Carbon isotopic composition of an isoprenoid-rich oil and its potential source rock

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Abstract

Despite the multiple controls on the carbon isotopic composition of organic matter, δ13C values for saturated and aromatic hydrocarbon fractions for over 300 Norwegian North Sea (Central Graben) oils range from −26 to −31‰ and from −24 to −30‰, respectively. However, for one oil (well 2/2-5), δ13C values for the saturate and aromatic fractions are −21.5‰ and −22‰, significantly enriched in 13C relative to the isotopic compositions of all oil fractions investigated. The saturate hydrocarbon fraction of the 2/2-5 oil is comprised predominantly of low-molecular-weight (<C20), primarily regular, acyclic isoprenoids. Also present are C36–C40 irregular acyclic isoprenoids; these compounds are composed of various combinations of tail-to-tail condensed C15–C20 regular isoprenoid subunits, resulting in pseudo-homologous series with six to nine methyl groups. The isotopic compositions of both high- and low-molecular-weight isoprenoids are around −19‰. This is in marked contrast to n-alkane δ13C values (ca. −31‰), and it is clear that isoprenoid δ13C values dictate the δ13C value of the saturate fraction. The same 13C-enriched isoprenoids are present in a thin (<1 m) immature oil shale facies (hudlestoni biozone) of the Kimmeridge clay formation (KCF; Dorset, UK). Moreover, the relatively immature KCF sediment contains unique high-molecular-weight isoprenoid thiophenes and thianes, whose structures indicate that sulfur has reacted with functional groups in the isoprenoid precursor’s interiors. This suggests that these novel high-molecular-weight isoprenoids were probably biosynthesized as such. Reasons for their profound 13C-enrichment and predominance are discussed. Although the KCF facies, located in the southern UK, cannot be a direct source of the North Sea 2/2-5 oil, a related North Sea facies seems plausible since an isotopically enriched TOC zone has been reported in a well also from the Central Graben. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Numerous factors govern the carbon-isotopic composition of algal and bacterial biomass. These include carbon source (Hayes, 1993), nutrient concentrations in

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organic matter (e.g. Hayes, 1993; Revill et al., 1994; Pancost et al., 1999) can strongly influence δ13C values of kerogen and oils. Because algal biomass δ13C values can range from −15 to −30‰ depending on physiological characteristics and growth conditions (Popp et al., 1998), significant variability in δ13C values could presumably occur even without dramatic source variations.

Nonetheless, the isotopic compositions of sedimentary organic matter generally range from −25 to −31‰ throughout the last 800 million years of Earth history (Hayes et al., 1999). Likely, this limited range is a consequence of the accumulation of organic matter from diverse sources and over extended intervals of time, which homogenizes inputs and averages out sources with extreme end-member δ13C values. For example, kerogen of Upper Devonian age in the Duvernay formation (Canada and Williston basins) contains abundant, 13C-enriched (ca. −15‰) biomarkers for green sulfur bacteria; nonetheless, the carbon isotopic composition of bulk organic matter is −29‰ (Hartgers et al., 1994) and similar to that observed for typical marine sediments of that age. Similarly, in the Green River shale, 13C-depleted methanogen biomarkers (as low as −80‰) are abundant but bulk organic matter δ13C values are ca. −30‰ (Collister et al., 1992).

However, numerous workers have shown that in some settings, kerogen consists of cell wall material predominantly derived from a single algal species (Tegelaar et al., 1989; Derenne et al., 1991; Gelin et al., 1996; de Leeuw et al., 1991; de Leeuw and Largeau, 1993). In some cases, these organisms apparently have unique physiologies that result in biomass and kerogen with carbon isotopic compositions significantly different from those generally observed for most marine or lacustrine oils. Examples include torbanites derived from selectively preserved Botryococcus braunii remains (Largeau et al., 1984, 1986; Dubreuil et al., 1989), tasmanites possibly derived from prasinophycean algae (Aquino-Neto et al., 1992; Revill et al., 1994), and organic matter-rich deposits of the extinct, organic-walled microfossil Gloeocapsomorpha prisca (Derenne et al., 1992; Foster et al., 1989; Pancost et al., 1999).

In this paper we present compound distributions and carbon isotope abundances for the Jurassic Kimmeridge Clay Formation (KCF) and a North Sea (Central Graben) oil. This work reveals that the bitumen in a KCF “oil shale” facies and the North Sea oil are profoundly enriched in 13C relative to adjacent stratigraphic units and related oils, respectively. Moreover, both are characterized by a predominance of similarly 13C-enriched isoprenoid compounds. Many of these isoprenoids have not previously been observed, indicating that although the source organism of these compounds is the vastly predominant source of total organic matter in these settings, it has yet to be identified in either modern or other ancient settings.

