Polyhydroxysterols from the Soft Coral Sarcophyton subviride of Andaman and Nicobar Coasts

B. Lakshmana Raju, Gottumukkala V. Subbaraju, M. C. Reddy, D. Venkata Rao, Ch. Bheemasankara Rao, and Vegesna S. Raju

J. Nat. Prod., **1992**, 55 (7), 904-911• DOI: 10.1021/np50085a009 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 55, No. 7, pp. 904-911, July 1992

POLYHYDROXYSTEROLS FROM THE SOFT CORAL *SARCOPHYTON SUBVIRIDE* OF ANDAMAN AND NICOBAR COASTS[1]

B. LAKSHMANA RAJU, GOTTUMUKKALA V. SUBBARAJU,*[,2]

Department of Chemistry, School of Mathematical and Physical Sciences,

M.C. REDDY,

Department of Physics, Sri Venkateswara University, Tirupati 517502, India

D. VENKATA RAO,

Department of Pharmaceutical Sciences

CH. BHEEMASANKARA RAO,* and VEGESNA S. RAJU[2]

School of Chemistry, Andhra University, Visakhapatnam 530 003, India

ABSTRACT.—Four new polyhydroxysterols, (24*S*)-ergost-25-ene-1β,3β,5α,6β-tetraol [**12**], (24*S*)-ergostane-1β,3β,5α,6β,18,25-hexaol 25-monoacetate [**14**], (24*S*)-ergostane-3β,5α,6β,25ξ,26-pentaol 25-monoacetate [**16**], and gorgostane-1β,3β,5α,6β,25-pentaol [**19**], besides the known polyhydroxysterols **1**, **3**, **5**, **7**, and **10**, were isolated from the soft coral *Sarcophyton subviride* of Katchal Island of Andaman and Nicobar coasts. Structure elucidation of the new compounds was performed through spectral analysis of their peracetyl derivatives **13**, **15**, **17**, and **20**; therefore the possibility of partial acetylation in natural sterols could not be ruled out.

Extensive studies on sterols from marine invertebrates during the past two decades have resulted in the identification of a plethora of unusual side chains and highly oxygenated forms (1–5). A variety of polyhydroxysterols have been reported from the soft corals of the genus *Sarcophyton* (6–11). As a part of our continuing study on the steroid metabolites of marine invertebrates of the Indian Ocean (12–15), the sterol composition of the soft coral *Sarcophyton subviride* Tixer Durivault (Coelenterata) was examined. The soft coral was collected from the Katchal Island of Andaman and Nicobar Coasts during December 1986. To the best of our knowledge, this is the first report of chemical examination on this soft coral.

RESULTS AND DISCUSSION

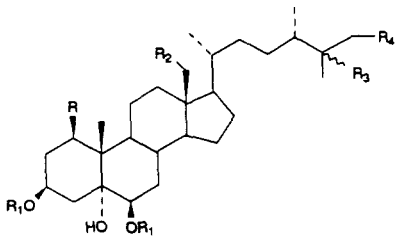
Si gel chromatography of the EtOAc-soluble part of the residue from an aqueous 95% EtOH extract of *S. subviride* gave batyl alcohol (16), a monohydroxy sterol fraction, and a mixture of polyhydroxy sterols.

The monohydroxy sterol fraction was acetylated using pyridine/Ac_2O under normal conditions. Gas chromatographic analysis of the acetylated material showed the presence of acetyl derivatives of cholesterol, 24ξ-methylcholesterol, and 24ξ-methyl-22-dehydrocholesterol (17).

Rechromatography of the polyhydroxysterols fraction gave (24*S*)-ergostane-3β,5α,6β,25-tetraol [**1**] (8,9,12,15) and (24*S*)-ergostane-3β,5α,6β,25-tetraol 25-monoacetate [**3**] (6,8,9,12,15), in addition to non-homogeneous fractions containing several polyhydroxysterols. These fractions were combined and acetylated using

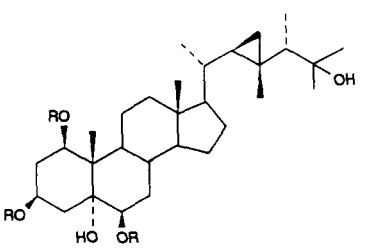
[1]Preliminary results on part of the work were presented at the 17th IUPAC International Symposium on the Chemistry of Natural Products, 4–9 February 1990, New Delhi, India, Abstracts, p. 76.

[2]Currently at the Department of Chemistry and Chemical Engineering, Stevens Institute of Technology, Hoboken, NJ 07030.



