

Lipid constituents of marine sponge *Suberites carnosus*

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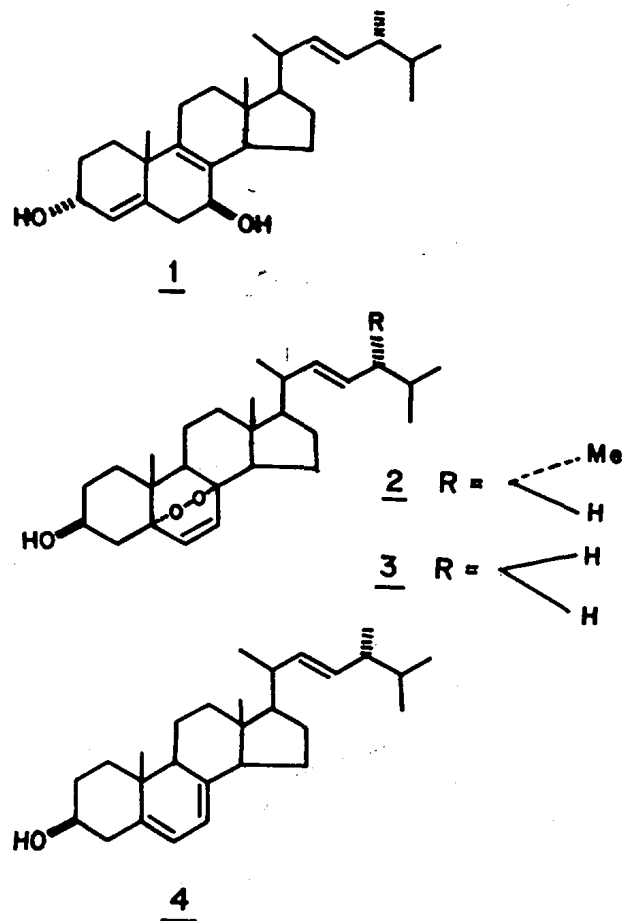
Sterols (22*E*) - 24 α - methyl-cholest - 4, 8(9), 22 (23) - triene - 3 α , 7 β - diol (**1**) and (22*E*)-24 α -methyl-ergost-6, 22(23)-diene-5 α , 8 α -epidioxy-3 β -ol (**2**), a fatty acid nonadecanoic acid and a fatty ester methyl nonadecanoate have been isolated from the sponge *Suberites carnosus*. Minor amounts of (22*E*)-ergost-6, 22(23), 24(28)-triene-5 α , 8 α -epidioxy-3 β -ol (**3**) and ergosterol (**4**) are also present. The structures have been elucidated on the basis of spectral data.

During the course of our continuing investigation on the isolation of bioactive substances from marine organisms the methanolic extract of the marine desmosponge *Suberites carnosus* belonging to the order Hadromerida, family Suberitidae, exhibited cytotoxic, diuretic and antihistaminic activities.

The alcoholic extract of the sponge *Suberites carnosus* was fractionated with chloroform and *n*-butanol. The chloroform fraction was chromatographed over a silica gel column by gradual elution with pet. ether-ethyl acetate. This afforded four compounds, which include sterols **1** and **2**, fatty acid **6** and fatty ester **5** in decreasing order of elution.

Compound **1** was obtained as a solid. Its IR spectrum exhibited absorption due to hydroxyl group (3382 and 1034 cm^{-1}), carbon-carbon double bond (1658, 970 cm^{-1}). EI mass spectrum of **1** gave a molecular ion peak at m/z 412 in agreement with the formula $\text{C}_{28}\text{H}_{44}\text{O}_2$. Further analysis of mass spectrum showed diagnostic peaks at m/z 394 ($\text{C}_{28}\text{H}_{42}\text{O}$) and m/z 376 ($\text{C}_{28}\text{H}_{40}$) corresponding to two successive losses of 18 amu from molecular ion peak, thus indicating the presence of two hydroxyl groups. Its ^1H NMR spectrum was suggestive of a sterol nucleus. These data indicated that compound **1** had C_{28} triene sterol with two hydroxyl groups.

