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Evidence for the rapid incorporation of hopanoids into kerogen

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Abstract—Hopanoids bound into the insoluble organic matter (kerogen) of Recent sediments from a freshwater lake (Priest Pot) and an anoxic sulphidic fjord (Framvaren) were released by hydropyrolysis and examined by gas chromatography-mass spectrometry. Bound hopanoids are present in high concentration (190–1400 μg/g TOC) and represent 22 to 86% of the total analysable hopanoids (i.e., bound and solvent-soluble), this proportion increasing with depth in Framvaren Fjord. The hopanes generated by hydropyrolysis contain higher amounts of the C_{35}, C_{32}, and C_{30} homologues, reflecting the carbon number distribution of the bound hopanoids and indicating that both biohopanoids (C_{30} and C_{35}) and their diagenetic products (dominated by C_{32}) are incorporated into the kerogen on a timescale of only 0 to 350 years. Sequential (multiple temperature) hydropyrolysis experiments gave an indication of the relative strengths of bonds being cleaved in association with hopane generation: The hopanoids of a sediment from Priest Pot are almost entirely bound by strong covalent bonds, interpreted to be mainly ether linkages, whilst a Framvaren sediment contains hopanoids that are bound by a mixture of weak di-/polysulphide linkages and stronger ether bonds. Labelling with deuterium indicated that the strong covalent linkages dominate, even for the Framvaren sediment. *Copyright © 2003 Elsevier Science Ltd*

1. INTRODUCTION

Lipids derived from organisms contributing organic matter to depositional environments can be preserved in the sedimentary record where they act as biological markers (biomarkers) for both the organisms and the environmental conditions prevailing at the time of deposition. However, only a fraction of the lipids produced by their source organisms survive in sediments, and most of those that do survive undergo a range of diagenetic reactions that modify their chemical structure. Studies of the effects of diagenesis on biomarker composition have typically focused on transformations between individual precursors and products in the solvent-soluble organic matter (bitumen) of sediments and sedimentary rocks (e.g., Mackenzie et al., 1982). However, with increasing understanding of the origin and composition of macromolecular sedimentary organic matter (resins, asphaltenes, and kerogen), it has become apparent that kerogen is an important sink for the preservation of biomarkers in the geosphere (Eglinton and Douglas, 1988; Love et al., 1995).

Hopanoids comprise one of the most abundant groups of organic molecules in sedimentary organic matter (Ourisson et al., 1984; Ourisson and Albrecht, 1992). Their biological precursors include bacteriohopanetetrol and similar highly functionalised components, which are synthesised as membrane lipids by bacteria of diverse taxonomic groups (Rohmer et al., 1984). In Recent sediments, biohopanoids are subject to a wide range of early diagenetic reactions, including loss or alteration of functional groups, structural modification and rearrangement, stereochemical changes, aromatisation, and, potentially, incorporation into macromolecular organic matter. The most frequently reported geohopanoid products in modern environments are hopanoic acids and hopanols (generally dominated by the C_{32} $17\beta(H),21\beta(H)$ components) and C_{30} hopenes (e.g., Dastillung et al., 1980a; Ries-Kautt and Albrecht, 1989; Buchholz et al., 1993; Innes et al., 1997, 1998). Bacteriohopanediols and triols, representing incomplete side-chain cleavage reactions, have been observed in Recent sediments (Rodier et al., 1999; Watson and Farrimond, 2000). Hopanoidal aldehydes, ketones, and hopanes also occur, although usually only as minor components (Dastillung et al., 1980a, 1980b; Robinson et al., 1986; Cranwell et al., 1987).

It is well known that fatty acids, isoprenoids, sterols, and other lipids can be bound within macromolecular material, which comprises the bulk of the organic matter in Recent sediments (e.g., Kawamura and Ishiwatari, 1984; Barouxis et al., 1988; Fukushima and Ishiwatari, 1988; Goossens et al., 1989; Lajat et al., 1990; Putschew et al., 1996). Hopanoids have been released from the bound fraction of Recent sediments by alkaline hydrolysis (Barouxis et al., 1988). It is likely, therefore, that hopanoids can become incorporated into macromolecular material at an early diagenetic stage and that these bound molecules may comprise a major pool of geohopanoids in Recent sediments. It has long been recognised that hopanoids occur within the kerogens of ancient samples (Gallegos, 1975; Seifert, 1978; Mycke et al., 1987) where they can comprise a major component of the sedimentary biomarkers (e.g., Eglinton and Douglas, 1988; Love et al., 1995), which progressively modify the hopane composition of the bitumen as they are released during maturation (e.g., Bishop and Abbott, 1993; Requejo, 1994; Farrimond et al., 1996, 1998).

The binding of hopanoids into macromolecular organic mat-

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ter has been examined by various chemical degradation methods targeting the cleavage of bonds that link biomarkers to the macromolecular structure. Mycke et al. (1987) released a bound C_{35} hopanoid (as bacteriohopanetetrol) from the kerogen of Messel oil shale using catalytic hydrogenolysis. By labelling with deuterium, they identified the linkages to be in the side chain of the hopanoids, presumed to be in the form of ether bonds. A wider range of hopanoids was released from the kerogen of Green River Shale by oxidative degradation using sodium dichromate in glacial acetic acid (Barakat and Yen, 1990). Cleavage of sulphur-bound hopanoids from kerogen and asphaltene fractions of source rocks has been performed using different chemolysis methods, and both Hofmann et al. (1992) and Richnow et al. (1992) showed that hopanoids can be bound into these macromolecules by one or more sulphur linkages in their side chains. This has also been demonstrated for the asphaltenes of oils (Hofmann et al., 1992; Richnow et al., 1992; Adam et al., 1993). Stepwise chemical degradation has further shown that hopanoids can be bound by a combination of sulphur and ether linkages at the multiple sites of functionality in their side chain (Richnow et al., 1992).

The results of chemical degradation studies generally indicate a predominance of C_{35} hopanoids in sulphur-bound organic fractions (e.g., Richnow et al., 1992; Sinninghe Damsté et al., 1995; Köster et al., 1997), recording the diagenetic incorporation of intact biohopanoids. Richnow et al. (1992) further noted that oxygen-linked hopanoids showed a greater proportion of side-chain shortened homologues, which is consistent with early diagenetic products that had become incorporated into the macromolecular structure.

To access a high proportion of the biomarkers found covalently linked within kerogen, a degradation regime must be used which results in high yields of soluble and gc-amenable products from insoluble geopolymers. While chemolysis methods can be used to target specific covalent bond types, these reagents have been mostly applied in organic geochemistry for releasing biomarkers from solvent-soluble polar organic fractions in oils and sediments, often neglecting the biomarker content of kerogen in sediments (e.g., Sinninghe Damsté et al., 1989; Kohnen et al., 1992; Kenig et al., 1995; Grice et al., 1996; Huang et al., 2000).

