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Molecular palaeontology of the earliest Danian at Geulhemmerberg (the Netherlands)

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Abstract

Organic compound distributions in extracts of three selected clay samples from the lowermost Danian section at Geulhemmerberg were analysed in order to enhance the understanding of the depositional environment immediately after the Cretaceous/Tertiary (K/T) boundary. A highly dominant $C_{40:2}$ ethyl ketone is identified. This compound is probably derived from specific, highly abundant non-coccolithophorid Prymnesiophyte algae which may already have been present in late Maastrichtian times.

Fatty acids as well as their randomly oxidized hydroxy- and keto counterparts are also abundant. These compounds probably indicate bacterially transformed biochemicals of terrestrial origin, although they are probably not derived from the Bryophyte moss spores abundantly present in these sediments. Their distributions are, however, strikingly similar to those of fatty acids in Antarctic soils.

ω^{16}-, ω^{17}-, and ω^{22}- keto- and hydroxy fatty acids with highly specific distribution patterns and a clear even over odd carbon number preference are thought to be of marine origin. The biochemical relationships between these compounds and the $C_{40:2}$ ethyl ketone suggest that they may originate from the same algae.

This presence of highly functionalized organic compounds demonstrates the extreme immaturity and excellent preservation of the unique Geulhemmerberg K/T boundary sediments.

Introduction

Molecular organic geochemistry is a useful tool for the reconstruction of palaeoenvironments. Molecular biomarkers or chemical fossils preserved in sedimentary organic matter can provide information complementary to that obtained by palynology and micropalaeontology. Biomarkers contain information concerning ancient environments and processes, and thus molecular organic geochemistry can be regarded as another, complementary palaeontological approach.

The origins of the sedimentary organic matter can be determined from the molecular geochemistry of sediments. Specific organic compounds (biomarkers) are indicative of certain sources. For example, long-chain unsaturated ketones derive from some, often coccolith-producing, Prymnesiophyte algae, and dinoflagellates are a source of a unique steroid, dinosterol. Organic molecules also can give an indication of sediment maturity as functionality is lost with diagenesis. Therefore, the more functionalized the compounds, the less mature the sediment. The unsaturation ratio of the C_{37} long-chain ketones is used to reconstruct palaeo-sea-surface-water temperatures, thus providing information unobtainable by other palaeontological methods (e.g. Brassell et al. 1986; Prahl and Wakeham 1987). Since not all Prymnesiophyte algae produce coccoliths, the presence of long-chain ketones merely confirms the presence of such algae as a source of organic matter. Thus, molecular geochemistry can provide additional information and confirm or complement other palaeontological findings.

The organic matter falls into two categories: that which can be extracted by organic solvents (extracta-

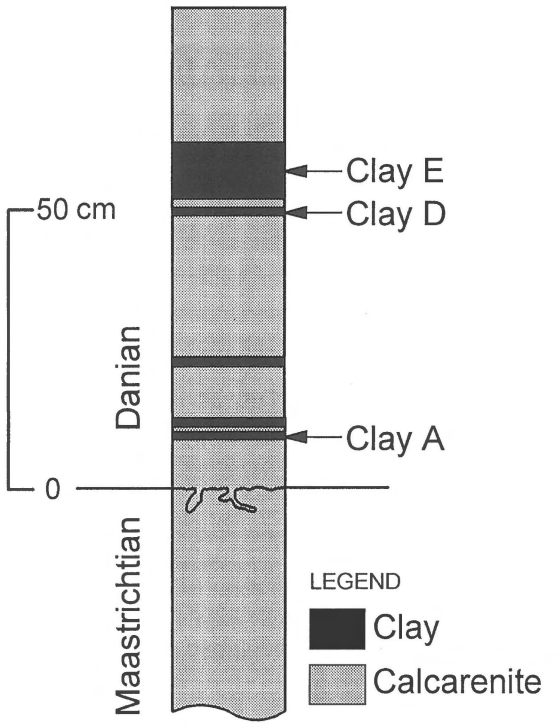

Figure 1. Schematic column showing sampling points of the earliest Danian section at Geulhemmerberg (for full details see Brinkhuis and Smit, this issue).

bles), and that which cannot (kerogen). In order to aid identification, the extractable organic matter is often separated into several fractions by chromatography. The distributions of the compounds can be determined by gas chromatography (GC) and the compounds themselves can be identified by gas chromatography-mass spectrometry (GC-MS). Once identified, the compound class distributions and the significance of individual compounds can be interpreted. This paper is concerned with the extractable organic matter of the Cretaceous/Tertiary (K/T) boundary section at Geulhemmerberg, the Netherlands (Figure 1; see also Brinkhuis and Smit, this issue).

Experimental

Three freeze-dried samples from the A, D and E clays (Figure 1) were homogenized and Soxhlet-extracted with DCM/MeOH (7:1 v/v). The extracts were methylated with diazomethane, chromatographed over silica gel with ethyl acetate (to remove very polar compounds) and silylated with BSTFA in pyridine at 60 °C for 30 minutes.

Gas chromatographic analyses were performed with a Carlo Erba HRGC 5300 instrument (on column injection, 25 m × 0.32 mm i.d. CP Sil-5 column, flame ionization detection). The typical temperature program was 70–130 °C at 20 °C/min, 130–320 °C at 4 °C/min and then isothermal at 320 °C for 20–100 minutes. The carrier gas was helium.

GC-MS analyses were performed with a Hewlett Packard 5890 Series II GC connected to an Autospec Ultima MS by direct insertion of the capillary column into the ion source. The column and the temperature program were identical to those used in the GC analyses. The mass spectrometer was operated in the EI mode at 70 eV. Cycle time: 1.8 s, range *m/z* 50–800.

Hydrogenation

An aliquot of the total extract was dissolved in c. 3 ml ethyl acetate and a small amount of platinum oxide was added. Hydrogen gas was bubbled gently through the mixture for an hour, after which the extract was chromatographed over silica gel with ethyl acetate to remove the catalytic residue.

DMDS derivatization

An aliquot of the total extract was dissolved in 100 μl hexane and reacted with 100 μl dimethyldisulphide (DMDS) and 50 μl iodine solution (60 mg iodine in 1 ml diethyl ether). The mixture was kept at 40 °C for 12 hours and then diluted with 2 ml hexane. A small amount of sodium thiosulphate (5% in water) was added and the hexane layer pipetted off. The residue was washed twice with a small amount of hexane.

