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Methylhopanoids: Molecular indicators of ancient bacteria and a petroleum correlation tool

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Abstract—Methylhopanoids are organic compounds synthesized by certain bacteria, that when preserved in sediments act as molecular fossils or biomarkers for organic matter inputs from specific bacterial sources. Two series of methylhopanoids occur, each mainly deriving from a distinct bacterial source: cyanobacteria (2-methyl) and methanotrophic bacteria (3-methyl). The abundance and composition of methylhopanoids within sediments of modern depositional environments varies widely, apparently due to different bacterial communities contributing to the sedimentary organic matter. Comparable molecular characteristics are found in oils and their source rocks. Consequently, methylhopanoids are valuable in oil-oil and oil-source rock correlations, distinguishing between samples related to different depositional environments. In particular, abundant 3β-methylhopanoids (from methanotrophic bacteria or an additional unknown bacterial source) are characteristic of some modern alkaline saline lake environments. Comparable compositional features in the methylhopanes of oils allow the assignment of lacustrine oils offshore West Africa to two distinct lacustrine source rock facies, and to distinguish between different marine source facies, thus refining oil-source rock correlation. *Copyright © 2004 Elsevier Ltd*

1. INTRODUCTION

Bacteria in modern sedimentary environments contribute organic matter to accumulating sediments; ancient bacteria did likewise, leaving recognizable organic compounds in sedimentary rocks to act as molecular fossils of past bacterial communities. In fact, the geological record of recognizably bacterially-derived organic matter extends back to the Archean (at least 2.7 billion years ago; Brocks et al., 1999; Summons et al., 1999). Foremost of the biologic marker compounds used for tracing bacteria in the geological record are the hopanoids, which are biosynthesized by a broad range of modern bacteria (Rohmer et al., 1984; Farrimond et al., 1998), and are ubiquitous in sediments and sedimentary rocks (Ourisson and Albrecht, 1992). In bacterial membranes these compounds take the form of bacteriohopanepolyols (BHPs), in which the C_{30} pentacyclic hopanoid skeleton is linked to a polyfunctional C_5 sugar-derived side chain (Fig. 1).

A relatively unusual structural feature of the BHPs of some bacteria is the presence of an additional methyl group (at C-2 or C-3) in the A-ring of the hopanoid skeleton (e.g., Rohmer et al., 1992; Summons and Jahnke, 1992; Fig. 1). 2β-MethylBHPs occur in many hopanoid-producing cyanobacteria, for which they seem to be good marker compounds (Summons et al., 1999), although 2-methylhopanoids lacking the C_5 side chain (i.e., 2β-methyldiploptene and/or 2β-methyldiplopterol) have been found in a Type II methanotrophic bacterium (*Methylobacterium organophilum;* Renoux and Rohmer, 1985) and in three nitrogen-fixing bacteria (Vilchèze et al., 1994; Bravo et al., 2001). 3β-MethylBHPs are absent from these bacteria, but have been reported in many Type I methanotrophs (Neunlist and Rohmer, 1985; Cvejic et al., 2000), and in some acetic acid bacteria (Simonin et al., 1994). Type I and II methanotrophic bacteria are phylogenetically distinct groups of methane-oxidizing bacteria that tend to predominate in different environments; Type I methanotrophs are dominant at low methane concentrations, such as in aquatic environments, with Type II methanotrophs generally being favored in soils and ground waters (Hanson and Hanson, 1996).

2-Methyl and 3-methylBHPs are present in modern sediments, but with increasing burial the reactive functional groups on the side chain are lost, leading to the formation of more stable methylhopanols and methylhopanoic acids, and ultimately the C_{28}-C_{36} methylhopanes (Fig. 1) that are reported in the geological record (Summons and Jahnke, 1990). In addition, the 2β-methyl stereochemistry inherited from the source bacteria undergoes rearrangement to the more stable 2α-methyl form. 2α-Methylhopanes have been reported in many ancient sediments, and have been used as indicators of cyanobacterial activity extending back to 2.7 billion years (Brocks et al., 1999). 3β-Methylhopanes have been less commonly reported in sediments, but when found they are typically ascribed to a methanotrophic bacterial origin, often supported by their light carbon isotopic composition (e.g., Ruble et al., 1994; Burhan et al., 2002). Summons and Jahnke (1992) noted that both methylhopane series are widespread (and possibly ubiquitous) in the sedimentary record, and noted that "methylhopanes will be particularly useful for oil-source rock correlation." Until now, this potential has gone largely unrealized.

Here we show that the composition of methylhopanes varies widely in oils and source rocks, and demonstrate their application in distinguishing between different oils, and in correlating oils with their source rocks. Furthermore, by comparison with the precursor methylBHPs in sediments of modern environments, we can relate methylhopanoid composition to spe-

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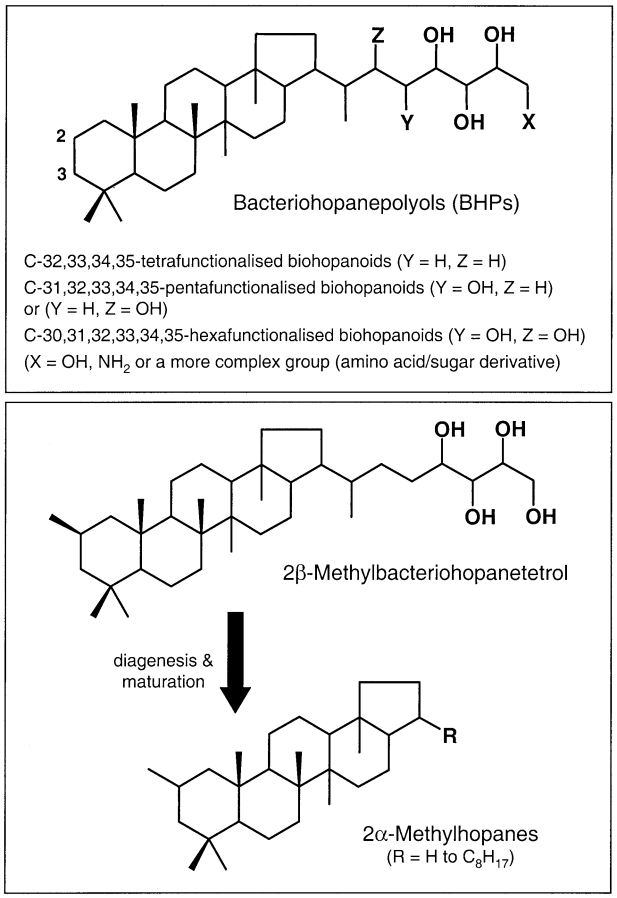

Fig. 1. Generalized structure of the bacteriohopanepolyols (top), with the carbon number positions of methylation (C-2 and C-3) for the methylhopanoids shown. Specific structure of 2β-methylbacteriohopanetetrol and the generalized structure of the 2α-methylhopanes (bottom).

cific depositional settings and the bacterial communities contributing to sedimentary organic matter.

