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Structural identification of steryl alkyl ethers in marine sediments

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Abstract

Steryl alkyl ethers have been reported to occur in immature marine sediments up to Cretaceous age. Here, we report their unambiguous structural identification in Holocene sediments from the Arabian Sea, Pleistocene sediments from the southeast Atlantic and a Miocene sediment from the Monterey formation through chemical degradation experiments and synthesis. We show that the main steryl alkyl ethers in sediments from the southeast Atlantic are cholest-5-enyl 3β-(2-dodecanyl) ether and cholest-5-enyl 3β-(3-dodecanyl) ether together with their 24-methyl and 24-ethyl pseudo-homologues. Steryl alkyl ethers in sediments from the Arabian Sea are dominated by cholest-5-enyl 3β-(5-decanyl) ether and cholest-5-enyl 3β-(4-decanyl) ether together with their 24-methyl and 24-ethyl pseudo-homologues. The structures suggest that these compounds are formed by direct biosynthesis by as yet unknown marine organisms.
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1. Introduction

Sterols are ubiquitous compounds in eukaryotic organisms. They and their diagenetic derivatives are frequently encountered in sediments and ancient rocks. They are often used to infer the contribution of specific groups of algae, heterotrophs or higher plants (e.g., Volkman, 1986, 1999). In immature sediments, sterols are predominantly present in both free and ester-bound form. Sedimentary compounds also containing steryl moieties include steryl chlorins, where sterols are esterified with chlorophyll breakdown products through herbivory (Eckardt et al., 1992; Talbot et al., 2000).

An unusual group of compounds containing steryl moieties in immature sediments are the steryl alkyl ethers. They were first reported by Boon and de Leeuw (1979) in sediments from Walvis Bay, based on comparison with a published mass spectrum of cholesteryl 1-hexadecyl ether (Funasaki and Gilbertson, 1968). Mass spectral characteristics indicate that the steryl alkyl ethers in Walvis Bay consist of C_{27}–C_{29} sterols with 1-2 double bonds, that are ether-bound to C_8–C_9 alkyl chains. Schouten et al. (2000) reported the presence of C_{27}–C_{29} sterols ether-bound predominantly with C_{10} alkyl chains in sediments from the Arabian Sea. Based on the different carbon number distributions of the free sterols and the sterol moieties of the alkyl ethers these authors suggested that the compounds represented a direct input, rather than products of secondary metabolism, as is the case for steryl chlorins (Eckardt et al.,

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1992). Since steryl alkyl ethers are mainly found in sediments with high diatom inputs, Schouten et al. (2000) suggested that diatoms may be the source. Indeed, Schefuß et al. (2001) and Marlow et al. (2001) found steryl alkyl ethers in relatively high abundance in sediments deposited in highly productive upwelling areas in the southeast Atlantic. They have also been found in more ancient immature sediments. For example, they are present in immature Pleistocene to Miocene sediments from the Japan Trench (Brassell et al., 1980). Recently, their presence in immature early Cretaceous samples from the Pacific Ocean was reported (Brassell, 2003).

Despite the fact that these compounds have been regularly encountered in sediments, their exact structures have not been determined. Here, we report the detailed characterization of the structures of some of the dominant sedimentary steryl alkyl ethers, which are substantially different from structures previously proposed tentatively.

2. Methods

2.1. Samples

The sediments were: a Holocene sediment from the Arabian Sea, Pleistocene sediments from the southeast Atlantic and a Miocene sediment from the Monterey formation. The Arabian Sea sediment is a composite of the top 50 cm of a box core taken at NIOP Site 309 in the Arabian Sea taken during the 1992–1993 expedition of the R.V. Tyro in the Indian Ocean (Netherlands

