Variations in origin and composition of kerogen constituents as revealed by analytical pyrolysis of immature kerogens before and after desulphurization*

FRANÇOIS GELIN[1], JAAP S. SINNINGHE DAMSTÉ[2], WAYNE N. HARRISON[2],
CHRISTINE REISS[2], JAMES R. MAXWELL[2] and JAN W. DE LEEUW[1]

[1]Department of Marine Biogeochemistry and Toxicology, Netherlands Institute for Sea Research
(NIOZ), PO Box 59, 1790 AB, Den Burg, The Netherlands and [2]Organic Geochemistry Unit,
University of Bristol, School of Chemistry, Cantock's Close, Bristol BS8 1TS, U.K.

Abstract—Kerogens isolated from nine samples from a single marl bed of the Gessoso-solfifera formation
in the Vena del Gesso basin (Upper Miocene, Italy) were treated with $CrCl_2$ and $Li/EtNH_2$ to remove
inorganic and organic sulphur, respectively. The untreated and "desulphurized" ($CrCl_2$ and $Li/EtNH_2$)
kerogens were qualitatively and quantitatively analyzed by flash pyrolysis–gas chromatography–mass
spectrometry. Typically, significant variations in the relative contributions and abundances of
n-alk-1-enes/n-alkanes, alkylated pyrroles, thiophenes and supposedly S-bound moieties released as
phytenes and sterenes were observed. The first series of products (n-hydrocarbons) contributed relatively
more to the pyrolyzates of the desulphurized kerogens, suggesting an enrichment of microalgal-derived
aliphatic macromolecules. These marine micro-organisms are thought to belong in part to the class of
Eustigmatophyceae. Alkylated pyrroles were barely detectable in the untreated kerogen pyrolyzates,
whereas they contributed significantly to those from the treated kerogens. Similar treatment of a
porphyrin, octaethylporphyrin, revealed that alkyl porphyrins are thermally too stable to be cleaved upon
pyrolysis. However, $Li/EtNH_2$ treatment reduced the porphyrin standard, such that it could generate
mainly monopyrroles upon pyrolysis. It is concluded that $Li/EtNH_2$ treatment reduced tetrapyrrole
moieties bound to the desulphurized kerogen network. Copyright © 1996 Elsevier Science Ltd

Key words—kerogen desulphurization, quantitative flash pyrolysis, evaporites, algaenan, porphyrins, alkyl
pyrroles

INTRODUCTION

In marine evaporitic systems, sulphate is often
abundant and, under anaerobic conditions, the
development of sulphate reducing bacteria in the top
layers of the sediment is favoured. These bacteria are
thought to affect significantly the preservation of
organic matter (OM) by way of two opposing
processes. Bacterial reworking and mineralization of
the OM generates reduced inorganic sulphur species
(H_2S, polysulphides), that may subsequently react
with lipids to form low- and high-molecular weight
organic constituents which have a much higher
preservation potential since they are thought to be
resistant to further biodegradation. Consequently,
kerogens from marine evaporitic environments often
contain significant amounts of sulphur-bound or-
ganic moieties (for a review see Sinninghe Damsté
and de Leeuw, 1990).

To determine the importance and nature of the
organic sulphur in such kerogens, to estimate the
degree of sulphurization and to identify contributions
from source organisms, qualitative and quantitative
analyses of the products released by flash pyrolysis
of kerogens before and after desulphurization
have been performed on nine sub-samples from a
marl bed from the Vena del Gesso evaporitic basin
(Italy). This study is part of a wider investigation
which aims to understand better the processes of
kerogen formation and to reconstruct the deposi-
tional setting of this particular Miocene marl bed
(Schaeffer *et al.*, 1995; Gelin *et al.*, 1995; Kenig
et al., 1995). Studies concerning the free lipids and the
lipids released after desulphurization of the polar,
asphaltene and kerogen fractions have resulted to
some extent in the palaeoreconstruction of the
depositional environment of these sediments (Kenig
et al., 1995; Schaeffer *et al.*, 1995). The aim of the
present study is to discriminate diagenetically
incorporated kerogen constituents from the preserved
biomacromolecules and to try to estimate the
contribution of these two processes during the
depositional period in question.

EXPERIMENTAL

Sample descriptions

Cycle IV of the Messinian evaporitic sequence from the Vena del Gesso sediments (see Vai and Ricci Lucchi, 1977 and Sinninghe Damsté *et al.*, 1995 for geological background) consists of a 1.3 m thick marl layer deposited under slow sedimentation rates and overlaid by a stromatolitic bed and a thick gypsum bed. The marl layer was split into ten samples, each representing a thickness of ca. 13 cm, and which are numbered 1–10 from base to top. Together they cover the entire marl bed of cycle IV. Sample 9 was not analyzed in the present study.

Isolation and desulphurization of kerogens

Detailed procedures for the isolation and desulphurization processes have been described previously in detail (Schaeffer *et al.*, 1995). In summary, the samples were Soxhlet-extracted and the residues were sequentially treated with HCl, HCl/HF and KOH. The residues were extracted and dried. The kerogens thus obtained were treated with chromous chloride ($CrCl_2$) to remove inorganic sulphur (i.e. pyrite) following the method of Canfield *et al.* (1986), as outlined previously. The resulting residues were then treated with lithium/ethylamine ($Li/EtNH_2$) according to the method of Hofmann *et al.* (1992).

Flash pyrolyses

The nine samples were analyzed by Curie-point pyrolysis–gas chromatography (Py–GC) and Curie-point pyrolysis–gas chromatography–mass spectrometry (Py–GC–MS). Aliquots were pressed on to a flattened ferromagnetic wire and heated by inductive heating (Curie temperature 610°C) for 10 s using a Curie point high frequency generator (Fischer 9425). The gas chromatograph (Hewlett Packard HP-5890) was equipped with a cryogenic unit and programmed from 0°C (5 min) to 320°C (10 min) at a rate of 3°C/min. Separation was achieved using a 25 m fused silica capillary column coated with CP-Sil 5 (0.32 mm I.D.; film thickness 0.45 μm). Helium was used as the carrier gas and the temperature of the flame ionization detector (FID) was 320°C. Py–GC–MS was performed using the same conditions as described above. The GC column was coupled to the electron impact ion source of a VG Autospec Ultima mass spectrometer (mass range m/z 40–800 at a resolution of 1000; cycle time 1.8 s; ionisation energy 70 eV). To a weighed aliquot (ca. 20 mg) of kerogen, 20.6 μg of an internal standard (2,3-dimethyl-5-(1′,1′-d_2-hexadecyl)thiophene) was added to allow quantification of the pyrolysis products. The determination of the concentrations and the validity of this quantification method have been discussed previously (Gelin *et al.*, 1995). The authors indicated that the relative standard deviation of 10 measurements of 16 peaks in the FID trace varied from 17 to 31%. Such

a deviation has been taken into account in the discussion of the results presented here.

