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Early diagenesis of bacteriohopanepolyol derivatives: Formation of fossil homohopanoids

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Abstract—Diagenetic pathways of bacteriohopanepolyol derivatives are proposed based on the concentrations and ¹³C contents of homohopanes, homohop-17(21)-enes, benzohopanes, hopanoid thiophenes and sulphides, and macromolecularly S-bound homohopanes present in the extracts of twelve composite one metre samples from a 120 m core recovered from the Upper Cretaceous Jurf ed Darawish Oil Shale (Jordan). A large part (>80–95%) of the pentakishomohopane skeleton occurs in a S-bound form. This reveals the selective preservation of the C₃₅ hopane skeleton by sulphur sequestration and provides a theoretical basis for the homohopane index as an indicator of anoxia in past depositional environments. A smaller part (>50–80%) of the total extended hopane skeletons (C₃₁–C₃₅) occurs in a S-bound form. Of the non-sulphur-containing hopanoids the homohop-17(21)-enes dominate. These latter components show a gradual increase of 22S epimers with depth (45–52%) towards the thermodynamic equilibrium (52–53%) as calculated by molecular mechanics. Molecular mechanic calculations indicate that this increase can be explained by either isomerisation of 22R hop-17(21)-enes or by isomerisation of double bonds of homohopanes formed by dehydration of bacteriohopanepolyols ‘en passant’ isomerising the chiral centre at C-22. A combination of these two pathways is also possible and provides an explanation for different ^δ¹³C values of pairs of 22R and 22S epimers. Isomerisation of 17β,21β(H)-homohopane to 17α,21β(H)-homohopane carbon skeletons occurs for all compound classes in a very narrow depth span (ca. 20 m) and is probably induced by small differences in thermal history. Compound-specific carbon isotope analyses indicated that the series of homohop-17(21)-enes have in some cases significant differences in ¹³C content, indicating that at least two different sources have contributed to this series of components. Differences with macromolecularly S-bound C₃₅ hopane skeletons and free C₃₁ hopanes ¹³C contents are in some cases even larger. These data show that the diagenetic pathways of bacteriohopanepolyol derivatives are more complex than previously recognized and reveal that multiple precursor bacteriohopanepolyol derivatives prone to different diagenetic pathways have to be envisaged to account for the differences observed.

1. INTRODUCTION

C₃₅ bacteriohopane derivatives (I; see Appendix A for structures) are exclusively biosynthesized by prokaryotic organisms basically as membrane rigidifiers, a surrogate of sterols used by eukaryotes for that purpose (for a review see Rohmer et al., 1992). These compounds are thought to be precursor molecules of extensive series of extended hopanoids (or ‘homohopanoids’) in sediments and crude oils (for a review see Ourisson et al., 1979; Ourisson and Albrecht, 1992). These include the extended hopanes II (C₃₁–C₃₅; van Dorsselaer et al., 1977; Armanios et al., 1994), extended hop-17(21)-enes III (C₃₁–C₃₅; Ensminger, 1977; Ourisson et al., 1979; McEvoy, 1983), aromatized hopanes such as ring B aromatized hopanes IV (C₃₁–C₃₂; Hauke et al., 1992), the hexacyclic hydrocarbons V–VII probably formed by internal cyclisation reactions (C₃₁–C₃₅; Oustroukov et al., 1983; Hussler, 1984a,b; Connan and Dessort, 1987; Schaeffer et al., 1995), ring-C opened 8,14-secohopanes VIII (C₃₁–C₃₅; Schmitter et al., 1982; Fazeelat et al., 1994), ring D monoaromatic 8,14-secohopanes IX (C₃₁–C₃₅; Hussler et al., 1984a), 8,14-secohexahydro benzo-

hopanes X (C₃₁–C₃₅; Dessort and Connan, 1993), and 8,14-seco-benzohopanes XI (C₃₂–C₃₅; Dessort and Connan, 1993). Oxygen-containing homohopanoids such as homohopanoic acids XIIa (van Dorsselaer, 1974), homohopanoic alcohols XIIb (Dastilung et al., 1980b), homohopanoic aldehydes XIc (Dastilung et al., 1980a), and homohopanoic ketones XIId and XIII (Dastilung et al., 1980a; Sinninghe Damsté et al., 1989b) are early diagenetic products of bacteriohopanepolyols. Sulphur-containing derivatives such as the hopanoid thiophenes XIV (C₃₃–C₃₅; Valisolalao et al., 1984; Sinninghe Damsté et al., 1989b; ten Haven et al., 1990; de Lemos Scofield, 1990; Köster et al., 1993; F. X. C. de Las Heras et al., unpubl. data), C₃₁–C₃₅ hopanoid sulphides XV–XX (Cyr et al., 1986; Schmid, 1986; Kohnen et al., 1991a; van Kaam-Peters et al., 1995b; F. X. C. de Las Heras et al., unpubl. data), C₃₅ hopanoid disulphide XXI (Kohnen et al., 1991a), hopanoid benzothiophenes XXII and XXIII (van Kaam-Peters et al., 1995a), and a C₃₅ hopanoid sulphoxide XXIV (Schouten et al., 1995) have also been identified and are formed by sulphur incorporation into functionalised homohopanoids during early diagenesis.

In addition, homohopanoids are sequestered in high-molecular-weight fractions of fossil organic matter and can be released by selective chemical degradations. Mycke et al.

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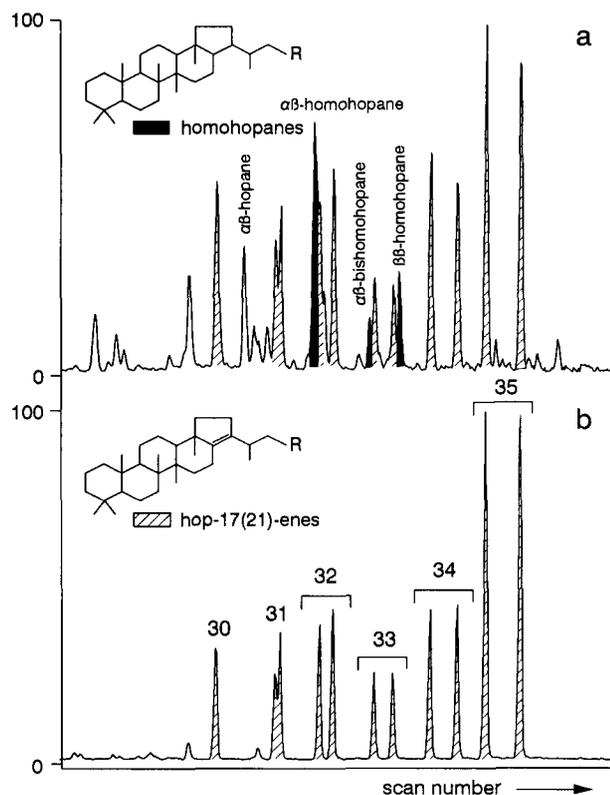


FIG. 1. Partial mass chromatograms of (a) m/z 191 and (b) m/z 367 of the saturated hydrocarbon fraction of JED-137 revealing the distributions of hopanes and hop-17(21)-enes. Numbers refer to total number of carbon atoms.

(1987) released intact bacteriohopanetetrol **Ia** from the kerogen of the Messel Oil Shale by hydrogenolysis on a rhodium/charcoal catalyst. Pentakishomohopane (**II**, R = pentyl) is often the dominant hopanoid released upon desulphurisation of high-molecular-weight fractions of bitumen and crude oil

(Sinninghe Damsté et al., 1988, 1990a, 1993; de Leeuw and Sinninghe Damsté, 1990; Kohnen et al., 1991b; Schoell et al., 1992, 1994; Adam et al., 1993; Richnow et al., 1992, 1993; Hofmann et al., 1992; Schouten et al., 1993). This is thought to be due to an excellent preservation of the carbon skeleton of bacteriohopanepolyols (or derivatives thereof) due to incorporation of inorganic sulphur species during early diagenesis. The use of deuteriated desulphurisation catalysts revealed that the side chain of pentakishomohopane can be linked to the macromolecular matrix by up to four (poly)sulphide linkages (Richnow et al., 1992, 1993; Hofmann et al., 1992; Adam et al., 1993; Schouten et al., 1993). Using a stepwise chemical degradation pathway, Richnow et al. (1992, 1993) demonstrated that homohopanoids are bound into macromolecular aggregates by oxygen and simultaneously by sulphur and oxygen linkages. Trifilieff et al. (1992) and Richnow et al. (1992, 1993) used RuO_4 oxidation on high-molecular-weight fractions of fossil organic matter to show that C_{31} - C_{35} homohopanes are attached at the ω -carbon of the side-chain to an aromatic ring system via a C-C bond. However, recent studies on model compounds indicate that RuO_4 oxidation is not so selective as previously thought and can cleave ether linkages as well (S. Schouten et al., unpubl. data).

Intact free bacteriohopanetetrol has only been reported to occur in Recent freshwater muds (Rohmer et al., 1980), cyanobacterial mats (Boon et al., 1983), and in an organic-rich soil (Ries-Knaut and Albrecht, 1989), but not in any older sediment. These data all hint to a complex diagenetic pathway of bacteriohopanepolyol derivatives which include defunctionalisation, oxidation, incorporation, and possibly Friedel-Crafts reactions, cleavage of C-C bonds both in the side chain and in the ring system (i.e., ring opening) and natural sulphurisation. However, the precise routes of transformations of homohopanoids in Recent and ancient sediments is largely unknown.

A new technique which is highly valuable to decipher diagenetic pathways of sedimentary organic constituents is isotope-ratio-monitoring gas chromatography-mass spectrometry

Table 1. Stable carbon isotopic composition of homohopanoids in the Jurf ed Darawish Oil Shale^a

	JED-50	JED-59	JED-83	JED-120	JED-137	JED-156	JED-165
17 α ,21 β (H)-22R-homohopane	n.d.	-30.5 \pm 0.3	n.d.	n.d.	-34.4 \pm 2.5	n.d.	-27.7 \pm 0.4
17 β ,21 β (H)-22R-homohopane	-27.2 \pm 1.0	-29.4 \pm 0.8	-26.9 \pm 0.4	-32.0 \pm 0.8	-33.4 \pm 1.0	n.d.	-24.0 \pm 0.1
22S-bishomohop-17,21-ene	-27.6 \pm 1.1	-29.9 \pm 0.6	-28.0 \pm 0.9	-32.7 \pm 1.1	-27.4 \pm 0.3	-30.8 \pm 0.6	-27.5 \pm 0.2
22R-bishomohop-17,21-ene	-27.7 \pm 1.3	-30.5 \pm 0.1	-27.9 \pm 0.5	-31.3 \pm 1.4	-29.6 \pm 0.7	-29.3 \pm 0.7	-26.0 \pm 0.1
22S-trishomohop-17,21-ene	-27.7 \pm 1.3	-30.6 \pm 0.7	-28.6 \pm 0.7	-34.6 \pm 0.1	-29.5 \pm 0.6	-29.6 \pm 0.7	-27.7 \pm 0.3
22R-trishomohop-17,21-ene	-29.7 \pm 0.2	-30.4 \pm 1.1	-30.2 \pm 0.5	-30.1 \pm 0.8	-29.8 \pm 0.4	-28.6 \pm 0.2	-28.6 \pm 0.6
22S-tetrakishomohop-17,21-ene	-27.0	-29.5 \pm 0.2	-27.7 \pm 0.3	-32.7 \pm 0.5	-29.4 \pm 0.0	-31.5 \pm 0.3	-26.7 \pm 0.1
22R-tetrakishomohop-17,21-ene	-26.8	-29.3 \pm 0.7	-27.4 \pm 0.5	-30.9 \pm 0.1	-30.0 \pm 0.5	-31.4 \pm 0.1	-26.9 \pm 0.1
22S-pentakishomohop-17,21-ene	-28.7 \pm 1.4	-30.5 \pm 0.8	-28.1 \pm 0.0	-31.0 \pm 0.0	-30.0 \pm 0.6	-29.7	-24.9 \pm 0.5
22R-pentakishomohop-17,21-ene	-29.6 \pm 1.4	-31.0 \pm 1.7	-27.9 \pm 0.8	-32.0 \pm 0.7	-29.1 \pm 0.5	-30.0 \pm 0.7	-26.4 \pm 0.5
17 α ,21 β (H)-22R-pentakishomohopane ^b	-28.5 \pm 0.6	-26.1 \pm 1.0	-23.9 \pm 1.3	-28.8 \pm 0.4	-29.6 \pm 0.8	-29.9 \pm 0.4	n.d.
17 β ,21 α (H)-22R-pentakishomohopane ^b	n.d.	n.d.	-24.0 \pm 1.5	-29.2 \pm 0.9	n.d.	n.d.	n.d.
17 β ,21 β (H)-22R-pentakishomohopane ^b	-28.0	-27.9 \pm 0.4	-22.2 \pm 0.6	-28.6 \pm 0.7	-29.5 \pm 0.2	n.d.	-31.0 \pm 0.4

