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LETTER

A novel sterane, 27-nor-24-methyl-5α-cholestane, in sediments

STEFAN SCHOUTEN,[1] JAAP S. SINNINGHE DAMSTÉ,[1] MARTIN SCHOELL,[2] and JAN W. DE LEEUW[1]

[1] Division of Marine Biogeochemistry, Netherlands Institute for Sea Research (NIOZ),
P. O. Box 59, 1790 AB Den Burg, Texel, The Netherlands
[2] Chevron Petroleum Technology Company, 1300 Beach Boulevard, La Habra, California 90631, USA

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Abstract—A novel sterane, 27-nor-24-methyl-5α-cholestane, has been identified in sedimentary hydrocarbon mixtures by coinjection with an authentic standard. The C_{27} sterane occurs in silica-rich sediments from the Monterey Formation, USA, the Onnagawa Formation, Japan, and the Menilite Shale, Poland, and in bituminous marls from the Vena del Gesso basin, Italy. The compound seems to be biosynthetically related to 24-norcholestanes which co-occurs in sometimes relatively high amounts. The ^{13}C-content of the sterane in the Monterey Formation indicates an algal source similar to other steranes present in this sediment. A diatom or a dinoflagellate source is suggested for this compound.

INTRODUCTION

NUMEROUS SEDIMENTARY sterols, sterenes, and steranes have been synthesized and identified in order to obtain information on the biota present in the depositional environment. Most of the steroids encountered are quite common (e.g., cholestane, 24-methylcholestane, 24-ethylcholestane, etc.) and are not very specific as biomarkers (VOLKMAN, 1986). Some compounds are, however, specific products of certain algal species. All are used as biomarkers. For instance, steroids with a dinosterane carbon skeleton [**I**] (BOON et al., 1979; SUMMONS et al., 1987) are specific biomarkers for dinoflagellates, though the sterol is also present in low amounts in a marine diatom species (VOLKMAN et al., 1993). MOLDOWAN et al. (1990) reported the presence of cholestanes with a propyl group at C-24 [**II**] in oils and bitumens derived from marine source rocks. These compounds are characteristic for marine Chrysophyte algae. MCCAFFREY et al. (1994) reported the presence of cholestane with an isopropyl group at C-24 in Precambrian to Cenozoic sediments and oils and suggested specific sponges as the organisms which produced these compounds. Other steranes with unusual side-chain patterns may thus have potential to provide more information on the biota present in the depositional environment.

Here we report the identification, using a synthesized authentic standard, of a novel C_{27}-steroid, 27-nor-24-methyl-5α-cholestane [**III**] present as such in a Miocene sediment from the Onnagawa Formation (northern Japan), in a sample from the Oligocene Menilite shale (southeast Poland) and in a S-bound form in a Miocene sediment from the Monterey Formation (California, USA) and the Miocene Vena del Gesso basin (northern Apennines, Italy).

EXPERIMENTAL

Samples

The samples from the Miocene Monterey Formation were taken from an outcrop at Shell Beach (California, USA). Stratigraphic descriptions of this outcrop are given by OMARZAI (1992). The total organic carbon (TOC) of samples varies from 3 to 8%.

The Onnagawa Formation of the northern Honshu Island, Japan, is considered to be an equivalent of the Monterey Formation since it is Miocene in age and rich in diatomaceous silica. The TOC content of the studied sample is 1.5%. Extensive descriptions of the lithology and of the compounds in the extract of the Japanese Onnagawa sediments are given by YAMAMOTO and WATANABE (1992).

The Vena del Gesso basin is Miocene in age and located in the northern Apennines, Italy. The samples studied were from a bituminous marl of an evaporitic cycle. The lithology is described by VAI and RICCI LUCCHI (1977). The compounds present in the extract are described by KENIG et al. (1994).

The Menilite black shale is from the Carpathian mountains in southeastern Poland and Oligocene in age. KRUGE et al. (1991) described some of the biomarkers present in outcrop samples from the Melinite black shale.

Synthesis of 27-nor-24-methyl-5α-cholestane

Patinosterol (27-nor-24-methyl-5α-cholest-22-en-3β-ol; **IV**) was tosylated by stirring with *para*-toluenesulfonylchloride in pyridine for 24 h at room temperature. The tosylgroup was eliminated by refluxing with $LiAlH_4$ in dioxane for 2 h. The residual double bond was hydrogenated with H_2/PtO_2 for 1 h at room temperature.