RESULTS AND DISCUSSION

The flash pyrolyzates from the untreated and desulphurized kerogens contain complex mixtures of products, including homologous series of components (e.g. sample 7 in Fig. 1). They are dominated by C_1-C_2 alkylated benzenes and thiophenes (Fig. 1a); n-alk-1-enes and n-alkanes are also abundant and range over the entire TIC chromatogram. Isoprenoid hydrocarbons, i.e. prist-1-ene, phytenes and thiophenes with a phytanyl carbon skeleton, are major products, though in different relative abundances depending on the sample. Polycyclic compounds, mainly steranes, sterenes and hopenes, are the most abundant high-molecular weight products and can be the dominant components, as observed for samples 1 and 2.

As determined for sample 2, the pyrolyzate of the residue after $CrCl_2$ treatment was very similar to the pyrolyzate of the untreated kerogen. Although $CrCl_2$ treatment released up to 60 mg/g_{TOC} (45 mg/g_{TOC} for sample 2) of organic products (Schaeffer *et al.*, 1995), this does not appear to have affected the relative composition of the pyrolyzate. Significantly, however, relative contributions of the main series of pyrolysis products changed dramatically after $Li/EtNH_2$ treatment. Whereas aromatic, isoprenoid and steroid products dominate the pyrolyzate of the untreated kerogens, the series of n-alk-1-enes and n-alkanes become by far the most dominant products in the desulphurized kerogen pyrolyzate, as shown for sample 7 (Fig. 1b). Hence, if we assume that the GC-amenable pyrolysis products are representative of the whole kerogen (Horsfield, 1989; Larter and Horsfield, 1993), it appears that it becomes more aliphatic in nature after $Li/EtNH_2$ treatment and, as suggested by the flash pyrolysis, its structure is dominantly composed of polymethylenic units linked by C–C or C–O bonds (Lattimer, 1994). Also, alkylated pyrroles are observed in high abundance in most of the desulphurized kerogen pyrolyzates, while they are minor in, or absent from, the products from the untreated kerogens. The unresolved complex mixture underlying the n-alk-1-ene/n-alkane envelope in the pyrolyzate of the treated kerogen also occurs for the untreated kerogen, although it is less apparent since the main pyrolysis products are not the n-alk-1-ene/n-alkane but toluene and methyl-thiophene.

Variations in abundance of organic sulphur components

The organic sulphur content can be estimated by Py–GC by using the ratio [1,2-dimethylthiophene]/ ([1,2-dimethylbenzene] + [n-non-1-ene]), termed the thiophene ratio (*TR*) (Eglinton *et al.*, 1990). The authors showed that this ratio correlates with atomic

S_{org}/C ratios for most of the bitumens, asphaltenes and kerogens investigated. Hence, TR values were used here to obtain information on both the efficiency of the $Li/EtNH_2$ treatment, i.e. the relative amount of sulphur-bound constituents left in the treated kerogens, and the degree of sulphurization. Elemental analyses of a representative sample of the marl layer of cycle IV allowed calculation of the atomic

S_{org}/C ratio of this kerogen (Koopmans *et al.*, 1995). The value obtained (0.08) corresponds to a Type II-S kerogen (Orr, 1986). The TR calculated from the pyrolyzate of the "untreated" VdG kerogens ranges from 0.19 to 0.40, with no particular depth trend (Fig. 2). These values are consistent with those of most Type II immature marine kerogens (Eglinton *et al.*, 1990). The discrepancy noted between the
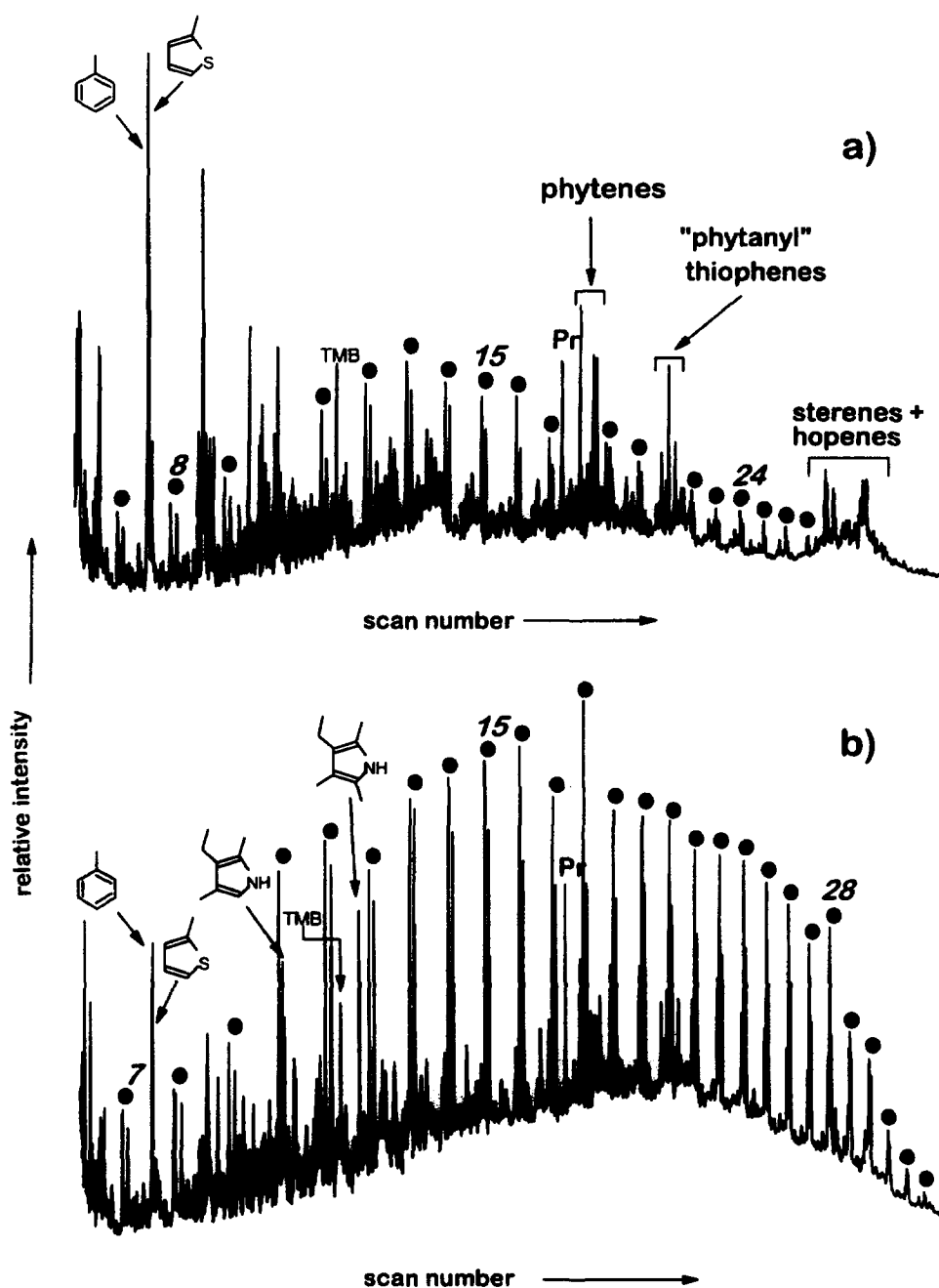