^a δ values (‰ versus PDB), errors indicate standard deviation from duplicate analyses ^b released from the polar fraction by Raney Ni desulphurisation

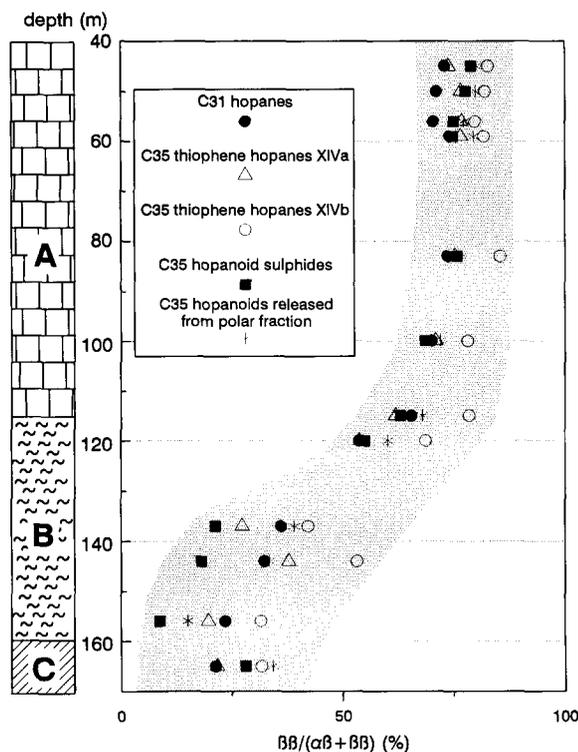


FIG. 2. The ratio of $17\beta,21\beta(\text{H})$ -hopanoids/(the sum of $17\alpha,21\beta(\text{H})$ - and $17\beta,21\beta(\text{H})$ -hopanoids) vs. depth for homohopane, C_{35} hopanoid thiophenes, C_{35} hopanoid sulphides (determined after desulphurisation and, thus, representing the average of all desulphurised hopanoid sulphides) and C_{35} hopanes released after desulphurisation of the polar fractions.

try (irm-GC-MS). This analytical technique enables the determination of the ^{13}C contents of individual molecules (Hayes et al., 1990). The $\delta^{13}\text{C}$ values of biolipids do vary since they depend on (1) the isotopic composition of the carbon source assimilated by the organism, (2) isotope effects associated with the assimilation of carbon, and (3) the biochemical processes involved in its synthesis (Hayes, 1993). Since diagenetic reactions of geolipids only take place at a small number of specific positions in the carbon skeleton, the fractionation effects associated with these reactions are buffered by the isotopic composition of the large number of other carbon atoms and the $\delta^{13}\text{C}$ value of the precursor molecule is, in most cases, similar to that of the diagenetic product (Hayes et al., 1990). For example, Freeman et al. (1994) reported that the triterpenoid-derived polycyclic aromatic hydrocarbons with varying degrees of aromatisation in the Eocene Messel Oil Shale had similar $\delta^{13}\text{C}$ values as their precursors. Similarly, Hauke et al. (1992) reported ^{13}C contents of a series of C_{31} - C_{35} benzohopanes VI in a Triassic black shale from Italy in the range -32.4 to -33.4% , suggesting that they derive from a common precursor.

In this paper the abundances and carbon isotopic compositions of sedimentary homohopaneoids in twelve samples from a sediment core from the relatively immature, Upper Cretaceous Jurf ed Darawish Oils Shale are reported and these data are used to obtain a better insight into the complex diagenetic pathways of bacteriohopanepolyol derivatives.

2. SAMPLES: SOURCE AND DESCRIPTION

The Jurf ed Darawish Oil Shale (thickness ca. 120 m; depth 45–165 m) from Jordan was deposited during the Campanian/Maastrichtian (Late Cretaceous) in a small basin on the edge of the Tethys (Hufnagel, 1984; Wehner and Hufnagel, 1987). It can be divided into three facies: the bituminous limestone facies (facies A; 45–115 m), the bituminous calcareous marl facies (facies B; 115–160 m), and the phosphorite facies (facies C; 160–165 m) (Hufnagel, 1984; Kohnen et al., 1990). These sediments contain high amounts (TOC = 4–17%; see Kohnen et al., 1990) of thermally immature organic matter ($R_o = 0.28$ – 0.31% over the depth range studied). Twelve composite 1-m samples of a core from a drill hole were investigated. These are the same samples as studied by Kohnen et al. (1990).

3. EXPERIMENTAL

3.1. Preparation of Samples

Samples were ultrasonically extracted with mixtures of methanol and dichloromethane and fractionated as reported previously (Kohnen et al., 1990). Briefly, the extracts were separated into polar and apolar fractions by column chromatography and the apolar fraction was subsequently separated by argentation thin layer chromatography into a saturated hydrocarbon, an alkylthiophene, and an alkylsulphide fraction. Compounds were quantified by the use of internal standards as reported elsewhere (Kohnen et al., 1990). A known amount of the polar fraction with a known amount of 2,3-dimethyl-5-(1',1'-d₂-hexadecyl)-thiophene were desulphurised with Raney Ni and the hydrocarbons obtained were isolated and hydrogenated with PtO_2 as reported previously (Sinninghe Damsté et al., 1988). The alkylsulphide fraction was desulphurised with nickel boride (Schouten et al., 1993). Branched and cyclic hydrocarbons were isolated from the saturated hydrocarbons and the released hydrocarbons from the polar fraction by activated 5Å sieves. TOC values were determined previously (Kohnen et al., 1990).

3.2. Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS)

The conditions have been described previously (Kohnen et al., 1990).

3.3. Quantitation

Homohopanes and homohop-17(21)-enes were quantified by integration of m/z 367 and 191 mass chromatograms and appropriate peaks in the FID chromatograms. Benzohopanes and hopanoid thiophenes were quantified by integration of appropriate peak areas in m/z 191 and 107 (for the internal standard) mass chromatograms. Corrections were made for the abundance of the fragment ions used in the total ion yields of the components. The homohopanes released from the polar and alkylsulphide fractions upon desulphurisation were quantified by integration of appropriate peak areas in the FID chromatograms. Concentrations are normalized on TOC and expressed as $\mu\text{g/g}$ TOC (ppm). Duplicates indicated good reproducibility ($>90\%$).

3.4. Isotope-Ratio-Monitoring Gas Chromatography-Mass Spectrometry (irm-GC-MS)

The irm-GC-MS system (DELTA-S) has been described previously (Hayes et al., 1990). A fused silica capillary column (50 m \times 0.32 μm) coated with crosslinked methyl silicone gum (Ultra-1, film thickness 0.52 μm) was used with helium as carrier gas. The samples were injected on column at 70°C and subsequently the oven was programmed to 130°C at 10°C min^{-1} , and then at 3°C min^{-1} to 320°C, at which it was held for 60 min. The isotopic values were obtained by integrating the mass 44, 45, and 46 ion currents from the carbon dioxide produced by the continuous combustion of the chromatographic effluent. The isotopic compositions are referred to the PDB ^{13}C standard by comparison to co-injected isotopic standards.

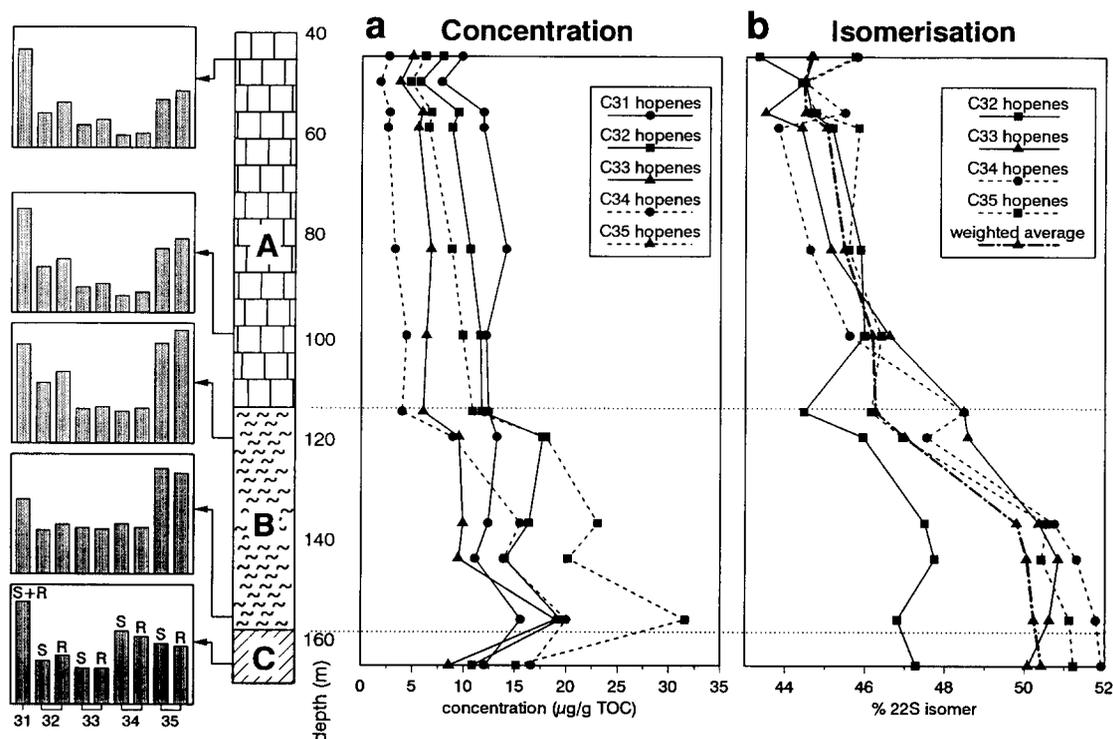


FIG. 3. (a) Concentration (sum of 22R and 22S isomers) and (b) degree of isomerisation (% of 22S isomer) of extended hop-17(21)-enes vs. depth. The fat dashed line in (b) represents the concentration-weighted average of the abundance of the 22S isomer. Bar plots represent typical examples of extended hop-17(21)-ene distributions at various positions in the bed.

3.5. Molecular Mechanics

Molecular mechanics calculations were performed on a Silicon Graphics Indigo XZ400 computer. For selected hopenes and hopane cations the heats of formation and entropies were calculated. Since, due to the presence of an alkyl chain in these compounds, many local minimum conformations are possible, a conformational search was performed for each compound to identify its global minimum. By assuming that the energy of the corresponding tertiary carbocation is representative for the energy barrier between two hopenes the calculated thermodynamic data could be combined into a set of linear differential equations describing the mixture composition in time. These equations were solved, using the MATLAB-program. By varying the initial concentrations of several compounds in the isomerisation scheme and determining the values of the frequency factors present in the differential equations the fit between the observed and calculated data was optimised.

4. RESULTS

The Jurf ed Darawish Oil Shale contains abundant homohopaneoids, such as homohopanes and extended hop-17(21)-enes (Wehner and Hufnagel, 1987; Sinninghe Damsté et al., 1988; Kohnen et al., 1990), hopanoid thiophenes (Sinninghe Damsté et al., 1989b, 1990b; Kohnen et al., 1990), hopanoid sulphides (Sinninghe Damsté et al., 1989b), and macromolecularly sulphur-bound C_{35} hopane units (Sinninghe Damsté et al., 1990a). Therefore, the set of twelve core samples from a drill hole were thought to be highly suitable for studying the diagenetic pathways of bacteriohopanetetrol derivatives. To this end the important classes of homohopaneoids in these sediments were quantified and, in a number of cases, their carbon

isotopic compositions were determined in seven representative samples from the core. We have only taken C_{31} - C_{35} hopaneoids into consideration since it is quite probable that they all derive from bacteriohopanepolyol derivatives, whereas this is not the case for lower molecular weight components which may also derive from C_{30} hopaneoids.

