Steroids
Recent sediments
Diagenesis
Organic geochemistry

Stéroïdes
Sédiments récents
Diagenèse
Géochimie organique

Aspects of the steroid geochemistry of a recent diatomaceous sediment from the Namibian Shelf

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ABSTRACT

Five consecutive 2 cm thick sections, from a core taken on the South-West African shelf, have been analysed for various classes of steroidal compounds. Fifty-two different sterols were identified, ranging from C_{26}-C_{30}, in concentrations up to 38 µg/g dry weight of sediment. Steroidal ketones (sterones) and alkenes (sterenes) were also present, in concentrations up to 8.6 µg/g and 1.8 µg/g dry weight of sediment respectively. Many of the sterols can be related to the known large input of diatoms, but there is also evidence for a contribution from coccolithophorids and coelenterates. The major sterol in all sections was 4α, 23, 24-trimethyl-5α-cholest-22-en-3β-ol (dinosterol) which, together with several other 4-methyl-sterols, might be expected to be explained by a dinoflagellate input. However, no recognisable dinoflagellate remains were observed under the light microscope and a major, as yet uncharacterised, phytoplankton source is postulated. Nuclear saturated stanols with both 5α- and 5β(H)-configurations were abundant, comprising 43-77% of the total 4-desmethyl sterols. A depth profile of the stanol/stenol ratio suggests that reduction of the Δ^5-double bond of stenols is occurring in the sediment. The presence of suites of stanones corresponding to all the major stenols and stanols, together with several Δ^4-stenones, provides indirect evidence for the operation of the diagenetic pathway: stenol → stenone → stanone → stanol. Direct biological input may also contribute to the occurrence of stanols. C_{27}-C_{30} sterenes, present in the sediment, probably arise by dehydration of the sterols.

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RÉSUMÉ

Aspects de la géochimie des stéroïdes dans un sédiment récent à diatomées provenant du plateau namibien.

Cinq sections consécutives, de 2 cm d'épaisseur, ont été réalisées dans une carotte en provenance du plateau continental sud-ouest africain; leur teneur en différentes classes de composés stéroïdes a été analysée. Cinquante deux stérols — de C_{26} à C_{30} — ont été identifiés à des concentrations atteignant 38 µg/g de poids sec de sédiment. On a trouvé également des cétones stéroïdes et des alcènes à des concentrations respectives de 8,6 et 1,8 µg/g de poids sec de sédiment. La présence de bon nombre de stérols est liée à la contribution importante des diatomées, mais d'évidence aussi à celle des coccolithophorides et des coelentères. Le stérol le plus important dans chaque section est le 4α. 23. 24-trimethyl-5α-cholest-22-en-3β-ol (dinostérol), dont la présence, au même titre que celle d'autres 4-méthyl stérols, peut s'expliquer par la contribution des dinoflagellés. Toutefois, aucun résidu de dinoflagellés n'a été observé au microscope, et on pense, même si on ne l'a pas encore caractérisée, à une origine phytoplanctonique. Des stanols cycliques saturés, avec une double configuration en 5α et 5β (H) sont abondants, comprenant de 43 à 77% des 4-diméthyl stérols. Un profil selon la profondeur du rapport stanol/sténol suggère que la réduction de la double liaison Δ des

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sténols se produit dans le sédiment. La présence de séries de stanols correspondant à tous les principaux sténols et stanols, en compagnie de plusieurs Δ^4 sténones constitue, de manière indirecte, une évidence en faveur d'une série de relations diagénétiques : sténol → sténone → stanone → stanol. Une contribution biologique directe pourrait également jouer un rôle dans la présence de stanols. Les stérènes en C_{27}-C_{30}, présents dans le sédiment, pourraient avoir pour origine la déshydratation des stérols.

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INTRODUCTION

A great variety of sterol structures are found within the marine environment (Goad, 1976; 1978; Morris, Culkin, 1977), many of which are only known to be biosynthesised by a limited number of organisms. This, coupled with the comparatively high resistance of the sterol skeleton to extensive degradation, makes them valuable as biological markers (Philp *et al.*, 1976). Thus, studying the sterol distribution in a marine sediment may allow an assessment of the likely source organisms which contributed the organic matter to the sediment. Most biologically produced sterols are 3 β-hydroxy tetracyclic structures, commonly with a C_7-C_{11} side chain, and varying degrees and positions of unsaturation. They can be transported through the water column in particulate matter (e. g. dead organisms, faecal pellets) and a part of this flux may be incorporated into the sediments, where, over geological time, it is believed that sterols can undergo diagenetic transformations to form compounds such as sterenes and steranes (largely retaining the carbon skeleton), which are found in many sediments (Dastillung, Albrecht, 1977).

A number of geochemical studies of sterols have taken place in the Walvis Bay region. This is an area of upwelling, resulting in high primary productivity in the surface waters. Diatoms are thought to compose about 98% of the phytoplankton (Hart, Currie, 1960; Kollmer, 1963) and the settling of diatom blooms produces a very large flux of organic matter to the sediments and has resulted in the deposition of an extensive diatomaceous ooze. Studies by Bremner (1974; 1975) indicated the existence of zones of different sediment types, running virtually parallel to the coastline, with differing contents of organic matter. Cores within a zone of organic-rich diatomaceous ooze have been analysed by Morris and Calvert (1977), Boon (1978), Gagosian and Farrington (1978), Gagosian and Smith (1979) and Lee *et al.* (1980). A diagram of the area, showing some of the core locations, is given in Lee *et al.* (1980).

Decomposition of the large planktonic input of organic matter causes severe oxygen depletion and results in large areas of sediment being kept anoxic (Morris, Calvert, 1977); oxidative degradation of compounds within the sediments is minimal and benthic fauna are restricted to anaerobic microorganisms. This situation limits degradation and reworking of the organic input so that the less labile classes of compound, such as sterols, are comparatively well preserved. This, together with the lack of terrigenous run-off from the desert areas on the neighbouring coast, makes the area very suitable for marine geochemical studies, as described by Morris and Calvert (1977) and Boon (1978).

From the results of sterol analyses Wardroper *et al.* (1978) and Lee *et al.* (1980) implied diatoms to be the major source of sedimentary sterols, although there was evidence of contributions from dinoflagellates and coelenterates.

Studies of the sterol geochemistry in sedimentary environments such as Walvis Bay have suggested possible mechanisms for the diagenetic transformation of sterols into sterenes and Δ^5-stenols into stanols, the former involving dehydration reactions and the latter occurring *via* stenone and stanone intermediates (Fig. 1). From studies of radio-labelled cholesterol, evidence that stenol to stanol conversion occurs in anaerobic sediments and sewage sludge was provided by Gaskell and Eglinton (1975) and conversion of cholesterol to 5 β-cholestanol *via* cholest-4-en-3-one and 5 β-cholestan-3-one by anaerobic microbes, has been demonstrated by Bjorkhem and Gustaffson (1971) and Parmentier and Eyssen (1974). Microbial reduction of a Δ^5-stenol directly to a stanol, by a one-step reaction, however, has not been conclusively demonstrated. Stanones have been identified in Walvis Bay sediment by Gagosian and Smith (1979) and these may be intermediates in a stenol → stanol conversion process. However, they were not detected in Black Sea sediment samples, which did contain abundant stenols and stanols (Gagosian *et al.*, 1979 *b*).

The sediment samples used in this present study came from a core taken from the middle of the organic-rich diatomaceous ooze (core 1248). Previous work, based on ^{14}C-dates from a number of cores in this area, has indicated that the surface sediments in this area have accumulated with sedimentation rates of *ca.* 0.3 mm/yr and are relatively undisturbed (Morris, Calvert, 1977; Morris, Calvert, unpublished results). Such a rapid accumulation of organic material should allow some of the early diagenetic reactions of the sedimentary sterols to be followed by a detailed analysis of thin, sequential layers of the near-surface sediment. This present study presents the results of analyses, for steroidal compounds, on short (2 cm) sections from the upper 10 cm of core 1248. Assuming a sedimentation rate of 0.3 mm/yr (Morris, Calvert, unpublished results) these samples should cover a sedimentary period of approximately 350 years (2 cm *ca.* 70 yrs.).

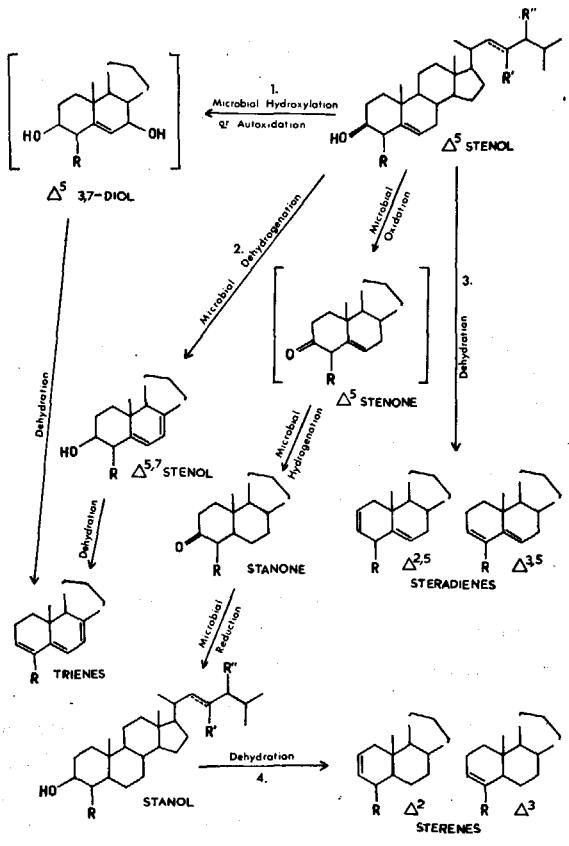

Figure 1

Proposed diagenetic routes for the conversion of biologically derived sterols into other steroidal compounds in sediments (compiled from Gagosian, Farrington, 1978; Gagosian, Smith, 1979 and Gagosian, Heinzer, 1979).

Notes: *Compounds enclosed in brackets are hypothetical intermediates, not yet isolated from sediments. Reactions* (1) *and* (2) *have been observed to occur in bacteria. Reactions* (3) *and* (4) *have been observed, for cholesterol and cholestanol respectively, in laboratory incubations of sterols with sedimentary material.*

The unsectioned upper 10 cm of this same core has already been the subject of sterol analysis by Wardroper *et al.* (1978), who reported the principal components of the 4-desmethyl sterols and related them to possible biological input. The main objective of this present study was to carry out more detailed analyses on sequential 2 cm sections of the core in order to assess (*a*) the biological input to the sediments and relate this to the present water column, and (*b*) the early diagenetic fate of sterols, to provide some possible confirmation of the diagenetic pathways proposed by Gagosian and Farrington (1978), Gagosian and Smith (1979) and Gagosian and Heinzer (1979), outlined in Figure 1.

METHODS

Sampling

The sediment was collected on 5th February, 1975, during cruise 1/75 of R.R.S. Shackleton. A core (10 cm diameter, 60 cm long) was taken from a site (22°35 O'S, 13°45 O'E) on the south west African shelf at a water depth of 127 m. A clean, stainless steel gravity corer with a cellulose acetate butyrate liner (pre-washed in $CHCl_3$) was used. Care was taken to minimise contamination. The core was sealed with pre-washed high density polyethylene caps and was immediately stored at −30°C. The upper 10 cm was taken and, after the outer rind of the core had been removed, was carefully sectioned into 2 cm layers.