Analysis of sediments

The isolation and desulphurization of the polar fraction of Shell Beach was performed as described by SCHOUTEN et al. (1994) resulting in a fraction of released hydrocarbons. A similar procedure has been used for the Vena del Gesso sediment (KENIG et al., 1994) to obtain a released hydrocarbon fraction. The isolation of the saturated hydrocarbon fraction of the Onnagawa sediment extract has been described by YAMAMOTO and WATANABE (1992). The fractions were analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). The released hydrocarbons of the desulphurized polar fraction of Shell Beach were separated in adduct and nonadduct subfractions using molecular sieves 5Å (O'CONNOR et al., 1962) and subsequently analyzed by compound specific isotope analysis using an isotope-ratio-monitoring gas chromatography–mass spectrometer (irm-GC–MS).

Gas Chromatography (GC)

GC was performed using a Carlo Erba 5300 instrument equipped with an on-column injector. A fused silica capillary column (25 m

x 0.32 mm) coated with CP Sil-5 (film thickness 0.12 μm) was used with helium as carrier gas. A flame ionization detector (FID) was used. The samples were dissolved in ethyl acetate and injected at 70°C. Subsequently the oven was programmed to 130°C at 20°C/min and then at 4°C/min to 320°C where the temperature was maintained for 10 min.

Gas Chromatography-Mass Spectrometry (GC–MS)

GC–MS was performed using a Hewlett-Packard 5880 gas chromatograph interfaced to a VG Autospec Ultima mass spectrometer operated at 70 eV with a mass range of *m/z* 40–800 and a cycle time of 1.8 s (resolution 1000). The gas chromatograph was equipped with a fused silica capillary column (25 m x 0.32 mm) coated with CP Sil-5 (film thickness = 0.2 μm) or with an HP Ultra 1 column (50 m x 0.32 mm) coated with crosslinked methyl silicone (film thickness = 0.5 μm). The carrier gas was helium. The samples were on column injected at 50°C and, in the case of a CP Sil-5 column, the oven was subsequently programmed to 130°C at 20°C/min and then at 4°C/min to 300°C at which it was held for 10 min. In the case of the Ultra 1 column the oven was programmed to 130°C at 10°C/min, and then at 3°C/min to 320°C/min at which it was held for 30 min.

Isotope-Ratio-Monitoring Gas Chromatography-Mass Spectrometry

The DELTA-S irm-GC–MS system has been described previously (HAYES et al., 1990; SCHOELL et al., 1992). In short, the gas chromatographic column was a 50 m Hewlett Packard Ultra 1 column with 0.32 mm internal diameter and a 0.5 μm stationary phase (crosslinked methyl silicone). The carrier gas was helium at a flow rate of 1.5 mL/min. The samples (dissolved in cyclohexane) were on-column injected at 50°C and subsequently the oven was programmed to 130°C at 10°C/min, and then at 3°C/min to 320°C/min at which it was held for 40 min. The isotopic values were calculated by integrating the mass 44, 45, and 46 ion currents of the peaks produced by combustion of the chromatographically separated compounds and that of CO_2 spikes produced by admitting CO_2 with a known ^{13}C content at regular intervals via the dual inlet system into the mass spectrometer. All values reported were determined by at least two analyses and the results were averaged to obtain a mean value and to calculate the standard deviation. The stable carbon isotope compositions are reported in the delta notation against the PDB ^{13}C standard.

RESULTS AND DISCUSSION

The apolar fraction of the Monterey sample contains only traces of steranes. However, desulphurization of the polar fraction with nickel boride (SCHOUTEN et al., 1993) released substantial amounts of steranes. The predominant steranes are C_{27}-C_{29} 5α- and 5β-steranes with the 5α-steranes dominating (Fig. 1). Furthermore, relatively high amounts of isomeric compounds with a dinosterane carbon skeleton [**I**] (SUMMONS et al., 1987) and 24-nor-5α-cholestane [**V**] (MOLDOWAN et al., 1991; SUZUKI et al., 1993) are present. Interestingly, an unknown compound present in relatively high amounts elutes just after 5α-cholestane [**VI**] on the stationary phase used. The mass spectrum of this compound (Fig. 2) is indistinguishable from that of 5α-cholestane. Based on retention time it was suspected that the side-chain was different from that of 5α-cholestane and that the methylgroup normally at C-25 was at C-24. Thus, 27-nor-24-methyl-5α-cholestane [**III**] was synthesized from patinosterol (27-nor-24-methyl-5α-cholest-22-en-3-ol; **IV**). The mass spectrum of the synthetic compound is identical to that of the unknown sterane. A coinjection experiment revealed that the unknown compound was indeed 27-nor-24-methyl-5α-cholestane (Fig. 1).

