variety of publications as has the Green River Shale and related oils (Burlingame et al., 1969; Tissot et al., 1978; Moldowan et al., 1984; Curiale et al., 1985; McKay and Blanche, 1987; Curiale and Odermatt, 1989; Collister et al., 1992; Schouten et al., 1997; Ruble et al., 2001). The tar balls from the Seychelles islands have been previously characterized geochemically and the results described by Plummer (1996).

### 2.2. Extraction of acids from source rocks and oil samples

The Monterey shale was extracted with dichloromethane:methanol (v:v 50:50) for 72 h. The extract was concentrated and the acids isolated using a modification of the method described previously (Ramljak et al., 1977). The acids were subsequently isolated in the manner described below.

The oil, or rock extract (2 g), was loaded onto KOH impregnated silica gel (60–100 mesh, 20 g) and transferred to a pre-extracted Soxhlet thimble. The neutral fraction was extracted from the silica gel with dichloromethane for 4 h and the acid fraction with 20% formic acid in diethyl ether for 4 h. After extraction, the solvent was removed and acids quantified.

Hopanoic acid concentrations were determined using a similar procedure to that previously published by Watson et al. (2002). 5β-Cholanic acid was added to the methylated acid fraction prior to analyses by GC and/or GCMS and the hopanoic acid concentrations were measured by comparison of their peak areas in the $m/z$ 191 mass chromatograms with that of the 5β-cholanic acid in the $m/z$ 217 mass chromatogram, assuming equal response factors for the standard and the hopanoic acids. Concentrations of the hopanes in the saturate hydrocarbon fraction were determined using $n$-$C_{24}D_{50}$ as an internal standard and again assuming a similar response factor for the standard and the hopanes.

#### 2.2.1. Acid purification

The acids were methylated using 14% (w/v) $BF_3$ in methanol and purified on an alumina column (10 g) to remove polar components. The esters were eluted with 10% diethyl ether in hexane (100 ml) and quantified. If excessive amounts of polar components were present HPLC was used for an additional purification step. The methyl esters (30 mg) were dissolved in hexane and injected onto a normal phase column (Whatman, Partisil 5 Pac, 9.4 mm i.d., 25 cm long) and eluted using the following solvent program: hexane 100% 2 min; DCM increased from 0% to 20% (hexane 100–80%) from 2 to 3 min; then from 20% to 40% (hexane from 80% to 60%) between 3 and 21 min; DCM increased to 100% for 5 min, then 100% ethyl acetate for 5 min, then DCM 100% for 5 min, and finally hexane 100%

Table 1
Sample locations

| Samples and locations of oils and rocks | | | | | | |
|---|---|---|---|---|---|---|
| Sample name | Sample location | Geological age | Lithology | Depth (m) | Source | |
| Novolabitovskaya 37 | Siberia, Russia | Frasnian, upper Devonian (D3f) | Carbonate | 1938–1992 | Anoxic marine, terrestrial | |
| Van-Egan P-109 | Siberia, Russia | Cenomanian (K2s) | Sand stone | 976–978 | Anoxic marine | |
| Monterey shale | California | Miocene | Mudstone: brown gray, fissile | 1222 | Marine | |
| Green River Shale | Uinta Basin, Utah | Eocene | Mud-supported carbonate | Outcrop | Lacustrine | |
| | Seychelles tar balls | Seychelles islands | Late Cretaceous | Clastic | Surface | Marine |

| Samples of Monterey oils and degraded residues | | |
|---|---|---|
| Sample ID | Description | Location |
| 00-128 | Tar on terrace | Santa Rosa I., Lobo Canyon |
| 00-130 | Fresh tar on rock | Santa Rosa I., Sandy Pt. |
| SM-10 | Tar on beach | San Miguel I., W. Simonton Beach |
| L33-13 | Degraded crude oil | Santa Maria Valley Field, California |

for 12 min to equilibrate the column. The methyl esters were collected from 2 to 24.5 min.

### 2.3. GC and GC/MS of the esters

The purified esters were analyzed on a Varian GC 3300 equipped with an on-column injector and a fused silica capillary column (30 m × 0.25 mm i.d.) coated with DB-1 (film thickness 0.25 μm) and programmed from 40 to 310 °C at 4 °C/min and held at 310 °C for 32 min.

GC–MS analyses of the esters were performed on a Varian 3400 GC with a DB-5 MS fused silica capillary column (60 m × 0.32 mm i.d., J&W Scientific) interfaced to a Finnigan MAT triple stage quadrupole mass spectrometer (TSQ-70). The GC column was programmed from 40 to 310 °C at 4 °C/min, and held isothermal at 310 °C for 52 min. The MS was operated at 70 eV, source temperature 200 °C, and data collected in either the full-scan or multiple ion detection (MID) mode.

## 3. Results and discussion

Hopanoic acids have been reported previously to occur in a number of crude oils and rock extracts (i.e., Jaffé and Gallardo, 1993; Farrimond et al., 2002). For orientation purposes, a typical distribution of hopanoic acids observed in a non-degraded crude oil from the Uinta Basin is shown in Fig. 1a and the peaks identified in Table 2. The hopanoic acid distribution from an immature shale sample, the Green River Shale (GRS), is shown in Fig. 1b. The identity of these acids is primarily based on comparison with previously published *m/z* 191 chromatograms illustrating the hopanoic acid distributions and comparison with concentrates of previously identified hopanoic acids. The identity of the gammaceranoic acid is tentative and based on a comparison with previously published data (Peng et al., 1999). The purpose of this paper is not to focus on the regular hopanoic acids but to describe a number of acids not previously reported in the literature, or discussed only in limited detail, namely the 17,21-secohopanoic acids, 25-norhopanoic acids, and 28-norhopanoic acids. Quantitative data have been used in previous studies to suggest that in some shales, free hopanoic acids could be one potential source, among others, of the hopanes that are generated during contact metamorphism (Bennett and Abbott, 1999). While such a relationship may be true in many cases, particularly in the case of shales or source rocks of increasing maturity, it is felt that there are many situations where this acid/hydrocarbon relationship has not been clearly established. As mentioned above in certain cases, biodegradation for example, a certain proportion of the acids may be formed via oxidation of the hydrocarbons (Watson et al., 2002). The unusual nature, and apparent limited occurrence of the acids described in the current paper, may assist in establishing acid/hydrocarbon or hydrocarbon/acid relationships.