	R	R_1	R_2	R_3	R_4
1	H	H	H	OH	H
2	H	Ac	H	OH	H
3	H	H	H	OAc	H
4	H	Ac	H	OAc	H
5	OH	H	H	OAc	H
6	OAc	Ac	H	OAc	H
7	OH	H	H	OH	H
8	OAc	Ac	H	OH	H
9	OH	Ac	H	OAc	H
10	OH	H	H	H	H
11	OH	Ac	H	H	H
12	OH	H	H	DOUBLE BOND	
13	OAc	Ac	H	DOUBLE BOND	
14	OH	H	OH	OAc	H
15	OAc	Ac	OAc	OAc	H
16	H	H	H	OAc	OH
17	H	Ac	H	OAc	OAc
18	H	H	H	OH	OH

pyridine/Ac_2O. The chromatography of acetylated material gave eight compounds: (24*S*)-ergostane-1β,3β,5α,6β,25-pentaol 1,3,6,25-tetraacetate [**6**] (8,9,12), (24*S*)-ergostane-1β,3β,5α,6β,25-pentaol 1,3,6-triacetate [**8**] (8,9), (24*S*)-ergostane-1β,3β,5α,6β,25-pentaol 3,6,25-triacetate [**9**], (24*S*)-ergostane-1β,3β,5α,6β-tetraol 3,6-diacetate [**11**], and acetyl derivatives of four new polyhydroxysterols, (24*S*)-ergot-25-ene-1β,3β,5α,6β-tetraol 1,3,6-triacetate [**13**], (24*S*)-ergostane-1β,3β,5α,6β,18,25-hexaol 1,3,6,18,25-pentaacetate [**15**], (24*S*)-ergostane-3β,5α,6β,25ξ,26-pentaol 3,6,25,26-tetraacetate [**17**] and gorgostane-1β,3β,5α,6β,25-pentaol 1,3,6-triacetate [**20**].



19 R=H
20 R=Ac

Compound **13** was obtained as a semisolid and analyzed for $C_{34}H_{54}O_7$, $[M+H]^+$ 575, $[\alpha]_D$ −23.6° (*c* = 0.9, $CHCl_3$). A comparison of its ^1H- and ^{13}C-nmr data (Tables 1 and 2) with those of **6** reveals that the 2-acetoxypropyl unit of the side chain in **6** is replaced by an isopropylidene moiety in **13** based on the following observations: (i) the

TABLE 1. ^{13}C-nmr Data of Compounds **13**, **15**, **17**, and **20**.[a]

Carbon	Compound			
	13	**15**	**17**	**20**[b]
C-1	75.2	75.1	31.8	75.2
C-2	33.0	32.9	26.6	33.0
C-3	67.6	67.4	70.7	67.6
C-4	36.4	36.5	36.7	36.4
C-5	75.2	75.4	74.7	75.3
C-6	76.5	76.2	76.2	76.5
C-7	31.3	31.4	31.4	31.3
C-8	31.0	31.2	30.7	31.1
C-9	44.7	44.8	45.0	44.8
C-10	43.1	43.1	38.5	43.2
C-11	22.9	22.8	21.0	23.0
C-12	40.1	41.8	39.8	40.2
C-13	41.9	43.1	42.7	42.4
C-14	55.4	54.9	55.7	55.4
C-15	24.4	24.1	24.1	24.7
C-16	27.9	27.5	28.1	27.9
C-17	56.1	56.2	55.8	57.9
C-18	11.9	62.9	12.2	12.0
C-19	10.2	10.4	16.3	10.3
C-20	35.7	35.3	36.1	34.9
C-21	18.6	19.1	18.9	15.5
C-22	33.6	34.6	34.6	32.6
C-23	41.5	27.2	27.6	24.5
C-24	43.1	44.6	39.8	53.7
C-25	150.1	85.9	85.4	74.6
C-26	109.3	22.9	65.7	29.6
C-27	31.2	23.3	18.2	28.6
C-28	20.1	14.5	14.0	12.7
C-29				21.0
C-30				21.2
OAc	21.2	21.2	20.8	21.3
	21.4	21.5	21.4	21.5
	21.8	21.8	22.2	21.8
		22.5		
	170.0	170.0	170.2	170.1
	170.2	170.1	170.3	170.5
	170.4	170.4	170.7	
		170.5		
		171.6		

[a]Chemical shifts in δ (ppm). $CDCl_3$ solutions with TMS as an internal standard. Spectra of **15**, **17**, **20**, and **13** were recorded at 125.759 MHz or 50.168 MHz.
[b]Assignments are supported by DEPT experiments.

^{1}H-nmr spectrum of **6** contained a six-proton singlet at δ 1.36 and an acetate signal at δ 1.94 (3H, s), whereas in **13** these signals are replaced by an olefinic methyl at δ 1.63 (3H, s) and a two-proton singlet at δ 4.65 (brs); (ii) the ^{13}C-nmr spectrum of **6** exhibited signals at δ 85.9 (C-25), 23.0 (C-26), and 23.3 (C-27), and in **13** these signals are replaced by signals at δ 150.1 (C-25), 109.3 (C-26), and 31.2 (C-27); (iii) the secondary methyl (C-28) is deshielded in **13** by the influence of the double bond at C-25 and C-26 as expected. Thus, the structure of this peracetate could be derived as (24*S*)-ergost-25-ene-1β,3β,5α,6β-tetraol 1,3,6-triacetate [**13**], and that of the original sterol is assigned tentatively as **12**.