2. Experimental

2.1. Samples

The 2/2-5 oil from the North Sea, Central Graben, is one of several hundred North Sea oils collected by Norsk Hydro and for which saturate and aromatic δ13C values have been determined. The Kimmeridge clay formation (Dorset, UK) sample was collected from one of several narrow “oil-shale” intervals in the hudlestoni biozone (Cox and Gallois, 1981). The particular oil shale (carbonate content is 10%) was investigated by van Kaam-Peters et al. (1998; sample UK4), and throughout this paper, references to the KCF hudlestoni “oil shale” facies pertain only to that specific interval. This unit is characterized by profound 13C-enrichment (−22.9‰) of organic matter and high total organic carbon contents (TOC; 22.9%). Similarly, the organic sulfur content is elevated relative to adjacent units while the concentration of pyrite is similar to that throughout the KCF (van Kaam-Peters et al., 1998).

2.2. Extraction and fractionation

For the KCF sample, powdered rock (ca. 70 g) was Soxhlet extracted with methanol (MeOH)/dichloromethane (DCM) (1:7.5 v/v) for 24 h. Asphaltenes were removed from the extracts by precipitation in heptane. An aliquot of the maltene fraction (ca. 200 mg), to which a mixture of four standards was added for quantitative analysis (Kohnen et al., 1990), was separated into apolar and polar fractions using a column packed with alumina (van Kaam-Peters et al., 1998). An aliquot of the maltene fraction of the 2/2-5 oil was similarly prepared but during column chromatography was split into three fractions using hexane (saturate fraction), hexane:DCM (9:1 v/v; aromatic fraction), and DCM/MeOH (1:1 v/v; stripping) as eluents. Saturated hydrocarbon and aromatic hydrocarbon fractions were analyzed by GC and GC/MS. n-Alkanes were removed from aliquots (ca. 4 mg) of the saturated hydrocarbon fractions by molecular sieve adduction (van Kaam-Peters et al., 1998). An aliquot (ca. 10 mg) of the KCF apolar fraction was further separated by argentation TLC (thin layer chromatography) into four fractions, which were scraped off the TLC plate and ultrasonically extracted with ethyl acetate (van Kaam-Peters et al., 1998). Aliquots (ca. 15 mg) of the polar fraction and the A2 (thiophenes) and A4 (thianes) TLC fractions of the KCF were desulfurized using Raney nickel and subsequently hydrogenated (Sinninghe Damsté et al., 1988).

2.3. Off-line pyrolysis and fractionation

Off-line pyrolysis was performed with a weighed amount of decarbonated (6 N HCl), ultrasonically
extracted rock sample, equivalent to ca. 100 mg organic carbon to which 100 µg standard (3-methyl-6-dideuterohenicosenoic) was added. The sample was heated (400°C) for 1.5 h and the volatile products generated were trapped in cold traps and fractionated by column chromatography according to the methods described in van Kaam-Peters et al. (1998).

2.4. Analysis of biomarkers

Biomarker fractions were analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) for identification using previously described conditions. GC/MS with chemical ionization was performed using an HP 6890 gas chromatograph coupled to a HP 5973 mass spectrometer with a mass range m/z of 50–800 and a cycle time of 0.5 s. Curie-point pyrolysis-gas chromatography (Py-GC) was performed with a Hewlett-Packard 5890 gas chromatograph using a FOM-3LX unit for pyrolysis and according to the conditions published in Höld et al. (1998).

2.5. Isotope-ratio-monitoring gas chromatography-mass spectrometry (irm-GC/MS)

Irm-GC/MS was performed on a Finnigan Delta C and used to determine compound-specific δ13C values (Hayes et al., 1990; Merrit et al., 1994). The GC conditions are the same as those used during GC/MS analyses. δ13C values are expressed against the Vienna Peedee Belemnite standard and have an error of less than ±0.4‰ unless otherwise noted (error based on analytical accuracy and precision of measurements of co-injected standards —C30 and C24 perdeuterated n-alkanes). For high-molecular-weight isoprenoids, the errors are typically ±1.0‰ due to co-elution.

Carbon isotope abundances of the saturate and aromatic fractions of the North Sea oils were determined using the methods described by Bjorøy et al. (1994).