4.1. Homohopanes

The homohopanes are dominated by the C_{31} members as is evident from the mass chromatogram of m/z 191 of the saturated hydrocarbon fraction (Fig. 1a) of sample JED-137 (the number indicates the depth of the sample in the core). Bis-homohopanes were present in minor amounts and higher homologs were not detected or were only present in trace amounts. The abundance of the extended hop-17(21)-enes made it impossible to quantify these C_{32} homologs by integration of m/z 191 mass chromatograms. Three stereoisomers were identified for every structural isomer: the 22R-17 α ,21 β (H)-, 22R-17 β ,21 β (H)-, and in minor amounts, the 22R-17 β ,21 α (H)-isomer. A consistent increase in the relative abundance of the $\alpha\beta$ -isomer relative to the $\beta\beta$ -isomer was noted with increasing depth (Fig. 2). The carbon isotopic compositions of 22R-17 α ,21 β (H)- and 22R-17 β ,21 β (H)-homohopane are reported in Table 1.

4.2. Homohop-17(21)-enes

The extended hop-17(21)-enes are important constituents of the saturated hydrocarbon fractions (see also Kohnen et al.,

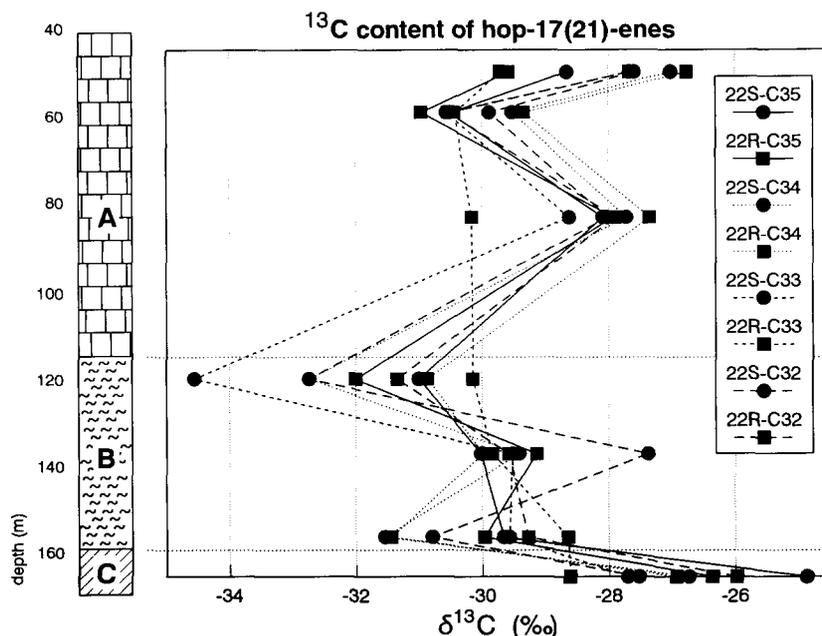


FIG. 4. ^{13}C content of C_{32} – C_{35} extended hop-17(21)-enes vs. depth. Standard deviations of measurements are indicated in Table 1.

1990): their total summed concentration can be as high as 120 ppm (Fig. 3). The extended hop-17(21)-enes occur as doublets of 22S and 22R isomers and have a distribution dominated by the C_{35} members (Fig. 1b). The percentage of C_{35} hop-17(21)-enes of the total C_{31} – C_{35} hop-17(21)-enes varies from ca. 30% in the uppermost beds to almost 37% in the lowermost beds of Facies B and then abruptly drops to 22.5% in the phosphorite facies (C). In the phosphorite facies the C_{34} hop-17(21)-enes are more abundant than their C_{35} counterparts (Fig. 3). There is also a small, but consistent, decrease in the abundance of the C_{31} – C_{35} 22R hop-17(21)-enes relative to the presumably thermodynamically more stable 22S counterparts (55% to 48%) independent of facies (Fig. 3). The carbon isotopic composition of the C_{32} – C_{35} hop-17(21)-enes are presented in Table 1 and plotted in Fig. 4. Significant changes between samples and for different members within one sample can be noted.

4.3. Benzohopanes

Benzohopanes (VI) are only important compounds in the lowermost beds of Facies B and in Facies C. Their total summed concentration varies from 0.4 to 21 ppm (Fig. 5). In the benzohopane-rich samples their distribution is dominated by the C_{35} member except in the phosphorite facies where the C_{34} component is more abundant (Fig. 5).

4.4. Thiophene Hopanoids

Thiophene hopanoids are present in all samples in a concentration of 30–70 ppm with a maximum at the facies boundary between Facies A and B (Fig. 6). They comprise two structural isomers (XIVa and b) and for every structural isomer there are three different stereoisomers: 22R-

$17\alpha,21\beta(\text{H})$ -, 22R- $17\beta,21\beta(\text{H})$ -, and in minor amounts, the 22R- $17\beta,21\alpha(\text{H})$ -isomer as reported previously (Sinninghe Damsté et al., 1989b). The ratio of the two structural isomers varies significantly (from 0.77 to 0.50) but not gradually. A large and gradual decrease is observed for the ratio of the $\beta\beta/(\alpha\beta+\beta\beta)$ isomer ratio for both structural isomers (Fig. 2) but is generally higher for hopanoid thiophene XIVb. No lower homologs ($<\text{C}_{35}$) were observed to be present.

4.5. Homohopanoïd Sulphides

The homohopanoïd sulphides comprise a complicated mixture of mainly C_{35} isomers. One dominant class of homohopanoïd sulphides is formed by the compounds in which the sulphur atom is incorporated into the side-chain. One member of this series was first tentatively identified by Schmid (1986) in Rozel Point Oil as 31-(2'-thiolanyl)- $17\alpha,21\beta(\text{H})$ -homohopane (XVIII) and is characterized by m/z 87, 101, 291, and 369 in its mass spectrum. Kohnen et al. (1991a) identified the corresponding $\beta\beta$ -isomer in an extract of the Vena del Gesso Basin. In the Jurf ed Darawish samples both isomers are present and a small $\beta\alpha$ -isomer is identified as well. Smaller amounts of homohopanoïd sulphide XVII previously tentatively identified by Schmid (1986) occur as well. In addition, two series of homohopanoïd sulphides are present which have not been previously identified. The first series is comprised of stereoisomers of 30-(2'-(5'-methylthiolanyl))-hopane (XIX) and is characterized by m/z 101, 141, 191, 291, and 369. Different isomers (probably *cis* and *trans*) of the $\alpha\beta$ -, $\beta\alpha$ -, and $\beta\beta$ -stereoisomers are present. The second series comprises C_{33} – C_{35} hopanoïd sulphides and has been identified as components (XX) in which the sulphur atom is attached to C-17 and C-30 of the hopanoïd carbon skeleton with the $17\alpha,21\beta(\text{H})$ -stereochemistry (van Kaam-Peters et al., 1995b).

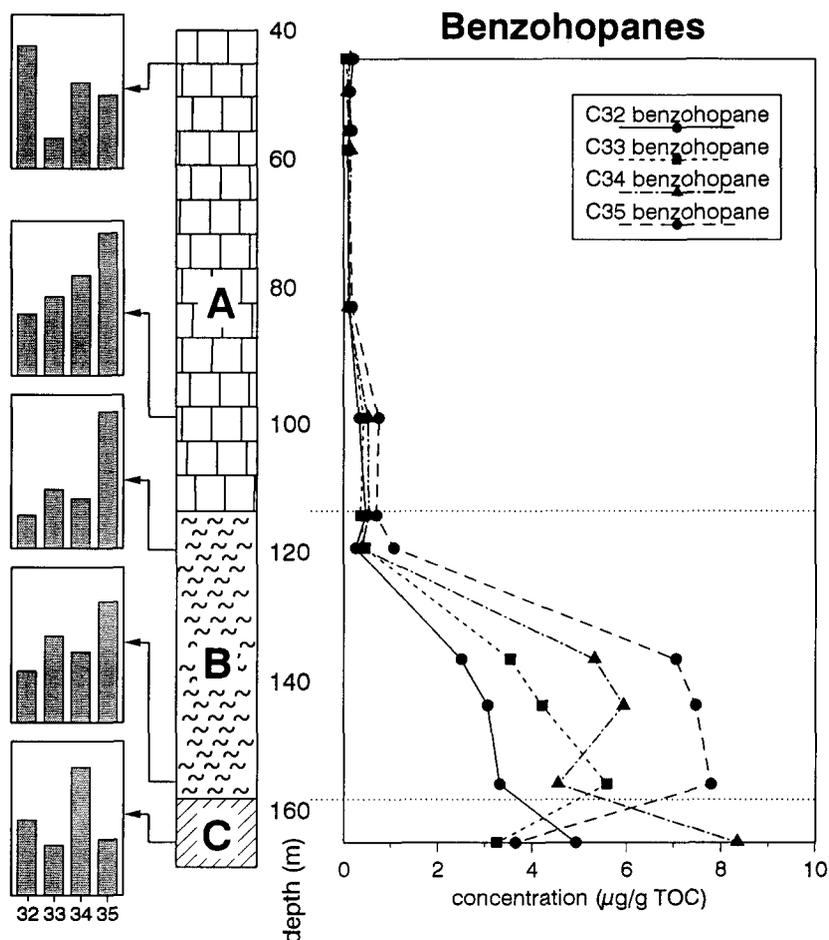


FIG. 5. Concentration of C_{32} - C_{35} benzohopanes vs. depth in the bed. Bar plots represent typical examples of benzohopane distributions at various positions in the bed.

These hopanoid sulphides were quantified by desulphurisation of the alkylsulphide fractions with nickel boride (Schouten et al., 1993) and subsequent isolation of the hydrocarbons formed. These fractions contained significant amounts (20–65 ppm) of C_{35} hopanes with the 22R-17 α ,21 β (H)-, 22R-17 β ,21 β (H)-, and to a much lesser extent the 22R-17 β ,21 α (H)-stereochemistry. As in the case of the hopanoid thiophenes a large and gradual decrease is observed for the $\beta\beta/(\alpha\beta+\beta\beta)$ isomer ratio (Fig. 2). This approach did not work for the novel series of C_{33} - C_{35} hopanoid sulphides XX since these components do not desulphurise under the conditions used (van Kaam-Peters et al., 1995b).

4.6. Homohopanooids in the Polar Fraction

Substantial amounts (30–147 ppm) of C_{35} hopanes were released upon desulphurisation of the polar fraction. These hopanes are formed from (1) macromolecular aggregates in which pentakishomohopane units are incorporated by one or more (poly)sulphide linkages and (2) C_{35} hopanoid sulphoxides and/or sulphones. Both these groups have a polarity which leads to their presence in the polar fraction. Schouten et al. (1995b) have recently demonstrated that both these groups of compounds are present in the polar fraction of sam-

ple JED-156. It is noteworthy that lower homologs are very minor relative to the C_{35} components. Three stereomers were identified, the 22R-17 α ,21 β (H)-, 22R-17 β ,21 β (H)-, and the 22R-17 β ,21 α (H)-isomer, with the first two dominating (Fig. 7). Again, a large and gradual decrease is observed for the $\beta\beta/(\alpha\beta+\beta\beta)$ isomer ratio (Fig. 2). The carbon isotopic compositions of 17 α ,21 β (H)- and 17 β ,21 β (H)-pentakishomohopane released upon desulphurisation are reported in Table 1 and plotted in Fig. 8.