TABLE 2. ^{1}H-nmr Data of Compounds **13**, **15**, **17**, and **20**.[a]

Proton	Compound			
	13	**15**	**17**	**20**
H-18	0.67 (s)	3.92 (d, 11.5) 4.15 (d, 11.5)	0.67 (s)	0.65 (s)
H-19	1.24 (s)	1.23 (s)	1.15 (s)	1.22 (s)
H-21	0.99 (d, 6.8)	1.01 (d, 6.1)	0.91 (d, 6.7)	1.01 (s)
H-22	—	—	—	0.15 (m)
H-26	4.65 (bs)	1.39 (s)	4.38 (d, 11.7) 4.46 (d, 11.7)	1.27 (s)
H-27	1.63 (s)	1.39 (s)	1.37 (s)	1.27 (s)
H-28	0.89 (d, 6.4)	0.86 (d, 6.8)	0.90 (d, 7.0)	1.05 (d, 7.0)
H-29	—	—	—	0.99 (s)
H-30	—	—	—	−0.05 (m) 0.58 (dd, 9.4, 3.9)
H-1α H-3α	5.15–5.30 (m)	5.10–5.25 (m)	5.16 (m)	5.10–5.25 (m)
H-6α	4.70 (bs)	4.69 (bs)	4.68 (bs)	4.70 (bs)
-OAc	2.00, 2.09 (s)	1.97, 2.00 2.01, 2.09, 2.11 (s)	2.00, 2.02, 2.07, 2.08 (s)	2.00, 2.08 (s)

[a]Chemical shifts in δ (ppm). $CDCl_3$ solutions with TMS as an internal standard. Coupling constants J, in parentheses, are expressed in Hz. Spectra of **15**, **17**, **20**, and **13** were recorded at 500.135 MHz or 199.495 MHz.

Compound **15** was obtained as a semisolid and analyzed for $C_{38}H_{60}O_{11}$, $[M-Ac]^+$ 649, $[\alpha]_D$ −18.0° (c = 0.8, $CHCl_3$). A comparison of its ^{1}H- and ^{13}C-nmr data (Tables 1 and 2) with those of **6** suggests that **15** contains an additional acetoxyl group. The position of this additional group was fixed at C-18 based on two findings. The first was the absence of the characteristic shielded methyl (C-18) signal around δ 0.70 and instead the presence of two AB doublets at δ 3.92 (1H, J = 11.5) and 4.16 (1H, J = 11.5) in the ^{1}H-nmr spectrum of **15**. Second, in the ^{13}C-nmr spectrum, **15** exhibited a signal at δ 62.9, and the usual C-18 methyl carbon signal at about δ 12.0 was absent. The structure of this peracetate could thus be deduced as (24*S* -ergostane-1β,3β,5α,6β,18,25-hexaol 1,3,6,18,25-pentaacetate [**15**], and that of the original sterol is tentatively assigned as **14**.

Steroid **17** was obtained as a low melting solid and analyzed for $C_{36}H_{58}O_9$, $[M-Ac]^+$ 591, $[\alpha]_D$ +23.92° (c = 0.5, $CHCl_3$). A careful study of its ^{1}H-nmr spectrum in comparison with that of **4** reveals that **17** contains the same oxygenation pattern but has an additional acetoxyl group. The nature of the additional acetoxyl was found to be primary based on the presence of two doublets at δ 4.38 (1H, J = 11.7) and 4.46 (1H, J = 11.7) in the ^{1}H-nmr spectrum of **17**. The placement of this acetoxyl on C-26 was favored by the presence of only one tertiary methyl at δ 1.37 (C-27). The alternate positions (C-18, C-19) possible for this acetoxyl were ruled out, as the ^{1}H-nmr spectrum of **17** showed signals for the C-18 and C-19 methyls at δ 0.67 and 1.15, respectively. As expected, the ^{13}C-nmr spectrum of **17** contained five oxygenated carbons at δ 65.7 (C-26), 70.7 (C-3), 74.7 (C-5), 76.2 (C-6), and 85.4 (C-25). Based on the above, the structure of this acetyl derivative was deduced as (24*S*)-ergostane-3β,5α,6β,25ξ,26-pentaol 3,6,25,26-tetraacetate [**17**], and the corresponding natural sterol is assigned tentative structure, **16**.

It may be noted that a closely related polyhydroxysterol **18**, which contains hydroxyl at C-25 instead of acetoxyl as found in **16**, was isolated from *Sarcophyton glaucum*

(8). The configuration at C-24 in the new sterols **12**, **14**, and **16** is considered to be the same (24*S*) as that of the known congeneric polyhydroxysterols **1**, **3**, and **6**, based on comparison of spectral data.

