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Presence of Geochemical *Botryococcus* and Methane Series in The Mid-Cretaceous Lokpanta Oil Shale, SE Nigeria

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ABSTRACT

In Nigeria, oil shale deposits are abundantly located mostly in Benue, Bornu, Adamawa, and Ebonyi States; and there are also potentials for shale gas in the Niger Delta region. The richest deposits, the mid-Cretaceous oil shale deposits, which exist in the Lokpanta area of the Abakiliki anticlinorium. The bulk hydrocarbons of two samples (IMA-23 and IMA-27) from the same section of the mid-Cretaceous terrestrial Lokpanta oil shale in SE Nigeria were used and to identified algal and bacterial main sources. Comparison of the two samples provided an information for telalginite to the bulk hydrocarbon profiles. Cells identified from microscopy as *Botryococcus* in the telalginite of IMA-27 were confirmed as belonging to the Lamalginite (L) race of B. braunii from the presence of monoaromatic lycopane derivatives and small amounts of several lycopadienes. Lycopane was abundant and was probably derived from biohydrogenation of lycopadienes and related lipids on the basis of $\delta^{13}C$ values. Hopane distributions showed a dominance of those with the biological 17β, 21β-stereochemistry, as expected for an immature shale, with low amounts of 17β, 21α-hopanes (moretanes) and 17α, 21β-hopanes. Two hopenes were also abundant and assigned as C_{29} and C_{30} neohop-13(18)-enes, which occurred together with the C_{29} and C_{30} hop-17(21)-enes. These had depleted carbon isotope values (-43.7‰ to -50.8‰), indicative of production by methane oxidizing bacteria (methanotrophs). The high proportion of hopanoids with carbon numbers < C_{32} indicates extensive post-depositional diagenetic alteration of bacteriohopanepolyols as well as a direct input of C_{30} hopanoids. The data clearly indicate that there was active utilization of methane in this lacustrine depositional setting, but isoprenoid hydrocarbon biomarkers for methanogens, such as pentamethylicosane (PMI) and squalane, were in surprisingly low abundance. It is possible that these bacterial contributions were present as polar lipids. The origins of an unusual C_{38} isoprenoid alkane assigned as bipristane are uncertain, but may be from methanogens. Steranes were relatively minor components, but abundant diasterenes and 4-methyldiasterenes were present, reflecting significant conversion of the original lipid composition by way of clay-catalysed diagenesis. The biomarker data suggest that the bottom waters in the original depositional environment had low O_2 content, but the sediments were probably neither sulfidic nor strongly reducing. The high content of organic matter in the shale likely reflects both high (but fluctuating) productivity due to eutrophic conditions in the overlying water and good preservation in the sediments.

Keywords: Fossil fuel; Pyrolysis; Diasterenes, *Botryococcus*; Carbon isotopes; Lycopane.

INTRODUCTION

The studied mid-Cretaceous Lokpanta oil shale is located within the area of latitudes 5°24'17" to 5°59'35"N and longitudes 6°52'18" to 7°37'53"E (Fig. 1). Interest in source rock characterization has increased with the growth in development of unconventional petroleum resources (Katz and Lin, 2014), including lacustrine source rocks, which dominate the petroleum reserves in regions such as China, Indonesia, Brazil and Nigeria (Katz and Lin, 2014; Osuji, 2015; Okon and Olagunju, 2017; Omoregie, 2017). An oil shale deposit, possibly of high economic value and corresponding to the Turonian Ezeaku shale (lower Nkalagu formation) of the lower Benue Trough, was found in a 1.5 x 1.0 km belt in Lokpanta near Okigwe in Imo State, Nigeria (Ekweozor and Unoma, 1990; Ehinola et al., 2005). The characteristically dark – grey, laminated and fissile marlstone contains total organic carbon (TOC) >7% in some locations and total extractable organic matter generally in excess of 10000 ppm (Ekweozor and Unoma, 1990). The kerogen is type I-II (oil-prone) and at the updip rim it has attained intermediate thermal maturity status. A total thickness of > 25m was established by drilling/coring for one location (Ekweozor and Unoma, 1990; George and Olugbenga, 2020). Moreover, it has become clear that certain biomarkers appear in the sediment record at a specific time, indicating that the biosynthesis of these compounds (or their precursor lipids as a diagenetic products) first evolved at that time. Such age-diagnostic biomarkers (Holba te al., 1998; Moldowan, 2000; Adam et al., 2006) have proven to be useful for establishing the age of a particular oil (Moldowan, 2000; Sinninghe Damsté et al., 2004a; Rampen et al., 2007; Barbanti et al., 2011) and show increasing promise for further understanding of the evolution of biosynthetic pathways over time (Pearson et al., 2003; Welander and Summons, 2012; Villanueva et al., 2014; Volkman et al., 2015).

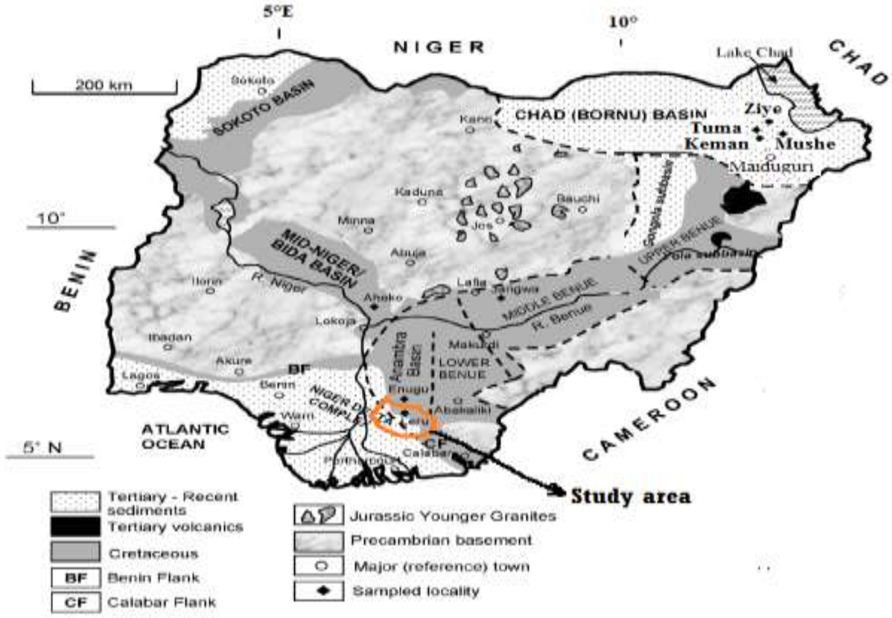

Fig. 1: Geological map of Nigeria showing the study location in the Lower Benue Trough (modified from Obaje et al., 2011).

Organic-rich (bituminous) shales (''oil shales'') have been of considerable interest to organic geochemists for many decades because they represent specific depositional environments that are possible analogues for more mature petroleum source rocks. From analysis of the biomarkers in a variety of rock types it has been possible to define biomarkers for distinguishing open ocean, deltaic or restricted marine environments and whether lacustrine systems are freshwater, brackish, saline or evaporitic (e.g. Brassell et al., 1986). Well known examples of immature oil shales include torbanites, kukersites, tasmanite shales and cannel coals (Hutton, 1987). Species such as Gloeocapsomorpha prisca (thought to be either a cyanobacterium or related to Botryococcus; Derenne et al., 1992; Blokker et al., 2001; Fowler et al., 2004), and green microalgae such as *Botryococcus braunii* (Derenne et al., 1992; Adam et al., 2006; Volkman, 2014) and Tasmanites punctatus (Revill et al., 1994; Dutta et al., 2006) are often associated with these organic rich shales and much has been learned about their unusual lipid biochemistry. A feature of these oil shales is that the organic matter (OM) is well preserved, indicating that the original sediments were deposited under low O_2 conditions and that the water column was likely to have been stratified. In a few cases, the presence of aromatic carotenoid derivatives indicative of sulfur oxidizing bacteria has provided evidence that the anoxic and sulfidic bottom waters extended into the photic zone (photic zone euxinia; e.g. Sinninghe Damsté et al., 2001; Grice et al., 2005).

Several of these shales were deposited in the Eocene, including the lacustrine Maoming oil shale that has been the subject of several biomarker studies (e.g. Brassell et al., 1986; Fu et al., 1990; Yu et al., 2000; Summons et al., 2002) and shown to contain botryococcanes and polymethylsqualanes from the B race of Botryococcus and 4-methylsteroids from dinoflagellates. Limited data on steranes and hopanes in the mid-Cretaceous Lokpanta oil shale have been reported by, Ekweozor and Unoma (1990); Meng et al. (2011); George and Olugbenga (2020) and a comparison made with data from the Green River Shale. Oil shale layers deposited in an overall deep terrestrial environment suggests frequent changes in fresh water level, controlled by tectonic movement, together with long term climatic cycles and variation in OM production (Sun et al., 2013). The Oil Shale Member has been dated to the mid-Cretaceous on the basis of a mammalian fauna (Beard and Wang, 1991). The distributions of biomarkers in two samples from oil shale layer 4 as defined by Sun et al. (2011; 2013) are the main objectives of this present work. Comparison between the two samples provided an opportunity to characterize the biomarkers in terrestrial algal-dominated oil shales and to explore the contribution from telalginite to the lipid profiles.

The Lokpanta oil shale provides an excellent example of a very organic-rich sediment where most of the OM is well preserved and maceral analysis shows a major contribution from terrestrial microalgae (Xie et al., 2014). Terrestrial plant input is present on the basis of maceral analysis but we can be reasonably confident that the major sources of the biomarkers found are either microalgae, bacteria or archaea. The OM is still immature, with vitrinite reflectance values only around 0.40 and Rock Eval Tmax values around 443 ± 3 °C (Xie et al., 2014; George and Olugbenga, 2020). The OM has been subjected to extensive diagenesis, probably due to the high clay content of the sediments (Huet al., 2012) but, since the sediments are terrestrial, the sulphur (S) content is low, so there has been little formation of organic sulfur compounds.

REGIONAL GEOLOGICAL SETTING

The tectonism in Southern Nigeria probably started in Early Cretaceous, with the separation of Africa from South America due to the opening of the Atlantic. This resulted in the development of the Benue Trough which stretched in a NE-SW direction (Fig. 1), resting unconformably upon the Pre-Cambrian basement complex. It extends from the Gulf of Guinea to the Chad Basin and is thought to have been formed by the Y-shaped (RRR) triple junction ridge system that initiated the breaking up and dispersion of the Afro-Brazilian plates in Early Cretaceous (Kogbe, 1989).

After the evolution of the Benue Trough, sediments started depositing in the Trough. Stages of sedimentations in the trough were in three cycles; the Pre-Cenomanian deposit of Asu River Group followed by the Cenomanian-Santonian sedimentation. According to Hogue (1977) the inversion tectonics of the Abakaliki anticlinoria which led to the evolution of both Afikpo Syncline and Anambra basin, represented the third cycle of sedimentation which produced the incipient Nkporo shale, Enugu shale and Owelli sandstone. The Nkporo group is overlain conformably by the Coal Group consisting of the Mamu, Ajali and Nsukka Formations that form the terminal units of the Cretaceous series.