Fig. 1. Total ion current chromatograms of the pyrolyzates of (a) untreated VdG-IV-7 kerogen and (b) desulphurized VdG-IV-7 kerogen. Italic numbers indicate the carbon chain length of the *n*-alk-1-ene/*n*-alkane doublets, indicated by filled circles. TMB and Pr represent 1,2,3,4-tetramethylbenzene and prist-1-ene, respectively.
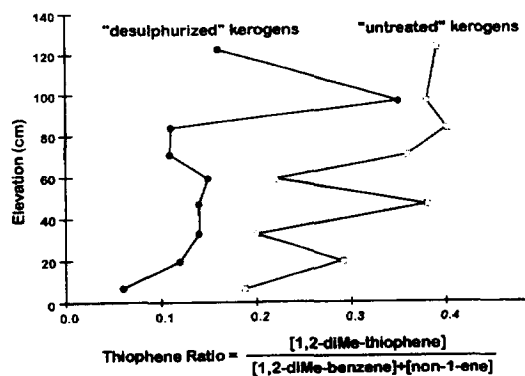
Fig. 2. Depth profiles of the thiophene ratio calculated from the pyrolysates of the untreated (□) and desulphurized (●) kerogens.

S_{org}/C and *TR* ratios may be explained by the presence of an unusually high contribution of intermolecular rather than intramolecular S-bonds. Cleavage of the former linkages leads to the formation of *n*-alk-1-enes/*n*-alkanes and H_2S whereas the latter are presumed to generate mainly alkylthiophenes upon pyrolysis.

After desulphurization, with the exception of VdG-8, the *TR* values are around 0.1 which corresponds to Type I kerogens (Eglinton et al., 1990). Therefore, according to these *TR* values, the $Li/EtNH_2$ treatment released a significant part of the S-bound lipids, i.e. those intermolecularly incorporated. Indeed, yields of the extracts obtained after $Li/EtNH_2$ treatment revealed that up to 30 wt% of the kerogen could be released, though some of the released material may have resulted from ester bond cleavage (Schaeffer et al., 1995).

Relative distributions of pyrolysis products

Although the relative abundances of the different series of components in the products vary significantly between the untreated and treated kerogens, the internal distributions within the series are similar (Fig. 3). For example, the *n*-alkan-2-one distribution, revealed by the *m/z* 58 chromatogram, of the pyrolyzate of the treated kerogen is virtually identical to that of the untreated kerogen, ranging from C_6 to C_{26} with a maximum at C_{16}. Likewise, the distributions of benzenes are similar and consist mainly of benzene itself and C_1-C_4 alkylated benzenes, although the relative abundance of 1,2,3,4-tetramethylbenzene (TMB) is slightly higher in the pyrolyzate from the treated samples. TMB is thought to be a pyrolysis product of macromolecularly-bound isorenieratene derivatives (Hartgers et al., 1991, 1994; Douglas et al., 1991; Requejo et al., 1992). Isorenieratene contains several sites for sulphur or oxygen linking and a fraction of this compound is probably more strongly linked to the kerogen network than the other aromatic moieties. It should be noted that recent investigations demonstrated that TMB may also be

derived from stable, unidentified macromolecular material from marine algae (Hoefs et al., 1995). Similarities in the distributions are also observed for the alkylthiophenes, the only significant difference being the presence of abundant thiophenes with a phytanyl carbon skeleton in the pyrolyzate of the untreated kerogen. These thiophenes are thought to be generated from S-bound phytanyl moieties (Koopmans et al., 1995) which were probably released by the chemical treatment. The *n*-alk-1-enes and *n*-alkanes, as revealed by the *m/z* 55 + 57 chromatogram, range from C_6 to C_{33} and maximize at C_{13}/C_{14} in both pyrolyzates. However, as observed for most of the samples, the *n*-alk-1-enes and *n*-alkanes with chain lengths greater than 14 carbons are relatively more abundant in the pyrolyzate from the desulphurized kerogen. This phenomenon may be partly explained by the fact that the S-incorporated lipids possess *n*-alkyl units with fewer than 15 carbons.