Core description

The sediment consisted of a fluid, dark olive green diatomaceous ooze, containing high concentrations of H_2S. There was no evidence of lithological variation over the full length (1 m) of the core. The core was also examined for the presence of any large benthic organisms but none were found.

Sample preparation

Sub-samples of each 2 cm layer (4 g) for mineralogical and chemical analyses were air dried at 100°C and ground to a fine powder in a tungsten carbide mill. The remainder of the sample was not air dried but was taken directly for smear slide analysis and the extraction of the component lipids.

Mineralogical and chemical analyses

Organic carbon and carbonate were determined gravimetrically using a Leco carbon analyser following methods given previously (Gaskell *et al.*, 1975). Opal (diatomaceous silica) and quartz were determined by X-ray diffraction techniques following methods given by Eisma and Van Der Gaast (1971) and Calvert (1966) respectively. The determinations were made on the same sample mixtures using α-Al_2O_3 as the internal standard and were carried out on a Philips 1410 XRF spectrometer with CuK_α radiation. Fusion beads were made according to the method of Harvey *et al.* (1973) and CaO was determined directly by XRF analysis.

The carbon, carbonate, opal and quartz data have been corrected for the diluting effect of sea salt in the dried samples by determining the Cl contents by X-ray emission methods (*see* Calvert, Morris 1977).

Clay peaks were seen during XRD analyses of some of the samples but they were only minor components and are included in the uncharacterised fraction (i.e., clay + others; *see* Table 1). Illite was the most abundant clay mineral present, kaolinite also being found, though as a lesser component. No chlorite or montmorillinite were detected.

Lipid extraction

The solvent-extractable organic material was obtained by addition of 20 volumes of a chloroform/methanol mixture (2 : 1 v/v; BDH Aristar Grade), to the sediment samples, which were then sonicated for 1 hour in a Mettler ultrasonic tank and then allowed to stand for 12 hours at ambient temperature under nitrogen. The organic and aqueous phases were separated by centrifugation and the organic layers removed. The sediment residues were again sonicated for 1 hour in chloroform/methanol (2 : 1 v/v) and the second organic

Table 1
Results of the chemical and mineralogical analyses

Sample	Water content (% wet wt)	Organic carbon (% dry wt)	$CaCO_3$ (by Leco) (% dry wt)	$CaCO_3$ (fusion bead) (% dry wt)	Opal (% dry wt)	Quartz (% dry wt)	Uncharacterized fraction (clay + others) (% dry wt)	Extractable lipid (% dry wt)
0-2 cm	91.7	7.6	5.1	–	–	–	–	1.9
2-4 cm	90.8	7.7	5.0	5.8	75.4	3.4	T	1.4
4-6 cm	92.3	8.2	5.2	6.9	70.0	1.0	T	1.5
6-8 cm	89.6	8.3	5.5	6.6	80.2	3.6	T	2.0
8-10 cm	89.4	7.3	5.5	7.0	85.0	5.1	T	1.0

[a] A precise carbonate value for low-carbonate samples is difficult by the Leco method; the results were therefore checked by use of the fusion bead method. T, less than 5% of the sample (dry weight).

extracts combined with the first. The total organic extracts were evaporated to dryness at 30°C, using a rotary vacuum evaporator, weighed, then redissolved in *ca.* 2 ml of dichloromethane and stored in glass vials at −20°C under nitrogen. The lipid extracts were maintained under an atmosphere of nitrogen during all operations.

Lipid analyses

A known portion of each total organic extract was saponified, using a 10% solution of KOH (previously heated at 500°C for 48 hours) in methanol (12 hours at ambient temperature plus 1 hour reflux). After addition of water, the total neutrals were extracted into hexane/diethyl ether (4 : 1 v/v), then stored in dichloromethane, as above. The remaining aqueous fraction was acidified to pH 2 with concentrated HCl, then extracted similarly to yield a total acid fraction; analysis of the fatty acids will be reported elsewhere.

An aliquot of each total neutral fraction was derivatised with bis (trimethylsilyl) trifluoroacetamide (BSTFA; Analabs Inc.) and analysed by GC and GC-MS. For more detailed examination, a further aliquot was separated into several classes by thin layer chromatography (0.4 mm thick Silica gel G, developed in 85% hexane/15% ethyl acetate). The following fractions (corresponding to the R_f values and standards given) were obtained: hydrocarbons (R_f = 0.9-1.0 = tetracosane), 4-methyl-stanones + 5 β-stanones (R_f = 0.65-0.75 = 5 β-cholestan-3-one), 5 α-stanones (R_f = 0.55-0.65 = 5 α-cholestan-3-one), stenones (R_f = 0.39-0.55 = cholest-4-en-3-one), 4-methyl-sterols + *n*-alcohols (R_f = 0.28-0.39 = octadecan-1-ol) and desmethyl-sterols (R_f = 0.17-0.28 = cholesterol).

The sterol and alcohol fractions were derivatised to their trimethylsilyl ethers, using BSTFA, for gas chromatographic (GC) analysis, which was performed on a Carlo Erba FTV 2150 instrument fitted with an FID. The column was a 25 m × 0.3 mm i.d. flexible silica capillary, coated with OV1 (Phase Separations Ltd.), temperature programmed from 80-280°C at 4°C. min^{-1}, and with helium as carrier gas at a flow rate of *ca.* 2 ml. min^{-1}. Computerised gas chromatography-mass spectrometry (C-GC-MS) was performed on a Finnigan 4000 GC-MS system with an on-line INCOS 2300 computer data system. The GC column was a 25 m × 0.3 mm i.d. flexible silica capillary, coated with a methyl-silicone polymer (Hewlett Packard), which passed directly from the GC oven through a heated interface box into the ion source of the mass spectrometer. GC-MS conditions were as previously described (Volkman *et al.*, 1981).

Blank analyses were carried out on all solvents and reagents used to check for contamination. No significant amounts of the compounds under investigation were found in these blanks.

Identification of compounds was achieved by consideration of GC retention times, coinjection of standards (where available), interpretations of MS data and comparison of mass spectra with those of authentic compounds or with published data (Gaskell, Eglinton, 1976; Wardroper *et al.*, 1978; Withers *et al.*, 1978; Boon *et al.*, 1979; Gagosian, Heinzer, 1979; Lee *et al.*, 1979; Wardroper, 1979; Brassell, 1980; Horning *et al.*, 1968; Edmonds *et al.*, 1977; Djerassi, 1978; Gagosian, Smith, 1979; Sheikh, Djerassi, 1974; Brooks, 1979; Gagosian, Farrington, 1978). Quantitation of components was by measurement of the peak area of the GC response, compared to that of a known quantity of the 5 α-cholestane added to samples as an internal standard. Where peaks were insufficiently well resolved for accurate quantitation by GC, mass fragmentography was employed to measure intensities of characteristic ions in the mass spectra of the components, which served as a basis for approximate quantitation.

RESULTS

Sediment mineralogy

All sections of the core showed similar mineralogy (Table 1), with high organic carbon values (7-8% dry weight). The major inorganic material present was opaline silica (70-85% dry weight), which is a characteristic component of diatoms. In contract, the quartz content was low (1-5% dry weight), which together with the very low quantities of clay, suggest that the terrigenous input is minor. Calcium carbonate was also present in only small amounts (5% dry weight).

Several smear slides were prepared from unextracted sediment from each section of the core and examined under the light microscope. Diatom frustules (predominantly species of *Coscinodiscus* and *Chaetoceros*), both intact and fragmented, were abundant, whereas

only a few coccoliths and foraminifera were observed (consistant with the high opal and low carbonate content shown in Table 1). No cysts or other recognisable remains of dinoflagellates were observed, neither was a significant quantity of sans evident (consistent with the low quartz content shown in Table 1). These data are in good agreement with the microscopical observations reported by Boon (1978); however, data presented by Gagosian, Farrington (1978) and Lee *et al.* (1980); partly derived from Bremner, 1974; 1975), on cores taken in the same sedimentary zone, indicate a rather different mineralogy. These authors give calcium carbonate content as 5-50%, clay 25-50%, silt 10-50%, sand 25-75% and organic carbon 6-11% of sediment weight. Another core from this zone, analysed by Gagosian, Smith (1979), required treatment with HCl, before extraction, to remove the large amount of carbonate present. Dinoflagellate cysts were also observed in the core examined by Lee *et al.* (1980).

Radioisotope dating was not carried out on core 1248; however, another core, taken on the same cruise from a nearby location (22°15'S, 13°45'E) with very similar mineralogy to that reported here, was subjected to ^{14}C dating. The data obtained indicated that the sediment had not been subject to significant disturbance during the time period under study.

Sterol distributions

The identities and distributions of the sterols observed are presented in Table 2 and Figure 2. Representative gas chromatograms of two sterol fractions are shown in Figure 3. A total of 52 different sterols were identified, although the precise structures of some of the minor components could not be unambiguously elucidated and some assignments are tentative. The overall distributions in the five sections are broadly similar, indicating that no major change in input has occurred during the course of deposition of the upper 10 cm. However, some trends with depth are evident. The ratio of 5α-stanols to Δ^5-stenols increases with depth (*see* Fig. 4) — a variation which is more

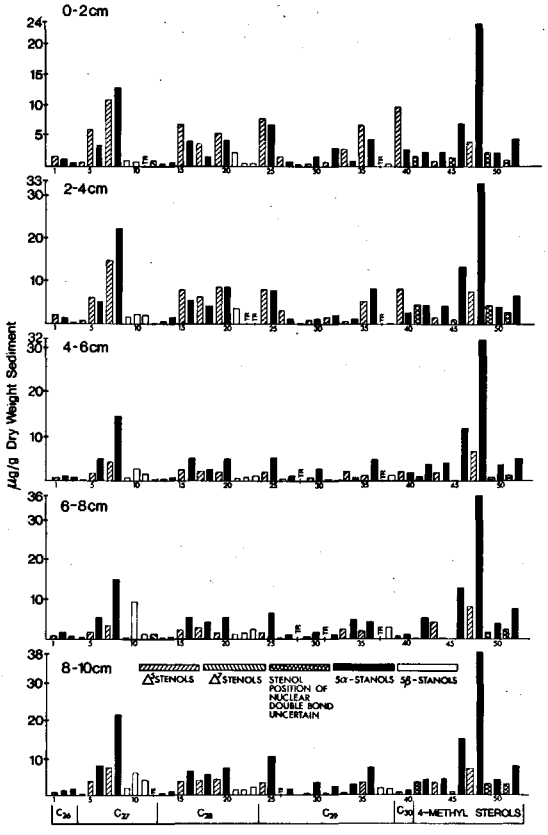

Figure 2

Distributions of total extractable sterols in core 1248, from the Namibian Shelf (compounds are numbered according to Table 2).