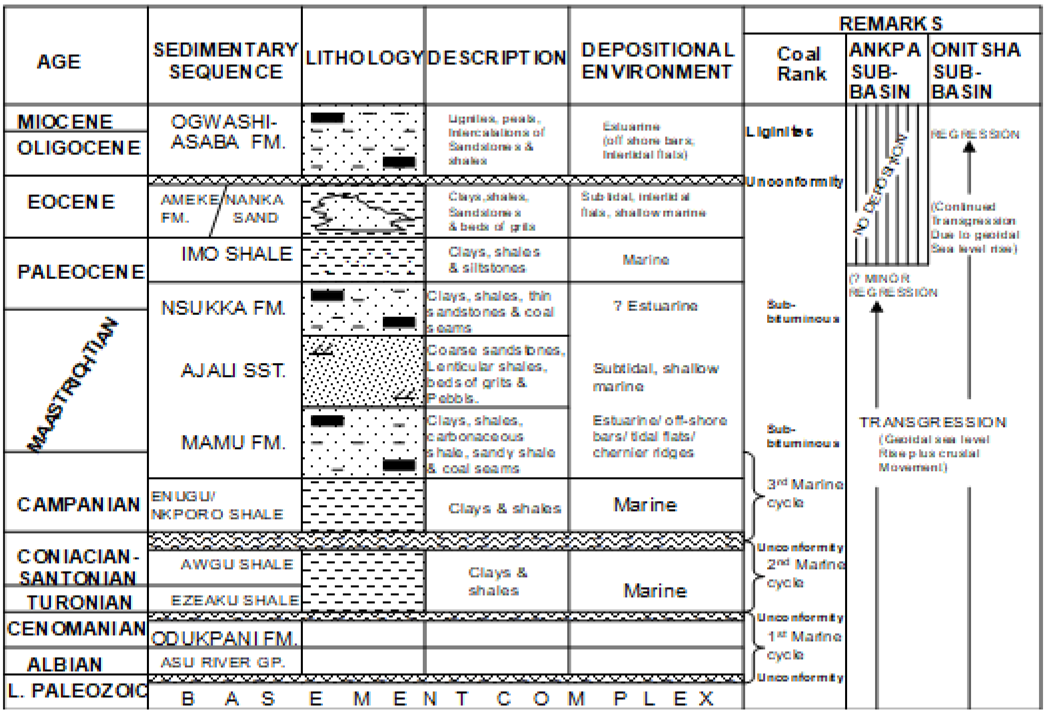

Fig. 2: The stratigraphy of the Anambra Basin southeastern Nigeria (Modified Uzoegbu et al., 2015).

Stratigraphic Setting

The sandstones which is about 330 m thick is an extensive stratigraphic unit conformably overlying the Lower Coal Measure (Mamu Formation) and Nkporo Formations that are 400 and 200 m thick, respectively and underlying the Upper Coal Measure (Nsukka Formation) in the Maastrichtian (Reyment, 1965; Nwajide, 1990) (Fig. 2). The Ajali Formation is typically characterized by white coloured sandstone (Reyment, 1965) while the Mamu Formation is essentially composed of sandy shale and some coal seams; the Nkporo Formation consists mainly of grey - blue mudstone and shale with lenses of sandstone (Obaje, 2009). According to Reyment (1965), the prevailing unit of Ajali Formation consists of thick, friable, poorly sorted sandstone.

MATERIALS AND METHODS

Sampling

Ehinola et al. (2005) examined the geologic mapping and Rock Eval characteristics of 17 samples of the Lokpanta oil shale recovered from drilling/coring. The studied drill-core samples are taken from sites located near the towns of Lokpanta, Onoli-Awgu and Acha (Fig. 1). Two samples from oil shale layer (IMA-23 and IMA-27) were selected for further study here for the maceral composition and biomarker composition of the aliphatic hydrocarbon fractions using gas chromatography–mass spectrometry (GC–MS) and isotope ratio monitoring GC-irm-MS.

Extraction and separation

Around 30 g of finely ground samples were Soxhlet extracted form 72 h with $CHCl_3$ and MeOH (87:13, v:v; pure $CHCl_3$ used for IMA-27). Activated Cu turnings were added to remove a small amount of elemental sulfur. After extraction, the solvent was removed using rotary evaporation. Asphaltene precipitation was carried out with n-hexane, followed by filtration through extracted cotton wool. Aliquots (ca. 50 mg) of the filtrate were fractionated via column chromatography (alumina:silica gel, 3:1, w:w; activated for at least 12 h at 120 °C), which had been pre-eluted with n-hexane.

Fractions were obtained using gradient elution with solvent of increasing polarity with two column volumes of solvent used to obtain each fraction. The ‘‘saturated’’ hydrocarbon fraction (which included alkanes, alkenes and some monoaromatics) was collected with n-hexane and the aromatic fraction was collected with dichloromethane (DCM): n-hexane (1:1, v:v), and the polar fraction was eluted with EtOH. For IMA-23, n-octadecene was added as an internal standard. The fractions were transferred to 4 ml vials and the eluting solvent was carefully removed using a gentle flow of N_2. The aliphatic hydrocarbon fraction was analysed using GC–MS and compound specific isotope analysis (CSIA using GC-irm-MS).

Isolation of branched/cyclic fractions using molecular sieve

Each aliphatic fraction was treated with ZSM-5 silicalite to yield the branched/cyclic alkane fraction, as reported by West et al. (1990). The molecular sieve was activated at 120 °C for ca. 2 h, after which a small column containing ca. 2 g silicalite was prepared with a Pasteur pipette plugged with a small piece of extracted cotton wool. Ca. 2 mg of the aliphatic fraction in 1 ml cyclohexane was placed on the top of silicalite and eluted with 3 bed volumes of cyclohexane. The branched/cyclic fraction was collected in a 4 ml vial. After concentration with a gentle N_2 flow, the residue was analysed using GC–MS and GC-irm-MS.

GC and GC–MS

GC with flame ionisation detection (GC-FID) was carried out with an Agilent 7890 GC instrument equipped with a HP-5 fused silica column (30 m x 0.2 mm i.d.). He was the carrier gas. The GC oven was programmed from 60 °C to 310 °C at 7 °C/min with a final hold of 20 min. GC–MS was carried out with an Agilent 6890 GC instrument interfaced with an Agilent 5973 mass spectrometer operated at 70 eV in full scan mode (m/z 50–800). A J&W DB-5MS column (30 m x 0.25 mm i.d.) was employed. Samples were injected in splitless mode with an injector temperature of 290 °C. The two samples were analysed some months apart, and different GC conditions were used. In a typical programme, the GC oven temperature was held for 2 min at 80 °C and then increased to 230 °C at 3 °C/min and then at 2 °C/min to 310 °C (held 15 min). A faster programme rate was used for IMA-27, where the temperature was increased to 180 °C at 20 °C/min and then at 4 °C/min to 320 °C (held 30 min). Compounds were assigned by comparing mass spectra and retention indices with laboratory standards and literature data.

CSIA (GC-irm-MS)

GC-irm-MS was performed on the aliphatic fractions before and after treatment with molecular sieve using an Agilent 7890 GC instrument equipped with a 30 m (0.25 mm i.d., 0.25 lm DB-5 phase) column, linked to a Thermo Scientific Delta Plus V isotope ratio monitoring mass spectrometer. The GC oven was increased from 80 to 320 °C (held 15 min) at 2 °C/min. Samples were injected using a splitless injector held at 305 °C.

Isotopic composition was calculated by integration of the m/z 44, 45 and 46 ion currents of the peaks produced by combustion (CuO quartz packed tube, 1000 °C) of the compounds. The values quoted are averages of three runs with a standard deviation for n-alkanes of ± 0.9‰. Due to considerable co-elution of hydrocarbons within the diasterene/ hopene/hopane regions, $\delta^{13}C$ values are only reported for major well resolved compounds.

RESULTS

Maceral compositional data and Rock Eval data for IMA-23 and IMA-27 taken as a guide from Xie et al. (2014) are shown in Table 1. TOC values were high for both samples (27.3% and 39.6%) and lamalginite was the dominant maceral in both cases (87.7% and 67.6%). Unlike IMA-23, the more organic-rich sample (IMA-27) contained a high proportion of telalginite (30%) associated with cells identified as the green alga *Botryococcus* and various acritarchs. The terms lamalginite and telalginite are used according to definitions by Hutton (1987), where both are forms of alginite. Telalginite is derived from large, thick walled unicellular algae with strong fluorescence at low rank and includes genera such as *Botryococcus*. Lamalginite (L) is derived from a variety of small unicellular planktic or benthic algae with weak to moderate fluorescence. Vitrinite in the form of detrovitrinite was present in both samples, but was much more abundant in IMA-23 (13.4% vs. 3.6%). Detrovitrinite consists of finely fragmented vitrinitized plant remains occurring either isolated or cemented by amorphous vitrinitic matter. Rock Eval measurements (Table 1) showed that both oil shale samples were immature but IMA-27 was more hydrogen rich (HI values 657 and 768 for IMA-23 and IMA-27) and had a greater hydrocarbon generating potential (S_2 values of 211 and 261 mgHC/g).

n-Alkanes

Both samples contained a suite of n-alkanes from C_{13} to C_{35}, but components above C_{29} were only trace constituents (Fig. 1). C_{21}–C_{29} *n*-alkanes predominated and showed a maximum at C_{27} with a small odd predominance > C_{22}. *n*-Alkenes were not detected.

Isoprenoid hydrocarbons

The major isoprenoid alkanes were pristane (Pr) and phytane (Ph) with Pr much more abundant (Pr/Ph 1.86 for IMA-23 and 1.91 for IMA-27). Shorter chain C_{16} and C_{18} isoprenoid alkanes were also present (Figs. 1 and 2). The C_{30} isoprenoid, squalane, was not detected using m/z 113 and 183 chromatograms, but minor amounts of 2,6,10,15,19-pentamethylicosane (PMI; ⁄ in Fig. 1) were detected in both sediments and assigned from retention time and comparison with mass spectra reported by Risatti et al. (1984). Unsaturated analogues of PMI (Schouten et al., 1997) were not detected. The main difference between the two distributions was the presence of a C_{40} isoprenoid alkane (Fig. 2), assigned as lycopane from its retention time (coelution with n-C_{35}; Sinninghe Damsté et al., 2003) and mass spectrum (Wakeham, 1990; Ogihara, 2014) in IMA-27; it was a very minor component in IMA-23; Fig. 1). the presence of a C_{40} isoprenoid alkane (Fig. 2), assigned as lycopane from its retention time (coelution with n-C_{35}; Sinninghe Damsté et al., 2003) and mass spectrum (Wakeham, 1990; Ogihara, 2014) in IMA-27; it was a very minor component in IMA-23; Fig. 1).

