Pergamon

Org. Geochem. Vol. 29, No. 1–3, pp. 649–669, 1998

Printed in Great Britain
PII: S0146-6380(98)00182-X
0146-6380/98/$ - see front matter

Biomarker geochemistry of a foreland basin: the Oligocene Menilite Formation in the Flysch Carpathians of Southeast Poland

J. KÖSTER[1]*, M. ROSPONDEK[2], S. SCHOUTEN[3], M. KOTARBA[4], A. ZUBRZYCKI[4] and J. S. SINNINGHE DAMSTÉ[3]

[1]Department of Petroleum Geology, Institute of Geology and Palaeontology, Technical University Clausthal, Leibnizstr. 10, 38678 Clausthal-Zellerfeld, Germany, [2]Institute of Geological Sciences, Jagellonian University, ul. Oleandry 2a, 30063 Krakow, Poland, [3]Department of Marine Biogeochemistry and Toxicology, Netherlands Institute for Sea Research (NIOZ), 1790 AB Den Burg, Texel, The Netherlands and [4]University of Mining and Metallurgy, Al. Mickiewicza 30, 30059 Krakow, Poland

Abstract—Black shales of the Menilite Formation, the source rock for oils in the Carpathian overthrust belt, display a large variability in their bulk and molecular geochemical parameters. Biomarker and stable carbon isotope analyses indicate a variable contribution from different algae (particularly dinoflagellates and diatoms) and cyanobacteria. This is reflected by specific, predominantly sulfurised biomarkers (e.g. C_{35} homohopanoids, C_{25} highly branched isoprenoids and marine *n*-alkanes) and by particular distributions of steranes and 4-methylsteranes comprising 24-nor- and 24-methyl-27-norcholestanes, and related, novel steranes with a methylation at C-23. The presence of hopanoids of methanotrophic origin ($\delta^{13}C$ up to −57‰) implies a temporarily enhanced full methane cycle in a marine environment which affected the isotopic composition of organisms dwelling in the upper photic zone. The presence of isorenieratene derivatives indicate periods of euxinic conditions within the photic zone in all investigated sub-basins. © 1998 Elsevier Science Ltd. All rights reserved

Key words—Carpathians, Oligocene, palaeoenvironment, steranes, hopanes, *n*-alkanes, stable carbon isotopes, organic sulfur compounds, highly branched isoprenoids, methane cycle, Poland

INTRODUCTION

The Carpathian basin is part of the foreland basin that was formed in front of the Alpide orogenic belt. It extends from the Czech Republic through Slovakia, Poland and the Ukraine to Romania. From the Upper Cretaceous to the Oligocene it was filled with up to 4000 m of flysch sediments. During the Oligocene large amounts of organic matter were buried in sediments of the Menilite Formation. The area investigated here is located in the SE part of Poland, where the Menilite Formation outcrops in the major overthrust units (from N to S: Skole, Silesian, Pre-Dukla and Dukla units; Fig. 1). These units correspond to former sub-basins and swells, which internally subdivided the Carpathian foredeep into several sub-basins (Unrug, 1979; Ellouz and Roca, 1994). It comprises mainly carbonate free, black and grey shales and claystones. Intercalations of turbidite sandstones and grey mudstones deposited on deep marine fans are common. Chert horizons and thin pelagic coccolith limestones are important as stratigraphic markers (Jucha, 1969; Haczewski, 1989). In the Skole unit, diatomites and diatomaceous shales and marlstones occur in the Lower Menilite Formation (Kotlarczyk and Lesniak, 1990). The total organic carbon (TOC) content of the black shales reaches *ca.* 3 to 10 wt% (average values of various sections; Köster *et al.*, 1998).

The base of the Menilite Formation is near the Eocene/Oligocene boundary, and is marked by the underlying Globigerina marlstone which is regarded to be synchronous in the Polish Carpathians (Bleicher, 1970; van Couvering *et al.*, 1981). To the top intercalations of calcareous turbidite sediments occur frequently and lead over to the Krosno Formation. This transition is diachronous and becomes increasingly older from the outer to the inner tectonic units as shown by the different positions of isochronous coccolith limestone horizons (Jucha, 1969; Haczewski, 1989; see Köster *et al.*, 1998, Fig. 2). The Menilite Formation is immature to marginally mature at outcrop over most of the area investigated (Köster *et al.*, 1998). A general increase in maturity from the outer to the inner units and lateral variations within the inner nappes are observed (Kruge *et al.*, 1996; Bessereau *et al.*, 1997; Köster *et al.*, 1998). Higher stages of matu-

---

*To whom correspondence should be addressed. Institute for Chemistry and Biology of the Marine Environment (ICBM), Organic Geochemistry Group, University of Oldenburg, P.O. Box 2503, 26111 Oldenburg, Germany. E-mail: j.koester@ogc.icbm.uni-oldenburg.de.

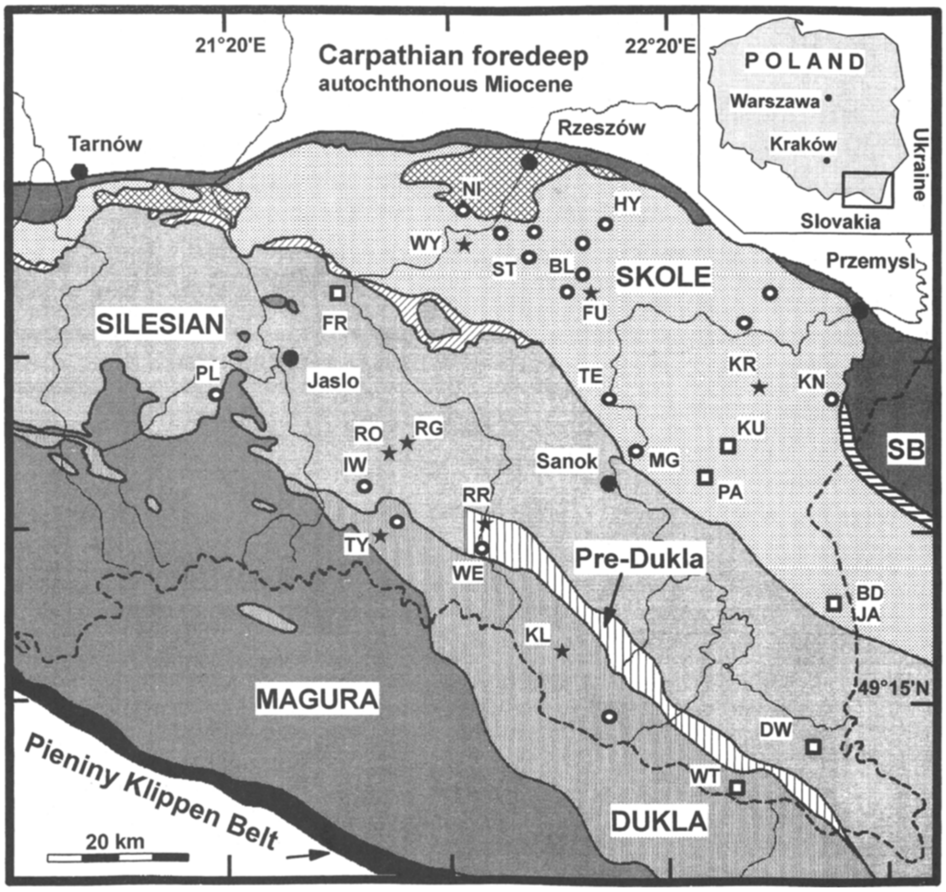

Fig. 1. Geological overview map of the eastern part of the Polish Carpathians (modified after Depowski, 1990 and Bessereau *et al.*, 1997) showing the location of outcrops (circles) and wells (squares) studied. The major tectonic units are indicated. Vertically hatched: Pre-Dukla unit; diagonally hatched: Sub-Silesian unit; cross hatched: Miocene sediments resting on the overthrust units; horizontally hatched: Borislav-Pokut unit; black: Peniny Klippen belt; SB: Stebnik and coeval units. Stars indicate locations of samples containing isorenieratene derivatives. See text for abbreviations of the outcrops and wells.

ration are reached only in the SE part of the Pre-Dukla and Dukla units.

Recently, increasing interest in the Carpathians and its hydrocarbon resources has led to a number of tectonic, geochemical and petroleum geological studies (Koltun, 1992; Roure *et al.*, 1993; ten Haven *et al.*, 1993; Lafargue *et al.*, 1994; Köster *et al.*, 1995; Roca *et al.*, 1995; Kruge *et al.*, 1996; Bessereau *et al.*, 1997). A study of crude oils and some potential source rocks has shown that most Carpathian oils are very likely derived from the Menilite Formation (ten Haven *et al.*, 1993; Bessereau *et al.*, 1997). These oils are characterised by the presence of 28,30-dinorhopane, a C_{25} highly branched isoprenoid alkane, oleanane, and other higher-plant derived triterpanes and a sometimes relatively high sulfur content (ten Haven *et al.*, 1993). It has been observed that the shales of Menilite Formation display a strong facies variability and that they are very inhomogenous in their geochemical composition (ten Haven *et al.*, 1993; Kruge *et al.*, 1996; Bessereau *et al.*, 1997; Köster *et al.*, 1998). This may have led to a complex system of petroleum generation and is probably responsible for the observed variety of crude oil types. The variability of the organic matter is indicated by the hydrogen index (HI) values which vary strongly within the sections and between the sub-basins investigated (Köster *et al.*, 1998). High HI values were found in black shale samples from the lower Menilite Formation in Skole unit ($HI > 350$ mg HC/g TOC) and in the Pre-Dukla unit throughout the whole section at Rudawka Rymanowska (RR in Fig. 1; $HI > 500$ mg HC/g TOC). Black shales with low HI values < 300 mg/g TOC occur in the upper part of the sequence in Skole unit.

Here, we present results of a detailed organic geochemical study of selected shale samples from the Menilite Formation based on biomarkers and their stable carbon isotope composition. It aims at a palaeoenvironmental reconstruction and the identification of sources of the organic matter to improve

Table 1. Location and basic geochemical data of selected samples

Section	Sample	Unit	TOC (wt%)	S_{tot} (wt%)	Organic S* (%)	Rock Eval			C_{31} homohopane ratios§		pristane/phytane	CPI (C_{25}–C_{31})	
						T_{max} (°C)	HI†	OI‡	$\beta\beta/(\beta\beta + \alpha\beta)$	$22S/(22S + 22R)$		free *n*-alkanes	S-bound *n*-alkanes
Krepak (KR)	KR93-08	Skole	5.3	2.8	18	414	242	32	0.47	0.12	1.3	3.5	0.7
Krepak (KR)	KR93-15	Skole	11.5	1.5	59	418	718	31	0.46	0	0.7	1.3	0.8
Straszydle (ST)	ST93-08	Skole	10.2	2.4	51	395	663	24	0.56	0.14	0.6	1.4	1.3
Futoma (FU)	E2-32	Skole	8.3	1.7	31	395	197	74	0.28	0.1	0.4	3.1	1.0
Wyzne (WY)	WY93-09A	Skole	14.6	5.2	32	403	581	21	0.18	0.12	0.7	4.1	1.6
Rudawka Rym. (RR)	RR90-21	Pre-Dukla	4.8	3.0	11	429	574	16	0	0.51	2.6	1.2	n.d.
Tylawa (TY)	TY91-11	Dukla	6.6	2.4	19	430	688	23	0	0.52	1.4	1.1	n.d.

*Organic sulfur as percent of total sulfur. †Hydrogen index (mg HC/g TOC). ‡Oxygen index (mg CO_2/g TOC). §Calculated from integration of *m/z* 191, not corrected for different intensities. n.d.: not determined.

our understanding of the formation and heterogeneity of these source rocks. Special attention is paid to the very immature black shales from the Skole unit. The samples discussed in this study were selected on the basis of the data obtained from an accompanying study of the source rock potential (Köster *et al.*, 1998).

EXPERIMENTAL

The analytical procedures applied are reported in detail by Kohnen *et al.* (1990b), Köster *et al.* (1997) and van Kaam-Peters and Sinninghe Damsté (1997). In brief, the extraction and fractionation comprises the following main steps: (1) Soxhlet extraction with a dichloromethane (DCM)/methanol (MeOH) mixture (7.5:1 v/v); (2) precipitation of asphaltenes in heptane; (3) fractionation of an aliquot of the maltenes (*ca.* 200 mg, after addition of four standards) by column chromatography over activated alumina with hexane/DCM (9:1 v/v) and DCM/MeOH (1:1 v/v) into an apolar and polar fraction, respectively; (4) fractionation of *ca.* 10 mg of the apolar fractions into four fractions (A1 to A4) by thin layer chromatography on Ag^+-impreganted silica plates according to the retention behaviour of the standards using hexane as developer; (5) desulfurisation of polar fractions by refluxing in ethanol under addition of Raney Nickel (after addition of 2,3-dimethyl-5-(1,1-dideutero-hexadecyl)-thiophene as internal standard), separation of released apolar hydrocarbons over a small alumina column with hexane/DCM (9:1 v/v), hydrogenation at room temperature with PtO_2 as catalyst and (6) separation of *n*-alkanes in saturated hydrocarbon fractions and desulfurised polar fractions for GC–IRMS by adduction on a molecular sieve (silicalite; West *et al.*, 1990) in a small column with dry cyclohexane as eluent, release of adducted *n*-alkanes by dissolution of the silicalite in HF, and collection in hexane (after neutralisation with a $NaCO_3$ solution). In some cases, TLC fractions were further separated to improve the resolution for GC–IRMS analyses.

The obtained fractions were analysed by gas chromatography on a Hewlett Packard 5890 instrument with on-column injector, flame ionisation detector (FID) and sulfur-selective flame photometric detector (fused silica capillary column 25 m x 0.32 mm coated with 0.12 μm CP-Sil 5, helium as carrier gas, oven programmed from 70 to 130°C at 20°/min and from 130 to 310°C at 4°C/min, final temperature held for 15 min). Analyses by gas chromatography–mass spectrometry (GC–MS) on a Hewlett Packard 5890 connected with VG Autospec Ultima Q mass spectrometer (operated at 70 eV, cycle time 1.8 s, range *m/z* 50–800, resolution 1000) were performed under the same chromatographic conditions as described above.