### 3.1. 17,21-Secohopanoic acids

#### 3.1.1. Novolabitovskaya oil from Novolabitovskoe oil field, West Siberia

There have been a number of geochemical studies related to oils from West Siberia, and in particular the Chevron group identified the source rocks

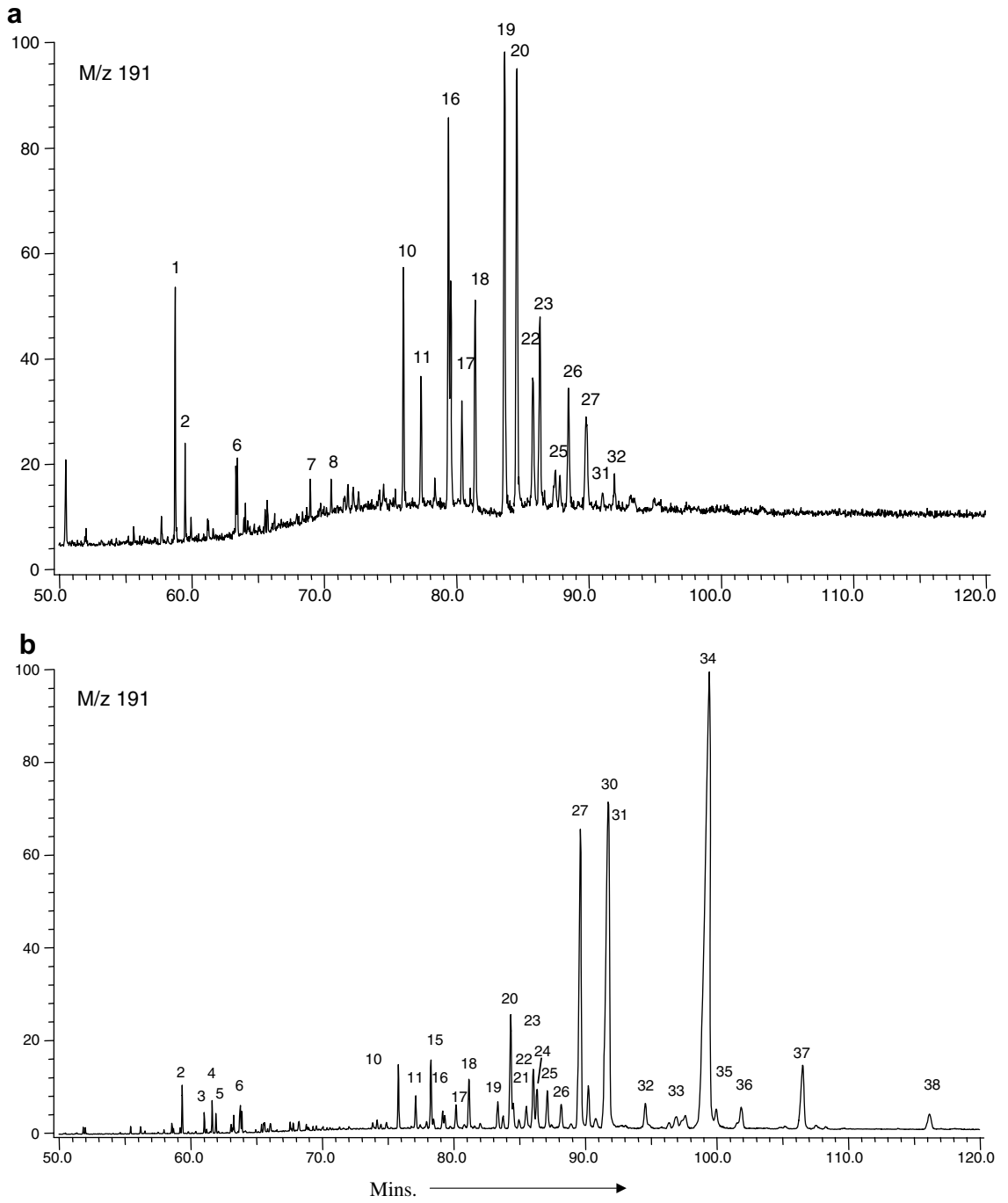


Fig. 1. (a) The distribution of hopanoic acids (as methyl esters in all figures) observed in a regular non-degraded crude oil from the Uinta Basin are shown in this *m/z* 191 chromatogram and the peaks, for this and all other chromatograms, are identified in Table 2. (b) The *m/z* 191 chromatogram in the figure shows the distribution of hopanoic acids from an immature Green River Shale extract.

of these oils and the relationship between the oil families in the region (Peters et al., 1991, 1993, 1994). The majority of these papers only discussed the use of saturate aliphatic and aromatic hydrocarbons for source, maturity and correlation purposes and did not make any reference to the presence of the carboxylic acids in these oils and source rocks. In the present study, carboxylic acids from a num-

Table 2
Peak identification in the hopanoic acid chromatograms shown in Figs 1–6

| Peak # | Compound identification |
|---|---|
| 1 | $C_{21}$ tricyclic acid |
| 2 | $C_{21}$ tricyclic acid |
| 3 | $C_{22}$ tricyclic acid |
| 4 | $C_{22}$ tricyclic acid |
| 5 | $C_{24}$ tricyclic acid |
| 6 | $C_{24}$ tricyclic acid |
| 7 | $C_{25}$ 17,21-secohopanoic acid |
| 8 | $C_{26}$ 17,21-secohopanoic acid |
| 9 | $C_{27}$ 17,21-secohopanoic acid |
| 10 | $C_{28}$ 17α(H),21β(H)-hopanoic acid |
| 11 | $C_{28}$ 17β(H),21α(H)-hopanoic acid |
| 12 | $C_{29}$ 17α(H),21β(H)-hopanoic acid |
| 13 | $C_{31}$ 17,21-secohopanoic acid (22S) |
| 14 | $C_{31}$ 17,21-secohopanoic acid (22R) |
| 15 | $C_{30}$ 17,21-secohopanoic acid |
| 16 | $C_{30}$ 17α(H),21β(H)-hopanoic acids (22S+22R) |
| 17 | $C_{30}$ 17β(H),21α(H)-hopanoic acid (22S) |
| 18 | $C_{30}$ 17β(H),21α(H)-hopanoic acid (22R) |
| 19 | $C_{31}$ 17α(H),21β(H)-homohopanoic acid (22S) |
| 20 | $C_{31}$ 17α(H),21β(H)-homohopanoic acid (22R) |
| 21 | $C_{32}$ bishomohopenoic acid |
| 22 | $C_{31}$ 17β(H),21α(H)-homohopanoic acid (22S) |
| 23 | $C_{31}$ 17β(H),21α(H)-homohopanoic acid (22R) |
| 24 | $C_{32}$ bishomohopenoic acid |
| 25 | $C_{31}$ gammaceranoic acid |
| 26 | $C_{32}$ 17α(H),21β(H)-bishomohopanoic acid (22S) |
| 27 | $C_{32}$ 17α(H),21β(H)-bishomohopanoic acid (22R) |
| 28 | $C_{31}$ 17β(H),21β(H)-homohopanoic acid (22S) |
| 29 | $C_{32}$ 17β(H),21α(H)-bishomohopanoic acid (22S) |
| 30 | $C_{31}$ 17β(H),21β(H)-homohopanoic acid (22R) |
| 31 | $C_{32}$ 17β(H),21α(H)-bishomohopanoic acid (22R) (co-eluting with $C_{31}$ 17β(H),21β(H)-homohopanoic acid (22R)) |
| 32 | $C_{33}$ 17α(H),21β(H)-trishomohopanoic acid (22S) |
| 33 | $C_{33}$ 17α(H),21β(H)-trishomohopanoic acid (22R) |
| 34 | $C_{32}$ 17β(H),21β(H)-bishomohopanoic acid (22R) |
| 35 | $C_{33}$ 17β(H),21α(H)-trishomohopanoic acid (22R) |
| 36 | $C_{34}$ 17α(H),21β(H)-tetrakishomohopanoic acid (22S) |
| 37 | $C_{33}$ 17β(H),21β(H)-trishomohopanoic acid (22R) |
| 38 | $C_{33}$ 17β(H),21β(H)-tetrakishomohopanoic acid (22R) |