Chemical reagents are generally inefficient at fragmenting kerogen due to the steric protection of reactive bond sites afforded by the highly cross-linked host macromolecular matrix. Most chemical degradation regimes, including low-temperature batch catalytic hydrogenolysis (Mycke and Michaelis, 1986; Mycke et al., 1987; Michaelis et al., 1990), produce low yields of soluble products from kerogen (typically < 40 wt.%, and in most cases, < 20 wt.%); clearly, a high proportion of the bound biomarker information is not being accessed (e.g., Boucher et al., 1991; Hofmann et al., 1992; Richnow et al., 1992; Schaeffer et al., 1995; Behrens et al., 1998; Peng et al., 1999). Although high conversions of immature kerogen to soluble organic material (up to 80 wt.%) have been reported previously from a detailed sequential chemical degradation regime involving the stepwise use of numerous reagents (Schaeffer-Reiss et al., 1998), the amount of useful readily analysable product released was very small (< 1 wt.%). The bulk of the product obtained was still macromolecular and not amenable to analysis by gas chromatography. Therefore, in general, the use of chemical degradation for the study of kerogens can potentially lead to biased bound molecular signals being used for biogeochemical interpretation. To generate representative biomarker profiles, a high proportion of the bound biomarkers must be accessed under reaction conditions that minimise the extent of structural and stereochemical rearrangements in products.

This paper reports evidence for the early incorporation of intact biohopanoids and their early diagenetic products into solvent-insoluble sedimentary organic matter (here termed “kerogen”) on a timescale of tens to hundreds of years. We have applied a mild pyrolysis technique known as catalytic hydropyrolysis (Love et al., 1995, 1996, 1997) to release the hopanoids bound into the kerogen of sediments from two modern environments: Priest Pot (a lake in the English Lake District; Innes et al., 1997) and Framvaren Fjord (Norway; Skei, 1988; Ficken and Farrimond, 1995). Catalytic hydropyrolysis involves temperature-programmed pyrolysis assisted by high hydrogen gas pressures and catalyst and is performed in a well-swept, open-system reactor configuration. A combination of slow heating rate (5–20°C/min), high hydrogen pressure (15 MPa), and the use of a dispersed sulphided molybdenum catalyst represents the best regime for achieving high conversions of kerogen—typically > 85 wt.% for pre to early oil-window maturity Type I-III source rocks (Lafferty et al., 1993; Roberts et al., 1995, Murray et al., 1998)—to principally dichloromethane-soluble products whilst still ensuring good preservation of the molecular features of the biomarker hydrocarbons released (Love et al., 1997). In this study, we determined the abundance and composition of kerogen-bound hopanoids relative to their solvent-soluble (free) counterparts and discuss their preservation and the timing and mode of their incorporation into macromolecular organic matter.

2. EXPERIMENTAL

2.1. Localities and Sample Details

Priest Pot is a small, highly productive hypereutrophic lake (4 m deep) in the English Lake District. A thermocline develops during the summer months, which results in anoxic bottom waters; the sediments remain anoxic after overturn of the water column in autumn, although the overlying bottom waters are oxygenated. Sedimentation rate is high (0.7–1 cm/yr; Cranwell et al., 1987; Cranwell and Koul, 1989), and the sediments are known to be rich in hopanoids derived from bacterial activity within the overlying water column and from the adjacent catchment area (Innes et al., 1997). The sediment core (24 cm long) was collected from the centre of the lake in 4 m water depth using a Jenkin corer.

Framvaren is a silled fjord in southern Norway characterised by a permanently stratified water column and anoxic, highly sulphidic bottom waters (7–8 mmol/L sulphide; Skei, 1988). The fjord is 8.5 km long, up to 1-km wide, and 183 m deep with a stable chemocline at ~20 m depth throughout the year. A sediment core (36 cm long) was collected from below the chemocline (44 m water depth) using a Hongue-type gravity corer. An approximate sedimentation rate of 0.1 cm/yr has been estimated from the recognition of a change of environment (at 14–15 cm depth) associated with the opening of the fjord entrance in 1850 (Innes, 1998).

The sediment cores from both environments were frozen immediately after collection. They were later cut into slices (1–6 cm in thickness), which were then homogenised, freeze dried, and ground to a fine powder before analysis.

TOC values were obtained by the method of Kröm and Berner (1983). One aliquot of sediment (~100 mg) was analysed directly using a Leco Carbon-Sulphur Analyser to determine the total carbon and sulphur content of the samples. A second aliquot was ashed before analysis to combust all the organic carbon in a furnace at 450 °C for 24 h. The TOC value was obtained by taking the difference between the total carbon and the carbon remaining after ashing.

2.2. Analysis of Solvent-Extractable Hopanoids

Solvent-soluble biohopanoids and geohopanoids were extracted and derivatised as outlined in Innes et al. (1997). Briefly, freeze-dried sediments were extracted with chloroform/methanol (2:1 v/v) using a Gerhardt Soxtherm apparatus, and separate aliquots of the extract were derivatised by means of three methods: One aliquot was methylated using boron trifluoride/methanol (14% BF_3) to convert hopanoic acids to methyl esters; a second aliquot was acetylated with acetic anhydride/pyridine (1:1 v/v) to convert hopanoidal alcohols to acetates; and a third aliquot underwent treatment with periodic acid and then sodium borohydride to cleave the hopanoid side chains between adjacent hydroxyl functional groups and reduce the resulting aldehydes to simple terminal alcohols, which were converted to acetates by acetylation (Rohmer et al., 1984).

GC-MS (gas chromatography-mass spectrometry) analysis of the derivatised extracts was conducted on a Hewlett-Packard 5890 II GC (split/splitless injector at 350 °C) linked to a Hewlett-Packard 5972MSD (ionisation energy 70 eV; filament current 220 μA; source temperature 270 °C; multiplier voltage 2000 V; interface temperature 350 °C). A DB5-HT column (15 m × 0.25 mm i.d.; 0.1-μm film thickness) was used with helium as the carrier gas. The oven temperature was programmed from 50 to 200 °C at 15 °C/min (held for 1 min), 200 to 250 °C at 10°C/min (held for 1 min), and 250 to 350 °C at 5 °C/min (held for 8 min). Samples were routinely analysed in SIM mode targeted at the appropriate ions for the standards and selected characteristic hopanoid ions (e.g., m/z 191, 205, 221, 243, 245, 258, 260). Selected samples were run in full scan mode (m/z 50–700) to obtain mass spectra of compounds.

Hopanoids were identified by their relative retention times in comparison with data from Innes (1998) and with authentic standards (e.g., diplopterol, bishomohopanol, aminobacteriohopanetetrol, and bacteriohopanetetrol). Identification was also confirmed by comparison with published mass spectra. Hopanoids were quantified using peak areas from the m/z 191 mass chromatograms relative to the m/z 221 response of a (20R)-5α,14α,17α-[2,2,4,4-d4]-cholestane standard.