Table 1: Relative amounts of identified different macerals (vol. %) and Rock Eval data for Lokpanta oil shales.

Sample N0.	TOC (%)	Alginite Telalginite	Lamalginite	Vitrinite Detrovitrinite	S_1	S_2	Tmax	HI	OI	TS (%)
IMA-23	26.4		87.7	13.4	0.96	211	443	657	11	4.66
IMA-27	40.3	30.0	67.6	3.6	1.28	261	446	768	7	4.97

The peak was broader than expected, probably due to partial resolution of different stereoisomers. This sample also contained a group of at least 4 minor compounds eluting just before C_{31} ββ-hopane (Fig. 2), which had mass spectra and retention times (Zhang et al., 2011) characteristic of lycopadienes (e.g. $M^{+\cdot}$ m/z 558, major ion at m/z 292). An additional isoprenoid alkane was present in IMA-27, eluting just before n-C_{33} alkane. The mass spectrum had major ions at m/z 183, 252/253, 308/309, 378/379 and 448/449 and thus was tentatively assigned as the C_{38} head-to-head isoprenoid, bipristane, based on the close similarity of its mass spectrum with that reported by Stefanova (2000) for the compound after NaH treatment of a Miocene coal. Isoprenoids with irregular head-to-head linkages of isoprenoid units, including bipristane, have been found in a number of oils and have been suggested to be derived from the isoprenoid ether lipids of methanogens (Petrov et al., 1990; Stefanova, 2000).

Steroidal compounds

The samples contained small amounts of steranes dominated by 20R isomers, as shown by m/z 217 and 218 chromatograms. We found a similar distribution to that reported by Meng et al. (2011), although the m/z 217 chromatogram also showed contributions from unrelated compounds not reported by these authors. A closer examination of the hydrocarbon extract revealed that diacholest-13(17)-enes (diasterenes) and 4-methyldiacholest-13(17)-enes (4-methyldiasterenes) were much more abundant than the steranes. Mass chromatograms for m/z 257 (diasterenes) and m/z 271 (4-methyldiasterenes) for IMA-23 are shown in Fig. 3 which also shows generalized structures. The distributions in IMA-27were almost identical to those IMA-23, but showed a slightly higher abundance of the 4-methyl,24-ethyldiasterenes (data not shown). Non-rearranged sterenes were only trace constituents and were not examined further.

Table 2: Assignment of peaks in m/z 257 (diasterenes) and m/z 271 (4-methyldiasterenes) chromatograms.

Peak[a]	C No: double bonds	Assignment
A	27:1	20S-10β-diacholest-13(17)-ene
B	27:1	20S-10α-diacholest-13(17)-ene
C	27:1	20R-10β-diacholest-13(17)-ene
D	27:1	20R-10α-diacholest-13(17)-ene
E	28:1	20S-10β-24-methyldiacholest-13(17)-ene
F	29:1	20S-10β-24-ethyldiacholest-13(17)-ene
G	29:1	20S-10α-24-ethyldiacholest-13(17)-ene[b]
H	29:1	20R-10β-24-ethyldiacholest-13(17)-ene
I	29:1	20R-10α-24-ethyldiacholest-13(17)-ene
J	28:1	20S-10α-4-methyldiacholest-13(17)-ene
K	28:1	20R-10α-4-methyldiacholest-13(17)-ene
L	29:1	20S-10α-4,24-dimethyldiacholest-13(17)-ene
M	30:1	20S-10α-4-methyl,24-ethyldiacholest-13(17)-ene
N	30:1	20R-10α-4-methyl,24-ethyldiacholest-13(17)-ene

[a] Labelled in Fig. 3. [b] Includes some 20R-10α-24-methyldiacholest-13(17)-ene.

In both diasterene and 4-methyldiasterene distributions, there were two main peaks for each carbon number assigned as the 20S and 20R 10α(H)-isomers (Brassell, 1984; Peakman et al., 1988, 1989). Note that the C_{28} 20R 10α(H)-diasterene coeluted with the C_{29} 20S 10α(H)-diasterene (although they can be separated on a DB-17 column; Peakman et al., 1989), so the 20S/20R ratio could only be accurately measured for the C_{27} components in the diasterenes and C_{28} components in the 4-methyldiasterenes using the mass chromatograms. An additional minor series of diasterenes eluting before the major 20R and 20S 10α(H)-diasterenes (peaks A, C, F and H in Fig. 3; Table 2) was assigned as the corresponding 10β(H)-isomers based on the fact that they had nearly identical mass spectra (Peakman et al., 1988) and expected retention times (Peakman et al., 1989). Only traces of compounds that might be 10β(H)-isomers of 4-methyldiasterenes were apparent in the m/z 271 chromatogram (Fig. 3). De Leeuw et al. (1993) have shown that, at thermodynamic equilibrium, the major sedimentary products are the 10α(H)-diasterenes.

In the diasterene series, $C_{29} > C_{27} >> C_{28}$, whereas in the 4-methyldiasterene series $C_{28} \sim C_{30} >> C_{29}$. The total amounts of diasterenes and 4-methyldiasterenes were very similar. In the diasterenes, the C_{29} compound was expected to have a 24-ethyl substituent, but in the 4-methyldiasterenes the C30 component may have either 24-ethyl or 23,24-dimethyl substitution.

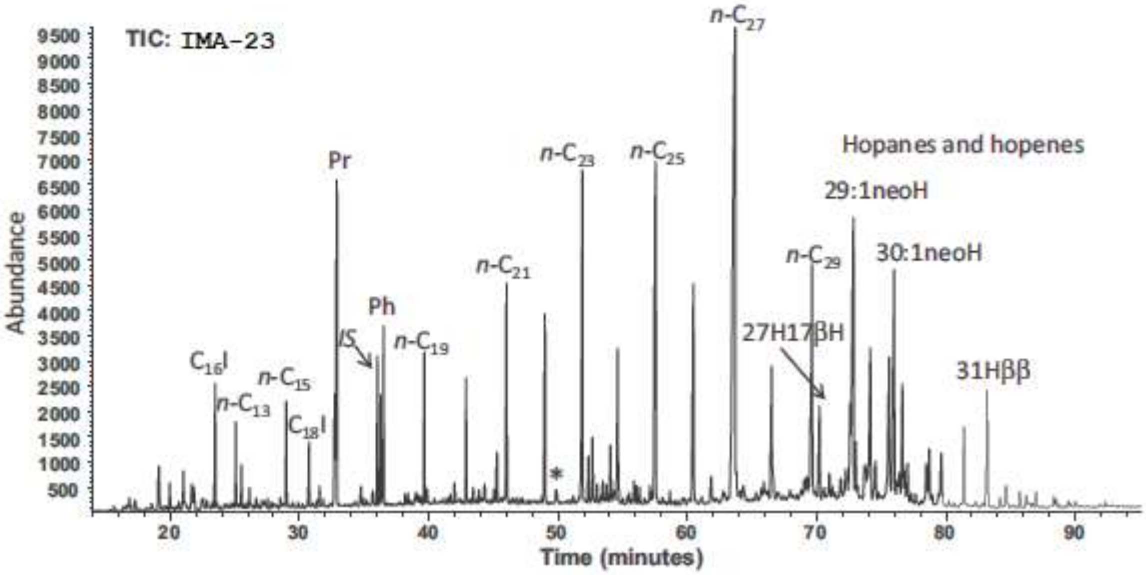

Fig. 1: TIC chromatogram for IMA-23 showing distributions of n-alkanes (n-Cx), C_{16} isoprenoid (16I), C_{18} isoprenoid (18I), pristane (Pr), phytane (Ph) and hopanoid hydrocarbons including C_{29} neohop-13(18)-ene (29:1neoH), C_{30} neohop-13(18)-ene (30:1neoH) and C_{31} 17β(H),21β(H)-homohopane (31βββ). The distribution in IMA-27 was very similar. A minor amount of 2,6,10,15,19-pentamethylicosane (PMI) is shown by ⁄. More detail on the distribution of hopenes and hopanes can be found in Fig. 4.

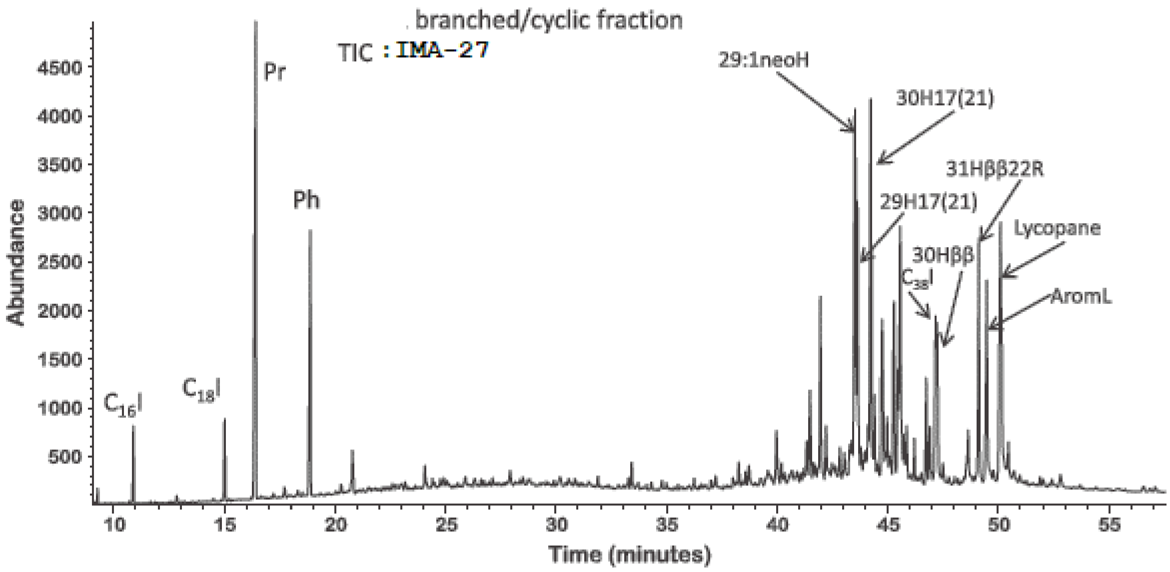

Fig. 2. Total ion chromatogram for branched/cyclic fraction isolated from IMA-27 showing the distributions of isoprenoids (C_{16} isoprenoid (16I), C_{18} isoprenoid (18I), pristine (Pr), phytane (Ph)) and hopanoid hydrocarbons including the C_{29} neohop-13(18)-ene (29:1neoH), C_{30} neohop-13(18)-ene (30:1neoH) and C_{31} 17β(H),21β(H)-homohopane (31Hββ22R). Relative abundances of hop-17(21)-enes were higher than in IMA-23. Unlike IMA-23, the hydrocarbon distribution shows high concentrations of a C_{40} monoaromatic lycopane-related compound (AromL), lycopane and a C38 isoprenoid tentatively assigned as bipristane (C_{38}I). Note that a faster temperature programme was used than that for IMA-23 (Section GC and GC-MS).