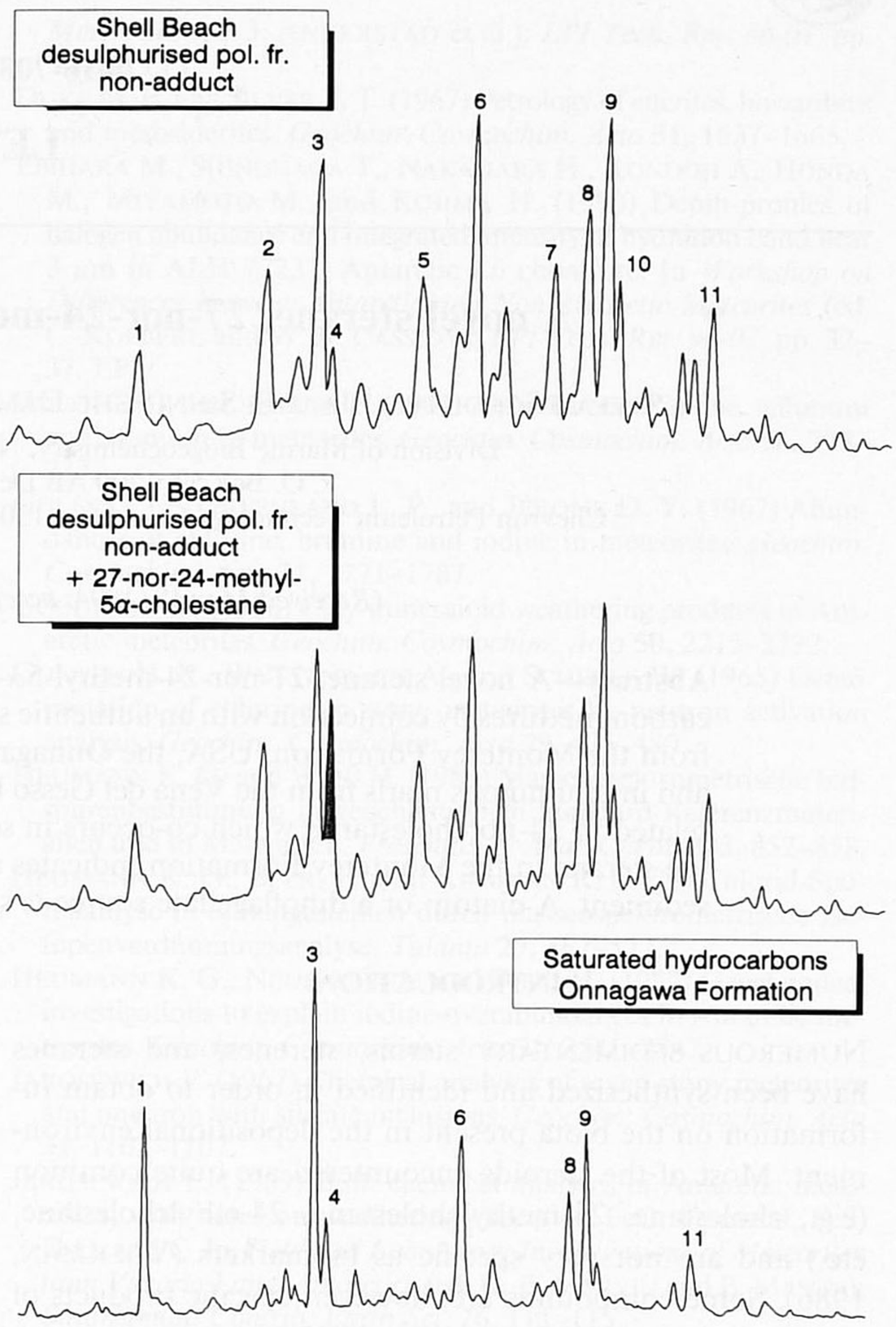

FIG. 1. Partial FID-trace of (top) the nonadduct of the desulphurized polar fraction of Shell Beach and (middle) the same fraction with coinjected 27-nor-24-methyl-5α-cholestane and (bottom) the saturated hydrocarbon fraction of an Onnagawa Formation sample.