3. Results

3.1. Geochemical analyses of the 2/2-5 North Sea oil

Extensive analyses of over 300 North Sea oils reveal a remarkable similarity of saturate and aromatic fraction δ13C values. Despite age or inferred source rock facies, relatively narrow ranges of −26 to −31‰ and −24 to −30‰ were obtained for all saturate and aromatic fractions, respectively (Fig. 1). This is essentially an extension and confirmation of previously published analyses of 72 Central Graben oils (Bjorøy et al., 1993). The sole exception is an oil from the 2/2-5 well, for which the saturate fraction has a δ13C value of −21.5‰ and the aromatic fraction has a δ13C value of −22‰. The reason for this difference is unclear and is exacerbated by uncertainty in the age of the 2/2-5 oil. The aromatic fraction contains isorenieratene alteration products (Koopmans et al., 1996a), which are biomarkers for green sulfur bacteria (Chlorobiaceae; Liaaen-Jensen, 1979). These compounds are enriched in 13C in the 2/2-5 oil, as is commonly observed for isorenieratene derivatives (Sinninghe Damsté et al., 1993; Koopmans et al., 1996a) due to this organism’s utilization of the reverse tricarboxylic acid cycle (Sirevåg and Ormerod, 1970; Quandt et al., 1977; Sirevåg et al., 1977). While this could explain the 13C-enrichment of the aromatic fraction, it cannot explain the enrichment observed for saturated hydrocarbons, and so we examined this fraction in greater detail.

3.1.1. Biomarker distributions of the 2/2-5 oil

The saturate hydrocarbon fraction of the 2/2-5 oil contains typical marine oil biomarkers, including steranes, hopanones, and n-alkanes with a distribution exhibiting no odd-over-even predominance and a maximum at n-C17. However, these compounds are of relatively low abundance compared to the profound predominance of acyclic isoprenoids (compounds a to l; Fig. 2a and structures are shown in the Appendix). The distribution of the isoprenoids ranges from C13 to C40 and is bimodal with the most abundant compounds containing 16–20 carbon atoms. The second maximum in the isoprenoid distribution profile occurs in the C36–40 range and is considerably more complex than that of the low-molecular-weight isoprenoids (Fig. 2a).

3.1.2. Identification of high-molecular-weight irregular isoprenoids

Chemical ionization (CI) MS was used to ascertain the molecular weight of the compounds eluting in the high-molecular-weight range and revealed that they consist of multiple isomers of C36–40 acyclic compounds. In all compounds, pronounced enhancements of the m/z 113 and 183 fragments in their electron impact (EI) mass spectra indicate that these compounds are isoprenoids. With the exception of lycopane (h–h; see Appendix for structures) and g–h (Philp, 1985), these are previously unreported compounds and thus, neither previously published mass spectra nor authentic standards are available. On the basis of molecular weights determined by GC/CI-MS and fragmentation patterns obtained by GC/EI-MS, structures were tentatively assigned.

These assignments were further confirmed by comparison to calculated retention (Kovats) indices (Table 1). Retention indices were calculated according to the method of Kissin et al. (1986) and are systematically offset from measured values by 20–30 units. Because the identifications of g–h and, particularly,
lycopane have been established, this offset is likely a consequence of our particular GC conditions. Correcting for this offset reveals that all measured retention indices are within 5 units of those calculated and supports our structural assignments.

In general, the compounds are C36–40 irregular acyclic isoprenoids composed of various combinations of C15–C20 regular isoprenoid subunits, resulting in pseudo-homologous series with six to nine methyl groups. For example, compound f–g is characterized by enhanced m/z 113, 183, and 253 fragments consistent with an acyclic isoprenoid structure (Fig. 3a). The larger mass fragments do not extend this pattern but instead exhibit enhanced fragments at m/z 295, 365, 435 indicating that the structure is an irregular isoprenoid. The specific fragmentation is consistent with a C37 isoprenoid containing an extended n-alkyl portion in the core of the molecule.

In all cases, the high-molecular-weight isoprenoids have terminal head groups, indicating that two C15–20 isoprenoid units have been joined together in a tail-to-tail linkage. Indeed all structures are consistent with a tail-to-tail condensation of two C15–20 isoprenoid units, and in most cases represent the joining of the two terminal carbon atoms. The sole exception is h–h*, a C40 compound that appears to be composed of a terminal carbon on one C20 isoprenoid unit joined to the ω-1 carbon of another. This compound, in particular, generates highly diagnostic fragments during GC/MS analyses. The preferred cleavage of the bond linking two tertiary carbon atoms results in pronounced m/z 252 and 308 peaks (Fig. 3b). Such cleavages are expected to generate the atypical even mass fragments (cf. Kohnen et al., 1990).

