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Lipids in a sulfur-rich lacustrine sediment from the Nördlinger Ries (southern Germany) with a focus on free and bound sterols

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The distribution of free sterols in a sulfur-rich lacustrine sediment of Miocene age deposited in the ancient crater lake of the Nördlinger Ries (southern Germany) was investigated and compared with the corresponding distributions of esterified and kerogen-bound sterols. The three fractions exhibited the same suite of principal sterols, with 4α,24-dimethyl-5α-cholestan-3β-ol, dinosterol and dinostanol displaying the highest concentrations and relative abundances. The distributions of sterols, steroidal ketones, *n*-alkanols and isoprenoid alcohols suggest a prevalent deposition of autochthonous aquatic organic matter under saline conditions. In particular, the high abundance of 4α,24-dimethyl-5α-cholestan-3-one, dinosterone and dinostanone and the corresponding 4-methyl sterols indicates the importance of dinoflagellate productivity in this former crater lake and its significant contribution to the sedimentary lipids in the Nördlinger Ries sediment. The similarity in structures and relative abundance of the major 4-methyl sterols and the corresponding steroidal ketones suggest that they are biosynthetically linked.

Keywords: Nördlinger Ries, lacustrine environment, core sediments, 4-methyl sterols, steroidal ketones, dinoflagellates

INTRODUCTION

Biological markers in extracts of organic matter (OM)-rich sediments and crude oils are routinely used to provide information on the source of the precursor organic material, the environmental conditions of deposition, the extent of thermal maturity of OM and to demonstrate the relationship between oils and their source rocks (e.g., Mackenzie *et al*., 1982; Brassell and Eglinton, 1986; Johns, 1986; Peters and Moldowan, 1993; Simoneit, 2004; Gaines *et al*., 2008; and references therein). The components include lipids with structural features indicative of their biological origin.

Sterols and their derivatives are among the most powerful biomarkers due to their resistance to degradation (Saliot *et al*., 1991) and wide variety of structures (Volkman, 1986). A great diversity has been found in microalgae (e.g., Patterson, 1991; Volkman *et al*., 1998; and references therein). Specific patterns of sterol composition in the geological record of marine and lacustrine environments provide useful biogeochemical information (Fernandes *et al*., 1999; Sangiorgi *et al*., 2005; Wang *et al*., 2008). Dinoflagellates produce an array of sterols not commonly found in other groups of algae. For example, the sterol composition of most dinoflagellates is dominated by 4α-methyl sterols including the C_{30} compound, dinosterol (4α,23,24-trimethyl-5α-cholest-22*E*-en-3β-ol; Withers, 1983, 1987). It is rarely found in other algae (Volkman *et al*., 1993) and hence has been used as an indicator of dinoflagellate OM contribution to marine sediments (Boon *et al*., 1979; Robinson *et al*., 1984).

Dinoflagellate sterol biomarkers can be either broad or limited in their scope (Leblond and Lasiter, 2012). For example, dinosterol is found in *ca*. two thirds of the dinoflagellates, covered by the studies examined through the data-mining approach of Leblond *et al*. (2010), whereas biosynthesis of gymnodinosterol and brevesterol [(24*R*)-4α-methyl-5α-ergosta-8(14),22-dien-3β-ol and its 27-nor derivative, respectively] is limited primarily to the dinoflagellate genera *Karenia*, *Karlodinium*, and *Takayama* (Leblond and Chapman, 2002). A few dinoflagellates also contain steroid ketones, usually with a predominance of dinosterone (Withers *et al*., 1978; Kokke *et al*., 1982).

Sterols can exist in different biochemical forms. Most occur mainly in the free (i.e., non-esterified) form, but there are also smaller amounts esterified to fatty acids (FAs) or bound to sugars, and in some organisms steryl sulfates have been found (Volkman *et al*., 1999). Sterols in sediments are also known to occur bound to the macromolecular structure of kerogen by ester, ether or

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Table 1. Total organic carbon (TOC), sulfur content and extract yield of free and bound alcohol fractions

Well	Depth (m)	TOC (%)	S_{tot} (%)	Extract (mg/g C_{org})	Fraction (%)*			
					Free alcohols	Neutrals (Esters + ketones)	Ester-bound alcohols	Kerogen-bound alcohols
NR-10	151.5	12.3	1.7	206	23	8.9	0.3	0.22

**Relative to the total extractable bitumen.*

sulfur bonds (Schaeffer *et al*., 1995; Amblès *et al*., 1996). In a tightly bound state, sterols are considered less susceptible to diagenetic transformation than free sterols and are thought to be better indicators of the original algal sterol source than the free sedimentary sterols (Pearce *et al*., 1998). The mode of occurrence of sterols may therefore also provide information on the diagenesis of sedimentary OM, as has been demonstrated for FAs (Barakat and Rullkötter, 1995a).

The present study complements previous investigations of sulfur-rich lacustrine sediments from the Nördlinger Ries, southern Germany. In this study, a single sample was selected to generally characterize a specific compound group (sterols) not analyzed before in the Nördlinger Ries black shales. The distributions of free sterols were compared with the corresponding distributions of esterified and kerogen-bound sterols. The bound sterols were liberated by alkaline hydrolysis of the neutral polar fraction and of the isolated kerogen. The purpose was to provide information on the extractable free sterols and on sterol entities bound through ester linkages to the periphery of the macromolecular structures of kerogen and bitumen-derived asphaltenes obtained from the same rock, and to gain some insight into the sources, pathways and diagenesis of these compounds.