Steroid **20** was also obtained as a low melting solid and analyzed for $C_{36}H_{58}O_8$, $[M]^+$ 618, $[\alpha]_D$ −31.3° (*c* = 0.4, $CHCl_3$). Its ^{1}H-nmr spectrum showed shielded protons at δ −0.05 (1H, m), 0.15 (1H, m), and 0.58 (1H, dd, *J* = 9.4, 3.9), which are not present in the ^{1}H-nmr spectra of other polyhydroxysterols isolated from this organism. These signals are characteristic of the cyclopropane ring found in the gorgostane skeleton (15, 18, 19). The ^{13}C-nmr spectrum of **20** showed five oxygenated carbons at δ 67.6, 74.6, 75.2, 75.3, and 76.5. Its ^{1}H-nmr spectrum exhibited three acetoxyls (δ 2.00, 6H, s and 2.08, 3H, s). A comparison of ^{13}C-nmr data of **20** with those of **6** suggests that **20** also contains acetoxyls at 1β,3β,6β and a hydroxyl at 5α. The remaining tertiary hydroxyl was placed at C-25 in view of the oxygenated methyls observed in the region δ 1.20–1.30 and the oxygenation at C-25 in congeneric polyhydroxysterols. Thus, the structure of this peracetate may be described as gorgostane-1β,3β,5α,6β,25-pentaol 1,3,6-triacetate [**20**], and that of the original sterol is assigned tentatively as **19**.

It is interesting to note that a gorgost-5-en-3β-ol isolated from *Sarcophyton* species was found to exhibit cytotoxic activity (20).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were determined on a Mel-Temp apparatus and are uncorrected. The ir spectra were recorded on a Perkin-Elmer 841 spectrometer. ^{1}H-nmr spectra were recorded on a Bruker 500 MHz or 250 MHz or Boilmaker NMR network 200 MHz NMR spectrometer in $CDCl_3$ using TMS as internal standard, operating at 500.135 MHz or 250.133 MHz or 199.495 MHz, respectively. ^{13}C-nmr and DEPT spectra were recorded on Bruker 500 MHz or Boilmaker NMR network 200 MHz NMR spectrometer operating at 125.759 MHz or 50.168 MHz, respectively. Cims spectra were recorded on a Biospect Mass spectrometer, optical rotations were measured on a Perkin-Elmer 141 Polarimeter, and glc analysis was performed on a Shimadzu GC-4CM instrument with flame ionization detector. Microanalyses were determined on a Carlo Erba EA 1108 elemental analyser.

COLLECTION, EXTRACTION, AND ISOLATION.—Specimens of the soft coral *S. subviride* (voucher No. MF/CBR-23) were collected during December 1986 at Katchal Island (E, 93°13′; N, 8°) off Andaman and Nicobar coasts. Voucher specimens are on deposit at the chemistry department, Andhra University, India and the Northern Territory Museum of Arts and Sciences, Darwin, Australia. Freshly collected specimens (wet wt ca. 20 kg) were soaked in 95% EtOH, and the concentrate from the alcoholic extract was partitioned with EtOAc. The EtOAc-soluble part was concentrated in vacuo, and the gummy residue (ca. 85 g) was chromatographed over Si gel (ACME, 100–200 mesh). Fractions eluted with hexane afforded batyl alcohol (200 mg). Elution with mixtures of hexane/C_6H_6 gave a monohydroxysteroid fraction, and C_6H_6/EtOAc yielded polyhydroxysterol fractions.

The monohydroxysterol fraction was acetylated using pyridine/Ac_2O under normal conditions. The glc analysis (30 m × 0.3 mm i.d. capillary column, column temperature 255°, injection temperature 280° of the acetylated fraction (150 mg) showed the presence of cholesteryl acetate (4.8%), 24ξ-methylcholesteryl acetate (30.9%) and 24ξ-methyl-22-dehydrocholesteryl acetate (5.1%).

Rechromatography of the polyhydroxysterol fractions yielded **1** and **3**, in addition to non-homogeneous fractions, which were combined and acetylated with pyridine/Ac_2O under normal conditions.

ACETYLATION OF POLYHYDROXYSTEROLS.—The combined non-homogeneous fractions (1.0 g) of polyhydroxysterols were acetylated with pyridine (4 ml) and Ac_2O (4 ml) at room temperature for 24 h. Usual workup followed by repeated chromatography over Si gel yielded **6** (150 mg), **8** (20 mg), **9** (50 mg), **11** (10 mg), **13** (100 mg), **15** (50 mg), **17** (40 mg), and **20** (60 mg).

(24S)-Ergostane-3β,5α,6β,25-tetraol [**1**].—Mp 255–258°. *Anal.* found C 74.6, H 11.0%; $C_{28}H_{50}O_4$ requires C 74.7, H 11.1%. $[\alpha]_D$ −12.6° (*c* = 0.61, MeOH) [lit. (8) $[\alpha]_D$ −12.5°, MeOH]; ir (KBr) ν max 3440, 1450, 1370 cm^{-1}; ^{1}H nmr (DMSO-d_6) δ 0.62 (3H, s, H-18), 0.80 (3H, d, *J* = 6.8, H-28), 0.89 (3H, d, *J* = 6.5, H-21), 0.99 (3H, s, H-19), 1.01 (3H, s, H-26), 1.02 (3H, s, H-27), 3.30 (1H, bs, H-6α), 3.80 (1H, m, H-3α), 3.62 (1H, s, 5α-OH), 3.97 (1H, s, 25-OH), 4.16 (1H, d, *J* = 5.7, 3β-OH), 4.38 (1H, d, *J* = 4.2, 6β-OH); ^{13}C nmr (DMSO-d_6) δ 31.1 (C-1), 32.0 (C-2), 65.7 (C-3),