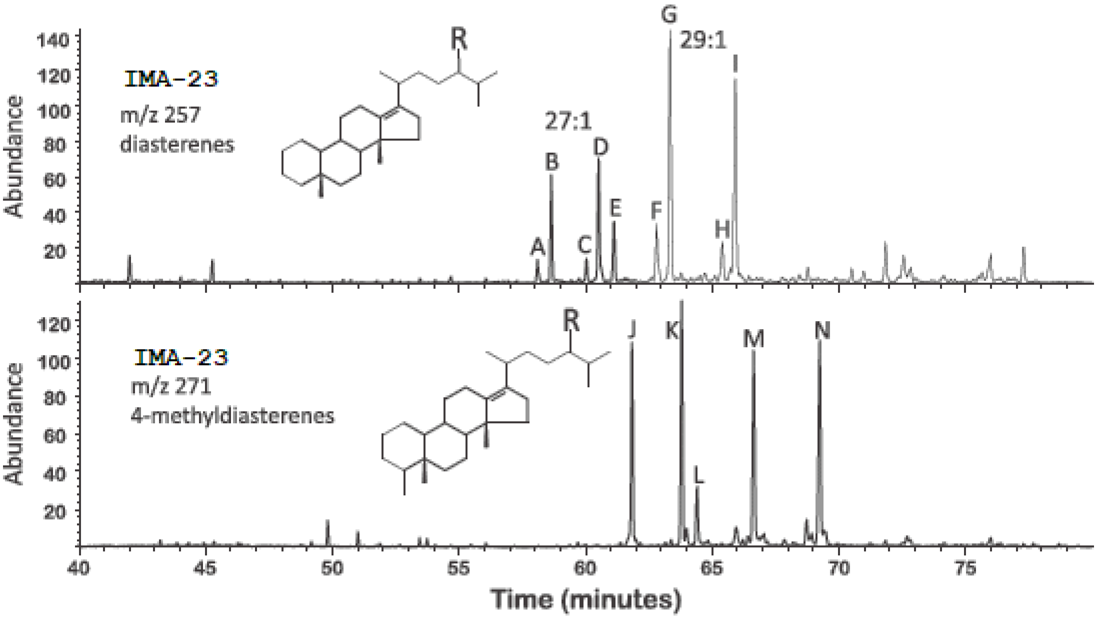

Fig. 3: Chromatograms of m/z 257 and 271 showing distributions of diasterenes and 4-methyldiasterenes in IMA-23. The distribution in IMA-27 was similar, but additional peaks occurred after the diasterenes due to contributions from unknown constituents. In the structures, R = H, CH_3 or C_2H_5. Compounds are assigned in Table 2.

Although regular steranes with these side chains can be distinguished from their mass spectra (Summons et al., 1987), mass spectra of standard C_{30} 4-methyldiasterenes were not available so we could not fully assign the side chain in these compounds. We note, however, that 4-methyl,24-ethylcholestanes seem to be associated with freshwater lacustrine sediments and 4,23,24-trimethylcholestanes with marine/saline/brackish sediments (Goodwin et al., 1988).

The single series of 4-methyldiasterenes suggested that the stereochemistry of the methyl group was 4a, as in the parent sterols, and that minimal rearrangement to the 4b isomer had occurred. This view has also been expressed by Ensminger et al. (1978) and Wolff et al. (1986a), although we are not aware that this assignment has been confirmed. The presence of a single series of 4-methyldiasterenes in a bituminous shale from Jouy-aux Arches (Paris Basin) was noted by Rubinstein et al. (1975), which led Rubinstein and Albrecht (1975) to speculate that 4b-methylsterenes do not undergo backbone rearrangement.

Hopanoids

The m/z 191 chromatograms for IMA-23 and IMA-27 (Fig. 4) were very similar and dominated by two major peaks assigned as C_{29} and C_{30} neohopenes (XX and XIX; Roman numbers refer to structures in Fig. 5), together with a suite of immature hopanes with 17β,21β-stereochemistry and minor amounts of 17α,21β and 17β),21α-hopanes (moretanes; Figs. 4 and 5). The structures of the major components are given in Fig. 5, together with possible interconnecting pathways. C_{27} hopanes were dominated by the 17β-hopane (III) with only minor amounts of Tm (17α-22,29,30-trisnorhopane) and traces of Ts (18α-22,29,30-trisnorneohopane).

C_{28} hopanes were below detection limit. These distributions are consistent with the low maturity inferred from the Rock Eval and vitrinite reflectance data (Xie et al., 2014). Meng et al. (2011) reported a very similar m/z 191 chromatogram for another sample of Huadian oil shale, but did not elaborate on the structure of the C_{29} and C_{30} hopenes. Mass spectra of the neohop-13(18)-enes (XX and XIX) showed strong $M^{+\cdot}$ at m/z 396 and 410 respectively, and m/z 191 ions. These peaks were assigned by comparison with published elution orders of the various possible hopene isomers (Douka et al., 2001; van Dongen et al., 2006) and mass spectral data (Masuda et al., 1983; Ageta et al., 1987; Sinninghe Damsté et al., 2014). Neither C_{27} neohop-13(18)-ene, nor neohop-13(18)-enes > C_{30} were detected in the extracts of the two samples. Note that in the neohop-13(18)- ene structure the methyl group that was at C-18 has migrated to C-17 (Moldowan et al., 1991).

The hopane distributions in IMA-23 and IMA-27 were very similar (see Fig. 4 for a typical distribution). However, some differences were apparent in the hopene distributions. The ratio of C_{29}/C_{30} neohop-13(18)-enes was much greater in IMA-27, which also contained a much higher proportion of hop-17(21)-enes (contrast Figs. 1 and 2). The major ion in hop-17(21)-enes is m/z 231 which is also the base peak in the mass spectra of 4-methylsteranes, so a clearer picture of their distribution can be obtained from chromatograms of m/z 367 (Fig. 6) (Sinninghe Damsté et al., 1995). In the Lokpanta oil shale, hop-17(21)-enes (e.g. XV and XVI; Fig. 5) from C_{29} to C_{34} (trace) dominated the m/z 231 and 367 chromatograms.

Note that, unlike the neohop-13(18)-enes, the distribution extended > C_{30}, implying that they were at least partly derived from diagenesis of bacteriohopanepolyols (Sinninghe Damsté et al., 1995) although, as with the neohop-13(18)-enes, their major source seems to be a C_{30} hopanoid precursor. These extended hop-17(21)-enes were also fully isomerized at C-22 (i.e. contain similar amounts of 22S and 22R isomers) as noted in studies of other immature sediments (e.g. ten Haven et al., 1986).

The total ion current (TIC) trace contained a number of minor compounds eluting between n-C23 and n-C_{25} (Fig. 1). Several of these gave mass spectra with M+_ m/z 328 that are similar to those reported by Hauke et al. (1993) for a series of des-E-hopanoids isolated from a Triassic black shale from Italy and identified using nuclear magnetic resonance (NMR) spectroscopy and comparison with synthetic des-E-D:C-friedohop-8-ene. Identification of this particular isomers was beyond the scope of the present work.

Monoaromatic hydrocarbons

Several high molecular weight monoaromatic hydrocarbons occurred in the aliphatic hydrocarbon fraction of IMA-27. The main component (peak 2; Fig. 7) was assigned as a monoaromatic lycopane derivative from comparison with mass spectral data presented by Derenne et al. (1994) and Adam et al. (2006). It had $M^{+\cdot}$ at m/z 554, a weak fragment ion at m/z 302 and base peak at m/z 119. An additional 3 minor peaks eluting just before and after the major peak also showed $M^{+\cdot}$ at m/z 554. Peak 3 had a base peak at m/z 133 (119 + 14), indicating an additional Me group on the aromatic ring. Peak 4 showed an intermediate ion at m/z 330 (302 + 28) and base peak at m/z 119. The distribution was remarkably similar to that found by Adam et al. (2006) in the Eocene Messel shale. Structures of the 4 components assigned according to Adam et al. (2006) are shown in Fig. 7.

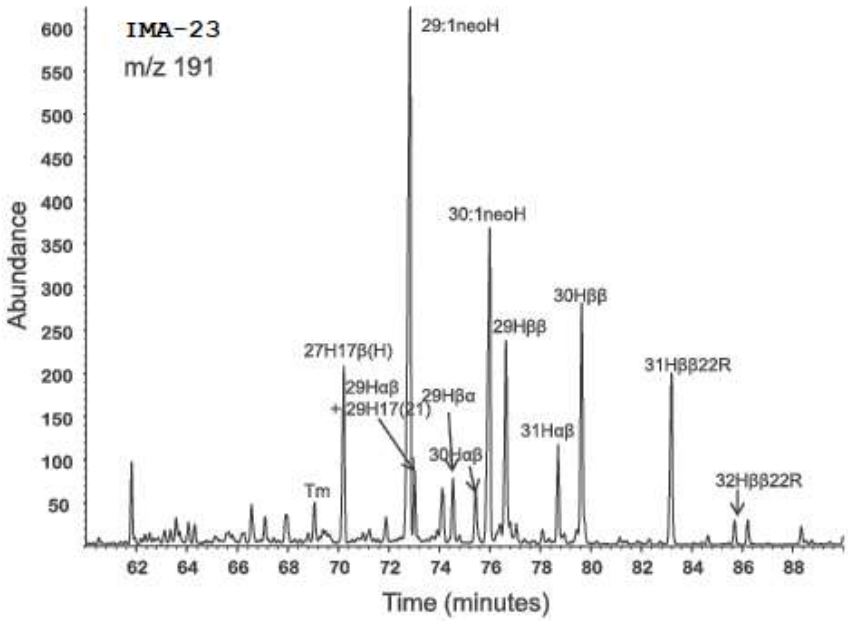

Fig. 4: Chromatogram of m/z 191 showing distribution of hopanes and hopenes in IMA-23. The C_{29} neohop-13(18)-ene is shown as 29:1neoH and C_{30} neohop-13(18)-ene as 30:1neoH. Hopane isomers are shown as αβ, βα or ββ; for example the $C_{31}17\beta(H),21\beta(H)$-homohopane is shown as 31Hββ. See Fig. 5 for structures.