ber of West Siberian oils have been examined, leading to the discovery of some unusual acid distributions. The acids from the Novolabitovskaya oil were characterized by the virtual absence of any regular hopanoic acids. In view of previously reported results for hopanoic acids in crude oils this is a rather unique observation. In the absence of the regular hopanoic acids, the *m/z* 191 chromatogram (Fig. 2) was dominated by two components, tentatively identified as the 22S and 22R epimers of the $C_{31}$ 17,21-secohopanoic acid. Mass spectral data (spectrum of one epimer shown in Fig. 3) suggested these components were $C_{31}$ 17,21-secohopanoic acids and based on a comparison with the relative retention times of the corresponding regular hopanoic acids it is tentatively proposed that the 22S epimer elutes prior to the 22R epimer. Three characteristic fragment ions in the spectrum support the 17,21-secohopanoic acid structural assignment. First, the molecular ion is at *m/z* 472 and since this is a methylated acid fraction, it is reasonable to assume that this molecular ion corresponds to a molecular formula $C_{32}H_{56}O_2$, equivalent to a tetracyclic structure with one carboxyl group, leading to the proposed 17,21-secohopane structure. This is further supported by the base peak at *m/z* 191 and a fragment ion at *m/z* 399 resulting from the loss of the $-CH_2COOCH_3$ fragment from the side chain.

The only previous report of ring-opened acids in crude oils appears to be from the work of Schmitter et al. (1981, 1982). An acid with ring A open was

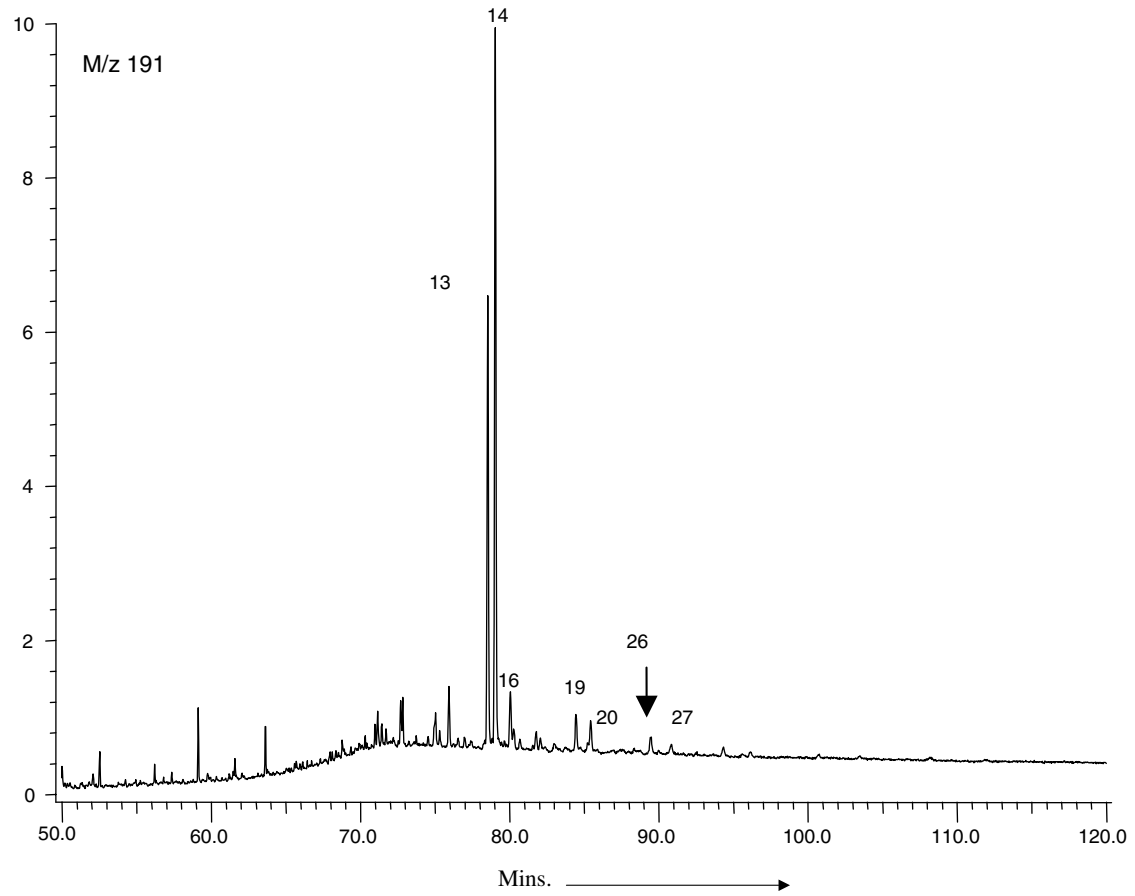


Fig. 2. The *m*/*z* 191 chromatogram illustrating the distribution of the acids, as methyl esters, in the Novolabitovskaya oil from W. Siberia. The major components, peaks 13 and 14, have been tentatively identified as the 22S and 22R epimers of the $C_{31}$ 17,21-secohopanoic acid, respectively (peak identifications are given in Table 2).

detected in an oil from Nigeria and proposed to form through a photomimetic degradation process (Corbet et al., 1980). While there are some similarities between the spectrum of the compounds tentatively identified as the 17,21-secohopanoic acids in this study and the ring A opened acids described by Schmitter et al. (1981) in terms of the fragmentation patterns, the relative intensity of the fragment ions is quite different, suggesting we are not looking at ring A opened acids in the present study.

While the Novolabitovskaya oil is the only one to date with this rather unique distribution of secohopanes, a number of oils containing minor amounts of secohopanoic acids over different carbon number ranges have been observed. For example, a tar ball sample from the beaches of the Seychelles islands was found to contain relatively low concentrations of 17,21-secohopanoic acids in the $C_{25}$–$C_{27}$ range (Fig. 4). This sample has been weathered to some degree during transportation in the ocean and the *n*-alkanes below $C_{32}$ have been degraded along with the isoprenoids. However, the steranes and terpanes do not show any signs of extensive alteration so it is unlikely that the secohopanoic acids have formed as a result of this weathering process, which occurred over a relatively short period of time.