40.9 (C-4), 74.3 (C-5), 74.1 (C-6), 34.5 (C-7), 30.0 (C-8), 44.5 (C-9), 37.8 (C-10), 20.7 (C-11), 39.8 (C-12), 42.2 (C-13), 55.6 (C-14), 23.9 (C-15), 27.5 (C-16), 55.8 (C-17), 11.9 (C-18), 16.3 (C-19), 35.9 (C-20), 18.9 (C-21), 34.6 (C-22), 27.8 (C-23), 44.6 (C-24), 71.3 (C-25), 26.0 (C-26), 27.1 (C-27), 14.8 (C-28). Assignments of ^{13}C-nmr data are supported by DEPT experiments.

Acetylation of **1** (20 mg) with pyridine/Ac_2O under normal conditions gave (24*S*)-ergostane-3β,5α,6β,25-tetraol 3,6-diacetate [**2**] (20 mg), colorless needles, mp 220°. *Anal.* found C 71.7, H 10.8%; $C_{32}H_{54}O_6$ requires C 71.9, H 10.1%. ^{1}H nmr ($CDCl_3$) δ 0.68 (3H, s, H-18), 0.89 (3H, d, J = 6.8, H-28), 0.93 (3H, d, J = 6.6, H-21), 1.15 (6H, s, H-26, -27), 1.17 (3H, s, H-19), 2.02 (3H, s, -OAc), 2.07 (3H, s, -OAc), 4.68 (1H, bs, H-6α), 5.13 (1H, m, H-3α); ^{13}C nmr ($CDCl_3$) δ 31.8 (C-1), 26.7 (C-2), 70.6 (C-3), 36.9 (C-4), 75.0 (C-5), 76.2 (C-6), 31.4 (C-7), 30.8 (C-8), 45.2 (C-9), 38.5 (C-10), 21.1 (C-11), 39.9 (C-12), 42.8 (C-13), 55.8 (C-14), 24.1 (C-15), 28.1 (C-16), 56.0 (C-17), 12.2 (C-18), 16.4 (C-19), 36.4 (C-20), 19.0 (C-21), 34.9 (C-22), 28.2 (C-23), 45.2 (C-24), 73.6 (C-25), 26.2 (C-26), 27.3 (C-27), 24.8 (C-28), 21.4 (2 × -OAc), 170.1 and 170.6 (2 × -OAc). Assignments of ^{13}C-nmr data are supported by DEPT experiments.

(24S)-Ergostane-3β,5α,6β,25-tetraol 25-monoacetate [**3**].—Crystalline solid, mp 235–238°. *Anal.* found C 73.4, H 10.7%; $C_{30}H_{52}O_5$ requires C 73.2, H 10.6%. [α]D −17.4° (c = 1.7, MeOH) [lit. (8) [α]D −17.4°, MeOH]; ir (KBr) ν max 3440, 3380, 1730, 1254 cm^{-1}; ^{1}H nmr (DMSO-d_6) δ 0.63 (3H, s, H-18), 0.83 (3H, d, J = 6.8, H-28), 0.90 (3H, d, J = 6.6, H-21), 1.03 (3H, s, H-19), 1.33 (6H, s, H-26, -27), 1.92 (3H, s, -OAc), 3.32 (1H, bs, H-6α), 3.65 (1H, s, 5α-OH), 3.80 (1H, m, 3α-H), 4.20 (1H, d, J = 6.0, 3β-OH), 4.40 (1H, d, J = 4.0, 6β-OH); ^{13}C nmr (DMSO-d_6) δ 31.0 (C-1), 32.0 (C-2), 65.6 (C-3), 40.8 (C-4), 74.2 (C-5), 73.9 (C-6), 34.4 (C-7), 30.0 (C-8), 44.6 (C-9), 37.7 (C-10), 20.7 (C-11), 39.8 (C-12), 42.2 (C-13), 55.4 (C-14), 23.9 (C-15), 27.1 (C-16), 55.8 (C-17), 12.0 (C-18), 16.3 (C-19), 35.7 (C-20), 18.9 (C-21), 34.3 (C-22), 27.7 (C-23), 41.3 (C-24), 85.0 (C-25), 22.7 (C-26), 23.2 (C-27), 14.3 (C-28), 22.2 (-OAc), 169.6 (-OAc). Assignments of ^{13}C-nmr data are supported by DEPT experiments. Cims *m/z* (%) [M − Ac]$^+$ 449 (34), 431 (23), 413 (85), 394 (100).