A reinspection of other sediment extracts revealed that this compound is also present as such (i.e., not S-bound) in the saturated hydrocarbon fractions of the Miocene silica-rich Onnagawa sediments from northern Japan (Fig. 1) and of the Oligocene Melinite black shale from southeastern Poland. Furthermore, it was detected in the desulphurized polar fraction of bituminous marl sediments from the Messininan Vena del Gesso basin. Since 27-nor-24-methyl-5α-cholestane elutes closely after 5α-cholestane and has an identical mass spectrum, this compound may have been overlooked in the past in other sediments especially when chromatographic conditions were not optimal or other columns have been used. A further close inspection of the mass chromatograms of *m/z* 217, characteristic for 4-desmethylsteranes, of the fractions studied revealed the presence of a compound eluting closely after 5β-cholestane and with an identical mass spectrum. Therefore this compound was tentatively identified as 27-nor-24-methyl-5β-cholestane (Table 1).

To shed more light on its origin, carbon isotopic analysis was performed on this compound and other steroids released by desulphurization from the polar fraction of a sediment from the Monterey Formation (Table 2). Since several of these compounds partially coelute the reported values have relatively high uncertainty values. The carbon isotopic com-

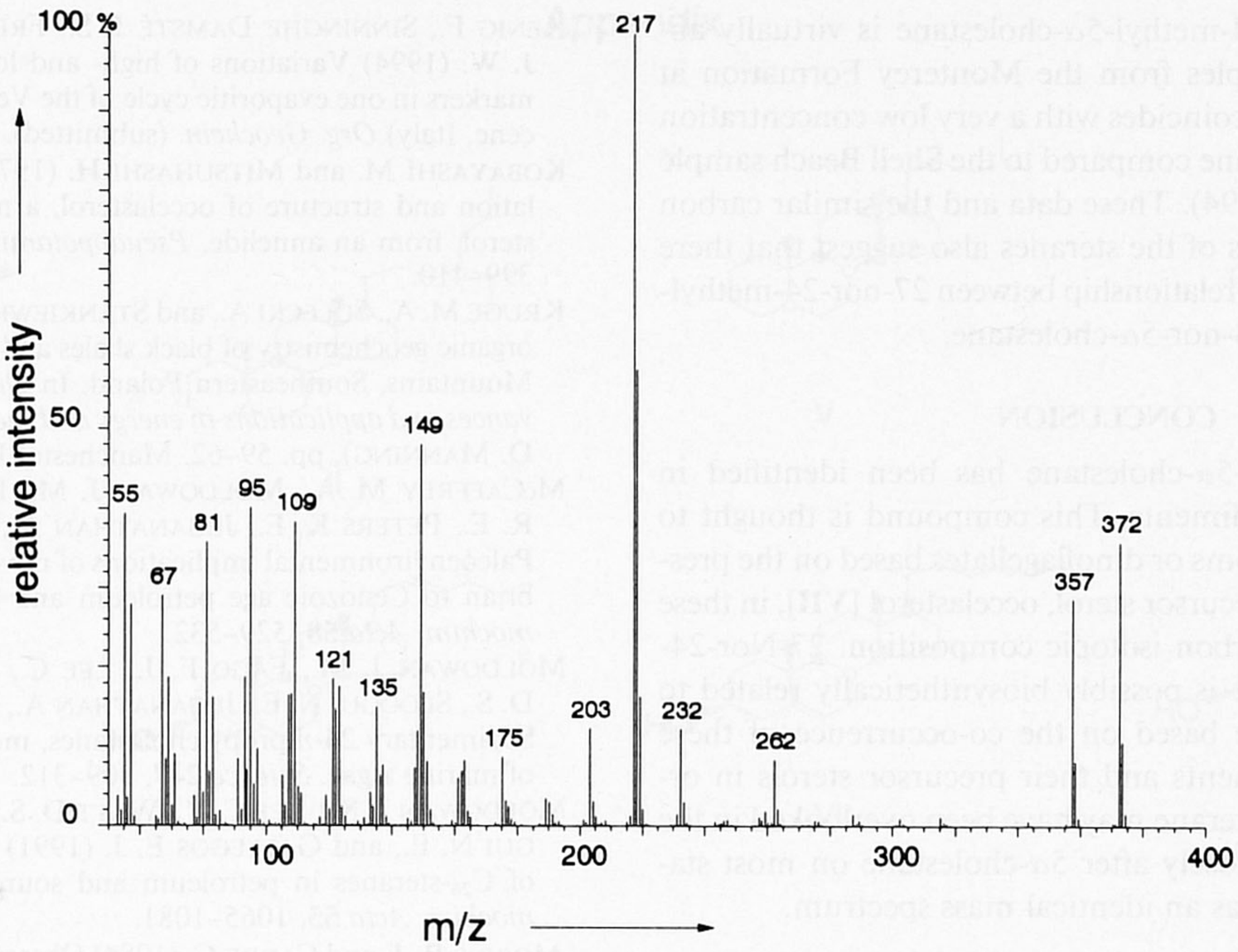

FIG. 2. Mass spectrum of synthetic 27-nor-24-methyl-5α-cholestane.

positions of the steroids are, however, similar to each other and indicate that they are derived from algae living in a similar habitat.

The precursor of 27-nor-24-methyl-5α-cholestane could be either occelasterol (27-nor-24-methylcholest-5,22E-dien-3β-ol; **VII**) or patinosterol (27-nor-24-methyl-5α-cholest-22E-en-3β-ol; **IV**). The presence of trace amounts of 27-nor-24-methyl-5β-cholestane indicates that isomerization has taken place at C-5 and thus a double bond must have been present (DE LEEUW et al., 1989). Occelasterol has been reported in several organisms; in marine worms (KOBAYASHI and MITSUHASHI, 1974), in cultures of the marine dinoflagellate *Gymnodinium simplex* (GOAD and WITHERS, 1982) and in field samples from a bloom of the diatom *Rhizosolenium spp.* (MORRIS and CARRE, 1984). Occelasterol has further been reported in sediments from the Japanese Trench (BRASSELL et al., 1980) and in Unit I of the Black Sea (DE LEEUW *et al.