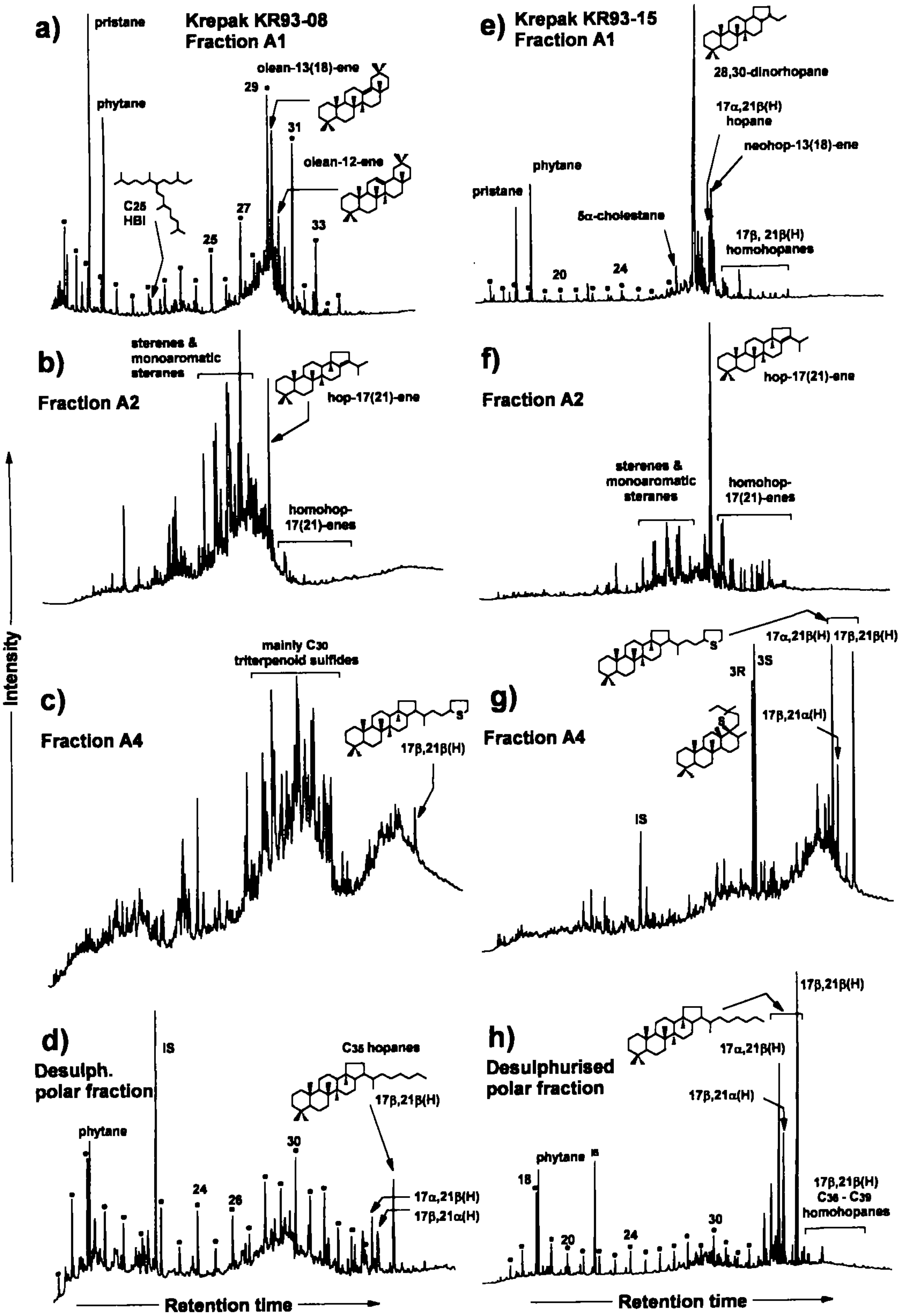

Fig. 2. Gas chromatograms (FID) of bitumen fractions of black shales from Krepak, Skole unit: (a–d) KR93-08; (e–h) KR93-15. The panels display (from top to bottom) fractions containing mainly saturated hydrocarbons [A1; (a) and (e)], alkenes, monoaromatic compounds and thiophenes [A2; (b) and (f)], sulfides [A4; (c) and (g)] and alkanes released by desulfurisation of the polar fractions (d and h). Key: IS: internal standard, filled circles: *n*-alkanes (numbers of carbon atoms for selected *n*-alkanes are given). Two major peaks in (g) belong to two stereoisomers of a C_{30} pentacyclic thiolane formed by cyclisation and sulfurisation of an all trans, regular polyprenol (Poinsot *et al.*, 1997).

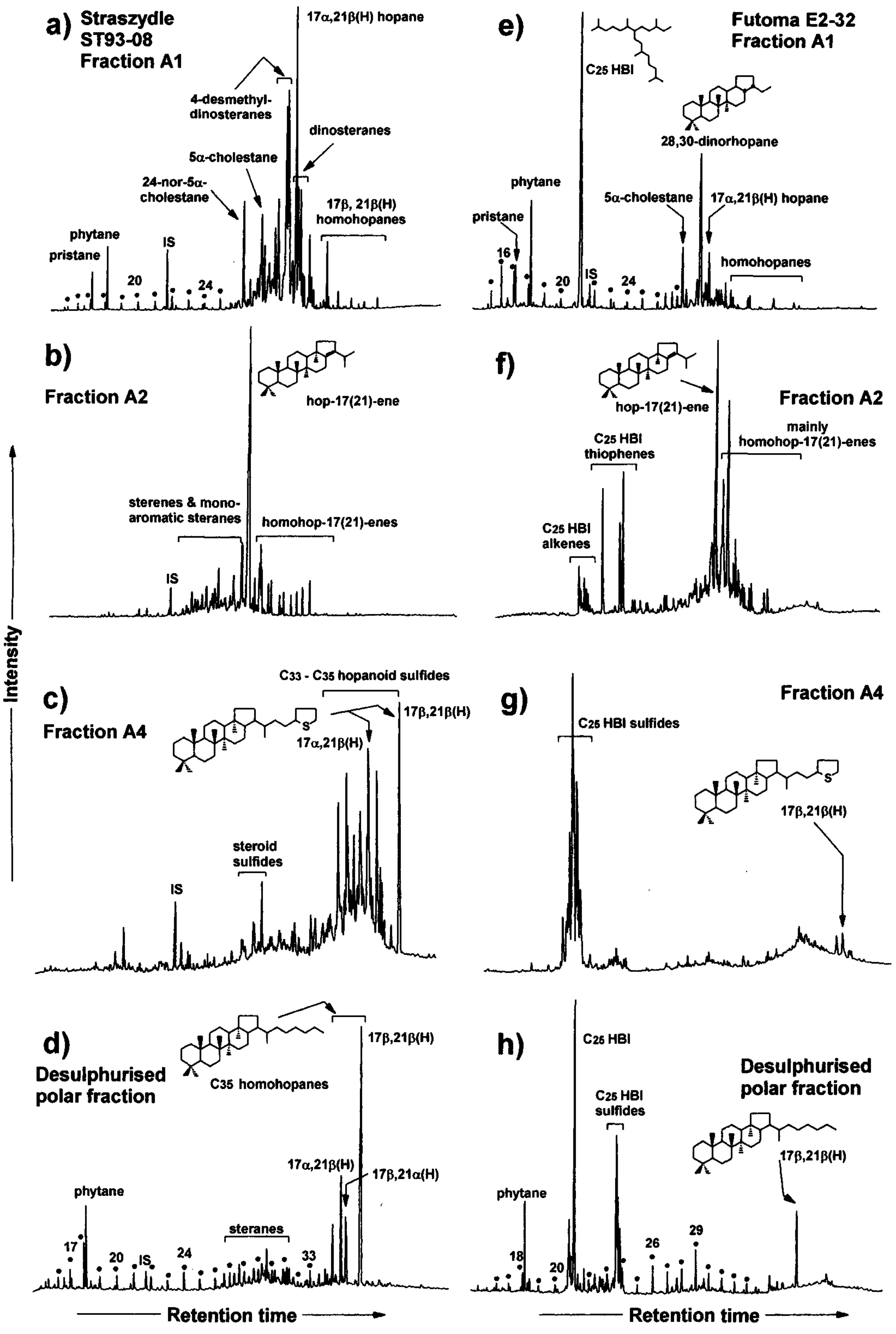

Fig. 3. Gas chromatograms (FID) of bitumen fractions of black shales from Straszydle [ST93-08, (a)–(d)] and Futoma [E3-32, (e)–(h)], Skole unit. The panels display (from top to bottom) fractions containing mainly saturated hydrocarbons [A1; (a) and (e)], alkenes, monoaromatic compounds and thiophenes [A2; (b) and (f)], sulfides [A4; (c) and (g)], and alkanes released by desulfurisation of the polar fractions (d and h). Key: IS: internal standard, filled circles: *n*-alkanes (numbers of carbon atoms for selected *n*-alkanes are given).

Compounds were quantified by comparison of their FID signal with that of an internal standard, or by integration of the corresponding signals in ion chromatograms if correction factors for the specific response in the mass spectrometer were available. For GC–MSMS analyses the gas chromatograph was equipped with a 60 m CP5 Sil-5CB-MS capillary column (ID 0.25 mm, 0.25 μm film thickness). The oven was programmed from 60 to 200°C at 15°C/min and from 200 to 310°C at 1.5°C/min (final temperature held for 10 min). Dissociation of parent ions was induced by argon, parent–daughter transitions were analysed with 20 ms settling and 80–100 ms sampling periods (total cycle time *ca.* 1 s).

For gas chromatography–isotope ratio monitoring mass spectrometry (GC–IRMS) the effluent from the gas chromatograph (chromatographic conditions as above) was directly transferred into a combustion oven. The carbon isotope composition of the CO_2 was monitored on-line by a Delta C GC–IRMS system (for detailed description see Hayes *et al.*, 1990). As standard, spikes of CO_2 with known ^{13}C content were directly let into the mass spectrometer. Data are reported in (^{13}C notation relative to the PDB standard.

RESULTS AND DISCUSSION

The samples described in this paper were selected from a large set of samples analysed for bulk geochemistry and source rock potential (Köster *et al.* 1998). Bulk data of selected samples are given in Table 1. The selected samples are rich in organic matter (TOC 4.8 to 14.6%). The total sulfur content varies between 1.5 and 5.2%, 18 to 59% of it being organic sulfur. The low maturity of samples from Skole unit is indicated by low Rock Eval T_{max} values (395 to 418°C). Subtle differences in maturity are shown by variations of the 17β,21β(H)/(17β,21β(H) + 17β,21β(H)) homohopane ratio. A higher maturity for the two samples from Pre-Dukla- and Dukla units (RR90-21 and TY91-11, Table 1) is indicated by the absence of 17β,21β(H) homohopanes, 22S/(22*S* + 22*R*) homohopane ratios near 0.5 and T_{max} values around 430°C. This paper concentrates on the comparison of immature black shales from the Skole unit with high (e.g. Krepak KR93-15, Straszydle ST93-08 and Wyzne WY93-09A; Fig. 1) and low HI values (Krepak KR93-08, Futoma E2-32), and with different characteristics in their bulk and molecular geochemistry. Figures 2–4 give an overview over the molecular composition of biomarker fractions obtained from the extract of the immature samples from Skole unit.

Free and sulfur-bound n-alkanes

Normal alkane skeletons are present both as free hydrocarbons and in macromolecularly sulfur-bound form. Their relative abundance in the free

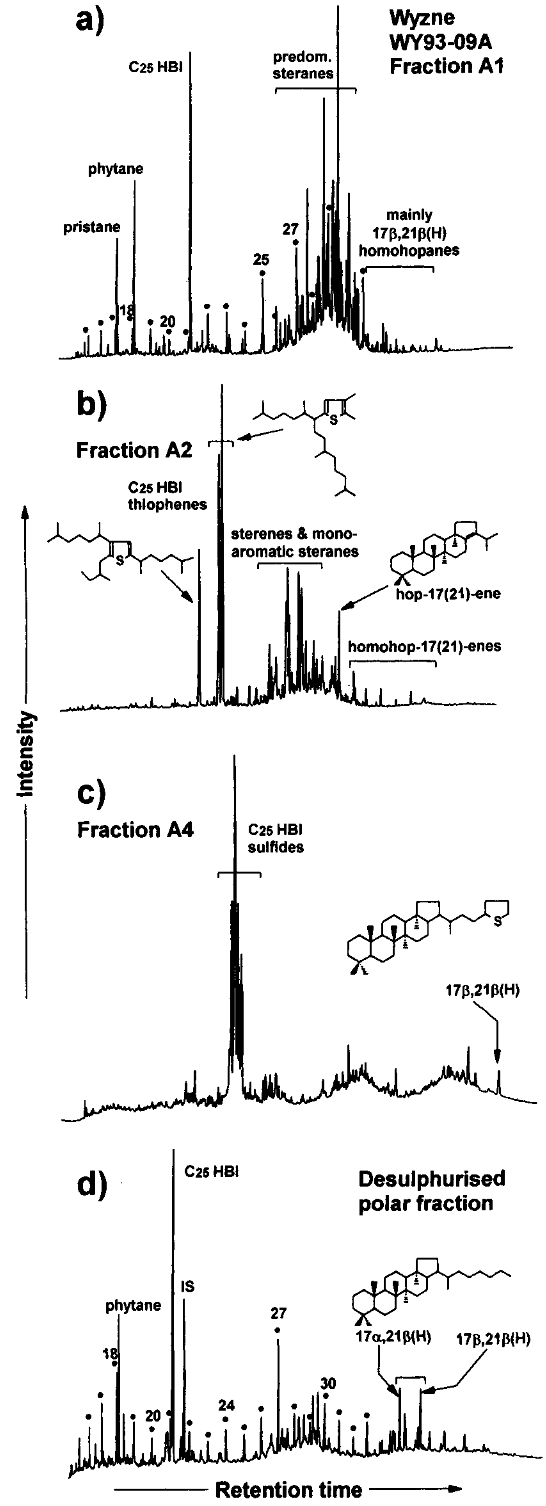

Fig. 4. Gas chromatograms (FID) of bitumen fractions of a black shales from Wyzne, Skole unit (WY93-09A). The panels display (from top to bottom) fractions containing mainly saturated hydrocarbons [A1; (a)], alkenes, monoaromatic compounds and thiophenes [A2; (b)], sulfides [A4; (c)] and alkanes released by desulfurisation of the polar fractions (d). Key: IS: internal standard, filled circles: *n*-alkanes (numbers of carbon atoms for selected *n*-alkanes are given).

saturated hydrocarbon fractions (A1) depends strongly on the maturity of the samples. In the very immature samples from the Skole unit they are present in relatively low abundance compared to the polycyclic hydrocarbon biomarkers (Figs 2–4). They are much more prominent in shale extracts from the Pre-Dukla and Dukla units as a result of the higher maturity of these samples (Fig. 5; see also ten Haven *et al.*, 1993, Kruge *et al.*, 1996 and Bessereau *et al.*, 1997).

