AN ACETOXYGENATED ANALOGUE OF ERGOSTEROL FROM A SOFT CORAL OF THE GENUS *LOBOPHYTUM*

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Chemical investigation of a soft coral of the genus *Lobophytum* of Andaman and Nicobar coasts resulted in the isolation of a new marine sterol acetate, (24*S*)-ergostane-3β,5α,6β,25-tetraol-3,6,25-triacetate (**1**) and of two known sterol glycosides 3β,4α-dihydroxypregn-20-ene-4-O-β-D-arabinopyranoside and 24-methylenecholest-5-ene-3β,7β,16β-triol-3-O-α-L-fucopyranoside-7β-acetate. The structures of the compounds were elucidated based on spectral studies and chemical conversions.

Keywords: Soft coral, *Lobophytum* sp., polyhydroxy sterol triacetate

INTRODUCTION

Soft corals (*Coelenterates*) contain terpenoids and a diversity of mono- and polyhydroxysterols[1-2], most of them being derivatives of (24*S*)-ergostanes.[3-5] In continuation of our search for bioactive natural products from marine soft corals,[6-8] we have now examined the soft coral *Lobophytum* sp. collected from the Indian Ocean.

RESULTS AND DISCUSSION

Extensive chromatography of *Lobophytum* sp. extracts delivered the polyhydroxysterol triacetate **1** which had not been isolated from nature so far, along with two known polyhydroxysterol glycosides, 3β,4α-dihydroxypregn-20-ene-4-O-β-D-arabinopyranoside[9] and 24-methylenecholest-5-ene-3β,7β,16β-triol-3-O-α-L-fucopyranoside-7β-acetate.[10] According to the EI HRMS value (exp. 576.4025, calcd. 576.4028), compound **1** analysed for the molecular formula $C_{34}H_{56}O_7$ which indicated the presence of seven degrees of unsaturation in the molecule. It was transparent to UV and showed absorptions in the IR spectrum at 3474 and 1734 cm^{-1} which pointed to the presence of acetyl and hydroxyl groups.

The ^1H NMR and ^{13}C NMR ($CDCl_3$) spectrum of Compound **1** corroborated the presence of four oxygenated carbon atoms and three acetyl groups of a triacetoxy sterol with an additional tertiary hydroxyl group. The ^1H shifts of the C-26 and C-27 methyl groups (δ 1.35) in **1** pointed to a substituent at C-25. The latter must be an acetoxy residue, as a free hydroxy group would give rise to a shift of δ ≈ 0.21. This was supported by a strong peak in the EI mass spectrum of compound **1** at *m/z* 289 which could be due to the loss of the acetoxylated side chain and two further acetate residues. Additional peaks at *m/z* 456 (M^+ - 2 AcOH) and 438 (M^+ - 2AcOH - H_2O) are in agreement with a tetrahydroxyergostan triacetate, however, are of little

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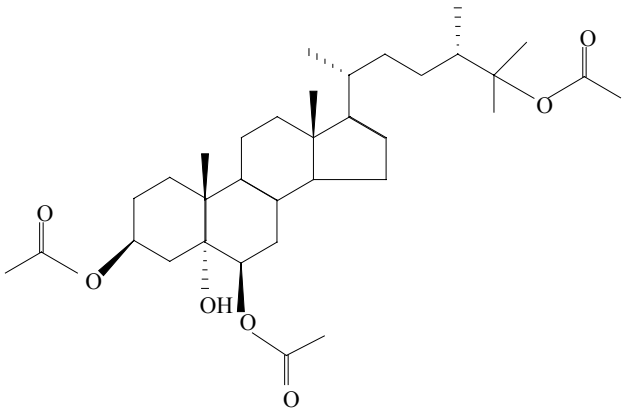
diagnostic value. Four double bond equivalents were attributed to the sterol skeleton and the remaining three are accounted for a tertiary and two secondary acetoxyl groups.