5. DISCUSSION

5.1. Distribution of Extended Hopanoid Classes

Figure 9 shows the combined quantitative data of all forms of C_{35} hopanoids. It reveals that, despite the relative abundance of C_{35} hop-17(21)-enes in the saturated hydrocarbon fractions, S-bound C_{35} hopanoids dominate; they represent 80–95% of the total quantified skeletons, with a consistent higher relative abundance in Facies A (>90%). The absolute concentration of the S-bound C_{35} hopanoids is relatively constant in Facies A and B (180–270 $\mu\text{g/g}$ TOC), but drops to ca. 100 $\mu\text{g/g}$ TOC in Facies C. No consistent variations can be observed in the relative abundance of the various classes

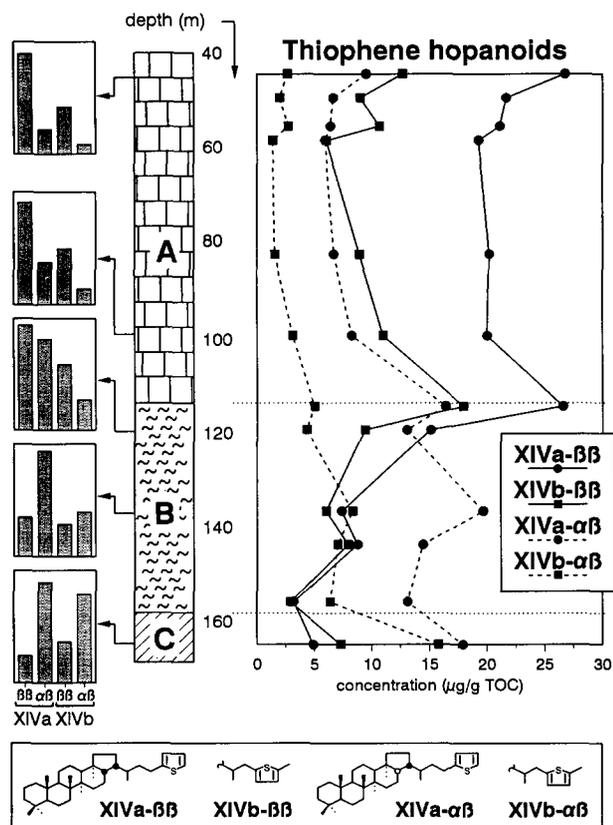


FIG. 6. Concentration of C_{35} hopanoid thiophenes vs. depth in the bed. Bar plots represent typical examples of distributions of the different structural and stereochemical isomers at various positions in the bed.

of S-bound hopanoids. If the other extended hopanoids are also taken into account, the picture changes slightly. A smaller percentage of the extended hopanoids occurs in a S-bound form (50–80%) with a consistent lower amount of sulphurised hopanoid skeletons with increasing depth (Fig. 10). It should be noted that, in fact, an even larger portion of the homohopane carbon skeleton probably occurs in a S-bound form since the asphaltene and kerogen fractions were not taken into consideration in this study. Indeed, Hofmann et al. (1992) released homohopaneoids from desulphurisation of the kerogen fraction of sample JED-156.

The reported preferential incorporation of inorganic sulphur species into bacteriohopanepolyol derivatives leading to preservation of the intact carbon skeleton (e.g., Sinnighe Damsté et al., 1989a, 1990a,b; de Leeuw and Sinnighe Damsté, 1990) can explain why the distributions of the S-bound hopanoid skeletons are dominated by the C_{35} member. This process obviously did not effect the distributions of the hopanoid hydrocarbons (extended hopanes, homohop-17(21)-enes and benzohopanes). The extended hopanes are dominated by the C_{31} member and only very minor amounts of higher homologs are present. The full range of structurally possible homologs of the homohop-17(21)-enes and benzohopanes are present. Both series are dominated by the C_{35} member in facies B and by the C_{34} member in the phosphorite facies. The large differences in carbon number distributions

of the various series of homohopaneoids suggests that they were formed by different diagenetic reactions of bacteriohopanepolyol derivatives.

5.2. Carbon Isotopic Composition of Extended Hopanoids

The relatively high concentrations of extended hop-17(21)-enes in the saturated hydrocarbon fractions allowed the determination of their $\delta^{13}C$ values to assess a possible common origin from a bacteriohopanepolyol precursor. Figure 4, however, demonstrates that there is quite some spread in the $\delta^{13}C$ values of the extended hop-17(21)-enes: 3‰ on average and in sample JED-120 almost 5‰ although standard deviations are sometimes relatively large (Table 1). Multivariate analysis of these data show that the differences between the carbon isotopic compositions of the homohop-17(21)-enes are statistically significant for sample JED-83 and samples lower in the section. The homohop-17(21)-enes from samples JED-50 and -59 can, on basis of this statistical treatment, be derived from one precursor. If we consider Fig. 4 in detail, it is apparent that the ^{13}C contents of all homohop-17(21)-enes, with one exception, follow a common trend which may be related to changes in water column characteristics (e.g., $[CO_2(aq)]$, $\delta^{13}C_{DIC}$). The exception is 22R-trishomohop-17(21)-ene, the $\delta^{13}C$ value of which remains relatively constant. The reason for this is not known. If we run our statistical test without the $\delta^{13}C$ values of this component, the differences observed for the homohop-17(21)-enes in sample JED-83 are no longer statistically significant. If the $\delta^{13}C$ value of 22R trishomohop-17(21)-ene is considered to be an outlier, the range of $\delta^{13}C$ values of the homohopene series shrinks from a minimum of 1.3‰ in JED-83 to a maximum of 3.8‰ in JED-120.

The biosynthesis of bacteriohopanepolyol exerts some effect on the ^{13}C content of extended hop-17(21)-enes derived from it. The C_5 side-chain is derived from a pentose which is attached to a C_{30} hopanoid derived from 6 isoprene units (Rohmer et al., 1992). It is well established that carbohydrate carbon is heavier than lipid carbon biosynthesized by the same organism (e.g., Hayes, 1993). If we estimate this difference to be 5‰ this would only lead to a difference in ^{13}C content between a C_{35} and a C_{31} component of ca. 0.5‰. Since (1) the higher-molecular-weight hopenes are not consistently enriched in ^{13}C relative to the lower molecular weight hopenes and (2) the statistically significant differences in samples of Facies B and C are much larger (Fig. 4), this biosynthetic effect cannot explain the differences observed. Since these differences are larger than those which can be expected starting from a single bacteriohopanepolyol precursor with a specific $\delta^{13}C$ value, these data indicate that there are multiple precursors for this series of homohop-17(21)-enes, with different chemical structures, leading to differences in diagenetic transformation pathways, ultimately generating homohop-17(21)-enes with different distributions. Since these different precursors are likely to have different initial ^{13}C contents, the $\delta^{13}C$ values of the ultimately formed series of homohop-17(21)-enes are likely to form a very complicated pattern. In cases where one precursor has dominated, the $\delta^{13}C$ values may be in a narrow range (e.g., sample JED-83); whereas in cases where a suite of isotopically distinct precursors was approximately equally abundant, a wide range in $\delta^{13}C$ values may

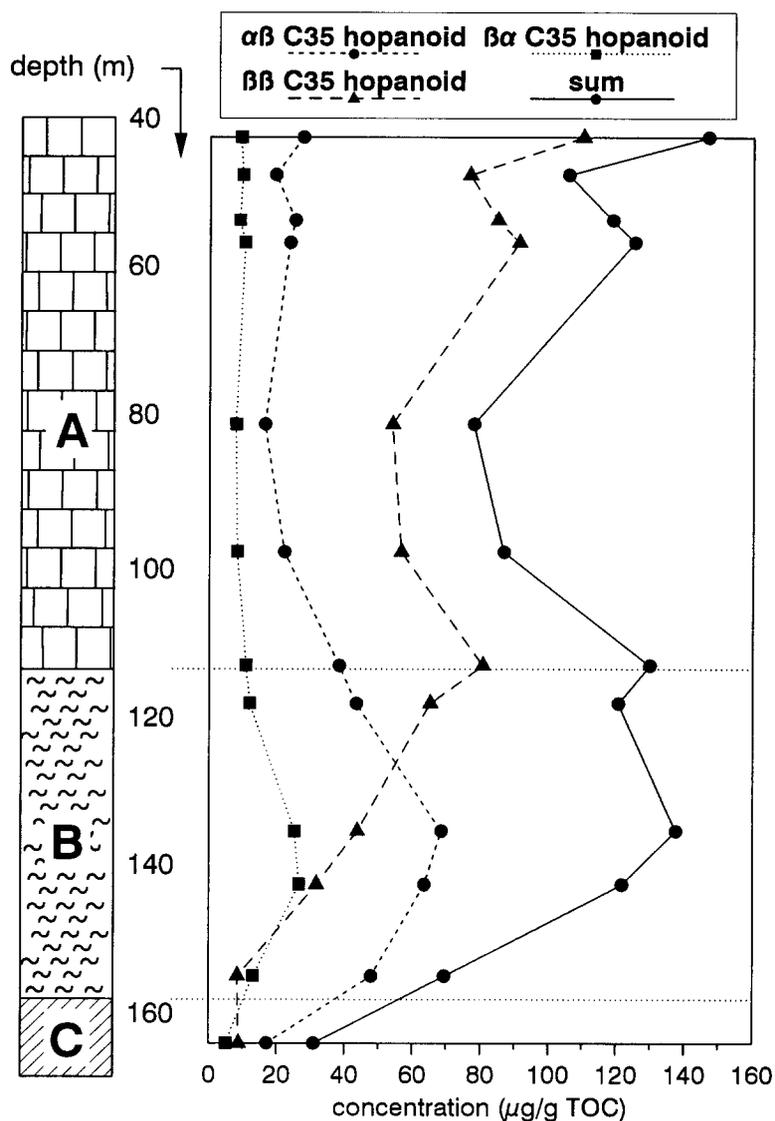


FIG. 7. Concentrations of the individual C₃₅ hopane epimers released by desulphurisation of the polar fractions and their summed concentration vs. depth.

result (e.g., sample JED-120). In view of the large variability in structure of bacteriohopanepolyol derivatives (**1a–i**; e.g., Rohmer et al., 1992) this scenario seems likely. The variability in $\delta^{13}\text{C}$ content in a series of homohopanoids is not restricted to the Jurf ed Darawish Oil shale. Ruble et al. (1994) reported a range in $\delta^{13}\text{C}$ values of C₃₁–C₃₅ homohopanoids of 5.6‰ in a native bitumen from the Uinta Basin.

When the carbon isotopic data of the C₃₁ hopanes and the C₃₅ hopanes released by desulphurisation from the polar fraction are taken into consideration, the picture becomes even more complicated (Fig. 8). Despite the large range in $\delta^{13}\text{C}$ values for the homohop-17(21)-enes, the $\delta^{13}\text{C}$ content of these hopanoids, also presumed to be derived from bacteriohopanepolyol derivatives, often falls outside this range. The most striking example in this respect are the C₃₅ hopanes released from the polar fraction of sample JED-83. Their $\delta^{13}\text{C}$ values are in the range -22.2 to -24.0‰ , a difference with the av-

erage value for the homohop-17(21)-enes of ca. 5‰. This, in our view, is another indication that multiple bacteriohopanepolyol precursors have to be invoked in the formation of fossil homohopanoids.

5.3. Isomerisation at C-17 and C-21

Independent of compound class, a large increase in the relative abundance of 17 α ,21 β (H)-epimers in the lower part of Facies B and Facies C is noted (Fig. 2). This increase in relative abundance of 17 α ,21 β (H)-epimers is not due to preferential destruction of the less stable 17 β ,21 β (H)-epimers as illustrated, for example, by the concentration profiles of the thiophene hopanoids (Fig. 6) and of the C₃₅ hopanes released upon desulphurisation of the polar fractions (Fig. 7). In Fig. 7 this is clearly evident from the sum of the three epimers of the C₃₅ hopane: in the depth interval where the largest change

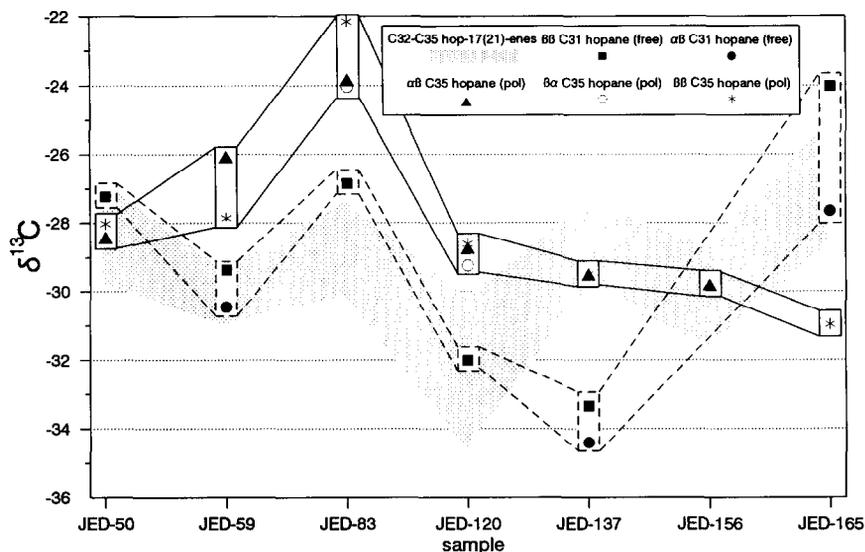


FIG. 8. $\delta^{13}\text{C}$ values of C_{35} hopanes released from the polar fraction by desulphurisation and free C_{31} homohopanes in six samples. Values for the extended hop-17(21)-enes (see also Fig. 4) are given for reference and are indicated as the stippled area.