Results

The extractable lipid contents of the A, D and E clay are 444, 290 and 189 ppm, respectively. The gas chromatograms of the derivatized total extracts of the three clay samples show that the extracts are highly complex (Figure 2). The total extract gas chromatograms of the three clays are almost identical and so only the E clay was chosen to be studied in greater detail. The major compounds identified in the extracts were a long-chain unsaturated ethyl ketone (I), *n*-fatty acids (II), keto-*n*-fatty acids (III) and hydroxy-*n*-fatty acids (IV). The Roman numerals mentioned between brackets refer to

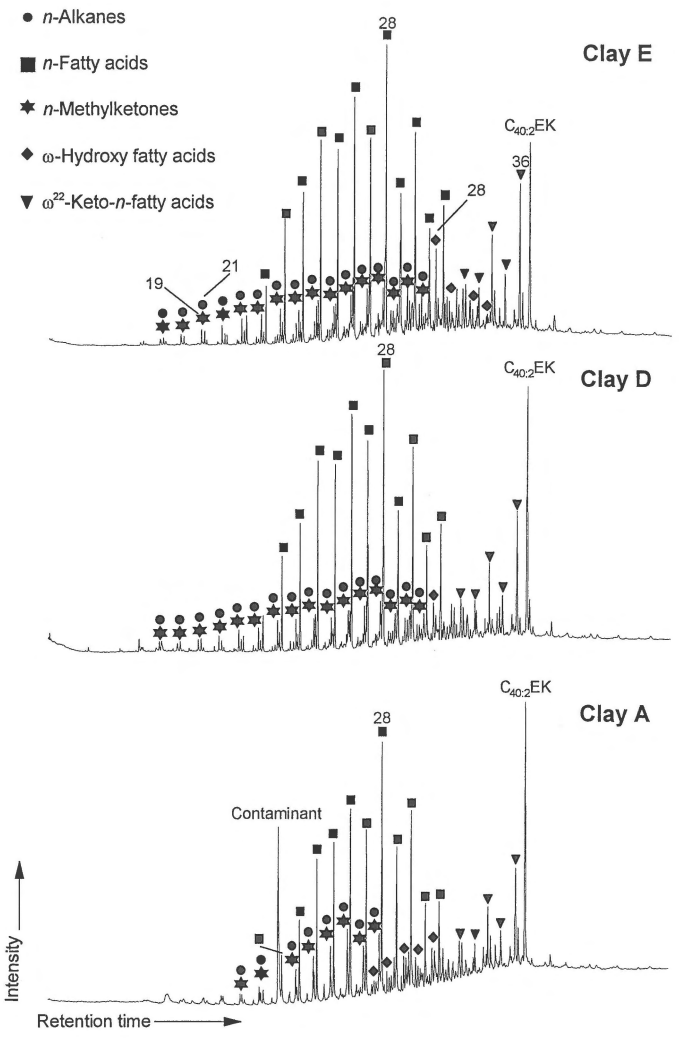

Figure 2. Gas chromatograms of freely extractable lipids in samples from the A, D and E clays in the Geulhemmerberg section. GC conditions are given in the experimental section.

the structures of the compound classes shown in Figure 3.

Long-chain ethyl ketone

A long-chain ketone was identified as a major compound in the extracts. The mass spectrum of this ketone suggested that it is a C_{40} twice unsaturated ethyl ketone (I) (Figure 4). Hydrogenation of the compound caused a shift of 4 daltons in the molecular ion from *m/z* 572 to *m/z* 576 (Figure 4), which confirmed the presence of two double bonds in the original molecule. In order to determine the position of these double bonds DMDS (dimethyldisulphide) treatment was performed. The mass spectrum of the most abundant compound after this treatment reflects a mixture of compounds with *m/z* 285 and *m/z* 381 dominant (Figure 5). This spectrum was assigned to a mixture of the partially methylsulphurized compounds 16,17-dimethylsulphide-*n*-tetraconta-23-ene-3-one and 23,24-dimethylsulphide-*n*-tetraconta-16-ene-3-one. This indicates that in the original structure the double-bonds are located at the ω^{17} and ω^{24} position, although whether they are cis or trans still has to be determined. These double-bond positions are clearly different to those previously determined for the