With the exception of lycopane and g–h, these are novel compounds. Regular isoprenoids extending up to C35 were reported by Albaiges (1980) and head-to-head isoprenoids ranging from C28 to C40 are specific biomarkers for archaea and are found in diverse oils and...
Lycopane and its unsaturated homologues, typically attributed to archaea but also observed in many microorganisms and higher plants (Schmidt, 1979), are the only previously reported high molecular weight (>C35) tail-to-tail isoprenoids. In addition, C36–40 isoprenoids with aromatic moieties near the center of the molecule are also present. Probably, because of the extended alkyl components, these compounds eluted in the saturate fraction during our preparation. These isoprenoids are present in abundances about one order of magnitude lower than that of 

Fig. 2. Gas chromatograms of the saturate hydrocarbon fractions of the 2/2-5 oil (a) and the KCF hudlestoni “oil shale” facies (b). The insets show the distributions of high-molecular-weight C35-C40 isoprenoids as revealed by an m/z 183 mass chromatogram. Isoprenoid compounds of interest are labeled (a–l; selected mass spectra are shown in Fig. 3 and structures are shown in the Appendix) and squares denote n-alkanes.
the acyclic isoprenoids and comprised of a very complex distribution of structural isomers. Nonetheless, their distribution and chain lengths are consistent with a genetic link to the acyclic isoprenoids.

3.1.3. Low-molecular-weight isoprenoids

In addition to the abundant regular isoprenoids, we also observe C₁₉ (g’) and C₂₀ (h’) isoprenoid compounds characterized by different retention times and fragmentation patterns than regular isoprenoids (Fig. 2a; structures shown in Appendix). Mass spectra suggest that these compounds are composed of three acyclic isoprenoid units with an additional four and five methylene units, respectively. The mass spectrum of compound g’ has been previously published (McCarthy and Calvin, 1967) and is in excellent agreement with our observations. Such compounds could have been generated by thermal cracking of the previously described high-molecular-weight isoprenoids. Specifically, cleavage at branch points in f–g and h–c will generate the C₁₉ isoprenoid with an extended n-alkyl tail and cleavage of h–d and h–f will generate the C₂₀ compound. Based on such considerations, higher molecular weight isoprenoids (C₂ₑ–₂₅) with extended n-alkyl tails are also expected (e.g., 2,6,10,14 tetramethylicosane [l] would also be generated by cleavage of f–g). Indeed, careful inspection reveals that such compounds probably are present in the saturate hydrocarbon fraction of the 2/2-5 oil; however, their abundances are too low relative to background to obtain unambiguous mass spectra.

3.1.4. Compound-specific δ¹³C values

All acyclic isoprenoids, including both low and high-molecular-weight components, have δ¹³C values ranging from −17 to −20‰ (Fig. 4a). The similarity of δ¹³C values for both high and low-molecular-weight isoprenoids suggests that they derive from the same source. These values are also identical to that of the C₁₉ isoprenoid with an extended n-alkyl tail (g’). In contrast, δ¹³C values of co-occurring n-alkanes range from −28 to −31‰, profoundly depleted in ¹³C relative to all acyclic isoprenoids.

3.2. Geochemical analyses of the KCF hudlestoni oil shale facies

3.2.1. Saturate hydrocarbons

During previous molecular and isotopic investigations of the KCF, van Kaam-Peters et al. (1998) observed that an interval of the hudlestoni “oil shale” facies (Cox and Gallois, 1981) is characterized by a profound abundance of C₁₆-₃₀ homologues, the most abundant components of the saturate hydrocarbon fraction (Fig. 2b). In contrast to adjacent KCF facies in which phytane concentrations are always <350 μg/g TOC and typically lower, in the hudlestoni “oil shale” facies the phytane concentration is >1200 μg/g TOC. n-Alkane concentrations, on the other hand, do not vary among the hudlestoni “oil shale” and adjacent facies, suggesting that inputs of these compounds did not vary substantially.

δ¹³C values of the isoprenoids in the hudlestoni “oil shale” facies range from −17 to −21.5‰ and are enriched in ¹³C relative to isoprenoids in all other analyzed KCF facies. In one facies, the mean isoprenoid δ¹³C value is −22.5‰, in another it is −25‰, and in all others, it is ca. −30‰ (van Kaam-Peters et al., 1998). The acyclic isoprenoids in the hudlestoni “oil shale” facies are also profoundly enriched in ¹³C relative to co-occurring n-alkanes, which are generally −28‰ (Fig. 4b). The only n-alkanes with δ¹³C values comparable to those of the isoprenoids are n-C₂₁ and n-C₂₅, but these enriched n-alkanes are characteristic of all other KCF facies (van Kaam-Peters et al., 1997, 1998).