2.3. Analysis of Kerogen-Bound Hopanoids: Hydropyrolysis

Thirteen sediment samples (seven from Priest Pot and six from Framvaren Fjord) were subjected to hydropyrolysis (Love et al., 1995) to release hopanoids from the solvent-insoluble macromolecular organic matter present in the sediments. Selected dried, extracted sediments were impregnated with an aqueous/methanol solution of ammonium dioxydithiomolybdate ($(NH_4)_2MoO_2S_2$), resulting in a nominal molybdenum catalyst loading of 1%. After being vacuum dried, the samples were then mixed with sand (1:2 w/w) and placed in the hydropyrolysis reactor tube. For routine hydropyrolysis, they were first heated from room temperature to 100 °C at 50 °C/min and then to 520 °C at 5 °C/min. Products generated from the heating process were quickly swept out of the heated reactor tube by high-pressure hydrogen gas and were collected in a cold trap, thus minimising secondary reactions. Additional experiments were conducted on selected samples in which heating stopped at 300 or 350 °C, and in one case, deuterium gas was used in place of hydrogen to label sites of bond cleavage.

Pyrolysates obtained from the hydropyrolysis procedure were weighed and then fractionated using silica gel thin layer chromatography (TLC; 20 × 20 cm; 0.5 mm thickness; Kieselgel 60G) and eluted using petroleum ether. The aliphatic and aromatic hydrocarbon fractions were recovered with petroleum ether, transferred to preweighed vials, and blown down under nitrogen before being weighed. A standard of (20R)-5α,14α,17α-[2,2,4,4-d4]-cholestane was added to the aliphatic hydrocarbon fraction. GC-MS was carried out on a Fisons 8060GC (on-column injection at 250 °C) interfaced to a Fisons TRIO 1000 mass spectrometer (ionisation energy 70 eV; filament current 4.1 A; source current 2000 μA; multiplier voltage 500 V). The interface temperature was 300 °C and the ion source was at 200 °C. The GC oven was programmed from 50 to 175 °C at 10 °C/min, from 175 to 225 °C at 6 °C/min, and then from 225 to 300 °C at 4 °C/min, followed by 32 min at 300 °C. Separation was performed using an HP-1 fused silica capillary column (30 m × 0.25 mm i.d.; 0.25 μm film thickness). The mass spectrometer was operated in SIM mode (30 ions, 40 ms dwell time). Hopanoids were quantified from peak areas in the m/z 191 chromatogram against the m/z 221 peak area of the d4-cholestane standard. In comparisons of free (solvent-soluble) and kerogen-bound (released by hydropyrolysis) hopanoid concentrations, no account is made for differing response factors of the different hopanoid species.

3. RESULTS AND DISCUSSION

The hydropyrolysis procedure (i.e., pyrolysis performed in an open system with high-pressure hydrogen) generates large quantities of aliphatic hydrocarbon products, and compounds bound into macromolecular organic matter are reductively cleaved with minimal alteration to their stereochemistry (Love et al., 1995, 1997; Bishop et al., 1998). The thermal reactivity of different organic functionalities under typical catalytic hydropyrolysis conditions has been modelled in detail previously by Snape and co-workers using solid calibrants, such as silica-immobilised phenolic model compounds and synthesised polymers, to mimic coal and kerogen behaviour (Mitchell et al., 1993; Ismail et al., 1995; Brown et al., 1997; McGinn, 2002). The use of high hydrogen gas pressure and a dispersed sulphided molybdenum catalyst promotes reductive cleavage of heteroatomic bonds (organic oxygen and organic sulphur cross-linkages and functional groups) in geomacrolecules through hydrodesulphurisation and hydrodeoxygenation at relatively low temperatures (< 450°C) in open-system pyrolysis terms (Rocha et al., 1997). Significant hydrocracking of C-C linkages only occurs above 400°C under catalytic hydropyrolysis reaction conditions (Mitchell et al., 1993) and can lead to scission of long alkyl chains and alkyl aromatic bonds but does not apparently cause destruction of stable polycyclic species such as the hopanoid pentacyclic ring system.

The fact that catalytic hydropyrolysis is a temperature-programmed pyrolysis method, employing a slow constant heating rate (in this case 5°C/min), means that covalent bonds within the kerogen structures can be cleaved at the minimum possible temperature in the heating cycle and that the final pyrolysis temperature is easily controllable. Therefore, in principle it is possible to differentiate between cleavage of thermally labile heteroatomic bonds (such as ester, S-S, and C-S), and stronger covalent bonds (principally C-O and C-C, as well as hydrodesulphurisation of thiophenic organic sulphur) by choosing a suitable cut-off temperature in sequential hydropyrolysis experiments (McGinn, 2002). The principal temperature window for cleavage of ether-bound compounds is typically between 330 and 400°C, while the weaker covalent linkages (ester, S-S, and C-S) are usually reductively cleaved at lower temperatures (McGinn, 2002). Note, however, that in some instances it is likely that some overlap of the temperature windows for C-S and C-O bond cleavage will occur (depending on the kerogen matrix used, which affects the chemical environment around the bond in question), since sulphide bond scission, particularly for aromatic sulphides, is not always complete at ~350°C for ancient sedimentary organic matter (Brown et al., 1997).

For the sulphur-rich Framvaren Recent sediments used in this study, however, direct evidence obtained from temperature-programmed reduction (TPR) of a representative bulked sample indicates that all labile organic sulphur functionalities

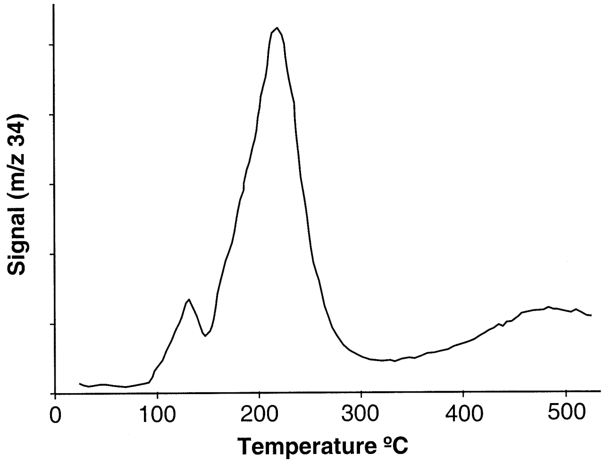

Fig. 1. Plot showing the mass spectrometric response (m/z 34) to hydrogen sulphide evolved during temperature programmed reduction (TPR) of an extracted sediment sample from Framvaren Fjord (27.5 cm).

in the proto-kerogen structure are efficiently cleaved by 300°C under identical catalytic hydropyrolysis conditions to those used in this investigation (Fig. 1). In TPR, a quadrupole mass spectrometer is used to monitor the release of hydrogen sulphide (H_2S) and other volatiles during hydropyrolysis as a function of time and hence temperature (Mitchell et al., 1994; Brown et al., 1997). It is based on the principle that different organic sulphur forms have different characteristic reduction temperatures, derived from their respective bond strengths, at which they will be converted to H_2S in a hydrogen-rich environment. The low-temperature evolution profile of H_2S (Tmax = 220°C) observed here provides direct evidence, based on previous TPR investigations using sulphur-containing model substrates to understand the thermal chemistry, that the organic sulphur functionalities present in the Framvaren sediment are composed predominantly of poly/disulphides and thiols with lesser contributions from mono-sulphide bonds (Brown et al., 1997). All sulphide cleavage is clearly complete by 300°C. As no significant cleavage of more stable ether and C-C bonds was likely to occur under hydropyrolysis at temperatures of 300°C and below (McGinn, 2002), this temperature was used as the cut-off point in sequential hydropyrolysis to discriminate between kerogen-bound hopanes linked exclusively by weak bonds (ester, S-S, and C-S) compared with those bound by at least one strong covalent bond (assumed to be an ether linkage from what is known about biohopanoid structure and macromolecular binding, although C-C linkages formed from diagenetic alterations cannot be discounted).