17,21-Secohopanes in the $C_{24}$–$C_{27}$ range were first reported by Trendel et al. (1982) but to date it does not appear that any 17,21-secohopanes with extended side chains have been reported. As far as can be ascertained, this is the first report of the 17,21-secohopanoic acids in a crude oil and their presence raises a number of interesting issues. First, in the saturate fraction of the Novolabitovskaya crude oil there was no anomalously high concentration of the corresponding 17,21-secohopanes suggesting the probable absence of any relationship between the acids and hydrocarbons in

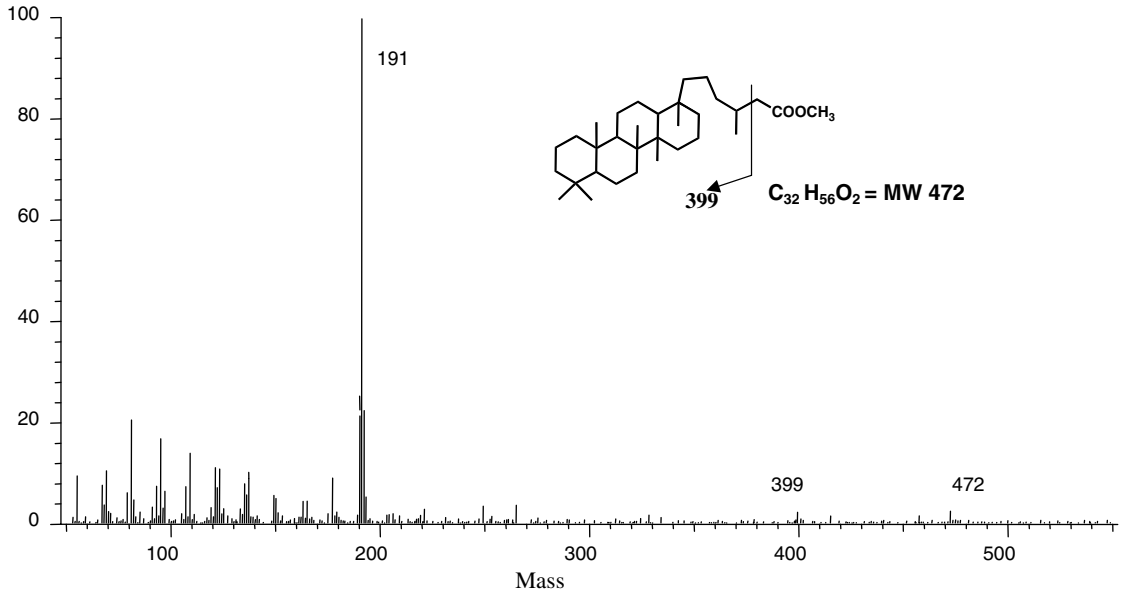


Fig. 3. Mass spectrum data for the compound tentatively identified as the 17,21-secohopanoic acid methyl ester (22S) in the Novolabitovskaya oil from W. Siberia, shown in Fig. 2, peak 13.

this oil. It should be noted that even the $C_{24}$ 17,21-secohopane, formed through cleavage of the side chain, was not present in any significant abundance. So what are the possible origin(s) for these compounds? It would appear that there are at least four possibilities:

1. Direct source input of secohopanoic acids.
2. Direct input of hopanoic acids followed by cleavage of the 17,21 bond.
3. Side chain oxidation of regular hopanes followed by cleavage of the 17,21 bond.
4. Initial cleavage of the 17,21 bond in the hopanes followed by oxidation of the side chain.

Examination of the acid and hydrocarbon data from the Novolabitovskaya oil permits several of these ideas to be eliminated. The possibility that these compounds are derived from the hopanoic acids by cleavage of the 17,21 bond seems unlikely simply through the absence of any regular hopanoic acids. Even if it is proposed that the hopanoic acids were converted to secohopanoic acids, why are only the $C_{31}$ secohopanoic acids present? Similar arguments apply for the third and fourth possibilities, since the complete suite of hopane hydrocarbons are present and therefore it is difficult to imagine how either of these processes could lead to the predominance of the acids by just one member of the series. Hence, although evidence of a direct source input, or formation during early diagenesis, is not available at this time, the circumstantial evidence seems to be pointing in that direction and certainly needs further investigation. In support of the source input concept, the extract of an immature sample of the GRS appeared to contain relatively minor amounts of the $C_{30}$ 17,21-secohopanoic acid, and the regular hopanoic acids were dominated by those with the 17β(H),21β(H) or 17α(H),21β(H) 22R immature stereochemistry (Fig. 1b).

A possible explanation for the predominance of the $C_{31}$ secohopanoic acids and absence of lower carbon number compounds may be related to the fact that if the lower members of the series are formed by side chain cleavage, the presence of the methyl group at the C22 may prevent or reduce the chances of cleavage at the C22 atom necessary for formation of the lower carbon members of the series. Alternatively it may again simply represent a single source input. In peat samples from a number of sources it was observed that the hopanoic acid distributions were dominated by the $C_{32}$ hopanoic acids and alcohols as well as a preponderance of the 17α(H),21β(H) homohopane (Quirk et al., 1984). It was suggested in that case that the specific distribution of the acids was probably associated with the bacterial activity in acidic nutrient deficient environments. While they did not observe any