Further investigation revealed that the high-molecular-weight isoprenoids observed in the 2/2-5 oil are also present in the KCF hudlestoni “oil shale” facies (Fig. 2b). Although there are some minor differences in compound distributions between the KCF and the 2/2-5 oil, the same compounds are present and the overall distribution is the same. These compounds are also enriched in ¹³C (Fig. 4b), although low signal to background ratios resulted in large accuracy errors for these determinations (±1.5‰). The KCF hudlestoni “oil shale” facies also contains cyclized and aromatized isoprenoids similar to those observed in the 2/2-5 oil.

3.2.2. Sulfur-containing compounds

In addition to the aromatic and acyclic isoprenoids, we also observed thiophenes, thianes, and thiolanes with C₃₆–₄₀ isoprenoid skeletons (Figs. 5a and 6a). Although
some low-molecular-weight sulfur-containing isoprenoids are present, the high-molecular-weight components are dominant and, based on mass spectra, appear to be directly related to the acyclic high-molecular-weight isoprenoids. The structural relationship between the acyclic isoprenoids and the thiophenes, thianes, and thiolanes was proven by desulfurization of the former compounds (Figs. 5b and 6b). In both cases, the previously described acyclic isoprenoids were generated. Based on mass spectra of the intact thiophenes, thianes, and thiolanes, sulfur was incorporated into the interior of the aforementioned high-molecular-weight isoprenoids (Fig. 7) — similar to the patterns expressed by the isoprenoids with aromatic moieties.

Although both the thiophenes and thianes/thiolanes are clearly structurally related to the high-molecular-weight isoprenoids, their distributions differ. In the case of isoprenoid thiophenes, the distribution of compounds
generated upon desulfurization is similar to the distribution of sulfur-free compounds. In contrast, the thiane/thiolane fraction contains predominantly C_{39} and C_{40} isoprenoids — specifically those compounds in which the thiane or thiolane moiety is joined to one of the isoprenoid chains via a quaternary carbon. It seems likely that the differences in distribution reflect the age and thermal maturity of the KCF. A more complex distribution of thiannes and thiolanes was probably originally present; however, with thermal maturity...
compounds lacking a quaternary carbon were transformed into more thermodynamically stable thiophenes (Sinninghe Damsté and de Leeuw, 1990).

In addition to isoprenoid compounds with intermolecular sulfur moieties, the presence of intramolecular sulfur incorporation was shown by desulfurization of the polar fraction (Fig. 8). Both low and high-molecular-weight isoprenoids were generated and the high-molecular-weight compounds have structures identical to those observed in the saturated hydrocarbon fraction and generated from the thiophene and thiane/thiolane fractions. The distribution of high-molecular-weight isoprenoids is similar to that observed in the free hydrocarbon fraction, although with somewhat elevated abundances of the C_{39} and C_{40} components.

$\delta^{13}$C values for thiophenes and thianes were not determined. However, $\delta^{13}$C values of the high-molecular-weight isoprenoids generated by desulfurization of the polar fraction have values ranging from $-18.5$ to $-20\%$ and are consistent with those determined for all other isoprenoids in the KCF hudlestoni “oil shale” facies.

3.2.3. Kerogen pyrolysis

GC/MS traces clearly show that the kerogen pyrolysate of the KCF hudlestoni “oil shale” facies is dominated by isoprenoids (Fig. 9) (Höld et al., 1998). Van Kaam-Peters et al. (1998) performed off-line pyrolysis to examine the $\delta^{13}$C values of these and other kerogen-bound compounds (Fig. 4b). In general, the kerogen-bound components exhibit the same isotope patterns as those expressed by the free lipids. Specifically, isoprenoids in the hudlestoni “oil shale” facies are enriched relative to isoprenoids in other facies and are enriched in $^{13}$C relative to n-alkanes in both the same and other facies.

4. Discussion

4.1. Relationship between the KCF hudlestoni “oil shale” facies and 2/2-5 oil

The KCF hudlestoni “oil shale” facies cannot be a direct source for the 2/2-5 oil. The location of the KCF is too far south to have served as a source rock for the Central Graben oil. Additionally, this southern UK KCF site is not sufficiently thermally mature to have generated oil. This is apparent from burial history and biomarker ratios in the KCF. In this study, the presence of abundant sulfur-containing compounds indicate that
the KCF is not even of sufficient thermal maturity for all C–S bonds to have been cleaved and further indicates that it is not a petroleum source rock.