Typical distributions of the hopanes generated from Priest Pot and Framvaren Fjord sediments (exhaustively solvent extracted before hydropyrolysis) are shown in Figure 2. They are dominated by $17\beta(H),21\beta(H)$ isomers, especially in the samples from Framvaren Fjord, although $17\alpha(H),21\beta(H)$ and $17\beta(H),21\alpha(H)$ hopanes are also present and particularly significant in the Priest Pot samples. The hopanes range in carbon

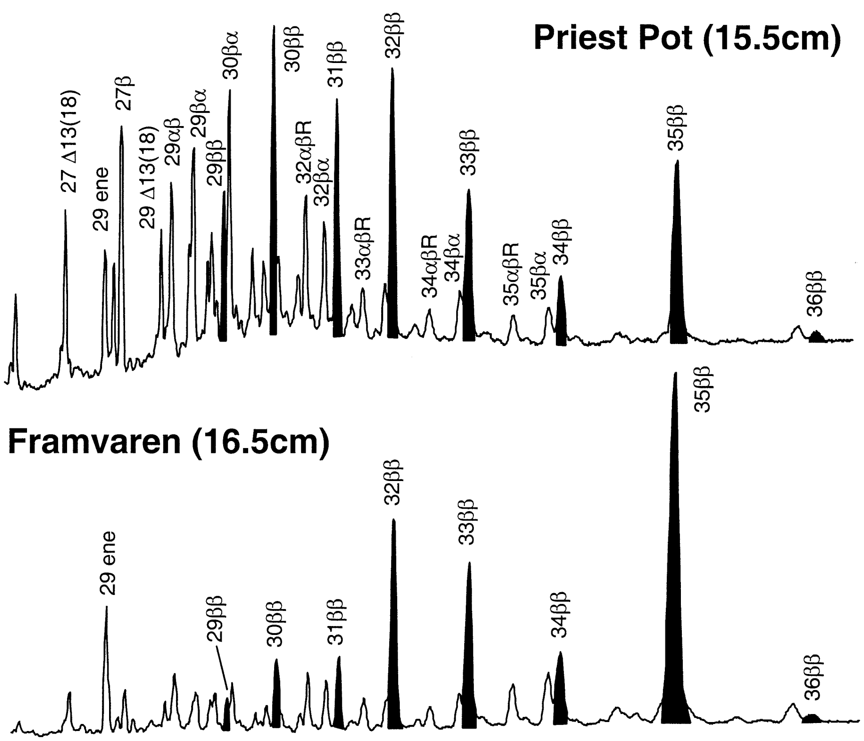

Fig. 2. M/z 191 mass chromatograms showing the distributions of hopanes and hopenes in the aliphatic hydrocarbon fractions of the hydropyrolysates of typical sediments from Priest Pot and Framvaren Fjord. $17\beta(H),21\beta(H)$-Hopane peaks are shaded. Selected hopanes and hopenes are labelled with their carbon number and stereochemistry (at C-17, C-21, and C-22); Δ13(18) = neohop-13(18)-ene.

Table 1. Total yield (in μg/g TOC) of free (solvent-soluble) biohopanoids and geohopanoids and bound (solvent insoluble) hopanoids released by hydropyrolysis of sediments from Priest Pot and Framvaren Fjord.

Framvaren Fjord

				Hopanoid concentrations (μg/g TOC)				
Depth (cm)	TOC (%)	TS (%)	% Geohops	Free Bio	Free Geo	Total Free	Total Bound	% Bound
3.5	2.91	1.68	–	–	–	–	340	–
7.5	6.56	1.68	–	–	–	–	380	–
12.5	9.99	2.73	40	160	110	270	350	57
16.5	13.2	3.68	39	170	110	280	280	50
21.5	13.8	3.89	–	–	–	–	710	–
33.5	10.4	4.12	35	150	80	230	1400	86

Priest Pot

				Hopanoid concentrations (μg/g TOC)				
Depth (cm)	TOC (%)	TS (%)	% Geohops	Free Bio	Free Geo	Total Free	Total Bound	% Bound
0.5	25.1	1.28	–	–	–	–	190	–
4.5	23.9	1.41	–	–	–	–	310	–
7.5	23.5	1.45	75	220	650	870	290	25
11.5	22.4	1.58	–	–	–	–	270	–
15.5	18.2	1.54	79	230	830	1060	300	22
18.5	19.6	1.37	–	–	–	–	260	–
21.5	18.4	1.44	71	220	550	770	330	30

Free Bio = concentration of bacteriohopanepolyols determined by periodic acid/sodium borohydride treatment of solvent extracts. Free Geo = concentration of geohopanoids, comprising hopanoic acids, hopanols (including diols and triols) and hopenes. % Geohops = Free Geo/(Free Geo + Free Bio) %. Total bound hopanoids represent the total concentration of hopanes and hopenes in the aliphatic fraction of the hydropyrolysates of extracted sediments. % Bound = Total Bound/(Total Bound + Total Free) %. TOC = total organic carbon. TS = total sulphur; values for Priest Pot are from a duplicate core. – = not determined.

number from C_{27} to C_{35} (no C_{28} components), with C_{35} $\beta\beta$ hopanes often dominant (Fig. 2). C_{36} $\beta\beta$-hopane was identified from its mass spectrum in low relative amounts. C_{27} and C_{29} neohop-13(18)-enes and an unknown C_{29} hopene are also found in the aliphatic fractions. Analysis of one hydropyrolysate from both Framvaren and Priest Pot, using the derivatisation methods (methylation, acetylation, and periodic acid/sodium borohydride) to target hopanoic acids, hopanols, and hopanepolyols, failed to detect any functionalised hopanoid products.