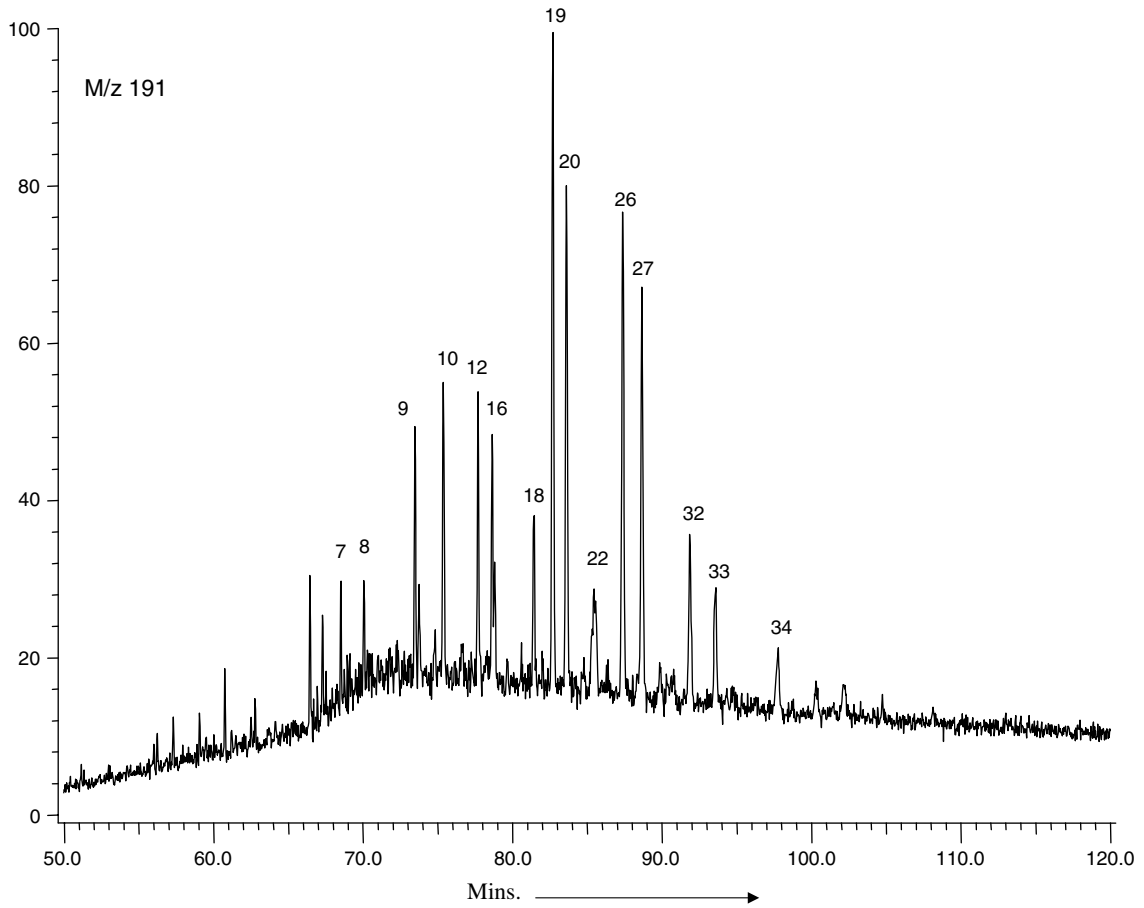


Fig. 4. The *m*/*z* 191 chromatogram for the cyclic carboxylic acids, as methyl esters, from a tar ball sample from the beaches of the Seychelles islands (peak identifications are given in Table 2).

17,21-secohopanoic acids they did establish a precedent for the predominance of hopanes with a specific carbon number rather than a complete homologous series of hopanes.

### 3.2. 25-Norhopanoic acids

#### 3.2.1. van Egan crude oil

The van Egan oil is one of six oils from West Siberia, previously described in detail, along with a number of source rocks from the same area (Peters et al., 1991, 1993, 1994). The primary focus of these publications was to discuss the geochemical characteristics and relationships between the oils and source rocks. Geochemical data for the van Egan, which is a very heavily biodegraded (level 8 on Peters and Moldowan (1993) scale), included isotope data, saturate aliphatic and aromatic biomarker distributions including the hopanes and norhopanes. The 25-norhopanes are characterized by the presence of the $C_{28}$–$C_{33}$ 25-norhopanes as shown in the *m*/*z* 177 chromatogram (Fig. 5a). The hopanes are characterized by relatively low concentrations of the $C_{27}$–$C_{29}$ hopanes, the virtual absence of the $C_{30}$–$C_{32}$ hopanes, and relatively low concentrations of the extended $C_{33}$–$C_{35}$ hopanes as shown in the *m*/*z* 191 chromatogram (Fig. 5b, *m*/*z* 191). The corresponding acids are characterized by the presence of $C_{29}$–$C_{33}$ 25-norhopanoic acids (Fig. 5c, *m*/*z* 177) and the absence of any regular hopanoic acids (Fig. 5d, *m*/*z* 191). As far as can be ascertained the only other paper that has reported the presence of the 25-norhopanoic acids was that by Nascimento et al. (1999) in their study of acidic biomarkers from Albacora oils in the Campos Basin. The distribution of the 25-norhopanoic acids reported in the Albacora oils was remarkably similar to that observed in