EXPERIMENTAL

Samples

The geological setting and stratigraphy of the Nördlinger Ries sediments have been reported (Rullkötter *et al*., 1990). Black shales in the Miocene crater lake of the Nördlinger Ries were deposited under largely stagnant conditions in slightly saline, sulfate-rich water in an arid climate. The sample for this study was selected from well NR-10 in the center of the small basin and contained 12.3% organic carbon and 1.7% total sulfur, of which a significant portion was bound to OM (Table 1).

Analysis

The pulverized sediment (140 mesh) was exhaustively extracted in a Soxhlet apparatus with CH_2Cl_2–MeOH (99/1 v/v). The extractable lipids were separated via medium pressure liquid chromatography (MPLC) over silica gel in three steps into the following classes: non-aromatic hydrocarbons, aromatic hydrocarbons, free alcohols, free carboxylic acids, bases, free alcohols, neutral polar fraction (esters + ketones) and a highly polar fraction (Radke *et al*., 1980; Barakat and Rullkötter, 1995a). The neutral polar fraction was saponified using 40 ml of 0.2% KOH (w/v) in MeOH/H_2O (97:3 by stirring (1 h) at reflux temperature under N_2. The extract was separated using MPLC to afford ketones, ester-bound acids and ester-bound alcohols. The procedures for isolation of the kerogen concentrate, kerogen saponification and chromatographic isolation of kerogen-bound alcohols have been described (Barakat and Rullkötter, 1995a).

Derivatization

The free alcohols, ester-bound alcohols and kerogen-bound alcohol fractions were derivatized with a mixture of 50 μl of bis-*N*,*O*-(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS; Supelco) and 50 μl acetone for 2 h at 70°C. Immediately prior to analysis, the derivatizing agent was evaporated under N_2 and the residue was taken up in a mixture of EtOAc and BSTFA and analyzed via gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).

Instrumentation

GC analysis was carried out with a Hewlett Packard 5890 series II instrument equipped with a temperature programmable injector system (Gerstel KAS 3) and a flame ionization detector (FID). A DB-5 (J&W) fused silica column (30 m × 0.25 mm i.d., *df* = 0.25 pm) was used with He as carrier gas. Samples (in EtOAc were injected at 60°C. The temperature in the GC oven was programmed from 60 (held 1 min) to 300°C (held 30 min) at 3°C min^{-1}. The injector temperature was programmed from 60°C (5 s hold time) to 300°C (60 s hold time) at 8°C s^{-1}.

GC/MS measurements were performed with the same type of GC system under the above conditions except that an Ultrix (Hewlett-Packard) column (50 m × 0.32 mm i.d., *df* = 0.17 μm) was used. The gas chromatograph was coupled to a Finnigan SSQ 710B mass spectrometer operated at 70 eV with a scan range *m*/*z* 50 to 600 and scan

time of 1 scan s^{-1}. The temperature program was identical to that described before.

Sterol TMS ethers were tentatively assigned by comparison of relative retention times and mass spectra with those reported in the literature (Yamauchi and Matsushita, 1979; McEvoy, 1983; Volkman, 1986; Bayona *et al*., 1989; Hudson *et al*., 2001; Rontani and Volkman, 2003; Sangiorgi *et al*., 2005) and by MS interpretation. In particular, for sterols and steroidal ketones of dinoflagellates, comparison was made with GC/MS data reported by Harvey *et al*. (1988), Mansour *et al*. (1999) and Leblond and Chapman (2002, 2004).

Results and Discussion

Backgound information from previous studies

The total organic carbon (TOC) and sulfur contents, as well as extract yield of free and bound alcohol fractions for the lacustrine source rock sample are summarized in Table 1. The TOC content and the total extract yield, normalized to TOC, are considerably higher than would commonly be expected for immature OM at shallow depth. Such elevated bitumen content is common for OM in carbonates, evaporites and diatomites, all depleted in clay minerals, and is associated with the high sulfur content of fossil OM in sediments deposited under strongly reducing conditions, when microbial sulfate reduction provides an excess of reactive inorganic sulfur species and Fe supply is limited (Type II-S kerogen). Studies have indicated that the Nördlinger Ries sediment sample displays a unique chemistry. This includes abnormally high concentration of free FAs (nearly 28% of total extractable bitumen) with an abundant *n*-C_{16} acid followed by *n*-C_{18} acid, and very strong even/odd carbon number predominance (Barakat and Rullkötter, 1994) consistent with an origin mainly from plankton and bacteria and an early diagenetic stage. The non-aromatic hydrocarbon fraction was dominated by the *n*-C_{15} and *n*-C_{17} alkanes from submerged higher plants, phytane (Pr/Ph 0.5), cholest-4- and -5-enes and the C_{30}–C_{35} series of extended hop-17(21)-enes. In addition, three less common series of bound 3β-carboxy steroids (including a C_{29}–C_{31} 4-methyl carboxy steroid series) were identified in the kerogen hydrolysate and extractable polar fraction (Barakat and Rullkötter, 1995a). Aromatic hydrocarbons were characterized by a distinct distribution of sulfur-bearing compounds, dominated by thiolanes and thiophenes possessing the normal or isoprenoid carbon skeletons (Barakat and Rullkötter, 1995b). The "neutral polar" fraction, representing *ca*. 10% of the extractable lipids, was enriched in isoprenoid and steroid ketones (mainly 3- or 7-ketosteroids) and contained abundant steroid thiols (including C_{28}–C_{30} 4-methyl steroid thiols), methylated chromans, tocopherols and related compounds confirming deposition under saline conditions (Barakat and Rullkötter, 1997, 1999). The occurrence of 4-methyl steroid thiols and the 4-methyl carboxy steroid series provided initial evidence that dinoflagellates may have been among the source organisms for the OM. The Nördlinger Ries black shale also contained several series of alkylated phenols not reported before to occur in sediments (Barakat *et al*., 2012). Nevertheless, kerogen microscopy did not identify the type of algae in the black shale kerogen, which consisted of brightly fluorescing alginite mostly <20 μm, and it was not clear if the alginite was derived from algal blooms or benthic algal mats (Rullkötter *et al*., 1990).