Nonetheless, the bitumen in the KCF hudlestoni “oil shale” facies and the 2/2-5 oil share several marked similarities that clearly suggest a relationship between the organic matter source for each and distinguish them from other oils and bitumens. In both, 13C-enriched isoprenoids are the dominant components in the saturate hydrocarbon fraction. Moreover, both fractions contain 13C-enriched high-molecular-weight isoprenoids with structures that have not previously been reported for any other oil or source rock. Nor have possible precursor compounds been observed in any modern environment or extant organism. Other biomolecular characteristics, such as the presence of isorenieratane in both, reinforce this similarity.

There is a thin zone of source rocks with a carbon isotopic enrichment (−24‰ versus −28‰ on average) in both the kerogen and the kerogen pyrolysate of the Kimmeridgian of the Central Graben (Bailey et al., 1990). Thus, this zone could be the source rock of the Central Graben 2/2-5 oil. If this is indeed true, then a thin isotopically enriched zone in the middle of several hundred meters of potential source rock has generated and expelled hydrocarbons, whereas no significant contribution from the rest of the source rock section is noted in the 2/2-5 oil. This could be explained by the fact that, like the KCF oil shale facies, the Central Graben facies originally contained abundant sulfur-bound isoprenoids, resulting in a much earlier oil generation. Artificial maturation experiments of organic sulfur-rich source rocks in combination with the analysis of sulfur-bound biomarkers have demonstrated that generation of hydrocarbons can indeed occur at low levels of thermal stress in rocks where organic sulfur contents are high (Koopmans et al., 1996b, 1998).

4.2. Relationship amongst isoprenoid components

There are relatively few cases in which isoprenoids comprise a significant component of either an organism’s biosynthesized or preserved biomass. Here, not only is a predominance of isoprenoids observed but also significant quantities of novel high-molecular-weight components. In some instances, it has been shown that
Fig. 7. Structures of thiophene, thiane, and thiolane isoprenoids present in the A2 and A4 fractions of the KCF *hudlestoni* “oil shale” facies and their products formed upon Raney nickel desulfurization.

Fig. 8. Gas chromatogram showing the desulfurized and hydrogenated polar fraction of the KCF *hudlestoni* “oil shale” facies. Labels denote structures shown in the Appendix and Fig. 7 and squares denote *n*-alkanes.
the distribution of biomarkers in the extractable fraction of ancient rocks is not representative of the source contributions to the total preserved organic matter (Goth et al., 1988; Tegelaar et al., 1989; Hartgers et al., 1994; Sinninghe Damsté and Schouten, 1997). However, both pyrolysis and off-line pyrolysis clearly reveal the predominance of isoprenoids in the kerogen of the KCF hudlestoni ‘‘oil shale’’ facies.

It seems likely that all isoprenoids in the KCF hudlestoni ‘‘oil shale’’ facies are related. The δ13C values of all high- and low-molecular-weight isoprenoids in the free hydrocarbon and polar desulfurized fractions and generated during off-line pyrolysis of the kerogen are similar and distinct from other hydrocarbons. However, it is unclear whether both the low- and high-molecular-weight isoprenoids were biosynthesized by the precursor organism or if only the high-molecular-weight compounds were biosynthesized and low-molecular-weight components were generated upon thermal cracking. It seems likely that the latter explanation is largely true. The subordinate series of low-molecular-weight acyclic isoprenoids with extended n-alkyl tails are likely products of thermal cracking of the high-molecular-weight compounds.

The location of the sulfur atoms in the high-molecular-weight isoprenoid sulfur compounds provides further clues to the structure of the original biological compounds. Sulfur incorporation into sedimentary organic matter is known to occur relatively rapidly (Kok et al., 2000; Werne et al., 2000), and thus, is expected to react with pre-existing (i.e. biosynthesized) functionalities. Thus, the location of the thiophene, thiane, and thiolane moieties suggests that the novel high-molecular-weight isoprenoids were biosynthesized as such and with interior functional moieties, such as double bonds or keto groups (Schouten et al., 1993; de Graaf et al., 1992).

As stated previously, the majority of the compounds reported here are novel; however, there are some similarities to previously reported regular and irregular isoprenoids and linearly extended phytane skeletons containing thiophene moieties (Peakman et al., 1989a). Specifically noteworthy similarities are the extended n-alkyl tails and thiophene moieties in these structures. In contrast to the isoprenoids reported here these compounds are less abundant than co-occurring n-alkanes, but they have been observed in a variety of bitumens and oils (Peakman et al., 1989b) and could have a related source.