3.1. Quantitative Significance of Insoluble Bound Hopanoids

The yield of hopanes and hopenes released by hydropyrolysis of the Priest Pot sediments is relatively constant (260–330 μg/g TOC) except for the lower concentration (190 μg/g TOC) in the surface (0–1 cm) sample (Table 1; Fig. 3). The sediment samples from the upper part of the Framvaren Fjord core released similar amounts of hopanoids by hydropyrolysis (280–380 μg/g TOC) as the Priest Pot sediments, but the two deepest samples (21.5 and 33.5 cm) exhibit a substantial increase in bound hopanoid concentration (Fig. 3) of up to 1400 μg/g TOC.

Three sediments from each core were analysed for their free (solvent-soluble) hopanoids, allowing a comparison of both free and bound hopanoid concentrations (Table 1). In Priest Pot, hopanoids released after hydropyrolysis represent 22 to 30% of the total measured hopanoids and show no trend with

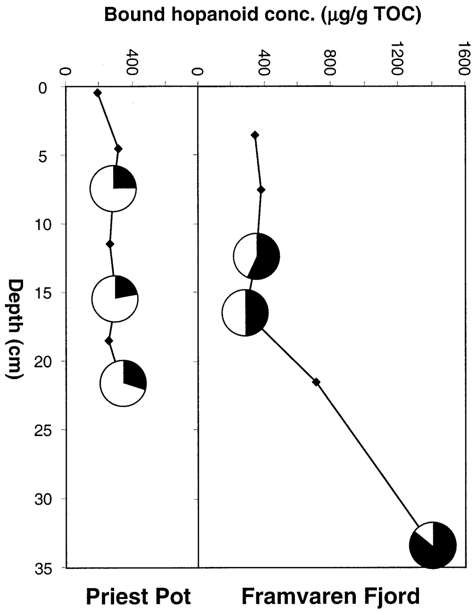

Fig. 3. Downcore concentrations (μg/g TOC) of hopanoids (hopanes + hopenes) released by hydropyrolysis ("bound hopanoids") of extracted sediments from Priest Pot and Framvaren Fjord. The proportion of characterisable hopanoids that are released during hydropyrolysis (i.e., total bound/(total bound + total free)) is shown as a pie chart for three samples from each core (the bound fraction shown in black). See Table 1 for numerical data.

depth (Fig. 3; Table 1). These bound hopanoids could be associated with bacterial cell material, as it is known that some biohopanoids are not easily extracted (Herrmann et al., 1996). However, data shown later indicate that the bulk of these compounds are strongly covalently bound (see Section 3.3.1), and have a distinct carbon number distribution (see Section 3.2) that make them more likely to represent diagenetic incorporation of hopanoids. In Framvaren, the proportion of kerogen-bound hopanoids is higher (50% or more) and reaches 86% in the deepest sample (33.5 cm; Table 1; Fig. 3). This marked increase in the proportion of hopanoids that are bound and the increase in absolute concentration of bound hopanoids are interpreted as recording the early diagenetic incorporation of hopanoids into the insoluble macromolecular organic matter (kerogen) within this shallow core. The Framvaren core represents a time interval of ~250 to 350 yr (Innes, 1998), so a major portion of the sedimentary hopanoids are undergoing incorporation into kerogen on a timescale of tens to hundreds of years. This finding is in general agreement with observations of rapid formation (hundreds to thousands of years) of intermolecular sulphur linkages between steroids (Kok et al., 2000) or tricyclic terpenoids (Werne et al., 2000) in polar fractions of solvent soluble organic matter but extends the finding to kerogen and indicates that the incorporation can be more rapid than previously shown. The nature of the linkages between hopanoids and the macromolecular structure is addressed in Section 3.3.

3.2. Composition of Bound Hopanoids

In all cases, the hopanes released by hydropyrolysis of the extracted sediments are dominated by $\beta\beta$-hopanes (Fig. 2). This dominance is particularly marked in the Framvaren sediments where $\beta\beta$ isomers represent 68 to 76% of released hopanes (except the uppermost sample, which contains only 55% $\beta\beta$-hopanes). The sediments from Priest Pot released lower relative amounts of $\beta\beta$-hopanes upon pyrolysis (55–65%); this observation is consistent with the high relative abundance of solvent-soluble $\alpha\beta$-hopanoic acids and hopanols previously reported in these sediments (Innes et al., 1997), which are interpreted to be derived from soils and/or peat in the lake catchment. Previous studies have demonstrated that hopanes produced by hydropyrolysis closely reflect the isomeric composition of the bound hopanoids, showing that the hydropyrolysis conditions cause little isomerisation of the generated compounds (Love et al., 1997; Bishop et al., 1998). The principal temperature window for cleavage of ether-bound compounds is between 330 and 400 °C irrespective of the maturity level of the sample, but modern sediments generally release hopanes that are dominated by $\beta\beta$ isomers (this study), whilst hopanes generated from the kerogen of ancient sediments are dominated by $\alpha\beta$ isomers (Love et al., 1995; Bishop et al., 1998). Furthermore, the hopanes released by hydropyrolysis of extracted sedimentary rocks comprising a maturity suite showed systematic changes in their isomeric distributions such that maturity parameters increased with burial depth (Murray et al., 1998).

The carbon number distributions of the hopanes released by hydropyrolysis from Priest Pot and Framvaren sediments show maxima at C_{35}, C_{32}/C_{33}, and C_{30} (Figs. 2, 4). For the Framvaren samples, C_{35} hopanes (mainly C_{35} $\beta\beta$ hopane) are consistently the most abundant homologues and are particularly dominant towards the bottom of the core (where the proportion of bound hopanoids is greatest). The C_{35} $\beta\beta$ hopane in the hydropyrolysates must be derived from intact C_{35} biohopanoids that were not solvent extracted and are interpreted to be bound by covalent linkages into the kerogen structure of these young sediments. Although C_{35} $\beta\beta$-hopane is an abundant constituent of the Priest Pot bound hopanoids, the predominance is less marked (Fig. 4).

The C_{30} hopanes generated by hydropyrolysis, which are particularly prominent in the Priest Pot samples and the uppermost Framvaren sediment (Fig. 4), may also record bound biohopanoids in the form of diplopterol and/or diploptene, which are commonly synthesised by hopanoid-producing bacteria (e.g., Rohmer et al., 1984) though in generally low concentrations. Although diplopterol has not been detected in the extracts of Priest Pot sediments (Innes et al., 1997), either diploptene (Watson, 2002) or hop-17(21)-ene, which may be derived from the rearrangement of diploptene (Ageta et al., 1987), occurs in high abundance. Alternatively, the C_{30} hopanes in the hydropyrolysates may reflect C_{30} hopanols and hopanoic acids formed during early diagenesis (from hexafunctionalised biohopanoids, which have been shown to be abundant in Priest Pot sediments; Innes et al., 1997; Farrimond et al., 2000) that have become incorporated into the macromolecular organic matter.