The *n*-alkane distributions of the black shales from the Skole unit differ not only between the samples, but also between the free and sulfur-bound moieties. The samples KR93-08 [Fig. 2(a) and Fig. 6(a)], WY93-09A [Fig. 4(a)] and E2-32 show a distinctive odd-over-even carbon number predominance with carbon preference index (CPI) values >3 (after Bray and Evans, 1961), indicating a strong prevalence of vascular plant derived *n*-alkanes (Eglinton and Hamilton, 1963) with a minor contribution of *n*-alkanes of marine origin. The samples KR93-15 [Fig. 6(c)] and ST93-08 contain a much higher contribution of *n*-alkanes of a marine origin. This is revealed by the higher abun-

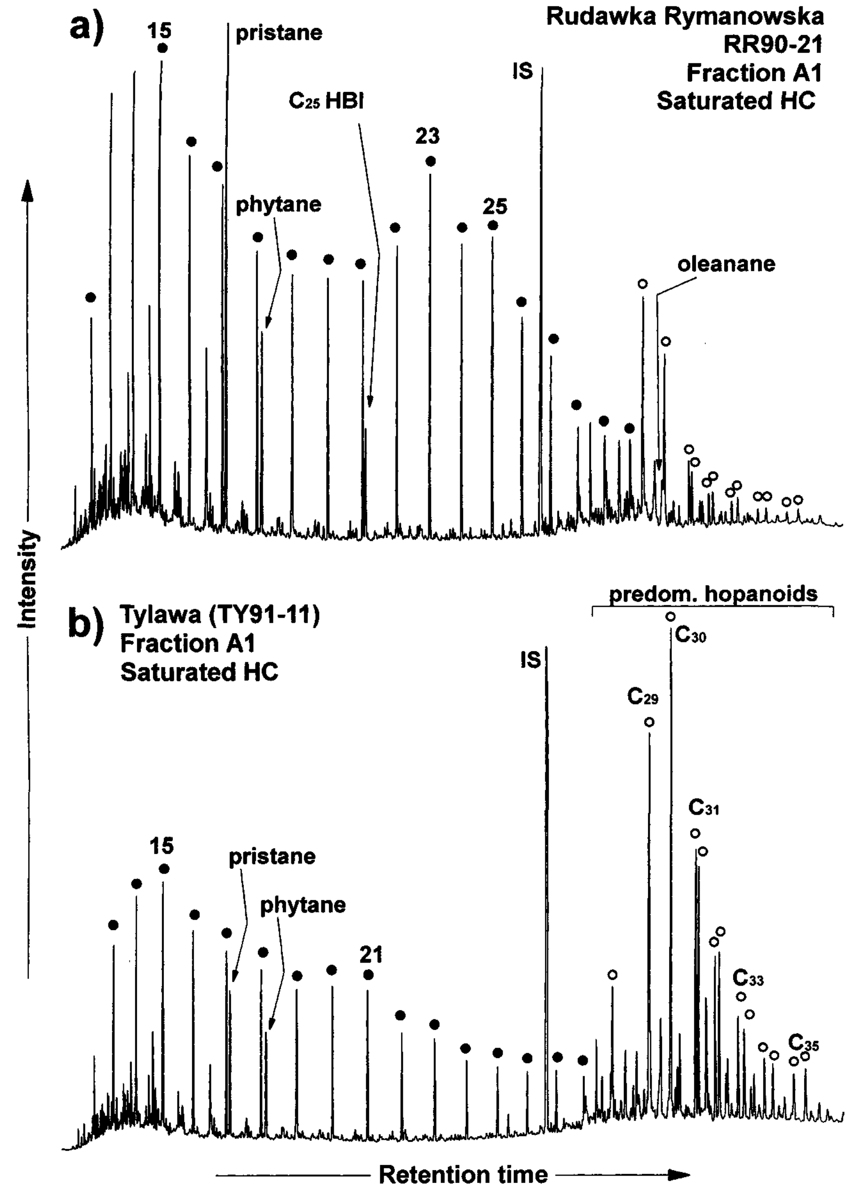

Fig. 5. Gas chromatograms (FID) of saturated hydrocarbon fractions (A1) of black shales: (a) Pre-Dukla unit, Rudawka Rymanowska (sample RR90-21: 4.8% TOC, HI 574 mg HC/g TOC); (b) Dukla unit, Tylawa (sample TY91-11: 6.6% TOC, HI 688 mg HC/g TOC); filled circles: *n*-alkanes (numbers of carbon atoms for selected *n*-alkanes are given), open circles: 17α,21β(H) hopanes.

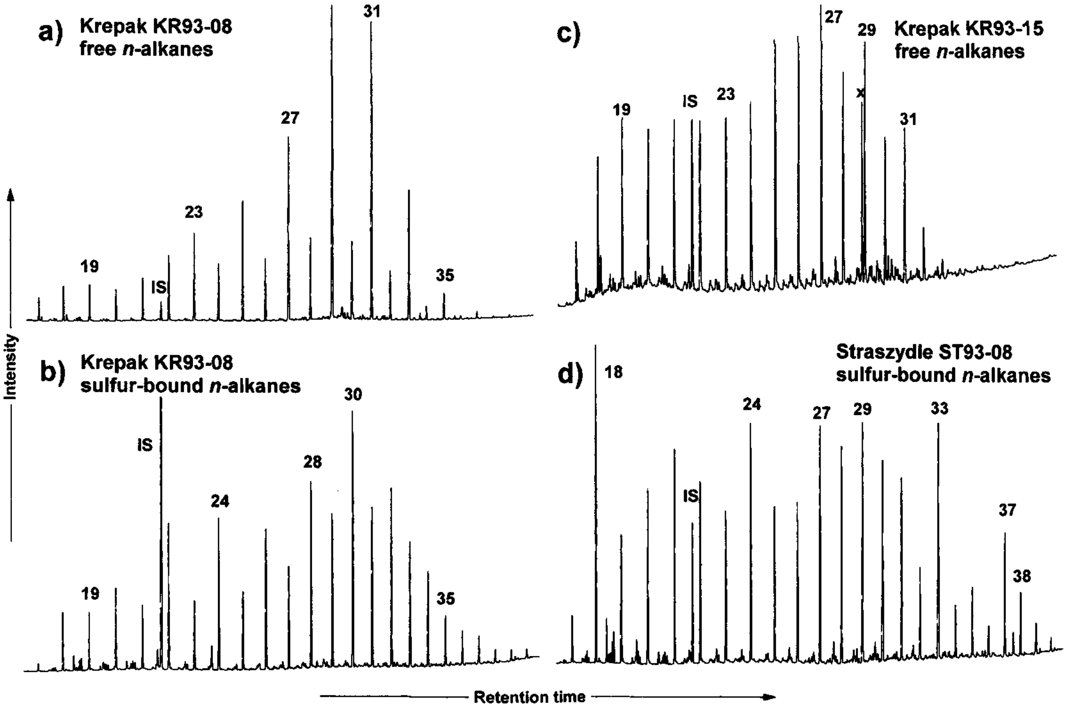

Fig. 6. Gas chromatograms (FID) of free and sulfur-bound *n*-alkanes (silicalite adducts) of black shales from Krepak (a and b: sample KR93-08; c: sample KR93-15) and Straszydle (d: sample ST93-08); major peaks are *n*-alkanes, selected homologues are marked by their carbon number; IS: internal standard, x: 28,30-dinorhopane (partially in adducted fraction).

dance of homologues with low carbon numbers and by the lower CPI values < 1.4. In contrast, *n*-alkanes released by desulfurisation of the polar fractions of the KR samples show an even-over-odd carbon number predominance [CPI values of 0.71 and 0.82; Fig. 6(b)]. This suggests a predominantly marine origin from functionalised precursor molecules, which have become sulfurised during early diagenesis. Numerous straight chain fatty acids, alcohols and alkenes have been found in microalgae (reviewed by Volkman *et al.*, this volume) many of them possessing an even-numbered carbon chain. Different contributions to free and sulfur-bound *n*-alkanes are evident in the case of the samples from Futoma (E2-32) and Wyzne (WY93-09A) as shown by the large difference in CPI values (Table 1). These samples have elevated concentrations of sulfur-bound C_{29} and C_{27} homologues, respectively [Fig. 2(h) and Fig. 3(d)]. The pattern of sulfur-bound *n*-alkanes of sample ST93-08 [Fig. 6(d)] is even more irregular, with C_{27} and C_{29} *n*-alkanes slightly dominating over the neighbouring homologues and a higher relative abundance of C_{18}, C_{21}, C_{24}, C_{33}, C_{37} and C_{38} *n*-alkanes. This indicates an input of functionalised *n*-alkane skeletons with specific chain lengths. Possible sources are *n*-alkenes which have been found in several microalgae (reviewed by Volkman *et al.*, this volume). For example, the precursors for the sulfur-bound C_{37} and C_{38} *n*-alkane skeletons are probably C_{37} and C_{38} alkenones and alkenes biosynthesised by prymnesiophyte algae (e.g. de Leeuw *et al.*, 1980; Volkman *et al.*, 1980) which can become sulfur-bound to the kerogen (Sinninghe Damsté *et al.*, 1988; Schaeffer *et al.*, 1995; Koopmans *et al.*, 1997).

The different sources for *n*-alkanes are also confirmed by their stable carbon isotope compositions. In KR93-08 [Fig. 7(a)] the ^{13}C contents of C_{22} to C_{31} *n*-alkanes shows a smooth decrease with increasing chain length from *ca.* 28 to 30.5‰. Such distributions have been found in leaf lipids of plants (Collister *et al.*, 1994) which is in agreement with the high CPI values of the *n*-alkanes in this sample. In contrast, the carbon isotopic composition of the sulfur-bound *n*-alkanes in this range show a zigzag pattern. The $\delta^{13}C$ values of odd-carbon-numbered homologues are nearly identical with those of free *n*-alkanes, whereas $\delta^{13}C$ values of the even-carbon-numbered compounds ($< C_{31}$) are less negative (between $\delta^{13}C$ −26 and −28‰). This difference increases with increasing chain length from *ca.* 1 to 4‰. The *n*-alkanes from the Monterey Formation studied by Schouten *et al.* (1998) show a similar relationship of isotope values like sample KR93-08. According to these authors it results from a mixture of sulfur-bound *n*-alkanes from marine and terrigenous sources. They propose *n*-alkanones with an odd-over-even predominance as precursors of the sulfur-bound alkanes. These alkanones can be formed by oxidation of terrestrial *n*-alkanes

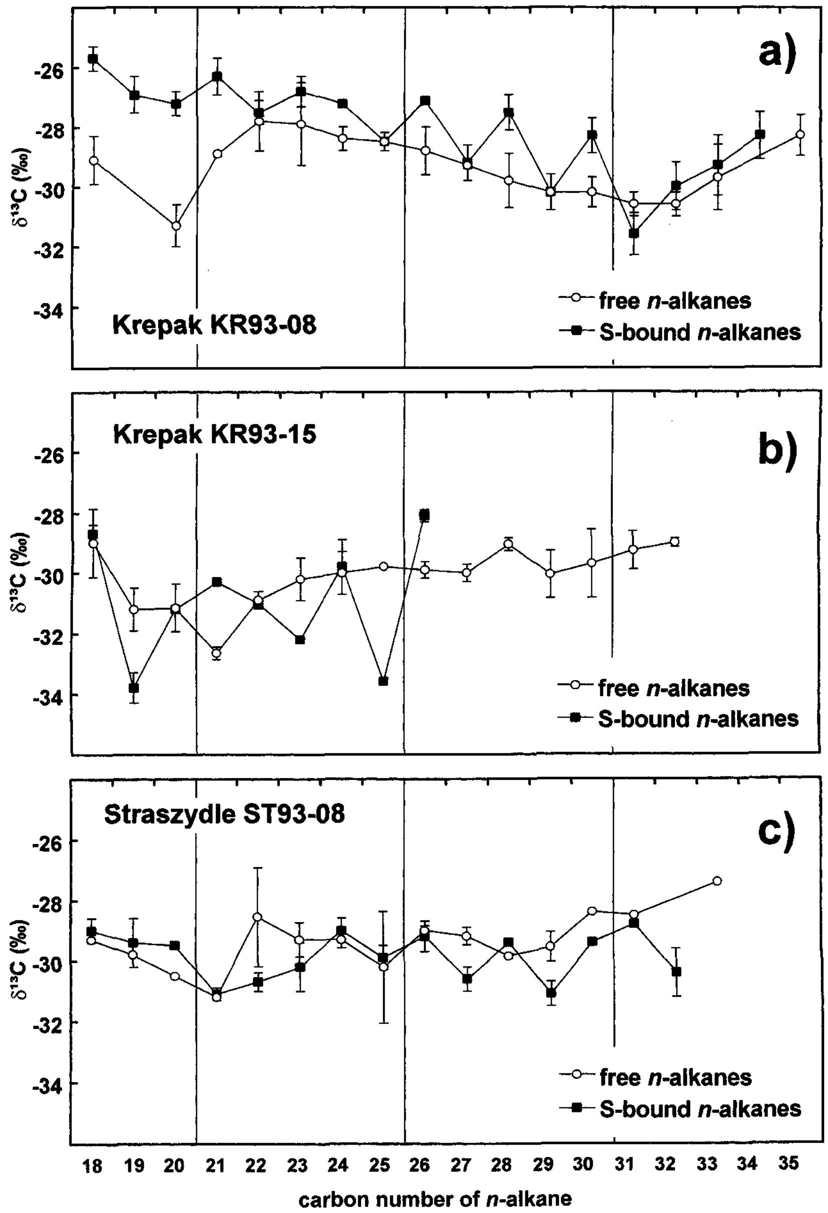

Fig. 7. Stable carbon isotope composition of free and sulfur-bound *n*-alkanes of black shales from the Skole unit: (a) Krepak KR93-08, (b) Krepak KR93-15 and (c) Straszydle ST93-08.