4.3. Sources of isoprenoids

In the saturate fractions of both the KCF hudlestoni ‘‘oil shale’’ facies and the 2/2-5 oil, the predominant compounds are 13C-enriched isoprenoids. This is in marked contrast to typical marine oils and sediments, in which a mixture of components derived from diverse phytoplankton, bacteria, and possibly terrigenous inputs are present, and it seems likely that the primary source of organic matter to the KCF facies and 2/2-5 oil is a single isoprenoid-rich organism. However, the nature of this organism and reason for its imprint on both the 2/2-5 oil and the KCF is difficult to ascertain.

One explanation for the predominance of acyclic isoprenoids is enhanced preservation of precursor compounds via sulfur incorporation. In the KCF, the release of high-molecular-weight irregular isoprenoids from the thiane/thiolane, thiophene, and polar fractions provides
direct evidence that such processes did occur. Indeed, unsaturated analogs to lycopane (h–h) have been shown to react with sulfur and aromatize, resulting in compounds similar (VIII) or identical (IX) to those observed here (Grice et al., 1998a). The presence of isorenieratane, a biomarker for green sulfur bacteria, which require both light and sulfide, throughout the KCF (van Kaam et al., 1997, 1998), clearly indicates that the availability of reduced sulfur was not restricted to the “oil shale” facies. Moreover, Sinninghe Damsté et al. (1998) showed that the abundance of low-molecular-weight thiophenes released upon pyrolysis is high throughout the KCF and correlated to the %TOC. These observations reveal that sulfurization of organic matter was not restricted to the “oil shale” facies and is apparently unrelated to the presence or absence of the 13C-enriched isoprenoids. Thus, the occurrence of these compounds in restricted intervals of the KCF is due not to changes in conditions favoring preservation but to the emergence of an isoprenoid-rich organism into a setting ideal for preserving its biomarkers.

The nature of such an organism is unclear. Lycopene is a carotenoid common in diverse microorganisms and higher plants (Schmidt, 1978 and references therein); however, because it is unsaturated at thirteen positions, sulfurization of lycopene would be expected to generate compounds in which sulfur moieties are not restricted to the interior of the molecule. The structures we observe are consistent with an origin from lycopadienes. Lycopadienes have only been observed in the L strain of B. braunii (Metzger et al., 1991) and certain thermophilic archaea (Lattuati et al., 1998). Although neither a freshwater alga nor a thermophilic archaea can be the direct source of the isoprenoids in these samples, it is tempting to suggest that a marine, mesophilic analog to one of the above organisms was present in the Jurassic epicontinental seas. Indeed, recent work (DeLong et al., 1998; Hoeft et al., 1997; Schouten et al., 1998) has highlighted the ubiquity of archaea-derived compounds in marine waters and sediments. Moreover, the isoprenoid chains of such pelagic crenarchaeota are typically enriched in 13C relative to compounds derived from other organisms (Hoeft et al., 1997). In addition, C21–25 isoprenoidal biomarkers isolated from Miocene/Pliocene deposits of the Dead Sea basin (Sdom formation) and inferred to be derived from halophilic archaea also exhibit a strong enrichment in 13C (7‰) relative to co-occurring phytoplankton biomarkers (Grice, 1998b). Although unsaturated analogs of most of the C36–40 irregular isoprenoids observed here have never been reported, it is possible that a poorly studied or even non-extant archaeon could have thrived during this brief interval and served as a source for these compounds.

An alternative explanation is that the isoprenoids derive from a resistant isoprenoidal biopolymer (cf. Hold et al., 1998). Indeed, the presence of functional groups in the interior of the isoprenoids is suggestive of the oxidative cross-linking that occurs in biological macromolecules such as algaenan (Blokker et al., 1998). Such an isoprenoidal biopolymer — but not an isoprenoidal algaenan — has been isolated from B. braunii race L (Berthéas et al., 1997). However, it is highly unlikely that this lacustrine alga contributed isoprenoids to the marine 2/2-5 oil and KCF sediments. Also, the isoprenoidal biopolymer in B. braunii race L would not be as resistant to degradation as the co-existing aliphatic algaenan (Derenne et al., 1989), and such an organism is not expected to generate sedimentary organic matter dominated by isoprenoids. Other than the L race of B. braunii, there are no other modern organisms known to biosynthesize isoprenoidal biopolymers. Nonetheless, it remains possible that an organism living during the Jurassic could have synthesized such a biopolymer, which would have been preferentially preserved relative to other sources of organic matter as has been observed for aliphatic algaenans (Tegelaar et al., 1989; de Leeuw and Largeau, 1993).