2. SAMPLES AND METHODS

2.1. Samples

Two sample suites were analyzed: (1) a set of 64 modern sediment samples, and (2) an ancient sample suite comprising 58 oils and 42 source rocks. The modern sediments were from 38 localities, comprising 26 lakes (from UK, Germany, Norway, Switzerland, Spain, Kenya, Nigeria, Mexico and Antarctica; Talbot et al., 2003), 10 marine sites (in the Arctic Ocean, Gulf of Mexico, offshore Peru and the Benguela Upwelling; Watson, 2002) and two "transitional" settings (a fjord and a coastal lagoon; Farrimond et al., 2000; Talbot et al., 2003). These sediments are generally from near the sediment surface (sampling depths are given in Talbot et al., 2003), ranging between 0 and 26 cm subsurface for the lacustrine samples, 12–27 cm for the transitional environments, and 0–260 cm for the marine samples. The ancient sample set covered a wide range of marine and lacustrine environments, with oils from offshore West Africa (16), Germany (6), offshore Norway (10), Iran (20), and others (6; China, Java, Australia, Gulf of Suez, USA), and source rocks from offshore West Africa (21), offshore Norway (7) and others (14; Switzerland, Italy, Tunisia, UK, USA). The modern and ancient samples were analyzed using different protocols, outlined below.

2.2. Analysis of BHPs and methylBHPs

BHPs and methylBHPs were solvent-extracted from modern sediments using the method of Innes et al. (1997). They are not generally amenable to analysis by gas chromatography-mass spectrometry (GC-MS), even after derivatization (although bacteriohopanetetrol and 2β-methylbacteriohopanetetrol [Fig. 1] were able to be determined directly by GC-MS of acetylated extracts; Innes et al., 1997). Accordingly, we applied the technique of periodic acid oxidative side chain cleavage followed by sodium borohydride reduction (Rohmer et al., 1984; Innes et al., 1997) to convert the BHPs and methylBHPs to simple hopanols and methylhopanols that were easily analyzed by GC-MS following acetylation (acetic anhydride/pyridine; 1:1 v/v; 50°C for 1 h). This periodic acid/sodium borohydride treatment yields C_{32}, C_{31} and C_{30} hopanols from tetra-, penta- and hexafunctionalized BHPs, respectively (and corresponding methylhopanols from methylBHPs; see Innes et al., 1997). Preexisting hopanols and methylhopanols in the sediment samples were determined by analysis of a second aliquot of the extract that was merely acetylated before analysis; BHPs and methylBHPs were quantified by subtracting the preexisting hopanol or methylhopanol concentrations from those obtained after the periodic acid/sodium borohydride treatment. Hopanols and methylhopanols (as acetates) were analyzed by GC-MS using a Hewlett-Packard 5890 II GC linked to a Hewlett-Packard 5972 MSD operating in selected ion monitoring (SIM) mode (7 ions; m/z 191, 205, 221, 243, 245, 258, 260). Analytical conditions have been reported previously (Talbot et al., 2003). Compounds were identified by their mass chromatographic responses and relative retention times by comparison with samples

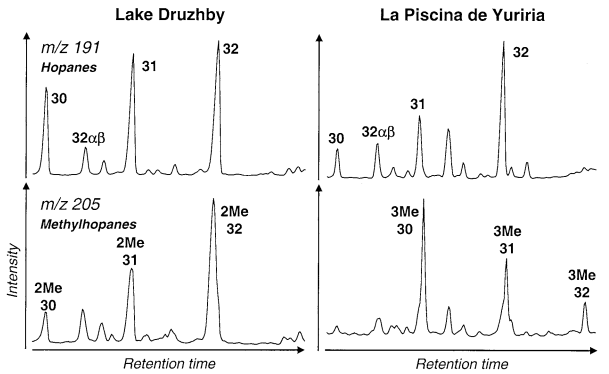

Fig. 2. M/z 191 and 205 mass chromatograms showing the distributions of hopanols and 2β- or 3β-methylhopanols (as acetates), respectively, following periodic acid/sodium borohydride treatment of solvent extracts of sediment samples from Lake Druzhby (Antarctica; 40–49 cm) and La Piscina de Yuriria (Mexico; 20–22 cm). Hopanols are labeled with their carbon number (17β(H),21β(H) isomers); 32$\alpha\beta$ = C_{32} 17α(H),21β(H) isomer; 2Me = 2β-methyl component; 3Me = 3β-methyl component. Note: Partial coelution of hopanols with some methylhopanols contributes to the m/z 205 response, raising the detection limit to around 0.1 μg/g TOC (Table 1).

Table 1. Ranges of bacteriohopanepolyol (BHP) and methylBHP concentration and composition in modern sediments.