Sterols and steroidal ketones

Total ion chromatograms (TICs) of the silylated products of extractable free alcohols as well as bound alcohols released by saponification from the neutral polar fraction and the kerogen are shown in Fig. 1. The structural attribution of the principal GC-MS peaks is presented in Table 2. The relative proportions of steroids in the three fractions are compared in Fig. 2. The results in Fig. 1 show that there is a general similarity in the predominance of steroidal components in the free, and ester- and kerogen-bound alcohol fractions. With our liquid chromatography method, both sterols and steroid ketones occurred in the so-called free alcohol fraction. Similar observations were made in a survey of the sterol composition of marine dinoflagellates (Leblond and Chapman, 2002, 2004) and in the work of Robinson *et al*. (1987), who noted that steroid ketones chromatographically fractionate between sterol esters and the more polar free sterols. In addition, Withers *et al*. (1978) reported that steroid ketones migrate between hydrocarbons and more polar free sterols in a TLC separation system and Kokke *et al*. (1982) noted the elution of steroid ketones before free sterols using an HPLC separation system.

As shown in Fig. 1a, the most intense peaks in the free sterol fraction, besides diethylene glycol bis-trimethylsilyl ether, are associated with 4-methyl steroid ketones. They are dominated by 4α,24-dimethyl-5α-cholestan-3-one (compound V), followed by 4α,23,24-trimethyl-5α-cholestan-3-one (dinostanone, VII) and 4α,23,24-trimethyl-5α-cholest-22*E*-en-3-one (dinosterone, VI). In addition, 5β-cholestan-3-one (I), 5α-cholestan-3-one (II), 24-methyl-5α-cholestan-3-one (III) and 23,24-dimethyl-5α-cholest-22*E*-en-3-one (IV) were present in minor amounts. On the other hand, three major sterols were found: 4α,24-dimethyl-5α-cholestan-3β-ol (L, Fig. 1a), 4α,23,24-trimethyl-5α-cholest-22*E*-en-3β-ol (dinosterol, M), and 4α,23,24-trimethyl-5α-cholestan-3β-ol (dinostanol, N). Of the three, 4α,24-dimethyl-5α-cholestan-3β-ol was the most abundant component, while dinostanol was the second-most abundant one (Fig. 2). No other sterols were detected.