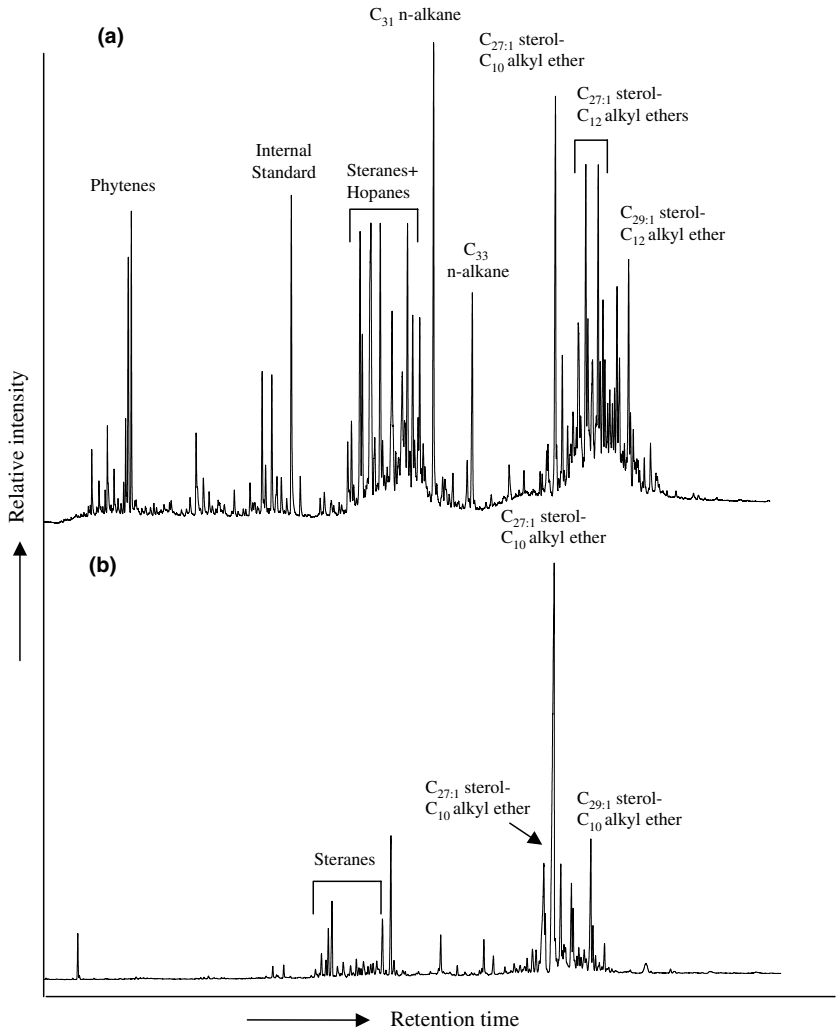

Fig. 1. Gas chromatograms of: (a) the apolar fraction of sediment extract from ODP 1084 core at 386.9 mbsf depth and (b) steryl alkyl ether fraction of the extract of the NIOP 309 sediment. Numbers with the sterol moiety of the steryl alkyl ethers indicate carbon number and number of double bonds, respectively. Number with alkyl moiety indicates number of carbon atoms.

Indian Ocean Program; Van Weering et al., 1997). The southeast Atlantic sediments are from ODP leg 175, site 1084A at Lüderitz Bay (Schefuß et al., 2001). The Miocene sediment from the Monterey formation is sample KG-1 from the Cooperative Monterey Organic Geochemistry Study taken at an outcrop section at Naples Beach (Isaacs and Rullkötter, 2001).

2.2. Extraction and fractionation of soluble organic matter

Sediment samples were freeze-dried and ground using a mortar and pestle. The NIOP 309 sediment and Monterey formation sediment were Soxhlet extracted with dichloromethane (DCM)/methanol (7.5:1, v/v) for 24 h, whilst the ODP 1084 sample was ultrasonically extracted using DCM (×3), methanol/DCM (1:1, v/v, ×3) and methanol (×3). The extracts were concentrated with a rotary evaporator at 30 °C. A part of each extract was separated using a column (25 cm × 2 cm i.d.; column volume 35 mL) packed with alumina (activated for 2.5 h at 120 °C). Apolar fractions were obtained by elution with hexane/DCM (9:1, v/v). The apolar fraction of NIOP 309 was subsequently separated by first eluting with hexane to yield the aliphatic fraction and then eluting with hexane/DCM (9:1, v/v) to obtain a more polar fraction which contained the steryl alkyl ethers. The apolar fraction of the extract from the Monterey formation sediment was separated using argentation thin layer chromatography with hexane as developing solvent as described by Schouten et al. (2001). The fractions which contained the sterol ethers were analysed using gas chromatography (GC) and GC–mass spectrometry (MS) and subjected to chemical degradation.

2.3. Chemical degradation

Fractions containing high amounts of steryl alkyl ethers were hydrogenated with H_2/PtO_2 in ethyl acetate containing a few drops of acetic acid for 1 h at room temperature. Acetic acid and catalyst were removed by filtration over a column filled with $MgSO_4$ and Na_2CO_3. The fractions were analysed using GC and GC–MS to confirm the full hydrogenation of the double bonds of the steryl alkyl ethers. The hydrogenated fractions were subsequently refluxed for 1 h in 56 wt% (in H_2O) HI to cleave the ether bond of the steryl ethers. The reaction mixture was washed with water and a 5% solution of NaS_2O_3 in water and taken up in hexane. The reaction mixture was filtered over a column filled with $MgSO_4$ to remove water. The alkyl iodides were then converted to alkyl methyl thioethers by stirring for 24 h with $NaSCH_3$ in methanol. Hexane was added and the reaction mixture was washed with water and filtered over a column filled with $MgSO_4$ to remove water. The compounds were then analyzed using GC and GC–MS.