Environment group	No.	BHP concentration[a] (μg/g TOC)			MethylBHP parameters		
		BHPs	2-Methyl BHPs	3-Methyl BHPs	2Me/BHPs (%)[b]	3Me/BHPs (%)[c]	3Me/2Me[d]
Lacustrine environments							
Temperate lakes	5	150–2100	2.4–37	<0.1–1.8	0.1–6.6	<0.1–0.3	<0.1–0.15
Spanish saline lakes							
Fresh-brackish	2	98–370	7.7–14	<0.1	3.8–7.8	<0.1	<0.1
Mesosaline	4	66–980	3.4–1100	<0.1	5.2–110	<0.1	<0.1
Hypersaline	5	10–200	0.7–44	<0.1	5.0–22	<0.1	<0.1
Playa lakes	1	460	4.4	50	1.0	11	11
Mount Kenya lakes	4	130–1300	2.9–13	<0.1	0.2–6.8	<0.1	<0.1
Mexican lakes	2	480–730	2.5–7.8	<0.1–170	0.5–1.1	<0.1–36	0–68
Antarctic lakes							
Eutrophic	2	180–250	10–11	<0.1	4.2–6.3	<0.1	<0.1
Ultraoligotrophic	1	510	53	<0.1	10	<0.1	<0.1
Transitional environments							
Lagoons	1	220	<0.1	<0.1	<0.1	<0.1	n.m.[e]
Fjords	1	290	2.2	<0.1	0.8	<0.1	<0.1
Marine environments							
Coastal upwellings	2	18–130	<0.1–1.0	<0.1	<0.1–0.7	<0.1	n.m.[e]
Gulf of Mexico	3	6.4–44	0.2–1.6	<0.1	2.9–3.7	<0.1	n.m.[e]
Arctic Ocean	5	4.0–45	<0.1–1.8	<0.1	<0.1–4.9	<0.1	n.m.[e]

Note: Data are for the surface or the most near-surface sample only for each locality (Talbot et al., 2003), shown as ranges for groups of samples comprising related depositional environments.

[a] Total concentration (relative to total organic carbon) of bacteriohopanepolyols (BHPs) lacking methylation at C-2 or C-3 or with methylation at C-2 (2-methyl) or C-3 (3-methyl). Detection limit ranges from 0.01 (hopanols) to 0.1 μg/g TOC.

[b] Total 2-methylBHPs expressed as a percentage relative to BHPs.

[c] Total 3-methylBHPs expressed as a percentage relative to BHPs.

[d] Total 3-methylBHPs divided by total 2-methylBHPs.

[e] n.m. = not measurable as compounds were not detected, or in very low abundance.

that were characterized using mass spectra. They were quantified using peak areas in the m/z 191 (hopanols) and 205 (methylhopanols) mass chromatograms, respectively, relative to the peak area response of the 5α-androstan-3β-ol internal standard in the m/z 243 mass chromatogram without applying relative response factors. The detection limit for methylBHPs (as methylhopanols after periodic acid/sodium borohydride treatment) is around 0.01 to 0.05 μg/g TOC, although this increases to around 0.1 μg/g TOC for the compounds that partially coelute with hopanols (due to contribution of the latter to the m/z 205 response). Bacteriohopanetetrol and 2β-methylbacteriohopanetetrol were quantified directly in the same way (but will also comprise part of the BHPs and 2β-methylBHPs determined after the periodic acid/sodium borohydride treatment).

2.3. Analysis of Hopanes and Methylhopanes

Powdered source rock samples were extracted using a Soxtec auto avanti 2050 system (dichloromethane/methanol, 93:7; immersion in boiling solvent for 1 h, followed by rinsing under reflux for 3 h). Oils and source rock extracts were deasphaltened using *n*-pentane before being fractionated using a semi-automated medium pressure liquid chromatography (MPLC) technique to provide aliphatic hydrocarbon fractions. These were analyzed by gas chromatography-mass spectrometry (GC-MS) in selected ion monitoring mode using a HP1901A-105 mass selective detector. The HP6890 GC was fitted with an Agilent split/splitless injector (300°C; constant helium flow) and two flexible silica capillary columns (50 m × 0.2 mm i.d. methyl silicone (HP-1); 0.33 μm film thickness), one leading to the mass spectrometer, and the other to a flame ionization detector. The carrier gas was helium. The oven was temperature programmed from 70°C (held for 2 min) to 150°C at 5°C/min (held for 1 min), then 150 to 325°C at 2°C/min with a final hold for 10 min. Methylhopanes were identified by their mass chromatographic responses and relative retention times in comparison with samples analyzed by full scan GC-MS and by GC-MS-MS (monitoring molecular ion to m/z 205 transitions; Varian CP3800/1200 Triple Quadrupole system fitted with a 60 m HP-1MS column) and with previous literature (e.g., Summons and Jahnke, 1990). Hopanes and methylhopanes were quantified from their peak area responses in the m/z 191 and 205 mass chromatograms (obtained by SIM analysis), respectively, relative to the peak area response of the 5β(H)-cholane internal standard (m/z 217).

3. RESULTS AND DISCUSSION

In this paper we focus on the abundance, composition and significance of the 2-methyl and 3-methylhopanoids in the

Table 2. Ranges of hopane and methylhopane concentration and composition in oils and source rocks.

Sample group	No.	Hopane or methylhopane concentration[a] (μg/g oil or extract)			Methylhopane parameters		
		Hopanes	2-Methyl hopanes	3-Methyl hopanes	2Me/hops (%)[b]	3Me/hops (%)[c]	3Me/2Me[d]
Oils							
Lacustrine							
Offshore West Africa	7	920–2600	27–160	31–210	1.6–8.9	2.4–11	0.5–4.9
China	1	760	20	17	2.6	4.3	0.8
Marine (or mixed)[e]							
Offshore West Africa	9	200–2700	15–100	12–72	2.8–7.3	1.8–6.4	0.3–1.1
Offshore Norway	10[f]	76–1100	2–35	1–14	2.4–5.0	1.1–2.5	0.4–0.8
Germany	6	300–1600	11–60	10–38	1.5–5.6	1.8–4.4	0.6–1.2
Iran	20	200–1000	17–170	9–31	6.1–23	2.5–6.9	0.1–0.8
Others	5	240–760	5–37	6–24	1.0–5.4	1.3–4.3	0.5–1.7
Source rocks							
Lacustrine							
Offshore West Africa	5	1300–2900	18–57	70–300	1.4–2.9	3.3–10	2.2–6.9
Green River (USA)	1	1400	18	200	1.3	14	11
Marine							
Offshore West Africa	16	270–2000	8–220	8–64	2.4–13	1.5–4.8	0.2–1.5
Offshore Norway	7	300–940	9–22	8–13	1.4–3.8	1.2–3.0	0.6–0.9
Kimmeridge Clay (UK)	1	110	3.0	1.8	2.7	1.7	0.6
Marl Slate (UK)	1	550	26	10	4.6	1.7	0.4
Toarcian (UK)	2	870–1100	15–20	16–22	1.7–1.8	1.9–2.1	1.1
Toarcian (Italy)	4	550–1000	13–130	5–9	2.4–14	0.6–1.1	0.1–0.5
Livello Selli (Italy)	1	70	2.2	1.9	3.1	2.7	0.9
Serpiano (Switzerland)	2	1100–2400	260–970	16–35	24–41	1.4–1.5	0.1
Bahloul Fm. (Tunisia)	2	1700–2200	130	32–38	6.0–7.5	1.8–1.9	0.2–0.3