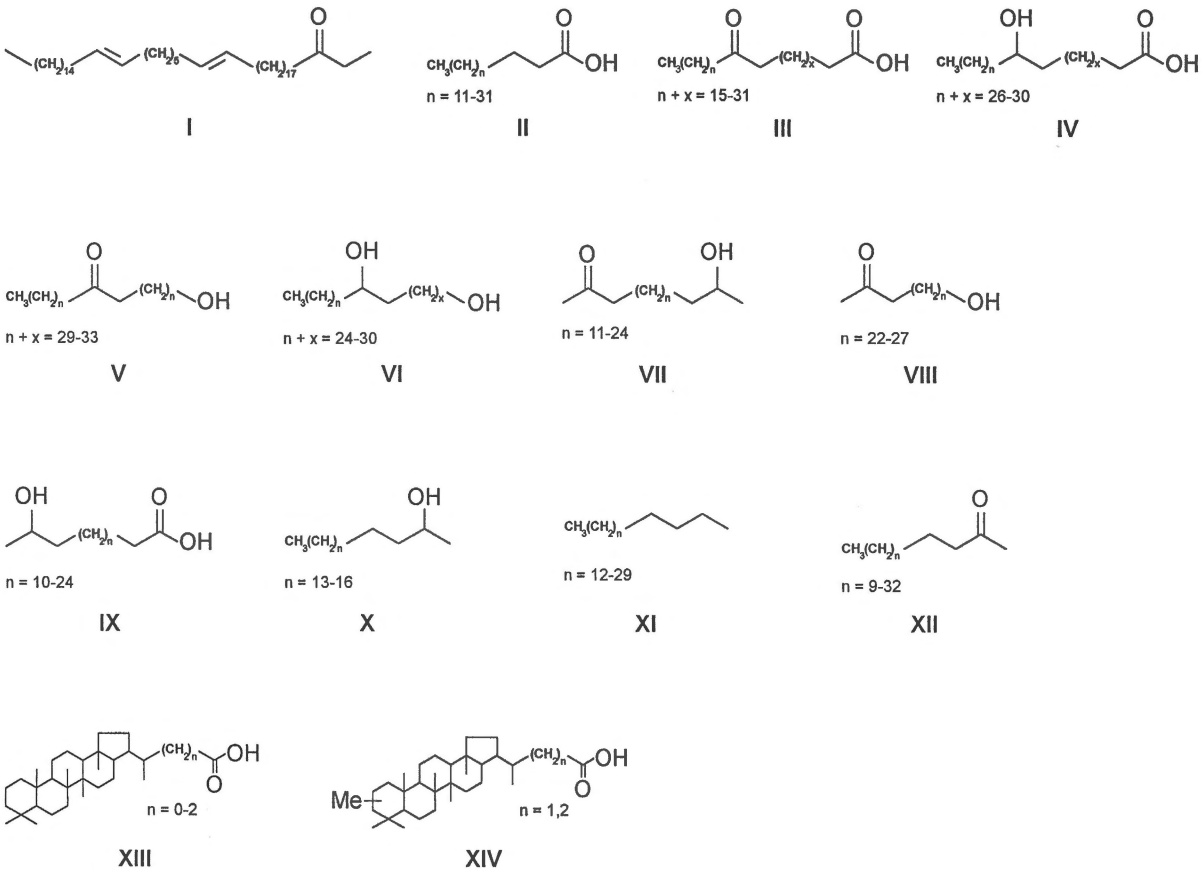

Figure 3. Schematic structures of the organic compounds encountered in samples from the A, D and E clays in the Geulhemmerberg section. Roman numerals serve as references in the text and figure captions.

diunsaturated C_{37} long-chain ketone (ω^{15} and ω^{22}; De Leeuw et al. 1980).

n-*Fatty acids*

The *n*-fatty acids (II) represent a major compound class in the samples and range from C_{14} to C_{34} (Figure 6). The distribution pattern is monomodal with a maximum at C_{28}. The higher homologues are more abundant than the lower carbon number homologues and there is a weak even carbon number preference.

Functionalized fatty acids

Among the functionalized fatty acids several dominant series are present which do not appear to be related to the fatty acids (II) in terms of distribution profiles. The highly specific distribution patterns of the ω^{16}-, ω^{17}- and ω^{22}-keto- (III) and ω^{16}-, ω^{17}- and ω^{22}-hydroxy-fatty acids (IV) are shown in Figure 6. The higher homologues have a strong even carbon number preference. The carbon number of the most abundant homologue is fixed in all samples for each series, e.g. C_{30} for the ω^{16}-series, C_{28} for the ω^{17}-series and C_{36} for the ω^{22}- series. The most abundant homologue of each keto- and hydroxy series has its keto-*n*-alkan-1-ol (V) and *n*-alkyldiol (VI) counterpart, respectively (Figure 7).

Minor compounds

Minor series of compounds include (ω-1)-keto-*n*-alkan-2-ols (VII), (ω-1)-keto-*n*-alkan-1-ols (VIII), (ω-1)-hydroxy-*n*-fatty acids (IX) and keto- (III) and

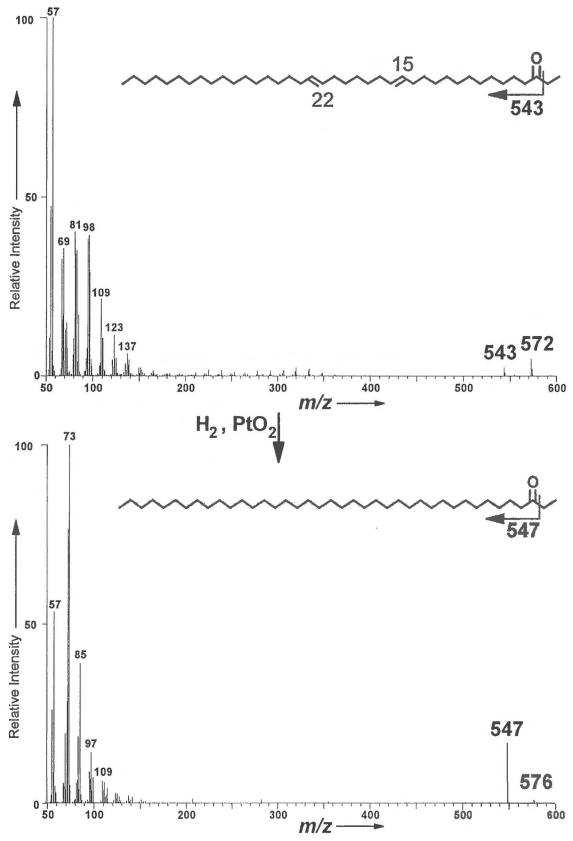

Figure 4. Mass spectra of the $C_{40:2}$ ethyl ketone (I) and the $C_{40:0}$ ethyl ketone, produced from the $C_{40:2}$ ethyl ketone by hydrogenation (E-clay, Geulhemmerberg).

hydroxy-*n*-fatty acids (IV) with keto- and hydroxy groups at other positions than ω^{16}, ω^{17} and ω^{22} (Figures 8, 9). These compounds all show a distribution which is almost identical to that of the *n*-fatty acids (II), i.e. monomodal, maximizing at C_{28} and a weak even carbon number preference.

Other series of minor compounds are the *n*-alkano-2-ols (X) and *n*-alkanes (XI), which range from C_{16} to C_{33} (Figure 9). They have a monomodal distribution maximizing at C_{29} and a slight odd carbon number preference. The *n*-methylketones (XII) have a very similar distribution pattern to that of the *n*-alkanes and have possibly formed by the oxidation of the *n*-alkanes.

Other minor compound classes in the clay samples are the hopanoic (XIII) and methylhopanoic acids (XIV). They range from C_{31} to C_{33}, the dominant isomer having the $\beta\beta$-configuration (Figure 9).

Discussion

Three-dimensional plots of the keto- (III) and hydroxy (IV) fatty acids plotted against carbon chain length and the position of the keto- or hydroxy group show several major series (Figures 10, 11). The first series shows the similarity between the distributions of the randomly oxidized fatty acids (keto- and hydroxy fatty acids) and the normal fatty acids (II), especially for the 4-, 5- and 6- keto- and hydroxy fatty acids. These distributions maximize at C_{28} and have a weak even over odd carbon number preference.

The other dominant series consists of the ω^{16}-, ω^{17}- and ω^{22}- keto- (III) and hydroxy fatty acids (IV) (Figures 10 and 11, respectively). These series have a strong even over odd carbon number preference, unlike the other series. Since the distributions of these series of compounds are completely different to those of the fatty acids (II), it may be suggested that these ω^{16}-, ω^{17}- and ω^{22}-series of compounds derive from a source different to that of the fatty acids.

