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Isolation of Polyhydroxysterols from a Species of *Sarcophyton* of the Indian Ocean

V. CHANDRA SEKHAR†, CH. BHEEMASANKARA RAO†,
D. VENKATA RAO*, B. SARVANI and D.K.M. LAKSHMI
Department of Pharmaceutical Sciences
Andhra University, Visakhapatnam-530 003, India
E-mail dvrao50@hotmail.com

A new trihydroxysterol, (24S)-4α,24-dimethylcholestane-3β,6α, 25-triol (**1**) was isolated from a soft coral *Sarcophyton* sp. collected from the coasts of Gulf of Mannar, Indian Ocean together with four known polyhydroxy sterols **3**, **4**, **5** and **6**, that was earlier reported as its pentaacetate. The structures were derived by spectral data.

Key Words: Polyhydroxysterols, *Sarcophyton* sp., Soft coral, Indian Ocean.

INTRODUCTION

The soft corals of the genus *Sarcophyton* are a rich source of a variety of polyhydroxysterols[1–7]. In continuation with our work on this genus of the Indian Ocean, we have isolated five polyhydroxysterols and elucidated their structure.

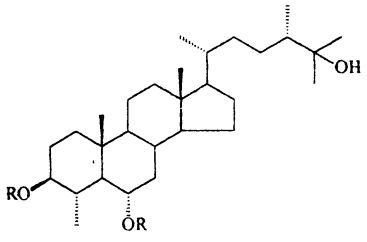
EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. The IR spectra were recorded on a Perkin-Elmer 841 spectrometer. ^{1}H NMR spectra were recorded on a Bruker VM-400 (400 MHz FT nmr) or Jeol, JMN GX-270 spectrometer, with $CDCl_3$ and pyridine-d^5 using TMS as internal standard. ^{13}C NMR and DEPT spectra were recorded on Bruker VM-400 MHz or Jeol EX-90 spectrometer operating at 100 MHz or 22.5 MHz respectively. Mass spectra were determined on a Jeol D-300 mass spectrometer. Elemental analyses were determined on a Carlo-Erba EA-1108 instrument.

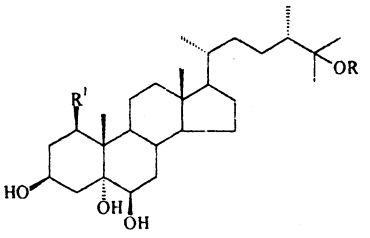
The soft coral *Sacrophyton* sp. was collected (dry weight 1.9 kg) on the coasts of Gulf of Mannar of Indian Ocean near Mandapam (9°20′ E, 19°10′ N) in Tamilnadu, India. The identification was carried out upto genus level and the specimen is available in the Department of Pharmaceutical Sciences, Andhra University with code No. MD-90-22. Freshly collected organism was washed

†Department of Organic Chemistry, School of Chemistry, Andhra University, Visakhapatnam-530 003, India

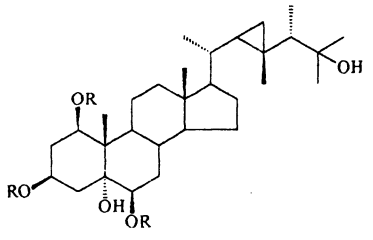
with fresh water, cut into thin slices and soaked in 95% EtOH. The extraction of the soft coral was carried out using EtOH at room temperature by percolation. The alcoholic concentrate was fractionated with EtOAc and concentrated. The combined EtOAc extract (*ca.* 35 g) was chromatographed over silica gel (ACME, 100–200 #) using eluents with increasing polarity starting from pet. ether through benzene to ethyl acetate. Fractions eluted with 40–80% EtOAc in benzene yielded polyhydroxysterols. Repeated column chromatography followed by recrystallization gave **1** (35 mg), **3** (430 mg), **4** (70 mg), **5** (210 mg) and **6** (160 mg).