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The presence of two secondary and four tertiary methyl groups suggested an ergostane skeleton,[11] which was further confirmed by H,H COSY, HMQC and HMBC experiments. The carbon shifts of C-23 and C-28 agreed within Δδ 0.2 with those of (24*S*)-24-methylcholestane-3β,5α,6β-25-tetrol.[12] All known 24-methylsterols from soft corals bear, without exception, the same configuration, as has been shown by X-ray crystallography or by correlation with (24*S*)-22,23-dihydrobrassicasterol. The latter is the predominant monohydroxysterol of soft corals and is distinguishable, by ^{1}H and ^{13}C NMR spectra, from the C-24 epimer campestrol. We assume therefore that Compound **1** has the same configuration and is (24*S*)-ergostane-3β,5α,6β-25-tetraol 3,6,25-triacetate (**1**). Mild alkaline hydrolysis of compound **1** gave a deacetylated product which was found by co-TLC and ^{1}H NMR spectrum to be identical with (24*S*)-ergostane-3β,5α,6β-25-tetraol-25-monoacetate.[13] In the agar diffusion test, Compound **1** was biologically inactive against the bacteria *Escherichia coli*, *Streptococcus aureus*, *Bacillus subtilis* and the fungus *Mucor miehei.*

The literature survey revealed that Raju *et al.* had obtained compound **1** by acetylation of the corresponding 25-acetoxy-ergostane-triol[13] isolated from the polyhydroxysterol fraction from the soft coral *Sarcophyton subviridae*. The published data of their sterol acetate were identical with our spectral data of Compound **1**. To the best of our knowledge, however, **1** was not isolated previously from natural sources. As transesterifications with ethyl acetate do not occur under usual work-up conditions, **1** is a new natural product.

EXPERIMENTAL SECTION

Materials and methods were used as described previously.[6]

COLLECTION, EXTRACTION AND ISOLATION

The soft coral, (1.0 kg after dehydration) was collected by hand picking in inter-tidal rocky region during March 1993 on the coasts of the Andaman and Nicobar Islands (Diglipur Island 13°20' N, 93°02' E). It was identified as *Lobophytum* sp. by Dr. B. Grebnev, Biologist, PIBOC, Vladivostok-22, Russia. The voucher specimen was preserved at the above museum, and at the Department of Organic Chemistry, Andhra University, Visakhapatnam, India as MF-VA/35.

Table 1. ^{1}H, ^{13}C NMR, H,H COSY and HMBC data of (24*S*)-ergostane-3β,5α,6β-25-tetraol 3,6,25-triacetate (**1**) ($CDCl_3$, 300 MHz)

No.	^{13}C NMR	^{1}H NMR	HMBC	H,H COSY
1	31.7	1.38, 1.42	--	H-2
2	26.5	1.83	C-3	H-1, H-3
3	70.8	5.15(br m, 1H)	C-3-OAc	H-2, H -4
4	36.5	1.6,1.88(br t, 1H, 12.0)	--	H-3
5	74.5	--	--	--
6	76.1	4.65 (br s, 1H)	C-7, C-5, C-8, C-6-OAc	H-7
7	31.2	1.55, 1.61	--	H-6, H-8
8	30.6	1.59	--	H-7
9	44.7	1.36	--	--
10	38.3	--	--	--
11	20.9	1.35	--	H-12
12	39.7	1.95, 1.18	--	H-11, H-17
13	42.6	--	--	--
14	55.8	1.10	--	H-15
15	24.0	1.52, 1.05	--	H-14, H-16
16	28.0	1.25, 1.22	--	H-15
17	55.6	1.08	C-21	--
18	12.1	0.68 (S, 3H)	C-12, C-14	--
19	16.2	1.18(S, 3H)	C-1, C-5, C-9	--
20	36.2	1.32	--	H-21, H-23
21	18.8	0.92 (d, 3H, 6.8)	C-13, C-17	H-20
22	34.5	1.5, 0.9	--	H-20, H-23
23	27.7	1.55, 0.75	--	H-22
24	41.8	1.91	--	H-23
25	85.8	--	--	--
26	23.2	1.35(S, 3H)	C-25	--
27	22.8	1.35(S, 3H)	C-25	--
28	14.4	0.87 (d, 3H, 7.0)	C-24, C-23, C-25	--
-OAc	21.4	2.05 (S, 3H)	C-6-OAc	--
	21.4	2.02 (S, 3H)	C-3-OAc	--
	22.5	1.98 (S, 3H)	C-25-OAc	--
C-3-OAc	170.7	--	--	--
C-6-OAc	170.4	--	--	--
C-25-OAc	170.2	--	--	--