Acetylation of **3** (30 mg) with pyridine/Ac_2O under normal conditions gave (24*S*)-ergostane-3β,5α,6β,25-tetraol 3,6,25-triacetate [**4**] (30 mg). *Anal.* found C 70.7, H 9.8%; $C_{34}H_{56}O_7$ requires C 70.8, H 9.7%. ^{1}H nmr ($CDCl_3$) δ 0.68 (3H, s, H-18), 0.86 (3H, d, J = 6.8, H-28), 0.92 (3H, d, J = 6.6, H-21), 1.15 (3H, s, H-19), 1.39 (6H, s, H-26, -27), 1.97, 2.02, 2.07 (3H each, s, 3 × -OAc), 4.70 (1H, bs, H-6α), 5.14 (1H, m, H-3α); ^{13}C nmr ($CDCl_3$) δ 31.8 (C-1), 26.6 (C-2), 70.6 (C-3), 36.8 (C-4), 75.0 (C-5), 76.2 (C-6), 31.3 (C-7), 30.7 (C-8), 45.1 (C-9), 38.5 (C-10), 21.0 (C-11), 39.8 (C-12), 42.7 (C-13), 55.7 (C-14), 24.1 (C-15), 28.1 (C-16), 55.9 (C-17), 12.2 (C-18), 16.4 (C-19), 36.2 (C-20), 19.0 (C-21), 34.6 (C-22), 27.7 (C-23), 42.0 (C-24), 85.9 (C-25), 23.0 (C-26), 23.3 (C-27), 14.5 (C-28), 21.4, 21.5, 22.6 (3 × -OAc), 170.2, 170.5, 170.7 (3 × -OAc). Assignments of ^{13}C-nmr data are supported by DEPT experiments.

(24S)-Ergostane-1β,3β,5α,6β,25-pentaol 1,3,6,25-tetraacetate [**6**].—Colorless crystals, mp 170–173° [lit. (8) oil]. *Anal.* found C 68.2, H 9.1%; $C_{36}H_{58}O_9$ requires C 68.1, H 9.2%. [α]D −37.87° (c = 1.5, $CHCl_3$) [lit. (8) [α]D −35.1° (MeOH)]; ir (KBr) ν max 3450, 1750, 1375, 1250 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 0.64 (3H, s, H-18), 0.83 (3H, d, J = 6.8, H-28), 0.88 (3H, d, J = 6.5, H-21), 1.21 (3H, s, H-19), 1.36 (6H, s, H-26, -27), 1.94, 1.98, 2.06 (each s, 4 × -OAc), 4.69 (1H, bs, H-6α), 5.10–5.20 (2H, m, H-1α, -3α); ^{13}C nmr ($CDCl_3$) δ 75.2 (C-1), 33.0 (C-2), 67.5 (C-3), 36.5 (C-4), 75.5 (C-5), 76.5 (C-6), 31.4 (C-7), 31.1 (C-8), 44.8 (C-9), 43.2 (C-10), 22.9 (C-11), 40.1 (C-12), 41.9 (C-13), 55.4 (C-14), 24.4 (C-15), 27.8 (C-16), 56.0 (C-17), 12.0 (C-18), 10.3 (C-19), 36.2 (C-20), 18.8 (C-21), 34.6 (C-22), 27.9 (C-23), 41.9 (C-24), 85.9 (C-25), 23.0 (C-26), 23.3 (C-27), 14.5 (C-28), 21.2, 21.4, 21.8, 22.5 (4 × -OAc), 170.0, 170.4, 170.5, 171.1 (4 × -OAc). Assignments of ^{13}C-nmr data are supported by DEPT experiments. Cims *m/z* (%) [M + H − HOAc]$^+$ 575 (1), 557 (2), 515 (15), 497 (8), 455 (34), 437 (22), 395 (62), 377 (100), 311 (9), 271 (24), 253 (23).

(24S)-Ergostane-1β,3β,5α,6β,25-pentaol 1,3,6-triacetate [**8**].—*Anal.* found C 68.8, H 9.5%; $C_{34}H_{56}O_8$ requires C 68.9, H 9.5%. [α]D +4.7° (c = 0.4, $CHCl_3$); ir ($CHCl_3$) ν max 3455, 1745, 1370, 1250 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 0.67 (3H, s, H-18), 0.88 (3H, d, J = 6.9, H-28), 0.92 (3H, d, J = 6.5), 1.14 (3H, s, H-26), 1.17 (3H, s, H-27), 1.24 (3H, s, H-19), 2.01, 2.09 (each s, 3 × -OAc), 4.67 (1H, bs, H-6α), 5.18 (2H, m, H-1α, -3α); ^{13}C nmr ($CDCl_3$) δ 75.2 (C-1), 33.0 (C-2), 67.5 (C-3), 36.6 (C-4), 75.5 (C-5), 76.6 (C-6), 31.4 (C-7), 31.1 (C-8), 44.9 (C-9), 43.2 (C-10), 23.0 (C-11), 40.2 (C-12), 42.0 (C-13), 55.5 (C-14), 24.5 (C-15), 27.9 (C-16), 56.2 (C-17), 12.0 (C-18), 10.3 (C-19), 36.6 (C-20), 18.8 (C-21), 34.8 (C-22), 28.1 (C-23), 45.1 (C-24), 73.7 (C-25), 25.9 (C-26), 27.4 (C-27), 14.9 (C-28), 21.2, 21.4, 21.8 (3 × -OAc), 170.0, 170.4 (3 × -OAc).