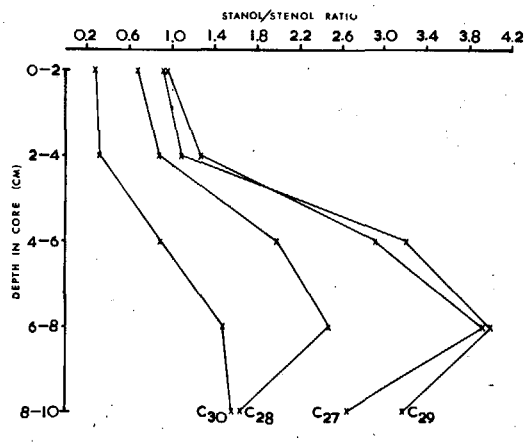

Figure 4

Depth profiles of the stanol : stenol ratio for the 4-desmethyl sterols in the top 10 cm of core 1248 from the Namibian Shelf. The $(\Delta^{5,22}+\Delta^5$ *stenols)/*$(\Delta^{22}+\Delta^0$ *5α-stanols) ratio is shown, for the five 2 cm thick sections of the core examined, for* C_{27}, C_{28}, C_{29} *and* C_{30} *4-desmethyl-sterols.*

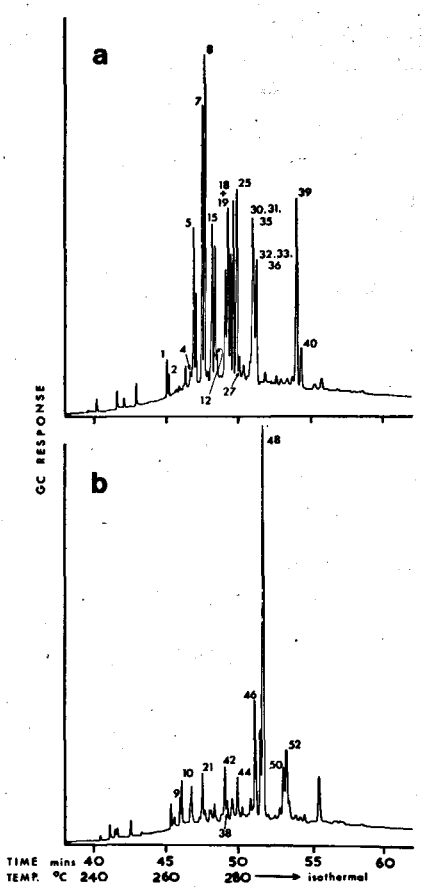

Figure 3

Capillary gas chromatograms of sterol fractions (as trimethylsilyl ethers) from the 0-2 cm section of core 1248 from the Namibian Shelf. For GC conditions see "Experimental" section. Peaks are numbered according to Table 2. (a) 4-desmethylsterolTMS ethers; (b) 4-methylsterolTMS ethers plus 4-desmethyl-5 -stanolTMS ethers.

Table 2
Total sterols identified in core 1248 from the Namibian Shelf.

	Component	Structure [a]	R_T [b]	Characteristic MS ions [c] (relative intensity)	Identification [d]	Concentration [μg/g of sediment (dry weight)]				
						0-2 cm	2-4 cm	4-6 cm	6-8 cm	8-10 cm
	4-desmethyl-sterols									
1	24-nor-cholesta-5, 22-dien-3 β-ol	$N_1\Delta^5$ R_1	1.046	442(M^+, 25), 352(25), 337(12), 313(20), 255(15), 97(100)	*a, b*	1.7	2.0	0.7	0.6	0.8
2	24-nor-5 α-cholest-22-en-3 β-ol	$N_1\Delta^0$ R_1	1.050	444(M^+, 32), 429(22), 374(45), 345(47), 257(65), 55(100)	*a, b*	1.1	1.1	1.3	1.5	1.4
3	24-nor-5 α-cholestan-3 β-ol	$N_1\Delta^0$ R_1	1.071	446(M^+, 28), 431(30), 257(30), 255(27), 215(35), 55(100)	*a*	0.3	0.4	0.9	0.7	1.6
4	27-nor-24-methylcholesta-5, 22(E)-dien-3 β-ol	$N_1\Delta^5$ R_3	1.089	456(M^+, 25), 366(30), 327(29), 255(20), 129(40), 69(100)	*a, b, c*	0.6	0.6	0.2	0.2	0.2
5	cholesta-5, 22(E)-dien-3 β-ol	$N_1\Delta^5$ R_4	1.096	456(M^+, 40), 366(45), 327(35), 255(30), 129(60), 69(100)	*a, b, c*	6.2	5.9	1.8	1.5	3.8
6	5 α-cholest-22(E)-en-3 β-ol	$N_1\Delta^0$ R_4	1.100	458(M^+, 65), 443(39), 374(90), 359(25), 345(60), 257(100)	*a, b*	3.6	5.1	5.2	5.4	8.3
7	cholest-5-en-3 β-ol	$N_1\Delta^5$ R_5	1.112	458(M^+, 60), 443(20), 368(95), 353(45), 329(100), 129(90)	*a, b, c*	11.2	14.6	4.8	3.6	7.2
8	5 α-cholestan-3 β-ol	$N_1\Delta^0$ R_5	1.116	460(M^+, 80), 445(95), 370(35), 355(50), 215(85), 75(100)	*a, b, c*	13.4	21.5	14.6	15.4	21.4
9	5 β-cholestan-3 β-ol	$N_1\Delta^0$ R_5	1.078	460(M^+, −), 370(100), 355(40), 257(5), 215(25)	*a, b*	0.8	1.5	0.8	0.4	2.5
10	C_{27} 5 β-stanol	?	1.082	460(M^+, −), 370(50), 355(75), 215(95), 55(100)	*a*	0.7	2.2	2.9	9.5	6.0
11	5 β-cholestan-3 α-ol	$N_1\Delta^0$ R_5	1.086	460(M^+, −), 370(100), 355(35), 257(15), 215(70)	*a, b*	T	1.8	1.6	1.2	4.1
12	cholest-7-en-3 β-ol	$N_1\Delta^7$ R_5	1.139	458(M^+, 95), 255(100), 229(70), 213(30)	*a, b*	0.9	1.3	0.3	1.2	T
13	C_{28} steradienol	?	1.123	470(M^+, 30), 388(15), 372(25), 298(49), 255(45), 69(100)	*a*	0.4	0.4	0.3	0.2	0.6
14	C_{28} ster-22-enol	?	1.129	472(M^+, 40), 457(40), 384(85), 374(90), 257(100)	*a*	0.6	1.5	0.7	0.7	0.9
15	24-methylcholesta-5, 22-dien-3 β-ol	$N_1\Delta^5$ R_6	1.130	470(M^+, 40), 380(40), 341(20), 255(39), 129(45), 69(100)	*a, b*	6.3	7.6	2.7	2.1	3.7
16	24-methyl-5 α-cholest-22-en-3 β-ol	$N_1\Delta^0$ R_6	1.136	472(M^+, 100), 457(20), 374(60), 345(65), 257(90), 69(95)	*a, b*	4.3	5.5	5.1	5.3	6.5
17	24-methylcholesta-5, 24(28)-dien-3 β-ol	$N_1\Delta^5$ R_8	1.154	470(M^+, 45), 386(100), 380(65), 341(55), 296(70), 129(90)	*a, b*	3.8	5.9	2.1	2.6	3.9
18	24-methyl-5 α-cholest-24(28)-en-3 β-ol	$N_1\Delta^0$ R_8	1.160	472(M^+), 457, 388, 382, 367	*a, b*	1.3	4.1	2.8	3.8	5.6
19	24-methylcholest-5-en-3 β-ol	$N_1\Delta^5$ R_7	1.160	472(M^+), 457, 343, 255, 129	*a, b*	5.5	8.2	2.0	1.3	4.1
20	24-methyl-5 α-cholestan-3 β-ol	$N_1\Delta^0$ R_7	1.165	474(M^+, 85), 459(100), 417(25), 384(40), 369(60), 215(80)	*a, b*	4.5	8.2	5.5	5.2	7.2
21	24-methyl-5 β-cholestan-3 β-ol	$N_1\Delta^0$ R_7	1.117	474(M^+, −), 384(50), 369(3), 255(10), 215(20), 75(100)	*a*	2.2	3.5	0.5	0.8	1.3
22	C_{28} 5 β-stanol	?	1.121	474(M^+, −), 384(25), 369(10), 255(10), 215(80), 75(100)	*a*	0.4	T	0.8	1.2	1.5
23	24-methyl-5 β-cholestan-3 α-ol	$N_1\Delta^0$ R_7	1.125	474(M^+, −), 384(100), 369(10), 257(10), 215(70), 75(75)	*a*	0.4	T	1.0	2.3	2.0
24	23, 24-dimethylcholesta-5, 22-dien-3 β-ol	$N_1\Delta^5$ R_9	1.171	484(M^+, 10), 394(7), 372(12), 343(15), 255(29), 69(100)	*a, b*	8.1	7.5	2.1	1.6	3.4
25	23, 24-dimethyl-5 α-cholest-22-en-3-ol	$N_1\Delta^0$ R_9	1.177	486(M^+), 471, 374, 345, 257, 69	*a, b*	6.2	7.4	5.0	6.3	10.2
26	24-ethylcholesta-5, 22-dien-3 β-ol	$N_1\Delta^5$ R_{11}	1.177	484(M^+), 394, 355, 255, 213, 69	*a, b*	1.5	2.7	0.5	0.3	T
27	24-ethyl-5 α-cholest-22-en-3 β-ol	$N_1\Delta^0$ R_{11}	1.182	486(M^+, 75), 374(12), 353(40), 345(25), 257(60), 55(100)	*a, b*	0.9	1.1	1.1	1.0	1.4
28	C_{29} steradienol	?	1.190	484(M^+, 80), 394(40), 355(55), 343(100), 213(25)	*a*	0.3	0.3	T	T	–
29	23, 24-dimethylcolest-5-en-3 β-ol	$N_1\Delta^5$ R_{10}	1.202	486(M^+, 90), 396(80), 357(55), 255(35), 129(80), 57(100)	*a, b*	0.6	0.6	0.4	0.3	0.3
30	23, 24-dimethyl-5 α-cholestan-3 β-ol	$N_1\Delta^0$ R_{10}	1.208	488(M^+), 473, 398, 383, 257	*a, b*	1.7	1.9	2.7	1.8	3.5
31	24-ethylcholesta-5, 24(28) E-dien-3 β-ol	$N_1\Delta^5$ R_{13}	1.208	484(M^+), 386, 355, 213, 129	*a, b*	0.7	1.3	0.1	T	0.3
32	24-ethyl-5 α-cholest-24(28) e-en-3 β-ol	$N_1\Delta^0$ R_{13}	1.214	486(M^+), 388	*a*	3.1	1.6	1.3	1.3	2.3
33	24-ethylcholesta-5, 24(28) z-dien-3 β-ol	$N_1\Delta^5$ R_{14}	1.216	484(M^+), 386	*a, b*	2.4	1.2	0.1	T_R	0.5
34	24-ethyl-5 α-cholest-24(28) z-en-3 β-ol	$N_1\Delta^0$ R_{14}	1.220	486(M^+, 2), 471(5), 388(100), 373(29), 345(10), 283(10)	*a*	0.8	1.6	0.9	1.3	2.3
35	24-ethylcholest-5-en-3 β-ol	$N_1\Delta^5$ R_{12}	1.208	486(M^+), 471, 396, 381, 357, 255, 129	*a, b*	6.0	6.7	2.0	1.9	3.9
36	24-ethyl-5 α-cholestan-3 β-ol	$N_1\Delta^0$ R_{12}	1.214	488(M^+), 473, 431, 398, 385, 305, 215	*a, b*	4.2	8.1	5.3	4.9	7.1
37	C_{29} 5 β, 3 β-stanol	$N_1\Delta^0$ R_{10} or R_{12}	1.161	488(M^+, −), 398, 383, 215	*a*	T	T	T	T	1.7
38	C_{29} 5 β, 3 α-stanol	$N_1\Delta^0$ R_{10} or R_{12}	1.165	488(M^+, −), 398(95), 383(10), 257(10), 215(80), 75(100)	*a*	0.4	T	1.0	2.9	1.5
39	22(23)-methylene-23, 24-dimethylcholest-5-en-3 β-ol	$N_1\Delta^5$ R_{15}	1.287	498(M^+, 35), 408(40), 386(30), 343(25), 129(85), 83(100)	*a, b*	10.3	7.9	1.8	0.7	0.6