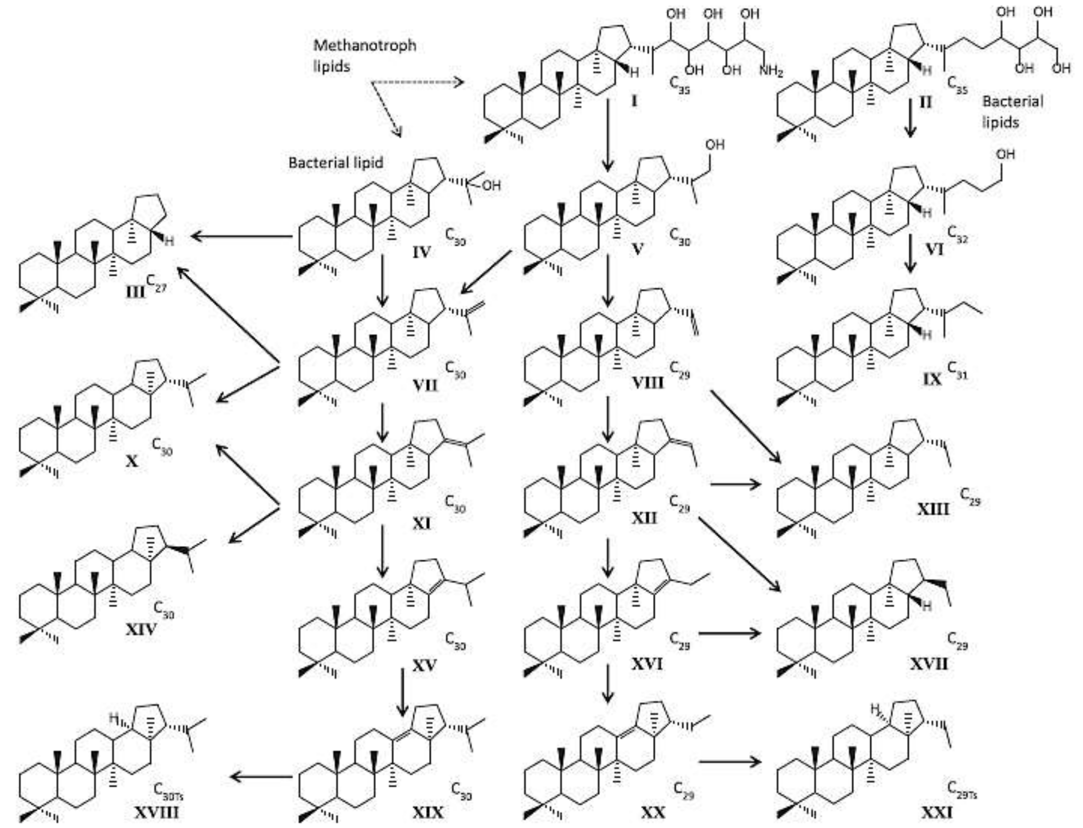

Fig. 5: Scheme showing major hopanoids in the Huadian oil shales and proposed diagenetic relationships between the major hopenes, neohopenes and hopanes.

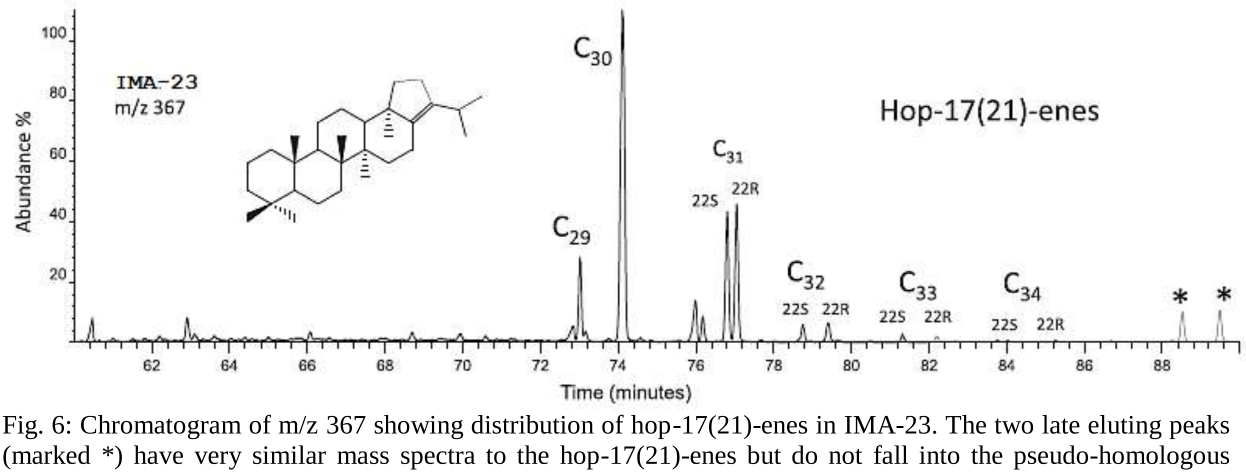

Fig. 6: Chromatogram of m/z 367 showing distribution of hop-17(21)-enes in IMA-23. The two late eluting peaks (marked *) have very similar mass spectra to the hop-17(21)-enes but do not fall into the pseudo-homologous series and have $M^{+\cdot}$ at m/z 464 rather than the expected m/z 480 for C_{35} hop-17(21)-enes.

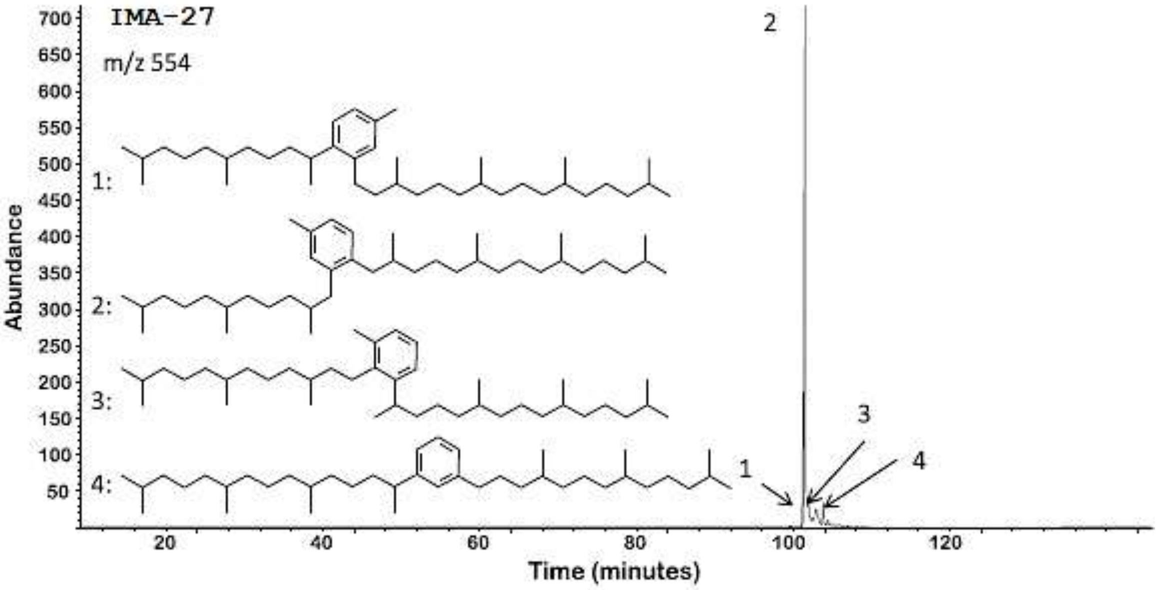

Fig. 7: Chromatogram of m/z 554 showing distributions and structures of aromatic lycopane derivatives in IMA-27. Compounds were assigned by comparison with data presented by Adam et al. (2006).

Compound-specific stable carbon isotopes

Some δ13C values were obtained from the total aliphatic hydrocarbon fraction and the branched/cyclic fraction after molecular sieving. The values for *n*-alkanes, Pr, Ph, neohop-13(18)-enes, hop-17(21)-enes and $C_{31}\beta\beta$-hopane are shown in Table 3.

Their low abundance made it not possible to obtain reliable isotope data for the rearranged sterenes and the complexity of the distributions limited isotopic analysis to the major compounds.

DISCUSSION

Nigeria has had a substantial unconventional petroleum industry based on exploitation of oil shales since the 1960s and the industry has been identified for significant growth by Nigeria in its future plans (Ekweozor and Unoma, 1990). The total Nigerian oil shale resource has been estimated at 500 billion tonnes deposits spread over Benue, Adamawa, Bornu, Ebonyi, Imo and Abia States, which is equivalent to 35 billion tonnes of oil with proven reserves of ca. 30 billion tonnes (Qian et al., 2006). The principal oil shale deposits are Imo (Okigwe province), Ebonyi (Abakaliki province), Abia (Lokpanta province), Abia (Leru province) and Benue (Makurdi province). The Okigwe and Lokpanta shales have the highest oil content (avg. ca. 14% and 10% respectively, but with individual samples up to 40%), but are the smallest reserves, having 0.1 and 0.3 billion tonnes of oil equivalent in place (Okon and Olagunju, 2017). Biomarkers in the Tertiary oil shales at Imo and Abia States in SE Nigeria have also received attention (Ekweozor and Unoma, 1990; Okon and Olagunju, 2017; George and Olugbenga, 2020) and provide an interesting contrast to those in the contemporaneous Niger Delta oil shale located on the same fault zone.

Biomarker analysis combined with compound specific δ13C measurements can provide a wealth of information on the sources of the OM in the Lokpanta oil shales, and features of the depositional environment as well as transformations due to diagenesis. Maceral analysis indicates that microalgae were a dominant source of the OM, although the proportions of different algal-derived macerals varies (Table 1; Xie et al., 2014).

Table 3: δ1[3]C values for bulk fractions, n-alkanes, isoprenoids, hopanoids and lycopene derivatives in IMA-27.[a]

Bulk fraction	**$\delta^{13}C$ (‰)**	**Branched/cyclic**	**$\delta^{13}C$ (‰)**
Total extract	-26.1	Isoprenoids	
Saturated HCs	-26.3	C_{16}	-21.5
Aromatic HCs	-24.4	C_{18}	-23.6
Non-HCs	-25.7	Pr	-21.5
Asphaltenes	-26.5	Ph	-23.3
n-Alkanes		Hopanoids	
n-C_{17}	-26.6	C_{29} neohop-13(18)-ene[a]	-50.8
n-C_18	-29.2	C_{29} hop-17(21)-ene[b]	
n-C_{19}	-29.4	C_{30} neohop-13(18)-ene[c]	nm
n-C_{20}	-29.0	C_{30} hop-17(21)-ene	-43.7
n-C_{21}	-28.3	$C_{31}\beta\beta$-hopane	-34.8
n-C_{22}	-28.4	Lycopane derivative	
n-C_{23}	-28.3	Monoaromatic lycopane	-18.7
n-C_{24}	-30.9	Lycopane	-17.2
n-C_{25}	-30.4		
n-C_{26}	-30.1		
n-C_{27}	-29.3		
n-C_{28}	-30.6		
n-C_{29}	-31.9		

[a] Mean of 3 analyses (standard deviation ± 0.9‰). [b] C_{29} neohop-13(18)-ene and C_{29} hop-17(21)-ene were insufficiently resolved and the value of -50.8‰ is the average over the combined peak; deconvolution suggests an imprecise value of - 45.4‰ for the C_{29} neohop-13(18)-ene. [c] nm, accurate data not available due to coelution with other hydrocarbons.