[a] Total concentration (relative to oil or solvent extract weight) of hopanes and C-2 or C-3 methylhopanes for the sum of C_{29} to C_{33} $\alpha\beta$ (22S + 22R) homologues (or their methylated equivalents), expressed as ranges for groups of samples.
[b] Total 2-methylhopanes expressed as a percentage relative to total hopanes (C_{29} to C_{33} $\alpha\beta$ 22S + 22R).
[c] Total 3-methylhopanes expressed as a percentage relative to total hopanes (C_{29} to C_{33} $\alpha\beta$ 22S + 22R).
[d] Total 3-methylhopanes divided by total 2-methylhopanes.
[e] Four of the West African oils are inferred to have a mixed marine and lacustrine origin based on other parameters (Fig. 6).
[f] Not including replicate analyses of the standard oil NSO-1.

modern and ancient sample sets; information relating to the hopanoids is presented where appropriate for comparative purposes, but not discussed in detail. A more detailed discussion of the BHPs in many of the modern sediment samples can be found in earlier papers (Farrimond et al., 2000; Talbot et al., 2003).

3.1. Methylhopanoids in Modern Sediments

2β-MethylBHPs were detected (as 2β-methylhopanols following periodic acid side-chain cleavage; Fig. 2) in almost every modern sediment sample, including those from lakes, marine environments and a fjord (Table 1). They were most abundant in many of the lake sediments, both in terms of absolute concentration and relative to the BHPs. In the most extreme case (L. Grande, Spain) 2β-methylhopanoids were more abundant than BHPs, with 2β-methylbacteriohopanetetrol being the most abundant hopanoid in the sediment (Talbot et al., 2003). Other saline lakes from Spain (Muerte and Chica), and the ultraoligotrophic Lake Druzhby from Antarctica (Fig. 2), also contained high relative proportions of 2β-methylBHPs. Analysis of BHPs in bacterial cultures (Summons et al., 1999, and references therein) indicates a likely cyanobacterial origin for these 2β-methylBHPs. Indeed, cyanobacterial mats have been reported in both L. Druzhby (Laybourn-Parry and Bayliss, 1996) and Salina de la Muerte (Guerrero and de Wit, 1992), and the stratified nature of Laguna Grande (and other saline lakes in Spain) will favor development of cyanobacteria such as *Oscillatoria* at the oxic/anoxic interface (Miracle et al., 1992). Talbot et al. (2003) reported abundant 2β-methylBHPs in *Oscillatoria amphigranulata*.

In contrast to the wide distribution of 2β-methylBHPs in modern sediments, 3β-methylBHPs were detected at just three localities, and were only abundant in an alkaline playa lake (Jikariya, Nigeria) and an alkaline crater lake (La Piscina de Yuriria, Mexico; Table 1; Fig. 2). However, another alkaline crater lake from Mexico (Laguna de Zempoala) and the four Spanish lakes noted to be alkaline did not contain detectable 3β-methylBHPs. The most likely known source of these compounds in environmental settings are the Type I methanotrophic bacteria (Cvejic et al., 2000) that utilize methane generated in lake sediments by methanogenic bacteria. Such bacteria might be expected to be widespread in the lakes studied here. Indeed, significant contribution to the sedimentary organic matter of many of the