^1H NMR spectrum of compound **1** supported the existence of a trisubstituted double bond (δ 5.3, a doublet) as well as a terminal isopropyl group in the side chain [δ 0.83 (3H, d, $J=6.8$ Hz, $\text{C}_{26/27}\text{-Me}$), δ 0.85 (3H, d, $J=6.8$ Hz, $\text{C}_{27/26}\text{-Me}$)], also present were two singlets at δ 0.61 and 1.12 corresponding to C_{18} and C_{19} methyl protons respectively, two doublets integrating for 3H ap-



peared at δ 0.91 ($J = 6.6$ Hz) and 1.02 ($J=6.6$ Hz) due to C_{28} and C_{21} methyl protons respectively. The nonequivalence of C_{28} methyl and one of the isopropyl methyl groups suggested 24*R* configuration¹. Signals due to protons attached to carbon bearing -OH group were observed at δ 4.09 and 3.5. Signal at δ 3.5 (triplet in appear-

ance) was quite narrow as would be expected for an equatorial proton (3 α -OH) and the other signal at 4.09 had a complexity (quintet) usually seen for β -carbinol (7 β -OH). This compound also exhibited a two-proton multiplet characteristic of $\Delta^{22(23)}$ at δ 5.2. A second double bond was placed at C₄ as the olefinic proton at δ 5.3 appeared as a doublet. The absence of any additional olefinic proton in ¹H NMR limited the possible position of the third double bond to $\Delta^{8(9)}$ or $\Delta^{8(14)}$. Based on mass fragmentation pattern (Figure 1) the third double bond was placed at $\Delta^{8(9)}$ position, thus establishing the structure as (22*E*)-24 α -methyl-cholest-4, 8(9), 22(23)-triene-3 α , 7 β -diol.

The mass spectrum of **2** gave a molecular ion peak at *m/z* 428 corresponding to C₂₈H₄₄O₃ and indicating the presence of six double bond equivalents; four were assigned to the four rings of carbocyclic nucleus and remaining two to unsaturated bonds. Its IR spectrum displayed characteristic bands at 3525 (-OH group), 968 (a trans C=C) and 1180 cm⁻¹ (peroxide group), the presence of this function was supported by the loss of oxygen from the molecular ion peak in the mass spectrum (*m/z* 396).

The EI mass spectrum in addition to M⁺ showed diagnostic peaks at *m/z* 410 (M⁺ - H₂O), 303 [M⁺ - SC (C₉H₁₇)], 285 [M⁺ - (SC + H₂O)], 271 [M⁺ - (SC + O₂)], 253 [M⁺ - (SC + H₂O + O₂)], 211 [253 - ring D fusion (42)].

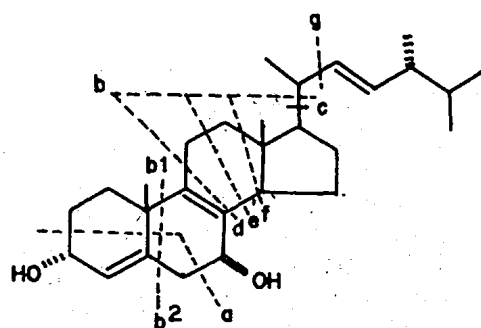
These fragments suggested that the compound was a C₂₈ sterol possessing a hydroxyl group and two double bonds, one in the nucleus and one in the side chain.

The ¹H NMR spectrum of **2** displayed C₁₈ and C₁₉ methyl protons at δ 0.81 and δ 0.88 as singlets and four doublets integrating 3H each at δ 0.82 (*J*=6.8 Hz), 0.83 (*J*=6.8 Hz), 0.94 (*J*=6.4 Hz) and δ 1.02 (*J*=6.4 Hz) were due to C₂₆, C₂₇, C₂₈ and C₂₁ secondary methyl protons respectively.

The nonequivalence of the C₂₈ methyl and one of the isopropyl methyl groups and a characteristic downfield shift in the doublet resonance for the protons of C₂₇ methyl group was indicative of 24*R* methyl configuration. The downfield shift of the C-3 carbinol proton at δ 3.96 as compared to ergosterol (δ 3.6) indicated some disturbance in the vicinity of C-3 position. ¹H NMR exhibited characteristic eight line pattern centered at δ 5.18 (2H, m) for C₂₂ and C₂₃ protons with *J*=7.5 Hz (coupling between 22-20 H and 23-24 H) and *J*=15 Hz (coupling between 22-23 H, *trans*) indicating a $\Delta^{22(23)}$ double bond. The signals at δ 6.24 (1H, d, *J*=8.5 Hz) and 6.5 (1H, d, *J*=8.5 Hz) were assigned to the protons of nuclear double bond; the peak at *m/z* 396 in the mass spectrum and the signals at δ 6.24 and δ 6.5 in ¹H NMR spectrum were characteristic of ergosterol peroxide. These data suggested the structure of (22*E*)-24 α -methyl-ergost-6, 22(23)-diene-5 α -8 α -epidioxy-3 β -ol for compound **2**.