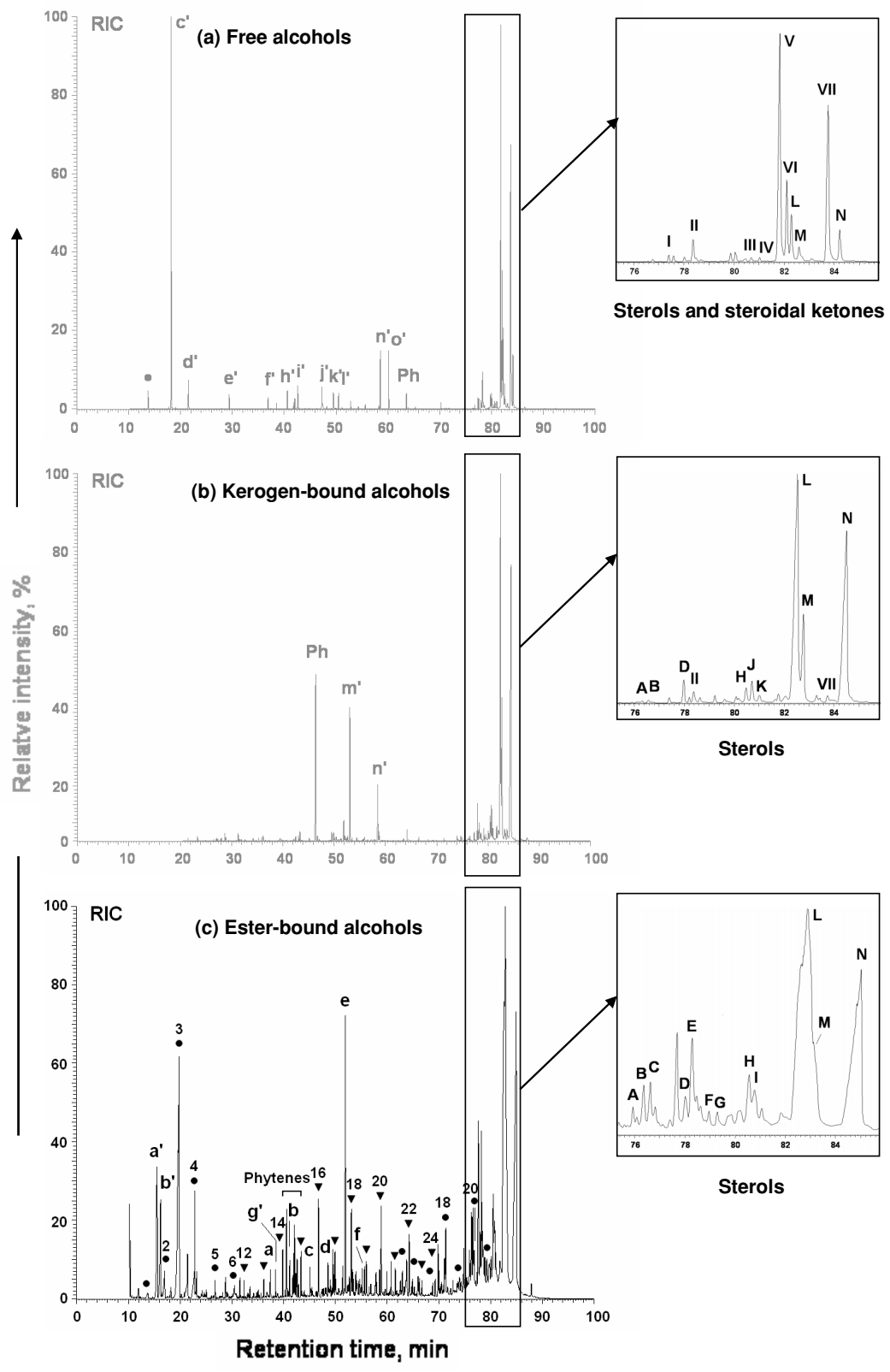

Fig. 1. TICs of silylated (a) free alcohol fraction; (b) kerogen-bound alcohol fraction and (c) ester-bound alcohol fraction of a Nördlinger Ries sediment from 151.5 m depth of well NR-10. Triangles and circles indicate the homologous series of n-alkanols and alkylated phenols, respectively; numbers correspond to the number of carbon atoms. Lettered peaks refer to compounds listed in Table 2 (Ph, phthalate).

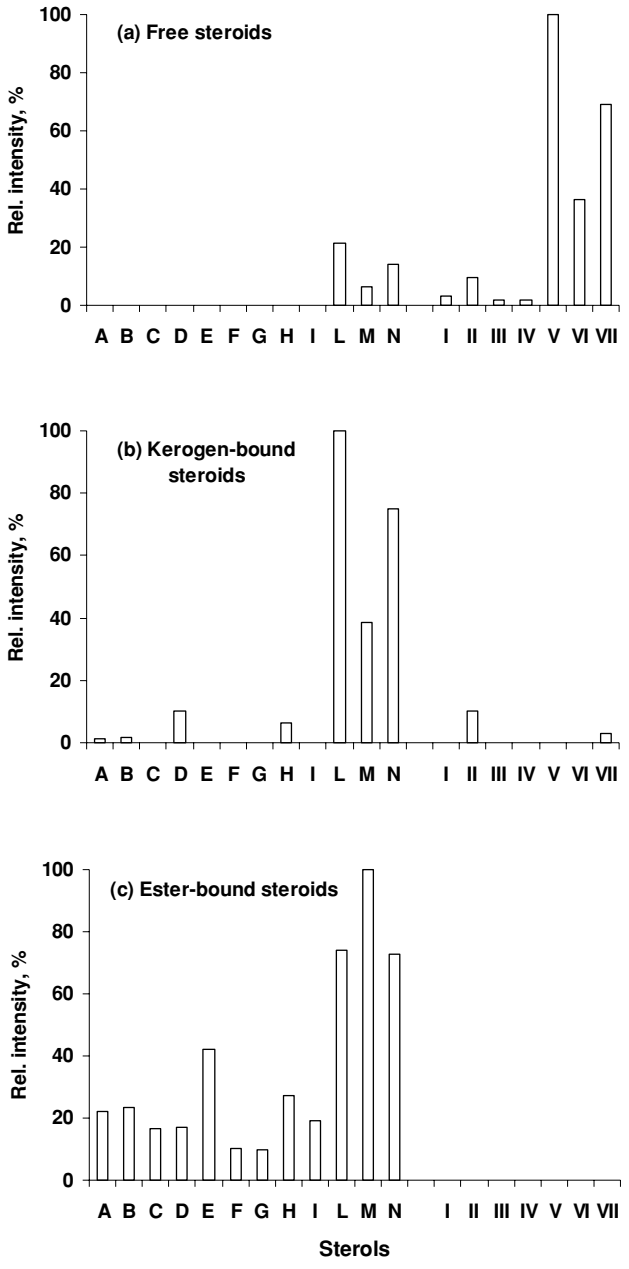

Fig. 2. Comparison of relative composition of steroid alcohols in the three geolipid fractions in a Nördlinger Ries sediment from 151.5 m depth of well NR-10. Labeled peaks refer to compounds in Table 2.