Table 2

	Component	Structure [a]	R_T [b]	Characteristic MS ions [c] (relative intensity)	Identification [d]	Concentration [μg/g of sediment (dry weight)] 0-2 cm	2-4 cm	4-6 cm	6-8 cm	8-10 cm
40	22(23)-methylene-23, 24-dimethyl-5α-cholestan-3β-ol 4-Methyl-sterols	$N_1\Delta^0$ R_{15}	1.296	500($M^{+\cdot}$, 15), 388(50), 345(60), 339(15), 285(30), 75(100)	*a, b*	2.9	2.4	1.6	1.0	1.1
41	4α-methylcholest-?-en-3β-ol	$N_2\Delta?$ R_5	1.154	472($M^{+\cdot}$, 100), 457(3), 382(10), 367(12), 269(10), 227(15)	*a*	1.8	4.3	0.9	0.4	3.5
42	4α-methyl-5α-cholestan-3β-ol	$N_2\Delta^0$ R_5	1.161	474($M^{+\cdot}$, 5), 459(50), 384, 369, 345, 229	*a*	2.9	4.2	3.8	5.4	3.8
43	4α, 24-dimethylcholesta-5, 22-dien-3β-ol	$N_2\Delta^5$ R_6		484($M^{+\cdot}$, 1), 388(2), 359(4), 355(2), 271(20), 269(15), 69(100)	*a*	0.9	1.3	2.0	4.1	3.0
44	4α, 24-dimethyl-5α-cholest-22-en-3β-ol	$N_2\Delta^5$ R_6	1.183	486($M^{+\cdot}$, 15), 388(10), 359(15), 271(40), 229(5), 69(100)	*a*	2.4	4.1	3.5	4.0	4.4
45	4α, 24-dimethylcholest-5?-en-3β-ol	$N_2\Delta^5?$ R_7	1.207	486($M^{+\cdot}$, 40), 396(5), 357(2), 269(20), 229(10), 81(100)	*a*	1.3	0.9	0.3	–	1.0
46	4α, 24-dimethyl-5α-cholestan-3β-ol	$N_2\Delta^0$ R_7	1.219	488($M^{+\cdot}$, 8), 473(15), 398(5), 359(85), 229(40), 75(100)	*a*	7.7	13.1	11.9	12.8	14.6
47	4α, 23, 24-trimethylcholesta-5, 22-dien-3β-ol	$N_2\Delta^5$ R_9	1.229	498($M^{+\cdot}$, 12), 369(4), 363(8), 269(8), 129(25), 69(100)	*a, b*	4.4	8.1	7.0	8.3	6.9
48	4α, 23, 24-trimethyl-5α-cholest-22-en-3β-ol	$N_2\Delta^0$ R_9	1.234	500($M^{+\cdot}$, 5), 485(2), 388(12), 359(25), 271(25), 69(100)	*a, b*	24.2	32.7	32.2	36.4	37.7
49	C_{30} 4-methyl-stenol	?	1.262	500($M^{+\cdot}$, 100), 485(2), 410(5), 395(5), 269(15), 227(20)	*a*	2.5	4.3	0.9	1.6	2.9
50	C_0 4-methyl-stanol	?	1.272	502($M^{+\cdot}$, 1), 412(25), 373(15), 261(25), 229(25), 75(100)	*a*	2.4	3.9	3.7	3.7	3.8
51	C_{30} 4-methyl-stenol	$N_2\Delta?$ $R_{10}?$	1.265	500($M^{+\cdot}$, 100), 485(1), 410(5), 395(5), 269(15), 227(15)	*a*	1.0	2.4	1.1	2.5	2.6
52	4α, 23, 24-trimethyl-5α-cholestan-3β-ol	$N_2\Delta0$ R_{10}	1.279	502($M^{+\cdot}$, 5), 412(50), 373(60), 261(15), 229(30), 75(100)	*a*	3.6	6.3	5.2	7.4	7.6

Notes: [a], structures as given in Figure 6. Δ refers to the position of the nuclear double bond; Δ0 indicates a saturated nucleus. [b], GC retention time, on OV1, relative to 5α-cholestane (internal standard). [c], principal characteristic ions in mass spectrum. Where two or more components co-elute, the relative intensities are not given, as some ions are common to the mass spectra of more than one component. [d], Identification based on: (a) consideration of GC retention time and interpretation of mass spectrum; (b) comparison of mass spectrum with that of the authentic compound and/or with published data; (c) co-injection with authentic standard.

noticeable among the 4-desmethyl-sterols than the 4-methyl sterols. Assuming that stanol/stenol ratios are related to residence time in the sediment (Gaskell, Eglinton, 1976; Nishimura, Koyama, 1977), then these data appear to support the stability of the upper sediment in core 1248 and so justify more detailed examination for other possible diagenetic products.

The 5α-stanols tend to show an increase in the 3α-ol epimer compared to the 3β-ol with depth. 5β-stanols can be separated from their 5α-analogues by virtue of their higher R_f value in the TLC system used and they also have shorter retention times in the GC analyses. 5β, 3β and 5β, 3α can be distinguished by GC retention time (5β, 3β ⩽ 5β, 3α) and differences in relative intensities of certain MS ions. Structural assignments followed Brassell (1980). Peaks (10) and (22) were a C_{27} 5β-stanol and C_{28} 5β-stanol respectively, of unknown structure. They were assigned as 3β-ols on the basis of their MS fragmentation pattern.

An interesting feature is the steady reduction in the concentration of 22(23)-methylene-23, 24-dimethylcholest-5-en-3β-ol (gorgosterol, 39) and its nuclear saturated analogue with depth. Possible reasons for this are discussed later.

In all sections, the dominant sterol was 4α, 23, 24-trimethyl-5α-cholest-22-en-3β-ol (dinosterol 48), which has been reported as an important component of the sterols from various other sediments (e. g. Brassell, 1980; Boon *et al.*, 1979). In these samples it is accompanied by its Δ^5 analogue. Components (49) and (51) had virtually identical mass spectra, with the molecular ion at *m/z* 500 as base peak, indicating a monounsaturated C_{30} structure. Other prominent ions at *m/z* 227 and 269 indicated a mono-unsaturated nucleus possessing an additional methyl group, probably at C-4; however, the position of the nuclear double bond cannot be deduced from the fragmentation. Compound (41) may be an analogous C_{28} sterol, as its mass spectrum shows a similar fragmentation pattern. Components (50) and (52) also had virtually identical mass spectra and are almost certainly the fully saturated stanols corresponding to (49) and (51) respectively. A peak with identical mass spectrum and GC retention time to compound (52) co-injected with a standard prepared by hydrogenation of dinosterol (J. K. Volkman, unpublished), indicating that (52) is probably 4α, 23, 24-trimethyl-5α-cholestan-3β-ol (dinostanol). Compound (50) may therefore be 4α, 22, 24-trimethyl-5α-cholestan-3β-ol or 4α, 22, 23-trimethyl-5α-cholestan-3β-ol. The other main possibility, 4α-methyl-24-ethyl-5α-cholestan-3β-ol, would be expected to have a greater retention time than (50) or (52) by analogy with the corresponding 4-desmethyl sterols. Indeed, in some GC runs a second, later eluting (minor) component was partially resolved as a "shoulder" on peak (52), which was indistinguishable from (52) by GC-MS. This second component may be 4α-methyl-24-ethyl-5α-cholestan-3β-ol. C_{28} and C_{29} 4-methyl sterols were also present, the most abundant of these being 4α, 24-dimethyl-5α-cholestan-3β-ol (46).

The second most abundant sterol in all sections was 5α-cholestan-3β-ol (8). This was accompanied by

cholest-5-en-3 β-ol (cholesterol, 7), 5 α-cholest-22-en-3 β-ol (6) and cholesta-5, 22-dien-3 β-ol (5). A variety of C_{26}, C_{28} and C_{29} 4-desmethyl-sterols were also present, encompassing $\Delta^{5,22}$, $\Delta^{5,24(28)}$, $\Delta^{24(28)}$, Δ^{22}, Δ^{5} and Δ^{0} unsaturation.

Sterone distributions

Steroidal ketones, with both saturated nuclei and Δ^4-unsaturation, were identified in all core sections. The distributions of the stanones are given in Figure 5 and Table 3, and those of the Δ^4-stenones in Table 4.

The stanones show a very similar distribution in all sections, being dominated by 4 α, 23, 24-trimethyl-5 α-cholest-22-en-3-one (dinosterone), with 5 α-cholestan-3-one as the second most abundant component. C_{26}, C_{27}, C_{28}, C_{29} and C_{30} stanones, corresponding to, and in similar proportions to, all the major 4-methyl and 4-desmethyl-sterol structures, are present. Compound (1) is probably 27-nor-24-methyl-5 α-cholest-22-en-3-one. The 5 α-epimers are generally more abundant than the 5 β-epimers, although an increase in the proportion of 5 β relative to 5 α is observed, for some stanones, in the lower sections compared to the upper.

Δ^4-stenones were found in each section of the core, but were less abundant than the nuclear saturated stanones (Table 4). Structures corresponding to the principal sterols and stanones were identified and their distributions show a similar pattern. Compounds (5) and (6) each showed a molecular ion at *m/z* 410, indicating a C_{29} diunsaturated structure, and a base peak at *m/z* 69, suggesting Δ^{22} unsaturation however, the assignment as $\Delta^{4,22}$ is only tentative. By analogy with the sterols, one of them presumably has a 23, 24-dimethyl side chain and the other a 24-ethyl side chain. Similarly, the side