Higher plant biomarkers

Detrovitrinite occurs as a minor maceral in IMA-27 (3.6%, Table 1), but the amount in IMA-23 is much higher (13.4%). No evidence for any saturated pentacyclic higher plant markers such as oleanoid or lupane derivatives could be seen in the m/z 191 chromatograms (Fig. 4), but the aromatic fraction was found to contain numerous aromatics derived from both higher plant triterpenoids and bacterial hopanoids (Volkman et al., 2015). Microalgae, bacteria and archaea are the main sources of the polycyclic alkanes and alkenes identified, but a study of non-aromatic hydrocarbons in isolation could give the false impression that higher plant contributions were minor. Notably, the Fushun oil shales of similar age contain abundant biomarkers attributable to higher plants (Strobl et al., 2014).

Origins of n-alkanes and isoprenoid alkanes

A contribution from higher plants should be most apparent in the alkane distributions and is usually manifested as a high proportion of long chain *n*-alkanes maximizing at C_{29} or C_{31} with a strong odd/even predominance typical of terrestrial leaf wax (Eglinton et al., 1962; Smith et al., 2007). The *n*-alkanes do indeed show the presence of long chain n-alkanes, but the major constituent is C_{27} and longer chain n-alkanes are much less abundant. Moreover, the predominance of odd chain alkanes is much less than in most vascular plant waxes and this also varies with carbon number unlike most plant waxes (Fig. 1). The distribution of n-alkanes is identical in both samples despite their very different content of detrovitrinite (Table 1).

The low abundance of chain lengths > C_{29} suggests that terrestrial plant leaf wax might not be the only major source of n-alkanes and additional sources including sphagnum moss and aquatic plants need to be considered. For example, the *n*-alkanes in sphagnum moss maximize at lower carbon numbers (e.g. n-C_{23} or n-C_{25}; and have lower even/odd ratio values of 5–9 compared with terrestrial wax (typically > 10; Corrigan et al., 1973). Analysis of modern bog vegetation containing Sphagnum species has shown enhanced abundance of lower chain length homologues (C_{21}–C_{25}; Nott et al., 2000). Those distributions are not very similar to those in the Lokpanta shales.

Aichner et al. (2010a) showed that emergent aquatic macrophytes contain mainly n-C_{27} and *n*-C_{29} alkanes, while Gao et al. (2011) and Wang and Liu (2012) have noted that C_{21}–C_{23} *n*-alkanes and C_{20}–C_{24} n-alkanoic acids are particularly abundant in floating and submerged aquatic plants. Ficken et al. (2000) reported an average n-alkane distribution from 5 submerged and floating plants ranging from n-C_{19} to n-C_{33}, maximizing at n-C_{25}, with a very low content of C_{29}–C_{31} *n*-alkanes, as in the Lokpanta oil shale.

These authors noted that high relative concentrations of C_{23} and C_{25} *n*-alkanes in cores from Kenyan lakes corresponded to high counts of aquatic pollen taxa, notably Nymphaea (Ficken et al., 2000). The *n*-alkane patterns in the Lokpanta oil shale are thus consistent with an environment with abundant macrophytes found in the fresh water swamp, as is common in lakes in China (Volkman et al., 2015). This is also consistent with the presence of coaly layers above the oil shales in these sedimentary sequences (Sun et al., 2013) formed from swampy areas when the river was shallower.

A comparison with the alkanes in the contemporaneous Niger Delta oil shales is instructive since these are located in the same fault zone and represent similar depositional environments (Meng et al., 2012; Strobl et al., 2014). While both oil shales were deposited in shallow marine, the Imo Formation of the Okigwe deposit consists of a single thick layer (Strobl et al., 2014), whereas the

Lokpanta oil shales occur as many layers (Sun et al., 2013), indicating a far more dynamic depositional environment. The OM in the lower unit of the Okigwe shale is characterized by land plant derived macerals giving rise to Type II kerogen. The upper unit is similar to the Lokpanta, containing Type I kerogen with HI values up to 600 mg HC/g TOC) and TOC content up to 26.4 wt.% (Strobl et al., 2014). The *n*-alkanes in Okigwe also show a maximum at C_{27} with a weak odd predominance, as in the Lokpanta shales, but extend to much longer chain length, clearly due to terrestrial higher plant input, as seen in the presence of higher plant-derived diterpenoids (Strobl et al., 2014). These diterpenoids were not observed in the Lokpanta shale, so it seems likely that multiple sources of n-alkanes occur in these shales, with chain length > C_{29} associated mainly with vascular plant waxes.

The $\delta^{13}C$ values for the n-alkanes show a gradual ^{13}C depletion with increasing carbon number (Table 3), but almost all of the values fall in a narrow range, implying a common source. The $\delta^{13}C$ values are substantially lighter than those of the isoprenoid alkanes, but similar to those reported for plant wax hydrocarbons in post-PETM Eocene sediments analysed by Smith et al. (2007). Mead et al. (2005) had some success in differentiating terrestrial/emergent and submerged aquatic sources of *n*-alkanes in Florida coastal everglades using both chain length distribution and $\delta^{13}C$ values. Ficken et al. (2000) showed that many aquatic plants use the C_3 photosynthetic pathway with a few, such as the emergent sedge Cyperus and the submerged Hydrilla, which utilize the C_4 pathway, and emergent Crassula which utilizes the CAM pathway and hence have more enriched $\delta^{13}C$ values. From their data it is not clear that $\delta^{13}C$ values can be used to distinguish between terrestrial and submerged plants without a good knowledge of the species present. For example, Yu et al. (2000) reported similar distributions of *n*-alkanes in the Maoming and Jiangshan oil shales, but longer chain alkanes of presumed higher plant wax origin were much more abundant in the Maoming oil shale. The average carbon isotope values of *n*-alkanes reported by those authors were -25.5‰ and -26.9‰ respectively, even though sources other than higher plants were proposed for the alkanes in the Jiangshan oil shale. This highlights the idea that $\delta^{13}C$ values might not always be sufficient to differentiate between algal/bacterial and higher plant sources of *n*-alkanes (e.g. Spooner et al., 1994), or between terrestrial and aquatic plants. In the case of the Lokpanta shales, our suggestion of a significant contribution of *n*-alkanes from macrophytes must remain circumstantial.

The high abundance of C_{16}–C_{20} isoprenoid alkanes is consistent with high algal productivity in the overlying water column and the $\delta^{13}C$ values accord with their origin from the phytyl side chain of chlorophyll (Volkman and Maxwell, 1986). The presence of shorter chain isoprenoids indicates significant post-depositional diagenetic alteration of phytol. It is commonly observed that biological and sedimentary acyclic isoprenoids such as phytol, steroids and triterpenoids will be enriched in ^{13}C compared with the fatty acids and *n*-alkanes from the same organism (Schouten et al., 1998), although the difference in $\delta^{13}C$ values between the isoprenoid alkanes and n-alkanes in the Huadian oil shales is at the far end of the range commonly found and suggestive of different sources for the n-alkanes (plants) and isoprenoid alkanes (microalgae). For example, Schouten et al. (1998) found that phytol was consistently enriched in ^{13}C by 2–5‰ compared with the C_{16} fatty acid in the microalgae they studied. Schouten et al. (2008) noted that, due to intramolecular isotopic differences within phytol, diagenetic products formed from it by loss of a C atom, such as Pr, may be naturally depleted in ^{13}C by several ‰ relative to Ph. In the Huadian shales the $\delta^{13}C$ value for Pr (-21.5‰) is enriched compared with Ph (-23.3‰), suggesting an additional contribution of Pr and/or Ph from non-algal sources.

Steroidal biomarkers for an algal contribution

The presence of diasterenes and 4-methyldiasterenes is consistent with the low maturity (Ro ~ 0.4) since these compounds are later stage diagenetic products of sterols and 4-methylsterols. It is well established that sterols are converted to Δ^2-, Δ^4- and Δ^5- sterenes (the latter likely formed by reduction of cholesta-3,5-dienes; de Leeuw et al., 1989) during early diagenesis, which in turn can be rearranged to diasterenes and then reduced to steranes at a later diagenetic stage (Mackenzie et al., 1982; Macquaker et al., 1986; Wolff et al., 1986a,b; Peakman et al., 1988; van Kaam-Peters et al., 1998). Note that non-rearranged sterenes were not detected in IMA-23 or IMA-27 and steranes were only minor components, so these sediments provide an opportunity to study distributions of mid-stage diagenetic products. Diasterenes with 20R stereochemistry undergo isomerization at C-20 to eventually produce similar amounts of the 20S isomer (Peakman et al., 1988) as found in here for both the diasterenes and 4-methyldiasterenes (Fig. 3).

Peakman et al. (1988) proposed that rearranged sterenes with a Δ^9 double bond are likely intermediates in diasterene rearrangements since both 10α(H) and 10β(H) isomers are found. Formation of the minor 10β(H) rearrangement products requires protonation from the more hindered b-face of this intermediate. The ratio of 10β(H)- to 10α(H)isomers is identical for each carbon number (Fig. 3) in IMA-23 and IMA-27 which is consistent with the involvement of such an intermediate. The predominance of C_{29} diasterenes provides yet another example of sediments in which algae are likely to be major contributors of C_{29} sterols (Volkman, 1986), as well as vascular plants. Potential algal contributors include freshwater green microalgae including *Botryococcus* (Metzger et al., 1990; Kodner et al., 2008), some diatoms (Volkman, 1986), some eustigmatophytes (Volkman et al., 1999) and some haptophytes (Volkman et al., 1997). Living Botryococcus contains very small amounts of sterols (0.1–0.2% of dry biomass; Metzger et al., 1990) and is thus probably not a major source of steroidal compounds in the Lokpanta environment. Moreover, the distributions in *B. braunii* are dominated by 24-ethylcholesterol and 24-methylcholesterol (Metzger et al., 1990) and yet C_{28} diasterenes are relatively minor constituents. Diatoms were observed from scanning electron spectroscopy (SEM; Xie et al., 2014), but the very low abundance of C_{28} diasterenes here is surprising given the high proportion of diatoms, which contain abundant C_{28} sterols (Rampen et al., 2010). We have no independent evidence for eustigmatophytes or haptophytes since these cells do not preserve well. Of interest is that Collister et al. (1992) also concluded that the C_{29} steranes in the Green River Shale must also have an aquatic source based on their abundance and $\delta^{13}C$ values.

In sediments where methane oxidation can be demonstrated one must also consider a possible input of steroidal compounds from methanotrophs (e.g. Volkman, 2003). 4α-Methylcholest- 8(14)-en-3β-ol and 4α-methylcholesta-8(14),24-dien-3β-ol with distinctively light $\delta^{13}C$ values have been found in methane-venting marine mud volcanoes (Elvert and Niemann, 2008) and a stratified Antarctic lake (Coolen et al., 2004) and attributed to methanotrophs.