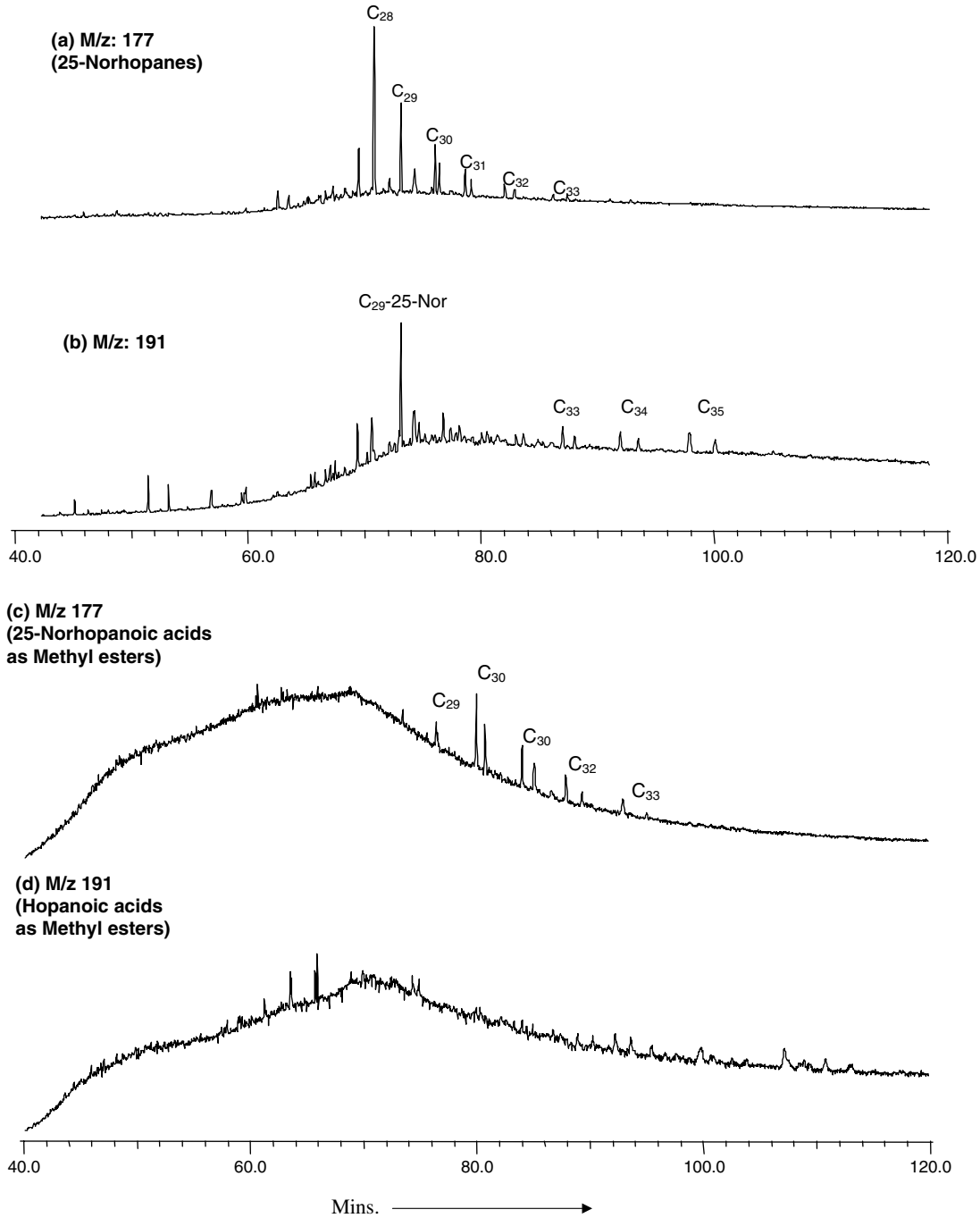


Fig. 5. The *m*/*z* 191 chromatogram for the hydrocarbons and carboxylic acids, as methyl esters, in the van Egan oil, from West Siberia. (a) The *m*/*z* 177 chromatogram illustrating the 25-norhopanes series; (b) *m*/*z* 191 showing the regular hopanes; (c) *m*/*z* 177 showing the 25-norhopanoic acids as methyl esters and (d) the *m*/*z* 191 chromatogram showing the regular hopanoic acids, as methyl esters.

the van Egan oil with the series of 25-norhopanoic acids ranging from $C_{29}$ to $C_{32}$.

Some of the possible relationships between the regular and 25-norhopanes and the corresponding acids are illustrated in Fig. 6. This scheme, along with previously proposed reaction pathways, requires the formation of an intermediate with a carboxyl group, or some other functionalized

group, at C10. In previous studies of North Sea oils and Albacora oils from the Campos Basin containing the 25-norhopanes it was proposed that the mechanism for removal of the methyl group at C10 was via oxidation and decarboxylation (Mason et al., 1995; Nascimento et al., 1999). The shift in relative distributions between the hopanes and 25-norhopanes with increasing biodegradation in degraded van Egan oils and the overall conservation of the original homohopane distribution has been proposed to indicate in-reservoir conversion via C10 demethylation (Peters et al., 1994). But to date the only report of such a functionalized C10 intermediate is the tentative identification of the 28,30-bisnorhopan-25-oic acid (de Lemos Scofield, 1990). It seems somewhat unusual that since the original report no one else has reported these intermediate acids if they are such a critical component in the reaction mechanism. It would seem that the oil originally characterized by de Lemos Scofield (1990) should be re-examined if possible in an attempt to confirm the identity of the intermediate.

A molecular mechanics approach has been used to explain how the rate of C25 demethylation varies as a result of molecular size and epimer conformation (Peters et al., 1996). While their study indicated

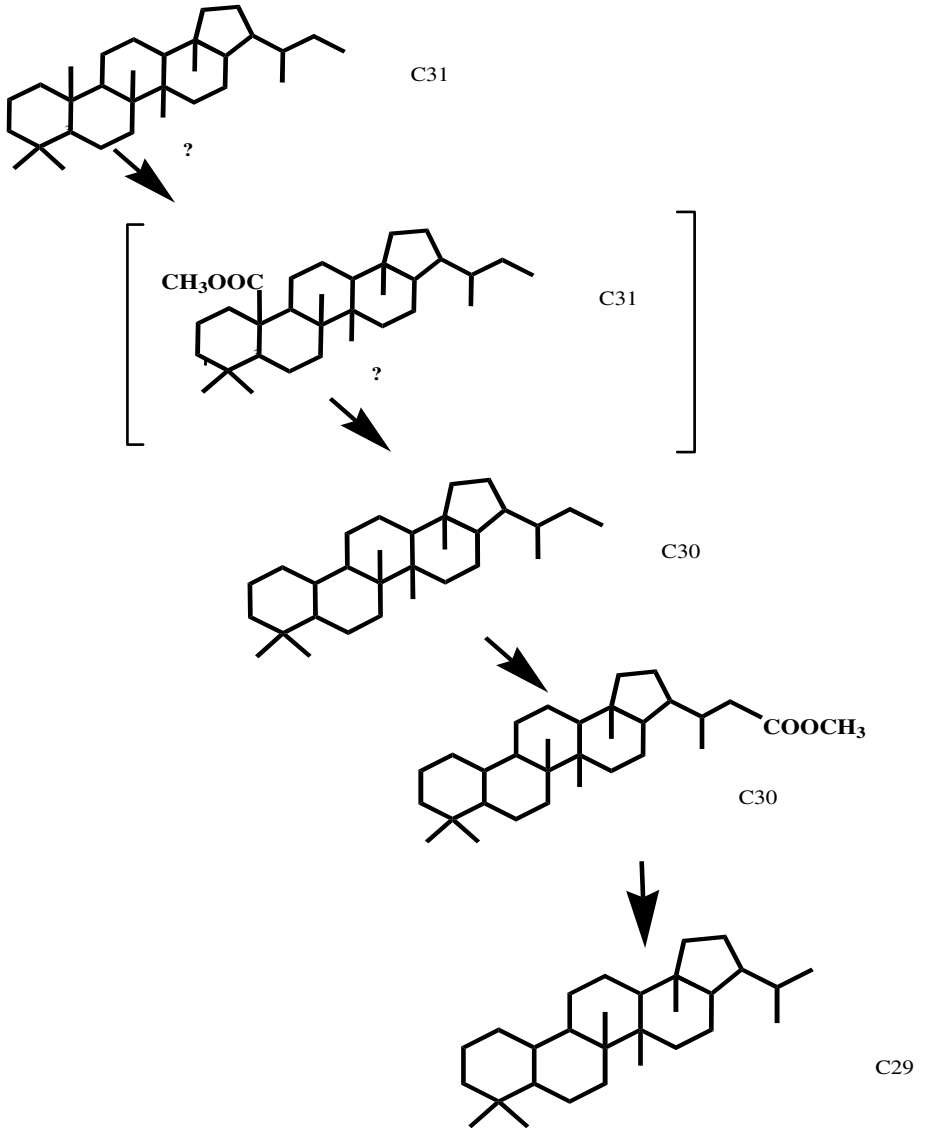


Fig. 6. Possible relationship between the 25-norhopanoic acids and 25-norhopanes.

that differences in the susceptibility of different members of the homologous hopane series to demethylation, they did not address the potential stability of the acid intermediates. The 22S and 22R epimers both had a decreasing susceptibility towards demethylation with increasing carbon number (Peters et al., 1996). The 22S epimers of the extended hopanes tend to favor a so-called "scorpion" conformation whereby the tail folds back towards to the C25 methyl group shielding it from enzymatic attack. The 22R has the so-called "rail" conformation where this shielding effect is not in place. As the length of the side chain is extended so the shielding becomes more pronounced and this explains the selective preservation of the $C_{35}$ extended hopanes as observed for example in the Pt. Arena tar sands (Requejo and Halpern, 1989).