Table 3
Total stanones identified in core 1248 *from the Namibian Ridge.*

	Component	R_T[a]	Characteristic ions in mass spectrum (relative intensity)	Identification[b]	Concentration [μg/g of sediment (dry weight)] 0-2 cm	2-4 cm	4-6 cm	6-8 cm	8-10 cm
	(a) 4-desmethyl-stanones								
1	C_{27} Δ^{22} 5 α-stanone	1.076	384($M^{+\cdot}$, 4), 300(25), 271(20), 255(40), 55(100)	*a*	T	T	0.1	0.1	–
2	Cholest-22-en-2-one	1.088	384($M^{+\cdot}$, 45), 300(95), 271(50), 255(45), 55(100)	*a, b*	0.1	0.4	0.7	0.5	0.1
3	5 α-cholestan-3-one	1.103	386($M^{+\cdot}$, 65), 371(20), 231(100), 217(25)	*a, b, c*	0.5	1.9	2.6	2.4	2.2
4	5 β-cholestan-3-one	1.087	386($M^{+\cdot}$, 100), 371(10), 316(55), 231(70)	*a, b, c*	T	0.4	0.9	1.1	1.4
5	C_{28} Δ^{22} 5-stanone	1.118	398($M^{+\cdot}$, 5), 300(20), 285(5), 273(5), 69(100)	*a*	T	0.1	0.2	0.1	0.2
6	24-methyl-5 α-cholest-22-en-3-one	1.124	398($M^{+\cdot}$, 75), 300(65), 285(30), 271(70), 55(100)	*a, b*	0.1	0.6	1.0	0.6	0.6
7	24-methyl-5 α-cholest-24(28)-en-3-one	1.146	398($M^{+\cdot}$, 10), 383(30), 314(100), 299(30), 271(35)	*a, b*	0.1	0.4	0.7	0.5	0.4
8	24-methyl-5 β-cholest-24(28)-en-3-one	1.126	398($M^{+\cdot}$, 7), 383(10), 314(100), 299(40), 281(40)	*a*	–	T	0.1	–	0.5
9	24-methyl-5 α-cholestan-3-one	1.151	400($M^{+\cdot}$, 80), 385(15), 231(100), 217(25)	*a, b*	0.1	0.7	0.9	0.5	0.6
10	24-methyl-5 β-cholestan-3-one	1.128	400($M^{+\cdot}$, 100), 330(40), 283(10), 231(55)	*a, b*	T	0.1	0.1	0.1	0.4
11	23, 24-dimethyl- or 24-ethyl- 5 α-cholest-22-en-3-one	1.163	412($M^{+\cdot}$, 5), 300(15), 285(10), 271(40), 69(100)	*a*	0.2	0.8	1.4	1.1	0.8
12	C_{29} 5 α-stanone	1.168	412($M^{+\cdot}$, 40), 369(20), 300(30), 271(55), 55(100)	*a, b*	T	0.1	0.2	0.1	0.2
13	24-ethyl-5 α-cholest-24(28)-en-3-one	1.206	412($M^{+\cdot}$, –),, 324(100), 299(10), 271(3)	*a, b*	T	T	0.2	0.1	0.3
14	24-ethyl-5 β-cholest-24(28)-en-3-one	1.182	412($M^{+\cdot}$, 5), 314(100), 299(30), 281(35), 273(15)	*a*	–	T	–	–	0.2
15	23, 24-dimethyl-5 α-cholestan-3-one	1.194	414($M^{+\cdot}$, 50), 317(25), 231(100), 217(20), 98(75)	*a*	T	0.4	0.4	0.3	1.3
16	24-ethyl-5 α-cholestan-3-one	1.196	414($M^{+\cdot}$, 85), 399(15), 314(15), 231(100), 217(25)	*a, b*	0.1	0.7	0.8	0.5	0.6
17	C_{29} Δ^0 5 β-stanone	1.172	414($M^{+\cdot}$, 100), 344(45), 328(25), 231(80)	*a, b*	T	0.2	0.2	0.3	0.5
18	C_{30} stanone	1.271	426($M^{+\cdot}$, 20), 397(15), 314(40), 271(60), 55(100)	*a*	0.1	0.2	0.2	T	0.1
	(b) 4-Methyl-stanones								
19	4-methyl-5 α-cholestan-3-one	1.127	400($M^{+\cdot}$, 55), 385(25), 245(100), 231(15)	*a*	0.1	0.3	0.4	0.6	0.5
20	4, 24-dimethyl-5 α-cholest-22-en-3-one	1.147	412($M^{+\cdot}$, 75), 314(65), 299(30), 285(75), 55(100)	*a*	0.1	0.3	0.7	0.8	0.7
21	4, 24-dimethyl-5 α-cholestan-3-one	1.176	414($M^{+\cdot}$, 80), 399(30), 245(100), 231(20)	*a, b*	0.3	1.0	1.4	1.5	1.1
22	4, 23, 24-trimethyl-5 α-cholest-22-en-3-one	1.188	426($M^{+\cdot}$, 5), 283(8), 314(15), 285(35), 69(100)	*a, b*	1.4	5.1	8.6	6.9	6.1
23	C_{30} 4-methyl Δ^0 5 α-stanone	1.223	428($M^{+\cdot}$, 65), 413(25), 331(30), 245(100), 231(25)	*a*	0.2	0.4	1.0	0.9	0.6
24	C_{30} 4-methyl Δ^0 5 α-stanone	1.228	428($M^{+\cdot}$, 70), 413(30), 331(25), 245(100), 231(25)	*a*	0.2	0.9	1.5	1.4	1.1

[a] GC retention time, on OV1, relative to 5 α-cholestane (internal standard).
[b] (*a*) GC retention time and interpretation of mass spectrum. (*b*) Comparison of mass spectrum with that of the authentic compound and/or with published data. (*c*) GC co-injection with authentic standard.

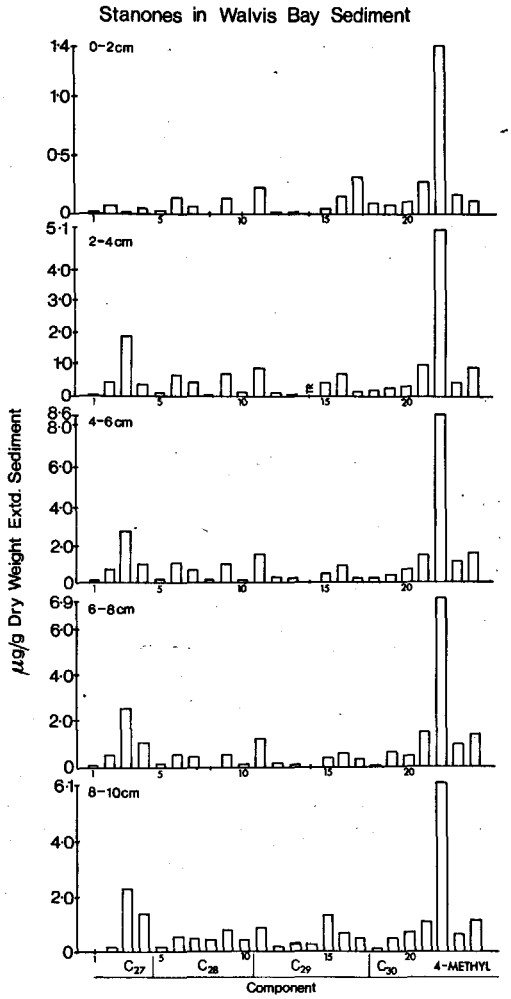

Figure 5
Distribution of stanones in core 1248 *from the Namibian Shelf (compounds are numbered according to Table* 3).

chain structures of compounds (7) and (8) are unknown, but again, 23, 24-dimethyl- is most likely for one and 24-ethyl- for the other.

Sterene distributions

Low levels of sterenes were found in all core sections (Table 5) with cholest-2-ene being the most abundant component. Two other C_{27} sterenes were identified, cholesta-3, 5-diene and very small amounts of cholestatriene. A variety of C_{28} and C_{29} mono-, di- and trienes were present, along with an unidentified C_{30} steradiene (the mass spectrum of which indicated both double bonds to be in the nucleus) in the 8-10 cm section. The only other C_{30} steroidal alkene found had a very similar mass spectrum to the compound tentatively identified as 22(23)-methylene-23, 24-dimethylcholestadiene (gorgostadiene) by Wardroper (1979), as is assigned as such in Table 5. The concentrations of sterenes generally tend to increase with depth in the core. No 4-methyl-sterenes were detected in any sample.

DISCUSSION

Sterol composition in relation to input

The major identifiable contributors of organic matter to these Walvis Bay sediments are diatoms, remains of which are readily recognisable in the sediment (*see* earlier). Many of the sedimentary sterols are indeed known to occur in diatoms, in particular cholest-5-en-3 β-ol, 24-methyl-cholesta-5, 22-dien-3 β-ol, 24-methylene-cholesta-5, 24 (28)-dien-3 β-ol (24-cholesterol), 24-methylcholest-5-en-3 β-ol, 24-ethylene-cholesta-5, 24 (28)-dien-3 β-ol (24-ethylidene-cholesterol) and 24-ethyl-cholest-5-en-3 β-ol (Orcutt, Patterson, 1975; Ballantine *et al.*, 1979 *b*). Several of these, however, are widely occurring throughout the biosphere and cannot be specifically related to a diatomaceous source. In addition, the C_{26} sterol, 24-nor-cholesta-5, 22(E)-dien-3 β-ol (1), has been identified in a phytoplankton sample composed principally of diatoms (Boutry *et al.*, 1971). Cholesta-5, 22(E)-dien-3 β-ol (5) is the major sterol of the diatom *Biddulphia sinensis* (Volkman *et al.*, 1980). This diatom also contains, as minor components of its sterols, two unusual compounds – cholest-7-en-3 β-ol (12) and 23, 24-dimethyl-cholesta-5, 22-dien-3 β-ol (24). Δ^7 sterols in the marine environment are mainly found in two classes of echinoderms (asteroids and holoturians), one class of molluscs (chitons) and some tunicates (*see* Morris, Culkin, 1977). These are normally benthic organisms and hence believed to be absent in these anoxic sediments – the azoic zone of Copenhagen, 1934, 1953).

Table 4
Total stenones identified in core 1248 *from the Namibian Shelf. Concentrations in µg/g dry weight sediment.*

Component [a]	core sections (depth in cm)				
	0-2	2-4	4-6	6-8	8-10
Cholesta-4, 22-dien-3-one	0.1	0.1	0.2	0.4	–
Cholest-4-en-3-one [b]	0.6	0.6	1.2	1.9	1.3
24-methylcholesta-4, 22-dien-3-one	0.3	0.2	0.6	1.0	–
24-methylcholest-4-en-3-one	0.3	0.4	0.7	1.1	1.0
$C_{29}\Delta^{4,22}$-dien-3-one	0.6	0.4	0.8	0.8	–
$C_{29}\Delta^{4,22}$-dien-3-one	T	T	0.2	0.1	–
$C_{29}\Delta^{4}$-en-3-one	T	T	0.2	0.2	0.1
$C_{29}\Delta^{4}$-en-3-one	0.6	0.6	0.9	1.3	0.6
4, 23, 24-trimethylcholesta-4, 22-dien-3-one	0.3	0.8	1.2	1.1	1.4

T = trace component (<0.1 µg/g); – = not detected; [a] Identification by GC retention times and MS data; [b] Co-injected with authentic standard.

Table 5
Total sterenes identified in core 1248 *from the Namibian Shelf. Concentrations in µg/g dry weight sediment.*

Component [a]	Core sections (depth in cm)				
	0-2	2-4	4-6	6-8	8-10
Cholest-2-ene	0.5	0.8	1.1	1.3	1.8
Cholest-3, 5-diene [b]	0.3	0.3	0.6	0.8	1.0
Cholestatriene	0.1	T	–	T	T
24-methylcholest-2-ene	0.1	0.1	–	–	–
24-methylcholestadiene	0.1	0.1	0.2	0.2	0.8
C_{28} steratriene	–	0.2	0.4	0.5	0.3
C_{28} steratriene	–	–	0.2	0.5	0.5
$C_{29}\Delta^{2}$-sterene	T	0.1	–	–	T
C_{29} steradiene	0.1	0.1	0.3	0.2	0.6
C_{29} steratriene	0.4	0.3	0.4	0.7	0.7
C_{30} steradiene	–	–	–	–	0.6
22(23)-methylene-23, 24-dimethyl-cholestadiene?	0.5	0.3	0.5	0.4	0.3

T = trace component (<0.1 µg/g); – = not detected; [a] identifications by GC retention time and MS data; [b] co-injected with authentic standard.