in epimer composition occurs (115–144 m; see Fig. 2) the concentration of S-bound C_{35} hopanes in the polar fraction (which is depending on input and degree of sulphurisation) stays relatively constant, which makes preferential thermal destruction rather unlikely. Preferential thermal destruction has been demonstrated to explain the “isomerisation” of ster-

anes at C-20 (Requejo, 1992) and of $17\beta,21\alpha(\text{H})$ - into $17\alpha,21\beta(\text{H})$ -hopanes (ten Haven et al., 1992). The ^{13}C content of the epimers in this instance is quite similar (Fig. 8), in agreement with the idea that isomerisation does take place. It is well established that the biological $17\beta,21\beta(\text{H})$ -configuration of hopanoids in organisms is unstable and is readily con-

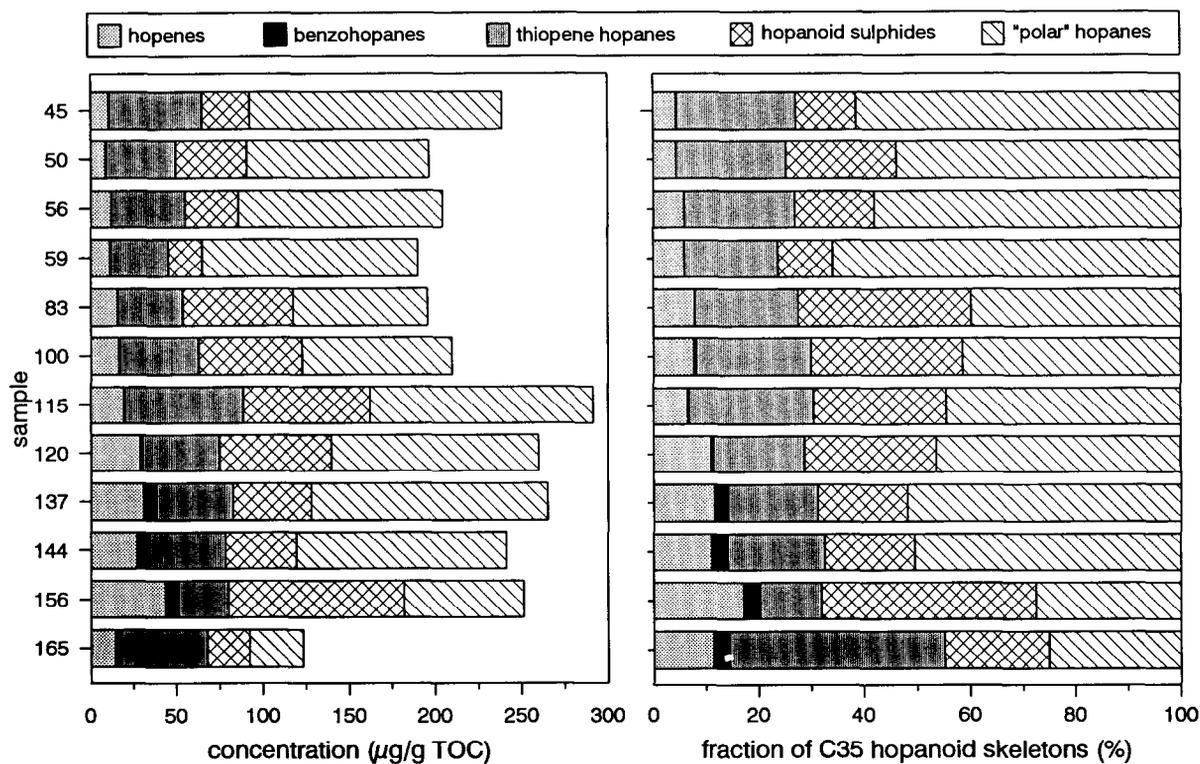


FIG. 9. Concentrations (absolute and relative) of C_{35} homohopanooid carbon skeletons as present in the compound classes indicated.

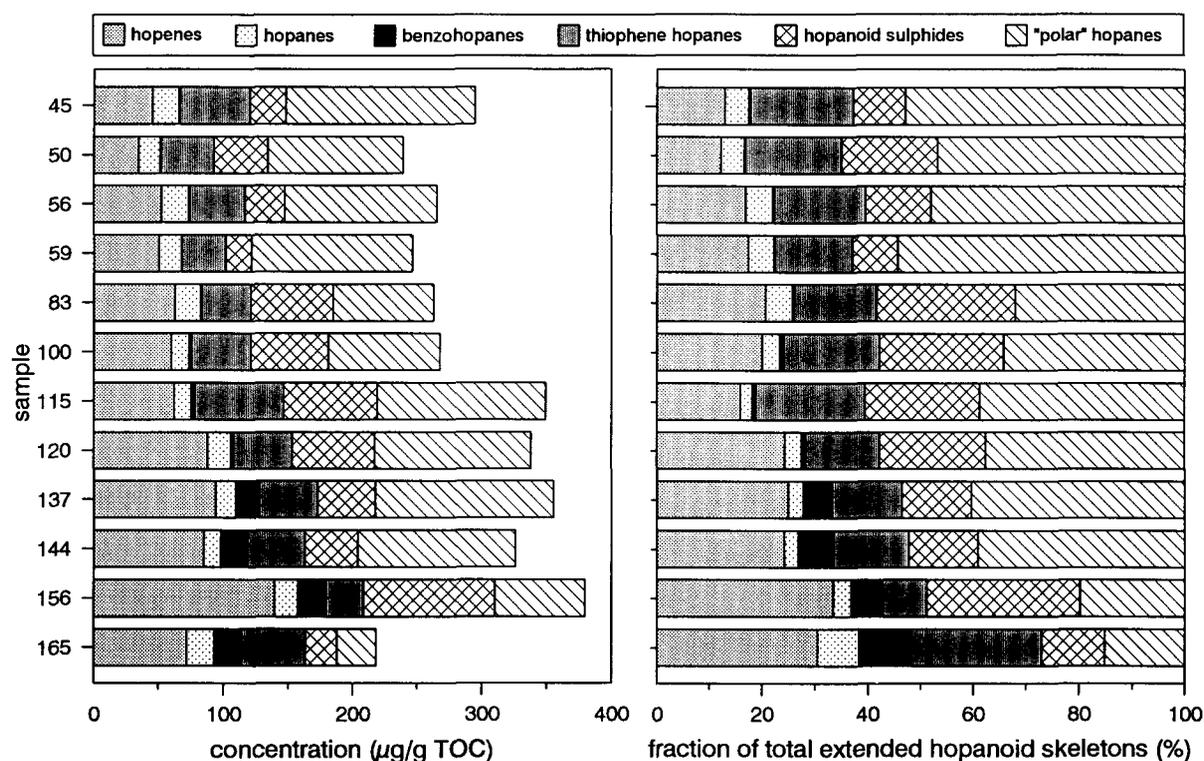


FIG. 10. Concentrations (absolute and relative) of C_{31} - C_{35} homohopanooid carbon skeletons as present in the compound classes indicated.

verted into more stable epimers (e.g., Seifert and Moldowan, 1980; Kolaczowska et al., 1990; Peters and Moldowan, 1993). Therefore, $\beta\beta$ -hopanes are not found in sediments which experienced some diagenesis ($R_0 > 0.4\%$) and not in crude oils (Peters and Moldowan, 1993). The isomerisation of $17\beta,21\beta(H)$ -hopanooids into their $17\alpha,21\beta(H)$ and $17\beta,21\alpha(H)$ counterparts occurs in a very narrow depth span in the sediment core studied and seems not to be affected by the inorganic composition of the sediments: the largest change in epimer composition occurs between sample JED-120 and -137, which have similar mineral assemblages (Kohnen et al., 1990). It is, therefore, concluded that slight differences in thermal histories have to explain this isomerisation. This would require that the isomerisation reaction has a very high frequency factor: if enough energy is available to overcome to activation energy the reaction is very fast. This would in-

dicating that the chance to find a suite of sediments where this isomerisation can be followed is only small.

It should be noted that the formation of benzohopanes correlates with epimerisation of homohopanooids at C-17; in the depth interval where 17α -homohopanooids are formed (>120 m; Fig. 2), a significant increase in the benzohopane concentrations is noted (Fig. 5). Formation of benzohopanes by cyclisation and aromatisation reactions requires precursors with the 17α -hopanooid carbon skeleton since 17β -benzohopanes are very strained molecules (Hussler et al., 1984a). Our results seem to indicate that the isomerisation at C-17 does not occur during the cyclisation process as suggested by Hussler et al. (1984a) but is a prerequisite for the formation of benzohopanes.

5.3. Isomerisation at C-22

All homohopanooid classes except the series of homohop-17(21)-enes possess the biological 22R configuration only. This confirms that isomerisation at C-17 (and C-21) occurs more readily than isomerisation at C-22 (Peters and Moldowan, 1993). A general increase in isomerisation at C-22 of the series of homohop-17(21)-enes can be noted with increasing depth (Fig. 3b). The isomerisation endpoint for homohopanes is ca. 60% 22S (Peters and Moldowan, 1993), but is not known for homohop-17(21)-enes. Calculations by molecular mechanics using a new force field (van Duin et al., 1994) indicate that the isomerisation endpoint for homohop-17(21)-enes is at 52–53% 22S, although some variations with the length of the alkyl side-chain are noted (Table 2). Calculations

Table 2. Calculated ΔG values of 22R and 22S homohop-17(21)-enes and composition (% 22S) of the thermodynamic equilibrium mixture at 50°C

	ΔG 22R	ΔG 22S	% 22S
homohop-17(21)-ene	-147.12	-147.16	51.92
bishomohop-17(21)-ene	-155.34	-155.41	52.70
trishomohop-17(21)-ene	-163.53	-163.60	52.70
tetrakishomohop-17(21)-ene	-171.68	-171.76	53.21
pentakishomohop-17(21)-ene	-179.83	-179.91	53.21

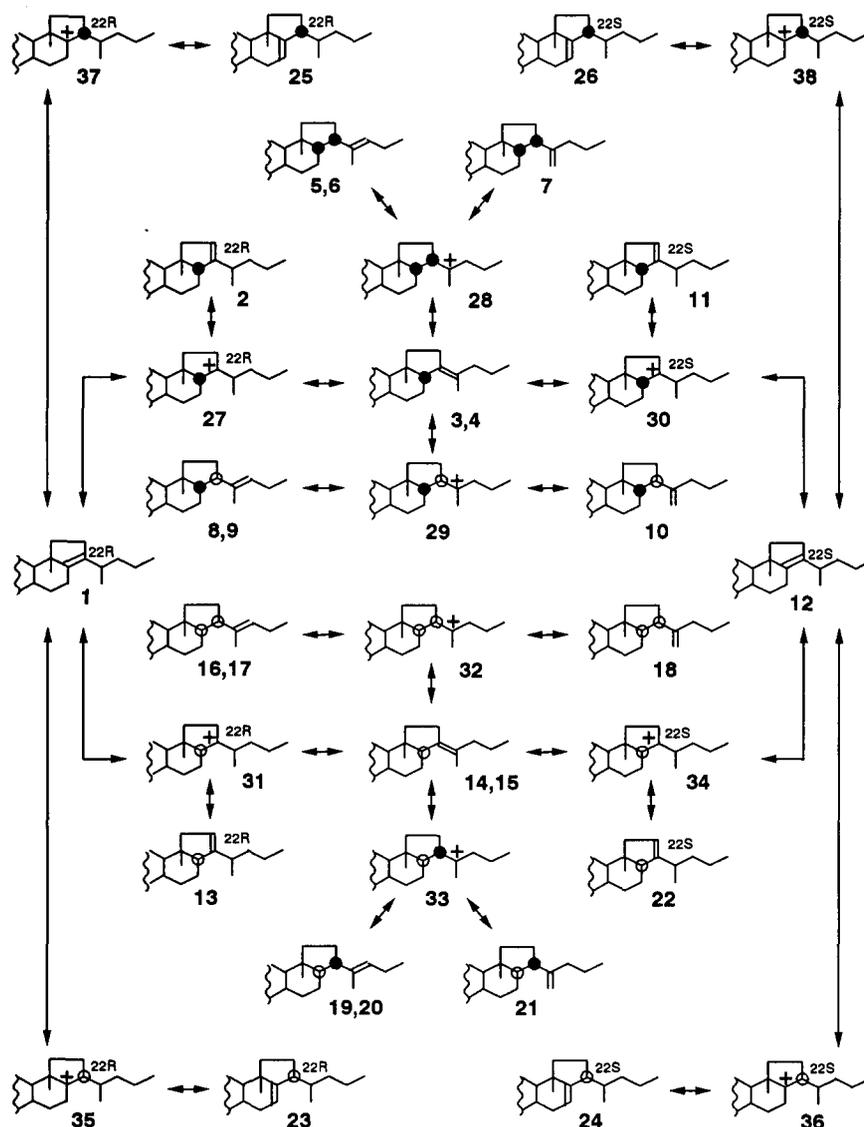


FIG. 11. Isomerisation scheme for homohopanes. Heats of formation and entropy of numbered bishomohopanes and intermediate carbocations are listed in Appendix B.