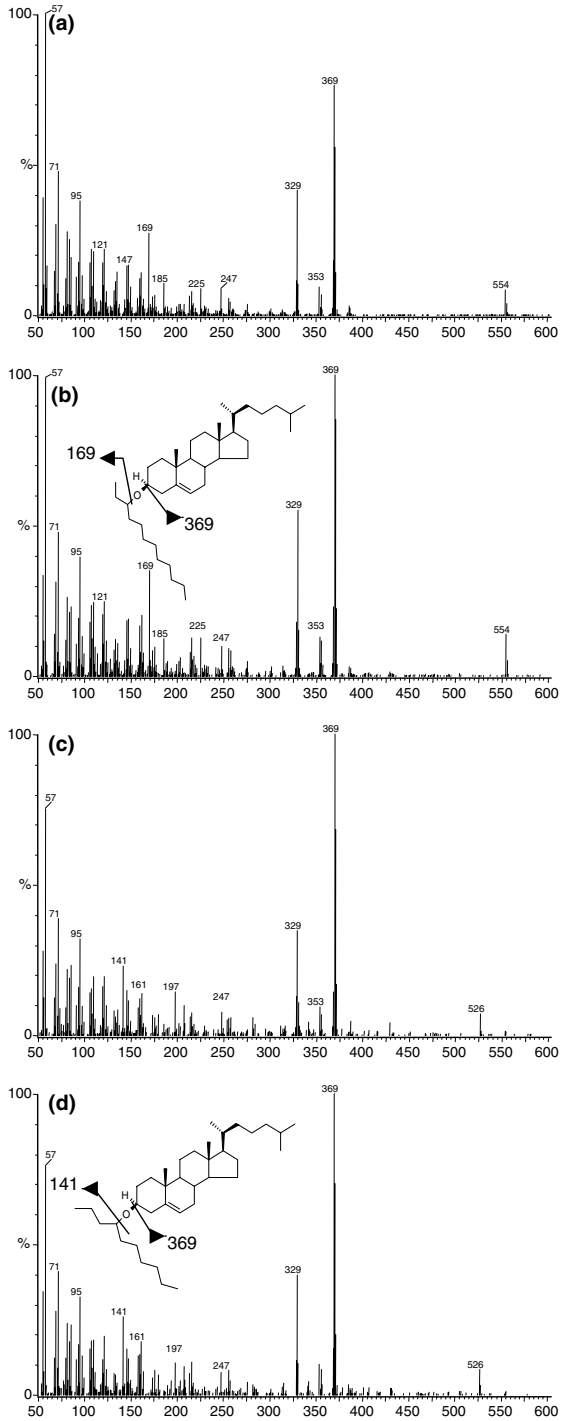

Fig. 2. Mass spectrum of: (a) cholest-5-enyl 3β-(3′-dodecanyl) ether in ODP 1084 sediment; (b) synthetic cholest-5-enyl 3β-(3′-dodecanyl) ether; (c) cholest-5-enyl 3β-(5′-decanyl) ether present in the NIOP 309 sediment; (d) synthetic cholest-5-enyl 3β-(5′-decanyl) ether.

2.4. Synthesis of sterol ethers

Four steryl ethers, cholest-5-enyl 3β-(2′-dodecanyl) ether (**Ia**; for structures see Appendix), cholest-5-enyl 3β-(3′-dodecanyl) ether (**IIa**), cholest-5-enyl 3β-(5′-decanyl) ether (**IIIa**) and cholest-5-enyl 3β-(1′-dodecanyl) ether (**IV**) were synthesized as described by Bills and McDonald (1926); 60 mg cholesterol and 350 mg of dried kaolin ("China clay") were added to 1.4 mL xylene and 0.5 mL of either 2-dodecanol, 3-dodecanol, 5-decanol and 1-dodecanol (Daniels Fine Chemicals Ltd., Alberta, Canada). The reaction mixture was refluxed for 8 h and products were purified using column chromatography, i.e., they were transferred to a column of activated Al_2O_3 with a volume of 50 mL and eluted with 200 mL hexane, 150 mL hexane/DCM (9:1, v/v) and 150 mL DCM/MeOH (1:1 v/v), respectively. The products of the reaction with 5-decanol was retrieved in the last 50 mL of hexane/DCM fraction with a yield of 1.1 mg (1.4%), whilst the products of the reactions with 1-decanol, 2-dodecanol and 3-dodecanol were recovered from the first 50 mL of the DCM/MeOH eluate with yields of 4.2 and 0.9 mg, respectively (4.9 and 1.1%, respectively). The reason for the different elution behaviour of the steryl alkyl ethers may be that **IIIa** is relatively less polar, due to the shielding of the oxygen functionality by the alkyl chain.

2.5. GC and GC–MS

GC analysis and co-injection experiments were performed using a Hewlett Packard 6890 series chromatograph equipped with an on-column injector and fitted with a 25 m × 0.32 mm fused silica capillary column coated with CP-Sil 5 (film thickness 0.12 μm). Helium was used as carrier gas and the oven was programmed from 70 to 130 °C at 20 °C/min, followed by an increase of 4 °C/min to 320 °C (10 min hold). Detection was performed with a flame ionization detector (FID).

GC–MS analysis was performed using a Hewlett–Packard 5890 series II chromatograph using conditions as described for GC. The column was directly inserted into the electron impact ion source of a VG – Autospec Ultima mass spectrometer, operated with a mass range of *m/z* 40–800, a cycle time of 1.8 s and ionization energy of 70 eV.

Table 1
Composition and relative retention times of dominant steryl alkyl ethers in synthetic products and sediments. n.d., not determined

Sample	Steroid moiety[a]	Alkyl moiety[b]	Position O-bond on alkyl chain	Pseudo-Kovats index[c]
Synthetic standards				
Ia	$C_{27:1}$	C_{12}	2	4063
IIa	$C_{27:1}$	C_{12}	3	4035
IIIa	$C_{27:1}$	C_{10}	5	3776
IV	$C_{27:1}$	C_{12}	1	4198
Sediments				
NIOP 309	$C_{27:1}$	C_{10}	5	3776
	$C_{28:1}$	C_{10}	5	3885
	$C_{29:1}$	C_{10}	5	3975
ODP 1084, depth 389.6 mbsf (175-1084A-43X-3, 20–23 cm)	$C_{27:1}$	C_{10}	5	3776
	$C_{28:1}$	C_{10}	5	3887
	$C_{29:1}$	C_{10}	5	3977
	$C_{27:1}$	C_{12}	3	4039
	$C_{28:1}$	C_{12}	3	4146
	$C_{29:1}$	C_{12}	3	4252
ODP 1084, depth 34.7 mbsf (175-1084A-5H-3, 20–23 cm)	$C_{27:1}$	C_8	n.d.	3608
	$C_{27:1}$	C_9	n.d.	3686
	$C_{27:1}$	C_{10}	n.d.	3790
	$C_{27:1}$	C_{11}	n.d.	3834
	$C_{27:1}$	C_{11}	n.d.	3855
	$C_{27:1}$	C_{11}	n.d.	3869
Monterey Fm. KG-1	$C_{27:1}$	C_{10}	n.d.	3725
	$C_{28:1}$	C_{10}	n.d.	3930
	$C_{29:1}$	C_{10}	n.d.	4162

[a] Numbers indicate number of carbon atoms and number of double bonds.
[b] Number indicates number of carbon atoms.
[c] Determined on a CPSil 5 column using the retention times of C_{38} and C_{40} *n*-alkanes with pseudo-Kovats indices of 3800 and 4000, respectively.