Quantitative variations in pyrolysis products

Figure 4 shows the depth concentration profiles of the main compounds or series of compounds released and detected by Py–GC analyses. The untreated kerogens of samples 3 and 5 released by far the highest amounts of *n*-alkanes, *n*-alk-1-enes (from 70 to 80 mg/g_{TOC}; Gelin et al., 1995), but these decreased dramatically (75%) in the pyrolyzates of the treated samples. However, the amounts of solvent-extractable material released after $Li/EtNH_2$ treatment (and column chromatography) were not particularly high for these two samples (Schaeffer et al., 1995). We assume, therefore, that desulphurization of samples 3 and 5 released abundant extractable aliphatic macromolecules which were originally attached to the kerogen via S-bonds. Soluble macromolecules have been shown to occur as polyaldehydes in the green microalga *Botryococcus braunii* A race and account for ca. 5% of the dry algal biomass (Metzger et al., 1993). Conversely, the amount of *n*-alk-1-enes/*n*-alkanes in the pyrolyzates of samples 4 and 10 showed, if anything, an increase after desulphurization. This can be explained by the 30% error estimated for quantitative flash pyrolysis analyses. The yields of phytenes, sterenes, thiophenes with a phytanyl skeleton and thiophenes are substantially lower in the pyrolyzates of the treated samples. This is probably a result of cleavage by $Li/EtNH_2$ of sulphur and possibly ester linkages which bound the species containing these moieties to the matrix. The phytane concentrations in the apolar fraction of the extracts released after $Li/EtNH_2$ treatment ranged from 0.1 to 1.1 mg/g_{TOC} (Schaeffer et al., 1995), whereas the differences in phytenes concentrations between the pyrolyzates from the untreated and treated kerogens ranged from 14 to 1 mg/g_{TOC}. This suggests that a significant proportion of the phytenes in the pyrolyzates of the untreated

kerogens derives from the cleavage of O-bound phytanyl moieties. Therefore, these phytanyl moieties were probably present in the polar fraction of the extract after Li/EtNH$_2$ treatment. Similar observations and explanations can be made for the steranes (and sterenes). In the pyrolyzates of the treated
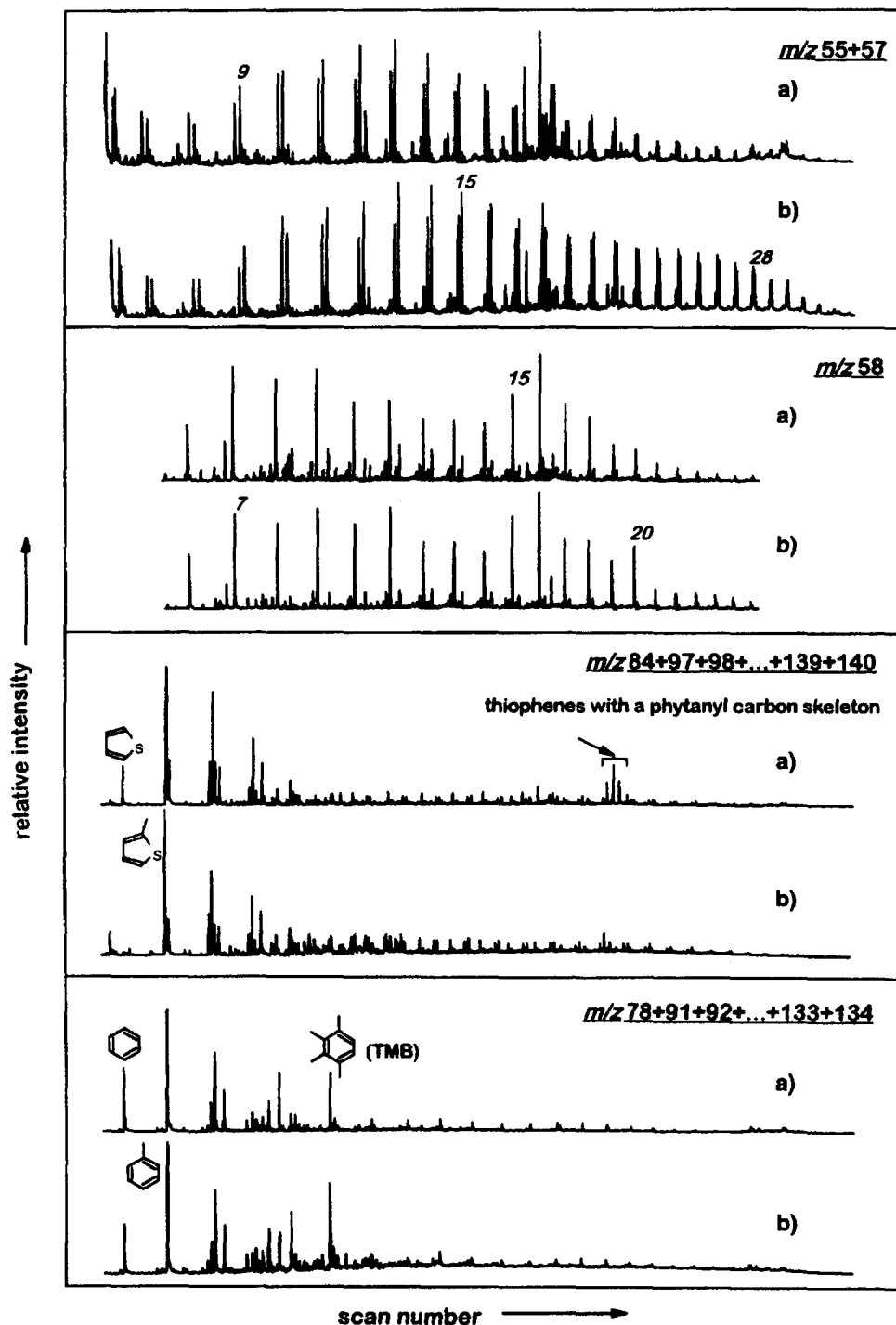