If, for the moment, it is assumed that the 25-norhopanes are formed from the hopanes via elimination of the C10 methyl group, by some still unknown mechanism, or if the 25-norhopanes are a source related input, the next step would be the relatively facile oxidation of the side chain to form the 25-norhopanoic acids. Formation of the acids through oxidation of the side chain with increasing biodegradation has been demonstrated in a number of situations described above. It should be noted that using the molecular mechanics approach applied to the regular hopanes (Peters et al., 1996) it might be possible to explain why the concentrations of the higher hopanoic acid homologues are lower in some cases than may be anticipated. A similar explanation may be used to indicate why the relative concentrations of the higher homologues of the 22S regular hopanoic acids are lower than those of the corresponding 22R epimers. The "scorpion" tail of the higher homologues not only shields the C10 methyl group but will shield the side chain from enzymatic activity.

Following formation of the 25-norhopanes, the side chain can be oxidized to form the 25-norhopanoic acids. Side chain oxidation after demethylation explains why only the extended 25-norhopanoic acids are observed. Formation of the $C_{26}$–$C_{28}$ 25-norhopanoic acids would involve oxidation of the more stable tertiary carbon at C22, a reaction less likely to occur than oxidation of the extended side chain (Fig. 6).

To summarize this section, the results from the present study have extended the occurrence of the 25-norhopanoic acids to another biodegraded oil following their initial discovery in the Albacora oils from the Campos Basin. The mechanism(s) for their formation still remains unclear. Are they formed from the regular hopanes via demethylation and then side chain oxidation? Do they reflect a direct source input? Are they derived from the 25-norhopanes which themselves may reflect a direct source input? Alternatively since the only two reported occurrences have been in heavily biodegraded oils could these 25-norhopanoic acids represent remnants of the microbial consortia responsible for the degradation of the oil.

### 3.3. 28-Norhopanoic acids

The presence of 28,30-bisnorhopane in extracts of the Monterey Shale and associated oils as well as oils from certain areas of the North Sea has been well documented (Seifert et al., 1978; Moldowan et al., 1984; Schoell et al., 1992; Curiale and Odermatt, 1989; Nytoft et al., 2000; Dahl, 2004). The origin of this compound has been assumed to reflect deposition of the organic matter under anoxic conditions and in the Monterey it has been hypothesized to reflect an origin from bacterial mats. In the course of examining the acids from a number of oils and tar balls from California, a component, tentatively identified as the 28-norhopanoic acid, was observed to be present in many of these samples (Fig. 7). The samples examined were ranked in terms of biodegradation using Peters and Moldowan (1993) scale but there was no apparent correlation between the concentrations of the acids or hydrocarbons and the level of degradation (Table 3). The concentrations of the hopanoic acids were several orders of magnitude lower than the concentrations of the hopanes in the tar ball extracts. In the extract of the Monterey shale sample used in this study, the concentration of the 28-norhopanoic acid was approximately 27 μg/g compared to concentrations of less than 1 μg/g in the degraded oil samples.

It was later noted that the peak identified as 28-norhopanoic acid was actually two closely eluting peaks and it is has been assumed that these peaks are derived from the two isomers of the 28-norhopanoic acids, analogous to the two isomers observed for the 28,30-bisnorhopanes (17α,21β and 17β,21α; Moldowan et al., 1984). The 28-norhopanoic acids were tentatively identified from the mass spectral information on the basis of their molecular ion at *m*/*z* 442; *m*/*z* 355 (ring fragment with loss of side chain); *m*/*z* 191 (AB ring fragment showing that methyl group was not lost from the A or B rings).

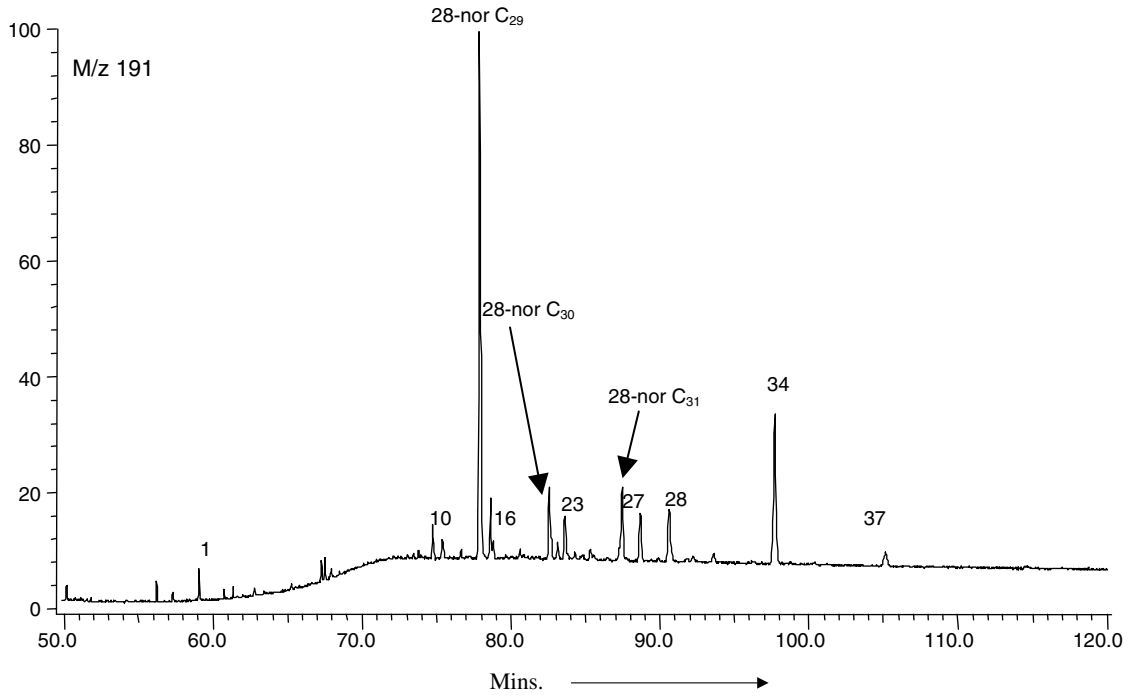


Fig. 7. The *m*/*z* 191 chromatogram for the acids, as methyl esters, from the extract of the Monterey Shale, dominated by the 28-norhopanoic acids (peak identifications are given in Table 2).