(Volkman *et al.*, 1983) and can be sulfurised under mild conditions by sulfurisation of the keto group (Schouten *et al.*, 1994b).

The ^{13}C content of the free *n*-alkanes in the samples KR93-15 and ST93-08 differ from that of sample KR93-08. It remains constantly close to −30‰ or even slightly increases with carbon number [Fig. 7(b) and (c)]. The carbon isotope values of sulfur-bound *n*-alkanes in ST93-08 again show a zig-zag pattern. However, in this samples the isotopically heavier, even carbon numbered homologues are close to the carbon isotope composition of the free *n*-alkanes. These data suggest that the free and the sulfur-bound even carbon numbered *n*-alkanes show hardly any terrestrial contribution, which confirms a predominantly marine source as inferred from the distributions of free *n*-alkanes. In KR93-15 the C_{19} and C_{25} *n*-alkanes are most depleted with $\delta^{13}C$ values near −34‰ [Fig. 7(b)]. On average the marine sulfur-bound *n*-alkanes of KR93-15 and ST93-08 are depleted by *ca.* 3 to 4‰ compared to those of KR93-08. This corresponds to the difference in $\delta^{13}C$ values of other marine biomarkers measured in these samples (see below; Fig. 8).

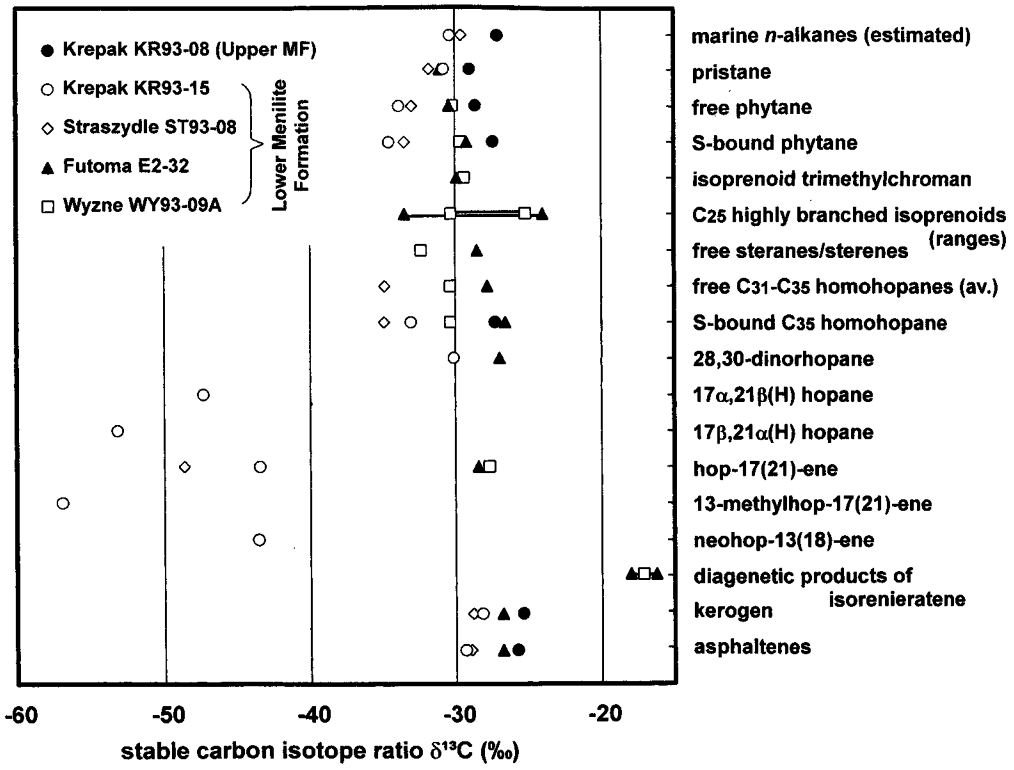

Fig. 8. Carbon isotope composition of biomarkers, kerogen and asphaltenes of black shales from Skole unit.

Acyclic isoprenoids

Pristane (Pr) and phytane (Ph) are abundant in the saturated hydrocarbon fractions of the immature samples investigated and dominate over the C_{17} and C_{18} *n*-alkanes. The Pr/Ph ratio is < 1 in the samples with high HI, but > 1 in the low HI black shale from Krepak and in most of the more mature samples from the Silesian, Pre-Dukla and Dukla units (Fig. 5; see also Kruge *et al.*, 1996). Upon desulfurisation of the polar fractions, Ph is released in relatively high amounts but Pr is almost absent. The carbon isotope composition of Pr, free Ph and sulfur-bound Ph varies between –33.9 (KR93-15) and –28.6‰ (KR93-08, Fig. 8). These compounds are more depleted in ^{13}C in samples KR93-15 and ST93-08 than in the other three immature black shales. The observed isotopic variation is largest for the sulfurised Ph skeletons, perhaps due to their origin from more specific, functionalised precursors. The δ-values of Pr and Ph are up to *ca.* 2‰ more depleted than the average δ-values of the marine *n*-alkanes. This can be explained by the carbon isotopic difference between isoprenoids and straight chain carbon skeletons (Monson and Hayes, 1982; Hayes, 1993; Schouten *et al.*, 1998).

C_{25} highly branched isoprenoids (HBIs) possessing a 2,6,10,14-tetramethyl-7-(3-methylpentyl)-pentadecane carbon skeleton are regarded as biomarkers for diatoms (e.g. Nichols *et al.*, 1988; Summons *et al.*, 1993; Volkman *et al.*, 1994). Sulfurisation is a major diagenetic pathway that preserves the carbon skeletons of the (poly-)unsaturated precursor molecules (Sinninghe Damsté *et al.*, 1989; Kohnen *et al.*, 1990a; Köster *et al.*, 1995). In two samples discussed in this paper (Futoma E2-32 and Wyzne WY93-09A) C_{25} HBI skeletons are dominant constituents of the bitumen fractions [Fig. 3(e)–(h) and Fig. 4]. They occur as saturated hydrocarbons, thiophenes, sulfides and in macromolecularly bound form. Sample RR90-21 contains the saturated C_{25} HBI (Fig. 5). Köster *et al.* (1995) have shown that C_{25} HBIs are abundant in black shale samples from Lower Menilite Formation in the Skole unit which are associated with diatomites or contain biogenic silica (present as opal-CT). In these samples concentrations of C_{25} HBIs are up to 3 mg/g TOC. About 90% of these HBI skeletons are sulfurised and occur predominantly as cyclic sulfides. Recently, a novel C_{26} HBI alkane and C_{26} HBI thiophenes have also been identified in these samples (Rospondek *et al.*, 1997).

A detailed study of free and sulfurised C_{25} HBIs in Menilite shale samples revealed a broad range of isotope values between –33.4 and –24‰ (Fig. 8). The difference within a single sample is largest between the free alkane (–33.5‰) and thiophenes (–24 to 25.5‰) of sample E2-32. Also in the other samples investigated the alkane is always most depleted in ^{13}C and the thiophenes are most enriched. This points to an obviously systematic variation of the carbon isotope values among the different HBI moieties (Köster, unpublished data). It suggests that the HBI alkane was biosynthesised as such and that the variable abundance and isotopic composition of different HBI species may be due to different unsaturated HBI precursor molecules which differed in the number and position of the double bonds. Our knowledge concerning the occurrence and isotopic composition of (poly-)unsaturated C_{25} HBIs and other pseudohomologues in different diatom species or strains (e.g. Volkman *et al.*, this volume) or during different life phases and growth conditions (e.g. Hird and Rowland, 1995; Rowland *et al.*, 1995) is still too limited to further interpret these data.

Steranes

Steranes and sterenes are present in all A1 and A2 fractions, but the relative concentrations vary considerably. They are especially abundant in sample WY93-09A. The saturated hydrocarbon fraction is dominated by a complex mixture of C_{26} to C_{30} 5α- and 5β-(20*R*)-steranes, 4α- and 4β-steranes and dinosteranes [Fig. 4(a)]. In case of sample ST93-08 [Fig. 3(a)] 4α-desmethyldinosteranes and 24-ethyl-5α-cholestane are the most abundant steranes. Remarkably, they are followed by unusually abundant 24-nor-5α-cholestane and 24-methyl-27-nor-5α-cholestane and by dinosteranes. Due to the low maturity of these samples, the dominant steranes all possess 14α,17α(H)-20*R* stereochemistry. 5β isomers are present but occur in low concentrations.

The very particular sterane composition of ST93-08 sample has been studied in detail by GC–MSMS analyses (Fig. 9 and Table 2). The assignment of the dominant C_{26} sterane to 24-nor-5α-cholestane (**1** in Fig. 9) is based on the retention behaviour relative to the other steranes (Moldowan *et al.*, 1991; Peters and Moldowan, 1993). Traces of 27-nor-5α-cholestane are also present. According to Holba *et al.* (1997) the occurrence of 24-norsteranes in oils and sedimentary rocks is age related and maximises in Oligocene or younger, diatom-derived siliceous source rocks deposited in high latitudes. Precursors for 24-norsteranes were found in sponges (e.g. Itoh *et al.*, 1983) and extant marine algae, e.g. a diatom (Morris and Carre, 1984), which points to an origin from eukaryotes. In a dinoflagellate (Goad and Withers, 1982) large amounts of 24β-27-norergostenol are accompanied by small amounts of 24-norcholestrol, suggesting a common biosynthetic pathway of these compounds (Giner, 1993).

The most abundant C_{27} sterane in sample ST93-08 is 24-methyl-27-nor-5α-cholestane (**4** in Fig. 9). Schouten *et al.* (1994a) identified this sterane in several silica-rich Miocene sediments. They discuss an origin from occelasterol or patinosterol which possess the same side chain as 24-methyl-27-nor-5α-cholestane. Dinoflagellates or diatoms are possible sources for this sterane and a biosynthetic relationship with 24-nor-5α-cholestane has been suggested. These compounds have been reported from two Miocene diatomaceous sediments, the Monterey Formation (U.S.A.) and the Onnagawa Formation (Japan; see Schouten *et al.*, 1994a). In good agreement, they occur in Menilite black shales associated with diatomites. The investigated samples also contain dinosteranes and C_{25} HBIs suggesting the presence of both dinoflagellates and diatoms. There are two additional C_{27} steranes present in sample ST93-08 which are assigned to isomers of 23-methyl-24-nor-5α-cholestane (**2** in Fig. 9). The elution order before 5α-cholestane (**3** in Fig. 9) is consistent with a shorter, branched side chain. However, this identification is tentative and has to be confirmed. Interestingly, diasteranes are almost absent despite the carbonate-free, siliciclastic lithology of this sample.

The trace for the C_{28} desmethylsteranes (transition 386 → 217 in Fig. 9) shows four compounds in addition to 24-methyl-5α-cholestane. They are tentatively identified as stereoisomers of 23,24-dimethyl-27-nor-5α-cholestane (**5** in Fig. 9). This compound contains two chiral centres in the side chain which (in analogy to the side chain of dinosteranes) may explain the gas chromatographic resolution of four 23 and 24 *S* and *R* isomers. Additionally, the relatively early elution (two of these compounds elute before the 24-methyl-5α-cholestane and shortly after the 24-methyl-27-nor-5α-cholestane) is consistent with the presence of a methyl group in the inner part of the side chain. The C_{29} steranes (Fig. 9, transition 400 → 217) comprise 24-ethyl-5α-cholestane and four isomers of 4-desmethyldinosteranes (**8** and **7** in Fig. 9, respectively). An origin from dinoflagellates living above the chemocline has been suggested by Putschew *et al.* (1995) who tentatively identified a 23,24-dimethylcholesta-3,5,22-triene in sediments of Lake Cadagno (Switzerland). Furthermore, 24-*n*-propyl-5α-cholestane (**9** in Fig. 9) is found in small amounts. This compound is considered to identify an input from marine Chrysophyte algae (Moldowan *et al.*, 1990).

The C_{27} to C_{29} steranes described above all have 4α-methyl counterparts as shown in the traces of $M^+ \rightarrow 231$ transitions (Fig. 9). Dinosteranes (four epimers of 4,23,24-trimethylcholestanes; **10**–**13** in the 414 → 231 trace) are known as specific biomarkers of dinoflagellates (Summons *et al.*, 1987), whereas other 4-methyl-24-alkylsteranes can also originate from other groups of algae (Peters and Moldowan, 1993 and references therein).