4.4. Causes of 13C-enrichments

The high δ13C values are a striking characteristic of the isoprenoids in both the 2/2-5 oil and the KCF “oil shale” facies. Low CO2 or high algal growth rates typically result in low carbon isotope fractionation (εp) by marine phytoplankton (Goericke et al., 1994; Laws et al., 1995; Popp et al., 1998) and, thus, 13C-enriched biomass. In such a situation, most phytoplankton are expected to respond to the environmental conditions, and compounds derived from all organisms should exhibit similar enrichment. However, in the KCF oil shale facies n-alkanes show no enrichment in 13C relative to those in adjacent units. Based on this as well as the carbon isotopic compositions of other biomarkers and carbonate, it seems unlikely that either CO2aq concentrations or DIC δ13C values varied (van Kaam-Peters et al., 1998). Thus, the 13C-enrichment of the acyclic isoprenoids is specific to an organism rather than solely the result of surface-water nutrient concentrations.

The observed 13C-enrichment of acyclic isoprenoids relative to n-alkanes could indicate that the source of the isoprenoids grew during episodic blooms. In this situation, the isoprenoid-rich organism would have grown under environmental conditions decoupled from “normal” conditions during which other organisms grow. Moreover, bloom conditions — high growth rates and lowered CO2aq concentrations — are expected to cause 13C-enrichment in algal biomass. Such blooms, because they represent episodic high productivity events, also might be preferentially preserved relative to non-blooming organisms and account for the high abundance of isoprenoids in our samples.

An alternative possibility is that the isoprenoid-rich organism is physiologically distinct from the algae that...
predominate during most of the time of KCF deposition. For example, CO$_2$ diffusion into the cell of the isoprenoid-rich organism could have been limited. Theoretical considerations indicate that physiological limitations to CO$_2$ diffusion can exert a large control on algal $\delta^{13}$C values (Rau et al., 1996). Such an explanation has been invoked to explain the observed $^{13}$C-enrichment of algae such as *B. braunii* that biosynthesize algaenan and are characterized by a thick cell wall (Boreham et al., 1994). Another physiological difference that could affect the carbon isotopic composition of an organism is the use of a carbon fixation pathway other than the Calvin cycle. Fractionation during carbon assimilation via either the reverse TCA cycle (Sir-evåg et al., 1977) or the 3-hydroxypropionate pathway (van der Meer et al., submitted) can be significantly lower than that during the Calvin cycle. However, all known algae utilize the Calvin cycle during photosynthesis, and thus, this explanation implies that the source of the isoprenoids was either an archaeon or a bacterium.

5. Conclusions

The 2/2-5 oil and the bitumen of the hudlestoni “oil shale” facies of the KCF share profound and diagnostic characteristics that clearly suggest a relationship. Both the oil and the bitumen contain novel high-molecular-weight isoprenoids and the saturate hydrocarbon fractions of both are dominated by $^{13}$C-enriched isoprenoids. Although the latter cannot be a source rock for the 2/2-5 oil, the presence of an isotopically enriched source rock facies in the Central Graben (Burkwood et al., 1990) suggests that an equivalent facies is.

There are multiple explanations for the unique characteristics of the 2/2-5 oil and the bitumen of the KCF “oil shale” facies. The high abundances of regular isoprenoids and irregular isoprenoids with unprecedented structures, both of which are characterized by elevated $\delta^{13}$C values indicate that the source organism is uncommon and if extant, has not been studied. Consequently, we cannot state with certainty the ecology or physiology of this organism. The sum of the evidence is consistent with either an algae that grew in strong and episodic blooms or biosynthesized a resistant cell wall or an archaeon rich in lycopadienes and related unsaturated irregular isoprenoids. Both explanations explain the predominance of isoprenoids in the KCF “oil shale” facies and the 2/2-5 oil and the fact that those isoprenoids are enriched in $^{13}$C. Regardless of source, the organism is clearly uncommon in both ancient and modern settings. Nonetheless, in these samples it is the predominant source of organic carbon, is largely responsible for high TOC contents in the KCF facies, and governs the unique carbon isotopic characteristics of the saturate hydrocarbon fraction of the 2/2-5 oil.

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Appendix

| f–d | g' |
| h–c | h' |
| f–e | f' |
| h–d | g |
| f–g | h |
| g–g | g' |
| h–f | h' |
| g–h | f' |
| h–h* | h–h |
References


Huang, Y., Peakman, T.M., Murray, M., 1997. $\delta^{13}$C, $\delta^{18}$O, and $\delta^{15}$N of a novel, optically active tricyclic hydrocarbon of algal origin. Tetrahedron Letters 38, 5363–5366.