of the compositions of the equilibrium mixture at other temperatures (25–150°C) revealed only changes <1% from the data shown in Table 2. The good fit between the calculated and geological data (Fig. 3) indicate that at the base of the section the homohop-17(21)-enes have almost reached thermodynamic equilibrium.

ten Haven et al. (1986, 1988) reported that many bitumens from immature rocks (most of them deposited under hypersaline conditions) contain suites of homohop-17(21)-enes and $17\alpha,21\beta(H)$ -homohopanes fully isomerized at C-22. These authors attributed this to either isomerisation of hop-17(21)-enes through formation of a stabilized carbocation at C-22 or to a defunctionalisation of the precursor hopanoids followed by a double bond migration from the side chain to the 17(21)-position, “en passant” isomerizing the chiral centre in the side chain. ten Haven et al. (1986) also proposed that the series of homohopanes was formed by hydrogenation of the homohop-17(21)-enes. Such a hydrogenation has cer-

tainly not occurred in the rocks investigated here since the distribution of the homohopanes is completely different from that of the homohop-17(21)-enes (i.e., strongly dominated by the C₃₁ member) and no isomerisation at C-22 is apparent.

To test the two hypotheses concerning the isomerisation at C-22 of homohopanes an isomerisation scheme (Fig. 11) was developed. This scheme takes only tertiary intermediate carbocations into consideration because earlier investigations have indicated that double bond isomerisations *via* secondary carbocations are unlikely to occur during early diagenesis (de Leeuw et al., 1989). The heat of formation and entropy of a number of C₃₂ hopanes and intermediate carbocations in this scheme were determined using newly developed force fields (van Duin et al., 1994, 1995). These data are compiled in Appendix B and reveal that 22R and 22S bishomohop-17(21)-enes are by far the most stable. This explains why these components are often dominant in geological samples which have undergone some diagenesis. It is interesting to note, however,

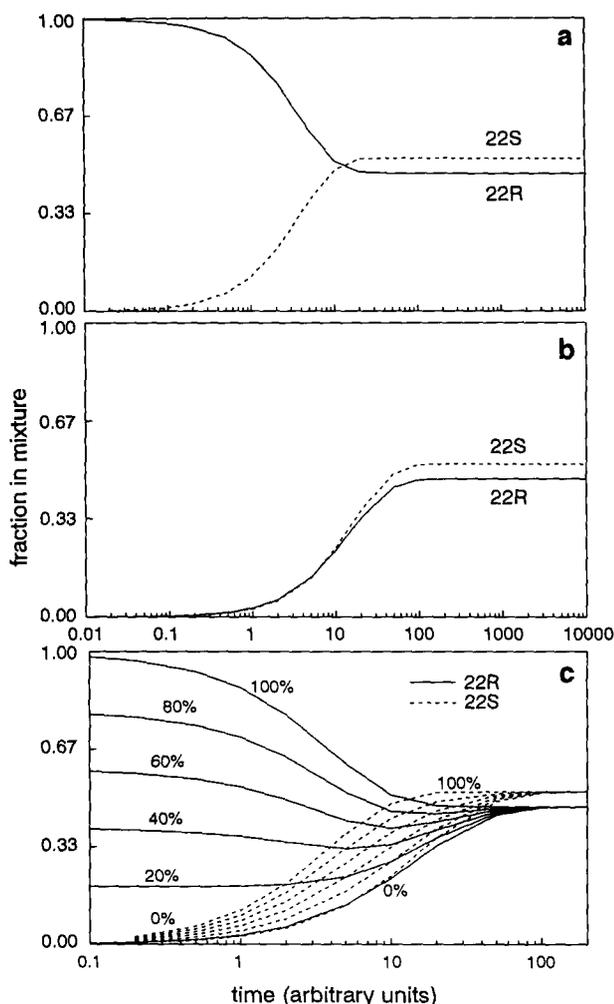


FIG. 12. Extent of the isomerisation reaction of (a) 22R-bishomohop-17(21)-ene and (b) 50/50 mixture of (Z)- and (E)-17 β ,21 β (H)-bishomohop-22(29)-ene calculated for $T = 70^\circ\text{C}$ and (c) mixtures of these two starting materials with % values showing the amount of hop-17(21)-ene starting material. Continued and interrupted lines indicate 22R and 22S hop-17(21)-ene epimers, respectively.

that 17 α -bishomohop-20(21)-enes and 21 α -bishomohop-16(17)-enes are the next most stable homohopenes. This may explain why hopanoid sulphides **XV** and **XVI** (where the sulphur is attached at C-20 and C-16 of the hopanoid skeleton, respectively) and benzohopanes **VI** and **VII** are formed in sediments.

The data compiled in Appendix B allowed the calculation of the thermodynamic equilibrium mixture starting with 22R-bishomohop-17(21)-ene and 22R-17 β ,21 β (H)-bishomohop-22(29)-ene and mixtures of these components. By estimation of frequency factors for the various reactions, it was also possible to follow the extent of the reaction on an arbitrary time scale (Fig. 12). If 22R-bishomohop-17(21)-ene (**1** in Fig. 11) functions as the starting component for the isomerisation reaction, a relatively simple profile results since only isomerisation to 22S-bishomohop-17(21)-ene occurs (Fig. 12a).

17 β ,21 β (H)-Bishomohop-22(29)-ene [50/50 mixture of (Z)- and (E)-isomers; **5** and **6** in Fig. 11] was also taken as a

starting component since it is a good model component for hopenes which might have been formed by dehydration reactions of bacteriohopanepolyols. In this case both 22R and 22S bishomohop-17(21)-enes are formed with the 22S isomer in slightly enhanced concentrations during the last phase of the reaction (Fig. 12b). It is noteworthy that in this case during all stages of the reaction approximately equal amounts of the 22R and 22S isomer are present (cf. Fig. 12a,b). Since in the Jurf ed Darawish oil shale the homohop-17(21)-ene composition (45–52% 22S) occurs in a range which can be explained by both starting materials, these molecular mechanics calculation cannot discriminate between direct isomerisation of 22R-homohop-17(21)-enes or isomerisation of hopenes formed from dehydration reactions of bacteriohopanepolyols.

It should be noted, however, that the ^{13}C contents of some of the pairs of the 22R and S epimers in some of the samples have significantly different δ values (Table 1). This is not consistent with either one of these possibilities since identical δ values for the pairs of 22R and 22S epimers would be expected. To explain these differences formation of 22S and 22R homohop-17(21)-enes can also be explained by a combination of both pathways presented above. Figure 12c shows the extent of the isomerisation reactions if mixtures of 22R homohop-17(21)-ene and 17 β ,21 β (H)-homohop-22(29)-ene [50/50 mixture of (Z) and (E)-isomers] are taken as starting material. These calculations indicate that a combination of these starting materials also results in mixtures of 22R and 22S homohop-17(21)-enes similar to those encountered in the Jurf ed Darawish Oil Shale (45–52% 22S). If different $\delta^{13}\text{C}$ values of the starting materials are assumed, different δ values for the 22R and 22S homohop-17(21)-enes before the isomerisation endpoint result (see Table 3 for an example), which may explain, at least in part, these differences observed in some of the samples.

Table 3. Calculated $\delta^{13}\text{C}$ values of 22R and 22S epimers during isomerisation of a mixture of 22R bishomohop-17(21)-ene (20%) and 17 β ,21 β (H)-bishomohop-22(29)-ene (80%)^a

time (arbitrary units)	% 22S	$\delta^{13}\text{C}$ 22R (‰)	$\delta^{13}\text{C}$ 22S (‰)
0	0	-30.0	n.a.
1	8	-27.0	-25.3
10	39	-26.3	-24.4
100	51	-22.1	-22.2
1000	53	-22.0	-22.0

^a assumed $\delta^{13}\text{C}$ starting materials: -30‰ for 22R bishomohop-17(21)-ene and -20‰ for bishomohop-22(29)-ene

5.5. Application in Geochemistry: Homohopane Index

Peters and Moldowan (1991) have advocated the use of the distributions of C_{31} - C_{35} $17\alpha,21\beta(H)$ -homohopanes in marine petroleum to describe redox conditions in the source rock depositional environments. They associate high C_{35} homohopane indices ($C_{35}/C_{31-C_{35}}$) with anoxic marine conditions, where the C_{35} backbone of the precursor bacteriohopanepolyol derivatives is preferentially preserved. Deposition under oxic conditions leads to oxidation of the side-chain of the precursor polyol derivatives and formation of predominantly lower members of the homohopane series. De Leeuw and Sinninghe Damsté (1990) and Peters and Moldowan (1993) related the preservation of the C_{35} carbon skeleton to cleavage of relatively weak C-S bonds of S-rich aggregates during diagenesis and catagenesis. These S-rich aggregates contain S-bound homohopanes dominated by C_{35} members (Schmid, 1986; Trifilieff, 1987; Sinninghe Damsté et al., 1988, 1990a, 1993; de Leeuw and Sinninghe Damsté, 1990; Kohnen et al., 1991c, 1992a,b; Richnow et al., 1992, 1993; Hofmann et al., 1992; Adam et al., 1993; Schouten et al., 1993; Schoell et al., 1994) due to natural sulphurisation of intact bacteriohopanepolyol precursors with retention of the intact carbon skeleton. This study shows that, indeed, the preservation of the intact C_{35} carbon skeleton is associated with the sulphurisation process since only S-containing homohopaneoids show this phenomenon. Thermal degradation of S-rich macromolecular aggregates leads to formation of C_{35} homohopanes (Koopmans, unpubl. results) but it should be noted that the dominance of the C_{35} member will certainly be less strong than in the S-bound homohopaneoids since, for example, hopaneoid thiophenes will certainly not release the intact C_{35} homohopane carbon skeleton upon thermal stress.

6. CONCLUSIONS

- 1) The diagenetic pathways leading to formation of fossil homohopaneoids from bacteriohopanepolyol derivatives is more complex than previously recognized. Carbon isotope compositions of homohopanes, individual members of the homohop-17(21)-ene series, and macromolecularly S-bound C_{35} hopaneoids indicate that these components are often not derived from one precursor bacteriohopanepolyol derivative.
- 2) Isomerisation at C-17 of both free and S-bound homohopaneoids occurs in a very narrow depth span in the Jurf ed Darawish Oil Shale. This is confirmed by the similar isotopic compositions of the two epimers and by quantitation of the concentration of the epimers, revealing no selective degradation of one of these components. Benzohopanes are only present if $\beta\beta$ -hopanes have isomerised to $\alpha\beta$ -epimers, suggesting that this conformation is essential for the formation of benzohopanes.
- 3) A slight increase of 22S homohop-17(21)-enes relative to their 22R isomers is observed down section. At the base of the section the thermodynamic equilibrium (52–53% 22S isomer) as calculated by molecular mechanics is reached. Molecular mechanic calculations indicate that this isomerisation behaviour can be due to either isomerisation of homohopanes from dehydration of bacteriohop-

anepolyol derivatives or from direct isomerisation of 22R homohop-17(21)-enes.

- 4) Natural sulphurisation of bacteriohopanepolyol derivatives leads to preservation of the intact skeleton. 80–95% of the pentakishomohopane and 50–80% of the total extended hopaneoid skeletons in the JED oil shale occurs in a S-bound form. This preservation mechanism provides a rationale for the application of the so-called homohopane index often applied in the assessment of redox potential of past depositional environments.