The discussed tentative identification the of novel steranes and 4-methylsteranes provides a rational,

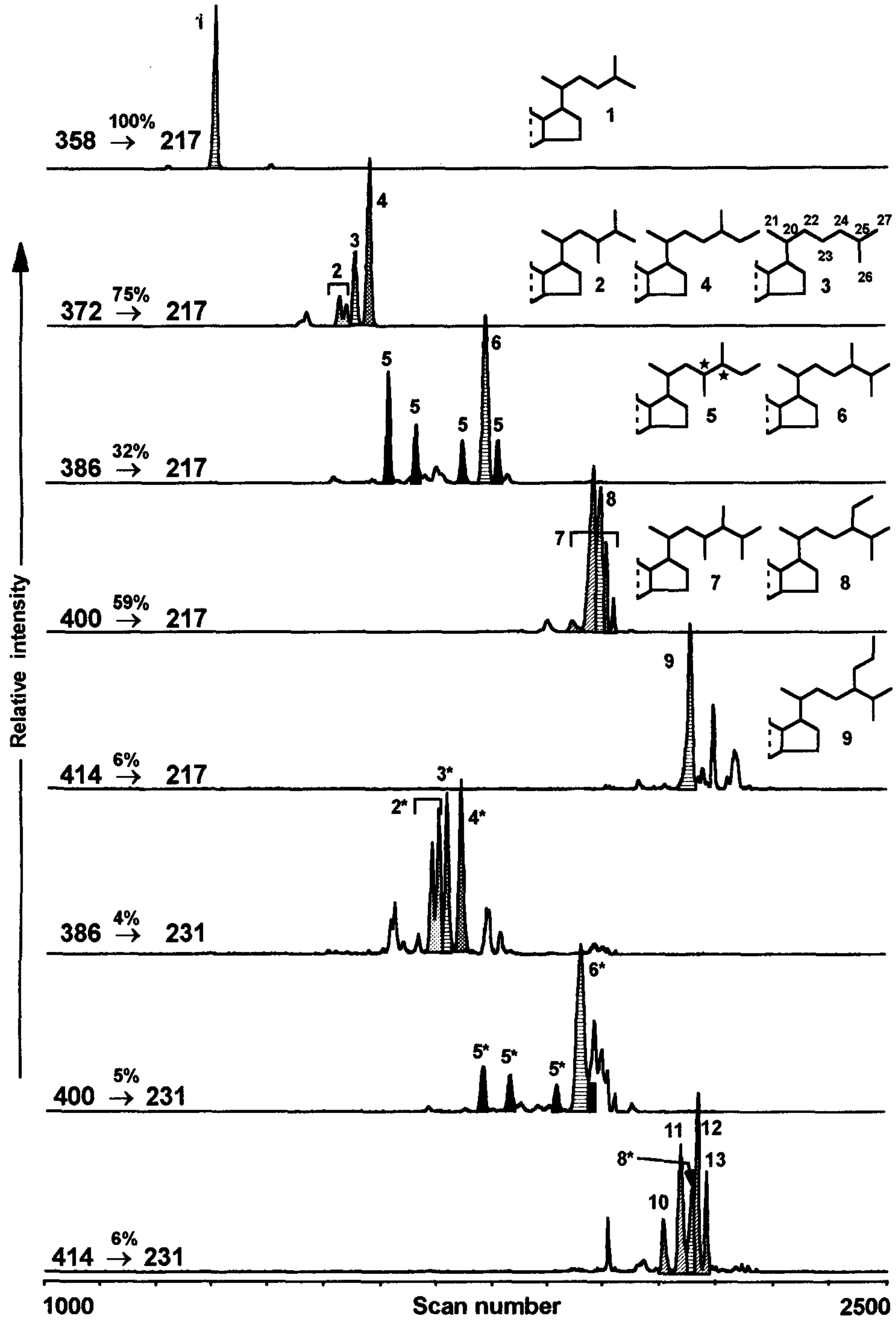

Fig. 9. GC–MSMS traces of steranes and 4-methylsteranes of an immature black shale from Skole unit (Straszydle ST93-08). Structures of side chains are indicated. See Table 2 for the designation of the compounds; *denotes 4α-methylated counterparts of the structures indicated.

systematic explanation of the observed distributions. It is also consistent with the abundancies of 24-nor- and 24-methyl-27-norsteranes. These classes of steranes are expanded by novel, possibly biosynthetically related compounds by virtue of additional methyl groups at C-23 and C-4. The geological setting of the samples suggest a common source from diatoms. An origin from dinoflagellates has to be considered as well since a methylation at C-23 is believed to be restricted to these algae (Giner, 1993). The fact, that the sample with the highest occurrence of these steranes (ST93-08) almost lacks C_{25} HBIs, but contains abundantly dinosteranes, supports this possibility.

Table 2. Identification of steranes in an immature black shale from Skole unit (Fig. 9). The 4α-methyl counterparts of the listed compounds are marked in Fig. 9 by an asterisk. All steranes possess 14α,21α(H) configuration; stereochemistry of dinosteranes after Peters and Moldowan (1993, p. 195).

Peak	Compound
1	24-nor-5α-cholestane 20*R*
2	23-methyl-24-nor-5α-cholestane 20*R**
3	5α-cholestane 20*R*
4	24-methyl-27-nor-5α-cholestane 20*R*
5	23,24-dimethyl-27-nor-5α-cholestanes 20*R**
6	24-methyl-5α-cholestane 20*R*
7	23,24-dimethyl-5α-cholestanes 20*R* (4-desmethyldinosteranes)
8	24-ethyl-5α-cholestane 20*R*
9	24-*n*-propyl-5α-cholestane 20*R*
Dinosteranes	
10	4α,23*S*,24*S*-trimethyl-5α-cholestane 20*R*
11	4α,23*S*,24*R*-trimethyl-5α-cholestane 20*R*
12	4α,23*R*,24*R*-trimethyl-5α-cholestane 20*R*
13	4α,23*R*,24*S*-trimethyl-5α-cholestane 20*R*

*Tentative identifications.

Similar sterane distributions, as described for sample ST93-08, were also found in other black shales from the Skole unit associated with diatomaceous sediments. The most immature samples contain, in addition, 4β-methylsteranes as well as higher amounts of 5β-steranes and 4-methyl-5β-steranes. More simple distributions lacking 24-methyl-27-norsteranes and related compounds were also found. The sterane compositions are thus highly variable, even between lithologically and stratigraphically related samples.

Due to the complexity of the fractions only few reliable carbon isotope values of steroids are available. 5α-cholestane (20R) in E2-32 has a $\delta^{13}C$ value of −28.5‰ (Fig. 8). In WY93-09A the $\delta^{13}C$ value of sterenes range from −33.2 to −31.9‰ (average −32.4‰).

Hopanoids

The immature black shales from the Skole unit are characterised by the only moderate to low abundance of free homohopanes compared to other biomarkers. In contrast, the C_{35} homohopane skeleton is selectively preserved in sulfurised form and often dominates the sulfide and desulfurised polar fractions, e.g. in sample KR93-15 [Fig. 2(g) and (h)] and ST93-08 [Fig. 3(c) and (d)]. Due to the large number of functionalities in the side chain, the precursor bacteriohopanepolyols (Ourisson *et al.*, 1979; Rohmer *et al.*, 1992) can react intramolecularly with inorganic sulfur species yielding a variety of low-molecular-weight organic sulfur compounds (see Sinninghe Damsté *et al.*, 1995; Köster *et al.*, 1997 and references therein). Intermolecular sulfur-bonds can link these biomarkers to macromolecular organic matter (kerogen, asphaltenes and macromolecules contained in polar fractions). In the case of the sample KR93-15 total concentration of C35 homohopane skeletons is *ca.* 200 μg/g TOC. Only *ca.* 10% of these compounds is present as saturated hydrocarbon, whereas *ca.* 39% occur as hopanoid sulfides, *ca.* 22% as thiophenes and *ca.* 29% are sequestered in the polar fraction. The hopanoid thiophenes (Valisolalao *et al.*, 1984) and thiolanes (Schmid, 1986) with the sulfur atom incorporated at the end of the side chain [Fig. 2(g)] are the by far most abundant species. They are accompanied by smaller amounts of compounds with the sulfur in the side chain attached to the C-34 position. Lower homologues of sulfurised hopanoids are in most cases absent except for ST93-08 where C_{33} and C_{34} homologues are relatively abundant [Fig. 3(c)]. In KR93-15, minor amounts of C_{36} and C_{37} homohopanes were released from polar fractions [Fig. 2(h)]. The selective preservation of homohopanes with an intact side chain shows that sulfurisation took place prior to any extensive oxidative degradation. Thus, it points to anoxic conditions (Moldowan *et al.*, 1992; Sinninghe Damsté *et al.*, 1995) and activity of sulfate reducing bacteria in the water column and/or the sediment. Free homohopanes and homohop-17(21)-enes in the black shales from Skole unit do not show a strong predominance of the C_{35} homohopane. The saturated hydrocarbon fractions (A1) of the samples from Rudawka Rymanowska (RR90-21) and Tylawa (TY92-11) contain abundant C_{29} to C_{35} hopanes predominantly with the 17α,21β(H) configuration (Fig. 5). This is explained by the higher maturity of these samples. It is likely that these free homohopanes result from initial sulfurisation of functionalised C_{35} homohopanes during early diagenesis and subsequent release by desulfurisation and side chain cleavage without yielding a prevalence of C_{35} homologues (see Köster *et al.*, 1997, for a detailed discussion).

The carbon isotope composition of sulfur-bound C_{35} homohopanes varies considerably among the samples between $\delta^{13}C$ −36.2 and −26.1‰. These values correspond to the average $\delta^{13}C$ ratios of the free homohopanes (Fig. 8). A predominantly cyanobacterial origin of the extended hopanoids is likely since the carbon isotope ratios are in the same range as those found for biomarkers originating from organisms in the upper photic zone. However, a minor contribution from other prokaryotes (e.g. chemoautotrophic of methanotrophic bacteria) is possible.

17α,21β(H) Hopane and hop-17(21)-ene are dominant compounds in the alkane and alkene fractions [e.g. Fig. 2(a), (b) and (f), Fig. 3(a), (b) and (f) and Fig. 4(b)]. In the samples KR93-15 and ST93-08 the C_{30} hopane and hop-17(21)-ene are strongly depleted in ^{13}C compared to the biomarkers of an algal or cyanobacterial source with $\delta^{13}C$ values between −53.2 and −43.4‰ (Fig. 8). In contrast, the δ-value of the hop-17(21)-ene is *ca.* −28‰ in the black shales from Wyzne (WY93-09A)

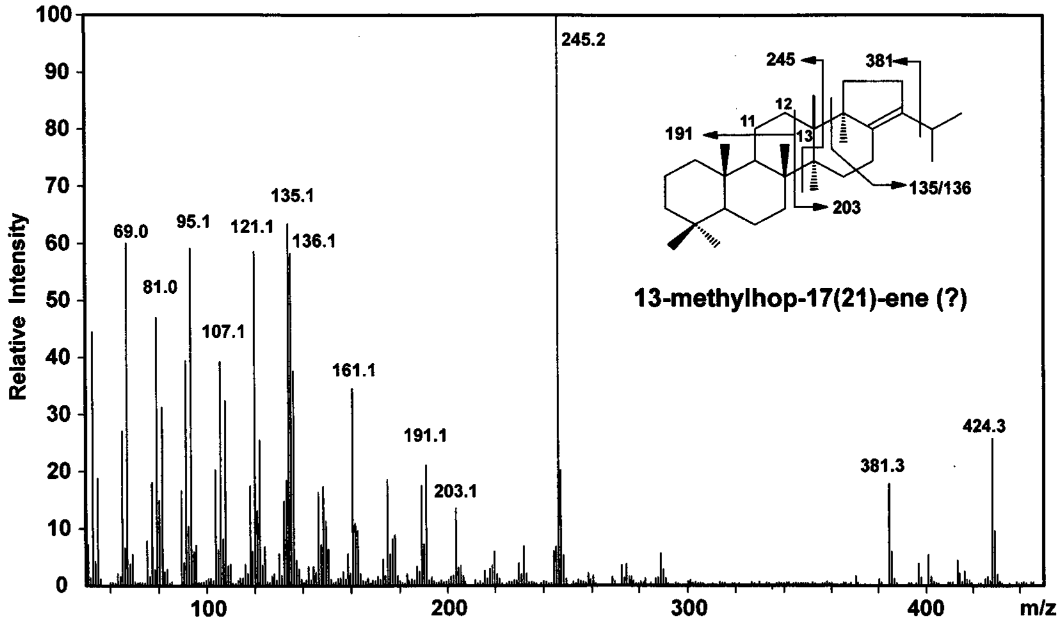

Fig. 10. Mass spectrum (background subtracted) of a compound strongly depleted in ^{13}C ($\delta^{13}C$ –56.9‰). It is tentatively identified as 13-methylhop-17(21)-ene. An origin from methanotrophic bacteria is suggested. Sample: Krepak KR93-15.

and Futoma (E2-32). This shows that this compound is probably derived from multiple sources. The abundance and isotopic composition of the C_{30} hopanes and hopenes clearly indicate that they are not related to the hopanoids with extended side chain. There are two other compounds in KR93-15 which are significantly depleted in ^{13}C (Fig. 8). Neohop-13(18)-ene has a $\delta^{13}C$ value of –43.5‰. The most negative $\delta^{13}C$ value of *ca.* –57‰ was found for a compound in the unsaturated hydrocarbon (A2) fraction which elutes just after hop-17(21)-ene, the most abundant alkene in this sample. This compound is tentatively identified as 13-methylhop 17(21)-ene, based mainly on the similarity of its mass spectrum (Fig. 10) with that of hop-17(21)-ene. The mass spectrum shows a molecular ion at *m/z* 424 and a fragment at *m/z* 381 resulting from the loss of the isopropyl group. Both are 14 Da higher than corresponding fragments of hop-17(21)-ene suggesting the presence of an additional methyl group. The AB-ring fragment at *m/z* 191 and the E-ring fragments at *m/z* 135 and 136 are not shifted. The base peak at *m/z* 245 corresponds to *m/z* 231 in the hop-17(21)-ene spectrum and results from the cleavage through the C- and D-ring. These fragmentations indicate an additional methyl group at C-11, C-12 or C-13 of the C-ring. The increased intensity of *m/z* 245 (compared to the *m/z* 231 fragment in the mass spectrum of hop-17(21)-ene) and the lower intensity of *m/z* 191 are best explained by locating the additional methyl group at C-13. In that case, only bonds between quaternary carbon atoms have to be cleaved to yield the *m/z* 245 fragment.

In sample ST93-08 the carbon isotope composition of only one of these hopanoid biomarkers could be measured due to the complexity of the bitumen fractions. However, the trace of the 45/44 mass ratio from GC–IRMS measurements indicates that the other hopanoid compounds discussed are significantly depleted in ^{13}C in this sample as well.

Possible sources for ^{13}C depleted biomarkers are chemoautotrophic bacteria or methanotrophic bacteria (e.g. Freeman *et al.*, 1990; Collister *et al.*, 1992; Summons *et al.*, 1994). Chemoautotrophic bacteria are depleted relative to their carbon source up to 27‰ (Popp *et al.*, 1989) depending on the CO_2 concentration. Assuming a ^{13}C depletion of only 20‰ (Freeman *et al.*, 1990) and considering the measured δ-value of –57‰ for the most depleted hopanoid, the CO_2 source should have a carbon isotope ratio in the order of $\delta^{13}C$ –34‰. This appears to be unlikely. Therefore, it is most plausible that the ^{13}C depleted compounds are derived from methanotrophs, especially since A-ring methylated hopanoids are rather specific for methanotrophs (Zundel and Rohmer, 1985; Summons and Jahnke, 1992; Summons *et al.*, 1994). In the marine environment, methanogenic bacteria produce methane with an extremely low ^{13}C content ($\delta^{13}C$ –110 to –60‰) predominantly via the CO_2 reduction pathway (Whiticar *et al.*, 1986). This methane serves as carbon source for methanotrophic bacteria which, therefore, also can be strongly depleted in ^{13}C. For hopanoid biomarkers derived from methanotrophs $\delta^{13}C$ values as low as −85‰ have been found (Collister *et al.*, 1992).

