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Hopanoic acids in Mesozoic sedimentary rocks: their origin and relationship with hopanes

Paul Farrimond*, Tony Griffiths, Efthymios Evdokiadis

Fossil Fuels and Environmental Geochemistry, Drummond Building, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, UK

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Abstract

Hopanoic acids were found in all but the two most mature of a suite of 30 Triassic to Cretaceous sedimentary rocks. Their carbon number distributions generally maximise at C_{32}, consistent with hopanoic acids found in modern sediments. The extent of isomerisation (measured by %ββ and %22S αβ parameters) is less for the acids than for associated hopanes, possibly due to increased isomerisation of hopanes in association with their generation from macromolecules and functionalised precursors. In immature samples the hopane carbon number distributions are consistent with an origin from defunctionalisation of hopanoic acids and other early diagenetically-formed hopanoids, whilst hopanes in more mature samples exhibit broader carbon number distributions indicative of side-chain cleavage during generation of macromolecularly-bound hopanoids.

1. Introduction

The hopanoids are a group of biological marker compounds that are both abundant and widespread in the environment and the geosphere (van Dorsselaer et al., 1974; Ourisson et al., 1979, 1984; Ourisson and Albrecht, 1992). Their ubiquity is due to the wide range of bacteria that synthesize hopanoids (Rohmer et al., 1984; Farrimond et al., 1998a), and the relative stability of the hopanoid hydrocarbon skeleton. These characteristics have resulted in hopanoids being extensively employed as bacterial marker compounds in molecular organic geochemical studies.

The hopanoids in environmental and geological samples are derived from bacteriohopanepolyols and related biological hopanoids (Rohmer et al., 1984, 1992). Although our knowledge of the diagenetic fate of these precursor compounds in the environment and the sedimentary record is incomplete, many diagenetic products are known, of which the hopanols, hopanoic acids, hopenes and hopanes are most usually encountered. Hopanols and hopanoic acids are typically the most abundant hopanoid species in the solvent-soluble organic matter of Recent sediments (e.g. Innes et al., 1997, 1998, and references therein), and are dominated by the C_{32} homologues with the biologically-inherited 17β(H),21β(H) 22R stereochemistry (e.g. Quirk et al., 1984; Ries-Kautt and Albrecht, 1989; Buchholz et al., 1993). Hopanes are usually minor constituents of environmental samples and modern sediments (Innes et al., 1997); any small amounts of hopanes formed from diagenesis of naturally-occurring hopanoids may be masked by hopanes derived from contamination by fossil fuels (e.g. Rowland and Maxwell, 1984). Consequently, molecular information regarding bacterial sources of organic matter in modern sediments typically focuses on hopanoic acids, hopanols, and their precursors, the bacteriohopanepolyols (Buchholz et al., 1993; Innes et al., 1997, 1998; Farrimond et al., 1998a). In contrast, in oils and ancient sedimentary rocks, it is typically only the hopanes which are examined, although hopanoic acids are known to occur in both.

Hopanoic acids have been reported in a number of ancient sediments and sedimentary rocks (Seifert, 1975 and references therein; Barnes et al., 1979; Jaffé et al., 1988a; Jaffé and Gardinali, 1990; Wolff et al., 1992; Barakat and Rullkötter, 1994; Ohkouchi et al., 1997;

* Corresponding author.
E-mail address: paul.farrimond@ncl.ac.uk (P. Farrimond).

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Bennett and Abbott, 1999). In immature samples, the hopanoic acids can be more abundant than the hopanes (Bennett and Abbott, 1999), but in mature samples hopanoic acids may be lacking or present only in very low concentration (Jaffé and Gardinali, 1990). Like the hopanes, the hopanoic acids occur as 17β(H),21β(H), 17β(H),21α(H) and 17α(H),21β(H) (22S and 22R) isomers, with progressive loss of the biologically-inherited ββ forms with increasing maturation. Changes in isomeric composition of the hopanoic acids appear to be related to the relative rates of generation and destruction (Bennett and Abbott, 1999), analogous to the equivalent changes in hopane composition (e.g. Bishop and Abbott, 1993; Farrimond et al., 1998b). Hopanoic acids have also been reported in oils, where their presence may be due to contamination of the oil during migration through immature sediments (Jaffé et al., 1988a,b; Jaffé and Gallardo, 1993) or formation during biodegradation, either from bacterial oxidation of hopanes (Watson et al., 1999) or from the biomass of the degrading bacteria (Meredith et al., 2000).

Here we present the results of a study investigating the abundance, composition and significance of hopanoic acids in a suite of thirty Cretaceous, Jurassic and Triassic sedimentary rocks of varying maturity. The paper documents the wide occurrence of these compounds in Mesozoic sedimentary rocks, their abundance in immature samples, and presents evidence that they are not the products of weathering of outcrop samples but represent preserved early diagenetic products of bacteriohopanepolyols. The relationships between hopanoic acids and the more commonly analysed hopane biomarkers are explored.

2. Materials and methods

2.1. Samples

Thirty sedimentary rock samples from eleven localities were used in this study (Table 1), selected to cover a range of ages (Cretaceous to Triassic) and maturity levels (immature to early oil window). Most of the samples were collected from outcrop (typically from ca. 10 cm subsurface). However, in order to study the effects of surface weathering upon hopanoic acids, two samples from a core (Aptian, Cismon, Italy) were compared with samples from the same interval (the Livello Selli) from a nearby outcrop. In addition, surface/subsurface (ca. 10 cm depth) outcrop sample pairs were collected from the Lias (Lyme Regis, Dorset, UK) and the Kimmeridgian (Kimmeridge, Dorset, UK).

Whole rock samples were cleaned of any weathered surfaces (except for the surface samples from Lyme Regis and Kimmeridge used in the weathering study), and crushed to a fine powder in a Tema disc mill. Total carbon, carbonate carbon and total organic carbon (TOC; measured after carbonate dissolution by HCl) were determined using a Leco WR12 Carbon Determinator.

2.2. Molecular analysis

Powdered rock samples (10–50 g) were solvent extracted in a Soxhlet apparatus (200 ml dichloromethane/methanol 93:7, 24 h) containing activated copper turnings to remove elemental sulphur. The extracts were rotary evaporated to near-dryness, transferred to vials and blown dry under nitrogen. Known amounts of two internal standards (5β-cholanic acid and androstane) were added to each extract.