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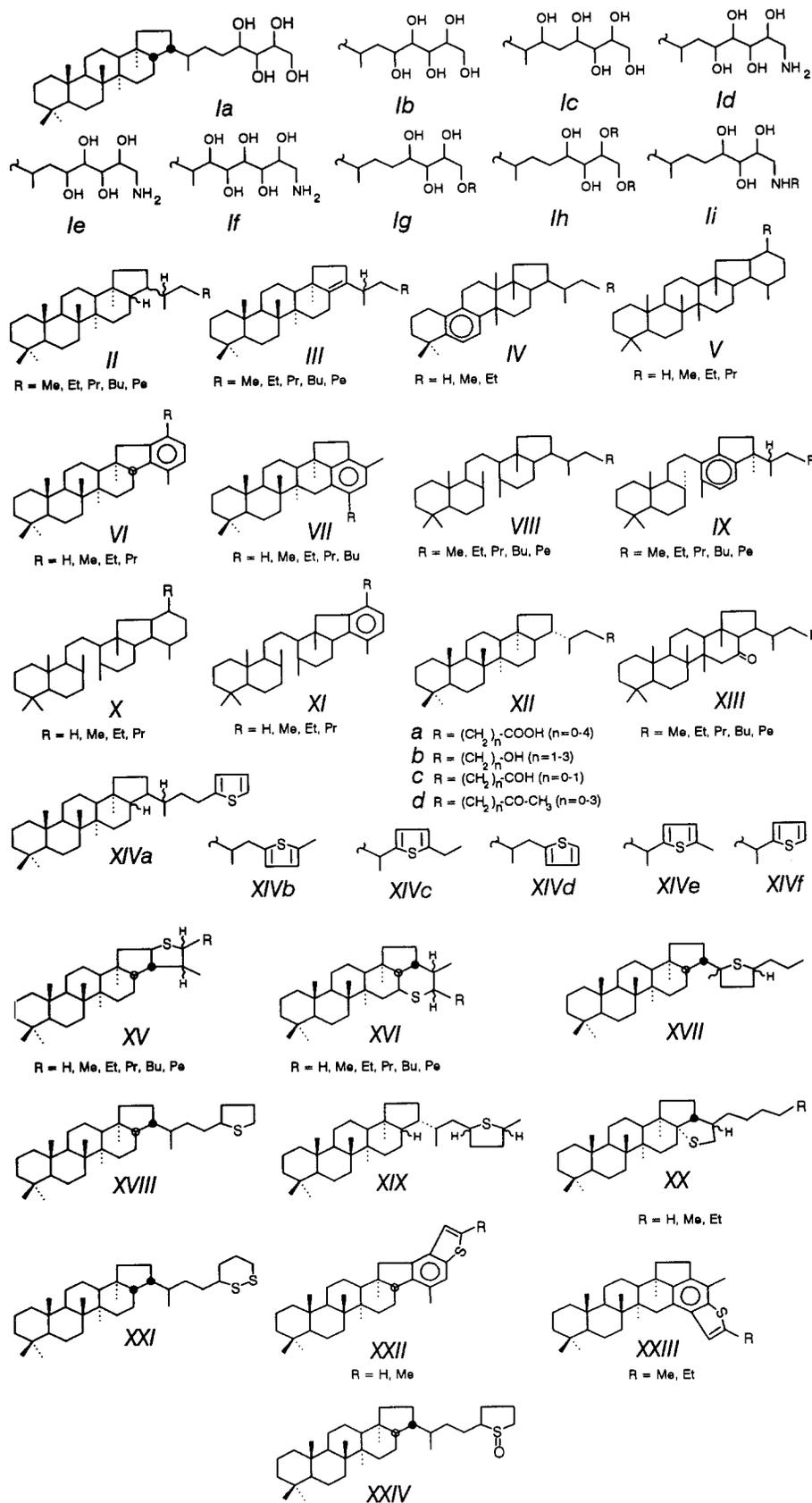
REFERENCES

- Adam P., Schmid J. C., Mycke B., Strazielle C., Connan J., Huc A., Riva A., and Albrecht P. (1993) Structural investigation of non-polar sulfur cross-linked macromolecules in petroleum. *Geochim. Cosmochim. Acta* **57**, 3395–3419.
- Armanios C., Alexander R., Kagi R. I., Sobolev A. N., and White A. H. (1994) The molecular structure of sedimentary (22R) $17\alpha,21\beta(H)$ -homohopane from an X-ray diffraction study. *Geochim. Cosmochim. Acta* **58**, 2941–2943.
- Boon J. J. et al. (1983) Organic geochemical studies of Solar Lake laminated cyanobacterial mats. In *Advances in Organic Geochemistry 1981* (ed. M. Bjorøy et al.), pp. 207–227. Wiley.
- Connan J. and Dessort D. (1987) Novel family of hexacyclic hopaneoid alkanes (C_{32} - C_{35}) occurring in sediments and oils from anoxic palaeoenvironments. *Org. Geochem.* **11**, 103–113.
- Cyr T. D., Payzant J. D., Montgomery D. S., and Strausz O. P. (1986) A homologous series of novel hopane sulfides in petroleum. *Org. Geochem.* **9**, 139–143.
- Dastillung M., Albrecht P., and Ourisson G. (1980a) Aliphatic and polycyclic ketones in sediments: C_{27} - C_{35} ketones and aldehydes of the hopane series. *J. Chem. Res. (S)*, 166–167.
- Dastillung M., Albrecht P., and Ourisson G. (1980b) Aliphatic and polycyclic alcohols in sediments: Hydroxylated derivatives of hopane and of 3-methylhopane. *J. Chem. Res. (S)*, 168–169.
- de Leeuw J. W. and Sinninghe Damsté J. S. (1990) Organic sulphur compounds and other biomarkers as indicators of palaeosalinity. In *Geochemistry of Sulfur in Fossil Fuels* (ed. W. L. Orr and C. M. White); ACS Symposium Series 429, pp. 417–443. Amer. Chem. Soc.
- de Leeuw J. W. et al. (1989) Limited double bond isomerisation and selective hydrogenation of sterenes during early diagenesis. *Geochim. Cosmochim. Acta* **53**, 903–909.
- de Lemos Scofield A. (1990) Nouveaux marqueurs biologiques de sédiments et pétroles riches en soufre: Identification et mode de formation. Ph.D. thesis, Univ. Strasbourg.
- Dessort D. and Connan J. (1993) Occurrence of novel C-ring opened hopaneoids in biodegraded oils from carbonate source rocks. In *Organic Geochemistry* (ed. Øygard K.), pp. 485–495. Falch Hurigtrykk.
- Ensminger A. (1977) Evolution de composés polycycliques sédimentaires. Ph.D. thesis, Univ. Strasbourg.
- Fazeelat T., Alexander R., and Kagi R. I. (1994) Extended 8,14-secohopanes in some seep oils from Pakistan. *Org. Geochem.* **21**, 249–264.
- Freeman K. H., Boreham C. J., Summons R. E., and Hayes J. M. (1994) The effect of aromatization on the isotopic compositions of hydrocarbons during early diagenesis. *Org. Geochem.* **21**, 1037–1049.

- Hauke V. et al. (1992) Novel triterpene-derived hydrocarbons of the arborane/fernane series in sediments: part II. *Geochim. Cosmochim. Acta* **56**, 3595–3602.
- Hayes J. M. (1993) Factors controlling ^{13}C contents of sedimentary organic compounds: Principles and evidence. *Mar. Geol.* **113**, 111–125.
- Hayes J. M., Freeman K. H., Popp B. N., and Hoham C. H. (1990) Compound-specific isotopic analyses: A novel tool for reconstruction of ancient biogeochemical processes. In *Advances in Organic Geochemistry 1989* (ed. B. Durand and F. Behar); *Org. Geochem.* **16**, 1115–1128.
- Hofmann I. C., Hutchinson J., Robson J. N., Chicarelli M. I., and Maxwell J. R. (1992) Evidence for sulphide links in a crude oil asphaltene and kerogens from reductive cleavage by lithium in ethylamine. In *Advances in Organic Geochemistry 1991* (ed. C. Eckardt et al.); *Org. Geochem.* **19**, 371–387.
- Hufnagel H. (1984) Oil shale in Jordan. *Geol. Jahrb., Rh A* **75**, 295–311.
- Hussler G., Connan J., and Albrecht P. (1984a) Novel families of tetra- and hexacyclic aromatic hopanoids predominant in carbonate rocks and crude oils. In *Advances in Organic Geochemistry 1983* (ed. P. A. Schenck et al.); *Org. Geochem.* **6**, 39–49.
- Hussler G., Albrecht P., Ourisson G., Cesario M., Guilhem J., and Pascard C. (1984b) Benzohopanes, a novel family of hexacyclic geomarkers in sediments and petroleum. *Tetrahedron Lett.* **25**, 1179–1182.
- Kohnen M. E. L., Sinninghe Damsté J. S., Rijpstra W. I. C., and de Leeuw J. W. (1990) Alkylthiophenes as sensitive indicators of paleoenvironmental changes: A study of a Cretaceous oil shale from Jordan. In *Geochemistry of Sulfur in Fossil Fuels* (ed. W. L. Orr and C. M. White); *ACS Symposium Series* **429**, pp. 444–485. Amer. Chem. Soc.
- Kohnen M. E. L., Sinninghe Damsté J. S., ten Haven H. L., Kock-Van Dalen A. C., Schouten S., and de Leeuw J. W. (1991a) Identification and geochemical significance of cyclic di- and trisulphides with linear and acyclic isoprenoid carbon skeletons in immature sediments. *Geochim. Cosmochim. Acta* **55**, 3685–3695.
- Kohnen M. E. L., Sinninghe Damsté J. S., Kock-Van Dalen A. C., and de Leeuw J. W. (1991b) Di- or polysulphide-bound biomarkers in sulphur-rich geomacromolecules as revealed by selective chemolysis. *Geochim. Cosmochim. Acta* **55**, 1375–1394.
- Kohnen M. E. L., Sinninghe Damsté J. S., and de Leeuw J. W. (1991c) Biases from natural sulphurization in palaeoenvironmental reconstruction based on hydrocarbon biomarkers distributions. *Nature* **349**, 777–778.
- Kohnen M. E. L., Schouten S., Sinninghe Damsté J. S., de Leeuw J. W., Merrit D., and Hayes J. M. (1992a) Improved recognition of palaeobiochemicals by a combined molecular sulphur and isotope geochemical approach. *Science* **256**, 358–362.
- Kohnen M. E. L., Schouten S., Sinninghe Damsté J. S., de Leeuw J. W., Merrit D., and Hayes J. M. (1992b) The combined application of organic sulphur and isotope geochemistry to assess multiple sources of palaeobiochemicals with identical carbon skeletons. In *Advances in Organic Geochemistry 1991* (ed. C. Eckardt et al.); *Org. Geochem.* **19**, 403–419.
- Kolaczowska E., Slougui N.-E., Watt D. S., Maruca R. E., and Moldowan J. M. (1990) Thermodynamic stability of various alkylated, dealkylated and rearranged 17 α - and 17 β -hopane isomers using molecular mechanics calculations. In *Advances in Organic Geochemistry 1989* (ed. B. Durand and F. Behar); *Org. Geochem.* **16**, 1033–1038.
- Köster J., Schouten S., de Leeuw J. W., and Sinninghe Damsté J. S. (1993) Palaeoenvironmental and maturity related variations in compositions of macromolecular organic matter and distributions of sulphur and non-sulphur biomarkers in Triassic organic-rich carbonate rocks. In *Organic Geochemistry* (ed. Øygard K.), pp. 402–406. Falch Huriqtrykk.
- McEvoy J. (1983) The origin and diagenesis of organic lipids in sediments from the San Miguel Gap. PhD thesis, Univ. Bristol.
- Mycke B., Narjes F., and Michaelis W. (1987) Bacteriohopanetetrol from chemical degradation of an oil shale kerogen. *Nature* **326**, 179–181.
- Ourisson G. and Albrecht P. (1992) Hopanoids. 1. Geohopanooids: The most abundant natural products on earth? *Acc. Chem. Res.* **25**, 398–402.
- Ourisson G., Albrecht P., and Rohmer M. (1979) The hopanoids, paleochemistry and biochemistry of a group of natural products. *Pure Appl. Chem* **51**, 709–729.
- Oustroukov S. B., Aref'yev O. A., and Petrov A. A. (1983) Hexacyclic monoaromatic hydrocarbons of petroleum. *Petrol. Chem. U. S. S. R.* **23**, 53–60.
- Peters K. E. and Moldowan J. M. (1991) Effects of source, thermal maturity and biodegradation on the distribution and isomerization of homohopanes in petroleum. *Org. Geochem.* **17**, 47–61.
- Peters K. E. and Moldowan J. M. (1993) *The Biomarker Guide*. Prentice-Hall.
- Requejo A. G. (1992) Quantitative analysis of triterpane and sterane biomarkers: Methodology and applications in molecular maturity studies. In *Biological Markers in Sediments and Petroleum* (ed. J. M. Moldowan et al.), pp.222–240. Prentice Hall.
- Richnow H. H., Jenisch A., and Michaelis W. (1992) Structural investigations of sulphur-rich kerogens and macromolecular oil fractions by sequential chemical degradation. In *Advances in Organic Geochemistry 1991* (ed. C. Eckardt et al.); *Org. Geochem.* **19**, 351–370.
- Richnow H. H., Jenisch A., and Michaelis W. (1993) The chemical structure of macromolecular fractions of a sulfur-rich crude oil. *Geochim. Cosmochim. Acta* **57**, 2767–2780.
- Ries-Kaut M. and Albrecht P. (1989) Hopane derived triterpenes in soils. *Chem. Geol.* **76**, 143–151.
- Rohmer M., Dastillung M., and Ourisson G. (1980) Hopanoids from C₃₀ to C₃₅ in recent muds: Chemical markers for bacterial activity. *Naturwissenschaften* **67**, 456–458.
- Rohmer M., Bissere P., and Neunlist S. (1992) The hopanoids, prokaryotic triterpenoids and precursors of ubiquitous molecular fossils. In *Biological Markers in Sediments and Petroleum* (ed. J. M. Moldowan et al.), pp. 1–17. Prentice Hall.
- Ruble T. E., Bakel A. J., and Philp R. P. (1994) Compound specific isotopic variability in Uinta Basin native bitumens: Palaeoenvironmental implications. *Org. Geochem.* **21**, 661–671.
- Schaeffer P., Adam P., Trendel J.-M., Albrecht P., and Connan J. (1995) A novel series of benzohopanes widespread in sediments. *Org. Geochem.* **23**, 87–93.
- Schmid J. C. (1986) Marqués biologiques souffrés dans les pétroles. Ph.D dissertation, Univ. Strasbourg.
- Schmitter J. M., Sucrow W., and Arpino P. J. (1982) Occurrence of novel tetracyclic geochemical markers: 8,14 secohopanes in a Nigerian crude oil. *Geochim. Cosmochim. Acta* **46**, 2345–2350.
- Schoell M., Mccaffrey M. A., Fago F. J., and Moldowan J. M. (1992) Carbon isotopic compositions of 28,30-bisnorhopanes and other biological markers in Monterey crude oil. *Geochim. Cosmochim. Acta* **56**, 1391–1399.
- Schoell M., Schouten S., Sinninghe Damsté J. S., de Leeuw J. W., and Summons R. E. (1994) A molecular organic carbon isotope record of Miocene climate changes. *Science* **263**, 1122–1125.
- Schouten S., Pavlovic D., Sinninghe Damsté J. S., and de Leeuw, J. W. (1993) Nickel Boride: An improved desulphurization agent for sulphur-rich geomacromolecules in polar and asphaltene fractions. *Org. Geochem.* **20**, 901–909.
- Schouten S., Sinninghe Damsté J. S., and de Leeuw J. W. (1995) The occurrence and distribution of low-molecular-weight sulphoxides in polar fractions of sediment extracts and petroleum. *Org. Geochem.* **23**, 129–138.
- Seifert W. K. and Moldowan J. M. (1980) The effect of thermal stress on source rock quality as measured by hopane stereochemistry. *Phys. Chem. Earth* **12**, 229–237.
- Sinninghe Damsté J. S., Rijpstra W. I. C., de Leeuw J. W., and Schenck P. A. (1988) Origin of organic sulphur compounds and sulphur-containing high molecular weight substances in sediments and immature crude oils. In *Advances in Organic Geochemistry 1987* (ed. L. Matavelli and L. Novelli); *Org. Geochem.* **13**, 593–606.
- Sinninghe Damsté J. S., Rijpstra W. I. C., Kock-Van Dalen A. C., de Leeuw J. W., and Schenck P. A. (1989a) Quenching of labile functionalized lipids by inorganic sulphur species: Evidence for