The ^{13}C content of the lipids of methanotrophs will strongly depend on the efficiency of methane utilisation and its isotopic composition. In case of sample KR93-15 the δ-value of *ca.* −57‰ appears to be the best estimate of the isotopic composition of biomarkers derived from methanotrophs since the particular structure of the 13-methylhop-13(21)-ene makes a mixing of this compound from multiple sources rather unlikely. It cannot be excluded that the other biomarkers depleted in ^{13}C result partly from a mixture of compounds having multiple biological sources and very different carbon isotope compositions as shown for the hop-17(21)-ene.

In saturated hydrocarbon (A1) fractions, 28,30-dinorhopane is dominant in sample KR93-15 [Fig. 2(e)] and a major compound in E2-32 [Fig. 3(e)]. Its carbon isotope composition is $\delta^{13}C$ −30.1‰ and −27.8‰, respectively. 28,30-Dinorhopane is often found in palaeoenvironments with anoxic bottom water and a major diatom input. The source of this compound is not known since a biological precursor has not yet been identified. Schoell *et al.* (1992) suggested chemoautotrophic bacterial lipids as precursors for the dinorhopane present in a Monterey oil since this compound is depleted in ^{13}C by *ca.* 6‰ compared to algal compounds. In the case of the two Menilite black shale samples analysed the carbon isotope composition of 28,30-dinorhopane is even slightly heavier than that of algal biomarkers (Fig. 8) and does not support this suggestion. Schouten *et al.* (1998) observed highly variable carbon isotopic compositions of this compound. As source organisms they proposed sediment dwelling bacteria using pore water CO_2. They suggested that ^{13}C enriched dinorhopane was biosynthesised during periods when the pore water CO_2 became enriched in ^{13}C due to intensified methanogenesis. Since the carbon isotope composition of dinorhopane could only be analysed in two samples and $\delta^{13}C$ values compared to other compounds are not exceptional, it is not possible to support or reject this interpretation. Like in other studies, dinorhopane does not occur in sulfur-bound form. This strongly supports that it was biosynthesised as saturated hydrocarbon since the presence of a functional group would make the precursor prone to sulfurisation in this type of sediments.

Terrigenous triterpenoids

The presence higher plant-derived triterpanes has been used (among others) as an argument to establish the Menilite Formation as source rock for most of the Carpathian overthrust oils (ten Haven *et al.*, 1993). In the samples studied oleanenes are abundant in KR93-08 [Fig. 2(a)]. The sulfide fraction of this sample [Fig. 2(c)] contains a poorly resolved, complex mixture of C_{30} compounds which are most likely triterpenoid sulfides. The interpretation of a terrigenous source of these compounds is in accordance with the high CPI value of free *n*-alkanes, an increased Pr/Ph ratio and a low HI indicative for the increased contribution of higher land plant-derived organic matter to this sample. Small amounts of 18α(H)-oleanane are frequently present in the samples from the Pre-Dukla and Dukla units [e.g. in RR90-21, Fig. 5(a); see also Kruge *et al.*, 1996] indicating that a minor contribution from angiosperms was almost ubiquitous.

Isorenieratene derivatives

Isorenieratene is a very specific biomarker derived from the brown coloured strain of Chlorobiaceae (Overmann *et al.*, 1992; Koopmans *et al.*, 1996 and references therein). These photoautotrophic green sulfur bacteria require both light and free hydrogen sulfide. Therefore, their habitat is at the chemocline within the lower photic zone. The specific pathway of CO_2 fixation via the reversed tricarboxylic acid cycle leads to an anomalous enrichment of their biomass in ^{13}C (e.g. Quandt *et al.*, 1977). Intramolecular reactions and sulfur incorporation yields a large number of diagenetic products of isorenieratene found in sedimentary rocks (Koopmans *et al.*, 1996; van Kaam-Peters *et al.*, 1997a). In the polyaromatic fraction of the black shale from Futoma (E2-32; Fig. 11), the main isorenieratene derivatives are isorenieratane, two triaromatic C_{40} carotenoids, C_{32} and C_{33} diarylisoprenoids (resulting from the expulsion of xylene and toluene, respectively, from isoprenoid chain of the C_{40} precursor molecule), and triaromatic C_{32} and C_{33} compounds. Their carbon isotope ratio of *ca.* $\delta^{13}C$ −17 to −19‰ is *ca.* 11 to 13‰ higher compared to compounds of an algal origin, e.g. the isoprenoid trimethylchroman (Sinninghe Damsté *et al.*, 1987), steranes or Ph (Fig. 8). This, together with the structural evidence, verifies an origin from photoautotrophic green sulfur bacteria. Isorenieratene derivatives have been found also in other samples from locations in all of the tectonic units investigated (marked by stars in Fig. 1).

Palaeoenvironmental implications

The biomarker composition of the black shales studies shows that algae were important primary producers. The contribution of different groups to the sedimentary organic matter apparently varied widely as shown by the variable abundance of dinosteranes and C_{25} HBIs identifying dinoflagellates and diatoms, respectively. The presence of different other groups of algae is indicated, for example, by the abundance of sulfur-bound *n*-alkane skeletons with specific carbon numbers. Additionally, cyanobacteria have played an import-

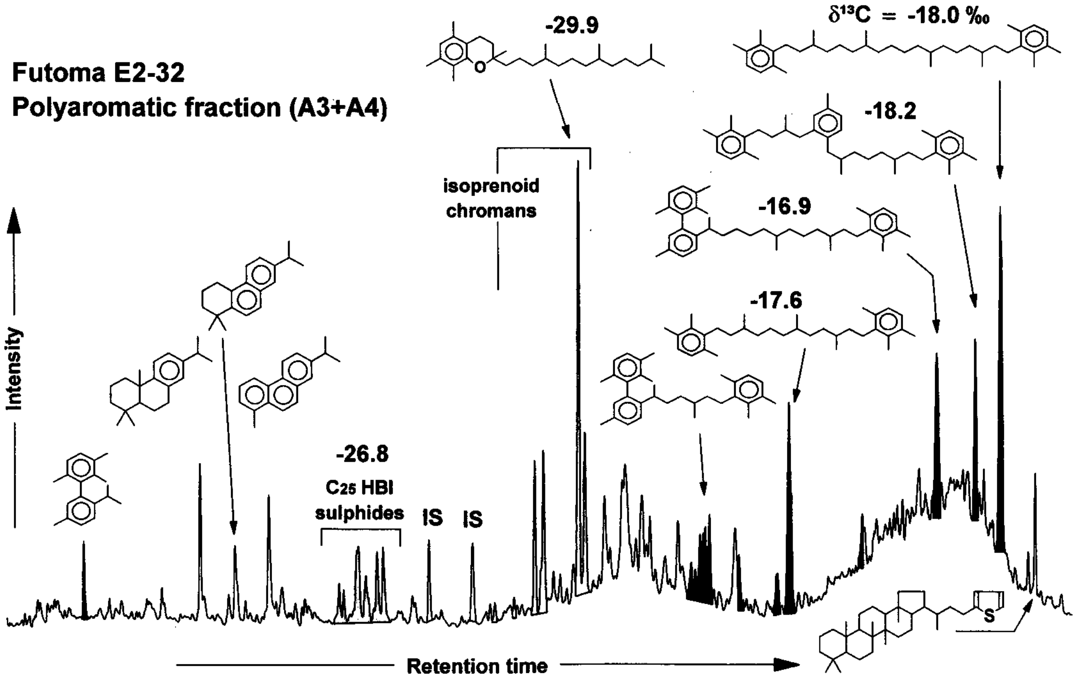

Fig. 11. Partial gas chromatogram of a polyaromatic fraction (A3 + A4) of a black shales from Skole unit (Futoma E2-32). Indicated carbon isotopic compositions of individual compounds were measured separately on subfractions.

ant role. Their significance is not apparent from saturated hydrocarbon fractions of the immature shales, since their biomarkers, C_{35} homohopanoids, are largely sequestered into the more polar fractions.

The selective preservation of C_{35} homohopane with intact side chain, C_{25} HBIs, *n*-alkanes of predominantly marine origin and Ph by inter- and intramolecular sulfurisation give evidence for an intensive bacterial sulfur cycle and presence of sulfur-reducing bacteria in an anoxic environment. Despite the fact that sedimentary rocks are carbonate-free and the clay mineral composition is dominated by smectite, a relatively high percentage (i.e. 30 to 60%) of the sulfur is present in an organic form. It is suggested that low sedimentation rates in starved slope or basin situations were favourable for this intense sulfurisation. In such sedimentary settings autochthonous, reactive organic matter can become concentrated resulting in the deposition of very TOC-rich sediments with high hydrogen index values.

Isorenieratene derivatives were found in black shales from all overthrust units. The presence of these very specific biomarkers for green sulfur bacteria indicates that euxinic conditions extended, at least temporarily, into the photic zone. It favours the model of topographically restricted sub-basins rather than upwelling conditions, and suggests that preservation played an important role in the accumulation of organic matter (Sinninghe Damsté and Köster, 1998).

The distribution and stable carbon isotope composition of individual biomarkers provides additional information on the sources of organic matter and has important implications for the palaeoenvironmental reconstruction. The δ-values found for biomarkers in the Menilite Formation range widely from *ca.* –57 to –17‰ (Fig. 8). The majority of the biomarkers studied fall in a range between *ca.* –27 and *ca.* –35‰. Most of them are only slightly more depleted in ^{13}C than the corresponding kerogens and asphaltene fractions. The carbon isotope ratio of free steranes and the isoprenoid trimethylchroman (Sinninghe Damsté *et al.*, 1987) are near –30‰ which is a good estimate for the average carbon isotope composition of algal lipids.

The hopanoid biomarkers depleted in ^{13}C ($\delta^{13}C$ *ca.* –57 to –43‰) are attributed to methanotrophic bacteria. Interestingly, in the two samples containing ^{13}C depleted hopanoids (KR93-15 and ST93-08) most other biomarkers are depleted up to 7‰ compared to the same compounds in the three other immature black shales studied (Fig. 8). The difference is largest for sulfur-bound Ph and C_{35} homohopane, but is also observed for free Ph, homohopanes, 28,30-dinorhopane and the sulfur-bound (marine) *n*-alkanes. Also the extract fractions, asphaltenes and kerogens of KR93-15 and ST93-08 are significantly depleted in ^{13}C compared to all other samples from Menilite Formation studied by Köster *et al.* (this volume) and to the Carpathian overthrust oils (ten Haven *et al.*, 1993). In the case of Ph an origin from various marine and terrigenous sources (ten Haven *et al.*, 1987) is possible. Among many other sources (see e.g. Volkman and Maxwell, 1986), Pr and Ph have also been found in pyrolysis products (Rowland, 1990) and complex lipids (Volkman and Maxwell, 1986 and references therein) of methanogens, but an algal source appears to be most common and likely. The C_{35} homohopanes and 28,30-dinorhopane however are likely derived from a more specific prokaryotic source.

To explain these differences between samples, a change in the isotope effect associated with the fixation of inorganic carbon has to be considered. This depends mainly on the concentration and the ^{13}C content of the aquatic CO_2 (Hayes, 1993). It has been suggested that a decrease of atmospheric CO_2 led to increasing $\delta^{13}C$ values of Oligocene-Miocene kerogens (Popp *et al.*, 1989). Firstly, this would drive the carbon isotope composition of the organic matter in opposite direction and, secondly, a global control on the CO_2 budget is expected to be long lasting and therefore should have affected the ^{13}C content of the organic matter in all samples simultaneously. In case of the Menilite Formation, more temporary, occasional or local effects have to be considered which influenced the carbon isotope composition.

A change in the ratio of marine to terrestrial organic matter is not a plausible explanation either. The hydrogen index is an adequate parameter to characterise the bulk organic matter composition of Menilite shale samples. The HI values show a negative correlation with the $\delta^{13}C$ values of the saturated hydrocarbon fractions suggesting to represent a mixing and/or oxidation trend (Köster *et al.*, this volume), but the range of δ-values covers 2‰ only (−28.7 to −26.7‰) . However, the saturated hydrocarbon fractions of KR93-15 and ST93-08 do not follow this trend. They are 3–5‰ more depleted in ^{13}C compared to alkane fractions of other black shales with similar high HI, which clearly demonstrates the exceptional character of these two samples.

Recycling of isotopically light CO_2 derived from oxidation of organic matter under conditions of restricted water circulation has been proposed to cause the ^{13}C depletion of the organic matter and carbonate from the Toarcian in SW Germany (Küspert, 1982; Küspert, 1983). This idea has been supported recently by results of a molecular geochemical and stable carbon isotope study (van Kaam-Peters *et al.*, 1997b). In a palaeoenvironmental model for the Toarcian Whitby Mudstone Formation, Sælen *et al.* (1995) proposed that sulfide and methane oxidising bacteria may have thrived at the chemocline, assimilated isotopically light CO_2 and contributed significantly to the sedimentary organic matter. They further suggest, that the occasional introduction of large amounts of CO_2 into the upper water column may have increased the carbon isotope fractionation and triggered phytoplankton blooms. However, this increase will indispensably counterbalanced by a decrease when CO_2 becomes depleted in course of a bloom, which drives the isotope composition towards higher values. In recent environments a significant depletion in ^{13}C by intense recycling of CO_2 (Rau, 1978) appears to be restricted to lakes, thus water bodies with a shallow water column, a limited volume and a very shallow chemocline. Examples are described from Lake Cadagno, Switzerland, (Putschew *et al.*, 1995) and Lake Gościąz, Poland; (Wachniew and Rozanski, 1997). Santos Neto *et al.* (1998) found ^{13}C-depleted hopane ($\delta^{13}C$ up to −50.3‰), C_{31} homohopanes and a methyl hopane in Cretaceous lacustrine shales and suggested a common origin from chemo- or methanotrophs. In that case, the isotopic composition of pristane, phytane, and carotanes remained unaffected. van Kaam-Peters *et al.* (1997b) pointed out that in the Black Sea, the classical example for a marine anoxic basin with a shallow chemocline within the photic zone, cycling of CO_2 does not play a major role. The $\delta^{13}C$ of CO_2 decreases by 4‰ over the upper 65 m of the water column (Freeman *et al.*, 1994). A large increase of the CO_2 concentration below 40 m is accompanied by a slight ^{13}C depletion of 0.6‰ only. The (relatively) high $\delta^{13}C$ values of phytoplanktonic lipids in the Recent sediments (Freeman *et al.*, 1994) indicate that the deposited organic matter was produced predominantly in the uppermost part of the water column where the isotopic composition of the dissolved inorganic carbon is controlled by the exchange with atmospheric CO_2.