The extracts were fractionated into neutral and acid fractions using the method of McCarthy and Duthie (1962). The column was prepared using silicic acid (5 g), isopropanol-KOH (10 ml) and diethyl ether (30 ml). The extract, dissolved in a small amount of dichloromethane and diethyl ether, was added to the column, and the neutral fraction eluted through with diethyl ether (150 ml). The acid fraction, retained on the column, was then eluted using 2% formic acid in diethyl ether (50 or 100 ml) followed by diethyl ether (100 or 50 ml). Both fractions were reduced by rotary evaporation, transferred to vials and blown dry under nitrogen. [Note: for one batch of samples (comprising half of the suite) chloroform was substituted for diethyl ether throughout the above procedure; reproducibility was confirmed by two samples that were run through both methods.]

The neutral fraction was further separated by thin layer chromatography (TLC; Kieselgel 60G, 0.5 mm thick, 20×20 cm). Half of the samples were separated using dichloromethane as developer to obtain "hydrocarbon", "ketone" and "alcohol" fractions; this was to allow examination of the samples for the presence of hopanols. No hopanols were found, in even the most immature samples, so the remaining samples were separated using petroleum ether as developer, to provide "aliphatic" and "aromatic" hydrocarbon fractions. If the mass of neutral fraction was small (<10 mg) an aliphatic hydrocarbon fraction was simply obtained using a miniature packed silica gel column made in a Pasteur pipette, eluting through with a few ml of petroleum ether.

The acid fraction was methylated prior to analysis, by refluxing with boron trifluoride/methanol (15 ml, 14% BF_3) in chloroform/methanol (2:1, 10 ml) for 1 h. Distilled water (25 ml) was added, and the mixture extracted in a separating funnel using chloroform (3×15 ml). The extracts were rotary evaporated, transferred to vials and blown dry ready for analysis.

The methylated acid and aliphatic (or total) hydrocarbon fractions were analysed by gas chromatography–mass spectrometry (GC–MS) using a Hewlett-Packard 5890 II GC equipped with a 7673 autosampler and

linked to a Hewlett-Packard 5972 MSD (electron voltage 70 eV; filament current 220 μA; source temperature 160 °C; interface temperature 300 °C) operated in selected ion monitoring mode. The hydrocarbon fractions were analysed using an HP-1 column (60 m×0.32 mm i.d.; 0.25 μm film thickness), whilst the methylated acid fractions were analysed using either a DB5-HT column (15 m×0.25 mm i.d.; 0.1 μm film thickness) or the same HP-1 column as for the hydrocarbons. Two samples were analysed in full scan mode to support the compound identifications made from mass chromatographic responses and relative retention times in comparison with the literature. Individual hopanoids were quantified from their peak area responses in the *m/z* 191 mass chromatogram relative to the responses of androstane (for hopanes) or 5β-cholanic acid (for hopanoic acids) in the m/z 217 mass chromatogram, assuming relative response factors of unity (except for C_{30} hopanes; these were divided by two to allow for the approximately double *m/z* 191 response).

3. Results and discussion

3.1. Hopanoic acid composition and abundance

Although the samples vary widely in organic carbon contents (<0.5–36%), maturity and geological age (ca. 89–235 Ma), hopanoic acids were detected in all but the two most mature samples. They typically range in carbon number from C_{28} to C_{35} (no C_{29}), with variable proportions of 17β(H),21β(H), 17β(H),21α(H) and 17α(H),21β(H) isomers, and 22S and 22R isomers for $\geqslant C_{30}$ compounds (Figs. 1 and 2; Table 2). Total hopanoic acid abundances range up to 233 ppmTOC (67 ppm of rock; Table 3).

Table 1
Sample codes, ages, localities and bulk geochemical data

Code	Age	Locality	TOC (%)	$CaCO_3$
BC4	Cenomanian/Turonian (Cret.)	Buckton Cliff, Yorkshire, UK	2.4	71
SF6B	Cenomanian/Turonian (Cret.)	South Ferriby, Yorkshire, UK	2.1	62
OB7	Cenomanian/Turonian (Cret.)	Oued Bahloul, Tunisia	5.0	76
OB8	Cenomanian/Turonian (Cret.)	Oued Bahloul, Tunisia	2.2	64
DOY8	Cenomanian/Turonian (Cret.)	Dir Oulad Yahia, Tunisia	6.2	19
DOY25	Cenomanian/Turonian (Cret.)	Dir Oulad Yahia, Tunisia	1.5	75
AS19	Aptian (Cret.)	Apecchiese, Italy	7.0	3
AS27	Aptian (Cret.)	Apecchiese, Italy	11.1	2
CI10	Aptian (Cret.)	Cismon, Italy (outcrop)	2.4	41
CI13	Aptian (Cret.)	Cismon, Italy (core)	0.7	46
CI14	Aptian (Cret.)	Cismon, Italy (core)	0.8	49
CI33	Aptian (Cret.)	Cismon, Italy (outcrop)	4.8	45
K5	Kimmeridgian (Jur.)	Kimmeridge, Dorset, UK	14.1	17
K7	Kimmeridgian (Jur.)	Kimmeridge, Dorset, UK	8.2	4
K37	Kimmeridgian (Jur.)	Kimmeridge, Dorset, UK	36.1	18
KS[a]	Kimmeridgian (Jur.)	Kimmeridge, Dorset, UK	2.2	7
KF[b]	Kimmeridgian (Jur.)	Kimmeridge, Dorset, UK	2.3	9
KBS1[b]	Kimmeridgian (Jur.)	Kimmeridge, Dorset, UK	10.2	27
KBS2[a]	Kimmeridgian (Jur.)	Kimmeridge, Dorset, UK	10.1	27
X-BG	Aalenian (Jur.)	Bearreraig Bay, Isle of Skye	1.2	2
JR6	Toarcian (Jur.)	Port Mulgrave, Yorkshire, UK	3.9	24
JR11	Toarcian (Jur.)	Port Mulgrave, Yorkshire, UK	11.2	10
LR2	Blue Lias (Jur.)	Lyme Regis, Dorset, UK	3.4	4
LR3	Blue Lias (Jur.)	Lyme Regis, Dorset, UK	5.3	2
BLS[a]	Blue Lias (Jur.)	Lyme Regis, Dorset, UK	4.7	27
BLD[b]	Blue Lias (Jur.)	Lyme Regis, Dorset, UK	4.8	24
G84	Upper Anisian (Trias.)	Monte San Giorgio, Switzerland	0.2	38
G88	Upper Anisian (Trias.)	Monte San Giorgio, Switzerland	1.1	16
G115	Upper Anisian (Trias.)	Monte San Giorgio, Switzerland	24.9	9
G132	Upper Anisian (Trias.)	Monte San Giorgio, Switzerland	28.6	5

TOC = Total Organic Carbon (%); $CaCO_3$ = Calcium carbonate (%), calculated from carbonate carbon content; Cret. = Cretaceous; Jur. = Jurassic, Trias. = Triassic.