2.6. Nuclear magnetic resonance spectroscopy

^{1}H NMR spectra were obtained at 25 °C with a Varian Unity Inova spectrometer, operating at 500 MHz, equipped with a 5 mm pulsed field gradient indirect detection probe. Chemical shifts in ppm were determined relative to the solvent signal (^{1}H in $CDCl_3$) and converted to the TMS scale using $\delta_{CHCl3} = 7.24$ ppm for proton and 77.0 ppm for carbon. The ^{2}H resonance of $CDCl_3$ was used for field-frequency lock. Typical proton acquisition parameters were: sweep width of 8 kHz, relaxation delay of 2, 2.5 s acquisition time and a pulse width of 2.0 μs where the 90° flip angle corresponds to 4.25 μs.

Carbon experiments were carried out on a 5 mm pulsed field gradient switchable broadband probe. The conditions used were: sweep width 27560 Hz, relaxation delay 3, 1.3 s acquisition time and a pulse width of 4.0 μs where the 90° flip angle corresponds to 7 μs. Proton decoupling was performed with WALTZ-16 modulation during acquisition.

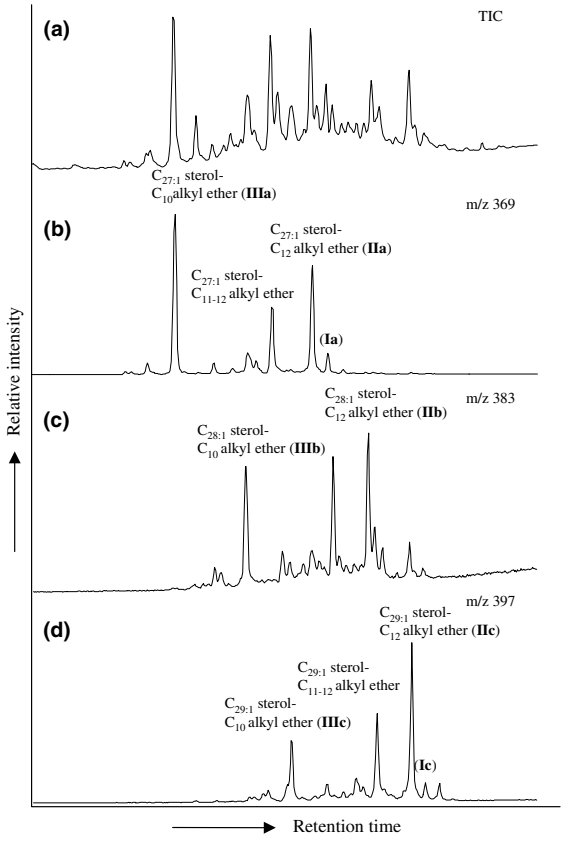

Fig. 3. Partial total ion chromatogram (a) and mass chromatograms: (b) *m/z* 369; (c) *m/z* 383; (d) *m/z* 397 of the apolar fraction isolated from Pleistocene ODP site 1084 sediment at 386.9 mbsf (175-1084A-43X-3, 20–23 cm) showing the distribution of steryl alkyl ethers with C_{27}, C_{28} and C_{29} sterol moieties, respectively. Structure numbers refer to structures shown in Appendix. Numbers with the sterol moiety of the steryl alkyl ethers indicate carbon number and number of double bonds, respectively. Number with alkyl moiety indicates number of carbon atoms.

3. Results and discussion

3.1. Occurrence and distribution of C_{27}–C_{29} steryl alkyl ethers

The apolar fractions from Pleistocene sediments from ODP site 1084 contained relatively high amounts of steryl alkyl ethers (e.g., Fig. 1a) as reported previously (Schefuß et al., 2001). Their mass spectra (e.g., Fig. 2a) reveal that they predominantly consist of C_{27}–C_{29} steroid moieties with one double bond, ether-bound to

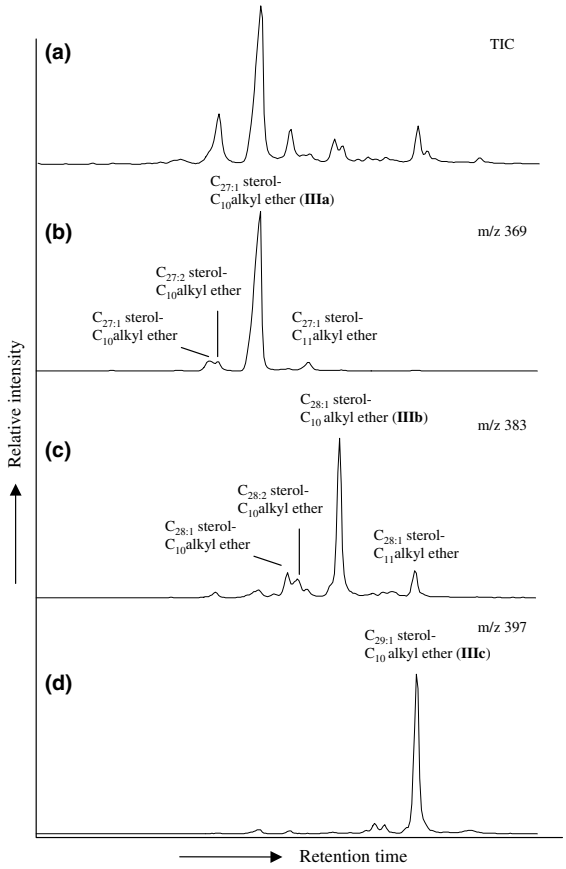

Fig. 4. Partial total ion chromatogram (a) and mass chromatograms: (b) *m/z* 369; (c) *m/z* 383; (d) *m/z* 397 of the steryl alkyl ether fraction of Holocene NIOP site 309 sediment showing the distribution of steryl alkyl ethers with C_{27}, C_{28} and C_{29} sterol moieties, respectively. Structure numbers refer to structures shown in Appendix.