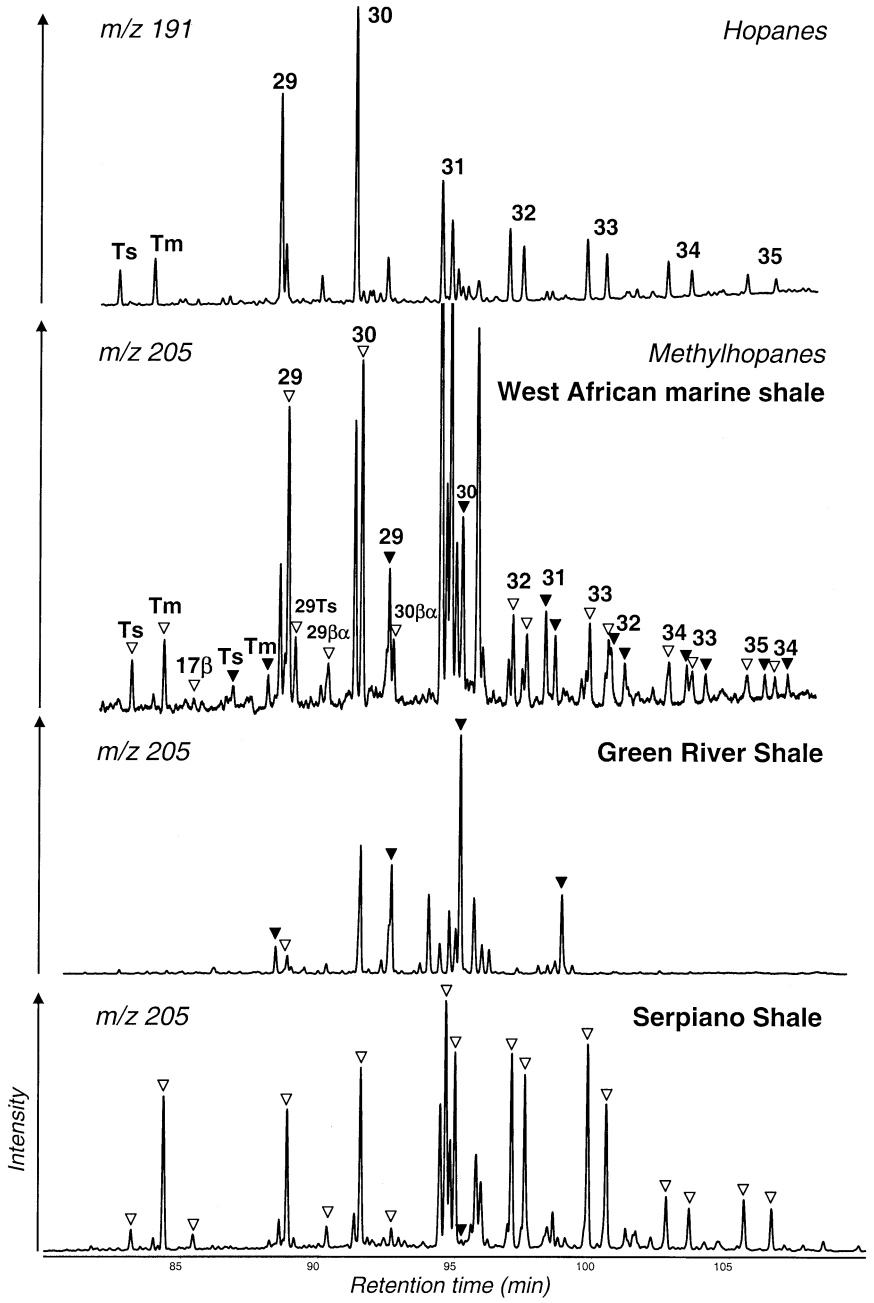

Fig. 3. M/z 191 and 205 mass chromatograms showing the hopane and 2α- and 3β-methylhopane distributions of a marine shale from offshore West Africa (upper two panels) and the 2α- and 3β-methylhopane distributions of samples of the Green River Shale (USA) and Serpiano Shale (Switzerland). The carbon numbers of the 17α(H)-hopanes are marked; Ts = 18α(H)-22,29,30-trisnorneohopane; Tm = 17α(H)-22,29,30-trisnorhopane; 17β(H) = 17β(H)-22,29,30-trisnorhopane. 2α-Methyl (open triangles) and 3β-methylhopanes (black triangles) are similarly labeled, with the carbon number not including the additional methyl group; $\beta\alpha$ = 17β(H),21α(H)-hopanes.

lakes studied here (including L. Zempoala) by Type I methanotrophic bacteria has been previously identified from abundant hexafunctionalized BHPs (Talbot et al., 2003)—yet most of these lake sediments do not contain 3β-methyl-BHPs. Methanotrophic bacteria live under microaerophilic conditions, and in aquatic sedimentary environments they will be most active around the interface between aerobic and anaerobic zones (Hanson and Hanson, 1996). At most of the sites studied here oxygen is present in only low concentration at or just below the sediment surface, so our surface or near-surface sampling would not bias against the detection of methanotroph hopanoids. Indeed, the three localities at which 3Me-BHPs were detected (Lakes Jikariya and Yuriria, and Loch Ness) were all sampled at the sediment surface (extending a maximum of 5 cm subsurface). Interestingly, the sediment samples from Jikariya and Yuriria that contain

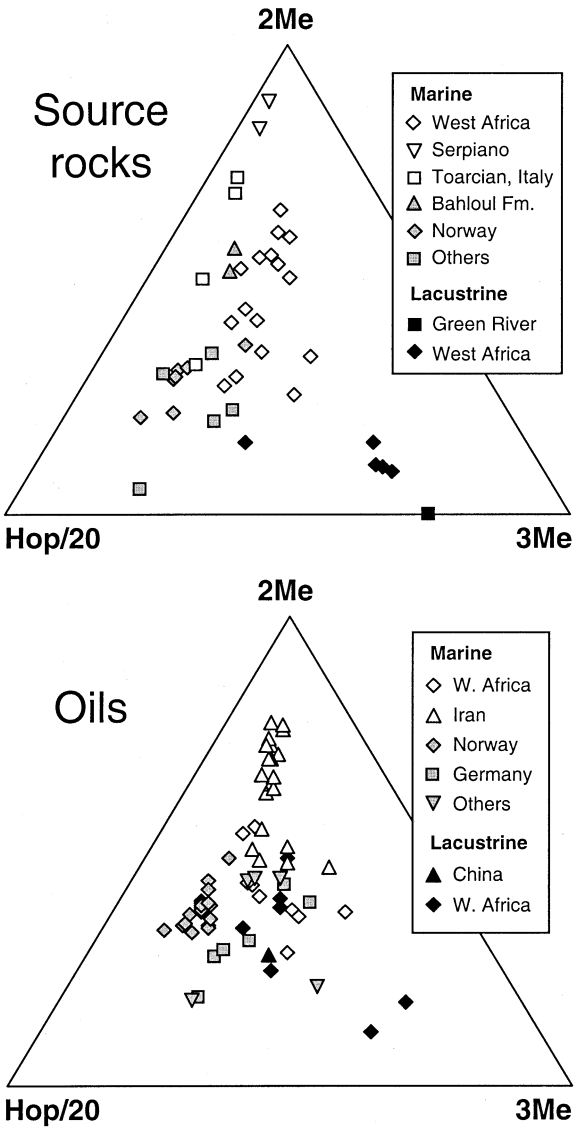

Fig. 4. Ternary plots showing the relative abundance of 2α-methyl-17α-hopane (2Me 30$\alpha\beta$), 3β-methyl-17α-hopane (3Me 30$\alpha\beta$) and 17α-hopane (30$\alpha\beta$) (divided by 20 for appropriate scaling of the diagram) in the source rock samples (top) and oils (bottom). The two methylhopanes are quantified from the m/z 205 mass chromatogram, and the hopane from the m/z 191 mass chromatogram.

3β-methylBHPs do not contain particularly abundant hexafunctionalized BHPs (Fig. 2). Accordingly, it seems likely that 3β-methyl BHPs and hexafunctionalized BHPs have different Type I methanotroph sources, with abundant 3β-methylBHPs characterizing a restricted range of Type I methanotrophic bacteria. Alternatively, they may have some additional unknown bacterial source; but in either case, a high concentration of 3β-methyl BHPs in sediments appears to be restricted to quite specific conditions (i.e., some alkaline lakes).