[a] Surface sample (0–2 cm depth) for weathering comparison.

[b] Subsurface sample (ca. 10 cm depth) for weathering comparison.

The more mature samples (%22S C_{31} αβ-hopane maturity parameter of >30%) contain lower amounts of hopanoic acids relative to the hopanes (hopanoic acids/hopane ratio; Fig. 3; Table 3; note that no account has been taken of differences in relative response of the internal standards and the different hopanoid species). Indeed, the two most mature samples (Toarcian, Yorkshire) were the only ones in which hopanoic acids were not detected. The immature samples (%22S C_{31} αβ-hopane maturity parameter of <30%) display a very wide range of acid/hopane ratios, from a dominance of hopanoic acids (e.g. SF6B, CI13, CI14; see Table 3) to a dominance of hopanes (e.g. LR2 and 3). The influence of maturity upon the concentration of hopanoic acids (ppm TOC) is less apparent (Fig. 3), with greater scatter in the concentration data than in the hopanoic acids/hopanes ratio. Furthermore, one of the Triassic samples from Switzerland displays an anomalously high concentration of hopanoic acids, which is matched by a high concentration of hopanes (not shown), resulting from unusually high bacterial contribution to the sedimentary organic matter (McEvoy and Giger, 1986). Previous studies documenting the response of hopanoic acid abundance to increasing maturity, either as a function of natural burial (Jaffé and Gardinali, 1990) or rapid heating by an igneous intrusion (Bennett and Abbott, 1999), differ in their observations. Bennett and Abbott (1999) documented a progressive decline in hopanoic acid concentration with maturation towards and through the equivalent of the oil window (and an initial increase in hopanes, before they also fell in concentration). In contrast, Jaffé and Gardinali (1990) reported evidence of an initial increase in hopanoic acid concentration with burial, prior to the oil window, possibly associated with the release of loosely bound hopanoic acids. The sample suite in the present study is not so well suited to study the effects of maturity, but it is clear that other factors in addition to maturity must influence the hopanoic acid concentration, at least at low maturities.

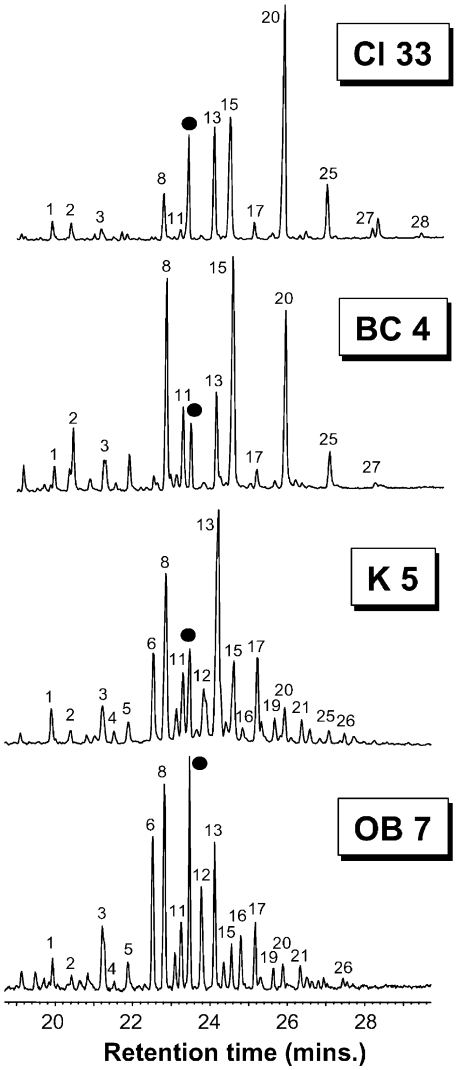

Fig. 1. *M*/*z* 191 mass chromatograms of the methylated acid fractions of four samples selected to show the compositional variability of the hopanoic acids (as methyl esters). Sample codes and compound assignments are given in Tables 1 and 2, respectively. ● = unidentified contaminant.

A-ring methylated hopanoic acids were detected in the Cenomanian/Turonian samples from Tunisia, and the Triassic samples from Switzerland. These sections have previously been shown to contain A-ring methylated hopanes (and methylated hopenes in the case of the Swiss Triassic; McEvoy and Giger, 1986; Farrimond et al., 1990), presumed to derive from cyanobacteria (Summons et al., 1999). Examination of the *m*/*z* 205 mass chromatogram, and appropriate molecular ion chromatograms (Fig. 4) shows that the methylated hopanoic acids coelute with their non-methylated analogues, and in accordance with our knowledge of the elution characteristics of A-ring methylated hopanes (Summons and Jahnke, 1990), we have assigned them as 2α-methyl hopanoic acids (αβ 22S & 22R; βα 22S & 22R isomers).

3.2. Effects of outcrop weathering upon hopanoic acid composition and abundance

Concern that hopanoic acids in the bitumen of outcrop samples could be at least partially derived from the effects of oxidative weathering [Petsch et al. (2001) observed an increase in carbonyl functional groups in the kerogen of heavily weathered shales], led us to compare the occurrence of hopanoic acids in a selected sub-suite of samples. Two sampling strategies were employed: firstly, exterior (surface) and interior (ca. 10 cm subsurface) pairs of samples from the Kimmeridgian

(Kimmeridge, UK; 2 pairs) and the Blue Lias (Lyme Regis, UK), and secondly two samples from a core (Aptian, Cismon, Italy; ca. 20 m subsurface) were compared with surface samples from the same interval (Livello Selli) from a nearby outcrop. The different extents of weathering were not quantified in any way, but the Kimmeridgian and Blue Lias samples were from relatively fresh coastal outcrops, with only thin (mm scale) oxidation surfaces. The Aptian (Cismon) outcrop samples were collected from a road cutting, beneath the zone of visible oxidation (i.e. a few cm subsurface), whilst the core samples represent totally unweathered material. None of these samples showed evidence of biodegradation effects on the hydrocarbon biomarker distributions (no elevated unresolved complex mixture, or unusual loss of lighter components); consequently,