The composition of the sterols bound to kerogen was very similar to that of the free sterols (Fig. 2). The occurrence of sterols in the saponification products of the kerogen most likely results from the cleavage of ester bonds (known to contribute to a large extent in the cross-linking of immature macromolecular material; e.g., Rullkötter and Michaelis, 1990). The relative proportions of the dominant sterols were similar to those in the free sterol fraction: 4α,24-dimethyl-5α-cholestan-3β-ol > 4α,23,24-trimethyl-5α-cholestan-3β-ol > 4α,23,24-trimethyl-5α-cholest-22*E*-en-3β-ol (Fig. 2), which are also the predominant components in many dinoflagellates. Worth noting in the kerogen-bound sterols is the presence of cholest-5-en-3β-ol (cholesterol, D, Fig. 1b) found to commonly occur in many dinoflagellates (Volkman *et al.*, 1998; Leblond and Chapman, 2002). The unsaturated analogue of L (4α,24-dimethyl-5α-cholest-22*E*-en-3β-ol, peak H, Fig. 1b) was found in small amount. Liberation of the kerogen-bound alcohol fraction also afforded small relative amounts of four steroid ketones, i.e., 5β-cholestan-3-one, 5α-cholestan-3-one, 4α,24-methyl-5α-cholestan-3-one, and 4α,23,24-trimethyl-5α-cholestan-3-one (I, II, V, and VII, Fig. 1b) which were most likely entrapped in the molecular sieve-type network of the kerogen.

The composition of the ester-bound sterols appeared to be more complex than those of the free and kerogen-bound sterols (Figs. 1c and 2). A diverse range of sterols and their corresponding stanols was identified. The distribution of the three most abundant sterols was different from those in the free and kerogen-bound sterols. The C_{29} sterol 4α,24-dimethyl-5α-cholest-22*E*-en-3β-ol was also present in small relative amount. In addition, a number of 4-desmethyl C_{27} and C_{29} sterols were present in low abundance. These included 5β-cholestan-3β-ol, 5β-cholestan-3α-ol, cholest-5-en-3β-ol (cholesterol), 5α-cholestan-3β-ol, 23,24-dimethyl-5α-cholest-22*E*-en-3β-ol, 24-ethyl-5α-cholest-22*E*-en-3β-ol, and 24-ethyl-5α-cholestan-3β-ol (see Fig. 1c and Table 2).

Acyclic alcohols and isoprenoid compounds

GC-MS analysis of the ester-bound alcohols revealed that the fraction was dominated by acyclic alcohols and several (pseudo)homologous series of bound alkylated phenols (Fig. 1c)—rather than sterols. The distribution and origin of the alkylated phenol series has been discussed (Barakat *et al.*, 2012). Acyclic alcohols included an abundant series of *n*-alkanols (C_{12}–C_{24}). Expanded sections of the *m/z* 271, 285, 299, 313, 327, 341, 355, and 369 chromatograms of the derivatized (TMS) "ester-bound alcohol" fraction representing the distribution of C_{14}–C_{21} *n*-alkanols is shown in Fig. 3. The series is characterized by a unimodal distribution around C_{16}, with a strong even predominance (Fig. 1c), which is indicative of an autochthonous origin of the OM (Fernandes *et al.*, 1999). On the other hand, the acyclic isoprenoid alcohols are dominated by 3,7,11,15-tetramethylhexadecan-1-ol (dihydrophytol, e, Fig. 1c). There is a discrepancy of views about the origin of this isoprenoid compound in sediments (for a review see Rontani and Volkman, 2003). It has often been considered a reduction product of free