1. R = H
2. R = Ac



3. R = Ac; R' = H
4. R = H; R' = H
5. R = Ac; R' = OH



6. R = H
7. R = Ac

(24S)-4α,24-Dimethylcholestane-3β,6α,25-triol (1): Colourless crystals (pet. ether-acetone) m.p. 232–234°C. Anal. found C 77.51, H 11.77%; $C_{29}H_{52}O_3$ requires C 77.68, H 11.61%. $[\alpha]_D^{25}$ + 15.2°(c = 0.26, $CHCl_3$). **IR** ($CHCl_3$): ν_{max} 3600–3500, 2960, 1420, 1060 and 940 cm^{-1}; **^{1}H NMR** ($CDCl_3$): δ 3.16 (1H, m, 3-H), 4.14 (1H, br s, 6-H), 0.69 (3H, s, 18-Me), 1.07 (3H, s, 19-Me) 0.93 (3H, d, J = 6.1 Hz 21-Me), 1.16 (3H, s, 26-Me), 1.16 (3H, s, 27-Me), 0.89 (3H, d, J = 6.6 Hz, 28-Me), 1.07 (3H, d, J = 6.2 Hz, 29-Me); **^{1}H NMR** (d^5-pyridine): δ 3.24 (1H, m, 3-H), 2.18 (1H, m, 4-H), 4.23 (1H, br s, 6-H), 0.47 (3H, s, 18-Me), 1.21 (3H, s, 19-Me), 0.86 (3H, d, J = 6.1 Hz, 21-Me), 1.18 (3 H, s, 26-Me), 1.16 (3H, s, 27-Me), 0.80 (3H, d, J = 6.6 Hz, 28-Me), 1.27 (3H, d, J = 6.2 Hz, 29-Me); **^{13}C NMR** ($CDCl_3$): 35.9 (C-1), 29.7 (C-2), 66.8 (C-3), 38.4 (C-4), 52.7 (C-5), 75.1 (C-6), 36.3 (C-7), 31.1 (C-8), 54.4 (C-9), 39.9 (C-10), 20.9 (C-11), 39.9 (C-12), 42.6 (C-13), 56.0 (C-14), 24.2 (C-15), 28.2 (C-16), 56.2 (C-17), 12.1 (C-18), 16.5 (C-19), 36.3 (C-20), 19.0 (C-21), 34.9 (C-22), 27.9 (C-23), 45.2 (C-24), 76.8 (C-25), 26.2 (C-26), 27.3 (C-27), 14.8 (C-28), 14.6 (C-29); **MS**: m/z (%)[M^+-H_2O] 430 (27), 412

(22), 394 (10), 379 (8), 346 (23), 331 (13), 313 (12), 303 (23), 295 (7), 285 (39), 267 (15), 260 (11), 245 (13), 227 (23), 109 (100), 69 (62).

Acetylation of **1** (10 mg) with pyridine-Ac_2O under normal conditions gave diacetyl derivative (24S)-4α,24-dimethylcholestane-3β,6α,25-triol-3,6-diacetate (**2**, 8 mg). **^{1}H NMR** ($CDCl_3$): δ 0.7 (3H, s), 0.85 (3H, d, J = 6.5 Hz), 0.95 (3H, d, J = 6.0 Hz), 1.05 (3H, s), 1.1 (3H, d, J = 6.5 Hz), 1.15(6H, s), 2.0 (6H, s), 4.40 (1H, m), 5.31 (1H, m).