C_8–C_{12} alkyl chains (Table 1). Mass chromatograms of *m/z* 369, 383 and 397 revealed the distribution of ethers with C_{27}, C_{28} and C_{29} sterol moieties, respectively (e.g., Fig. 3) and showed that at a depth of 389.6 mbsf, they are dominated by C_{27}–C_{29} steroid moieties ether-bound to C_{10} or C_{12} alkyl chains.

The Holocene NIOP 309 sediment contained relatively high amounts of C_{27}–C_{29} steryl ethers with a decyl moiety (Fig. 1b), as described previously for other Arabian Sea sediments (Schouten et al., 2000). Mass spectral analysis revealed that the dominant steryl alkyl ether consists of a cholesteryl moiety ether-bound to a C_{10} alkyl chain (Fig. 2c). Mass chromatograms of *m/z* 369, 383 and 397 (Fig. 4) revealed that the steryl alkyl ethers are mainly composed of C_{27}–C_{29} steroid moieties ether-bound to a C_{10} alkyl chain.

Steryl alkyl ethers are present in relatively low abundance in a Miocene sediment from the Monterey formation. Mass chromatography revealed that, like the Arabian Sea, they are mainly composed of C_{27}–C_{29} steroid moieties ether-bound to a C_{10} alkyl moiety (Fig. 5).

3.2. Chemical degradation

To elucidate the structures of the sterol ethers, chemical degradation experiments were performed. Fractions of sediment extracts containing high amounts of steryl alkyl ethers from the NIOP 309 and ODP 1084 were hydrogenated and refluxed with HI to cleave the ether bonds. Hydrogenation was performed prior to HI-cleavage as steroids containing double bonds undergo carbon skeleton rearrangements under the strongly acidic conditions during HI treatment. The released alkyl iodides were reacted with $NaSCH_3$ to replace the iodine atoms with a methylthio-group. Methyl thioethers yield characteristic fragments during mass spectrometric analysis, enabling identification of the original ether bond position (e.g., Kohnen et al., 1991; Schouten et al., 1998).

GC–MS analysis of the fraction from the NIOP 309 sterol alkyl ether fraction revealed, after chemical degradation, the presence of two groups of methyl thioethers: C_{27}–C_{29} steroids containing one methylthio-group and C_{10} alkyl chains containing one methylthio-group (Fig. 6a). Comparison of relative retention times and mass spectra of the methyl thiosteroids with those of authentic standards (Kohnen et al., 1991) revealed that they predominantly consist of C_{27}–C_{29} 3α- and 3β-methylthio-5α(H)-steranes with 3β-methylthiosteranes in higher abundance. This confirms that, as expected, the steryl moieties consist of regular C_{27}–C_{29} sterols with one double bond in the steroid carbon skeleton, being ether-bound at C-3 to the alkyl chain in the β-stereoisomeric configuration. The minor amounts of 3α-methylthiosteranes may reflect an enhanced Sn1 pathway under the strong acidic conditions during the HI degradation, which may have generated 3α-iodidosteranes and 3β-iodidosteranes. The composition of the methylthioalkanes, the second group of products released during chemical degradation of the steryl alkyl ethers, was, however, surprising (Fig. 6a). Mass spectral analysis revealed that the dominant methylthioalkane was 5-methylthiodecane with minor amounts of 4-methylthiodecane and not 1-methylthiodecane as might have been expected from previous tentative identifications (Boon and de Leeuw, 1979; Schouten et al., 2000). These data suggest that **IIIa-c** and **Va-c** are the structures of the dominant steryl alkyl ethers in the Arabian Sea samples, i.e., different from the structure we tentatively proposed previously (Schouten et al., 2000) (Table 3).

A similar sequence of degradation experiments performed on the sterol alkyl ether fraction of ODP 1084 sediment yielded predominantly C_{27}–C_{29} 3β-methylthio-5α(H)-steranes similar to the NIOP 309 sediment (Fig. 6b). The composition of the methylthioalkanes was dominated by C_{12} alkanes with one methylthio-group. Surprisingly, no C_{10} alkyl chain with a methylthio-group was detected, as might have been expected from the presence of steryl alkyl ethers with a C_{10} alkyl chain. These compounds are rather volatile and may have been lost during work up. Mass spectral analysis revealed that the dominant fragment in the two most

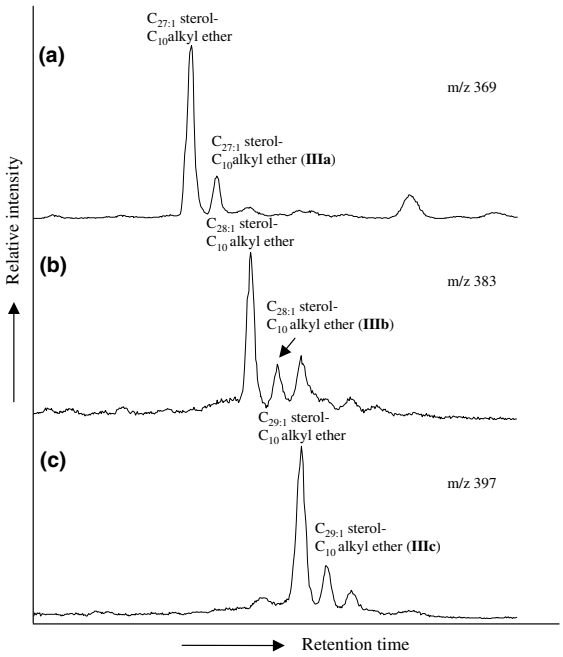

Fig. 5. Mass chromatograms of: (a) *m/z* 369; (b) *m/z* 383; (c) *m/z* 397 of the steryl alkyl ether fraction from Miocene Monterey formation sediment showing the distribution of steryl alkyl ethers with C_{27}, C_{28} and C_{29} sterol moieties, respectively. Structure numbers refer to structures shown in Appendix.