3.2. Methylhopanes in Oils and Source Rocks

Both 2α- and 3β-methylhopanes were found in each of the 58 oils and 42 source rocks analyzed (Table 2). The source rocks cover a broad range of depositional conditions from lacustrine through marine (clastic to carbonate-rich); the source rocks for the oils cover a similarly broad range, including a terrestrially-sourced oil from Java. In addition to the sample set presented here, both series of methylhopanes also occur in a Nigerian oil from a deltaic source rock (data not included here as the analysis was conduced in another laboratory without the addition of the internal standard). The occurrence of methylhopanes in all these samples, including various lacustrine, deltaic and various marine depositional environments, suggests that both 2-methyl and 3-methylhopanes may be as ubiquitous in the geological record as the hopanes. They occur as series extending from C_{28} through C_{36} with carbon number distributions comparable with those of the hopanes, including methylated analogues of the rearranged hopanes Ts and 29Ts (Fig. 3) and, when present, 28,30-bisnorhopane (see Farrimond et al., 1990). The occurrence of the 3β-methylhopanes in every oil and source rock sample analyzed contrasts markedly with the apparently very restricted distribution of 3β-methylBHPs in modern sediments; this is probably due to a lower detection limit for the hydrocarbons, suggesting that 3β-methylBHPs may actually occur widely in modern sedimentary environments, but in relatively low concentrations.

The concentrations of the two series of methylhopanes varied widely within the data set, as did their abundance relative to the hopanes (Table 2). Methylhopanes were always less abundant than the hopanes, and in general the 3β-methylhopanes (0.6 to 14% of the hopanes) were less abundant than the 2α-methylhopanes (1 to 41%). However, the relative proportions of the two series also varied widely, as shown by the ratio

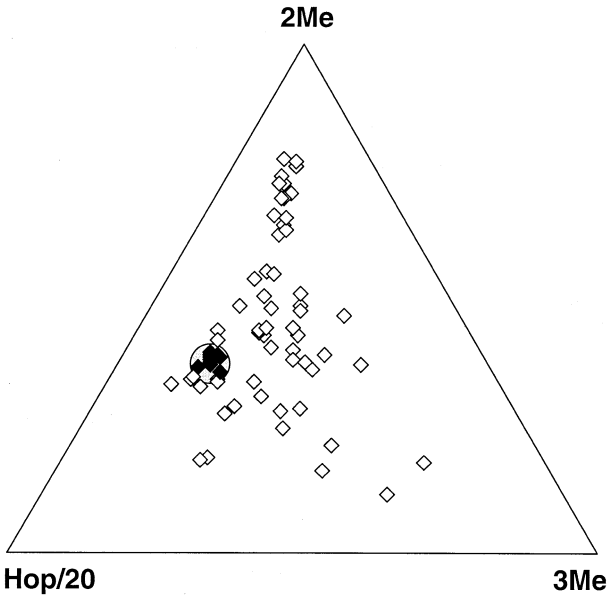

Fig. 5. A ternary plot showing the relative abundance of 2α-methyl-17α-hopane (2Me 30$\alpha\beta$), 3β-methyl-17α-hopane (3Me 30$\alpha\beta$) and 17α-hopane (30$\alpha\beta$) (divided by 20 for appropriate scaling of the diagram) in the ten replicate analyses of a reference oil (NSO-1; Oseburg field, North Sea; black diamonds) in comparison with the suite of studied oils (open diamonds). The two methylhopanes are quantified from the m/z 205 mass chromatogram, and the hopane from the m/z 191 mass chromatogram.

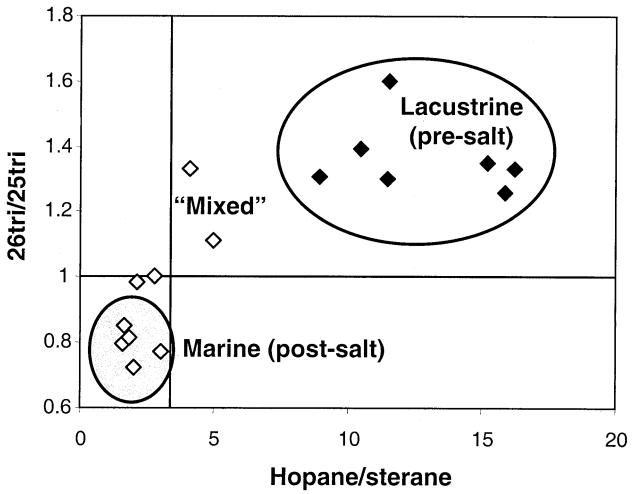

Fig. 6. Classification of the offshore West African oils using a cross-plot of the C_{26}/C_{25} tricyclic terpane and hopane/sterane ratios (Burwood, 1999; Schiefelbein et al., 1999). The hopane/sterane ratio uses Ts, Tm, 28,30-bisnorhopane, $C_{29}\alpha\beta$, $C_{29}\beta\alpha$, $C_{30}\alpha\beta$, $C_{30}\beta\alpha$, and C_{31} to C_{35} $\alpha\beta$ 22S+22R hopanes, and C_{27} to C_{30} $\alpha\beta\beta$ 20S+20R steranes. Several oils cannot be confidently assigned to either a lacustrine (presalt) or marine (postsalt) source, and are denoted as "mixed."

of 3β-methylhopanes/2α-methylhopanes (Table 2) and the ternary plots showing the relative abundance of C_{30} 17α-hopane and its 2α-methyl and 3β-methyl counterparts (Fig. 4). The significance of the observed differences in methylhopane composition between different samples becomes clear by comparison with ten replicate analyses (over a period of months) of a reference North Sea oil sample (NSO-1; Oseburg field); the replication of the data is remarkable (Fig. 5), especially considering the low concentration of methylhopanes in this oil (total 2-methyl + 3-methyl C_{29-33} hopanes = 33 μg/g oil).

Within the source rocks, those deposited in lacustrine environments (Green River Shale and five samples from offshore West Africa) contained high concentrations of 3β-methylhopanes (Fig. 3), with correspondingly high 3-methyl/2-methyl ratios (Table 2). This characteristic is reminiscent of the dominance of 3β-methylBHPs in sediments of two of the modern alkaline lakes. Indeed, some phases of deposition of the Green River Shale occurred under alkaline conditions (e.g., Horsfield et al., 1994), and abundant 3β-methylhopanes have been reported previously, having very light carbon isotopic compositions indicative of an origin from methanotrophic bacteria (Collister et al., 1992; Ruble et al., 1994). Furthermore, the Observatory Hill Formation of Australia, which was deposited in a Cambrian continental alkaline playa lake, is also characterized by abundant 3-methylhopanes (Logan et al., 1997). The depositional environments of the lacustrine source rocks from offshore West Africa studied here are not well known, but varied from freshwater to saline (Burwood et al., 1995; Cole et al., 2000), and are likely to have included alkaline facies.