EXTRACTION AND ISOLATION

The organism was washed with fresh water, cut into thin slices and preserved in ethanol until workup. After removal of ethanol, the soft coral was extracted with ethanol by percolation for 4 days. The process was repeated 7 times. The solvent was evaporated by distillation under reduced pressure, and the resulting crude extract was partitioned between ethyl acetate and water. Concentration of the organic layer resulted in a brownish gummy residue (30 g) which was passed over anhydrous $MgSO_4$. The extract was subjected to silica gel column chromatography (500 g, Acme 100-200 mesh) eluting with hexane through hexane-ethyl acetate to ethyl acetate and methanol. Three highly polar fractions were obtained by eluting with ethyl acetate and hexane in a ratio of 4.5:0.5, 4:1 and 3:2. Repeated column chromatography with the same solvent mixtures followed by recrystallisation from $CH_3OH/CHCl_3$, furnished (24*S*)-ergostane-3β,5α,6β-25-tetraol 3,6,25-triacetate (**1**) (20 mg), 3β,4α-dihydroxypregn-20-ene-4-O-β-D-arabinopyranoside[9] (10 mg) and 24-methylenecholest-5-ene-3β,7β,16β-triol-3-O-α-L-fucopyranoside 7β-acetate[10] (15 mg), respectively.

(24*S*)-Ergostane-3β,5α,6β-25-tetraol 3,6,25-triacetate (**1**):

Obtained as colourless needles, mp 175–177 °C; $[\alpha]_D$ –51.7° (c 0.968, CH_3OH). – IR (KBr) ν: 3474, 2941, 2869, 1734, 1471, 1445, 1369, 1252, 1161, 1139, 1032, 961, 610 cm^{-1}. – EI MS *m/z* (%): 576 ($[M]^+$, 8), 516 ($[M - AcOH]^+$, 60), 498 ($[M - AcOH - H_2O]^+$, 12), 456 ($[M - 2AcOH]^+$, 34), 438 ($[M - 2AcOH - H_2O]^+$, 56), 396 ($[M - 3AcOH]^+$, 52), 378 ($[M - 3AcOH - H_2O]^+$, 100), 363 ($[M - 2AcOH - H_2O - CH_3]^+$, 24), 312 (16), 289 (25), 251 (28). HR EIMS exp. 576.4025 (calcd. 576.4028 for $C_{34}H_{56}O_7$). – (+)-ESI MS *m/z*: 599.6 ($[M + Na]^+$, 50), 1175.5 ($[2M + Na]^+$, 100), 1751.6 ($[3M + Na]^+$, 96). – (-)-ESIMS *m/z*: 1151.2 ($[2M - H]^-$). The ^1H, ^{13}C, H,H COSY, HMQC and HMBC spectral data are given in Table 1.

Alkaline hydrolysis of **1**:

Compound **1** (10 mg) was refluxed with 10 % ethanolic KOH (15 ml) for 3 hrs. The reaction mixture was acidified with dilute HCl and extracted with ethyl acetate. The organic phase was washed with water, dried and evaporated. The residue was crystallized from CH_2Cl_2/CH_3OH to give a product which was identified by co-TLC and by comparison of the IR and ^1H NMR spectra as (24*S*)-ergostane-3β,5α,6β-25-tetraol 25-monoacetate.[13]

3β, 4α-Dihydroxypregn-20-ene-4-O-β-D-arabinopyranoside:

Colourless crystals, mp 283-285 °C (lit.[9] 279 °C), $[\alpha]_D^{25}$ –72° (c 0.20, pyridine) [lit.[9] –92° (c 0.20, pyridine]. The identification was made by comparison of the MS, ^1H, and ^{13}C-NMR data (pyridine-d_5) with those reported in the literature.[9]

24-Methylenecholest-5-ene-3β,7β,16β-triol-7β-acetoxy-3-O-α-L-fucopyranoside:

Colourless crystals, mp 188–200 °C (lit.[10]; 180–181 °C), $[\alpha]_D^{25}$ +8.8° (c 2.5, $CHCl_3$) [lit.[10] +11.32° (c 2.5, $CHCl_3$]. The identification was made by comparison of the MS, ^1H, and ^{13}C NMR data (pyridine-d_5) with those reported in the literature.[10]

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