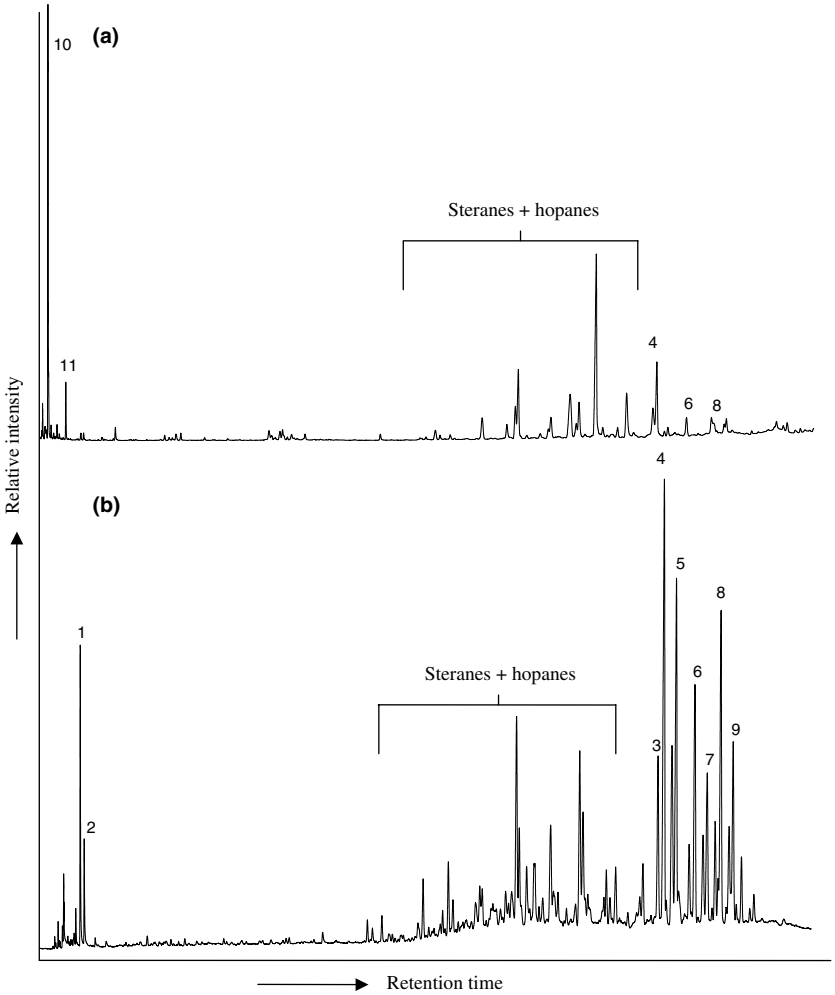

Fig. 6. Total ion current chromatograms of HI/$NaSCH_3$ degradation products of: (a) steryl alkyl ether fraction of NIOP 309 and (b) apolar fraction of ODP 1084. For compound identification see Table 3.

abundant C_{12} methylthioalkanes in the ODP 1084 sample was at *m/z* 75 and 89, respectively. Based on comparison with mass spectra of authentic standards (Kohnen et al., 1991), this suggests that the methylthioalkanes are 2-methylthiododecane and 3-methylthiododecane, respectively. This means that the alkyl moieties in the steryl alkyl ethers in the ODP 1084 sediment were bound at positions 2′ and 3′ to the sterol moiety and not at the terminal position 1′ as proposed earlier (e.g., Boon and de Leeuw, 1979; Schefuß et al., 2001). These results lead to structures **Ia-c** and **IIa-c** for the dominant steryl alkyl ethers in ODP 1084.

3.3. Synthesis of cholesteryl alkyl ethers

To confirm the results of the degradation experiments, we synthesized some of the most abundant steryl ethers encountered in the ODP 1084 and NIOP 309 sediments, i.e., cholest-5-enyl 3β-(2′-dodecanyl) ether (**Ia**), cholest-5-enyl 3β-(3′-dodecanyl) ether (**IIa**) and cholest-5-enyl 3β-(5′-decanyl) ether (**IIIa**), respectively. GC and GC–MS analysis of the product mixtures revealed that they all consisted of two closely eluting steryl alkyl ether isomers with identical mass spectra, but in varying ratios. Based on the synthetic procedure, it is likely that the two kinds of stereisomeric mixtures may have been formed: 3α- and 3β-O-alkyl stereoisomers or R and S stereoisomers at the C2′, C3′ or C5′ positions of the alkyl moieties of **Ia**, **IIa** and **IIIa**, respectively. To exclude the possibility that 3α- and 3β-O-alkyl stereoisomers were formed, we synthesized cholest-5-enyl 3β-(1′-dodecanyl) ether (**IV**) which does not possess a chiral centre in the O-alkyl side chain. GC and GC–MS analysis revealed only one peak with an identical mass spectrum to **Ia** and **IIa**. This suggests that the steryl alkyl ether isomers formed during synthesis are R/S