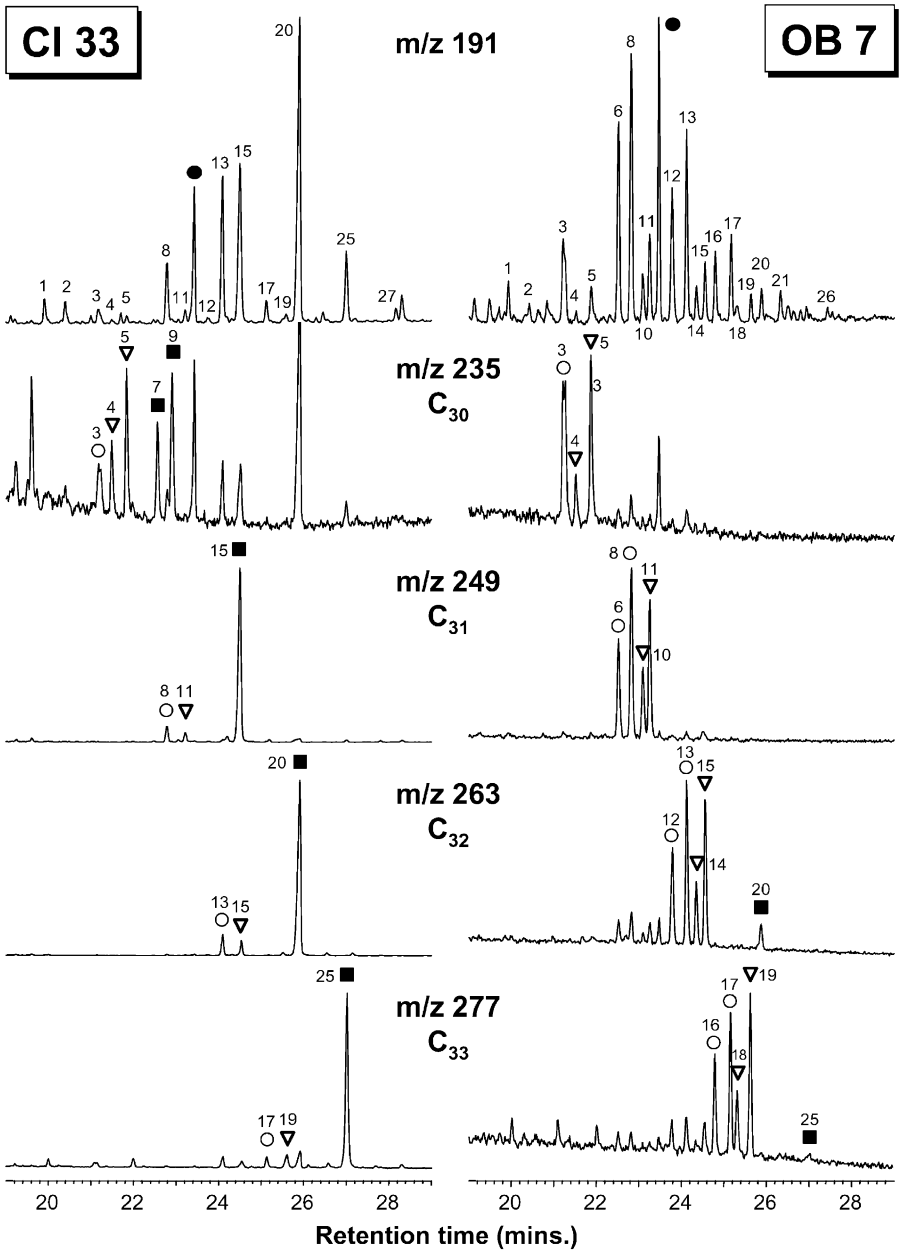

Fig. 2. M/z 191 mass chromatograms (top) showing the hopanoic acid distributions of two samples of differing maturity. The m/z 235, 249, 263 and 277 mass chromatograms represent the D/E-ring + side chain fragment ions of C_{30}, C_{31}, C_{32} and C_{33} hopanoic acid methyl esters, and the individual isomers of each carbon number are marked [○ = 17α(H),21β(H); (▽), = 17β(H),21α(H); ■ = 17β(H),21β(H)]. Peak assignments are given in Table 2. ● = unidentified contaminant.

we believe that none of the samples have been heavily weathered. In the following discussion, the more weathered samples will be termed "surface" and the less weathered equivalents will be termed "subsurface" for consistency.

If hopanoic acids are derived to a significant extent from oxidative effects of weathering, one would expect to see more acids in the surface samples. In fact, there are generally only minor differences in concentrations of hopanoic acids (ppm TOC) between the surface and subsurface samples (Fig. 5), the exception being the pair from the Lias, where the subsurface sample has a much lower concentration compared with its surface equivalent. The proportion of hopanoic acids to hopanes is also slightly higher in the surface Lias sample, compared to the subsurface equivalent (Fig. 5), although the difference is much less marked than for the hopanoic acid concentration. Comparison of the m/z 191 mass chromatograms (Fig. 6) for these two samples demonstrates that they are essentially identical in terms of hopanoic acid composition, which is confirmed by principal components analysis of the sample suite (not shown). This observation, coupled with the lack of an overall enrichment in hopanoic acids in the surface samples, is an indication that the hopanoic acids are not formed (to any recognisable extent) by subaerial weathering. The most convincing evidence of this comes from the core samples (Aptian, Cismon, Italy) that were collected from ca. 20 m below the surface, yet contain comparable hopanoic acid compositions and concentrations, and higher acid/hopane ratios, compared with similar samples collected from outcrop.

Table 2
Peak identifications for hopanoic acids

Hopanoic acids	
1.	C_{28} 17α(H),21β(H)
2.	C_{28} 17β(H),21α(H)
3.	C_{30} 17α(H),21β(H) 22S + 22R
4.	C_{30} 17β(H),21α(H) 22S
5.	C_{30} 17β(H),21α(H) 22R
6.	C_{31} 17α(H),21β(H) 22S
7.	C_{30} 17β(H),21β(H) 22S
8.	C_{31} 17α(H),21β(H) 22R
9.	C_{30} 17β(H),21β(H) 22R
10.	C_{31} 17β(H),21α(H) 22S
11.	C_{31} 17β(H),21α(H) 22R
12.	C_{32} 17α(H),21β(H) 22S
13.	C_{32} 17α(H),21β(H) 22R + C_{31} 17β(H),21β(H) 22S
14.	C_{32} 17β(H),21α(H) 22S
15.	C_{32} 17β(H),21α(H) 22R + C_{31} 17β(H),21β(H) 22R
16.	C_{33} 17α(H),21β(H) 22S
17.	C_{33} 17α(H),21β(H) 22R
18.	C_{33} 17β(H),21α(H) 22S
19.	C_{33} 17β(H),21α(H) 22R
20.	C_{34} 17α(H),21β(H) 22S + C_{32} 17β(H),21β(H) 22R
21.	C_{34} 17α(H),21β(H) 22R
22.	C_{34} 17β(H),21α(H) 22S
23.	C_{34} 17β(H),21α(H) 22R
24.	C_{35} 17α(H),21β(H) 22S
25.	C_{33} 17β(H),21β(H) 22R
26.	C_{35} 17α(H),21β(H) 22R
27.	C_{34} 17β(H),21β(H) 22R
28.	C_{35} 17β(H),21β(H) 22R