While sterols from methanotrophs are a potential contributor to the C_{28} 4-methyldiasterene (Coolen et al., 2004), this seems unlikely since 4,4-dimethylsteroids, also found in methanotrophs (Bouvier et al., 1976), were not detected. 4-Methylsterols having an alkyl group at C-24 have not been reported in methanotrophs or other bacteria (Volkman, 2003), indicating that these components in the Lokpanta shales must have an algal source. Several 4α-methyl sterols have been identified in haptophytes from the order Pavlovales (Volkman et al., 1997). Note that the major C_{30} 4-methyl sterol is 4α-methyl-24-ethyl-5α-cholest-22E-en-3β-ol, which has a conventional C-24 alkylated side chain rather than a 23,24-dimethyl structure. Dinoflagellates are well documented in lacustrine ecosystems and some of the acritarchs in the Huadian sediments (Xie et al., 2014) may indeed be dinoflagellates.

Biomarkers for Botryococcus

Fossil cells attributed to *B. braunii* have been identified in a variety of terrestrial sediments ranging from Precambrian to recent (Guy-Ohlson, 1992) and there are many examples where this alga has been a major source material for petroleum generation (Moldowan and Seifert, 1980; Glikson et al., 1989). Xie et al. (2014) identified Botryococcus cells in several samples from the

Lokpanta oil shale, including IMA-27, from microscopy. The cells were well preserved and retained their morphology. Our identification here of specific biomarkers associated with the L race of *Botryococcus*, including lycopadienes (albeit in small amounts), and monoaromatic lycopene derivatives confirms that the L race was abundant in the original depositional environment when IMA-27 was deposited but not at other times (IMA-23). Trace amounts of the monoaromatic lycopane derivative have been observed in pyrolysates of kerogen from IMA-27 (Zhang et al., 2014), but the extracts obtained here contained considerably higher amounts. This adds to the reports of *B. Braunii* race L occurring in Eocene sediments and adds weight to the suggestion that the lycopadiene biosynthesis pathway in Botryococcus likely first appeared in the Eocene (Volkman, 2014).

A number of sources have been suggested for lycopane in sediments, including methanogenic bacteria, unknown phototrophs and microalgae (reviewed by Volkman (2014)), but in this instance the very low abundance of hydrocarbon biomarkers for methanogens (Brassell et al., 1981) and the presence of Botryococcus suggests that lycopane is derived from the lipids of Botryococcus via diagenetic processes. In support of this suggestion, lycopene was only a trace constituent in IMA-23, which lacked Botryococcus remains. Further support for this contention is that the $\delta^{13}C$ values for the monoaromatic lycopane derivative (-18.7‰) and lycopene (-17.2‰) in IMA-27 are very similar and much more ^{13}C-enriched than the other biomarkers in the Lokpanta oil shale. It has been noted in other work that the biomarkers in Botryococcus are typically more enriched in ^{13}C than the lipids from other algae (Boreham et al., 1994; Summons et al., 2002), perhaps because of limited transfer of CO_2 from the surrounding water through the mucilage surrounding Botryococcus cells leading to enhanced intracellular carbon recycling.

Sources of hopanes and moretanes

The m/z 191 chromatograms here are very similar to that published by Meng et al. (2011) for a different sample of Lokpanta oil shale, although these authors did not identify the major hopenes.

Van Dongen et al. (2006) reported that some Early Eocene marine sediments from the Kilwa area, Tanzania contained high relative amounts of C_{30} and C_{31} ββ-hopanes and C_{29} hopenes. The distribution in their sample TDP 7a/54-1 (ca. 55.3 Ma) shows close similarities to those in the Huadian oil shales, with a predominance of the C_{29} neohop-13(18)-ene and smaller amount of the C_{30} neohop-13(18)-ene. Unlike the Lokpanta oil shale; that sample also contained the C_{27} neohop-13(18)-ene. Very similar distributions of neohop-13(18)-enes and ββ-hopanes were also reported by Lefort et al. (2012) for the Oxfordian-Kimmeridgian Flodigarry Shale Member and by Marynowski et al. (2007) for immature Middle Jurassic clays from Poland.

The major source of hopanoids > C_{30} in sediments is from diagenetic transformation of C_{31}–C_{35} bacteriohopanepolyols which occur in some cyanobacteria, and in heterotrophic and methanotrophic bacteria (e.g. Rˇezanka et al., 2010; van Winden et al., 2012; Eickhoff et al., 2014). It is intriguing that, despite the abundance of hopanoids in sediments, perhaps < 4% of bacteria contain the genes required for hopanoid biosynthesis (Pearson and Rusch, 2009). Hopanoid-producing bacteria also produce C_{30} hopanoids without an extended chain at C-22, including the C_{30} hopanoids diplopterol (hopan-22-ol; IV) and diploptene (hop-22(29)-ene; VII) (Fig. 5; Rohmer et al., 1984). Hopanoids were once considered as diagnostic markers for aerobic bacteria (e.g. Talbot and Farrimond, 2007 and references therein), but their biosynthesis does not require O_2. Hopanoids have now been found in several anaerobic bacteria (e.g. Sinninghe Damsté et al., 2004b; Hartner et al., 2005; Talbot and Farrimond, 2007), raising the possibility of a contribution of hopanoids from anaerobic bacteria to certain sediments. Nonetheless, in depositional environments such as the lacustrine setting of the Lokpanta oil shales an origin from aerobic bacteria in the water column seems more likely.

The presence of moretanes in immature sediments (e.g. XVII) is usually ascribed to partial isomerization at C-21 of naturally occurring 17β,21β-hopane isomers or alternatively (and perhaps more likely) due to reduction of the hop-21-enes (XI, XII; Fig. 5) and hop-17(21)-enes (ten Haven et al., 1986). Their low abundance is consistent with the low maturity of the Lokpanta oil shales, although we cannot rule out some direct contribution from bacteria (Rosa-Putra et al., 2001) since the hopane/moretane ratio values vary with carbon number.

Sources of hopenes and neohopenes

The presence of C_{27}, C_{29} and C_{30} neohop-13(18)-enes in marine and lacustrine sediments has been noted for many years [reviewed by Sinninghe Damsté et al. (2014)]. These authors proposed that a direct biological source of some neohopenes is likely based on their occurrence in particular sediments and from d13C data. C_{30} neohop-13(18)-ene has been reported as a very minor constituent of the purple non-sulfur bacterium Rhodomicrobium vannielii (Howard et al., 1984), in Alicyclobacillus acidocaldarius (Ageta et al., 1994), as a trace constituent of Frankia sp. (Rosa-Putra, 1998) and in the bacterium Zymonas mobilis (Douka et al., 2001). In none of these examples is the abundance of neohop-13(18)-ene particularly high relative to other hopenes and these reports cannot explain the predominance of neohop-13(18)-enes in the Lokpanta oil shale.

Methanotrophs contain a variety of hopanoid lipids, but neohop-13(18)-ene itself has not been reported. For example, *Methylococcus capsulatus* contains diploptene, diplopterol and 3β-methyl diplopterol as well as unusual 4-methyl and 4,4-dimethylsterols (Bird et al., 1971; Bouvier et al., 1976; Zundel and Rohmer, 1985). There are a number of reports of depleted $\delta^{13}C$ values for C_{27}– C_{30} hopanes and hopenes in sediments and in each case an origin from bacteria utilizing methane as carbon source (i.e. methylotrophic bacteria or methanotrophs) has been proposed (e.g. Freeman et al., 1990; Spooner et al., 1994; Köster et al., 1998; Burhan et al., 2002; Aichner et al., 2010b). The very light carbon isotope value of the C_{29} neohop-13(18)-ene and the C_{29} hop- 17(21)-ene (avg. -50.8‰) in IMA-27 (Table 3) indicates that the main source of these hopenes is methanotrophs. The C_{30} hop-17(21)-ene (-43.7‰) also shows a contribution from methanotrophs mixed with other bacterial sources. Sinninghe Damsté et al. (2014) have summarized $\delta^{13}C$ values reported for C_{27}–C_{30} neohop-13(18)-enes in sediments. In most cases, the values reported are not ^{13}C-depleted (e.g. for C_{30} neohopene reported values include: Arabian Sea: -20.7‰; Monterey: -22.2‰; Cape Verde: -22.6‰). However, Köster et al. (1998) reported depleted $\delta^{13}C$ values (-43.5‰) for both hop-17(21)-ene (XV) and neohop-13(18)-ene (XIX) in a black shale from the Oligocene Menilite Formation in Poland. In that case, these compounds were clearly from the same source and these authors also postulated a source methanotrophs. Aichner et al. (2010b) also reported isotopic depletion in diploptene (VII) and moretene (~ -60‰) from a sediment core from Lake Koucha in the eastern Tibetan Plateau, which they attributed to methanotrophs.

Aerobic methanotrophs are a diverse group of bacteria that can utilize CH_4 as a sole carbon and energy source. They are usually divided into Type I and Type II, and some authors also consider a third Type X. Each type contains abundant and distinctive distributions of bacteriohopanepolyols (Talbot et al., 2003; Rˇ ezanka et al., 2010), including distinctive hexafunctionalized 35-aminobacteriohopane-30R,31R,32R,33S,34S-pentol (I), which provides a useful biomarker for these bacteria in recent sediments (Neunlist and Rohmer, 1985; Cvejic et al., 2000; Talbot and Farrimond, 2007; Rˇ ezanka et al., 2010). Diagenesis of this biohopanoid can produce the hopanes and hopenes found in the Huadian shales (Fig. 5).

A distinguishing feature of the lipid profiles of some aerobic methanotrophs is the presence of bacteriohopanepolyols with a 3-methyl group (e.g. Neunlist and Rohmer, 1985; Summons and Jahnke, 1992; Burhan et al., 2002; Rˇ ezanka et al., 2010; Welander and Summons, 2012). On diagenesis, these give rise to 3β-methylhopanoids, which have been found in modern and fossil methane seep communities and used as biomarker proxies for aerobic methanotrophy (Welander and Summons, 2012). Only a trace of a C_{32} 2- or 3-methylhomohop-17(21)-ene was tentatively assigned in the Lokpanta shales, indicating that most of the methanotrophs in the original fresh water lacked the ability to synthesize 3-methylhopanoids.

Diagenetic factors determining the hopanoid distributions

Significant diagenetic transformation of the original lipids is apparent in the hydrocarbon distributions. This is clearly seen in the steroidal compounds, where clay catalysed transformation of steroidal compounds has led to diasterenes and 4-methyldiasterenes being the major steroidal compound class present. The hopanoids also show extensive transformation, with the high abundance of hopenes, neohopenes and the presence of des-Ehopanoids.