Table 2
^{13}C and selected ^{1}H NMR data for cholest-5-enyl 3β-(2′-dodecanyl) ether (**Ia**) and cholest-5-enyl 3β-(5′-decanyl) ether **IIIa**

Ia			**IIIa**	
C	^{1}H	^{13}C	^{1}H	^{13}C
1		37.50a (s)		37.47 (s)
2		29.75 (s)		29.37 (s)
3	3.18 (1H, m)	76.83, 76.97 (t)	3.18 (1H, m)	77.28a (t)
4		40.39 (s)		40.11 (s)
5		ca. 141.5 (q)		141.54 (q)
6	5.36 (1H, d, J = 4.8 Hz)	121.23 (t)	5.36 (1H, d, J = 4.9 Hz)	121.22 (t)
7		31.96 (s)		31.96 (s)
8		31.91		31.91 (t)
9		50.27 (t)		50.25 (t)
10		36.88 (q)		36.86 (q)
11		21.07 (s)		21.07 (s)
12		39.82 (s)		39.80 (s)
13		42.33 (s)		42.32 (s)
14		56.82 (t)		56.81 (t)
15		24.29 (s)		24.29 (s)
16		28.23 (s)		28.23 (s)
17		56.16 (t)		56.13 (t)
18	0.70 (3H, s)	11.86 (p)	0.70 (3H, s)	11.86 (p)
19	1.02 (3H, s)	19.40 (p)	1.02 (3H, s)	19.41 (p)
20		35.77 (t)		35.77 (t)
21	0.94 (3H, d, J = 6.5 Hz)	18.72 (p)		18.71 (p)
22		36.20 (s)		36.18 (s)
23		23.81 (s)		23.80 (s)
24		39.52 (s)		39.51 (s)
25		28.01 (t)		28.01 (t)
26		22.56 (p)		22.56 (p)
27		22.81 (p)		22.82 (p)
1′	1.13 (3H, d)	20.99 (p)		14.15 (p)
2′	3.50 (1H, m)	72.91 (t)		22.97 (s)
3′		37.42a (s)		27.88 (s)
4′		25.82 (s)		34.95b (s)
5′		29.00 (s)	3.30 (1H, m)	77.40a (t)
6′		29.65 (s)		34.70b (s)
7′		29.65 (s)		25.25 (s)
8′		29.65 (s)		32.14 (s)
9′		29.34 (s)		22.67 (s)
10′		31.96 (s)		14.15 (p)
11′		22.69 (s)		
12′		14.12 (p)		

Assignments of carbon and hydrogen atoms are based on comparison with literature data of 3β-methoxysterols (D'Auria et al., 1992).

stereoisomers and that the original 3β-O configuration of cholesterol was preserved.

Further confirmation came from the ^{1}H and ^{13}C NMR analyses which were performed on the stereoisomeric mixture of sterol ethers formed by the synthesis of cholest-5-enyl 3β-(2′-dodecanyl) ether (**Ia**) and cholest-5-enyl 3β-(5′-decanyl) ether (**IIIa**). The nuclear magnetic resonance (NMR) data (Table 2) are consistent with a 3β-O-alkyl configuration of the sterol ethers **Ia** and **IIIa** as the shift of the proton at C-3 (3.18 ppm, both for **Ia** and **IIIa**, Table 2) is in the same range as reported for 3β-methoxysterols (3.05–3.16 ppm; D'Auria et al., 1992). In contrast, the shift for the proton at C-3 for 3α -sterols is 0.5 ppm downfield compared to that for 3β-sterols (Kirk et al., 1990). In addition, the shift of the carbon atom C-3 (76.8–77.3 ppm, Table 2) is similar to that reported for 3β-tetrahydropyranyloxysterols (~76 ppm; Szendi et al., 2000). The NMR data thus confirm the structures of **Ia** and **IIIa**, but they do not allow elucidation of the R/S stereochemistry of the steryl alkyl ethers.

The mass spectra of the synthetic compounds are identical to those of the sedimentary compounds (Fig. 2a–d). Co-injection of **Ia** and **IIa** with the ODP 1084 steryl alkyl ether fraction showed that the sedimentary compounds co-eluted with the later eluting stereoisomer of the synthetic standards (see above), indicating that only one stereoisomer is present in the sediments

Table 3
Compounds identified after HI/$NaSCH_3$ degradation

Number[a]	Compound
1	3-Methylthiododecane
2	2-Methylthiododecane
3	3α-Methylthio-5α-cholestane
4	3β-Methylthio-5α-cholestane
5	3-Iodo-5α-cholestane
6	3β-Methylthio-24-methyl-5α-cholestane
7	3-Iodo-24-methyl-5α-cholestane
8	3β-Methylthio-24-ethyl-5α-cholestane
9	3-Iodo-24-ethyl-5α-cholestane
10	5-Methylthiodecane
11	4-Methylthiodecane

[a] Numbers refer to chromatogram in Fig. 6.

(Fig. 3b). Although we did not establish the configuration of the stereoisomers in the alkyl chain, the carbon skeleton of the sedimentary steryl alkyl ethers has now been unambiguously established. Co-injection of **IIIa** with the NIOP 309 fraction also confirmed the carbon skeleton of the dominant steryl alkyl ether as **IIIa** (Fig. 4, Table 1). Interestingly, co-injection of **IIIa** with the ODP 1084 steryl alkyl ether fraction showed that the steryl alkyl ethers with a C_{10} alkyl moiety were also attached at position 5 of the alkyl chain (Fig. 3(b)). In contrast, co-injection of **Ia** and comparison of relative retention times showed that **Ia** is only a minor isomer in the Miocene Monterey formation and that the dominant steryl alkyl ether with a C_{10} alkyl moiety eluted earlier, suggesting either a different position of the ether bond at the alkyl chain or, more likely considering the early retention time, that the alkyl chain is branched (Fig. 5a, Table 1). Remarkably, **IV** was not detected in the sediments.