The predominance of C_{32} hopanes over adjacent carbon numbers in the hydropyrolysates (Fig. 4) is interpreted as recording preferential binding of C_{32} hopanoids into the insoluble macromolecular organic matter. Although the hydropyrolysis procedure can cleave bonds in the hopanoid side chain, this does not explain the marked predominance at C_{32} in the distribution of generated hopanes, as hydropyrolysis of extracted bacterial cells with only C_{30} and C_{35} hopanoids produces a smooth distribution of $C_{31\text{-}35}$ hopanes (Bishop et al., 1998). However, many previous studies of the solvent-extractable hopanoids of modern sediments have recorded the dominance of C_{32} hopanoic acids and hopanols (e.g., Innes et al., 1997, 1998), and a proportion of these have clearly become incorporated into the kerogen of sediments in both Framvaren and Priest Pot. As the C_{32} hopanoids are diagenetic products of tetrafunctionalised biohopanoids (Innes et al., 1997), this is excellent evidence for diagenetic hopanoid incorporation into kerogen, as it cannot be confused with incomplete extraction of bacterial cell hopanoids.

In contrast with the obvious recognisable biological origin of the bound C_{35} hopanoids and the early diagenetic origin of bound C_{32} hopanoids, the origins of the C_{33} (in particular), C_{34}, and C_{31} hopanoids are more difficult to explain. A proportion of these released hopanes probably record incorporated C_{31}, C_{33}, and C_{34} diagenetic products (e.g., C_{31} hopanoic acids and hopanols and C_{33} and C_{34} hopanediols and triols; Rodier et al., 1999; Watson and Farrimond, 2000). In addition, it is likely that some are derived by side-chain cleavage of higher molecular weight bound hopanoids (C_{35} and/or C_{32}) during the hydropyrolysis procedure. Hydropyrolysis of bacterial cells containing only C_{35} and C_{30} biohopanoids produces hopanes covering the entire $C_{27\text{-}35}$ (no C_{28}) carbon number range but with a predominance of C_{35} and C_{30} hopanes representing the

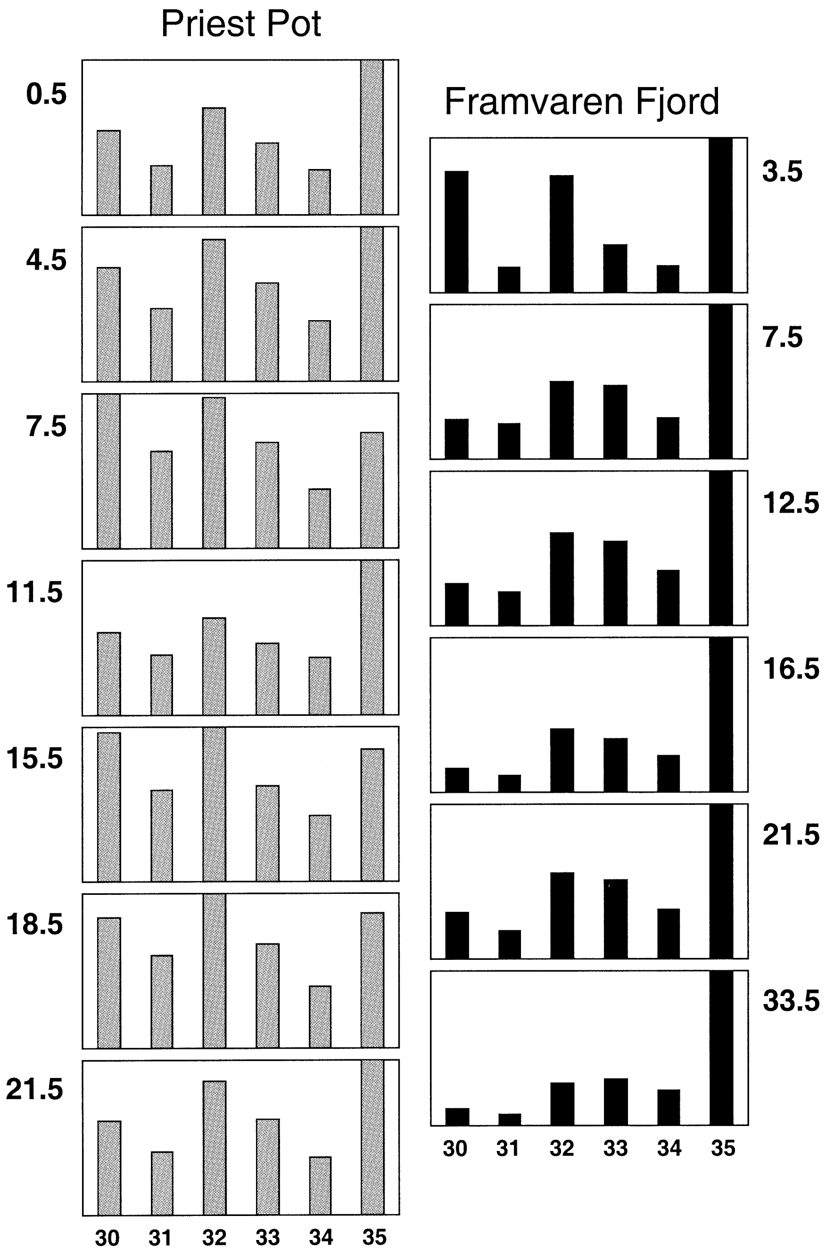

Fig. 4. Histograms showing the carbon number distributions ($C_{30\text{-}35}$) of the hopanes (all isomers summed) generated by hydropyrolysis of extracted sediments. Sediment depths (cm) are shown alongside the appropriate histogram.

intact biohopanoids (Bishop et al., 1998; Summons et al., 2001). Nevertheless, despite the likelihood of minor side-chain cleavage during hydropyrolysis, the generated hopanes in the present study preserve compositional evidence for both bound biohopanoids and bound geohopanoids in these very young sediments (0–350 yr).

3.3. Nature of Binding of Hopanoids into Macromolecular Organic Matter

The higher proportion of bound hopanoids in sediments from Framvaren Fjord (Fig. 3; Table 1) is interpreted to reflect, at least in part, an environmental control on hopanoid incorporation into kerogen. Previous work has shown that hopanoids (and other biomarkers) can be bound into macromolecular organic matter via sulphur linkages (Hofmann et al., 1992; Richnow et al., 1992) and that this process is favoured in sulphidic environments where reduced sulphur species are available for reaction with sedimentary organic matter (Hartgers et al., 1997; Kok et al., 2000; Werne et al., 2000). Framvaren Fjord is exceptionally rich in reduced sulphur species, with bottom water sulphide levels 25 times greater than those in the Black Sea (Skei, 1988). Although biomarker incorporation into macromolecules via sulphur linking is most significant in depositional settings with low iron inputs, as iron out competes