Thus, four main types of source and process indicators can be discriminated, as discussed below.

$C_{40:2}$ Ethyl ketone

This compound is the only homologue of this group of compounds seen in these sediments. The only known source for long-chain unsaturated ketones are marine algae belonging to the class Prymnesiophyceae. Therefore, this compound suggests an autochthonous source. However, its 'distribution' is quite unique compared to those previously reported for both pre- and post-K/T boundary sediments (Brassell et al. 1980; Cranwell 1985; Farrimond et al. 1986; Figure 12). It is possible that this compound was produced by a highly dominant Prymnesiophyte algae. Comparison of long-chain unsaturated ketones and nannofossil assemblages in oceanic sediments suggests that members of the family Gephyrocapsaceae have possessed the capability for alkenone biosynthesis for at least 45 Ma (Marlowe et al. 1990). The source of the alkenones in Cretaceous shales is speculated to be an earlier genus of the family Gephyrocapsaceae (Farrimond et al. 1986). However, no great abundance of coccoliths nor the dominance of one type of coccolith are revealed by microscopic studies of nannoplankton (Romein et al. this issue), and so it is suggested that this compound is derived from a non-coccolithophorid algae, therefore not visible in the sediments by microscopy. Due to this singular distribution, no reconstruction of palaeo-sea-surface-water

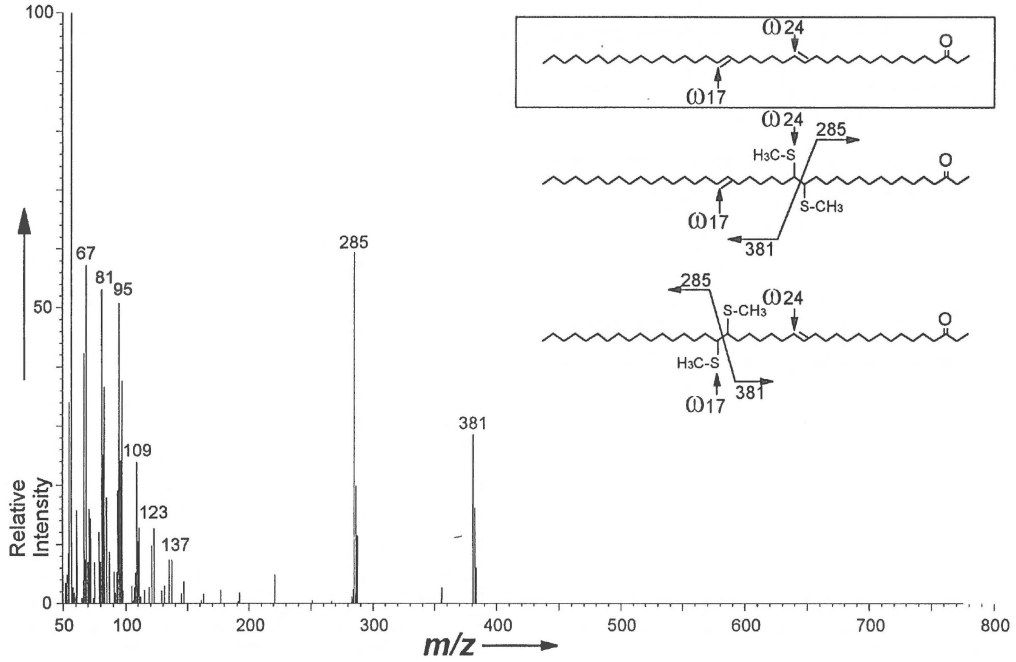

Figure 5. Mass spectrum of the mixture of 16,17-dimethylsulphide-*n*-tetraconta-23-ene-3-one and 23,24-dimethylsulphide-*n*-tetraconta-16-ene-3-one produced by dimethyldisulphide (DMDS) treatment from $C_{40:2}$ ethyl ketone (I) (E-clay, Geulhemmerberg).

temperatures can be calculated. It can even be speculated that the ketone did not function as a membrane fluidity regulator.

Fatty acids

The distribution pattern of the fatty acids (II) is unusual in that it has a weak even carbon number predominance and maximizes at C_{28}. Another unique point regarding these compounds is the number of randomly oxidized counterparts with the same distribution pattern as the fatty acids.

Fatty acids, ranging from C_{25} to C_{33} are thought to derive from higher plants, although the distribution pattern tends to have a strong even over odd carbon number predominance. However, palynological studies of the Geulhemmerberg clays have revealed an abundance of spores from Bryophytes (mosses), and a lack of higher plant pollen, which suggests that either the terrestrial vegetation was dominated by bryophytes or the bryophytes had conquered the coastal flats (Brinkhuis and Schiøler, this issue). Similar fatty acid distributions, with a weak even carbon number predominance were reported in soils and lacustrine sediments from Antarctica (Matsumoto et al. 1981, 1984; Volkman et al. 1988), where no higher plant vegetation exists. Thus it may be suggested that the long-chain fatty acids might derive from the Bryophyte spores. However, upon analysis, spores separated from extant Bryophytes did not yield long-chain fatty acids (unpublished data). Therefore, it is very unlikely that the long-chain acids are derived from these spores.

However, the source of these long-chain fatty acids must be terrestrial due to the many oxidation products with the same distribution patterns as the fatty acids. The hydroxy- and the keto fatty acids probably result from the hydroxylation by aerobic bacteria and fungi (Boon et al. 1977; Cranwell 1981) of the fatty acids. This oxidation of the fatty acids obviously occurred on land before they were deposited in the marine environment as no oxidation products of the autochthonous marine compounds (e.g. the long-chain ketone) were seen.

The weak even over odd carbon number predominance can be explained as a result of this oxidation. ω-Oxidation, possibly by fungi and/or aerobic bacteria, involves the sequential decrease in the carbon numbers of the fatty acids by decarboxylation (Figure 13). As a result odd carbon number fatty acids are produced from the even carbon numbered ones (Finnerty 1989).