Table 2. Assignment and MS characteristics of peaks labelled peaks in Fig. 1

	Compound	MS characteristics
C_{27} sterols (as TMS ethers)		
A	5β-Cholestan-3β-ol	M^+ 460(–), 370(100), 355(25), 257(14), 215(29), 75(38)
B	5β-Cholestan-3α-ol	M^+ 460(24), 370(97), 355(54), 257(25), 215(100), 75(67)
C	Isomer of *n*	M^+ 460(1), 370(100), 355(23), 257(20), 215(54), 75(40)
D	Cholest-5-en-3β-ol (cholesterol)	M^+ 458(75), 368(87), 329(80), 129(100), 353(34), 73(96)
E	5α-Cholestan-3β-ol + tocopherol + unidentified compound	M^+ 460(39), M^+ 430(19), 445(27), 370(14), 355(16), 221(100), 215(40), 178(80), 165(39), 75(52)
C_{29} sterols (as TMS ethers)		
F	23,24-Dimethyl-5α-cholest-22*E*-en-3β-ol + Tocopherol	M^+ 486(12), M^+ 430(16), 345(28), 374(24), 257(38), 165(25), 73(49), 69(100)
G	24-Ethyl-5α-cholest-22*E*-en-3β-ol	M^+ 486(7), 374(16), 345(23), 257(33), 73(59), 69(100)
H	4α,24-Dimethyl-5α-cholest-22*E*-en-3β-ol	M^+ 486(21), 388(31), 271(33), 69(100)
I	C_{29} Stera-22*E*-en-3β-ol + C_{29} steran-3β-ol	M^+ 488(5), M^+ 486(12), 398(38), 359(23), 345(25), 271(16), 257(20), 229(22), 73(60), 69(100)
J	C_{30} stera-5,22*E*-dienol + C_{29} sterol	M^+ 498(11), M^+ 488(5), 384(37), 398(67), 473(37), 359(39), 229(47), 129(44), 75(90), 69(100)
K	isomer of s	M^+ 486(7), 388(18), 359(22), 271(55), 69(100)
L	4α,24-Dimethyl-5α-cholestan-3β-ol	M^+ 488(26), 473(23), 398(76), 359(83), 229(39), 75(100)
C_{30} sterols		
M	4α,23,24-Trimethyl-5α-cholest-22*E*-en-3β-ol (dinosterol)	M^+ 500(13), 388(23), 359(53), 271(29), 69(100)
N	4α,23,24-Trimethyl-5α-cholestan-3β-ol (dinostanol)	M^+ 502(29), 487(23), 412(81), 373(77), 229(45), 75(100)
Steroidal ketones		
I	5β-Cholestan-3-one	M^+ 386(100), 316(33), 231(71)
II	5α-Cholestan-3-one	M^+ 386(59), 232(71), 231(100), 217(25)
III	24-Methyl-5α-cholestan-3-one	M^+ 400(69), 232(59), 231(100)
IV	23,24-Dimethyl-5α-cholest-22*E*-en-3-one	M^+ 412(2), 300(14), 273(20), 271(49), 69(100)
V	4α,24-Dimethyl-5α-cholestan-3-one	M^+ 414(68), 399(14), 246(67), 245(100)
VI	4α,23,24-Trimethyl-5α-cholest-22*E*-en-3-one (dinosterone)	M^+ 426(8), 383(4), 314(20), 287(28), 285(50), 69(100)
VII	4α,23,24-Trimethyl-5α-cholestan-3-one (dinostanone)	M^+ 428(82), 413(13), 246(60), 245(100), 231(24)
Isoprenoidyl alcohols (as TMS ethers)		
a	3,7,11-Trimethyldodecan-1-ol	M^+ 300(–), 285(42), 75(100), 73(98)
b	4,8,12-Trimethyltridecan-1-ol	M^+ 314(–), 299(44), 75(100), 73(91)
c	6,10,14-Trimethylpentadecan-2-ol	M^+ 342(–), 327(2), 117(100), 75(39), 73(38)
d	3,7,11,15-Tetramethylpentadecan-1-ol	M^+ 356(1), 341(85), 299(14), 103(100), 75(78), 73(83)
e	3,7,11,15-Tetramethylhexadecan-1-ol (dihydrophytol)	M^+ 370(–), 356(25), 355(100), 280(10), 57(97)
f	3,7,11,15-Tetramethylheptadecan-1-ol + *iso*- or *anteiso*-C_{19} *n*-alkanol	M^+ 384(1), 369(12), 341(100), 103(56), 75(83)

	Compound	MS characteristics
	Other compounds identified	
a′	1,2-Butanediol bis(trimethylsilyl)-ether*	M^+ 234(–), 245(8), 147(27), 132(21), 131(100), 75(21), 73(94)
b′	4-Methyl-2-penten-2,4-diol bis(trimethylsilyl)-ether)*	M^+ 260(1), 245(100), 151(24), 147(22), 75(15), 73(56)
c′	Diethylene glycol, ditrimethylsilyl ether	M^+ 250(–), 147(17), 117(44), 116(26), 73(100)
d′	Amine compound* (contaminant)	197(15), 196(100), 75(12), 67(9)
e′	Hydroxydibutyltoluene (contaminant)	M^+ 220(29), 206(14), 205(100), 145(7)
f′	Rubber compound (contaminant)	180(10), 123(12), 57(12), 97(100), 83(14), 57(53), 55(16)
g′	Pristene**	M^+ 266(5), 126(34), 83(47), 71(57), 70(75), 69(100)
h′	Phytene**	M^+ 280(12), 111(34), 83(42), 70(81), 69(76), 57(100)
i′	C_{15} unsaturated Me ketone*	M^+ 210(1), 71(70), 58(100), 57(67)
j′	Secondary aliphatic alcohol*	125(3), 111(3), 97(4), 83(6), 73(100), 57(14)
k′	Hexadecanoic acid, trimethylsilyl ester	M^+ 328(11), 313(74), 132(69), 117(76), 73(100)
l′	Polyethylene glycol trimethylsilyl ether*	172(58), 116(90), 112(69), 101(48), 73(100), 69(50)
m′	Unidentified	378(29), 327(29), 183(46), 125(100), 75(36), 73(84)
n′	4-Hydroxy-4,8,12,16-tetramethylheptadecanoic acid γ-lactone	M^+ 324(3), 99(100)
o′	Unidentified	299(20), 227(28), 199(44), 125(41), 57(100)
p′	α-tocopheryl quinone trimethylsilyl ether	341(62), 294(25), 293(100), 250(18), 75(40)