In case of the Menilite Formation we favour the explanation of a temporary overall shift in the marine inorganic carbon towards more ^{13}C depleted conditions. The occurrence of ^{13}C depleted hopanoid biomarkers derived from methanotrophic bacteria provides circumstantial evidence that bacterial methane was oxidised and recycled into the upper photic part water column to such an extent that the dissolved inorganic carbon and, thus, the organic matter and biomarkers of primary producers became ^{13}C-depleted. Probably, the enhanced operation of this full methane cycle was supported by a shallow chemocline. The fact that, so far, this has been observed only for some Lower Menilite black shales deposited in the Skole basin points out that this was a rather occasional phenomenon.

CONCLUSIONS

1. Biomarker distributions indicate that the contribution from different groups of primary producers to the organic matter varies widely. Dinoflagellates, diatoms and cyanobacteria were apparently the most import groups.
2. Inter- and intramolecular sulfurisation led to a selective preservation of specific biomarkers (C_{25} HBIs, C_{35} homohopanes, Ph, *n*-alkanes of marine origin) and indicates intense bacterial sulfate reduction. Differences in the distribution of free and sulfur-bound *n*-alkanes reveal a variable contribution from vascular-plant and marine (algal) sources to these fractions.
3. Specific sterane compositions comprise abundant 24-nor- and 24-methyl-27-norsteranes. Novel tentatively identified steranes with a methylation at C-23 are possibly biosynthetically related compounds. Their occurrence points to a common origin either from dinoflagellates or diatoms.
4. The hopanoids depleted in ^{13}C up to $\delta^{13}C$ −57‰ are most likely derived from methanotrophic bacteria. Their presence yields circumstantial evidence for a temporarily enhanced operation of a full methane cycle. The Menilite Formation seems to represent an exceptional case of a marine environment where CO_2 from methane oxidation affected the carbon isotope composition of dissolved organic carbon in the upper water column so that the biomass of primary producers and their biomarkers became partially depleted in ^{13}C.
5. Biomarker for green sulfur bacteria indicate that periods of euxinic conditions in photic zone existed in all investigated sub-basins of the Carpathian foredeep. It is suggested that this was a result of the individualisation of the sub-basins which favoured the accumulation and concentration of organic matter by enhanced preservation and partly low sedimentation rates.
6. The study has shown that highly variable sedimentological and palaeoenvironmental conditions existed within and between the Oligocene sub-basins of the Carpathian foreland which resulted in the inhomogeneity of black shales from the Menilite Formation concerning their bulk, molecular and isotope geochemistry and their source rock potential.

Acknowledgements—The study received financial support by grants from the German Research Foundation (DFG) to J. K. and by a PIONIER Grant from the Netherlands Organisation for Scientific Research (NWO) to J. S. S. D. Grants for travelling were given by the Federal Ministry of Education, Science, Research and Technology (BMBF) and the Polish Committee of Scientific Research in frame of a Polish-German co-operation agreement. Analytical assistance was provided by M. Dekker, Dr W. Pool, M. Baas and K. Grice (NIOZ) and A. Schulz (TUC). Logistic support during field work from enterprises of the Polish Oil and Gas Industry is gratefully acknowledged. This paper benefitted from the critical and constructive comments of W. Püttmann (Frankfurt) and an unknown reviewer. This is NIOZ contribution No. 3305.

REFERENCES

Bessereau, G., Roure, F., Kotarba, M., Kusmierek, J. and Strzetelski, W. (1997) Structure and hydrocarbon habitat of the Polish Carpathians. In *Structure and Prospects of Alpine Basins and Forelands*, eds. P. A. Ziegler and F. Horvàth, Peri-Tethys Memoir 2, Memoires du Museum National d'Histoire Naturelle Paris 170. Éditions du Muséum, Paris, pp. 343–373.

Bleicher, J. (1970) "Globigeryny" podmenilitowych margli globierinowych. "Globigerinae" of the Sub-Menilite Globigerina Marls. *Biuletyn Państwowy Instytut Geologiczny* **221**, 134–204 (In Polish with English summary).

Bray, E. E. and Evans, E. D. (1961) Distribution of *n*-paraffin as a clue to recognition of source beds. *Geochimica et Cosmochimica Acta* **22**, 2–15.

Collister, J. W., Rieley, G., Stern, B., Eglinton, G. and Fry, B. (1994) Compound-specific $\delta^{13}C$ analyses of leaf lipids from plants with differing carbon dioxide metabolism. *Organic Geochemistry* **21**, 619–627.

Collister, J. W., Summons, R. E., Lichtfouse, E. and Hayes, J. M. (1992) An isotopic biogeochemical study of the Green River Oil Shale. *Organic Geochemistry* **19**, 265–276.

de Leeuw, J. W., van de Meer, F. W., Rijpstra, W. I. C. and Schenck, P. A. (1980) On the occurrence and structural identification of long chain unsaturated ketones and hydrocarbons in sediments. In *Advances in Organic Geochemistry 1979*, eds. A. G. Douglas and J. R. Maxwell. Pergamon, pp. 211–217.

Depowski, S. (1990) Bituminous shales deposits. In *Geology of Poland*, ed. R. Osika, Vol. VI: Mineral deposits. Wydawnictwa Geologiczne, Warsaw, pp. 75–77.

Eglinton, G. and Hamilton, R. J. (1963) The distribution of *n*-alkanes. In *Chemical Plant Taxonomy*, ed. T. Swain. Academic Press, London, pp. 187–217.

Ellouz, N. and Roca, E. (1994) Kinematic reconstruction of the Carpathian fold belt and adjacent areas since Cretaceous: a quantitative approach. In *Peritethyan Platforms. Proceedings of the IFP-Eurotethys International Conference*, Arles, 1993, ed. F. Roure. Technip, Paris, pp. 51–58.

Freeman, K. H., Boreham, C. J., Summons, R. E. and Hayes, J. M. (1994) Predictive isotope biochemistry: Hydrocarbons from anoxic basins. *Organic Geochemistry* **21**, 629–644.

Freeman, K. H., Hayes, J. M., Trendel, J. M. and Albrecht, P. (1990) Evidence from carbon isotope measurements for diverse origins of sedimentary lipids. *Nature* **343**, 254–256.

Giner, J.-L. (1993) Biosynthesis of marine sterol side chains. *Chemical Reviews* **93**, 1735–1752.

Goad, L. J. and Withers, N. (1982) Identification of 27-nor-(24*R*)-24-methyl-cholesta-5,22-dien-3β-ol and brassicasterol as the major sterols of the marine dinoflagellate *Gymnodinium simplex*. *Lipids* **17**, 853–858.

Haczewski, G. (1989) Coccolith limestone horizons in the Menilite-Krosno Series (Oligocene, Carpathians): identification, correlation and origin. *Annales Societatis Geologorum Poloniae* **59**, 435–523. (In Polish with extended English summary).

Hayes, J. M. (1993) Factors controlling ^{13}C contents of sedimentary organic compounds: Principles and evidence. *Marine Geology* **133,** 111–126.

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. *Organic Geochemistry* **16,** 1115–1128.

Hird, S. J. and Rowland, S. J. (1995) An investigation of the sources and seasonal variations of highly branched isoprenoid hydrocarbons in intertidal sediments of the Tamar Estuary, U.K.. *Marine Environmental Research* **40,** 423–438.

Holba, A. G., Dzou, L. I. P., Masterson, W. D., Hughes, W. B., Huizinga, B. J., Singletary, M. S., Mello, M. R. and Tegelaar, E. W. (1997) Application of 24-norcholestanes, age-sensitive molecular fossils. In *18th International Meeting on Organic Geochemistry*, 22–26 Sept., Maastricht, The Netherlands. Abstracts.

Itoh, T., Sica, D. and Djerassi, C. (1983) Minor and trace sterols in marine invertebrates. Part 35. Isolation and structure elucidation of seventy-four sterols from the sponge *Axinello cannabina. Journal of the Chemical Society, Perkin Transactions I*, 147–152.

Jucha, S. (1969) *Lupki jasielkie, ich znacznie dla stratigrafii i sedimentologii serii Menilitowo-Krosnienskiej (Karpaty fliszowe). Les Shistes de Jaslo, leur importance pour la stratigraphie et la sédimentologie de la Série Ménilitique et les Couches de Krosno (Carpathes flyscheuses).* Wydawnictwa Geologiczne, Warsaw, 128 pp. (In Polish with French summary).

Kohnen, M. E. L., Sinninghe Damsté, J. S., Kock-Van Dalen, A. C., ten Haven, H. L., Rullkötter, J. and de Leeuw, J. W. (1990a) Origin and diagenetic transformations of C_{25} and C_{30} highly branched isoprenoid sulphur compounds: Further evidence for the formation of organically bound sulphur during early diagenesis. *Geochimica et Cosmochimica Acta* **54,** 3053–3063.

Kohnen, M. E. L., Sinninghe Damsté, J. S., Rijpstra, W. I. C. and de Leeuw, J. W. (1990b) Alkylthiophenes as sensitive indicators of paleoenvironmental changes: A study of a Cretaceous oil shale from Jordan. In *Geochemistry of Sulfur in Fossil Fuels*, eds. W. L. Orr and C. D. White, ACS Symposium Series 42. American Chemical Society, Washington D.C., pp. 444–485.

Koltun, Y. V. (1992) Organic matter in Oligocene Menilite Formation rocks of the Ukrainian Carpathians: Palaeoenvironment and geochemical evolution. *Organic Geochemistry* **18,** 423–430.

Koopmans, M. P., Köster, J., van Kaam-Peters, H. M. E., Kenig, F., Schouten, S., Hartgers, W. A., de Leeuw, J. W. and Sinninghe Damsté, J. S. (1996) Dia- and catagenetic products of isorenieratene: Molecular indicators of photic zone anoxia. *Geochimica et Cosmochimica Acta* **22,** 4467–4496.

Koopmans, M. P., Schaeffer-Reis, C., de Leeuw, J. W., Lewan, M. D., Maxwell, J. R., Schaeffer, P. and Sinninghe Damsté, J. S. (1997) Sulfur and oxygen sequestration of n-C_{37} and n-C_{38} unsaturated ketones in an immature kerogen and the release of their carbon skeletons during early stages of diagenesis. *Geochimica et Cosmochimica Acta* **61,** 2397–2408.

Köster, J., Rospondek, M. J., Zubrzycki, A., Kotarba, M., de Leeuw, J. W. and Sinninghe Damsté, J. S. (1995) A molecular organic geochemical study of black shales associated with diatomites from the Oligocene Menilite Shales (Flysch Carpathians, SE Poland). In *Organic Geochemistry: Developments and Applications to Energy, Climate, Environment and Human History*, eds. J. O. Grimalt and C. Dorronsoro. A.I.G.O.A., San Sebastian, pp. 87–89.

Köster, J., Kotarba, M., Lafargue, E. and Kosakowski, P. (1988) Source rock habitat and hydrocarbon potential of Oligocene Menilite Formation (Flysch Carpathians, Southeast Poland): an organic geochemical and isotope approach. In *Advances in Organic Geochemistry 1997*, eds. B. Horsfield, M. Radke, R. G. Schaefer and H. Wilkes. *Organic Geochemistry* **29,** 543–558.

Köster, J., van Kaam-Peters, H. M. E., Koopmans, M. P., de Leeuw, J. W. and Sinninghe Damsté, J. S. (1997) Sulphurisation of homohopanoids: Effects on carbon number distribution, speciation and 22*S*/22*R* epimer ratios. *Geochimica et Cosmochimica Acta* **61,** 2431–2452.

Kotlarczyk, J. and Lesniak, T. (1990) *Dolna czesc formacji menilitowej z poziomem diatomitów z Futomy w Jednostce skolskiej Polskich Karpat. Lower part of the Menilite Formation and related Futoma Diatomite Member in the Skole unit of the Polish Carpathians.* Wydawnictwo Akademii Górniczo-Hutniczej, Krakow, 74 pp. (In Polish with English summary).

Kruge, M. A., Mastalerz, M., Solecki, A. and Stankiewicz, B. A. (1996) Organic geochemistry and petrology of oil source rocks, Carpathian Overthrust region, southeastern Poland: implications for petroleum generation. *Organic Geochemistry* **24,** 897–912.

Küspert, W. (1982) Environment changes during oil shale deposition as deduced from stable isotope ratios. In *Cyclic and Event Stratification*, eds. G. Eisele and A. Seilacher. Springer, Berlin, pp. 482–501.

Küspert, W. (1983) *Faziestypen des Posidonienschiefers (Toarcium, Süddeutschland). Eine isotopengeologische, organisch-chemische und petrographische Studie.* University of Tübingen, Germany, Tübingen.

Lafargue, E., Ellouz, N. and Roure, F. (1994) Thrust-controlled exploration plays in the outer Carpathians and their foreland (Poland, Ukraine and Romania). *First Break* **12,** 69–79.

Moldowan, J. M., Fago, F. J., Lee, C. Y., Jacobsen, S. R., Watt, D. S., Slougui, N. E., Jeganathan, A. and Young, D. C. (1990) Sedimentary 24-*n*-cholestanes, molecular fossils diagnostic of marine algae. *Science* **247,** 309–312.