It is clear from our work that the mass spectra of the steryl alkyl ether isomers allow the identification of the steroid moieties and the carbon number of the alkyl chains, but not the position of the ether bond in the alkyl chain (Fig. 2). Thus, it is not possible to determine the exact structure of steryl alkyl ethers based on mass spectrometry alone, and relative retention times are necessary as well. Therefore, pseudo-Kovats indices of the synthetic standards and steryl alkyl ethers in sediments are reported in Table 1.

3.4. Origin of steryl alkyl ethers

To the best of our knowledge, the sedimentary steryl alkyl ethers have not been found in any organisms; 3β-methoxysterols containing different C_{27}–C_{29} sterol nuclei (mainly **VIa-c**) have been reported for a sponge from the Pacific Ocean (D'Auria et al., 1992). A methoxy methyl sterol ether, 24-methyl-5α-cholest-7-enyl 3β-methoxymethyl ether (**VII**) has been reported to occur in a deep water marine sponge (Gunasekera et al., 1996) and was shown to be cytotoxic. However, steryl ethers with longer alkyl chains have, to the best of our knowledge, not been reported. Based on their occurrence in sediments with a high diatom input, Schouten et al. (2000) suggested diatoms as a direct biological source. However, analysis of 120 strains of predominantly marine diatoms, distributed over the phylogenetic tree on the basis of the 18S RNA gene (Sinninghe Damsté et al., 2004), did not reveal the presence of steryl alkyl ethers (Rampen S.W, Schouten S. and Sinninghe Damsté J.S., unpublished results). This means that the diatom producing steryl alkyl ethers has not yet been analysed or that other sources have to be invoked.

Analysis of a set of samples of particulate matter recovered with sediment traps and covering the full annual cycle at NIOP site 905, located near NIOP site 309 (Brummer et al., 2002), did not reveal the presence of steryl alkyl ethers. However, they were present in the core top sediment (0–2.5 cm) directly underneath the sediment trap at NIOP site 905 in a distribution similar to those at NIOP site 309. Thus, herbivory processes may result in the production of steryl ethers in a similar way as has been suggested for steryl chlorins (Eckardt et al., 1992; Pearce et al., 1998). However, to the best of our knowledge the formation of ethers has not yet been shown to occur during ingestion of lipids. Furthermore, the distribution of free sterols from the same sediments is substantially different from that of the steroid moieties in the steryl alkyl ethers (cf. Schouten et al., 2000). However, this perhaps may also be caused by a selective preservation or selective incorporation of certain sterols as observed for steryl chlorins (Pearce et al., 1998; Talbot et al., 2000). Finally, since the alkyl chains are bound at specific positions to the sterols with stereochemical control, it seems more likely that biosynthetic pathways within a single type of organism are responsible for the production of steryl alkyl ethers. Further analysis of these compounds in different environments may shed more light on their distributions and origin.

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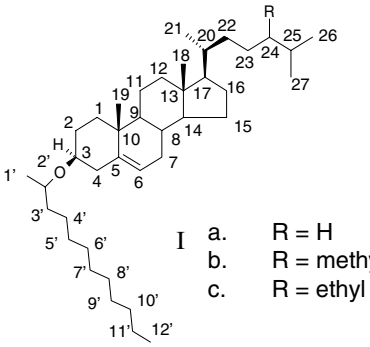
Appendix



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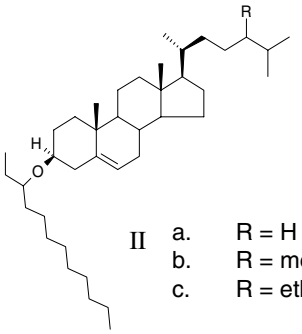
b. R = methyl
c. R = ethyl



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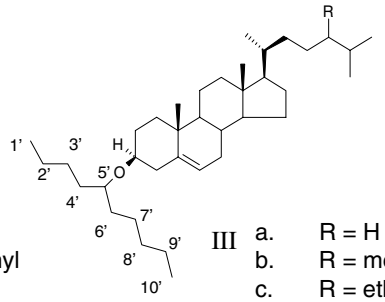
c. R = ethyl



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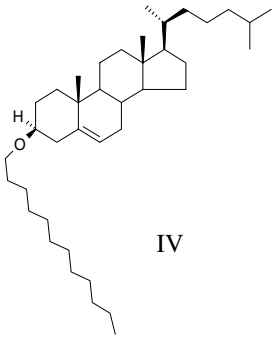
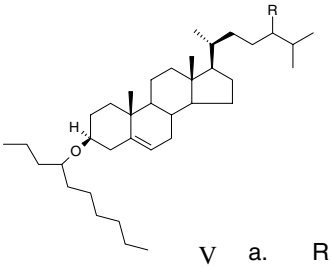


IV



V a. R = H
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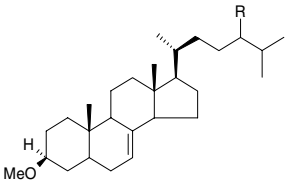
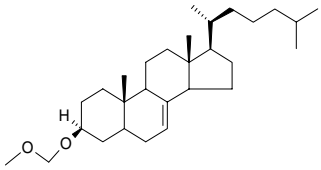
c. R = ethyl



VI a. R = H
b. R = methyl
c. R = ethyl



VII

Associate Editor—**P. Schaeffer**

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