Gorgostane-1β, 3β, 5α, 6β, 25-pentol (6): Colourless crystals (chloroform-methanol), m.p. 286–288°C. Anal. found C 72.98, H 10.69%; $C_{30}H_{52}O_5$ requires C 73.17, H 10.57%. $[\alpha]_D^{25}$ + 5.0° (c = 1.6, pyridine); **IR** (KBr): ν_{max} 3600–3500. 2990, 1370, 1190, 1040 and 960 cm^{-1}; **^{1}H NMR** (d^5-pyridine): δ 4.93 (1-H, dd, J = 5.0, 11.0 Hz, 1α-H), 4.98 (1-H, m, 3α-H), 3.08 (1H, t, J = 12.0 Hz, 4a-H), 4.23 (1H, br s, 6α-H), 0.81 (3H, s, 18-Me), 1.94 (3H, s, 19-Me), 1.32 (3H, d, J = 6.6 Hz, 21-Me), 0.20 (1H, m, 22-H), 1.43 (3H, s, 26-Me), 1.49 (3H, s, 27-Me), 1.09 (3H, s, 28-Me), –0.02 (1H, t, J = 4.9 Hz, 29-H), 0.66 (1H, dd, J = 9.5, 4.0 Hz, 29-H), 1.18 (3H, s, 30-Me); **^{13}C NMR** (d^5-pyridine): 73.8 (C-1), 44.1 (C-2), 65.4 (C-3), 43.2 (C-4), 77.2 (C-5), 77.0 (C-6), 35.7 (C-7), 32.0 (C-8), 47.3 (C-9), 44.9 (C-10) 25.0 (C-11), 41.6 (C-12), 43.2 (C-13), 58.7 (C-14), 25.3 (C-15), 28.6 (C-16), 56.8 (C-17), 12.6 (C-18), 10.7 (C-19), 35.3 (C-20), 15.7 (C-21), 33.2 (C-22), 25.3 (C-23), 54.5 (C-24), 73.4(C-25), 30.9 (C-26), 28.8 (C-27), 13.1 (C-28), 22.1 (C-29), 21.4 (C-30). Assignments of ^{13}C NMR data are supported by DEPT experiments; MS: m/z (%) $[M^+-H_2O]$ 474 (2), 456 (6), 438 (9), 420 (9), 405 (2), 402 (7), 364 (7), 351 (11), 346 (8), 328 (10), 323 (3), 321 (8), 310 (7), 303 (9), 297 (20), 292 (5), 285 (12), 281 (7), 267 (11), 227 (12), 151 (54), 147 (23), 123 (48), 110 (100).

Acetylation of **6** (15 mg) with pyridine-Ac_2O at room temperature gave triacetyl derivative gorgostane-1β,3β,5α,6β,25-pentol-1,3,6-triacetate (**7**, 12 mg) as low melting solid. **^{1}H NMR** ($CDCl_3$): δ 0.05 (1H, m), 0.15 (1H, m), 0.58 (1H, dd, J = 9.5, 4.0 Hz), 0.65 (3H, s), 1.0 (6H, s), 1.05 (3H, d, J = 7.0 Hz), 1.22 (3H, s), 1.27 (6H, s), 2.0 (6H, s), 2.08 (3H, s), 4.7 (1H, br, s), 5.10–5.26(2H, m): **^{13}C NMR** ($CDCl_3$): δ 75.3 (C-1), 33.0 (C-2), 67.6 (C-3), 36.3 (C-4), 75.3(C-5), 76.6 (C-6), 31.3 (C-7), 31.0 (C-8), 44.9 (C-9), 43.1 (C-10), 23.1 (C-11), 4.02 (C-12), 42.5 (C-13), 55.4 (C-14), 24.7 (C-15), 27.9 (C-16), 57.8 (C-17), 12.1 (C-18), 10.3 (C-19), 34.9 (C-20), 15.5 (C-21), 32.6 (C-22), 24.6 (C-23), 53.6 (C-24), 74.6 (C-25), 29.5 (C-26), 28.6 (C-27), 12.6 (C-28), 21.0 (C-29), 21.2 (C-30), 21.3, 21.5, 21.8(3x, -OAc), 170.2, 170.5 (3x -OAc); MS: m/z $[M]^+$ 618.