- the formation of sedimentary organic sulphur compounds at the early stages of diagenesis. *Geochim. Cosmochim. Acta* **53**, 1443–1455.
- Sinninghe Damsté J. S., Rijpstra W. I. C., de Leeuw J. W., and Schenck P. A. (1989b) The occurrence and identification of series of organic sulphur compounds in oils and sediment extracts. II. Their presence in samples from hypersaline and non-hypersaline depositional environments and their possible application as source, palaeoenvironmental and maturity indicators. *Geochim. Cosmochim. Acta* **53**, 1423–1441.
- Sinninghe Damsté J. S., Eglinton T. I., Rijpstra W. I. C., and de Leeuw J. W. (1990a) Molecular characterisation of organically-bound sulfur in high-molecular-weight sedimentary organic matter using flash pyrolysis and Raney Ni desulfurisation. In *Geochemistry of Sulfur in Fossil Fuels* (ed. W. L. Orr and C. M. White); *ACS Symposium Series* **429**, pp. 486–528. Amer. Chem. Soc.
- Sinninghe Damsté J. S., Kohnen M. E. L., and de Leeuw J. W. (1990b) Thiophenic biomarkers for palaeoenvironmental assessment and molecular stratigraphy. *Nature* **345**, 609–611.
- Sinninghe Damsté J. S., de Las Heras F. X. C., Van Bergen P. F., and de Leeuw J. W. (1993) Characterization of Tertiary Catalan lacustrine oil shales: Discovery of extremely organic sulphur-rich type I kerogens. *Geochim. Cosmochim. Acta* **57**, 389–415.
- ten Haven H. L., de Leeuw J. W., Peakman T. M., and Maxwell J. R. (1986) Anomalies in steroid and hopanoid maturity indices. *Geochim. Cosmochim. Acta* **50**, 853–855.
- ten Haven H. L., de Leeuw J. W., Sinninghe Damsté J. S., Schenck P. A., Palmer S. E., and Zumberge J. E. (1988) The application of biological markers in the recognition of palaeo hypersaline environments. In *Lacustrine Petroleum Source Rocks* (ed. K. Kelts et al.); *Geological Society Special Publication* **40**, 123–130.
- ten Haven H. L., Rullkötter J., Sinninghe Damsté J. S., and de Leeuw J. W. (1990) Distribution of organic sulphur compounds in Mesozoic and Cenozoic deep-sea sediments from the Atlantic and Pacific Oceans and the Gulf of California. In *Geochemistry of Sulfur in Fossil Fuels* (ed. W. L. Orr and C. M. White); *ACS Symposium Series* **429**, pp. 613–632. Amer. Chem. Soc.
- ten Haven H. L., Littke R., and Rullkötter J. (1992) Hydrocarbon biological markers in Carboniferous coals of different maturities. In *Biological Markers in Sediments and Petroleum* (ed. J. M. Moldowan et al.), pp. 142–155. Prentice Hall.
- Trifilieff S. (1987) Etude de la structure des fractions polaires de pétroles (résines et asphaltenes) par dégradations chimiques sélectives. Ph.D. thesis, Univ. Strasbourg.
- Trifilieff S., Sieskind O., and Albrecht P. (1992) Biological markers in petroleum asphaltene: Possible mode of incorporation. In *Biological Markers in Sediments and Petroleum* (ed. J. M. Moldowan et al.), pp. 350–369. Prentice Hall.
- Wehner H. and Hufnagel H. (1987) Some characteristics of the inorganic and organic composition of oil shales from Jordan. In *Biogeochemistry of Black Shales* (ed. E. T. Degens et al.); *Mitt. Geol. Palaeont. Inst. Univ. Hamburg* **60**, 381–395.
- Valisolalao J., Perakis N., Chappe B., and Albrecht P. (1984) A novel sulfur containing C₃₅ hopanoid in sediments. *Tetrahedron Lett.* **25**, 1183–1186.
- van Dorsseleer A. (1974) *Triterpènes de sédiments*. Ph.D. thesis. Univ. Strasbourg.
- van Dorsseleer A., Albrecht P. A., and Ourisson G. (1977) Identification of novel 17 α -hopanes in shales, coals, lignites, sediments and petroleum. *Bull. Soc. Chim. Fr.* 165–170.
- van Duin A. C. T., Baas J. M. A., and van de Graaf B. (1994) Delft molecular mechanics: A new approach to hydrocarbon force fields. Inclusion of a geometry-dependant charge calculation. *J. Chem. Soc. Faraday Trans.* **90**, 2881–2895.
- van Duin A. C. T., Baas J. M. A., and van de Graaf B. (1995) A new force field for tertiary carbo-cations. *J. Chem. Soc. Faraday Trans.* (in press).
- van Kaam-Peters H. E. L., Köster J., de Leeuw J. W., and Sinninghe Damsté J. S. (1995a) Occurrence of two novel families of benzo-thiophene hopanoids in sediments. *Org. Geochem.* (in press).
- Van Kaam-Peters H. E. L., Rijpstra W. I. C., and Sinninghe Damsté J. S. (1995b) Identification of a novel series of C₃₃-C₃₅ hopanoid sulphides in sediments. *Org. Geochem.* **23**, 607–616.

APPENDIX A



APPENDIX B: Heat of formation and entropy of C₃₂ 5 α ,8 β ,9 α ,14 α ,18 α -hopenes and hopane cations

Compound ^a	ΔH_f (kcal/mol)	S (cal/molK)
bishomohopenes		
1	22R, Δ 17,21	-89.69
2	17 β ,22R, Δ 20,21	-81.71
3	17 β , Δ 21,22 (E)	-82.17
4	17 β , Δ 21,22 (Z)	-81.87
5	17 β ,21 β (H), Δ 22,29 (E)	-76.49
6	17 β ,21 β (H), Δ 22,29 (Z)	-76.41
7	17 β ,21 β (H), Δ 22,31	-75.00
8	17 β ,21 α (H), Δ 22,29 (E)	-81.35
9	17 β ,21 α (H), Δ 22,29 (Z)	-81.46
10	17 β ,21 α (H), Δ 22,30	-78.64
11	17 β ,22S, Δ 20,21	-81.83
12	22S, Δ 17,21	-89.74
13	17 α ,22R, Δ 20,21	-87.61
14	17 α , Δ 21,22 (E)	-85.04
15	17 α , Δ 21,22 (Z)	-85.26
16	17 α ,21 α (H), Δ 22,29 (E)	-79.32
17	17 α ,21 α (H), Δ 22,29 (Z)	-80.09
18	17 α ,21 α (H), Δ 22,30	-78.60
19	17 α ,21 β (H), Δ 22,29 (E)	-84.48
20	17 α ,21 β (H), Δ 22,29 (Z)	-85.24
21	17 α ,21 β (H), Δ 22,30	-82.87
22	17 α ,22S, Δ 20,21	-87.55
23	21 α (H),22R, Δ 16,17	-86.52
24	21 α (H),22S, Δ 16,17	-86.80
25	21 β (H),22R, Δ 16,17	-84.73
26	21 β (H),22S, Δ 16,17	-84.49
bishomohopane carbocations		
27	17 β ,22R,c ²¹	55.52
28	17 β ,21 β (H),c ²²	62.56
29	17 β ,21 α (H),c ²²	56.72
30	17 β ,22S,c ²¹	55.78
31	17 α ,22R,c ²¹	49.89
32	17 α ,21 α (H),c ²²	60.15
33	17 α ,21 β (H),c ²²	54.11
34	17 α ,22S,c ²¹	49.90
35	21 α (H),22R,c ¹⁷	56.23
36	21 α (H),22S,c ¹⁷	56.19
37	21 β (H),22R,c ¹⁷	55.28
38	21 β (H),22S,c ¹⁷	55.33

^a compounds numbers refer to structures in Fig. 11