*, 1983). The occurrence of occelasterol in sediments known to have a significant input of dinoflagellates and diatoms suggests that either of these organisms may have been the source of the compound. The relatively high abundance of 27-nor-24-methyl-5α-cholestane in two diatomaceous sediments (Monterey and Onnagawa Formation) suggests a diatom source, though a dinoflagellate source cannot be excluded since dinosterane, likely derived from dinoflagellates (SUMMONS et al., 1987), is also present in the samples studied.

The Monterey and Onnagawa sediments, and to a lesser extent the Vena del Gesso and Melinite shale sediments, also contain relatively high amounts of 24-norcholestane [**V**]. BRASSELL and EGLINTON (1981) suggested that patinosterol is closely related to 24-nor-5α-cholestanols based on distribution patterns in several samples. Patinosterol was suggested by these authors to be formed by biological C-27 demethylation of 24-methyl-cholest-22-en-3β-ol [**VIII**]. A subsequent biological C-26 demethylation would then result in the formation of 24-nor-stanols. Indeed, the presence of occelasterol co-occurs with relatively high amounts of C_{26}-sterols both in sediments (BRASSELL and EGLINTON, 1981; DE LEEUW *et al.*, 1983) and in the studied diatom (MORRIS and CARRE, 1984) and dinoflagellate species (GOAD and WITHERS, 1982). In-

Table 1. Peak identifications

No.	Compound
1.	24-nor-5α-cholestane
2.	5ß-cholestane
3.	5α-cholestane
4.	27-nor-24-methyl-5α-cholestane
5.	24-methyl-5ß-cholestane
6.	24-methyl-5α-cholestane
7.	24-ethyl-5ß-cholestane
8.	23,24-dimethyl-5α-cholestane
9.	24-ethyl-5α-cholestane
10.	4,23,24-trimethyl-5α-cholest-22-ene (dinoster-22-ene)
11.	4,23,24-trimethyl-5α-cholestane (dinosterane)

Table 2. Carbon isotope data on steranes in Shell Beach

Compound	$\delta\ ^{13}C$ (‰)
24-nor-5α-cholestane	- 27.2 ± 0.5
27-nor-24-methyl-5α-cholestane	- 24.7 ± 2.0
5α-cholestane	- 26.2 ± 0.5
24-methyl-5α-cholestane	- 27.2 ± 1.0
24-ethyl-5α-cholestane	- 28.1 ± 1.5
dinosterene	- 26.0 ± 0.5

terestingly, 27-nor-24-methyl-5α-cholestane is virtually absent in outcrop samples from the Monterey Formation at Naples Beach which coincides with a very low concentration of 24-nor-5α-cholestane compared to the Shell Beach sample (SCHOUTEN *et al.*, 1994). These data and the similar carbon isotopic compositions of the steranes also suggest that there may be a biosynthetic relationship between 27-nor-24-methyl-5α-cholestane and 24-nor-5α-cholestane.

