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STUDIES ON MARINE CHEMICALS, PART IV.[1] ISOLATION OF CHOLESTEROL DERIVATIVES FROM THE MARINE SPONGE *SPIRASTRELLA INCONSTANS*[2]

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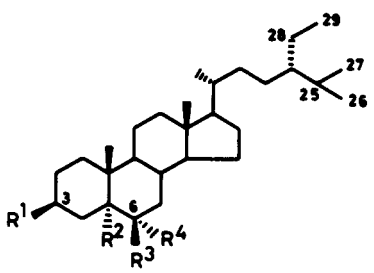
ABSTRACT.—Chemical investigation of the marine sponge *Spirastrella inconstans* resulted in the isolation of a new cholesterol derivative, (24*S*)-24-ethylcholesta-3β,5α-diol-6-one [**1**], along with cholesterol, clionasterol, and (24*S*)-24-ethylcholesta-3β,5α,6β-triol [**3**]. The structure of the new compound **1** was established from spectral data and confirmed via synthesis.

In continuation of our search (1–3) for new biologically active compounds from marine organisms, we investigated the chemical constituents of the marine sponge *Spirastrella inconstans* Dendy (order Tetraxonida, family Spirastrellidae). From the EtOAc extract of the sponge a new cholesterol derivative, (24*S*)-24-ethylcholesta-3β,5α-diol-6-one [**1**], has been isolated, along with cholesterol, clionasterol, and (24*S*)-24-ethylcholesta-3β,5α,6β-triol [**3**] (4). Here we report on the structure elucidation of the new compound **1**.

The new sterol, (24*S*)-24-ethylcholesta-3β,5α-diol-6-one [**1**], mp 253–254°, $[\alpha]^{25}D$ +1.34° (c = 0.258), showed ir absorption maxima at 3450, 3300, and 1705 cm^{-1} and uv absorption maximum at 302 nm (log ε 1.55). The ^{1}H-nmr spectrum revealed the presence of H-3α at δ 4.02 (1H, m, $W_{1/2}$ = 25.0 Hz) as well as six methyl groups at δ 0.65 (3H, s, Me-18), 0.80 (3H, s, Me-19), 0.81 (3H, d, J = 7.0 Hz, Me-27), 0.83 (3H, d, J = 7.0 Hz, Me-26), 0.85 (3H, t, J = 7.0 Hz, Me-28), and 0.91 (3H, d, J = 6.5 Hz, Me-21). The signals for the side chain methyl groups indicated the 24*S* configuration of the sterol (5,6). The structure of **1** was clearly settled from its ^{13}C-nmr spectral data (see Experimental). The reported spectral data of cholesta-3β,5α-diol-6-one (7) and clionasterol (6) were taken as references.



1 R^1=R^2=OH; R^3,R^4=O
2 R^1=OAc, R^2=OH; R^3,R^4=O
3 R^1=R^2=R^3=OH, R^4=H
4 R^1=R^3=OAc, R^2=OH, R^4=H

The sterol **1** formed a monoacetate, mp 275–276°, whose structure was established as 3β-acetoxy-(24*S*)-ethylcholestan-5α-ol-6-one [**2**] from its spectral analysis.

Finally, the selective oxidation of the known triol **3** at C-6 with *N*-bromosuccinimide in aqueous dioxane (8) afforded a ketosterol that was identical to the natural product **1** in all respects.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's are uncorrected. Spectra were recorded with the following instruments: ir, Beckman 4230 spectrophotometr; uv, Beckman DU 70 spectrophotometer; ^{1}H and ^{13}C nmr, Varian Gemini-200 MHz; ms, VG Micromass 7070H (70 eV). Optical rotations were measured in MeOH with a Jasco DIP 360 digital polarimeter. Ir spectra were recorded in KBr, uv spectra in $CHCl_3$, ^{1}H-nmr spectra in $CDCl_3$ with TMS as internal standard, and ^{13}C-nmr spectra in $CDCl_3$ with a few drops of CD_3OD. Cc was performed on Si gel (BDH, 100–200 mesh) and tlc with Si gel G.

COLLECTION OF SPONGE.—The sponge was

[1]For Part III, see Das and Srinivas (3).
[2]IICT Communication No. 2949.

collected from Mandapam Camp coast (17°N, 83°E) in the southeastern Indian peninsula during neap tides in November 1988. A voucher specimen is deposited in our laboratory.

ISOLATION OF STEROLS.—The sponge (5.25 kg dry wt after extraction) was chopped and extracted with C_6H_{14}, $CHCl_3$, EtOAc, and MeOH successively at room temperature. After removal of the solvent by a rotavapor (40°), the EtOAc extract gave a yellow mass (5.62 g). The extract (5 g) was chromatographed over Si gel. EtOAc/C_6H_6 (2% and 5%) eluates yielded, respectively, cholesterol, mp 146–148° (C_6H_6), yield 32 mg (0.64% of the crude EtOAc extract) and clionasterol, mp 138–139° (C_6H_6), yield 48 mg (0.96%). The EtOAc-C_6H_6 (1:1) eluate furnished a gummy solid which was purified by repeated cc (three times) and then by crystallization from MeOH to give (24*S*)-24-ethylcholesta-3β,5α-diol-6-one [**1**], yield 18 mg (0.36%): eims *m/z* (rel. int.) $[M]^+$ 446.3742 ($C_{29}H_{50}O_3$ requires 446.3760) (6), $[M-H_2O]^+$ 428 (7), $[M-2H_2O]^+$ 410 (10), $[M-H_2O-CO]^+$ 400 (15), $[400-Me]^+$ 385 (5), $[M-H_2O-C_{10}H_{21}]^+$ 287 (5), $[287-CO]^+$ 259 (4); ^{13}C nmr δ 36.01 (C-1), 29.63 (C-2), 66.04 (C-3), 37.09 (C-4), 80.64 (C-5), 212.21 (C-6), 41.45 (C-7), 29.63 (C-8), 44.27 (C-9), 41.99 (C-10), 21.46 (C-11), 39.09 (C-12), 42.99 (C-13), 56.04 (C-14), 22.79 (C-15), 27.55 (C-16), 55.14 (C-17), 11.58 (C-18), 14.23 (C-19), 35.64 (C-20), 18.16 (C-21), 33.80 (C-22), 23.76 (C-23), 45.58 (C-24), 27.96 (C-25), 18.60 (C-26), 18.95 (C-27), 22.53 (C-28), 11.98 (C-29).