(24S)-Ergostane-1β,3β,5α,6β,25-pentaol 3,6,25-triacetate [**9**].—Colorless needles, mp 198–200°. *Anal.* found C 68.7, H 9.4%; $C_{34}H_{56}O_8$ requires C 68.9, H 9.5%. [α]D −18.25° (c = 0.8, $CHCl_3$); ir ($CHCl_3$) ν max 3600, 3500, 1727, 1370, 1265 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 0.68 (3H, s, H-18), 0.86 (3H,

d, $J=6.8$, H-28), 0.91 (3H, d, $J=6.5$, H-21), 1.14 (3H, s, H-19), 1.39 (6H, s, H-26, -27), 1.97, 2.03, 2.09 (3H each, s, 3 × -OAc), 4.10 (1H, m, H-1α), 4.68 (1H, bs, H-6α), 5.14 (1H, m, H-3α); ^{13}C nmr ($CDCl_3$) δ 72.7 (C-1), 36.7 (C-2), 67.9 (C-3), 37.3 (C-4), 75.8 (C-5), 76.6 (C-6), 31.4 (C-7), 31.2 (C-8), 45.6 (C-9), 44.1 (C-10), 24.4 (C-11), 40.2 (C-12), 42.1 (C-13), 55.7 (C-14), 24.0 (C-15), 27.8 (C-16), 56.1 (C-17), 12.1 (C-18), 9.0 (C-19), 36.3 (C-20), 18.9 (C-21), 34.7 (C-22), 27.9 (C-23), 42.0 (C-24), 85.9 (C-25), 22.9 (C-26), 23.3 (C-27), 14.5 (C-28), 21.3, 21.4, 22.5 (3 × -OAc), 170.1, 170.5, 170.7 (3 × -OAc).

(24S)-Ergostane-1β,3β,5α,6β-tetraol 3,6-diacetate [**11**].—Oil. *Anal.* found C 72.0, H 10.2%; $C_{32}H_{54}O_6$ requires C 71.9, H 10.1%. ^{1}H nmr ($CDCl_3$) δ 0.68 (3H, s, H-18), 0.77 (3H, d, $J=6.7$), 0.78 (3H, d, $J=6.8$) 0.85 (3H, d, $J=7.5$), 0.90 (3H, d, $J=7.6$), 1.14 (3H, s, H-19), 2.02, 2.09 (3H each, s, 2 × -OAc), 4.09 (1H, dd, $J=11.3$, 4.9, H-1α), 4.66 (1H, bs, H-6α), 5.12 (1H, m, H-3α); ^{13}C nmr ($CDCl_3$) δ 72.7 (C-1), 36.7 (C-2), 67.8 (C-3), 37.3 (C-4), 75.9 (C-5), 76.6 (C-6), 31.5 (C-7), 31.2 (C-8), 45.6 (C-9), 44.1 (C-10), 24.5 (C-11), 40.2 (C-12), 42.1 (C-13), 55.7 (C-14), 24.1 (C-15), 28.0 (C-16), 56.2 (C-17), 12.1 (C-18), 9.1 (C-19), 36.3 (C-20), 18.8 (C-21), 33.7 (C-22), 29.7 (C-23), 39.1 (C-24), 30.6 (C-25), 17.6 (C-26), 20.5 (C-27), 15.5 (C-28), 21.3, 21.4 (2 × -OAc), 170.1, 170.6 (2 × -OAc).

(24S)-Ergost-25-ene-1β,3β,5α,6β-tetraol 1,3,6-triacetate [**13**].—Semisolid. *Anal.* found C 70.1, H 9.3%; $C_{34}H_{54}O_7$ requires C 71.1, H 9.4%. [α]D −23.6° ($c=0.9$, $CHCl_3$); ir (KBr) ν max 3450, 1735, 1370, 1235, 1025 cm^{-1}; ^{1}H nmr see Table 2; ^{13}C nmr see Table 1; cims *m/z* (%) $[M+H]^+$ 575 (1), 557 (2), 515 (21), 497 (12), 455 (41), 437 (23), 395 (60), 377 (100), 271 (32), 253 (32), 151 (36).

(24S)-Ergostane-1β,3β,5α,6β,18,25-hexaol 1,3,6,18,25-pentaacetate [**15**].—Semisolid. *Anal.* found C 65.8, H 8.8%; $C_{38}H_{60}O_{11}$ requires C 65.9, H 8.7%. [α]D −18.0° ($c=0.8$, $CHCl_3$); ir ($CHCl_3$) ν max 3462, 2932, 1730, 1371, 1248, 1035 cm^{-1}; ^{1}H nmr see Table 2; ^{13}C nmr see Table 1; cims *m/z* (%) $[M-Ac]^+$ 649 (24), 614 (17), 572 (64), 554 (21), 512 (100), 494 (36), 452 (59), 434 (64), 392 (36), 374 (36).