Table 3
Concentration of hopanes and hopanoic acids in Californian oils and source rocks

| Sample ID | Total hopanoic acids (μg/g) | Total hopanes (μg/g) | Biodegradation level after Peters and Moldowan (1993) |
|---|---|---|---|
| Monterey Shale | 61.30 | 501.49 | |
| Leroy 33-13 | 1.69 | 1458.28 | 5 |
| 00-128 | 4.39 | 998.43 | 6 |
| SM-10 | 1.60 | 2148.74 | 6–7 |
| 00-130 | 2.51 | 640.93 | 8 |

It was not possible to totally resolve the two co-eluting components of this peak to get spectra of the individual isomers. The fact that Moldowan et al. (1984) previously demonstrated the 28,30-bisnorhopanes, or precursors, are not bound into the kerogen but only occur in the bitumen, coupled with the relatively high abundance of the corresponding acids, leads us to suggest the acids are clearly the precursors of the hydrocarbons. A similar suggestion was also made based on quantitative results from the study of acids and hydrocarbons in the vicinity of an igneous intrusion (Bennett and Abbott, 1999). The quantitative results in the present study may not be as conclusive as those in the study of Bennett and Abbott (1999) but it can be observed in Table 3 that the proportion of acids/hydrocarbons is higher in the shale than in the oils, again suggesting conversion of acids to hydrocarbons through a decarboxylation mechanism with increasing maturity (Fig. 8). The acid/hydrocarbon ratio is two orders of magnitude higher for the shale than the oil and tar balls and with the exception of sample SM-10, the ratio is relatively similar for the oil and the tar ball samples (Table 3).

The carboxyl group in the acid is on the side chain and in this particular compound it is the C30 carbon which represents the carboxyl carbon and decarboxylation of this compound will produce 28,30-bisnorhopane. The 28-norhopanoic acid in turn reflects an original source input or a product of early diagenesis as previously suggested by Quirk et al. (1984) for the homohopanes in the peat

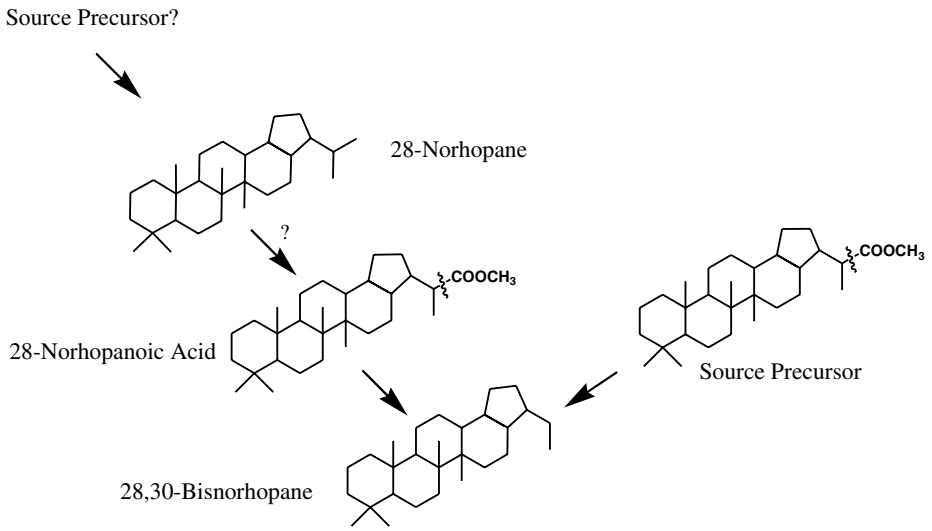


Fig. 8. Possible relationships between the 28,30-bisnorhopanes and 28-norhopanoic acids.

samples. The 28-norhopanoic acids have been previously reported in a number of sediments from the Onnagawa, Funakawa, and Tentokuji Formations from northeastern Japan and the Monterey Formation (Yamamoto et al., 1997, 2005). In the most recent report a combination of GCMS and carbon isotope values of individual compounds was used to suggest that since the $C_{29}$ and $C_{31}$ 28-norhopanoic acids were isotopically depleted relative to the $C_{30}$ and $C_{32}$ regular hopanoic acids, these demethylated and regular hopanoic acids were derived from different precursor organisms (Yamamoto et al., 2005). Similarities in the carbon isotope values of the $C_{30}$ 28-norhopanoic acid and the $C_{31}$ regular hopanoic acid are consistent with the hypothesis that C28 demethylation of regular hopanoic acids produces the 28-norhopanoic acids. The Upper Onnagawa Formation, which has abundant concentrations of the 28-norhopanoic acids, is also characterized by the *Calyptogena* sp. (Bivalvia). This organism lives in a specific chemoautotrophic ecosystem utilizing chemical substrates from hydrothermal vents or cold seeps. However, the organism does not appear to be present in the other formations in this study making the relationship between the 28-norhopanoic acids and the *Calyptogena* unclear at this time. In the Lower Onnagawa Formation the 28-norhopanoic acids were depleted by approximately 14 per mil in $^{13}C$ relative to the kerogen suggesting the acids were probably derived from bacteria utilizing $^{13}C$-depleted $CO_2$ as their carbon source. If decarboxylation of the $C_{29}$ 28-norhopanoic acid produced the 28,30-bisnorhopane then you would anticipate that the $C_{30}$ and $C_{31}$ 28-norhopanoic acids would be decarboxylated to form the $C_{29}$ and $C_{30}$ 28-norhopanes, respectively, but these homologues were not detected in the Neogene sediments (Yamamoto et al., 2005). Such variations illustrate the complexity of these acid/hydrocarbon relationships and possible conversion mechanisms. It appears that there are a number of possible origins for the carboxylic acids. The acids may be formed by oxidation of the corresponding hydrocarbons; they may reflect a direct source input; or they may be released from the kerogen. To further complicate the situation, the acids may be decarboxylated to the corresponding hydrocarbon. All of these variables make the acid/hydrocarbon relationships far more difficult to interpret than simply looking at the hydrocarbons alone.

## 4. Conclusions

17,21-Secohopanoic acids have been identified for the first time in a number of degraded and non-degraded oils. The predominance of the $C_{31}$ 17,21-secohopanoic acids in the Novolabitovskaya oil reflects a direct source input or acids formed by ring opening and side chain oxidation although it is not clear at this stage why only the $C_{31}$ 17,21-secohopanoic acids are found in this sample. In another West Siberian oil, the co-occurrence of the 25-norhopa-

noic acids and 25-norhopanes, suggested the 25-norhopanes were probably formed via a two stage reaction with removal of the C10 methyl group in the first step followed by oxidation of the side chain. It would appear that the 28-norhopanoic acids found in oils and the Monterey source rock may reflect a direct source contribution and that these acids are converted to the 28,30-bisnorhopanes, characteristic of the Monterey Shale. The presence of these compounds in the source rock make it unlikely the acids are oxidation products of the hydrocarbons unless the oxidation occurs during early diagenesis. In a series of Californian tar ball samples, degraded to varying degrees, based on hydrocarbon parameters, there did not appear to be any obvious correlation or relationship between the acid and hydrocarbon concentrations and degree of biodegradation based on the biodegradation scale as proposed by Peters and Moldowan (1993).

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