The high abundance of rearranged C_{29} and C_{30} neohop-13(18)-enes points to an origin from degradation and rearrangement of a C_{30} hopanoid precursor. Neohop-13(18)-enes can be formed from ubiquitous hopanols or hopenes through dehydration and isomerisation reactions (e.g. Sinninghe Damsté et al., 1995). For example, Ensminger (1977) showed that diploptene rearranges to neohop-13(18)-ene on treatment with active clay. Ageta et al. (1987) reported that neohop-12-ene was readily converted to neohop-13(18)-ene by mild acid and that the conversion could also occur on alumina. The key mechanism appears to be double bond migration out of the hopene side chain into ring D (Moldowan et al., 1991). A likely sequence of reactions (Sinninghe Damsté et al., 2014) is conversion of hop-22(29)-ene (diploptene; VII) to hop-21-ene (XI) to hop-17(21)-ene (XV) to neohop-13(18)-ene (XIX). The same sequence would apply to the C_{29} hopanoids (VIII to XII to XVI to XX; Fig. 5).

Hop-17(21)-enes (e.g. XV, XVI) are generally considered to be early diagenetic products from breakdown of bacteriohopanepolyols. For example, Sinninghe Damsté et al. (1995) found abundant C_{30}–C_{35} hop-17(21)-enes in the Jurf ed Darwish oil shale, with C_{35} 22R and 22S components dominating the distributions in that highly anoxic setting. This is in marked contrast to the Lokpanta oil shales where C_{29} and C_{30} components (XV and XVI) dominate the hop-17(21)-ene distributions, implying either a direct source from diagenesis of a C_{30} precursor hopanoid such as diplopterol (IV) and hopan-29-ol (V) or a contribution from diagenesis of longer chain bacteriohopanepolyols such as I and II (Fig. 5).

A scheme showing plausible pathways that account for all the hopanoids observed is shown in Fig. 5. It is not clear to which extent these reactions might have occurred when the OM was deposited, but extensive diagenesis would have occurred after burial. Indeed, Moldowan et al. (1991) have noted that it can be difficult to separate the effects of clay catalysis and those of partial oxidation in a sub-oxic (dysaerobic) depositional environment as found in many clay-rich source rocks. This would certainly apply to the Lokpanta oil shales. The scheme recognizes that there are direct inputs from C_{30} hopanoids such as hop-

22(29)-ene (VII) and diplopterol (IV) which are present in almost all hopanoid containing prokaryotes including cyanobacteria. The major compounds are always the C_{35} bacteriohopanepolyols, including I (Rohmer et al., 1984). A very high content of diplopterol has been noted in several Methylomonas species (230–1600 lg/g), although still less that the content of BHPs in the same species (3000–5800 lg/g; Rohmer et al., 1984). These species also contained diploptene.

On subsequent diagenesis these yield C_{27} and C_{30} hopanes, hopenes and neohopenes. Hopanoids > C_{30} are derived from side chain cleavage of bacteriohopanetetrols (typical examples are shown in Fig. 5, but there are many similar structures in bacteria; e.g. Talbot and Farrimond, 2007) that can also serve as precursors of the C_{27}–C_{29} hopanoids. The origin of C_{29} hopanoids has been much discussed because it is difficult to see how diagenesis of the C_{30} hopanoids diploptene and diplopterol would lose a single carbon to form C_{29} hopanoids.

However, a reasonable case can be made that the C_{29} hopenes and neohop-13(18)-ene originate from oxidative cleavage of hopan-29-ol (V), even though this is rarely found in organisms. Zundel and Rohmer (1985) noted that bacteriohopanepolyols in M. capsulatus and 3 Methylomonas species produced the hopan-29-ol and traces of the C_{29} hopanoid adiantol on HIO4 oxidation followed by NaBH4 reduction. They hypothesized that diagenesis of hopan-29-ol might account for the large amounts of hopanoids with an ethyl side chain (i.e. C_{29} hopanoids) commonly found in sediments and that these could represent chemical fossils of the hopanoids from methylotrophic bacteria. The $\delta^{13}C$ data are clearly consistent with the view that C_{29} and C_{30} precursor hopanoids in the Lokpanta oil shales are ultimately derived from methanotroph lipids via hop-17(21)-enes and neohop-13(18)-enes (Fig. 5). Isotopic evidence for such a pathway has also been obtained by Freeman et al. (1990) for the C_{29} hopane in the Eocene Messel shale, although in many sediments a source of C_{29} hopanoids from non-methanotrophs must occur since the hopanoids can show little difference in isotope values. This process is likely to be facilitated by contact with clays such as montmorillonite and kaolinite, as found in the Lokpanta oil shale (Hu et al., 2012).

Upon diagenesis, reduction of the neohop-13(18)-enes and further isomerisation can give rise to the series of rearranged hopanes found in many crude oils (Moldowan et al., 1991). For example, 30-norneohop-13(18)-ene (XX) would produce 18α(H),17α-methyl-28,30-dinorhopane [18α(H)-30-norneohopane, XXI], otherwise referred to as C_{29}Ts. This compound was not detected in the Lokpanta oil shales, reflecting their low maturity. The C_{30} neohopane (C_{30}Ts; XVIII) has not been detected in sediments or oils and yet the data reported here and elsewhere (Sinninghe Damsté et al., 2014) clearly show that the presumed precursor C_{30} neohop-13(18)-ene (XIX) occurs in a number of sediments.

Depositional environment

A heavily depleted $\delta^{13}C$ signal in the OM and lipids is typically taken as convincing evidence that the depositional environment contained an active CH_4 cycle. For example, Spooner et al. (1994) found that hop-22(29)-ene in a surficial sediment from a small eutrophic lake in the UK had a d13C value of -55.2‰, considerably lighter than the phytoplankton component (-32.7‰), attributed to cyanobacteria. In unusual environments, such as CH_4 seeps, where

CH_4 is the main carbon source for the microbial community, the $\delta^{13}C$ values of hopanoids and of bulk organic carbon can be in the -70‰ to -90‰ range (Burhan et al., 2002; Elvert and Niemann, 2008). It is clear from the range of isotope values found for the bulk hydrocarbon fractions and individual lipids in the Lokpanta oil shales (Table 3) that, while clearly CH4 oxidation was substantial, a microbial mat containing microorganisms using CH_4 as the main carbon source was not present. It is of interest that 3β-methylhopanes or other biomarkers representing methanotrophic bacterial activity were not detected by Strobl et al. (2014) in the contemporaneous Fushun shale, but isotopic data reported by Duan et al. (2004) suggest that CH_4 oxidation did occur in that setting. For example, they reported that the C_{27} 17β(H)-hopane had a $\delta^{13}C$ value of -46.3‰ and the C_{29} α,β-hopane a value of -42.6‰.

The biomarker data and maceral composition are consistent with a depositional environment with high algal productivity derived from a variety of species, including green algae, diatoms, dinoflagellates and other algal classes. Total phytoplankton production would have increased when the lake deepened (Sun et al., 2013). The absence of any biomarkers for photic zone euxinia indicates that bottom water sulfidic conditions did not reach the lit upper water. Indeed, the low content of sulfur (Xie et al., 2014) and trace amounts of organic sulfur compounds (av. 4.82) indicate that sulfidic conditions were not a feature of this depositional environment.

The good preservation of OM and presence of recognizable algal remains including telalginite is consistent with a low level of O_2 in the bottom water and the possibility of water column stratification, although we have no biomarker evidence to confirm the latter. However, it is unlikely that the ecosystem was continuously anoxic since the extended hopanoids have been substantially converted to shorter chain compounds and there is little preservation of the C_{31}–C_{35} hopanoids. Moreover, Pr/Ph is quite high (1.86 and 1.91) and this is usually attributed (with some caveats) to dysaerobic depositional environments (Didyk et al., 1978). In this case, the ratio probably reflects the importance of degradation of chlorophyll in the water column as well as in the sediments. The proposed depositional conditions are consistent with the work of Sun et al. (2013) who noted that their measured values of 0.53–0.82 for the V/(V + Ni) ratio in Huadian oil shales argued for strongly O_2-depleted, but not euxinic (sulfidic), conditions (Hatch and Leventhal, 1992). The lycopane/ n-C_{31} ratio has been suggested as a measure of palaeoxicity (Sinninghe Damsté et al., 2003), but in the Lokpanta depositional environment the high ratio simply reflects a low input of plant wax alkanes and good preservation of lipids from *Botryococcus*.

Ogihara (2014) also noted that the ratio was not an effective measure of anoxicity in bottom waters of the Japan Sea. Clearly there was substantial production of CH_4 in the river, implying that the sediments had low O_2 content, and the presence of isotopically depleted hopanoids, together with the absence of lipids associated with anoxic utilization of CH_4 (e.g. Thiel et al., 1999; Elvert et al., 2000), suggests that much of the CH_4 was assimilated in the water column by methanotrophs living there. Hydrocarbon biomarkers indicative of CH_4 producers, such as PMI (Fig. 1), were not abundant, which might lead one to propose that methanogen numbers were low. However, it may be that more polar constituents such as glycerol dialkyl glycerol tetraethers

(GDGTs) are present but these were outside the present study. The source of a sufficiently high level of CH_4 that could have supported extensive methane oxidation is presently unclear.

CONCLUSIONS

Aliphatic biomarker and petrology data for the Lokpanta oil shales provide a consistent picture of OM produced by a variety of microalgae including diatoms, Botryococcus and dinoflagellates in a deep lake with low O_2 content in the bottom water. No evidence for photic zone euxinia was obtained, and it is not clear whether the fresh water was stratified, although this would certainly be consistent with the good preservation of algal remains. The depositional environment clearly had an active methane cycle based on the abundance of hopanoid biomarkers for methane oxidizing bacteria. The high proportion of 4-methyldiasterenes is unusual and suggests a significant contribution from dinoflagellates and/or haptophytes in the lake phytoplankton. Definitive evidence for the importance of *B. braunii* race L was obtained from the presence of lycopadienes and monoaromatic lycopane derivatives in IMA-27, but these compounds were very minor in IMA-23, attesting to major changes in the abundance of this alga in the phytoplankton community during deposition. The high abundance of lycopane is attributed to reduction of lipids from Botryococcus given the similarity of its $\delta^{13}C$ isotope signature with that of the monoaromatic lycopane derivative. The hopane distributions show an abundance of β,β-hopanes indicative of a low level of maturity.

However, the predominance of C_{29} and C_{30} neohop-13(18)-enes is unusual and their depleted $\delta^{13}C$ values point clearly to an origin directly or indirectly from the lipids of methanotrophic bacteria. Significant diagenetic transformation of the original lipids is also apparent from the presence of rearranged hopenes and sterenes.

This is most obvious in the steroidal compounds where almost equal amounts of diasterenes and 4-methyldiasterenes were found but only minor amounts of steranes had been formed and precursor sterenes were only trace constituents. These biomarker characteristics share some features in common with other mid-Cretaceous fresh water oil shales in Nigeria, such as isotopically depleted hopanoids, but the low abundance of very long chain (> C_{29}) terrestrial leaf wax alkanes is distinctive. The main source of mid-chain *n*-alkanes may have been from aquatic macrophytes.

It is intriguing that, while Botryococcus appears to have been widespread and abundant both in lakes and fresh waters during the mid-Cretaceous, in the Lokpanta oil shale depositional environment the L race dominated.

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