Fig. 3. Mass chromatograms of the pyrolyzates of (a) untreated VdG-IV-7 kerogen and (b) desulphurized VdG-IV-7 kerogen. Mass chromatograms of m/z 55 + 57, 58, 84 + 97 + 98 + 111 + 112 + 125 + 126 + 139 + 140 and 78 + 91 + 92 + 105 + 106 + 119 + 120 + 133 + 134 reveal the distributions of n-alk-1-ene/n-alkanes, n-alkan-2-ones, alkylthiophenes and alkylbenzenes, respectively, italic numbers indicate carbon chain lengths.
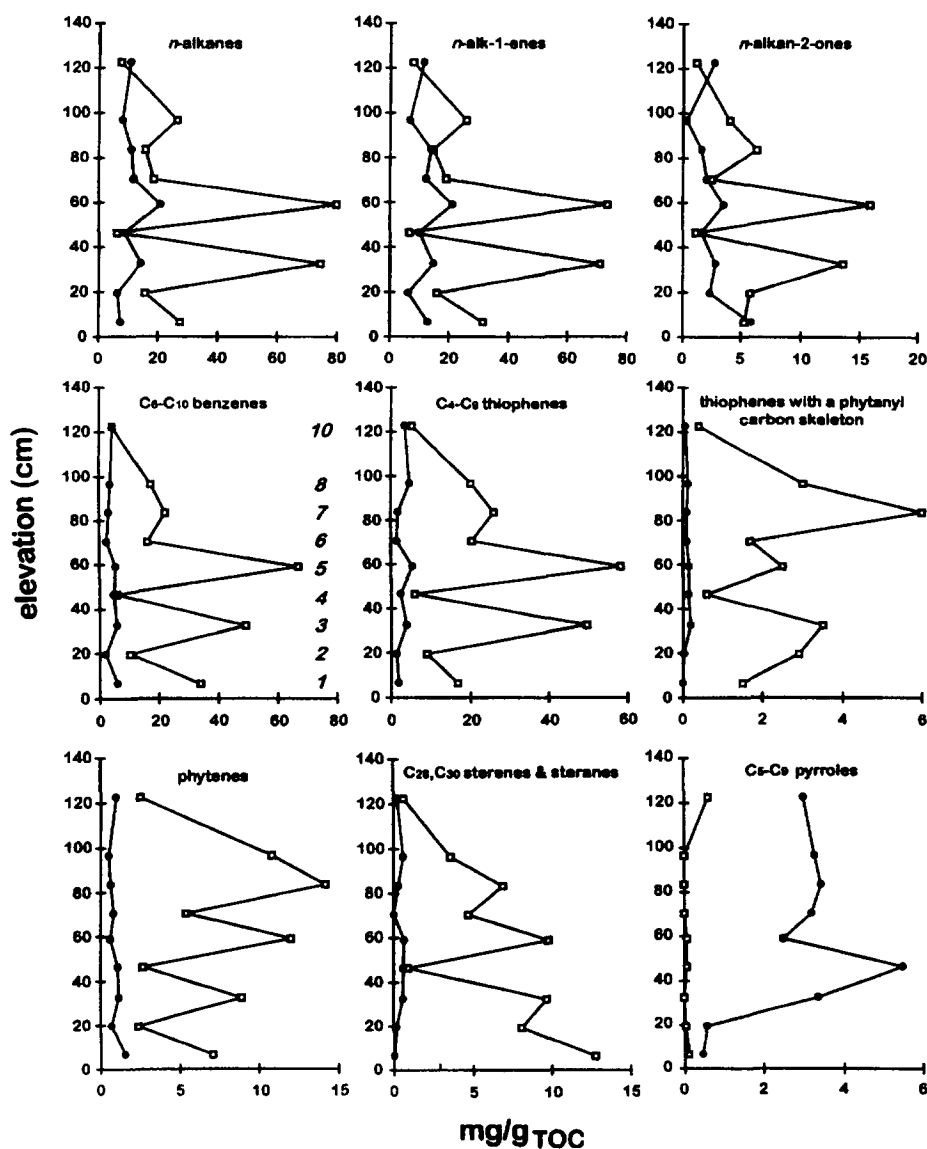
Fig. 4. Depth profiles of the concentration of the main products in the flash pyrolyzates of the untreated (□) and desulphurized (●) kerogens; italic numbers indicate samples VdG-IV-1 to VdG-IV-10.

kerogens of samples 1, 6 and 10, the amounts of these products were barely detectable.

Algaenan contribution

Recently, it has been shown that marine micro-algae, i.e. eustigmatophytes and some chlorophytes, biosynthesize highly aliphatic macromolecules, termed algaenans, which are probably located in the outer cell walls (Derenne *et al.*, 1992; Gelin *et al.*, 1996). Upon pyrolysis, these biomacromolecules release essentially *n*-alk-1-enes and *n*-alkanes. The predominance of *n*-alk-1-enes and *n*-alkanes in the pyrolysis products of the treated kerogens indicates that the Li/EtNH₂ treatment leads to a selective enrichment in such macromolecules. Thus, the polymethylenic moieties are less affected by the

LiEtNH₂ treatment than are the other macromolecularly-bound moieties (except for samples 3 and 5), i.e. aromatic, thiophenic, steroid and isoprenoid species. Circumstantial evidence for a contribution of these aliphatic macromolecules comes from the presence in the polar and asphaltene fractions of these sediments of C_{30}–C_{32} *n*-alkyl units which were S-bound, mainly at the C-15 position (Kenig *et al.*, 1995; Koopmans *et al.*, 1995). Although these C_{30}–C_{32} *n*-alkyl units do not clearly appear in the pyrolyzates of the untreated kerogens, C_{30} and C_{32} *n*-alkanes were among the most abundant aliphatic hydrocarbons released from Li/EtNH₂ treatment of the kerogens (Schaeffer *et al.*, 1995). These long-chain S-linked alkyl units are thought to be derived diagenetically from the sulphurization of C_{30}–C_{32} 1,15-diols produced by

microalgal *Nannochloropsis* species from the class Eustigmatophyceae (Volkman *et al.*, 1992; Gelin *et al.*, 1996). These diols and their diagenetic derivatives have also been found as such in extracts of many marine sediments (e.g. de Leeuw *et al.*, 1981; Morris and Brassell, 1988). Consequently, we conclude that a significant part of the kerogens from the Vena del Gesso marl sediments is composed of selectively preserved algaenans originating from marine nano-phytoplankton such as the eustigmatophytes. It is also likely that these algaenans favour the preservation of free lipids through their incorporation into the algaenan matrix as a result of sulphurization.