**Tentative assignment. **Resumably produced by thermal elimination of TMSOH from pristanol/phytanol during injection.*

Fig. 3. Expanded sections of m/z 271, 285, 299, 313, 327, 341, 355, and 369 ion chromatograms of the derivatized (TMS) "ester-bound alcohol" fraction showing the distribution of C_{14}–C_{21} n-alkanols. Numbers correspond to the number of carbon atoms. Labeled peaks correspond to isoprenoid alcohols (cf., Table 2 for identification).

phytol (van Vleet and Quinn, 1979) and was proposed to be a biomarker for reducing conditions during early diagenesis (de Leeuw *et al.*, 1977). It has also been suggested that its occurrence in sediments may be related to a direct input of lipids from archaea (Volkman and Maxwell, 1986) or to reduction of the chlorophyll *a* phytyl side chain during digestion by animals (e.g., Sun *et al.*, 1998). In view of the high salinity of the lake water during sediment deposition (Barakat and Rullkötter, 1997), the presence of dihydrophytol is likely to be the result of a direct input from halophilic archaea.

The isoprenoids 3,7,11-trimethyldodecan-1-ol, 4,8,12-trimethyltridecan-1-ol, 6,10,14-trimethylpentadecan-2-ol, 3,7,11,15-tetramethylpentadecan-1-ol, 3,7,11,15-tetramethylheptadecan-1-ol, isomeric phytenes, and pristene were found in the ester-bound alcohol fraction in relatively small amount (Fig. 1c and Table 2). Most of these compounds probably arise from degradation of phytol (Rontani and Volkman, 2003). Isomeric phytenes may be produced from phytol by clay-catalyzed degradation (de Leeuw *et al.*, 1977) or by biodegradation under

sulfate-reducing conditions (Grossi *et al*., 1998; Schulze *et al*., 2001). Considering the strong sulfate-reducing conditions in the Miocene crater lake of the Nördlinger Ries, we attribute formation of the phytenes to the biodegradation of phytol by sulfate reducers.

The presence of 6,10,14-trimethylpentadecan-2-ol is noteworthy. It was previously suggested that it is produced via reduction of the corresponding ketone, 6,10,14-trimethylpentadecan-2-one, produced during anaerobic phytol biodegradation (Brooks *et al*., 1978). This assumption was then well supported by the detection of this compound during anaerobic biodegradation of phytol and 6,10,14-trimethylpentadecan-2-one (Rontani *et al*., 1997, 1999). On the other hand, it was indicated that the likely route of formation of 4,8,12-trimethyltridecan-1-ol in sediments was via aerobic biodegradation of 6,10,14-trimethylpentadecan-2-one (Rontani *et al*., 1997) and phytol (Rontani *et al*., 1999) and was proposed to constitute a useful marker of aerobic bacterial biodegradation (Rontani and Volkman, 2005).

Surprisingly, we also detected a C_{21} γ-lactone (4-hydroxy-4,8,12,16-tetramethylheptadecanoic acid γ-lactone, or homophytanic acid γ-lactone), unrelated to phytol, in significant amounts in the free and kerogen-bound alcohol fractions (*n′*, Figs. 1a and b). The mass spectrum exhibits a base peak at *m/z* 99, M^+. at *m/z* 324 and a fragmentation pattern similar to that of the C_{21} lactone investigated by Rontani *et al*. (2007). The C_{21} isoprenoid γ-lactone was previously detected in various sediment (Schwarzbauer *et al*., 2000; Al-Mutlaq *et al*., 2008) and microbial mat samples (Rontani and Volkman, 2003) and obviously originates from isoprenoid species of a higher carbon number than phytol. The presence of this uncommon γ-lactone is in good agreement with the detection of α-tocopheryl quinone trimethylsilyl derivative (compound *p′* in Table 2); both are interpreted to be derived from the oxidation of vitamin E (Rontani *et al*., 2007).

Geochemical implications

The distribution of sterols in the three fractions was dominated by dinosterol, 4α,24-dimethyl-5α-cholestan-3β-ol, and dinostanol. 4-Methyl sterols are dominant components of both recent lacustrine (e.g., Robinson *et al*., 1984) and marine environments (e.g., de Leeuw *et al*., 1983), perhaps due to the wide salinity tolerance of dinoflagellates (Wall and Dale, 1974). For example, the sterol composition of *P. piscicida* consists almost entirely of a number of 4α-methyl sterols, such as dinosterol and dinostanol, widely distributed natural products within the class Dinophyceae (Leblond and Chapman, 2004).