3.3. Comparison of hopanoic acid and hopane compositions

3.3.1. Isomeric distributions

The hopanoic acids display similar isomeric distributions to the hopanes; the more mature samples are dominated by αβ-hopanes and αβ-hopanoic acids, whilst the least mature are dominated by the ββ-isomers of both compound types. The comparable behaviour of hopanoic acids and hopanes with respect to maturity has been reported previously by Bennett and Abbott (1999), and is apparent in the present study from cross plots of matching maturity parameters (Fig. 7; using C_{32} homologues for hopanoic acids and C_{31} homologues for the hopanes, because of their potential relationship via decarboxylation; Bennett and Abbott, 1999). However, the %ββ and %22S αβ maturity parameters exhibit consistently higher and lower values, respectively, for the hopanoic acids than for the hopanes, indicating that isomerisation has been relatively suppressed for the functionalised compounds [previously noted for nuclear isomerisation by Schaeffer et al. (1993) in Oligocene evaporites].

An explanation for the reduced extents of isomerisation of hopanoic acids compared to the hopanes may lie within the mechanisms by which these species are preserved or generated during sediment burial. Bennett and Abbott (1999) demonstrated a progressive decline in hopanoic acid concentration with increasing maturity, whilst hopane concentrations initially rose, suggesting that hopanes were being formed by decarboxylation. In contrast, Jaffé et al. (1992) observed a parallel increase in both hopane and hopanoic acid concentrations with increasing maturity in a well from Venezuela; although this observation might have been related to variation in facies, an alternative explanation was proposed to be the release of trapped hopanes and hopanoic acids during early stage maturation of the kerogen structure. Hopanes can be formed during maturation from a variety of hopanoid precursors, including hopanoic acids, but also more complex components such as asphaltenes and kerogen, through the cleavage of functional groups and/or heteroatomic (oxygen and/or sulphur) linkages to macromolecules. An important contribution to the isomerisation at C-22, and potentially C-17 and 21 in

the hopane E-ring, seems to be associated with the process of bond cleavage (Farrimond et al., 1998b), as kerogen-bound hopanoids have been shown to be less extensively isomerised in comparison with free hopanes in the same sample (Seifert and Moldowan, 1980; Peters et al., 1990; Murray et al., 1998). Similarly, decarboxylation may promote isomerisation through bond cleavage and the formation of a reactive intermediate. Thus, the lower extent of isomerisation of hopanoic acids observed in our suite of samples can be explained, if they mainly represent components preserved intact since their formation during early diagenesis (e.g. Innes et al., 1997, 1998 and references therein), rather than species that are released by bond cleavage (e.g. from macromolecular organic matter) during maturation. The clear and steady decline in hopanoic acids during maturation observed by Bennett and Abbott (1999) would support this suggestion, and the apparent release of trapped (immature) hopanoic acids at high maturity levels observed by Jaffé et al. (1992) is also consistent with our interpretation.

Hopanoic acids could be preserved in sediments (at least in part) through relatively weak ionic adsorption to mineral surfaces (e.g. Thomas et al., 1993; Edwards et al., 1996; Kubicki et al., 1999) and/or polar organic matter (e.g. asphaltenes and kerogen). Some of these adsorbed hopanoic acids are extractable in solvent, and are thus detected in our analytical protocol. The results of Jaffé et al. (1992) suggest that some hopanoic acids may be physically trapped within, or more tightly adsorbed onto, macromolecular organic matter, and that at high maturities (greater than the samples studied here) they may be released into the bitumen, preserving their immature isomeric composition. Preservation of the immature stereochemistry of trapped or tightly adsorbed hopanes has been observed in a study of stepwise supercritical carbon dioxide extraction (Jaffé et al., 1997). Even in an adsorbed form, hopanoic acids might

Table 3
Abundances of total (resolved and identified) hopanoic acids and hopanes, and calculated hopanoic acids/hopanes ratio

Code	Locality	Acids ppm rock	Acids ppm TOC	Hopanes ppm rock	Acids/ hopanes
BC4	Buckton Cliff, Yorkshire, UK	2.8	115	1.8	1.57
SF6B	South Ferriby, Yorkshire, UK	3.4	164	1.2	2.98
OB7	Oued Bahloul, Tunisia	3.4	68	174.4	0.02
OB8	Oued Bahloul, Tunisia	0.9	41	22.1	0.04
DOY8	Dir Oulad Yahia, Tunisia	3.0	48	154.4	0.02
DOY25	Dir Oulad Yahia, Tunisia	0.5	31	21.1	0.02
AS19	Apecchiese, Italy	6.9	99	11.3	0.61
AS27	Apecchiese, Italy	3.4	31	13.0	0.26
CI10	Cismon, Italy (outcrop)	1.9	79	2.2	0.86
CI13	Cismon, Italy (core)	0.5	65	0.2	2.74
CI14	Cismon, Italy (core)	0.4	54	0.2	2.19
CI33	Cismon, Italy (outcrop)	3.0	64	2.1	1.46
K5	Kimmeridge, Dorset, UK	6.0	43	55.1	0.11
K7	Kimmeridge, Dorset, UK	2.9	35	10.9	0.26
K37	Kimmeridge, Dorset, UK	4.9	14	23.9	0.21
KS[a]	Kimmeridge, Dorset, UK	1.9	86	1.6	1.18
KF[b]	Kimmeridge, Dorset, UK	1.7	76	1.1	1.57
KBS1[b]	Kimmeridge, Dorset, UK	4.4	44	19.1	0.23
KBS2[a]	Kimmeridge, Dorset, UK	5.0	49	5.4	0.93
X-BG	Bearreraig Bay, Isle of Skye	1.0	81	1.6	0.62
JR6	Port Mulgrave, Yorkshire, UK	n.d.	n.m.	18.3	n.m.
JR11	Port Mulgrave, Yorkshire, UK	n.d.	n.m.	58.6	n.m.
LR2	Lyme Regis, Dorset, UK	2.6	77	9.2	0.28
LR3	Lyme Regis, Dorset, UK	1.2	22	5.8	0.20
BLS[a]	Lyme Regis, Dorset, UK	2.6	56	2.0	1.30
BLD[b]	Lyme Regis, Dorset, UK	0.7	15	0.9	0.83
G84	Monte San Giorgio, Switzerland	4.9	n.m.	103.8	0.05
G88	Monte San Giorgio, Switzerland	0.8	70	3.3	0.23
G115	Monte San Giorgio, Switzerland	9.5	38	163.5	0.06
G132	Monte San Giorgio, Switzerland	66.6	233	2638.9	0.03

n.d. = Not detected; n.m. = not measurable.