Alkyl pyrroles

Alkyl pyrroles were also relatively abundant components in the pyrolyzates from all the treated kerogens (up to 6 mg/g$_{TOC}$ for sample 4), whereas they were present in trace amounts (0–0.6 mg/g$_{TOC}$) in the pyrolyzates of the untreated kerogens (Fig. 4). Alkyl pyrroles in pyrolyzates of kerogens (untreated) have been reported only twice, i.e. from sulphur-rich sediments from the Miocene Monterey Formation (Sinninghe Damsté *et al.*, 1992) and a Recent diatomaceous ooze from the Namibian Shelf (Klok *et al.*, 1984). The product distributions in the pyrolyzates in these two cases are very similar to those in the pyrolyzates of the treated kerogens from Vena del Gesso kerogens as shown in Fig. 5 for

sample 4, the main products being C$_1$–C$_5$ alkylated pyrroles, most notably 2,3-dimethylpyrrole, 3-ethyl-4-methylpyrrole and 2,4-dimethyl-3-ethylpyrrole. The distribution patterns indicate that these products originate from tetrapyrrole pigments ultimately derived from chlorophylls or bilirubin-type precursors, as suggested previously (Sinninghe Damsté *et al.*, 1992). Their presence in high abundance in the pyrolyzates of the "desulphurized" kerogens suggests that tetrapyrrole units were preserved in the sediments by incorporation into the macromolecular matrix. Although the presence of S-bound porphyrins in other sulphur-rich sediments has been reported (e.g. Schaeffer *et al.*, 1993), the fact that the alkyl pyrroles were found after Li/EtNH$_2$ treatment may suggest that the tetrapyrrole units were not bound by sulphur bonds but by other linkages such as oxygen bonds or by both sulphur and oxygen bonds. Whatever the mode of binding, it is perhaps surprising that alkyl pyrroles were only present in trace quantities in the pyrolyzates of the untreated kerogens. This suggested that Li/EtNH$_2$ might reduce double bonds in the cyclic tetrapyrrole system, decreasing the aromaticity and allowing the formation of alkyl pyrroles by pyrolysis. To investigate this hypothesis, a porphyrin standard, octaethylporphyrin, was subjected to Li/EtNH$_2$ treatment under the conditions used for the kerogen. Both the porphyrin and the reaction product were pyrolyzed at 610°C. Only the latter released GC-amenable
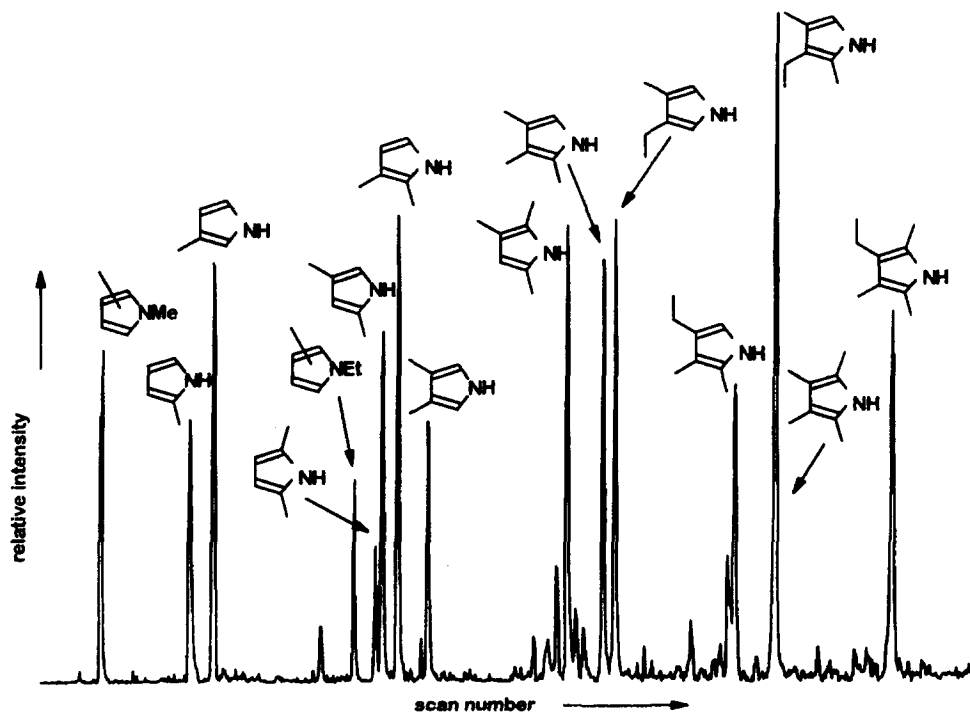

Fig. 5. Partial, accurate summed mass chromatogram of *m/z* 80.06 + 81.06 + 94.08 + 95.08 + 108.1 + 109.1 + 122.11 + 123.11 + 136.13 + 137.13 (mass window 0.02 Da) revealing the distribution of the C$_1$–C$_5$ alkylated pyrroles in the flash pyrolyzates of VdG-IV-4 desulphurized kerogen.
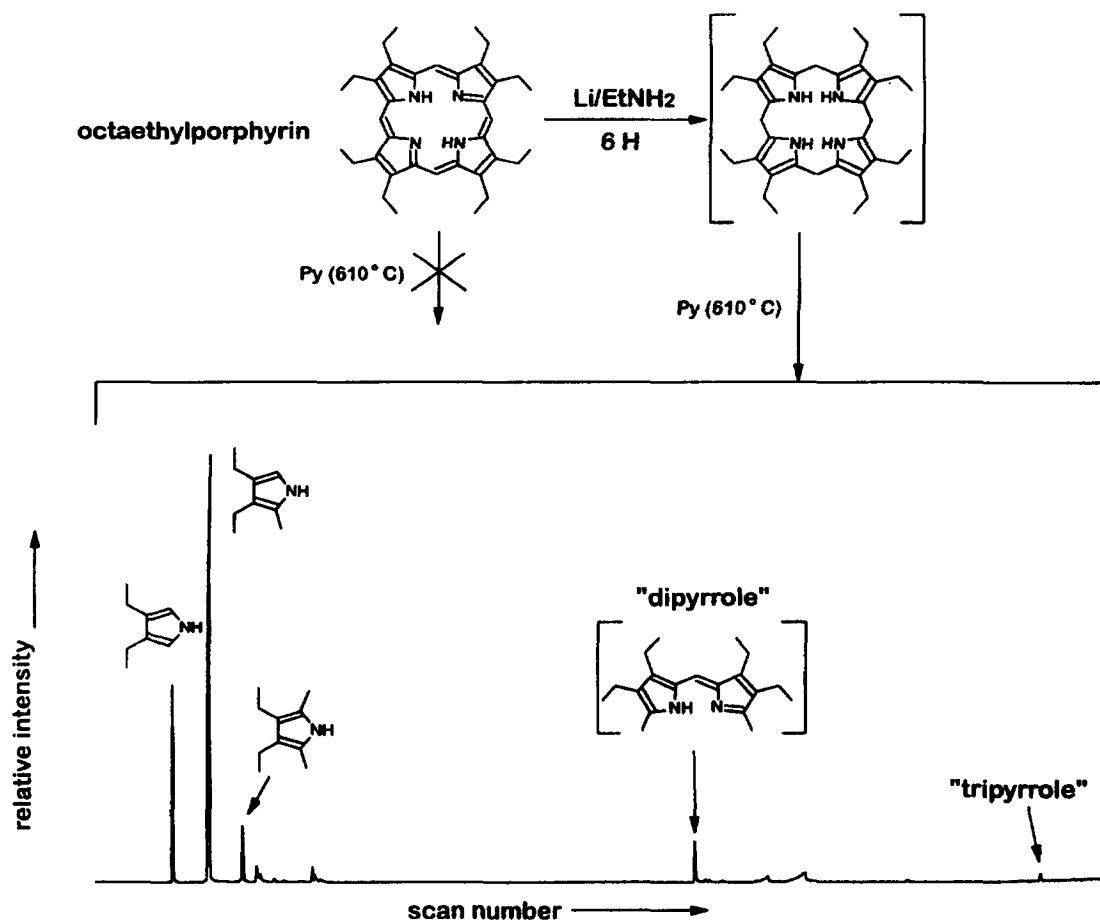
Fig. 6. Total ion current of the pyrolyzates of the products obtained after Li/EtNH₂ treatment of octaethylporphyrin. The structures of the dipyrrole and the reduced porphyrin that generated these products are tentative.