In an earlier study on this same core, a single class of compounds extracted from the 0-10 cm section, the 4-desmethyl-sterols, was analysed by Wardroper *et al.* (1978). They did not detect any Δ^7-sterols and associated their absence with lack of benthic macrofauna. In this more detailed study, covering several classes of steroidal compounds, a number of additional minor 4-desmethyl-sterols have been detected, including small amounts of cholest-7-en-3 β-ol, which may derive from diatoms such as *B. sinensis*. Although, as is to be expected, the distributions of 4-desmethyl-sterols in these two studies are similar, some differences are noteworthy. In particular, in this present work, the concentrations of sterols found are generally higher, whereas the proportions of certain sterols, most notably 23, 24-dimethyl-cholesta-5, 22-dien-3 β-ol and 22(23)-methylene-23, 24-dimethylcholest-5-en-3 β-ol, are lower than reported by Wardroper *et al.* (1978). Such dissimilarities are most likely attributable to the different extraction procedures used. Whereas Wardroper *et al.* (1978) extracted the solvent ultrasonically, for twenty minutes, into a propan-2-ol/hexane mixture (4:1 v/v), the methods employed in this present study involved more polar solvents and more prolonged extraction, and would therefore be expected to provide a more efficient extraction of the organic material.

A non-diatomaceous source can be invoked for a number of the sterols. 23, 24-dimethylcholest-5, 22-dien-3 β-ol (24) is only present in very low concentrations in the diatom *B. sinensis* (Volkman *et al.*, 1980) yet is quite abundant in the sediment, and so other sources are likely. This sterol has recently been found in *Hymenomonas carterae*, one of four species of coccolithophorid examined by Volkman *et al.* (1981). The major sterol of all four species was 24-methyl-cholesta-5, 22-dien-3 β-ol (15), which is quite abundant in the sediments (and which has previously been referred to by the trivial name of "diatomsterol" due to its common occurrence in diatoms). Also present in three of the species was 24-ethylcholesta-5, 22-dien-3 β-ol (26) – a minor component of these sedimentary sterols, which is not a common phytoplankton sterol. Further evidence for a (probably minor) coccolithophorid contribution to the sedimentary organic matter, is provided by the presence of small amounts of straight chain C_{37}-C_{39} methyl and ethyl ketones, compounds which so far have only been identified in one organism, *Emiliania huxleyi* (Volkman *et al.*, 1980), and the observation of a few coccolith structures in the sediment examined by microscopy.

In addition to its occurrence in *B. sinensis* and *H. carterae*, 23, 24-dimethylcholesta-5, 22-dien-3 β-ol has also been identified in a coral by Kanazawa *et al.* (1974), which led Wardroper *et al.* (1978) to suggest an input from coelenterates. Certainly, *Scyphozoa* jellyfish were observed in the area in huge numbers during the sampling cruise. From a survey of the published sterol compositions of oceanic jellyfish (Morris, Culkin, 1977), only one analysis appears to show the presence among the sterols of a possible marker compound for these organisms; analysis of *Cassipoea xamachana* by Ciereszko *et al.* (1968), revealed the presence of the unusual sterol 22(23)-methylene-23, 24-dimethyl-

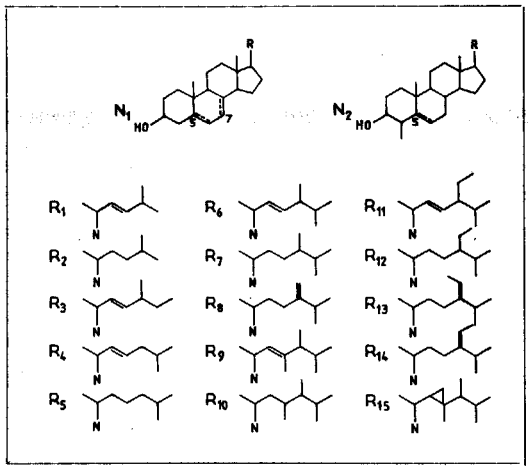

Figure 6
Structures of the sterols identified in core 1248 *from the Namibian Shelf, listed in Table* 2 *and with abundances shown in Figure* 2.

cholestan-3 β-ol (gorgostanol) which was believed to have been formed, during work-up, from the Δ^5 analogue (gorgosterol). The presence of both these compounds in the sediment thus may indicate a coelenterate contribution as suggested by Wardroper *et al.* (1978). Gorgosterol and gorgostanol have also both recently been identified, in small amounts, in dinoflagellates which occur as symbiotic zooxanthelae in coelenterates, and in heterotrophic phytoplanktonic dinoflagellates (Withers *et al.*, 1979). Possible dinoflagellate input is discussed below.

The steady reduction in the concentration of gorgosterol and gorgostanol with depth in the core, is of interest. This could reflect a changing input, particularly if its presence is due largely to the occurrence of jellyfish swarms which could well be only intermittently abundant.

Alternatively, it could be that gorgosterol/stanol is degraded more rapidly than the other sterols – it would not seem unreasonable that a structure containing a cyclopropane ring might be less stable than the other sterols. Since the overall concentration of sterols shows no decrease with depth, it seems unlikely that significant loss of the basic steroidal structure by degradation is occurring.

Of considerable interest is the major steroidal component in all the sediment sections -4 α, 23, 24-trimethyl-5 α-cholest-22-en-3 β-ol (48 dinosterol). This sterol has, so far, only been found in a number of dinoflagellate species (Shimizu *et al.*, 1976; Withers *et al.*, 1978; Alam *et al.*, 1979) and is generally regarded as a dinoflagellate marker (Boon *et al.*, 1979). Yet there was no microscopic evidence of dinoflagellate remains, although dinoflagellate cysts are known to be highly resistant and have been recognised in a number of sediments, e. g. in the Black Sea (Boon, 1978), with a geological record going back to the Cretaceous (Smayda, 1971). Dinoflagellates are reported to make up about 2% of the phytoplankton in the Walvis Bay area, although periodic "red-tide" blooms occur (Hart, Currie, 1960). Lee *et al.* (1980) observed dinoflagellate cysts in a core from Walvis Bay, whereas Boon (1978) did not and suggests dinoflagellate blooms are of a local nature.

A number of other 4-methyl sterols observed in these sediments have been identified in dinoflagellates; Withers *et al.* (1978) identified 4α, 23, 24-trimethylcholesta-5, 22-dien-3β-ol (47) and dinosterol, and tentatively identified 4α, 24-dimethyl-cholest-5-en-3β-ol (45?) and 4α, 24-dimethyl-cholestan-3β-ol (46) in *Crypthecodinium cohnii.*

Reasons why chemical indicators of dinoflagellates should be present when identifiable physical remains cannot be observed are open to speculation. Some dinoflagellates are "unarmoured", i. e. are enclosed only by a thin pellicle and probably would not survive in sediments in a recognisable form; whether or not all species can form highly resistant cysts is unknown. Although dinosterol and related sterols have not yet been identified in any group of organisms other than the Dinophyceae, there is a large group of the phytoplankton, collectively called "nannoplankton", whose chemical composition is virtually unknown. Their importance is just beginning to be recognised – studies of the waters off East Australia indicated nannoplankton as contributing 50-80% of the total plankton chlorophyll (Hallegraeff, 1981). Nannoplankton are a diverse group, commonly considered as phytoplankton with a cell size <15 μm and are very fragile, usually being destroyed by conventional plankton collection nets. Hence, the paucity of information available on them. Their delicacy also means they leave little or no fossil record (Jeffery, Hallegraeff, 1980). The species composition of nannoplankton is largely unknown, but it contains various types of small flagellates including non-thecate dinoflagellates (Hallegraeff, 1981). Thus it may have important implications for the assessment of contributions of organic matter to sediments (and, indeed, for the marine ecosystem generally) and it is to be hoped that improved collection methods (e. g. high speed continuous centrifugation as used by Jeffery, Hallegraeff, 1980) will enable more taxanomic and chemical data to be made available.

Sterol diagenesis

The diagenetic fate of sterols is of considerable interest in organic geochemistry, in particular the proposed conversions of Δ^5-stenols to stanols and their dehydration to form sterenes. Nuclear saturated stanols, whilst significantly increasing with depth, still form a high proportion of the total sterols in the uppermost section of sediment. This suggests that quite large quantities of stanols form part of the sedimentary input, or are formed rapidly in the upper sediment layers. One possibility is a direct biological input; significant amounts of natural product stanols have been found in a wide spectrum of phyla including molluscs, sponges, coelenterates, echinoderms, annelids and tunicates (Morris, Culkin, 1977 and references therein; Ballantine *et al.*, 1976; 1977; 1978; 1979 *a*; 1981; Voogt, 1976; Gupta *et al.*, 1979). In some instances the stanols have been found to comprise over 50% of the animals component sterols. Low levels of stanols have also been found in a freshwater diatom (Nishimura, Koyama, 1976). However, stanols are not recognised as very significant components of phytoplankton (with the exception of some 4-methyl sterols such as dinosterol). Thus, in view of the quantities present in these sediments and the likely biological input, plus the fact that major Δ^5 and $\Delta^{5,22}$ stenols in the sediments are present together with their Δ^0 and Δ^{22} analogues, it seems very likely that a diagenetic Δ^5-stenol → stanol conversion is the major source of the sedimentary stanols.

Studies on sterols in the water column of the Black Sea (Gagosian, Heinzer, 1979; Gagosian *et al.*, 1979 *a*) indicated that no significant stenol → stanol conversion was occurring in the water column. It thus appears that the abundance of stanols in the surface sediment is due to a combination of direct input, plus rapid reduction of stenols (probably microbially mediated) after deposition.

As discussed earlier, the stanol/stenol ratio tends to increase with depth in the core, as shown in Figure 4. Examination of Figure 2 shows that this trend occurs with all the 4-desmethyl $\Delta^{5,22}$-Δ^{22} and Δ^5-Δ^0 stenol-stanol pairs. In all cases the stanol/stenol ratio increases rapidly below the 2-4 cm section, reaching a maximum at the 6-8 cm level, then decreasing somewhat at the lowest (8-10 cm) level. The reasons for this behaviour are uncertain. However, the overall trend is consistent with a diagenetic conversion of stenols to stanols.

The mechanism of this reduction process is a source of much speculation. Figure 1 shows a summary of a proposed scheme. It is generally accepted that there is a microbiological role, as bacteria have been shown to convert stenols into stanols under anaerobic conditions (Björkhem, Gustafsson, 1971; Eyssen *et al.*, 1973; Parmentier, Eyssen, 1974). These studies indicated 3-keto-stanones and Δ^4-3-keto-stenones as intermediates in the major pathway for the conversion, but did not rule out the possibility of some direct reduction of the Δ^5-double bond occurring.

Whether or not a similar mechanism operates in sediments requires confirmation. A depth profile of the sterols in a lake sediment, carried out by Gaskell and Eglinton (1976), indicated that rapid conversion of stenols to stanols was occurring. Incubation of radiolabelled cholesterol with this sediment, and with anaerobic sewage sludge, showed formation of cholest-4-en-3-one and cholestan-3-one, as well as 5α- and 5β-cholestanol, from the labelled cholesterol (Gaskell, Eglinton, 1975). Indirect evidence for the operation of the stenol → stanol diagenetic pathway outlined in Figure 1 would be provided by the presence in the sediments of the intermediate stenones and stanones; a series of stanones have indeed been identified in Walvis Bay sediments by Gagosian and Smith (1979). Such compounds have also been found in other sediments (e. g. Brassell, 1980; Taylor *et al.*, 1981).