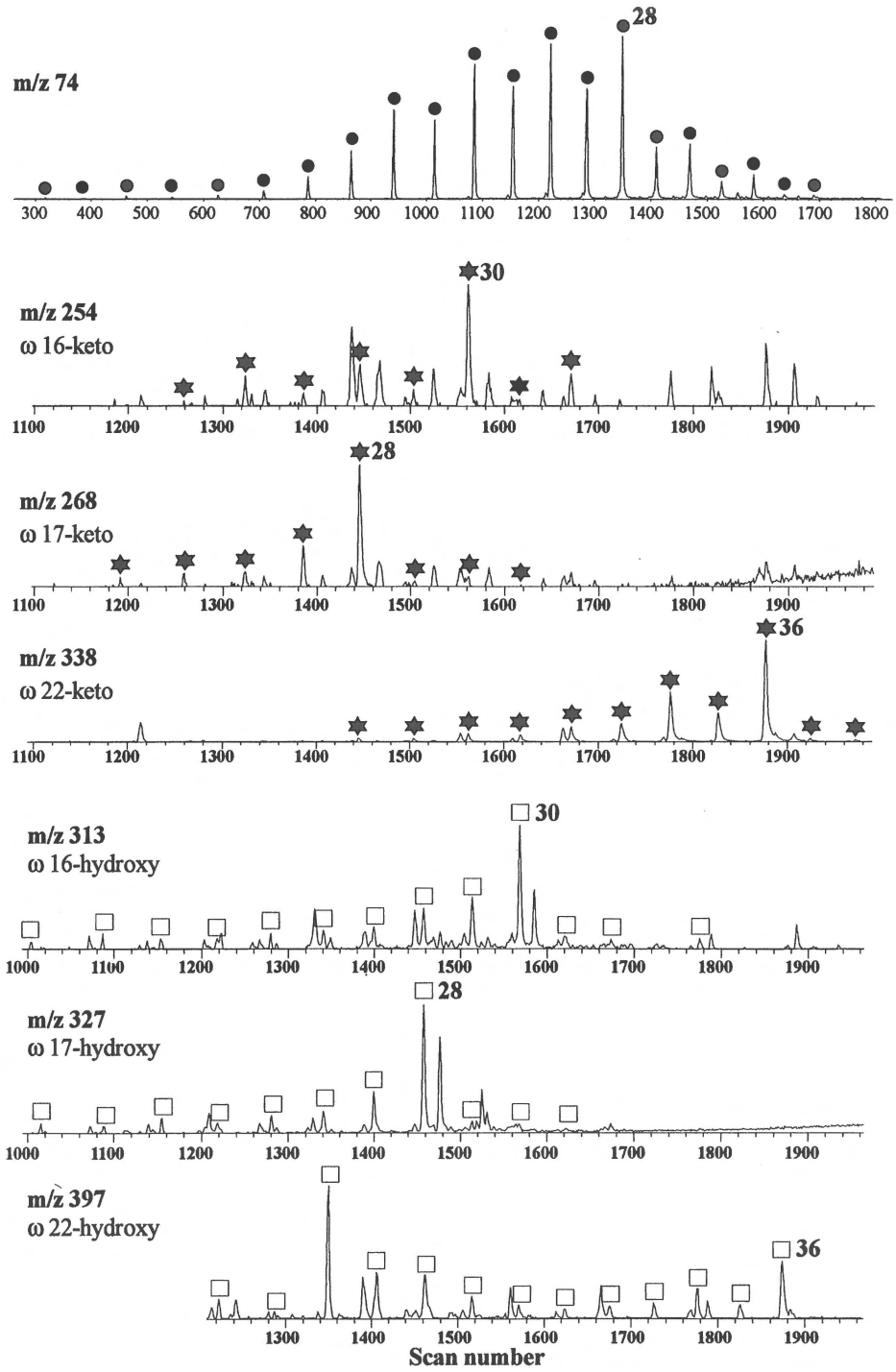

Figure 6. Mass chromatograms of the freely extractable lipids from the E-clay sample showing the distributions of *n*-fatty acids (II) (*m/z* 74), the dominant mid-chain keto-*n*-fatty acids (III): ω^{16}-, ω^{17}- and ω^{22}-keto *n*-fatty acids (*m/z* 254, 268 and 338), and the dominant mid-chain hydroxy-*n*- fatty acids (IV): ω^{16}-, ω^{17}- and ω^{22}-hydroxy *n*-fatty acids (*m/z* 313, 327 and 397).