The MeOH/EtOAc (2%) eluate furnished a viscous mass which after further cc gave a white solid. This was crystallized from MeOH to produce (24*S*)-24-ethylcholsta-3β,5α,6β-triol [**3**], mp 241–242° [lit. (4) mp 247–249° (C_6H_{14})]; $[\alpha]^{25}D$ +14.71° ($c=0.396$), yield 35 mg (0.70%); ir ν max 3360 cm^{-1}; ^1H nmr δ 0.68 (3H, s, Me-18), 0.81 (3H, d, $J=7.0$ Hz, Me-27), 0.83 (3H, d, $J=7.0$ Hz, Me-26), 0.85 (3H, t, $J=7.0$ Hz, Me-29), 0.90 (3H, d, $J=6.5$ Hz, Me-21), 1.17 (3H, s, Me-19); eims *m/z* (rel. int.) $[M-H_2O]^+$ 430 (15), $[M-2H_2O]^+$ 412 (10), $[M-2H_2O-Me]^+$ 397 (4), $[M-C_{10}H_{21}-H_2O]^+$ 289 (4), $[M-C_{10}H_{21}-2H_2O]^+$ 271 (10), $[M-C_{10}H_{21}-3H_2O]^+$ 253 (3); ^{13}C nmr δ 30.20 (C-1), 33.32 (C-2), 66.84 (C-3), 39.67 (C-4), 75.02 (C-5), 75.39 (C-6), 35.42 (C-7), 29.95 (C-8), 45.04 (C-9), 37.72 (C-10), 20.76 (C-11), 39.10 (C-12), 42.33 (C-13), 55.89 (C-14), 22.22 (C-15), 27.57 (C-16), 55.65 (C-17), 11.59 (C-18), 16.10 (C-19), 35.77 (C-20), 18.15 (C-21), 33.64 (C-22), 23.69 (C-23), 45.04 (C-24), 27.83 (C-25), 18.26 (C-26), 18.32 (C-27), 21.96 (C-28), 11.85 (C-29).

Acetylation (Ac_2O/pyridine) of the sterol **1** furnished the monoacetate **2**, mp 275–276° (MeOH); ir ν max 3500, 1710 cm^{-1}; ^1H nmr δ 0.65 (3H, s, Me-18), 0.80 (3H, s, Me-19), 0.81 (3H, d, $J=7.0$ Hz, Me-27), 0.83 (3H, d, $J=7.0$ Hz, Me-26), 0.85 (3H, t, $J=7.0$ Hz, Me-29), 0.90 (3H, d, $J=6.5$ Hz, Me-21), 1.98 (3H, s, Ac), 5.02 (1H, m, $W_{1/2}=24.0$ Hz, H-3α); eims *m/z* (rel. int.) $[M-HOAc]^+$ 428 (10), $[M-HOAc-H_2O]^+$ 410 (15), $[M-HOAc-CO]^+$ 400 (27), $[410-CO]^+$ 372 (12), $[M-HOAc-C_{10}H_{21}]^+$ 287 (10), $[400-C_{10}H_{21}]^+$ 259 (18).

Acetylation of triol **3** with Ac_2O/pyridine produced the diacetate **4**, mp 130–131° (MeOH), ir ν max 3460, 1730, 1710 cm^{-1}; ^1H nmr δ 0.65 (3H, s, Me-18), 0.81 (3H, d, $J=7.0$ Hz, Me-27), 0.83 (3H, d, $J=7.0$ Hz, Me-26), 0.85 (3H, t, $J=7.0$ Hz, Me-29), 0.90 (3H, d, $J=6.5$ Hz, Me-21), 1.15 (3H, s, Me-19), 1.98 (3H, s, Ac), 2.01 (3H, s, Ac), 4.65 (1H, brs, H-6α), 5.05 (1H, m, $W_{1/2}=25.0$ Hz, H-3α); eims *m/z* (rel. int.) $[M-HOAc]^+$ 472 (9), $[M-HOAc-H_2O]^+$ 454 (12), $[M-2HOAc]^+$ 412 (37), $[412-H_2O]^+$ 394 (45), $[394-C_{10}H_{21}]^+$ 253 (20).

SYNTHESIS OF **1**.—The triol **3** (10 mg) was oxidized with NBS (4.5 mg) in aqueous dioxane (0.5 ml dioxane and 0.1 ml H_2O) following the process described by Fieser and Rajagopalan (8). The product was washed with 50% aqueous MeOH and crystallized from MeOH to produce (24*S*)-24-ethylcholesta-3β,5α-diol-6-one, mp 251–252°, yield 8 mg, which was found to be identical to naturally occurring **1** in all respects.

SYNTHESIS OF **3**.—Clionasterol (20 mg) was treated with *m*-CPBA (20 mg) in CH_2Cl_2 (5 ml), and the resulting product was hydrolyzed with 20% H_2SO_4 (10 ml), following the conditions reported for the hydroxylation of cholesterol (9). The product, mp 239–240° (MeOH), yield 16 mg, was identical to the natural product **3**.

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