The structure was confirmed by comparison of the spectral data with literature data²⁻⁴. Further evidence for structure was provided by ¹³C NMR data which showed signals at δ 135.4, 135.2, 132.3 and 130.7 which were assigned to unsaturated carbon C₂₂ and C₂₃, C₆ and C₇ respectively. Signal due to C₃ carbinol carbon was observed at δ 66.4; signals at δ 51.7 and 51.2 were due to C₅ and C₈ attached to endoperoxide function.

Compound **2** has previously been detected in some sponges^{5,6}, tunicates^{7,8}, fungi^{9,10} and lichens⁵ but no ¹³C NMR data were reported. The mass spectrum of the compound also indicated trace amount of ergosterol **4** as evidenced by the presence of fragment ions at *m/z* 159, 158, 157 and 143 which are typical of $\Delta^{5,7}$ ring structure in sterols^{11,12}. The mass spectrum of **2** also suggested the presence of yet another ergosterol derivative, compound **3**, with M⁺ 426 and an important ion



<i>m/z</i>	Probable structure
412	M ⁺
394	M ⁺ - H ₂ O
376	M ⁺ - 2H ₂ O
315	g
287	c (M ⁺ - SC)
109	d
95	e
81	f
69	a
55	b ₁ & b ₂

Figure 1—Fragmentation of compound 1

at m/z 394 (426-O₂) indicative of peroxy linkage. The presence of the peroxide function was further supported by the signals in the ¹H NMR spectrum at δ 4.69 and 4.71 as a broad singlet, and a doublet at δ 6.3 and 6.6 due to $\Delta^{24(28)}$ and Δ^6 double bond respectively. The presence of C₂₁-methyl proton was also evident at δ 1.21. This led us to assign the structure of the steroid with M⁺ 426 tentatively as (22E)-ergost-6, 22(23), 24(28)-triene-5 α -8 α -epidioxy-3 β -ol.

Compound 5 was identified as aliphatic ester, methyl ester of nonadecanoic acid. It was obtained as liquid; IR spectrum showed bands at 1745, 1466, 1435, 1364, 1248, 1196 and 1170 cm⁻¹. High resolution ¹H NMR spectrum showed signals for a terminal CH₃ group at δ 0.89 (3H, t, $J=9.0$ Hz), a signal for 16 methylene units at δ 1.28 (32H, brs), a two-proton multiplet centered at δ 1.63 was assigned to β protons in the acid portion of the molecule and a sharp singlet at δ 3.68 (3H) for CH₃ group in alcohol portion.

Its mass spectrum showed M⁺ at m/z 312 (C₂₀H₄₀O₂), a base peak at m/z 74 which is characteristic of linear chain methyl esters which arises by McLafferty rearrangement. Besides, there were also fragments of the general formula [(CH₂)_nCOOCH₃]⁺ indicated by the fragment ions at m/z 87, 101, 129, 143, 157, 171, 185, 199, 213, 227, 270 and 298. In view of above evidence compound 5 was characterised as methyl nonadecanoate.

Compound 6 was an aliphatic acid, nonadecanoic acid, obtained as a colourless solid; molecular formula was assigned as C₁₉H₃₈O₂ based on EI mass spectrum (M⁺ 298). Its IR spectrum was characteristic of carboxylic acid with absorptions at 3000-2851, 1710, 1435, 1290 and 940 cm⁻¹ indicative of dimeric carboxylic acid; also bands at 1412 cm⁻¹ (CH₂ adjacent to -COOH group) and 721 cm⁻¹ of n-alkyl chain were observed. A uniform difference of 14 amu in fragmentation pattern of the molecule confirmed the presence of a long aliphatic chain. The absence of [M-15]⁺ ion peak indicated a linear chain skeleton¹³. Compound 6 thus was characterised as nonadecanoic acid. Its methyl ester was found to be identical with compound 5 in all respects.

Though literature indicates the steroids 24-methyl-5 α -cholest-22-en-3 β -ol, cholesterol, 24-methyl-5 α -cholestan-3 β -ol, 24-methyl-cholest-5-en-3 β -ol, 24-ethylcholest-5-en-3 β -ol and 24-methylidenecholesterol to be the constituents of the sponge *Suberites carnosus*^{14,15}, but no mention has been made on the isolation of the steroids reported in this paper. To our knowledge this is the first

report of natural occurrence of compound 1. Further, substitution at C₂₄ seems to be predominant in sterols of the family Suberitidae¹⁶.