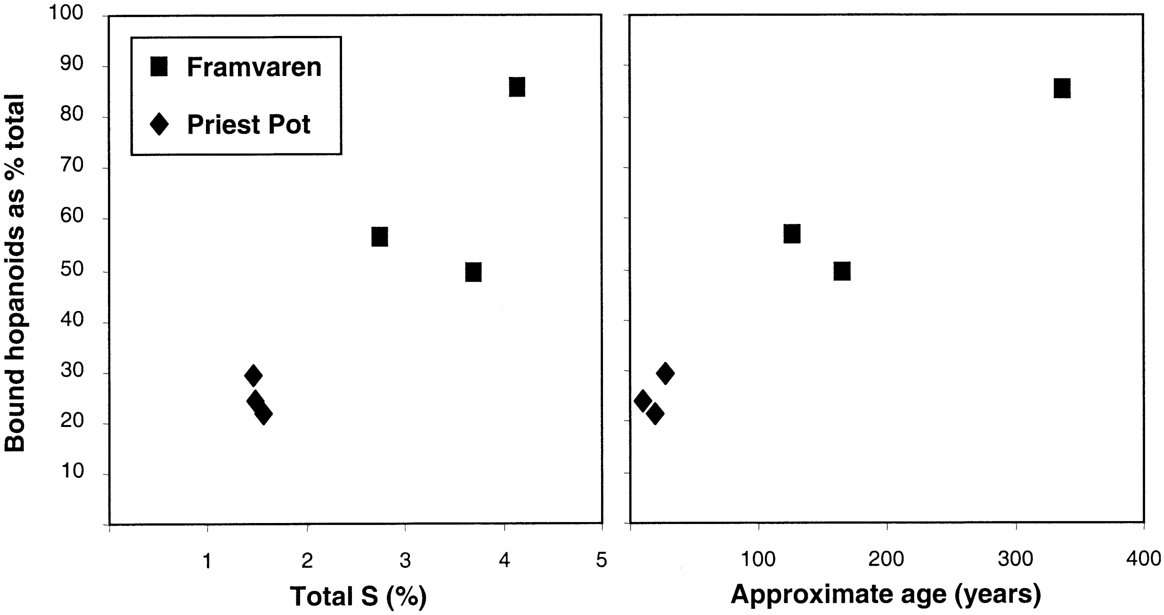

Fig. 5. Cross plots of %-bound hopanoids (i.e., total bound/(total bound + total free); see Table 1) vs. either total sulphur content (%) or calculated sediment age (using approximate sedimentation rates as discussed in the text).

organic matter in reactions with reduced sulphur (Hartgers et al., 1997), the extremely high sulphide concentrations in Framvaren may overcome this limitation. Total sulphur contents of Framvaren sediments are especially high (3.7–4.1%; Table 1) in the more deeply buried samples, which were deposited during a period of time when the fjord was isolated from the marine environment (i.e., a stagnant barred fjord with saline sulphidic bottom waters; Skei, 1988). However, how much of this sedimentary sulphur is accounted for by organic species is unknown.

The much lower proportions of bound hopanoids in the insoluble organic matter of the sediments from Priest Pot indicate that diagenetic incorporation of hopanoids is less important in this environment. This may be due to lower amounts of reduced sulphur species being available for reaction with sedimentary organic matter in Priest Pot (although total sulphur contents of the sediments range from 1.3 to 1.6%; Table 1) or the younger age of the sediments. Considering both Priest Pot and Framvaren samples together, there are positive relationships between the percentage of bound hopanoids and both total sulphur content and sediment age (inferred from a sedimentation rate of 0.85 and 0.1 cm/yr for Priest Pot and Framvaren, respectively; Cranwell et al., 1987; Cranwell and Koul, 1989; Innes, 1998; Fig. 5). As the older samples from Framvaren are also the most sulphur-rich, it is unclear which of these factors exerts a greater influence on hopanoid incorporation.

3.3.1. *Covalent bond-type determination by multiple temperature hydropyrolysis*

The nature of the linkages between the hopanoids and the macromolecular organic matter was investigated by hydropyrolysis over different temperature ranges (sequential hydropyrolysis), allowing the abundance and composition of generated hopanes to be determined as bonds of differing strength are cleaved. Hydropyrolysis to 300 °C of the deepest Priest Pot sample (21.5 cm; i.e., 20–30 yr old) released less than 5% of the hopanoids that were generated in the hydropyrolysis up to 520 °C. These hopanoids released at low temperatures are probably loosely adsorbed or trapped within the macromolecular organic matter, possibly including cell material, as it is known that a proportion of hopanoids can remain behind in extracted bacterial cells (e.g., Herrmann et al., 1996). Covalent labile sulphur bonds (di-/polysulphides) are also cleaved below 300 °C (Brown et al., 1997), although these are not likely to be abundant in this freshwater environment. Hydropyrolysis of another aliquot of this sediment to 350 °C released ~50% of the hopanoids; i.e., ~45% of the bound hopanoids are released between 300 and 350 °C, whilst the remaining 50% are only released above 350 °C. The hopanoids released above 350 °C are more strongly bound and represent hopanoids that have been incorporated into the kerogen structure by covalent bonds; at least some of the hopanoids released between 300 and 350 °C may also be covalently bound. Together, these data indicate incorporation of hopanoids into insoluble macromolecular organic matter in sediments of Priest Pot on a timescale of just years.

A sample from Framvaren Fjord (27.5-cm depth but not in the original sample suite) was also subjected to sequential hydropyrolysis in which 15% of $C_{32\text{-}35}$ hopane products were released below 300 °C and 85% above 300 °C. In this case, the bulk of the hopanoids are interpreted to be covalently bound, representing cleavage of weak C-S and/or S-S and strong ether bonds, respectively. Temperature programmed reduction (TPR) of a Framvaren sediment showed that all sulphur functionalities were reduced below 300 °C under standard hydropyrolysis conditions (Fig. 1), which is consistent with the organic sulphur functionalities comprising exclusively thermally labile thiols, poly/di- and possibly mono-sulphide bonds with no significant contribution from thiophenic sulphur (Brown et al., 1997). Our data thus indicate that even in the highly sulphidic environment of Framvaren Fjord, the bulk (85%) of bound C_{32-35} hopanoids contain at least one ether (or possibly C-C) linkage to the kerogen structure.

3.3.2. Labelling of binding sites: Hydropyrolysis with deuterium gas

To provide further detail about binding, the Framvaren sample from 27.5-cm depth was subjected to hydropyrolysis using deuterium instead of hydrogen for the low-temperature pyrolysis phase of a sequential hydropyrolysis experiment to label the sites of bond cleavage. The preextracted sediment was initially pyrolysed under high-pressure deuterium up to a temperature of 300 °C to allow selective cleavage of labile sulphur (mainly) and ester linkages. The generated products were recovered, then the unconverted residue was re-extracted with dichloromethane and subjected to further hydropyrolysis (this time under high-pressure hydrogen) up to a temperature of 500 °C to allow cleavage of the stronger ether (or C-C) linkages.