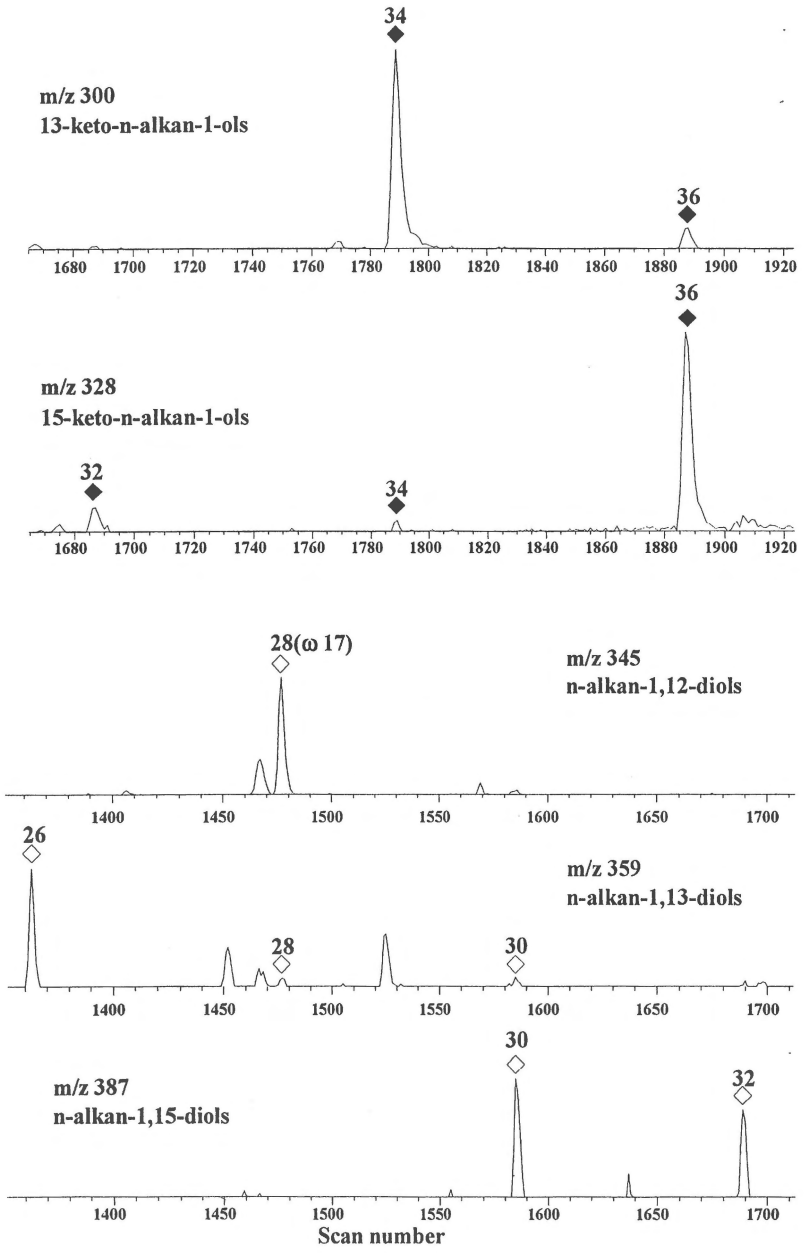

Figure 7. Mass chromatograms of the freely extractable lipids from the E-clay sample showing the distributions of mid-chain keto-*n*-alkan-1-ols (V): 13-keto-*n*-alkan-1-ols (*m/z* 300) and 15-keto-*n*-alkan-1-ols (*m/z* 328), and the *n*-alkyldiols (VI): *n*-alkan-1,12-diols (*m/z* 345), *n*-alkan-1,13-diols (*m/z* 359) and *n*-alkan-1,15-diols (*m/z* 387).

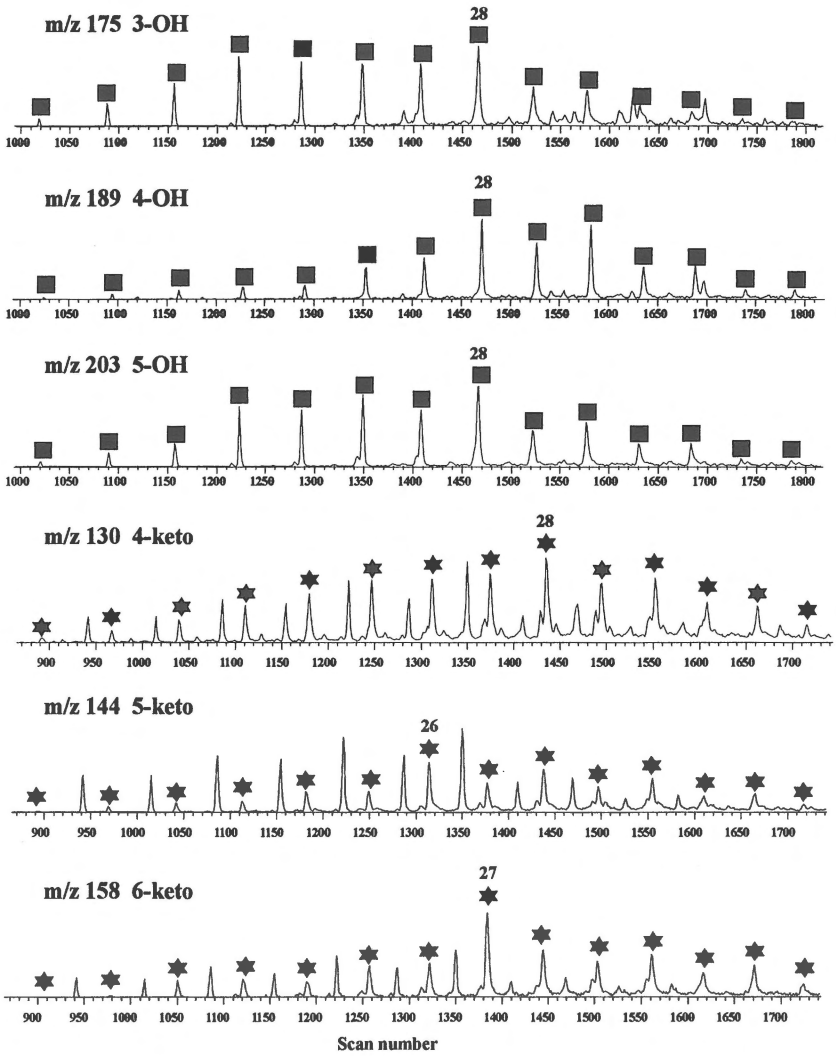

Figure 8. Mass chromatograms of the freely extractable lipids from the E-clay sample showing the distributions of 3-hydroxy-*n*-fatty acids (IV) (*m*/*z* 175), 4-hydroxy-*n*-fatty acids (*m*/*z* 189) and 5-hydroxy-*n*-fatty acids (*m*/*z* 203), and the 4-keto-*n*-fatty acids (III) (*m*/*z* 130), 5-keto-*n*-fatty acids (*m*/*z* 144) and 6-keto-*n*-fatty acids (*m*/*z* 158).