(24S)-Ergostane-3β,5α,6β,25ξ,26-pentaol 3,6,25,26-tetraacetate [**17**].—Low melting solid. *Anal.* found C 68.0, H 9.3%; $C_{36}H_{58}O_9$ requires C 68.1, H 9.2%. [α]D +23.9° ($c=0.5$, $CHCl_3$); ir ($CHCl_3$) ν max 3598, 2935, 1730, 1370, 1262, 1034 cm^{-1}; ^{1}H nmr see Table 2; ^{13}C nmr see Table 1; cims *m/z* $[M-Ac]^+$ 591.

Gorgostane-1β,3β,5α,6β,25-pentaol 1,3,6-triacetate [**20**].—Low melting solid. *Anal.* found C 70.0, H 9.3%; $C_{36}H_{58}O_8$ requires C 69.9, H 9.4%. [α]D −31.32° ($c=0.4$, $CHCl_3$); ir ($CHCl_3$) ν max 3597, 2932, 1730, 1371, 1186, 1036 cm^{-1}; ^{1}H nmr see Table 2; ^{13}C nmr see Table 1; cims *m/z* $[M]^+$ 618.

ACKNOWLEDGMENTS

The authors thank Dr. Phil Alderslade, Northern Territory Museum of Arts and Sciences, Darwin, Australia for the identification of the soft coral; Prof. T. Matsumoto, Nihon University, Tokyo, Japan for glc analysis of sterols; Dr. K. Suseela, Purdue University, Dr. D.R. Reddy, University of Pennsylvania, and Mr. C.V. Lakshmana Rao, RRL, Bhubaneswar, India for spectral data on some of the compounds; Mr. Joseph Angel, Chief Wildlife Warden, Andaman and Nicobar coasts for cooperation during the collection of organisms; and Prof. A.K. Bose, Stevens Institute of Technology, for encouragement. This research was partly funded by the Department of Science and Technology, Government of India to C.B.R.

LITERATURE CITED

1. F.J. Schmitz, in: "Marine Natural Products, Chemical and Biological Perspectives." Ed. by P.J. Scheuer, Acadamic Press, New York, 1978, Vol. 1, Chapter 5, p. 241.
2. C. Djerassi, in: "Natural Products and Drug Development." Ed. by P. Krogsgsard-Larsen, S.B. Christensen, and H. Kofod, Munksgaard, Copenhagen, 1984, p. 164.
3. G.A. Doss and C. Djerassi, *J. Am. Chem. Soc.*, **110**, 8124 (1988), and earlier papers in this series.
4. D.J. Faulkner, *Nat. Prod. Rep.*, **7**, 269 (1990), and earlier reviews in this series.
5. B. Tursch, *Pure Appl. Chem.*, **48**, 1 (1976).
6. J.M. Moldowan, B.M. Tursch, and C. Djerassi, *Steroids*, **24**, 387 (1974).
7. J.M. Moldowan, W.L. Tan, and C. Djerassi, *Steroids*, **26**, 107 (1975).
8. M. Kobayashi, T. Hayashi, F. Nakajima, and H. Mitsuhashi, *Steroids*, **34**, 285 (1979).
9. M. Kobayashi, T. Hayashi, K. Hayashi, M. Tanabe, T. Nakagawa, and H. Mitsuhashi, *Chem. Pharm. Bull.*, **31**, 1848 (1983).
10. M. Kobayashi and H. Mitsuhashi, *Chem. Pharm. Bull.*, **31**, 4127 (1983).
11. J. Su, T. Peng, K. Long, and L. Zeng, *Steroids*, **48**, 233 (1986).
12. M. Kobayashi, F. Kanda, C.V.L. Rao, S.M.D. Kumar, G. Trimurtulu, and C.B. Rao, *Chem. Pharm. Bull.*, **38**, 1724 (1990).

13. M. Kobayashi, F. Kanda, S.R. Damarla, D.V. Rao, and C.B. Rao, *Chem. Pharm. Bull.*, **38**, 2400 (1990).
14. M. Kobayashi, F. Kanda, C.V.L. Rao, S.M.D. Kumar, D.V. Rao, and C.B. Rao, *Chem. Pharm. Bull.*, **39**, 297 (1991).
15. M. Kobayashi, K. Kobayashi, K.V. Ramana, C.V.L. Rao, D.V. Rao, and C.B. Rao, *J. Chem. Soc., Perkin Trans. 1*, 493 (1991).
16. D.V. Rao, T.S. Rao, and C.B. Rao, *Indian J. Chem.*, **29B**, 683 (1990).
17. T. Itoh, H. Tanti, K. Fukushima, T. Tamura, and T. Matsumoto, *J. Chromatogr.*, **234**, 65 (1982).
18. B.M. Jagodzinska, J.S. Trimmer, W. Fenical, and C. Djerassi, *J. Org. Chem.*, **50**, 2988 (1988).
19. C.B. Rao, K.V. Ramana, D.V. Rao, E. Fahy, and D.J. Faulkner, *J. Nat. Prod.*, **51**, 954 (1988).
20. R. Li, Z. Lai, and K. Long, *Zhongshan Daxue, Xuebao Ziran Kexueban*, 78 (1982); *Chem. Abstr.*, **97**, 107457j (1982).

Received 26 November 1991