[a] Surface sample (0—2 cm depth) for weathering comparison.

[b] Subsurface sample (ca. 10 cm depth) for weathering comparison.

be expected to undergo some degree of isomerisation during burial, possibly associated with release/resorption processes, but not the high degree of isomerisation associated with cleavage of a bond in the side chain (as is inferred for the origin of the hopanes; see below). Ultimately (as in the case of the Toarcian samples from Yorkshire), the hopanoic acids will undergo thermally-induced decarboxylation (with associated isomerisation) to form hopanes (Bennett and Abbott, 1999), and conversion to other products, during progressive maturation.

3.3.2. Carbon number distributions

The C_{32} homologues are the most abundant hopanoic acids in the majority of the samples studied here, the exceptions being the Cenomanian/Turonian samples from Tunisia and Yorkshire, where the C_{31} compounds are most abundant (Fig. 8). The strong dominance of

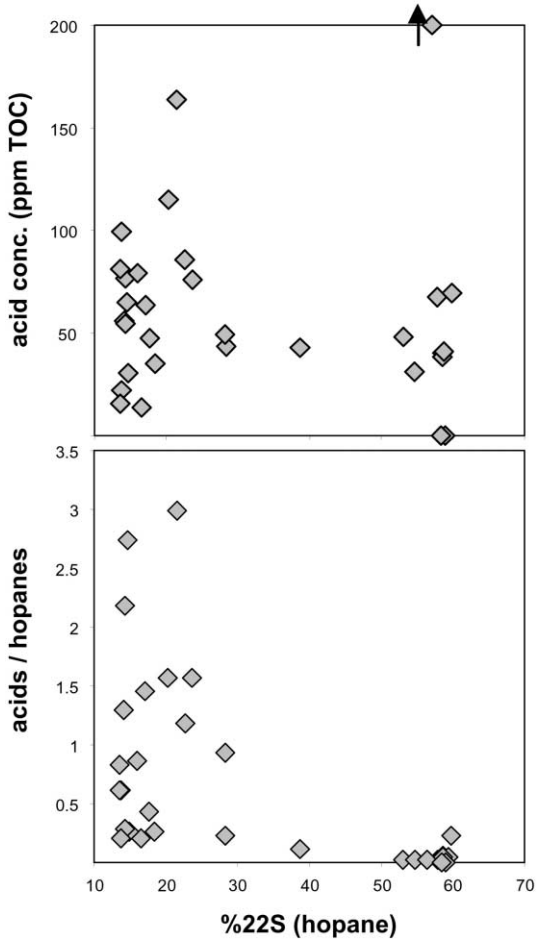

Fig. 3. Cross-plots showing the influence of sample maturity (measured using the %22S C_{31} αβ-hopane parameter) upon hopanoic acid concentration (ppm TOC; top) and the hopanoic acids/hopanes ratio (below).

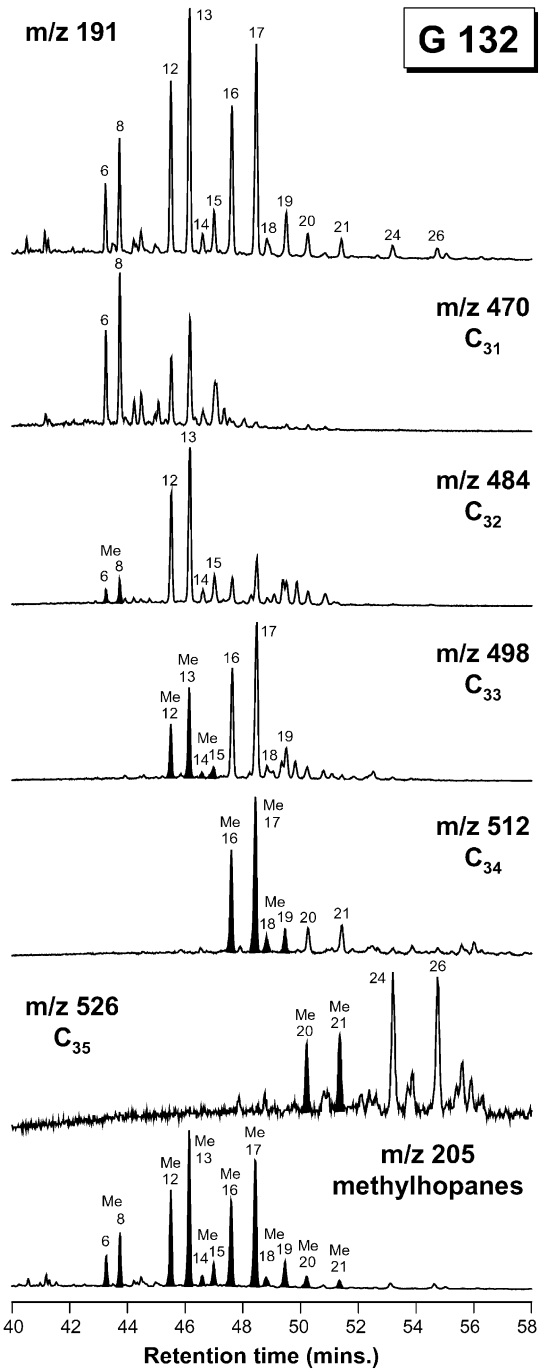

Fig. 4. An *m*/z 191 mass chromatogram (top) showing the distribution of hopanoic acids (as methyl esters) in a Triassic sample (Serpiano shale) from Switzerland. The *m*/*z* 470, 484, 498, 512 and 526 mass chromatograms represent the molecular ions of $C_{31–35}$ hopanoic acid methyl esters, including A-ring methylated hopanoic acids (shaded peaks; *m*/*z* 205) which are labelled "Me" and with the numerical code of their non-methylated equivalent hopanoic acid (Table 2).

the C_{32} homologues, particularly in the most immature samples, is in agreement with the carbon number distributions of hopanoic acids in modern sediments, where the C_{32} ββ component is usually dominant (e.g. Quirk et al., 1984; Buchholz et al., 1993; Innes et al., 1997, 1998), due to oxidative cleavage of the side chain of tetrafunctionalised biohopanoids between adjacent hydroxyl functionalities (Watson and Farrimond, 2000). Thus, the hopanoic acids extracted from the Mesozoic rock samples studied here are interpreted to represent preserved hopanoic acids formed during early diagenesis, that have undergone a degree of isomerisation, but little change in carbon number distribution. The greater proportion of C_{31} hopanoic acids in the Cenomanian/Turonian (Tunisia and Yorkshire) may relate to differences in hopanoid inputs to these sedimentary environments; biohopanoids with five or six functional groups in the side chain (e.g. from certain cyanobacteria and methanotrophs; Rohmer et al., 1984) would be expected to preferentially produce C_{31} and C_{30} hopanoic acids, respectively, during early diagenesis (rather than the dominant C_{32} hopanoic acid produced from tetrafunctionalised biohopanoids such as bacteriohopanetetrol; Innes et al., 1997).