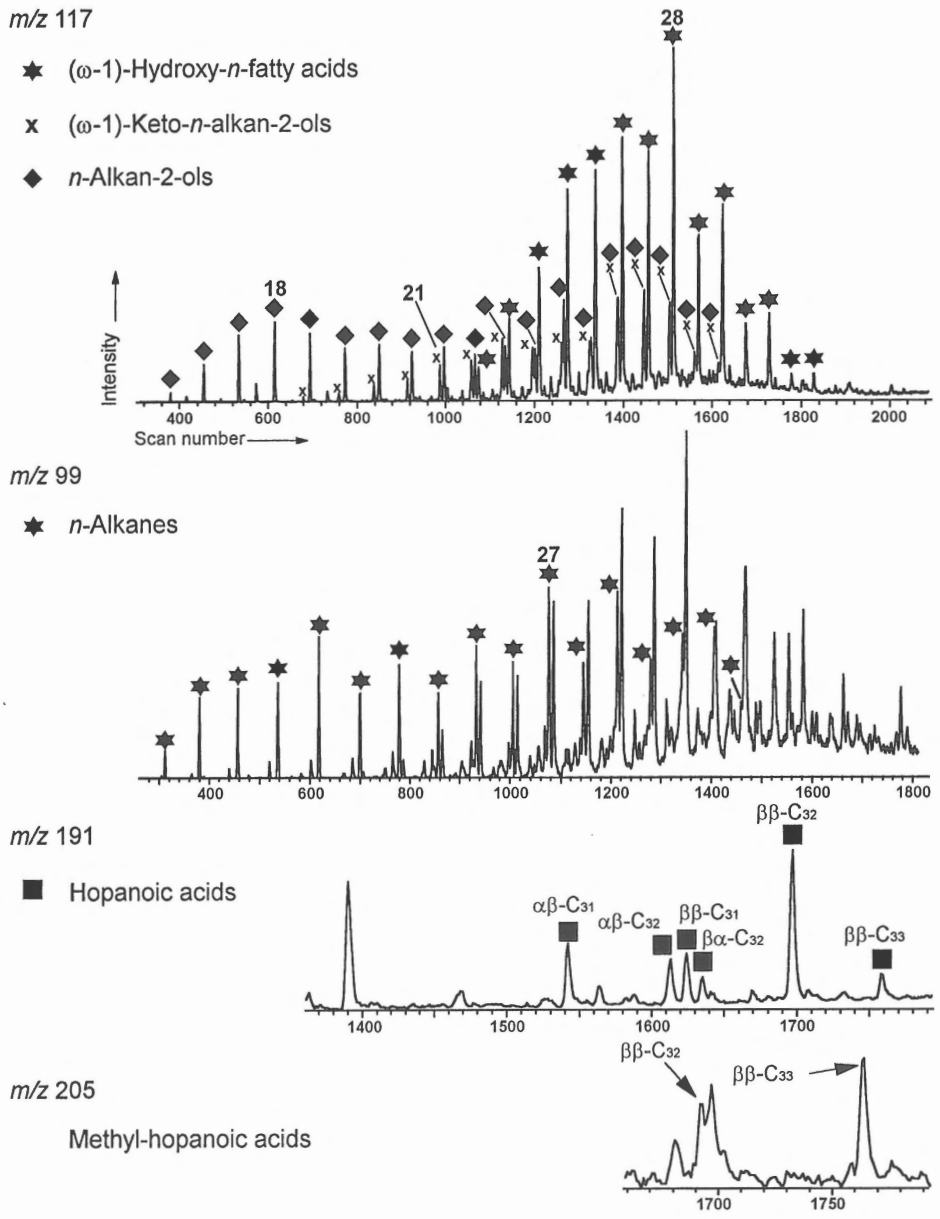

Figure 9. Mass chromatograms of freely extractable lipids from the E-clay sample showing the distributions of *n*-alkan-2-ols (X) (*m/z* 117), (ω-1)-keto-*n*-alkan-2-ols (VII) (*m/z* 117) and (ω-1)-hydroxy-*n*-fatty acids (IX) (*m/z* 117), the *n*-alkanes (XI) (*m/z* 99) and the hopanoic acids (XIII) and methyl hopanoic acids (XIV) (*m/z* 191 and 205).

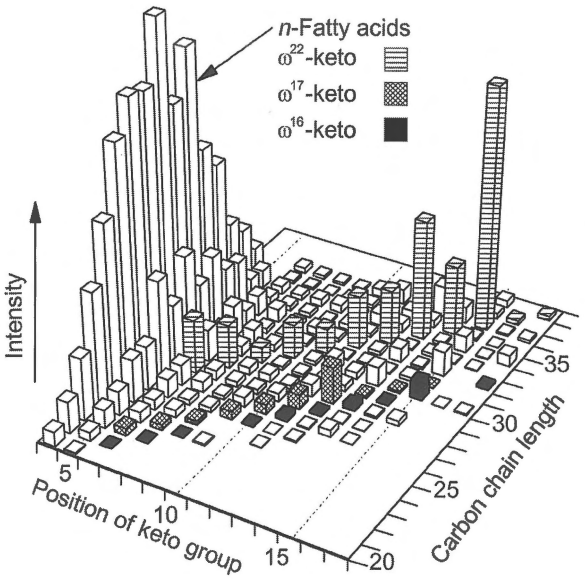

Figure 10. Three-dimensional plot showing the distributions of keto *n*-fatty acids (III) in the E-clay: relative intensities were calculated based on the intensity revealed from specific mass chromatograms (*m/z* 88 + 14n: n = position of keto group).

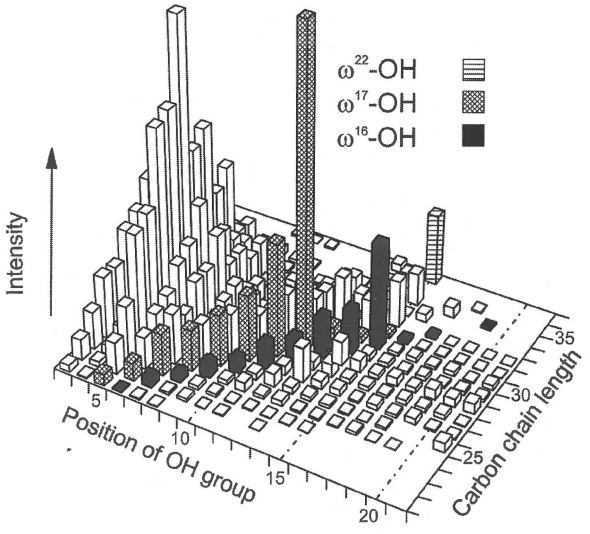

Figure 11. Three-dimensional plot showing the distributions of hydroxy-*n*-fatty acids (IV) in the E-clay: relative intensities were calculated based on the intensity on the specific mass chromatograms (*m/z* 133 + 14n: n = position of hydroxy group).

ω^{16}-, ω^{17}- and ω^{22}-keto- and hydroxy fatty acids

The relatively abundant ω^{16}-, ω^{17}- and ω^{22}-keto- (III) and hydroxy (IV) fatty acids have highly specific distribution patterns. They have a strong even over odd carbon number predominance, unlike the fatty acids, which suggests that they were derived from a different source. No signs of oxidation are observed and so it is suggested that these compounds derive from an autochthonous source.

Molecular relationships are found between the dominant mid-chain hydroxy- and keto compounds and the $C_{40:2}$ ethyl ketone (I). The position of the hydroxy- and keto group in the most abundant homologues of the ω^{16}- and ω^{22}- series is at the carbon atom 15. This is exactly the same position as the ω^{24} double bond position in the ketone group of the $C_{40:2}$ ethyl ketone (Figure 14). Furthermore, the ω^{17}-series of hydroxy fatty acids and *n*-alkyldiols (VI) have exactly the same position as the ω^{17} double-bond position in the $C_{40:2}$ ethyl ketone (Figure 14). This structural, possibly biochemical, relationship between the $C_{40:2}$ ethyl ketone and the relatively abundant mid-chain hydroxy- and keto compounds suggests that they may originate from the same source, i.e. Prymnesiophyte algae.