In contrast with the lacustrine source rocks, many of the marine source rocks were relatively enriched in 2α-methylhopanes (Fig. 4). Samples from the Triassic Serpiano shale (Fig. 3) contained the highest concentrations of these compounds (up to 970 μg/g extract; Table 2), an unusual characteristic that was previously noted by McEvoy and Giger (1986; although they wrongly assigned them as 3-methylhopanes). These shales are thought to have been deposited in a shallow reef-bound basin (McEvoy and Giger, 1986). 2α-Methylhopanes are also abundant in some of the Toarcian shales from Italy (Fig. 4). Previous workers have also reported 2-methylhopanes in Cenomanian/Turonian black shales of Italy (Farrimond et al., 1990) and the North Atlantic Ocean (Kuypers, 2002), with ratios relative to hopanes that are similar to those for the samples studied here (typically 10–15%, but up to at least 44%, not allowing for response factors of the different components used by different authors). By analogy with the distribution of 2-methylBHPs in modern sediments (this study; Talbot et al., 2003) and in bacteria, an origin from cyanobacteria (Summons et al., 1999; Kuypers, 2002) is most likely.

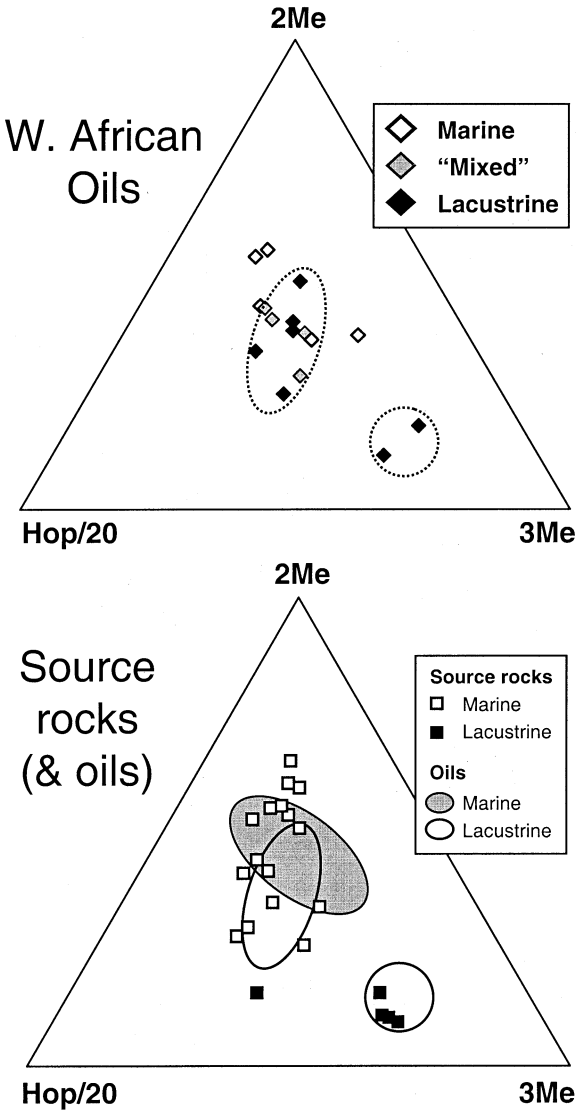

Fig. 7. Ternary plots showing the relative abundance of 2α-methyl-17α-hopane (2Me 30$\alpha\beta$), 3β-methyl-17α-hopane (3Me 30$\alpha\beta$) and 17α-hopane (30$\alpha\beta$) (divided by 20 for appropriate scaling of the diagram) in the West African oils (top) and source rocks (bottom). The lower plot also shows the areas of the lacustrine-sourced (open) and marine-sourced (shaded) oils for comparison with the source rocks.