For the hopanes, the immature samples have carbon number distributions maximising at C_{31} (Fig. 8; note that the C_{30} hopane response has been halved to compensate for the increased m/z 191 response due to the generation of two fragments of this mass in mass spectrometry). This compares well with the hopanoic acid distributions (which maximise at one carbon number higher), if we consider the potential relationship between hopanoic acids and hopanes via a decarboxylation reaction (i.e. including loss of a carbon atom; Bennett and Abbott, 1999), although the hopanes have a reduced predominance of the dominant homologue compared with the hopanoic acids, with a general shift towards lower carbon number hopanes (see Lias and Kimmeridge examples in Fig. 8). This observation of related carbon number distributions is at odds with the study by Barakat and Rullkötter (1994) where they found no match between the carbon number distributions of hopanoic acids and corresponding hydrocarbons in Miocene lacustrine sediments; however, their samples were highly immature and rich in sulphur, and previous studies have demonstrated the importance of sulphur incorporation in hopanoid diagenesis (Sinninghe Damsté et al., 1995; Köster et al., 1997). In the present study the hopane carbon number distributions of the immature samples (none of which are from particularly sulphur-rich environments) are consistent with a predominant origin *via* the defunctionalisation of early diagenetic hopanoids including C_{32} hopanoic acids and hopanols, C_{30} hopenes (diploptene and rearrangement products including hop-17(21)-ene) and C_{30}

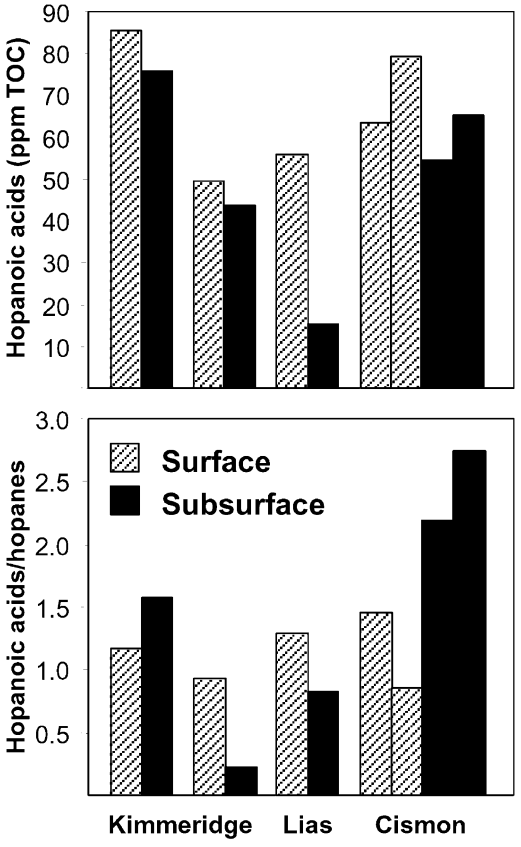

Fig. 5. Histograms comparing surface and subsurface samples in terms of their hopanoic acid concentrations (ppm TOC; top) and hopanoic acids/hopanes ratio (below).

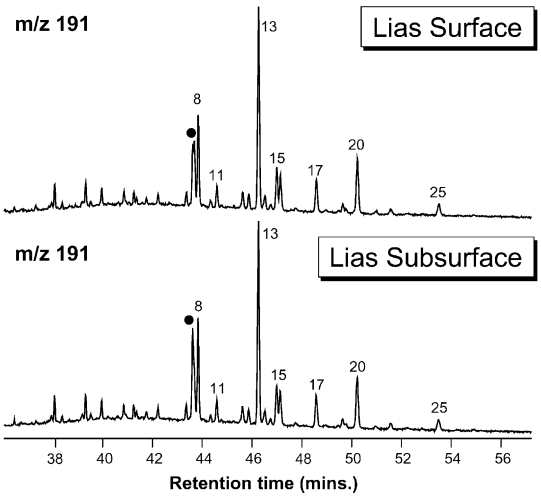

Fig. 6. M/z 191 mass chromatograms showing the hopanoic acid (methyl ester) distributions of surface and subsurface (ca. 10 cm depth) samples of the Blue Lias, Lyme Regis, UK. Peak assignments are given in Table 2. ● = unidentified contaminant.

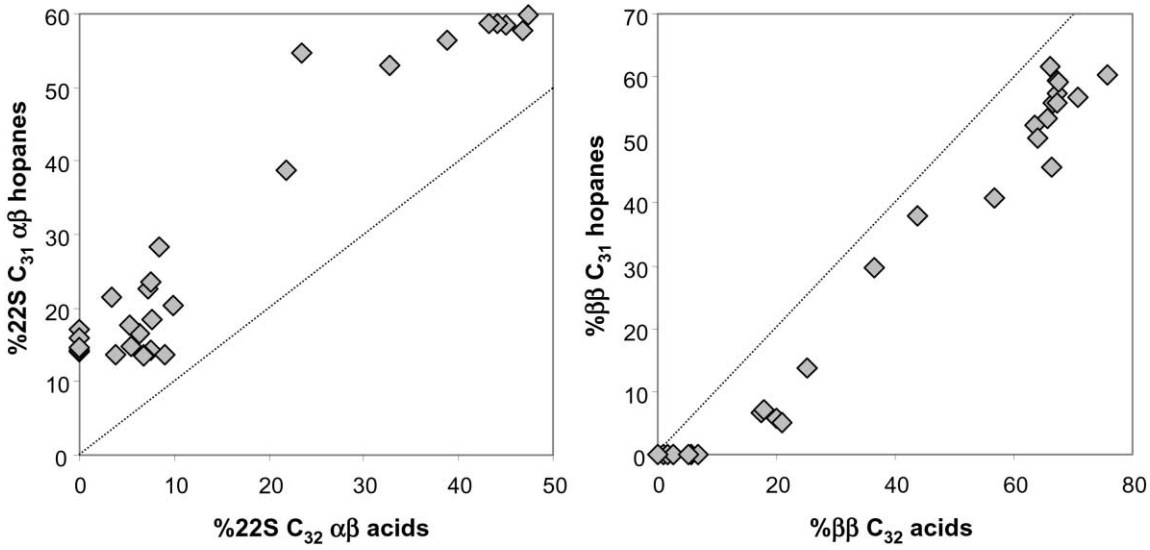

Fig. 7. Cross-plots of equivalent maturity parameters for the hopanoic acids and hopanes: %22S for C_{32} αβ hopanoic acids and C_{31} αβ hopanes (left), and %ββ for C_{32} hopanoic acids and C_{31} hopanes (right). Dashed lines indicate the trend for equal values of each parameter.