Experimental Section

General. Compounds were purified by repeated silica gel column chromatography and its purity was checked on TLC using different solvent systems. Melting points were determined on a Toshniwal melting point apparatus and are uncorrected. ¹H NMR spectra (TMS, CDCl₃) were recorded on a Bruker WM 400 MHz, FT NMR Spectrometer. IR spectra were taken on a Shimadzu FTIR-8001 (KBr pellet or neat sample). Mass spectra were recorded on a Jeol D-300 mass spectrometer at 70 eV. Column chromatography was performed over silica gel (60-120 mesh) and TLC on silica gel G (13% CaSO₄ as binder).

Extraction and isolation of the compounds. The sponge *Suberites carnosus*, collected in March 1994 from Malvan, West Coast of India, cut into small pieces, stored in methanol and transported to laboratory. The alcohol was decanted after a week and this process repeated twice. The decants were concentrated under reduced pressure to obtain a dark brown residue. This concentrated extract was resuspended in 10% aq. MeOH and successively partitioned with CHCl₃ and *n*-butanol. The residue from CHCl₃ fraction (7 g) was chromatographed over silica gel (200 g) using pet. ether-ethyl acetate as eluent yielding fractions 1, 2 and 3. Fraction 1 gave Compound 5 whereas compounds 2, 3 and 6 were obtained from fraction 2, Compound 1 was isolated from fraction 3.

Compound 1: Solid (5 mg) m.p. 155-60°; IR (KBr, cm⁻¹): 3382, 2953, 2871, 1658, 1456, 1380, 1237, 1161, 1034, 970 and 868; 400 MHz ¹H NMR (CDCl₃): δ 0.61 (3H, s, 18-CH₃), 0.83 (3H, d, $J=6.8$ Hz, C_{26/27}-CH₃), 0.85 (3H, d, $J=6.8$ Hz, C_{27/26}-CH₃), 0.91 (3H, d, $J=6.6$ Hz, 28-CH₃), 1.02 (3H, d, $J=6.6$ Hz, 21-CH₃), 3.5 (1H, t, H-3), 4.09 (1H, m, H-7), 5.2 (2H, m, H_{22/23}), 5.35 (1H, d, H-4); EIMS: m/z 412 (M⁺), 394 (M⁺-H₂O), 376 (M⁺-2H₂O), 287 (M⁺-SC), 269 [M⁺(SC+H₂O)], 251 [M⁺-(SC+2H₂O)].

Compound 2: Solid (15 mg) m.p. 157-62°; IR (KBr, cm⁻¹): 3525, 2872, 2955, 1460, 1380, 1180, 1074, 1043, 1028, 968, 935, 858, 779 and 275 cm⁻¹; 400 MHz ¹H NMR (CDCl₃): δ 0.81 (3H, s, 18-CH₃), 0.88 (3H, s, 19-CH₃); 0.82 (3H, d, $J=6.8$ Hz, C_{26/27}-CH₃), 0.83 (3H, d, $J=6.8$ Hz, C_{27/26}-CH₃), 0.94 (3H, d, $J=6.4$ Hz, C₂₈-CH₃).

1.02 (3H, d, $J=6.4$ Hz, $C_{21}-CH_3$), 3.96 (1H, m, $3\beta-H$), 5.18 (2H, m, $H_{22/23}$), 6.24 (1H, d, $J=8.5$ Hz, 6-H), 6.5 (1H, d, $J=8.5$ Hz, 7-H); ^{13}C NMR ($CDCl_3$): δ 135.4, 135.2, 132.37, 130.75, 66.4, 56.3, 51.7, 51.2, 44.59, 42.8, 39.6, 39.4, 37.0, 34.76, 33.09, 30.17, 28.57, 25.33, 23.42, 20.88, 20.65, 19.91, 19.62, 18.16, 17.55, 12.87 and 12.33; EIMS: m/z 428 (M^+) 410, 396, 377, 363, 337, 285, 253, 211, 185, 152, 145, 125, 107, 95, 81, 69 and 55.

Compound 5: Liquid, IR (KBr, cm^{-1}): 2941, 2850, 1745, 1460, 1435, 1363, 1248, 1146, and 1170; 1H NMR ($CDCl_3$): δ 0.89 (3H, t, $J=9.0$ Hz), 1.28 (32H, brs, $-CH_2$ groups), 1.63 (2H, m), 3.68 (3H, s); EIMS: m/z 312 (M^+), 298, 270, 227, 213, 199, 185, 171, 157, 143, 129, 101, 87 and 74 (base peak).

Compound 6: Solid, m.p. 125-30°; IR (KBr, cm^{-1}): 3000, 2851, 1710, 1460, 1435, 1412, 1375, 1290, 940 and 721, EIMS: m/z 298 (M^+), 284, 270, 256, 242, 228, 213, 199, 185, 171, 157, 143, 129, 115, 111, 97, 83, 73, 65, 55 and 44.

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