CONCLUSION

27-Nor-24-methyl-5α-cholestane has been identified in several immature sediments. This compound is thought to be derived from diatoms or dinoflagellates based on the presence of the alleged precursor sterol, occelasterol [**VII**], in these organisms and its carbon isotopic composition. 27-Nor-24-methyl-5α-cholestane is possibly biosynthetically related to 24-nor-5α-cholestane based on the co-occurrence of these compounds in sediments and their precursor sterols in organisms. The novel sterane may have been overlooked in the past since it elutes closely after 5α-cholestane on most stationary phases and has an identical mass spectrum.

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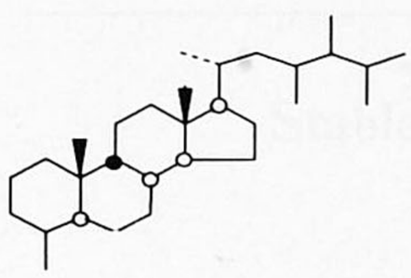
Appendix



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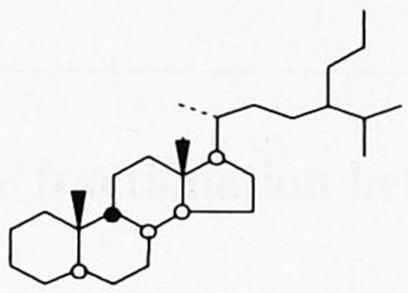

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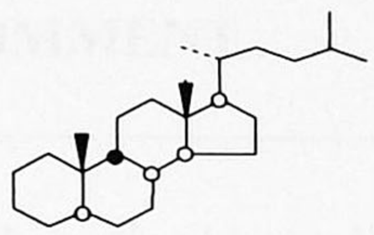

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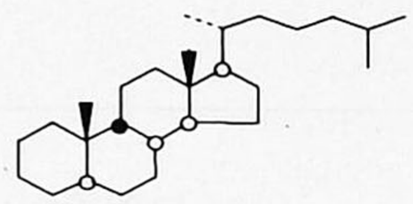

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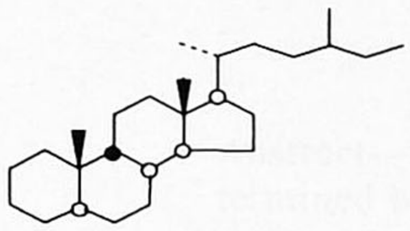

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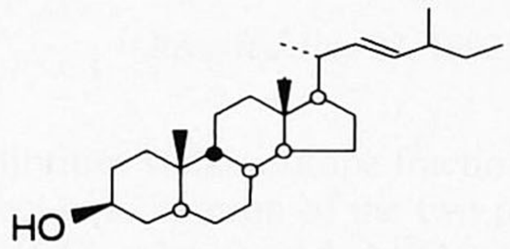

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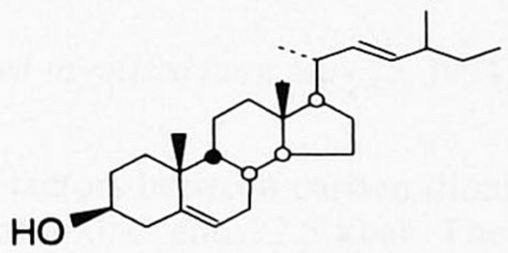

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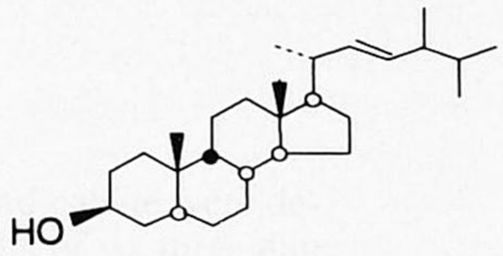

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