RESULTS AND DISCUSSION

Compound **1** was obtained as colourless crystals (pet. ether-acetone), m.p. 232–234°C, analyzed for $C_{29}H_{52}O_3$, $[M-H_2O]^+$ m/z 430. $[\alpha]_D^{25}$ + 15.2° (c = 0.26, $CHCl_3$). The ^{13}C NMR spectrum in $CDCl_3$ showed three oxygen bearing carbon signals at δ 66.8, 75.1 and 76.8. The ^{1}H NMR spectrum showed low field signals at δ 3.16 (1H, m) and 4.14 (1H, br s). These proton signals shifted to 4.2 and 5.19 in acetyl derivative (**2**) suggesting two acylable CHOH protons. The presence of one tertiary hydroxyl group and the deshielded six proton singlet signal at δ

1.16 (6H, s) suggests the position of hydroxyl group on C-25 (δ_c 76.8 ppm) methyl cholestane side chain. The ^{1}H NMR spectrum in $CDCl_3$ showed four tertiary and three secondary methyls. Since 24-methyl cholestane has only six methyls. The seventh methyl group is to be present in the nucleus. The presence of mass fragments at 303 (M^+-side chain, 2H), 285 (M^+-side chain, H_2O, 2H) and 267 (M^+-side chain, $2H_2O$, 2H) clearly indicates the presence of extra methyl group in the nucleus. If the cholestane skeleton is assumed, C-4 and C-14 positions are available for the seventh methyl group. The appearance of 3β-hydroxy methine proton at δ 3.16 (3.60 in case of C-14 methyl sterols) and additional secondary methyl proton signal at δ 1.07 (3H, d, J = 6.2 Hz) favours 4α-position for the additional methyl group. The pyridine induced deshielding effect of 6β-H (δ 4.23, br s), 19β-CH_3(δ 1.21, s) and 4α-CH_3(δ 1.27, d, J = 6.2 Hz). In the ^{1}H NMR spectrum in d^5-pyridine indicated the presence of 4α-methyl, 6α-hydroxy groups in the compound. Based on the above the structure, compound **1** could be derived as (24S)-4α,24-dimethylcholestane-3β,6α,25-triol and forms a new addition to the group.

The structures of polyhydroxy sterols (24S)-24-methylcholestane-3β,5α,6β,25-tetrol-25, monoacetate (**3**)[1], (24S)-24-methylcholestane-3β,5α,6β, 25-tetrol (**4**)[8], (24S)-24-methylcholestane-1β,3β,5α,25-pentol-25-monoacetate (**5**)[2] were deduced by comparison of their published spectral data.

Compound **6** was obtained as colourless crystals (chloroform-methanol), m.p. 286–288°C and analyzed for $C_{30}H_{52}O_5$,$[M-H_2O]^+$ m/z 474. $[\alpha]_D^{25}$ + 5° (c = 1.6, pyridine). The ^{13}C-DEPT spectrum showed thirty carbon signals. Of these signals at δ 65.4, 73.4, 73.8, 77.0 and 77.2 are assignable to oxygen bearing carbons. The molecular formula $C_{30}H_{52}O_5$ requires five degrees of unsaturation and there are no olefinic carbons. Hence a pentacyclic carbon skeleton was assumed. The ^{1}H NMR spectrum in d^5-pyridine showed three high field signals at δ 0.02 (1H, t, J = 4.9 Hz), 0.20 (1H, m) and 0.66 (1H, dd, J = 9.5, 4.0 Hz) indicates the presence of cyclopropane moiety in the molecule besides the presence of seven methyl groups. The above data prefers a gorgosterol type of skeleton[9, 10] for the molecule. Further C-28 methyl proton signal appear as a singlet (1.09, 3H, s) instead of a doublet due to the influence of the cyclopropyl ring[11]. This also suggests the presence of gorgosterol skeleton in the molecule. A close comparison of the ^{1}H NMR and ^{13}C NMR spectral data of A and B rings of the compound **6** with those of compound **5** suggests that the compound **6** contains 1β,3β,5α,6β-hydroxyl pattern. In view of the oxygenated methyls observed in the region δ 1.40–1.50 (1.43, s, 26-Me and 1.49, s, 27-Me) the remaining tertiary hydroxyl group was placed at C-25. Thus, the structure of the compound may be described as gorgostane-1β,3β,5α,6β,25-pentol (**6**) and the mass fragments at m/z 323 [M^+-s, chain], 267 [M^+-s, chain, $3H_2O$, 2H] also support the structure. The triacetate **7** has the same physical and spectral data of gorgostane-1β,3β,5α,6β,25-penta-acetate reported earlier from *Sarcophyton subviride*[3].

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