Moldowan, J. M., Lee, C. Y., Watt, D. S., Jeganathan, A., Slougui, N.-E. and Gallegos, E. J. (1991) Analysis and occurrence of C_{26}-steranes in petroleum and source rocks. *Geochimica et Cosmochimica Acta* **55,** 1065–1081.

Moldowan, J. M., Sundararaman, P., Salvatori, T., Alajbeg, A., Gjukic, B., Lee, C. Y. and Demaison, G. J. (1992) Source correlation and maturity assessment of select oils and rocks from the Central Adriatic Basin (Italy and Yugoslavia). In *Biological Markers in Sediments and Petroleum*, eds. J. M. Moldowan, P. Albrecht and R. P. Philp. Prentice Hall, Englewood Cliffs, NJ, pp. 370–401.

Monson, K. D. and Hayes, J. M. (1982) Carbon isotope fractionation in the synthesis of bacterial fatty acids. Ozonolysis of unsaturated fatty acids as a means of determining the intramolecular distribution of carbon isotopes. *Geochimica et Cosmochimica Acta* 46.

Morris, R. J. and Carre, C. (1984) Observation of the biochemical composition of a an unusual diatom bloom. *Journal of the Marine Biological Association of the United Kingdom* **64,** 721–738.

Nichols, P. D., Volkman, J. K., Palmisano, A. C., Smith, G. A. and White, D. C. (1988) Occurrence of an isoprenoid C_{25} diunsaturated alkene and a high neutral lipid content in Antarctic Sea-Ice diatom communities. *Journal of Phycology* **24,** 90–96.

Ourisson, G., Albrecht, P. and Rohmer, M. (1979) The hopanoids, paleochemistry and biochemistry of a group of natural products. *Pure and Applied Chemistry* **51,** 709–729.

Overmann, J., Cypionka, H. and Pfennig, N. (1992) An extremely low-light-adapted phototrophic sulfur bacterium from the Black Sea. *Limnology and Oceanography* **31,** 150–155.

Peters, K. E. and Moldowan, J. M. (1993) *The Biomarker Guide. Interpreting Molecular Fossils in Petroleum and Ancient Sediments.* Prentice Hall, Englewood Cliffs, 363 pp.

Poinsot, J., Schneckenburger, P., Adam, P., Schaeffer, P., Trendel, J. M. and Albrecht, P. (1997) Identification of novel sulfides derived from regular polycyclic isoprenoids. In *18th International Meeting on Organic Geochemistry,* 22–26 Sept., Maastricht, The Netherlands. Abstracts, pp. 707–708.

Popp, B. N., Takigiku, R., Hayes, J. M., Louda, J. W. and Baker, E. W. (1989) The post-Paleozoic chronology and mechanism of ^{13}C depletion in primary marine organic matter. *American Journal of Science* **289,** 436–454.

Putschew, A., Scholz-Böttcher, B. M. and Rullkötter, J. (1995) Organic geochemistry of sulphur-rich surface sediments of meromictic Lake Cadagno in the Swiss Alps. In *Geochemical Transformations of Sedimentary Sulfur,* eds. M. A. Vairavamurthy and M. A. Schoonen, ACS Symposium Series 612. American Chemical Society, Washington, D.C., pp. 59–79.

Quandt, L., Gottschalk, G., Ziegler, H. and Stichler, W. (1977) Isotope discrimination by photosynthetic bacteria. *FEMS Microbiology Letters* **1,** 125–128.

Rau, G. H. (1978) Carbon-13 depletion in a subalpine lake: carbon flow implications. *Science* **201,** 901–902.

Roca, E., Bessereau, G., Jawor, E., Kotarba, M. and Roure, F. (1995) Pre-Neogene evolution of the Western Carpathians: Constraints from the Bochnia-Tatra Mountains section (Polish Western Carpathians). *Tectonics* **14,** 855–873.

Rohmer, M., Bisseret, P. and Neunlist, S. (1992) The hopanoids, prokaryotic triterpenoids and precursors of ubiquitous molecular fossils. In *Biological Markers in Sediments and Petroleum,* eds. J. M. Moldowan, P. Albrecht and R. P. Philp. Prentice Hall, Englewood Cliff, pp. 1–17.

Rospondek, M. J., Köster, J. and Sinninghe Damsté, J. S. (1997) Novel C_{26} highly branched isoprenoid thiophenes and alkane from the Menilite Formation, Outer Carpathians, SE Poland. *Organic Geochemistry* **26,** 295–304.

Roure, F., Roca, E. and Sassi, W. (1993) The Neogene evolution of the Outer Carpathian flysch units (Poland, Ukraine and Romania): kinematics of a foreland/fold-and-thrust belt system. *Sedimentary Geology* **86,** 177–201.

Rowland, S. J. (1990) Production of acyclic isoprenoid hydrocarbons by laboratory simulation of methanogenic bacteria. *Organic Geochemistry* **15,** 9–16.

Rowland, S. J., Belt, S. T., Cooke, D. A., Hird, S. J., Neeley, S. and Robert, J.-M. (1995) Structural characterisation of saturated through heptaunsaturated C_{25} highly branched isoprenoids. In *Organic Geochemistry: Developments and Applications to Energy, Climate, Environment and Human History,* eds. J. O. Grimalt and C. Dorronsoro. A.I.G.O.A., San Sebastian, pp. 580–582.

Sælen, G., Telnæs, N. and Raiswell, R. (1995) Environmental conditions during deposition of organic-rich sediments in the Whitby Mudstone Formation (Toarcian), England. In *Organic Geochemistry: Developments and Applications to Energy, Climate, Environment and Human History,* eds. J. O. Grimalt and C. Dorronsoro. A.I.G.O.A., San Sebastian, pp. 224–226.

Santos Neto, E. V., Hayes, J. M. and Takaki, T. (1998) Isotopic biochemistry of the Neocomian lacustrine and Upper Aptian marine-evaporitic sediments of the Potiguar Basin, Northeastern Brazil. *Organic Geochemistry* **28,** 361–381.

Schaeffer, P., Harrison, W. N., Keely, B. J. and Maxwell, J. R. (1995) Product distributions from chemical degradation of kerogens from a marl from a Miocene evaporitic sequence (Vena del Gesso, N. Italy). *Organic Geochemistry* **23,** 541–554.

Schmid, J.-C. (1986) *Marqueurs biologiques soufrés dans les pétroles.* Université Louis Pasteur, Strasbourg, France, 263 pp.

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 a Monterey crude oil. *Geochimica et Cosmochimica Acta* **56,** 1391–1399.

Schouten, S., Schoell, M., Sinninghe Damsté, J. S., Summons, R. E. and de Leeuw, J. W. (1998) Molecular biogeochemistry of Monterey sediments (Naples Beach, U.S.A.). II: Carbon isotopic compositions of free and sulphur-bound carbon skeletons. In *The Monterey Formation: From Rock to Molecule,* eds. C. M. Isaacs and J. Rullkötter. In press.

Schouten, S., Sinninghe Damsté, J. S., Schoell, M. and de Leeuw, J. W. (1994a) A novel sterane, 27-nor-24-methyl-5α-cholestane, in sediments. *Geochimica et Cosmochimica Acta* **58,** 3741–3745.

Schouten, S., van Driel, G. B., Sinninghe Damsté, J. S. and de Leeuw, J. W. (1994b) Natural sulphurization of ketones and aldehydes: A key reaction in the formation of organic sulphur compounds. *Geochimica et Cosmochimica Acta* **58,** 5111–5116.

Sinninghe Damsté, J. S., Kock-Van Dalen, A. C., de Leeuw, J. W., Schenck, P. A., Guoying, S. and Brassell, S. C. (1987) The identification of mono-, di- and trimethyl 2-methyl-2-(4,8,12-trimethyltridecyl)-chromans and their occurrence in the geosphere. *Geochimica et Cosmochimica Acta* **51,** 2393–2400.

Sinninghe Damsté, J. S. and Köster, J. (1998) A euxinic southern North Atlantic Ocean during the Cenomanian/Turonian oceanic anoxic event. *Earth and Planetary Science Letters* **158,** 165–173.

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,* eds. L. Mattavelli and L. Novelli, Part II, Vol. 13. Pergamon, pp. 593–606.

Sinninghe Damsté, J. S., van Duin, A. C., Hollander, D., Kohnen, M. E. L. and de Leeuw, J. W. (1995) Early diagenesis of bacteriohopanepolyols derivates: Formation of fossil homohopanoids. *Geochimica et Cosmochimica Acta* **59,** 5141–5157.

Sinninghe Damsté, J. S., van Koert, E. R., Kock-Van Dalen, A. C., de Leeuw, J. W. and Schenck, P. A. (1989) Characterisation of highly branched isoprenoid thiophenes occurring in sediments and immature crude oils. *Organic Geochemistry* **15,** 555–567.

Summons, R. E., Barrow, R. A., Capton, R. J. and Hope, J. M. (1993) The structure of a new C_{25} isoprenoid alkene biomarker from diatomaceous microbial communities. *Australian Journal of Chemistry* **46,** 407–413.

Summons, R. E. and Jahnke, L. L. (1992) Hopenes and hopanes methylated in ring-A: Correlation of the hopanoids from extant methylotrophic bacteria with their fossil analogues. In *Biological Markers in Sediments and Petroleum,* eds. J. M. Moldowan, P. Albrecht and R. P. Philp. Prentice Hall, Englewood Cliffs, pp. 182–200.

Summons, R. E., Jahnke, L. L. and Rokasandic, Z. (1994) Carbon isotopic fractionation in lipids from methanotrophic bacteria: Relevance for interpretation of the geochemical record of biomarkers. *Geochimica et Cosmochimica Acta* **58,** 2853–2863.

Summons, R. E., Volkman, J. K. and Boreham, C. J. (1987) Dinosterane and other steroidal hydrocarbons of dinoflagellate origin in sediments and petroleum. *Geochimica et Cosmochimica Acta* **51,** 3075–3082.

ten Haven, H. L., de Leeuw, J. W., Rullkötter, J. and Sinninghe Damsté, J. S. (1987) Restricted utility of pristane/phytane ratios as a paleoenvironmental indicator. *Nature* **330,** 641–643.

ten Haven, H. L., Lafargue, E. and Kotarba, M. (1993) Oil/oil and oil/source rock correlations in the Carpathian Foredeep and overthrust, south-east Poland. *Organic Geochemistry* **20,** 935–959.

Unrug, R. (1979) Palinspastic reconstruction of the Carpathian Arc before the Neogene tectogenesis. *Annales Societatis Geologorum Poloniae* **49,** 3–21.

Valisolalao, J., Perakis, N., Chappe, B. and Albrecht, P. (1984) A novel sulphur-containing C_{35} hopanoid in sediments. *Tetrahedron Letters* **25,** 1183–1186.

van Couvering, J. A., Aubry, M.-P., Berggren, W. A., Bujak, J. P., Naeser, C. W. and Wieser, T. (1981) The terminal Eocene event and the Polish connection. *Palaeogeography, Palaeoclimatology, Palaeoecology* **36,** 321–362.

van Kaam-Peters, H. M. E., Schouten, S., de Leeuw, J. W. and Sinninghe Damsté, J. S. (1997a) A molecular and carbon isotope biogeochemical study of biomarkers and kerogen pyrolysates of the Kimmeridge Clay Formation: palaeoenvironmental implications. *Organic Geochemistry* **27,** 399–422.

van Kaam-Peters, H. M. E., Schouten, S., Schoell, M. and Sinninghe Damsté, J. S. (1997b) Biomarker and compound-specific stable carbon isotope analysis of the Early Toarcian shales in SW Germany. In *18th International Meeting on Organic Geochemistry*, 22–26 Sept., Maastricht, The Netherlands. Abstracts, pp. 129–130.

van Kaam-Peters, H. M. E. and Sinninghe Damsté, J. S. (1997) Characterisation of an extremely sulphur-rich, 150 Ma old carbonaceous rock: Palaeoenvironmental implications. *Organic Geochemistry* **27,** 371–397.

Volkman, J. K., Barrett, S. M., Blackburn, S. I., Mansour, M. P., Sikes, E. L. and Gelin, F. (1998) Microalgal biomarkers: a review of recent research developments. In *Advances in Organic Geochemistry 1997*, eds. B. Horsfield, M. Radke, R. G. Schaefer and H. Wilkes. *Organic Geochemistry*, in press.

Volkman, J. K., Barrett, S. M. and Dunstan, G. A. (1994) C_{25} and C_{30} highly branched isoprenoids in laboratory cultures of two marine diatoms. *Organic Geochemistry* **21,** 407–414.

Volkman, J. K., Eglinton, G., Corner, E. D. S. and Forsberg, T. E. V. (1980) Long chain alkenes and alkenones in the marine coccolithophorid *Emiliana huxleii. Phytochemistry* **19,** 2619–2622.

Volkman, J. K., Farrington, J. W., Gagosian, R. B. and Wakeham, S. G. (1983) Lipid composition of coastal marine sediments of Peru upwelling region. In *Advances in Organic Geochemistry 1981*, eds. M. Bjorøy *et al.* Wiley, Chichester, pp. 228–240.

Volkman, J. K. and Maxwell, J. R. (1986) Acyclic isoprenoids as biological markers. In *Biological Markers in the Sedimentary Record*, ed. R. B. Johns, Methods in Geochemistry and Geophysics, Vol. 24. Elsevier, Amsterdam, pp. 1–42.

Wachniew, P. and Rozanski, K. (1997) Carbon budget of a mid-latitude, groundwater-controlled lake: Isotopic evidence for the importance of dissolved inorganic carbon recycling. *Geochimica et Cosmochimica Acta* **61,** 2453–2465.

West, N., Alexander, R. and Kagi, R. I. (1990) The use of silicalite for rapid isolation of branched and cyclic alkane fractions of petroleum. *Organic Geochemistry* **15,** 499–501.

Whiticar, M. J., Faber, E. and Schoell, M. (1986) Biogenic methane formation in marine and freshwater environments: CO_2 reduction vs. acetate fermentation. Isotope evidence. *Geochimica et Cosmochimica Acta* **50,** 693–709.

Zundel, M. and Rohmer, M. (1985) Prokaryotic triterpenoids 3. The biosynthesis of 2-methylhopanoids and 3-methylhopanoids of *Methylobacterium organophilum* and *Acetobacter pasteurianus ssp. pasteurianus. European Journal of Biochemistry* **150,** 35–39.