compounds on pyrolysis (Fig. 6). The main products were alkyl pyrroles with ethyl substituents at C-3 and C-4, in agreement with the substitution pattern in the starting material. Di- and tripyrroles were also detected in the pyrolyzate but in much lower abundance. The absence of alkylated pyrroles in the pyrolyzate of the octaethylporphyrin is consistent with the findings of Meuzelaar *et al.* (1982) and Sinninghe Damsté *et al.* (1992) who performed Py–MS on tetraethyltetramethylporphyrin and Py–GC(MS) on metallo and free base octaethylporphyrins, respectively. This indicates that porphyrins are too stable to generate pyrolysis products which can be observed under our experimental conditions. Hence, the octaethylporphyrin probably simply evaporated as such, so we can consider that bound porphyrins in kerogens or asphaltenes are probably released as mainly intact tetrapyrroles upon pyrolysis. This is supported by studies of ETIO- and DPEP vanadyl porphyrins released after pyrolysis of Woodford and New Albany Shale kerogens (Sundararaman *et al.*, 1988).

Our results show that Li/EtNH₂ treatment affects the thermal stability of octaethylporphyrin. Probe mass spectra of the Li/EtNH₂-treated porphyrin suggested the presence of reduced products, including one with a molecular ion at m/z 540 (the molecular mass of octaethylporphyrin is 534 Da). Although the exact nature of these products was not examined, it is suggested that double bonds, presumably at the bridgehead carbons, were reduced, forming less stable cyclic products which were more readily cleaved upon flash pyrolysis.

CONCLUSIONS

The combined results of kerogen desulphurization and flash pyrolysis studies indicate the following:

1. The amounts and nature of originally low-molecular-weight compounds, i.e. C₂₀ isoprenoids, steroids and hopanoids vary significantly over cycle IV as judged from the differences in the compositions of the pyrolysis products before and after desulphurization.

2. Removal of a significant part of sulphur-bound lipids by Li/EtNH$_2$ treatment of the kerogens results in an enrichment of algaenan-derived organic matter in the "desulphurized" kerogens as revealed by the predominance of n-alk-1-enes and n-alkanes in the pyrolyzates of the latter.

3. Series of C$_5$–C$_9$ alkyl pyrroles in the pyrolyzates of the "desulphurized" kerogens revealed the presence of bound tetrapyrroles. The absence of these or trace abundance alkyl pyrroles in the pyrolyzates of the "untreated" kerogens indicate that the Li/EtNH$_2$ treatment reduced double bonds, thereby facilitating the generation of alkyl pyrroles upon pyrolysis.

REFERENCES

Canfield D. E., Raiswell R., Westrich J. T., Reaves C. M. and Berner R. A. (1986) The use of chromium reduction in the analysis of reduced inorganic sulphur in sediments and shales. *Chem. Geol.* **54**, 149–155.

Derenne S., Largeau C., Berkaloff C., Rousseau B., Wilhelm C. and Hatcher P. G. (1992) Non-hydrolysable macromolecular constituents from outer walls of *Chlorella fusca* and *Nanochlorum eucaryotum*. *Phytochemistry* **31**, 1923–1929.

Douglas A. G., Sinninghe Damsté J. S., Fowler M. G., Eglinton T. I. and de Leeuw J. W. (1991) Unique distributions of hydrocarbons and sulphur compounds released by flash pyrolysis from the fossilised alga *Gloeocapsomorpha prisca*, a major constituent in one of four Ordovician kerogens. *Geochim. Cosmochim. Acta* **55**, 1–17.

Eglinton T. I., Sinninghe Damsté J. S., Kohnen M. E. L. and de Leeuw J. W. (1990) Rapid estimation of the organic sulphur content of kerogens, coals and asphaltenes by pyrolysis-gas chromatography. *Fuel* **69**, 1394–1404.

Gelin F., Sinninghe Damsté J. S., Harrison W. H., Maxwell J. R. and de Leeuw J. W. (1995) Molecular indicators for palaeoenvironmental change in a Messinian evaporitic sequence (Vena del Gesso, Italy): III. Stratigraphic changes in the molecular structure of kerogen in a single marl bed as revealed by flash pyrolysis. *Org. Geochem.* **23**, 555–566.