Minor compounds

The 17β,21β(H)-hopanoic acids (XIII) are relatively more abundant than the other isomers. 17β,21β(H)-Hopanoic acids are readily converted to $\beta\alpha$- and $\alpha\beta$-hopanoic acids during diagenesis; thus the abundance of $\beta\beta$-hopanoic acids indicates the immaturity of the Geulhemmerberg samples. The hopanoic acids are thought to derive from Eubacteria (Ourisson et al. 1979).

Although Bryophyte spores and dinoflagellate cysts have been found in abundance in these samples (Brinkhuis and Schiøler, this issue), no molecular record of them has been observed. The absence of phytol (found in most plants) and sterols including dinosterol (a biomarker for dinoflagellates) is most unusual. Thus palynological studies and molecular geochemistry are highly complementary for these samples.

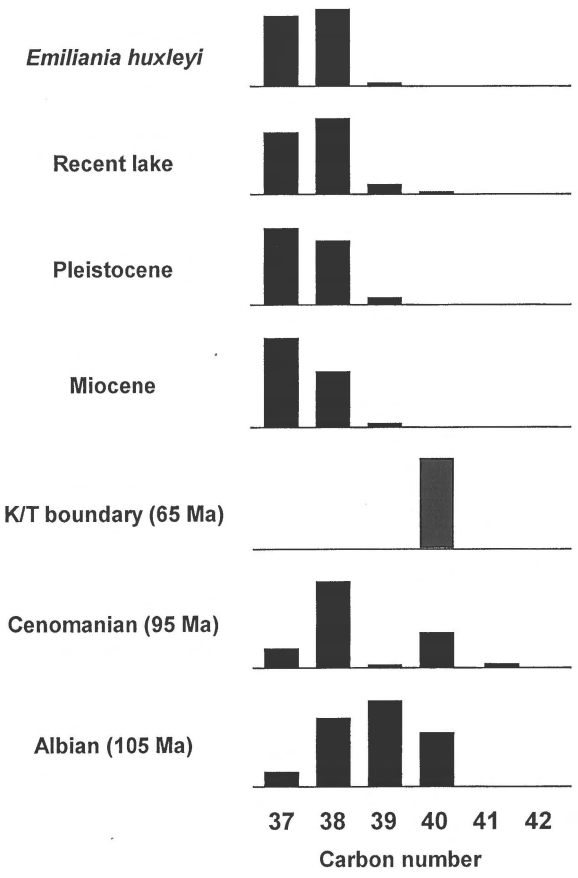

Figure 12. Carbon number distributions of long- chain unsaturated (LCU) ketones found in the coccolithophorid alga *Emiliania huxleyi* and in sediments from different geological ages and settings. Adapted from Farrimond et al. (1986).

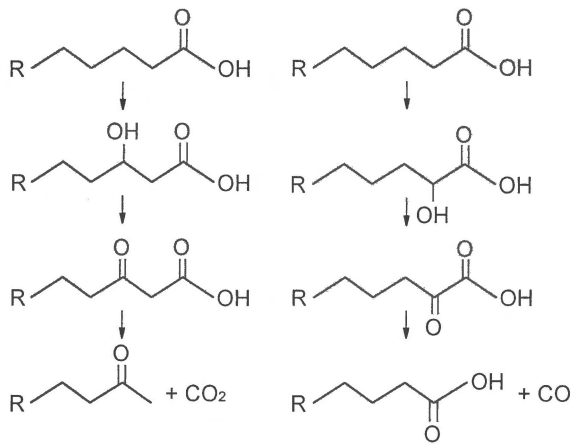

Figure 13. Oxidative degradation mechanisms of fatty acids proposed to explain the low even over odd carbon number preferences in the Geulhemmerberg samples.

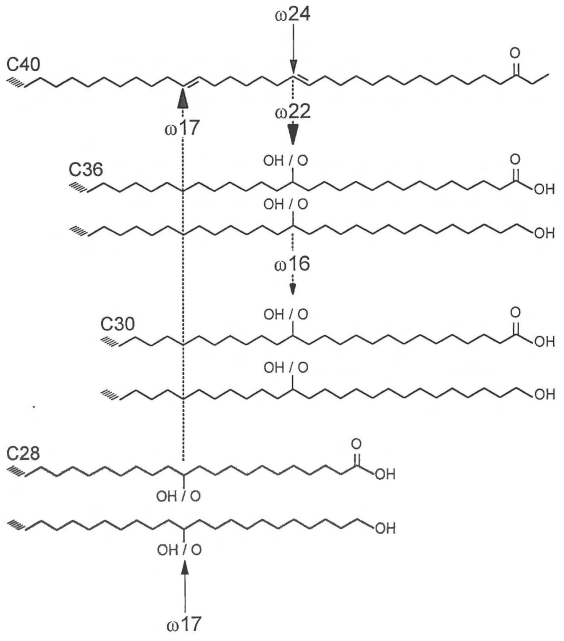

Figure 14. Structural relationships of the *n*-tetraconta-16,23-dien-3-one ($C_{40:2}$EK) with the dominant mid-chain keto- and hydroxy compounds: ω^{22}-keto-/hydroxy-*n*-C_{36} acids, ω^{22}-keto-*n*-C_{36}-1-ols/*n*-C_{36}-1, ω^{22}-diols, ω^{16}-keto-/hydroxy-*n*-C_{30} acids, *n*-C_{30}-1, ω^{16}-diols, ω^{17}-keto-/hydroxy-*n*-C_{28} acids and *n*-C_{28}-1, ω^{17}-diols.

Conclusions

The dominant $C_{40:2}$ ethyl ketone is probably derived from a specific, highly abundant non-coccolithophorid Prymnesiophyte algae, possibly already present in late Maastrichtian times.

The fatty acids, along with their randomly oxidized hydroxy- and keto fatty acid counterparts are probably indicative of bacterially transformed biochemicals of terrestrial origin transported into the depositional environment.

Relatively abundant ω^{16}-, ω^{17}- and ω^{22}-keto- and hydroxy fatty acids with specific distribution patterns and a clear even over odd carbon number predominance may be sourced by the same algae producing the $C_{40:2}$ ethyl ketone because of the biochemical relationships between these compounds.

The presence of highly functionalized organic compounds demonstrates the extreme immaturity and excellent preservation of the Geulhemmerberg clays.

Nannoplankton, palynological and molecular data are highly complementary.

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