The core examined in this study contained both Δ^4-stenones and 5α- and 5β-stanones in all sections. The distributions of stanones closely resemble those of the corresponding sterols, while their concentrations are considerably lower (Table 3, Fig. 5). Gagosian and Smith (1979) identified a similar series of stanones, with the exception of some of the minor components, and

also found 4α, 23, 24-trimethyl-5α-cholest-22-en-3-one to be the major compound. These authors, however, did not detect any stenones in their samples, whereas Δ^4-stenones were present, in low concentrations, in all core sections examined here (Table 4). Although the stenol stanol transformation, as previously proposed (Fig. 1) shows Δ^5 stenones as being the immediate precursors of stanones, such compounds have not yet been found in any sediments, to our knowledge. It seems much more likely that Δ^5-stenones, if formed initially, would very rapidly isomerise to the more stable, conjugated Δ^4-en-3-ones, such as are present in the sediments analysed here. Indeed, in experiments on bacterial hydrogenation of stenols, Eyssen *et al.* (1973), noted that under the conditions used, cholest-5-en-3-one spontaneously isomerised to cholest-4-en-3-one, while Björkhem and Gustafsson (1971) provided evidence, from radiolabelling, that isomerisation of the Δ^5- to a Δ^4-double bond did occur during microbial reduction of cholesterol to cholestanol. Hence, assuming the stanones and stenones detected in this core are intermediates in a stenol reduction process, the following diagenetic pathway is probably occurring in the sediment:

stenol → (Δ^5-stenone) → Δ^4-stenone → stanone → stanol.

The major stanone present is 4,23,24-trimethyl-5α-cholest-22-en-3-one (dinosterone); however, the sterol which would give rise to it by the above pathway, 4,23,24-trimethylcholesta-5,22-dien-3β-ol, is not of comparable abundance in the sediment. The most abundant sterol is 4α,23,24-trimethyl-5α-cholest-22-en-3β-ol (dinosterol), which does not possess a Δ^5-double bond and so cannot undergo hydrogenation as depicted above. It could, however, form dinosterone by an oxidation process, i. e. the reverse of the final step in the above pathway. Evidence that this could occur has been provided by Edmunds *et al.* (1979), who incubated radiolabelled 5α-cholestanol in an algal mat and found part of it was converted to 5α-cholestan-3-one. Additionally, radiolabelling experiments by Parmentier and Eyssen (1974) indicated that an anaerobic bacterium carries out reversible oxidation-reduction reactions on the 3β-hydroxy group of 5α-cholestanol. Thus it seems likely that the final step in the proposed stenol hydrogenation pathway is reversible (i. e. stanone ⇌ stanol), and so 4,23,24-trimethyl-5α-cholest-22-en-3-one may be formed from both 4α,23,-24-trimethylcholesta-5,22-dien-3β-ol and 4α,23,24-trimethyl-5α-cholest-22-en-3β-ol.

It is also necessary to consider the possibility of direct biological input contributing to the stanones in the sediment. 4α,23,24-trimethyl-cholest-22-en-3-one (dinosterone) has been identified in a dinoflagellate *Crypthecodinium cohnii*, by Withers *et al.* (1978), who suggested it was an intermediate in dinosterol biosynthesis. However, reports of 3-ketosteroids as significant components of biological systems are scarce and, as discussed by Gagosian and Smith (1979), it is unlikely that substantial amounts of the sedimentary sterones, unlike the sterols, arise from planktonic production.

In laboratory experiments by Björkhem and Gustafsson (1971), Eyssen *et al.* (1973) and Parmentier and Eyssen (1974), microbial biohydrogenation of stenols resulted in formation of 5β-stanols; chemical hydrogenation, however, produces the more stable 5α-stanols. In these sediments, all the stanols identified had predominantly the 5α(H) configuration, with 5β-stanols comprising only *ca.* 10% of the total. Thus if production of 5β-stanols is a general feature of microbial reduction and this is the major source of sterols in this sediment the results could imply a very rapid epimerisation of 5β to 5α stanols in the sediment. Alternatively, as suggested by Gaskell and Eglinton (1975), who obtained mixtures of 5α- and 5β-cholestanol from their incubations of cholesterol with sediment, the 5α: 5β ratio could be principally determined by the particular microbial population active in the sediment.

Several authors have attributed the presence of sterenes in sediments to their formation by diagenetic dehydration of sterols (e. g. Dastillung, Albrecht, 1977; Gagosian, Farrington, 1978). The concentrations of sterenes found in this study are lower than those reported by Gagosian and Farrington (1978), for other cores from the Walvis Bay region; the concentrations, however, do tend to increase with depth, suggesting that higher levels of sterenes result from longer residence times, which is consistent with diagenetic formation. Correlation between the structures of the sterenes and sterols is not as marked as between the sterones and sterols; in particular, no 4-methyl-sterenes were detected. This is in agreement with Gagosian *et al.* (1977*b*), who also found a lack of 4-methyl-sterenes compared with the 4-desmethyl-compounds, and suggested that this could be due either to their rapid degradation or steric inhibition of the sterol dehydration reaction by the methyl group at C−4, resulting in much slower formation of 4-methyl-sterenes.

Nevertheless, the most abundant sterene was cholest-2-ene, which could be formed by dehydration of the most abundant 4-desmethyl-stanol, 5α-cholestanol. C_{28} and C_{29} Δ^2-sterenes were also present, which would presumably be formed from the corresponding C_{28} and C_{29} stanols. Conversion of cholestanol to cholest-2-ene by incubation with clay, has been demonstrated by Rubinstein *et al.* (1975), while Δ^2-sterenes were the major components of the steroidal alkenes in sediments analysed by Dastillung and Albrecht (1977). Similarly, dehydration of the most abundant 4-desmethyl-stenol, cholest-5-en-3β-ol, could form cholesta-3,5-diene, which was the most abundant diunsaturated sterene. C_{28}, C_{29} and C_{30} steradienes were also present. Cholest-5-en-3β-ol has been incubated with Green River shale and found to produce cholesta-3,5-diene, along with Δ^2-, Δ^4- and Δ^5-cholestenes, plus 5α- and 5β-cholestane (Rhead *et al.*, 1971). The steratrienes may be formed either by dehydration of diunsaturated sterols, or by a pathway indicated in Figure 1, involving microbial hydroxylation or autoxidation of sterols.

The diagenetic transformations indicated by these results have taken place very rapidly in geological time. Stanols, steroidal ketones and sterenes, believed largely to be diagenetically derived from the original input of stenols, were all present in the topmost layer of the core, which we believe represents approximately the last 70 years of sediment accumulation, while the whole series of samples analysed covers a sedimentary period

of about 350 years (see earlier). These transformations can be illustrated and roughly quantified, by reference to a particular sterol. The major 4-desmethyl-stenol in these samples, cholest-5-en-3β-ol, occurs widely in the biosphere, and must be contributed to virtually all sediments from various biological sources. The probable early diagenetic fate of this compound, as indicated by the results of this study, can be summarised as follows:

Cholest-5-en-3β-ol → Cholesta-3,5-diene
(1) 40.9 (2) 19.0 (1) 1.1 (2) 2.6
↓
[Cholest-5-en-3-one]?
↓↑
Cholest-4-en-3-one
(1) 2.2 (2) 3.4
↓
5α + 5β-Cholestan-3-one
(1) 1.9 (2) 9.4
↓↑
5α + 5β-Cholestan-3β-ol → Cholest-2-ene
(1) 52.0 (2) 60.9 (1) 1.8 (2) 4.7

The amount of each component, present after (1) *ca.* 0-70 years (=0-2 cm depth), and (2) *ca.* 280-350 years (=8−10 cm depth) residence in the sediment, is given as a percentage of the total components shown.

The relative amounts of the suggested diagenetic components shown in the above scheme are, broadly, what might be expected in a stable sequence of sediment samples if the mechanisms for sterol diagenesis, as discussed earlier, are correct. A similar scheme can be drawn for a number of the other major sterols found in this study. Although such a series of reactions is believed to represent a significant part of the early stages of sterol diagenesis, it certainly will not include all the degradation pathways undergone by these compounds in sediments. Much further work will be required to elucidate more of what is undoubtedly a complex system.

CONCLUSIONS

1) Fifty-two sterols were idientified in five sections from the upper 10 cm of sediment in a core from the Namibian Shelf, Walvis Bay.

2) Many of the 4-desmethyl sterols can be related to the predominantly diatomaceous, phytoplankton input. However, there is probably also a contribution from coccolithophorids and coelenterates (most likely jellyfish).

3) The dominant component of all the sterol distributions is dinosterol which, together with other 4-methyl sterols, points to a dinoflagellate input. However, the lack of visible dinoflagellate remains may be an indication of a major role played by nannoplankton in contributing sedimentary organic matter and highlights the need for more data on this group of organisms.

4) Diagenetic transformation processes—namely dehydration of sterols to form sterenes and hydrogenation of stenols, *via* stenone and stanone intermediates, to stanols—have probably been occurring during the *ca.* 350 year history of the sediment samples.

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REFERENCES

Alam M., Sansing T. B., Busby E. L., Martiniz D. R., Ray S. M., 1979. Dinoflagellate sterols-I. Sterol composition of the dinoflagellates of *Gonyaulax* species, *Steroids*, **33**, 197-203.

Ballantine J. A., Roberts J. C., Morris R. J., 1976. Marine sterols-III. The sterol compositions of oceanic jellyfish. The use of gas chromatography-mass spectrometry techniques to identify unresolved components, *Biomed. Mass Spectrom.*, **3**, 14-20.

Ballantine J. A., Lavis A., Roberts J. C., Morris R. J., 1977. Marine sterols-V. Sterols of the Tunicata. The occurrence of saturated ring sterols in these filter-feeding organisms, *J. Exp. Mar. Biol. Ecol.*, **30**, 29-44.

Ballantine J. A., Lavis A., Roberts J. C., Morris R. J., Elsworth J. R., Cragg G. M. L., 1978. Marine sterols-VII. The sterol composition of oceanic and coastal marine annelida species, *Comp. Biochem. Physiol.*, **61 B**, 43-47.

Ballantine J. A., Lavis A., Morris R. J., 1979 *a*. Marine sterols-VIII. The sterol compositions of two marine sponges. Occurrence of new C_{26} and C_{30} stanols in an oceanic sponge, *Comp. Biochem. Physiol.*, **63 B**, 119-123.

Ballantine J. A., Lavis A., Morris R. J., 1979 *b*. Sterols of the phytoplankton-effects of illumination and growth stage, *Phytochemistry*, **18**, 1459-1466.

Ballantine J. A., Lavis A., Morris R. J., 1981. Marine sterols-XV. Sterols of some oceanic holothurians, *J. Exp. Mar. Biol. Ecol.*, **53**, 89-103.

Björkhem I., Gustaffson J.-A., 1971. Mechanism of microbial transformation of cholesterol into coprostanol, *Eur. J. Biochem.*, **21**, 428-432.

Boon J. J., 1978. Molecular biochemistry of lipids in four natural environments, *Ph. D. Thesis, Delft Univ. Press*, 215 p.

Boon J. J., Rijpstra W.I.C., De Lange F., De Leeuw J., 1979. Black Sea sterol, a molecular fossil for dinoflagellate blooms, *Nature*, **277**, 125-127.

Boutry J., Alcaide A., Barbier M., 1971. De la présence d'un stérol en C_{26} d'un plancton marin végétal, *C.R. Acad. Sci., Paris*, **272**, 1022-1023.

Brassell S. C., 1980. Lipids of deep-sea sediments: their origin and fate in the Japan Trench, *Ph. D. Thesis, Univ. Bristol.*

Bremner J. M., 1974. Texture and composition of surficial continental margin sediments between the Kunene River and Syvia Hill, SW Africa, *Tech. Rep. No.* 16, *Joint Geological Survey/Univ. of Capetown Marine Geology Programme*, Dep. Geology, Univ. Capetown, South Africa.