Gelin F., Boogers I., Noordeloos A. A. M., Sinninghe Damsté J. S., Hatcher P. G. and de Leeuw J. W. (1996) Novel, resistant microalgal polyethers: an important sink of organic carbon in the marine environment?. *Geochim. Cosmochim. Acta.* **60**, 1275–1280.

Hartgers W. A., Sinninghe Damsté J. S. and de Leeuw J. W. (1991) Mechanisms of hydrocarbon formation during flash pyrolysis of kerogen. *Prep. Am. Chem. Soc.-Div. Fuel Chem.* **36**, 790–795.

Hartgers W. A., Sinninghe Damsté J. S., Requejo A. G., Allan J., Hayes J. M. and de Leeuw J. W. (1994) Evidence for only minor contributions from bacteria to sedimentary organic carbon. *Nature* **369**, 224–227.

Hoefs M. J. L., van Heemst J. D. H., Gelin F., Koopmans M. P., van Kaam-Peters H. M. E., de Leeuw J. W. and Sinninghe Damsté J. S. (1995) Alternative biological sources for 1,2,3,4-tetramethylbenzene in flash pyrolysates of kerogen. *Org. Geochem.* **23**, 975–979.

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 (Edited by Eckardt C. B. *et al.*). *Org. Geochem.* **19**, 371–387.

Horsfield B. (1989) Practical criteria for classifying kerogens: some observations from pyrolysis-gas chromatography. *Geochim. Cosmochim. Acta* **53**, 891–901.

Kenig F., Sinninghe Damsté J. S., Frewin N., Hayes J. M. and de Leeuw J. W. (1995) Molecular indicators for palaeoenvironmental change in a Messinian evaporitic sequence (Vena del Gesso, Italy). II. Stratigraphic variations in abundances and ^{13}C contents of free and sulphur-bound skeletons in a single marl bed. *Org. Geochem.* **23**, 485–526.

Klok J., Baas M., Cox H. C., de Leeuw J. W., Rijpstra W. I. C. and Schenck P. A. (1984) Qualitative and quantitative characterization of the total organic matter in a recent sediment (Part II). In *Advances in Organic Geochemistry* 1983 (Edited by Schenck P. A. *et al.*). *Org. Geochem.* **6**, 265–278.

Koopmans M. P., Sinninghe Damsté J. S., Lewan M. D. and de Leeuw J. W. (1995) Thermal stability of thiophene biomarkers as studied by hydrous pyrolysis. *Org. Geochem.* **23**, 583–596.

Larter S. R. and Horsfield B. (1983) Determination of structural components of kerogens by the use of analytical pyrolysis methods. In *Organic Geochemistry, Principles and Applications* (Edited by Engel M. H. and Macko S. A.), pp. 271–287. Plenum Press.

Lattimer R. P. (1994) Pyrolysis field ionisation mass spectrometry (Py-FI–MS) of polyolefins. *J. Anal. Appl. Pyrolysis* **31**, 203–226.

de Leeuw J. W., Rijpstra W. I. C. and Schenck P. A. (1981) The occurrence and identifications of C$_{30}$, C$_{31}$ and C$_{32}$ alkan-1,15-diols and alkan-15-one-1-ols in Unit I and Unit II Black Sea sediments. *Geochim. Cosmochim. Acta* **45**, 2281–2285.

Metzger P., Pouet Y., Bischoff R. and Casadevall E. (1993) An aliphatic polyaldehyde from *Botryococcus braunii* (A race). *Phytochemistry* **32**, 875–883.

Meuzelaar H. L. C., Haverkamp J. and Hileman F. D. (1982) *Pyrolysis Mass Spectrometry of Recent and Fossil Biomaterials, Techniques and Instrumentation in Analytical Chemistry* 3, Elsevier.

Morris R. J. and Brassell S. C. (1988) Long-chain alkanediols: biological markers for cyanobacterial contributions to sediments. *Lipids* **23**, 256–258.

Orr W. L. (1986) Kerogen/asphaltene/sulphur relationships in sulphur-rich Monterey oils. In *Advances in Organic Geochemistry* 1985 (Edited by Leythauser D. and Rullkötter J.). *Org. Geochem.* **10**, 499–516.

François Gelin *et al.*

Requejo A. G., Allan J., Creaney S., Gray N. R. and Cole K. S. (1992) Aryl isoprenoids and diaromatic carotenoids in paleozoic source rocks and oils from the western Canada and Williston basins. *Org. Geochem.* **19,** 245–264.

Schaeffer P., Ocampo R., Callot H. J. and Albrecht P. (1993) Extraction of bound porphyrins from sulphur-rich sediments and their use for reconstruction of palaeoenvironments. *Nature* **364,** 133–136.

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

Sinninghe Damsté J. S. and de Leeuw J. W. (1990) Analysis, structures and geochemical significance of organically-bound sulphur in the geosphere: State of the art and future research. In *Advances in Organic Geochemistry 1989* (Edited by Durand B. and Behar F.) *Org. Geochem.* **16,** 1077–1101.

Sinninghe Damsté J. S., Eglinton T. I. and de Leeuw J. W. (1992) Alkylpyrroles in a kerogen pyrolysate: evidence for abundant tetrapyrrole pigments. *Geochim. Cosmochim. Acta* **56,** 1743–1751.

Sinninghe Damsté J. S., Frewin N., Kenig F. and de Leeuw J. W. (1995) Molecular indicators for palaeoenvironmental change in a Messinian evaporitic sequence (Vena del Gesso, Italy), 1: Variations in extractable organic matter of ten cyclically deposited marl beds. *Org. Geochem.* **23,** 471–483.

Sundararaman P., Biggs W. R., Reynolds G. J. and Fetzer J. C. (1988) Vanadylpoyrphyrins, indicators of kerogen breakdown and generation of petroleum. *Geochim. Cosmochim. Acta* **52,** 2337–2341.

Vai G. B. and Ricci Lucchi F. (1977) Algal crust, autochtonous and clastic gypsum in a cannibalistic evaporite basin: a case history from the Messinian of Northern Apennines. *Sedimentology* **24,** 211–244.

Volkman J. K., Barrett S. M., Dunstan G. A. and Jeffrey S. W. (1992) C_{30}–C_{32} alkyl diols and unsaturated alcohols in microalgae of the class Eustigmatophyceae. *Org. Geochem.* **18,** 131–138.