The composition of the free and kerogen-bound sterols was dominated by the C_{29} sterol, 4α,24-dimethyl-5α-cholestan-3β-ol. Its presence as a major sterol in gymnodinoid dinoflagellates was observed by Withers (1987) and Mansour *et al*. (1999). However, the dominant sterol released from hydrolysis of the neutral polar fraction was the C_{30} sterol, dinosterol. Because of its wide distribution in dinoflagellates, it has been suggested to be a biomarker representative of the class Dinophyceae as a whole (Leblond and Chapman, 2004). Dinostanol has been found as an abundant component of several dinoflagellates such as *Pfiesteria piscicida* (Leblond and Chapman, 2004), *L. chlorophorum* (Leblond and Lasiter, 2012), *Scrippsiella trochoidea* (Harvey *et al*., 1988; Leblond and Chapman, 2002), *Gonyaulax polygramma* (Volkman *et al*., 1984), and, most of all, *Gymnodinium* spp. (Piretti *et al*., 1997).

Other minor 4-methyl sterols typical of dinoflagellates, e.g., 4α,24-dimethyl-5α-cholest-22*E*-en-3β-ol, were tentatively assigned in the ester- and kerogen-bound fractions, confirming the contribution of dinoflagellates to the pool of sedimentary OM. This sterol was found in trace amounts in the free sterol fraction of *P. piscicida* (Leblond and Chapman, 2002). C_{27} 4-desmethyl sterols, such as cholest-5-en-3β-ol (cholesterol), commonly found in many dinoflagellates (Volkman *et al*., 1998; Leblond and Chapman, 2002; Mansour *et al*., 2003), were also released from the neutral polars and kerogen by hydrolysis. Notably absent, however, were C_{28} sterols and 4α,23,24-trimethyl-5α-cholest-8(14)-en-3-one, 4α,23,24-trimethyl-5α-cholest-24(28)-en-3β-ol found in *P. piscicida* (Leblond and Chapman, 2004). A surprising feature is the high proportion of species lacking a nuclear double bond, possibly a consequence of post-sedimentary diagenetic reduction of the original biogenic sterols. Other common features were the presence of 23,24-dimethyl alkylation and unsaturation at Δ^{22} in the side chain.

The predominance of 4-methyl sterols likely indicates a significant dinoflagellate contribution, although certain diatoms have been found to synthesize these sterols as well (Volkman *et al*., 1993). Dinosterol, dinostanol, and 4α,24-dimethyl-5α-cholestan-3β-ol have been identified in a laboratory culture of a marine diatom, *Navicula* sp. (Volkman *et al*., 1993). Given the diversity of sterols present in diatoms (e.g., Giner and Wikfors, 2011) and the importance of diatoms as a source of organic matter, one cannot rule out a minor contribution of 4-methyl sterols from diatoms to the OM in the Nördlinger Ries sediment.

Steroid ketones represented most of the compounds in the free sterol fraction (Fig. 1). Three major ones were 4α,24-dimethyl-5α-cholestan-3-one, dinosterone and dinostanone. All have been observed previously in the heterotrophic dinoflagellate *P. piscicida* (Dinophyceae) (Leblond and Chapman, 2004) as well as in a number of other dinoflagellates (Withers *et al*., 1978; Kokke *et al*.,

1982; Robinson *et al*., 1987; Withers, 1987; Harvey *et al*., 1988; Volkman *et al*., 1999; Mansour *et al*., 1999). The structures correspond to the major sterols in the three geolipid fractions, suggesting that the sterols and steroidal ketones are biosynthetically linked to each other. It is also interesting to note that their relative abundance appears to be strikingly similar to that of the corresponding methyl sterols in both the free and kerogen-bound sterol fractions. Assignment of the steroidal ketones provides further evidence that dinoflagellates make a significant contribution to the sedimentary lipids in the Nördlinger Ries sediment.

CONCLUSIONS

The profile of free, esterified and kerogen-bound sterols of the bituminous sediment from the Nördlinger Ries in southern Germany is characterized by a distinctive abundance of 4α,24-dimethyl-5α-cholestan-3α-ol, dinosterol, and dinostanol. The occurrence of sterols in the saponification products of the kerogen most likely results from the cleavage of ester bonds known to contribute to a large extent to the cross-linking of immature macromolecular organic material. A diverse range of esterified sterols from eukaryotic sources is also present. These include 5β-cholestan-3β-ol, 5β-cholestan-3α-ol, cholest-5-en-3β-ol (cholesterol), 23,24-dimethyl-5α-cholest-22*E*-en-3β-ol, and 4α,24-dimethyl-5α-cholest-22*E*-en-3β-ol. The high content of both 4-methyl sterols and the corresponding ketones attests to a prevalent deposition of autochthonous aquatic OM under saline conditions, as does the presence of *n*-alkanols and isoprenoid alcohol homologues. A surprising feature of the sterol distributions is the high proportion of species lacking a nuclear double bond, possibly a consequence of post-sedimentary diagenetic reduction of the original biogenic sterols. The overall fossil sterol assemblages are fully consistent with a lacustrine origin and a major contribution of dinoflagellates, although a minor addition from diatoms cannot be ruled out. Contributions from dinoflagellates are also displayed by a high abundance of 4α,24-dimethyl-5α-cholestan-3-one, dinosterone, and dinostanone in the kerogen-bound fraction, which were most likely entrapped in the molecular sieve-type network. The striking similarity of structures and relative abundance of major 4-methyl sterols and the corresponding ketones suggests that they are biosynthetically linked.

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