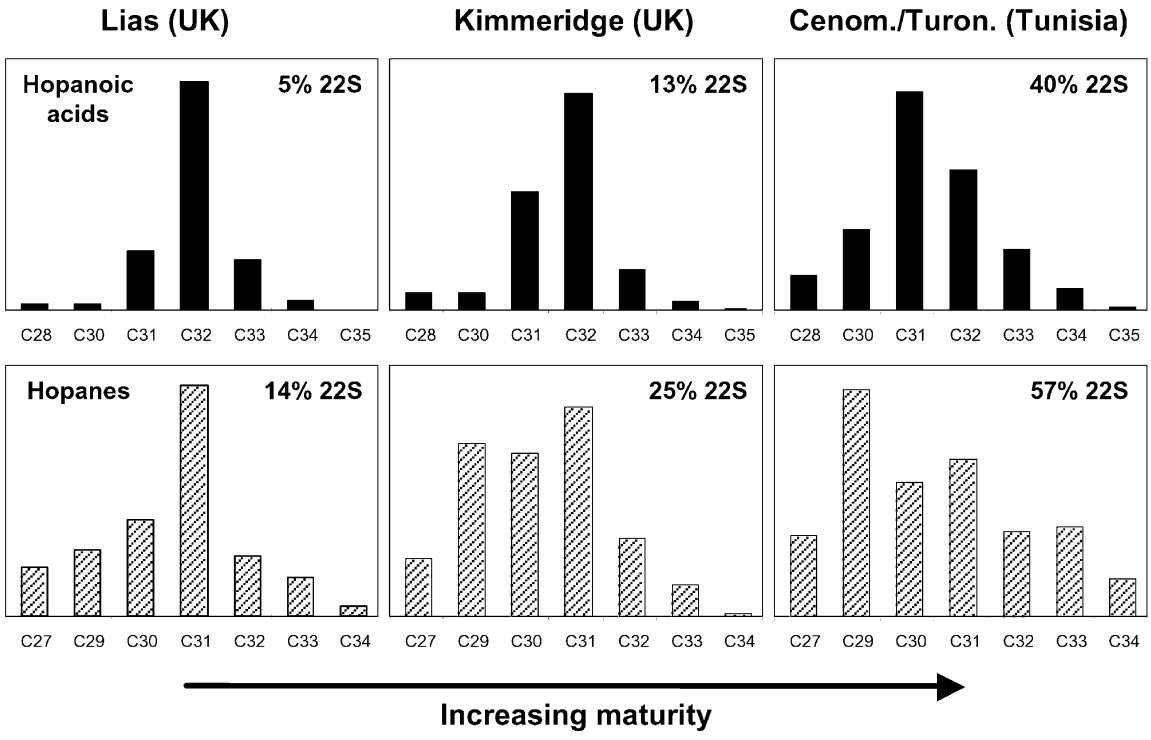

Fig. 8. Histograms showing the average carbon number distributions (sum of all isomers) for the hopanoic acids (top) and hopanes (below) in the Lias (4 samples), Kimmeridgian (7 samples) and Cenomanian/Turonian, Tunisia (4 samples). Average maturity levels are indicated by the %22S C_{32} αβ hopanoic acid and %22S C_{31} αβ hopane parameters.

hopanols (e.g. diplopterol), compounds that tend to dominate the geohopanoids of modern sediments (e.g. Innes et al., 1997, 1998 and references therein).

In the more mature samples (e.g. Cenomanian/Turonian, Tunisia; Fig. 8), the spread of hopane carbon numbers is greater, shifting to a dominance of C_{29} hopanes and a relative increase in higher homologues ($>C_{31}$). The hopane carbon number distribution no longer parallels that of the hopanoic acids, suggesting that the hopanes have been extensively derived from additional sources besides the hopanoic acids, hopanols and hopenes formed during early diagenesis. The most likely additional source of hopanes is the cleavage and release of hopanoids bound within macromolecular structures, mainly kerogen, and is consistent with their greater degree of isomerisation compared with the hopanoic acids. Previous workers have shown that hopanoids bound into macromolecules include abundant C_{35} components (e.g. Mycke et al., 1987; Hofmann et al., 1992; Richnow et al., 1992; Köster et al., 1997) representing incorporated biohopanoids; cleavage of these compounds from macromolecules, with a degree of associated side-chain shortening, would explain the increased proportion of C_{32+} hopanes in the mature samples (Fig. 8). The shorter hopane homologues ($<C_{31}$) can additionally be explained by side chain cleavage of bound C_{30} biohopanoids (e.g. diplopterol) and C_{32} geohopanoids during their release from macromolecules. The increased diversity in the carbon number distribution of hopanes with increasing maturity is consistent with an increasing degree of side chain cleavage associated with the generation of hopanes from macromolecularly-bound phases.

4. Conclusions

Free hopanoic acids were detected in all but the two most mature (near oil window) of the Mesozoic samples studied here, frequently in high abundance (up to 233 ppmTOC).

Comparison of the abundance and composition of hopanoic acids in samples from outcrop surface vs. subsurface (ca. 10 cm) or core (ca. 20 m subsurface) showed no notable differences, indicating that free hopanoic acids are not formed to any significant extent by subaerial weathering of these outcrops.

In each sample, the isomeric composition of the hopanoic acids is comparable to that for the hopanes, but molecular parameters (%ββ and %22S αβ) indicate that isomerisation is relatively enhanced for the hopanes compared with the hopanoic acids. The hopanes are interpreted to be formed predominantly by cleavage of bonds in the side chain of precursor species (e.g. decarboxylation of hopanoic acids and/or release of kerogen-bound hopanoids), with an associated increase in isomerisation.

The hopanoic acids of the least mature samples are dominated by C_{32} components, as seen in modern sediments, and are thus interpreted to represent preserved early diagenetic products.

Our data indicate that whilst the solvent-extractable hopanes in immature source rock samples may be extensively derived from hopanoic acids (and other early diagenetic products of biohopanoids, such as hopanols and hopenes), the hopanes of source rocks approaching oil window maturity have completely different carbon number distributions that owe more to side-chain cleavage during generation of macromolecularly-bound bio- and geohopanoids.

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