Bremner J. M., 1975. Faecal pellets, glauconite, phosphorite and bedrock from the Kunene-Walvis continental margin, *Tech. Rep. No.* 7, *Joint Geological Survey/Univ. Capetown, Mar. Geol. Progr.*

Brooks C. J. W., 1979. Some aspects of mass spectrometry in research on steroids, *Philos. Trans. R. Soc. London*, **A293**, 53-67.

Calvert S. E., 1966. Accumulation of diatomaceous silica in the sediments of the Gulf of California, *Geol. Soc. Am. Bull.*, **77**, 569-596.

Calvert S. E., Morris R. J., 1977. Geochemical studies of organic-rich sediments from the Namibian Shelf. II Metal-organic associations, in: *A voyage of discovery: Deacon anniversary volume, Deep-Sea Res. Suppl.*, edited by M. Angel, Pergamon Press, 667-680.

Ciereszko L. S., Johnson M. A., Schmidt R. W., Koons C. B., 1968. Chemistry of coelenterates. VI. Occurrence of gorgosterol, a C_{30} sterol, in coelenterates and their zooxanthellae, *Comp. Biochem. Physiol.*, **24**, 899-904.

Copenhagen W. J., 1934. Occurrence of sulphides in certain areas of the sea bottom on the South African coast, *Invest. Rep. Div. Fish. Union S. Africa*, **3**, 1-18.

Copenhagen W. J., 1953. The periodic mortality of fish in the Walvis Region, *Invest. Rep. Div. Fish. Union S. Africa*, **14**, 1-34.

Dastillung M., Albrecht P., 1977. Δ^2-sterenes as diagenetic intermediates in sediments, *Nature*, **269**, 678-679.

Djerassi C., 1978. Recent advances in the mass spectrometry of steroids, *Pure Appl. Chem.*, **50**, 171-184

Edmonds C. G., Smith A. G., Brooks C. J. W., 1977. Analysis of sponge sterols as the trimethylsilyl ethers and as the corresponding 5α- and Δ^4-3-ketosteroids using open-tubular gas chromatography-mass spectrometry. Application of selective enzymic oxidation, *J. Chromatogr.*, **133**, 372-377.

Edmunds K. L. H., Brassell S. C., Eglinton G., 1979. The short term diagenetic fate of 5α-cholestan-3β-ol: *in situ* radiolabelled incubation in algal mats, in: *Advances in Organic Geochemistry* 1979, edited by A. G. Douglas and J. R. Maxwell, Pergamon Press, 1980, 427-434.

Eisma D., Van der Gaast S. J., 1971. Determination of opal in marine sediments by X-ray diffraction, *Neth. J. Sea Res.*, **5**, 3, 382-389.

Eyssen H. J., Parmentier G. G., Compernolle F. C., De Pauw G., Piessens-Denef M., 1973. Biohydrogenation of sterols by *Eubacterium ATCC* 21,408, *Nova* species, *Eur. J. Biochem.*, **36**, 411-421.

Gagosian R. B., Farrington J. W., 1978. Sterenes in surface sediments from the southwest African shelf and slope, *Geochim. Cosmochim. Acta*, **42**, 1091-1100.

Gagosian R. B., Heinzer F., 1979. Stenols and stanols in the oxic and anoxic waters of the Black Sea, *Geochim. Cosmochim. Acta*, **43**, 471-486.

Gagosian R. B., Smith S. O., 1979. Steroids ketones in surface sediments from the south-west African shelf, *Nature*, **277**, 287-289.

Gagosian R. B., Lee C., Heinzer F., 1979 *a*. Processes controlling the stanol/stenol ratio in the Black Sea seawater and sediments, *Nature*, **280**, 574-576.

Gagosian R. B., Smith S. O., Lee C., Farrington J. W., Frew N. M., 1979 *b*. Steroid transformations in Recent marine sediments, in: *Advances in Organic Geochemistry* 1979, edited by A. G. Douglas and J. R. Maxwell, Pergamon Press, 1980, 407-419.

Gaskell S. J., Eglinton G., 1975. Rapid hydrogenation of sterols in a contemporary lacustrine sediment, *Nature*, **254**, 209-211.

Gaskell S. J., Eglinton G., 1976. Sterols of a contemporary lacustrine sediment, *Geochim. Cosmochim. Acta*, **40**, 1221-1228.

Gaskell S. J., Morris R. J., Eglinton G., Calvert S. E., 1975. The geochemistry of a recent marine sediment off north-west Africa. An assessment of source of input and early diagenesis, *Deep-Sea Res.*, **22**, 777-789.

Goad L. J., 1976. The steroids of marine algae and invertebrate animals, in: *Biochemical and biophysical perspectives in marine biology*, edited by D. C. Malins and J. R. Sargent, Academic Press, New York, 213-318.

Goad L. J., 1978. The sterols of marine invertebrates, in: *Marine natural products, chemical and biological perspectives*, Vol. 2, edited by P. J. Scherer, 75-172, New York, Academic Press.

Gupta K. C., Miller R. L., Williams J. R., Gagosian R. B., Heinzer F., 1979. Sterol composition of *Ciona intestinalis*, *J. Nat. Prod.*, **42**, 305-306.

Hallegraeff F. M., 1981. Seasonal study of phytoplankton pigments and species at a coastal station off Sydney: importance of diatoms and the nannoplankton, *Mar. Biol.*, **61**, 107-118.

Hart T. J., Currie R. I., 1960. The Benguela Current, *Discovery Rep.*, **31**, 123-298.

Harvey R. K., Taylor D. M., Hendry R. D., Bancroft T., 1973. An accurate fusion method for the analysis of rocks and chemically related materials by X-ray fluorescence spectrometry, *X-Ray Spectrom.*, **2**, 33-44.

Horning E. C., Brooks C. J. W., Vanden Heuvel W. J. A., 1968. Gas phase analytical methods for the study of steroids, *Adv. Lipid Res.*, **6**, 273-392.

Jeffery S. W., Hallegraeff G. M., 1980. Studies of phytoplankton species and photosynthetic pigments in a warm core eddy off the East Australian Current. I. Summer populations, *Mar. Ecol. Progr. Ser.*, **3**, 285-294.

Kanazawa A., Teshima S., Ando T., Tomita S., 1974. Occurrence of 23,24-dimethylcholesta-5,22-dien-3β-ol in a soft coral, *Sarcophyta elegans*, *Bull. Jpn Soc. Sci. Fish.*, **40**, 729.

Kollmer W. E., 1963. The pilchard of South West Africa (*Sardinops ocellata Pappe*); notes on zooplankton and phytoplankton collections made off Walvis Bay, *Invest. Rep. Mar. Res. Lab., SW Africa*, **8**, 1-78.

Lee C., Farrington J. W., Gagosian R. B., 1979. Sterol geochemistry of sediments from the western North Atlantic Ocean and adjacent coastal areas, *Geochim. Cosmochim. Acta*, **43**, 35-46.

Lee C., Gagosian R. B., Farrington J. W., 1980. Geochemistry of sterols in sediments from Black Sea and the southwest African shelf and slope, *Org. Geochem.*, **2**, 103-113.

Morris R. J., Calvert S. E., 1977. Geochemical studies of organic-rich sediments from the Namibian Shelf. I. The organic fractions. *Discovery: Deacon anniversary volume, Deep-Sea Res. Suppl.*, Pergamon, 647-665.

Morris R. J., Culkin F., 1977. Marine lipids; sterols, *Oceanogr. Mar. Biol. Ann. Rev.*, **15**, 73-102.

Nishimura M., Koyama T., 1976. Sterols and stanols in lake sediments and diatoms, *Chem. Geol.*, **17**, 229-239.

Nishimura M., Koyama T., 1977. The occurrence of stanols in various living organisms and the behaviour of sterols in contemporary sediments, *Geochim. Cosmochim. Acta*, **41**, 379-385.

Orcutt D. M., Patterson G. W., 1975. Sterol, fatty acid and elemental composition of diatoms grown on chemically defined media, *Comp. Biochem. Physiol.*, **50 B**, 579-583.

Parmentier G., Eyssen H., 1974. Mechanism of biohydrogenation of cholesterol to coprostanol by *Eubacterium* ATCC2 1408, *Biochim. Biophys. Acta*, **348**, 279-284.

Philp R. P., Maxwell J. R., Eglinton G., 1976. Environmental organic geochemistry of aquatic sediments, *Sci. Progr., Oxford*, **63**, 521-545.

Rhead M. M., Eglinton G., Draffan G. H., 1971. Hydrocarbons produced by the thermal alteration of cholesterol under conditions simulating the maturation of sediments, *Chem. Geol.*, **8**, 277-297.

Rubinstein I., Steskind O., Albrecht P., 1975. Rearranged sterenes in a shale; occurrence and simulated formation, *J. Chem. Soc. Perkin I.*, 1833-1836.

Sheikh Y. M., Djerassi C., 1974. Steroids from sponges, *Tetrahedron*, **30**, 4095-4103.

Shimizu Y., Alam M., Kobayashi A., 1976. Dinosterol, the major sterol with a unique side chain in the toxic dinoflagellate, *Gonyaulax tamarenis*, *J. Am. Chem. Soc.*, **98**, 1059.

Smayda T. J., 1971. The ocean flora, in: *Deep Oceans*, edited by P. J. Herring and M. R. Clarke, Arthur Barker Ltd., 130-149.

Taylor C. D., Smith S. O., Gagosian R. B., 1981. Use of microbial enrichments for the study of the anaerobic degradation of cholesterol, *Geochim. Cosmochim. Acta*, **45**, 2161-2168.

Wolkman J. K., Eglinton G., Corner E. D. S., 1980. Sterols and fatty acids of the marine diatom *Biddulphia sinensis*, *Phytochemistry*, **19**, 1809-1813.

Volkman J. K., Eglinton G., Corner E. D. S., Forsberg T. E. V., 1980. Long chain alkenes and alkenones in the marine coccolithophorid *Emiliania huxleyi Phytochem.*, **19**, 1809-1813.

Volkman J. K., Smith D. J., Eglinton G., Forsberg T. E. V., Corner E. D. S., 1981. Sterol and fatty acid composition of four marine haptophycean algae., *J. Mar. Biol. Assoc. UK*, **69**, 509-527.

Voogt P. A., 1976. Composition and biosynthesis of sterols in some sponges, *Neth. J. Zool.*, **26**, 84093.

Wardroper A. M. K., 1979. Aspects of the geochemistry of polycyclic isoprenoids, *Ph. D. Thesis, Univ. Bristol.*

Wardroper A. M. K., Maxwell J. R., Morris R. J., 1978. Sterols of a diatomaceous ooze from Walvis Bay, *Steroids*, **32**, 203-221.

Withers N. W., Tuttle R. C., Holz G. G., Beach D. H., Goad L. J., Goodwin T. W., 1978. Dehydrodinosterol, dinosterone and related sterols of a non-photosynthetic dinoflagellate, *Crypthecodinium cohnii*, *Phytochemistry*, **17**, 1987-1989.

Withers N. W., Kokke W. C. M. C., Rohmer M., Fenical W. H., Djerassi C., 1979. Isolation of sterols with cyclopropyl–containing side chains from the cultured marine alga *Peridinium foliaceum*, *Tetrahedran Lett.*, 3605-3608.