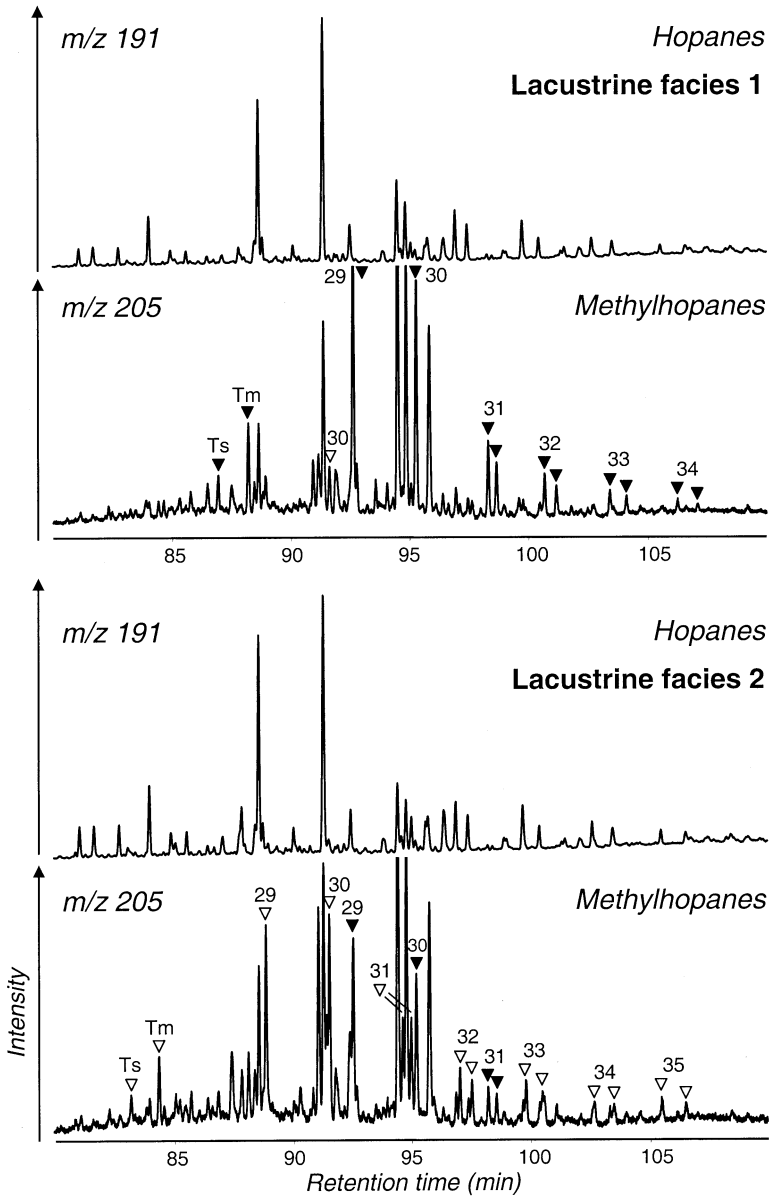

Fig. 8. M/z 191 and 205 mass chromatograms showing the distributions of hopanes and 2α- or 3β-methylhopanes in two oils from offshore West Africa: an oil from lacustrine facies 1 (upper two chromatograms) and an oil from lacustrine facies 2 (lower two chromatograms). The carbon numbers (excluding the additional methyl group) of the 2α-methyl-17α(H)-hopanes (open triangles) and 3β-methyl-17α(H)-hopanes (black triangles) are marked; Ts = 18α(H)-22,29,30-trisnorneohopane; Tm = 17α(H)-22,29,30-trisnorhopane.

Comparable variation in the composition of methylhopanes was seen in the oils (Fig. 4). Many of the oils from Iran contained high abundances of 2α-methylhopanes (both in absolute concentration and relative to 3β-methylhopanes and hopanes; Table 2); these oils are believed to have been sourced mainly from carbonate-rich rocks (Kazhdumi Formation; Bordenave and Burwood, 1990), where significant contributions from cyanobacteria to the sedimentary organic matter are likely. Several lacustrine-sourced oils from offshore West Africa were dominated by 3β-methylhopanes (Fig. 4), but interestingly, not all the West African lacustrine oils showed this dominance of 3β-methylhopanes; several contained equal, or slightly higher, amounts of 2α-methylhopanes (as did a Chinese lacustrine oil). Clearly, both marine and lacustrine-sourced oils show a range of methylhopane compositions, presumed to be dependent upon the nature of the bacterial communities contributing organic matter to their source rocks. These compositions, however, enable oil-oil and oil-source rock correlations to be made. For example, the oils from offshore Norway plot in the same area of the ternary plot (Fig. 4) and have comparable 3Me/2Me ratios (Table 2) as their Draupne/Spekk source rocks. Their potential as a petroleum correlation tool is further developed in a case study offshore West Africa in the following section.

3.3. Methylhopanes as a Correlation Tool: Offshore West Africa

Most of the oils and source rock samples from West Africa are relatively enriched in methylhopanes (Table 2). Furthermore, they display extremely diverse composition, as shown by wide-ranging 3Me/2Me ratios (Table 2) and their broad distribution in the ternary plots (Fig. 4). This is not surprising given the thick sequences of source rocks in this area, including presalt (Early Cretaceous syn-rift lacustrine) and postsalt (mid Cretaceous to Tertiary postrift marine) sources (Burwood, 1999). The oils are classified as being of marine, lacustrine or "mixed" source, on the basis of their C_{26}/C_{25} tricyclic terpane and hopane/sterane ratios (Fig. 6; Burwood, 1999; Schiefelbein et al., 1999). Of the oils studied here, five fall clearly within the marine field and seven within the lacustrine field. Four oils cannot be assigned with certainty, and have been classified as being of "mixed" (or uncertain) source.

In terms of methylhopane composition, only two of the oils proposed on the basis of tricyclic terpane and hopane/sterane ratios to be of lacustrine source, are notably enriched in 3β-methylhopanes (Fig. 7; 3Me/2Me = 2.4 and 4.9); the remaining five lacustrine-sourced oils plot in the middle of the diagram, having 3Me/2Me ratios between 0.5 and 1.1. These two distinct groups of lacustrine oils are interpreted to have been generated from two different lacustrine source rock facies—although their hopane distributions are similar, their methylhopane distributions are markedly different (Fig. 8). By analogy with the occurrence of abundant 3β-methylBHPs in two modern alkaline lakes, it is tempting to suggest that these facies may represent saline (possibly alkaline; with dominant 3-methylhopanes) and freshwater environments (documented by Burwood et al., 1995; Cole et al., 2000); however, the specific facies cannot be determined, but their depositional environments were surely characterized by different bacterial communities prevailing during source rock deposition.

Plotting the source rock samples on the same ternary plot (Fig. 7) shows that their methylhopane compositions and abundance relative to hopane, although widely variable, are very similar to those of the oils, demonstrating the utility of methylhopanes as correlation markers. In particular, four of the five presalt (lacustrine) source rocks correlate exactly with the two lacustrine oils ascribed to the source facies with predominant 3-methylhopanes (Fig. 7). The remaining presalt lacustrine source rock sample is significantly poorer in 3-methylhopanes, and may represent the second lacustrine facies. This definition of two distinct lacustrine source facies for the oils offers a significant refinement in oil-source rock correlation in this region of offshore West Africa. The postsalt (marine) source rock samples also show a wide range of composition, generally encompassing the marine-sourced oils, allowing contribution from specific oil-source rock intervals to be related to the different oil compositions.

4. CONCLUSIONS

Methylhopanes are ubiquitous in oils and source rocks, generally in the range of 1 to 10% of the abundance of the hopanes. Their composition varies widely due to differing bacterial inputs to the original depositional environments. Marine samples are generally characterized by higher proportions of 2α-methylhopanes, corresponding to an origin from cyanobacteria. Lacustrine oils and source rocks show a wide range of methylhopane composition, but some, representing a distinct source facies, are enriched in 3β-methylhopanes. Comparison with the composition of precursor methylhopanoids in modern sedimentary environments showed that some alkaline lake sediments can be highly enriched in 3β-methylhopanoids, that might represent either unusually high methanotrophic bacterial activity, or derivation from a currently unknown specific bacterial source.

The ubiquity of methylhopanes makes them excellent markers for ancient bacterial contributions to sedimentary organic matter. Furthermore, this bacterial input dependence makes them valuable tools for oil-oil and oil-source rock correlations, as demonstrated for a suite of oils and source rocks from offshore West Africa, within which two distinct lacustrine facies were recognized, with closely correlated oils and source rocks within each facies.

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