The hopanes generated from low-temperature pyrolysis (up to 300 °C) assisted by high deuterium pressure of the Framvaren sediment show extensive deuterium incorporation across the series of C_{31-35} hopanes (Fig. 6), indicating that covalent bonds must have been cleaved. The deuterium labels are present in the m/z 148+R fragment (where R is the mass of the alkyl side chain) and in the molecular ion, but not in the m/z 369 and 191 fragments produced during GC-MS analysis (Fig. 6). It is thus clear that deuterium incorporation is restricted exclusively to the side chain of the hopanoids, suggesting that the hopanoids were bound through multiple linkages coinciding with the positions of the functional groups of the parent biohopanoids (as demonstrated by Mycke et al., 1987; Hofmann et al., 1992; Richnow et al., 1992). These linkages must be relatively weak for cleavage to occur at the relatively low temperature employed ($<$ 300 °C), which is consistent with the TPR data (Fig. 1) that indicated cleavage of mainly di-/polysulphide bonds. Ester bonds are also possible but less likely given the relatively rare occurrence of hopanoic acids with more than 32 carbon atoms in sedimentary organic matter (e.g., Innes et al., 1997, 1998, and references therein).

The hopanes released upon subsequent high-temperature pyrolysis under hydrogen (up to 500 °C), after initial low-temperature pyrolysis under deuterium, still show evidence for significant incorporation of deuterium atoms (Fig. 6), although unlabelled hopanes are the dominant products. The labelled products released at this stage are interpreted to have been linked to the macromolecular organic matter by both weak (sulphur) and strong bonds, whilst the unlabelled compounds were probably linked exclusively by strong bonds, interpreted to be mainly ether linkages. This result is consistent with the sequential chemical degradation work of Richnow et al. (1992) that demonstrated simultaneous binding of some hopanoids by sulphur and oxygen linkages as well as purely sulphur-linked and oxygen-linked hopanoids in macromolecular fractions of a Monterey source rock or oil.

In terms of absolute compound yields, 15% of the C_{32-35} $\beta\beta$-hopanes were released from the Framvaren sediment during low-temperature hydropyrolysis ($<$ 300 °C), most probably arising from cleavage of hopanoids bound entirely by C-S or S-S bonds. For the C_{35} $\beta\beta$-hopane, ~47% seems to have been bound entirely by ether bonds (these being released during the high-temperature hydropyrolysis lacking any deuterium incorporation), whilst just under 40% of the C_{35} $\beta\beta$-hopane is generated from components bound by a combination of weak C-S or S-S bonds and stronger C-O bonds.

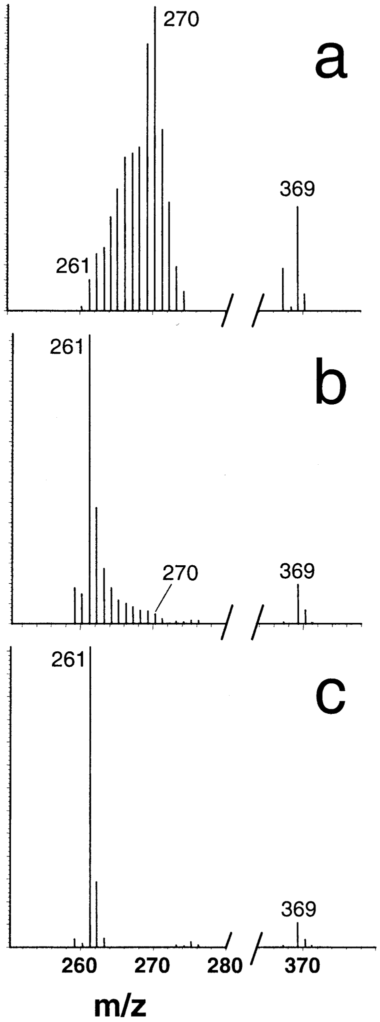

Fig. 6. Partial mass spectra of the C_{35} $\beta\beta$-hopane generated by hydropyrolysis of a Framvaren sediment (27.5 cm depth) in different experiments: (a) hydropyrolysis to 300 °C with deuterium, (b) hydropyrolysis to 500 °C with hydrogen of the residue (extracted) from the low-temperature deuterium experiment, and (c) hydropyrolysis to 500 °C under hydrogen to act as a control.

The deuterium incorporation approach was not used for any of the Priest Pot sediments, but a sequential hydropyrolysis experiment (21.5 cm Priest Pot sediment discussed earlier) demonstrated that $<$ 5% of the generated hopanes were released below 300 °C with only traces of C_{32} and C_{35} $\beta\beta$-hopanes being detected (only 2% of the C_{35} $\beta\beta$-hopane being generated below 300 °C). This is in agreement with a relatively minor component of C-S or S-S binding of hopanoids in the Priest Pot sediments, suggesting that the bound hopanoids are linked into the insoluble organic matter predominantly by ether bonds.

4. CONCLUSIONS

High concentrations (190–1400 μg/g TOC) of hopanoids were released from the insoluble sedimentary organic matter (kerogen) of Priest Pot and Framvaren Fjord sediments (0–350 yr in age) by hydropyrolysis. Bound hopanoids represent 22 to 86% of the total analysable hopanoids, being higher in the Framvaren Fjord sediments where both the absolute concentration and proportion of bound hopanoids increase with depth.

The hopanoids released by hydropyrolysis of all samples are dominated by 17β(H),21β(H) isomers, and the carbon number distributions exhibit maxima at C_{35}, C_{32}/C_{33}, and C_{30}, reflecting the carbon numbers of hopanoids bound into the kerogen. The bound C_{35} hopanoids represent incorporated biohopanoids (synthesised as C_{35} bacteriohopanepolyols), whilst the C_{30} hopanoids may represent either biohopanoids (diplopterol and/or diploptene) or diagenetic products of hexafunctionalised biohopanoids that have been bound into the kerogen. The predominance of C_{32} hopanes in the hydropyrolysates is due to the release of bound C_{32} hopanoic acids and hopanols, which are known to be the dominant diagenetic products of tetrafunctionalised biohopanoids such as bacteriohopanetetrol. Thus, both intact biohopanoids and geohopanoids produced during earliest stages of diagenesis have been incorporated into the kerogen, in both environments, on a timescale of between 0 and 350 yr.

Sequential (multiple temperature) hydropyrolysis experiments show that most bound hopanoids in Priest Pot sediments are linked by strong covalent bonds, which are probably ether linkages. In the sulphidic environment of Framvaren Fjord, sulphur linkages are more important but still not dominant. Approximately 15% of extended hopanoids ($C_{32\text{-}35}$) are bound entirely by weak sulphur bonds, which is confirmed by temperature programmed reduction (TPR) in which peak generation of H_2S below 300 °C indicates cleavage of di-/polysulphide (and possibly monosulphide) linkages. Labelling of binding sites with deuterium during low-temperature deuterium pyrolysis indicated that high proportions (e.g., 47% for the C_{35} $\beta\beta$-hopane) of the bound hopanoids were linked exclusively by strong bonds cleaved only above 300 °C (interpreted as ether linkages). A comparable proportion ($\sim$40% for the C_{35} $\beta\beta$-hopane) of hopanoids are linked by a combination of weak (sulphur) and strong (ether) bonds.

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