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STUDIES ON MARINE CHEMICALS, PART VI. A NEW CLIONASTEROL DERIVATIVE FROM THE MARINE SPONGE *SPIRASTRELLA INCONSTANS*[1]

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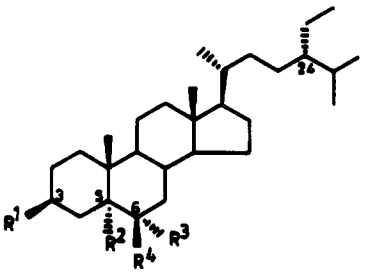
ABSTRACT.—A new clionasterol derivative, (24*S*)-24-ethylcholesta-3β,5α,6α-triol [**1**], has been isolated from the marine sponge *Spirastrella inconstans*. The structure of the new compound **1** was elucidated from spectral data and confirmed via semi-synthesis.

We reported previously (1) the occurrence of several clionasterol derivatives from the marine sponge *Spirastrella inconstans* Dendy (order Tetraxonida, family Spirastrellidae). In continuation of this work we have isolated one more new clionasterol derivative, (24*S*)-24-ethylcholesta-3β,5α,6α-triol [**1**] from the EtOAc extract of the same sponge. Here we report on the structure elucidation of the new compound **1**.

The new sterol, (24*S*)-24-ethylcholesta-3β,5α,6α-triol [**1**], mp 242–243°, $[\alpha]^{25}D$ +35.63° (*c*=0.247), showed ir absorption maxima at 3350 cm^{-1}. Its molecular formula was suggested as $C_{29}H_{52}O_3$ from the analysis of its DEPT ^{13}C-nmr spectrum, which showed the presence of twenty-nine carbon atoms, among which three are oxygenated (see Experimental). The ms did not exhibit the molecular ion peak and gave the highest mass peak at *m/z* 430 $[M-H_2O]^+$. The ^1H-nmr spectrum of **1** was very similar to that of (24*S*)-24-ethylcholesta-3β,5α,6β-triol [**3**] (1), previously reported by us from the same sponge, with the exception of the signals due to the H-6 and Me-19. The H-6β in **1** appeared at δ 3.74 (dd, *J*=11.5 and 4.5 Hz), whereas the H-6α in **3** [not reported in the previous paper (1)] appeared at δ 3.52 (brs). The coupling constants of the H-6β in **1** indicated the proton to be axial (2). The signal for Me-19 was observed at δ 1.05 (s) in **1**, highfield shifted with respect to the corresponding signal in **3** (δ 1.17, s) (1). The signals for the side chain methyl groups of both compounds **1** and **3** appeared at the same δ values (1), indicating the 24*S* configuration of **1**. This data is consistent (1,2) with the C-6 epimeric structure of **3** for the new sterol. The ^{13}C-nmr spectral data of **1** and comparison with reference **3** added support to this assignment. The upfield shift exhibited by C-6 [δ 67.01 in **1** and 75.39 in **3** (1)] and C-19 [δ 15.42 in **1** and 16.10 in **3** (1)] and the downfield shift exhibited by C-5 [δ 76.95 in **1** and 75.02 in **3** (1)] and C-7 [δ 38.12 in **1** and 35.42 in **3** (1)] clearly proved (2) the presence of 6α-OH in **1**. As the signals for the remaining carbons in **1** were very similar (nature and δ values) to those for the corresponding carbons in **3**, the structure for the new sterol was established as (24*S*)-24-ethylcholesta-3β,5α,6α-triol.



1 $R^1=R^2=R^3=OH$, $R^4=H$
2 $R^1=R^3=OAc$, $R^2=OH$, $R^4=H$
3 $R^1=R^2=R^4=OH$, $R^3=H$

The sterol **1** formed a diacetate, mp 152–153°, whose structure was established as 3β,6α-diacetoxy-(24*S*)-24-

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ethylcholesta-3β,5α,6α-triol [**2**] from its spectral analysis. The comparison of the ^{1}H-nmr spectral data of **2** and those reported for 3β,6α-diacetoxycholesta-3β,5α,6α-triol (3) in $CDCl_3$ clearly suggested the presence of the same nucleus in both these steroids.

Finally, the treatment of clionasterol with OsO_4 (4) afforded a triol 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 spectrophotometer; ^{1}H and ^{13}C nmr, Varian Gemini-200 MHz; ms, VG Micromass 7070 H (70 eV). Optical rotations were measured in MeOH with a Jasco DIP 360 digital polarimeter. Ir spectra were recorded in KBr, ^{1}H-nmr spectra in $CDCl_3$ with TMS as internal standard, and ^{13}C-nmr spectrum 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 collected from Mandapam Camp coast (17°N, 83°E) in the southeastern Indian peninsula during neap tides in November 1988. The sample was immediately stored in MeOH for transportation to the laboratory, and in the laboratory the MeOH was carefully decanted before extraction of the sponge. The sponge was stored in MeOH for 22 h after its collection. A voucher specimen of the animal 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. The EtOAc/C_6H_6 (2%, 5%, and 50%) eluates yielded, respectively, cholesterol (32 mg), clionasterol (48 mg), and (24*S*)-24-ethylcholesta-3β,5α-diol-6-one (1) (18 mg). The MeOH/EtOAc (2%) eluates furnished (24*S*)-24-ethylcholesta-3β,5α,6β-triol [**3**] (1) (35 mg).

The MeOH/EtOAc (3%) eluates afforded a gummy mass, which was purified by repeated cc (three times) and then by crystallization from MeOH to give (24*S*)-24-ethylcholesta-3β,5α,6α-triol [**1**], yield 15 mg: ^{1}H 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), 1.05 (3H, s, Me-19), 3.74 (dd, J=11.5 and 4.5 Hz, H-6β), 4.02 (m, 1H, H-3α); eims *m/z* (rel. int.) $[M-H_2O]^+$ 430 (21), $[M-2H_2O]^+$ 412 (12), $[M-2H_2O-Me]^+$ 397 (7), $[M-C_{10}H_{21}-H_2O]^+$ 289 (4), $[M-C_{10}H_{21}-2H_2O]^+$ 271 (17), $[M-C_{10}H_{21}-3H_2O]^+$ 253 (3); ^{13}C nmr δ 30.08 (C-1), 33.28 (C-2), 66.82 (C-3), 39.68 (C-4), 76.95 (C-5), 67.01 (C-6), 38.12 (C-7), 28.30 (C-8), 44.83 (C-9), 36.76 (C-10), 20.66 (C-11), 39.23 (C-12), 42.35 (C-13), 55.88 (C-14), 22.19 (C-15), 22.67 (C-16), 55.60 (C-17), 11.67 (C-18), 15.42 (C-19), 35.76 (C-20), 18.21 (C-21), 33.63 (C-22), 23.76 (C-23), 45.02 (C-24), 27.79 (C-25), 18.22 (C-26), 18.31 (C-27), 21.94 (C-28), 11.82 (C-29).

Acetylation (Ac_2O/pyridine) of the sterol **1** produced the diacetate **2**: mp 152–153° (MeOH); ir ν max 3450, 1730, 1710 cm^{-1}; ^{1}H-nmr δ 0.63 (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), 1.02 (3H, s, Me-19), 1.98 (3H, s, Ac), 2.01 (3H, s, Ac), 4.92 (1H, dd, 12.0 and 4.8 Hz, H-6β), 5.01 (1H, m, H-3α); eims *m/z* (rel. int.) $[M-HOAc]^+$ 472 (14), $[M-HOAc-H_2O]^+$ 454 (18), $[M-2HOAc]^+$ 412 (45), $[412-H_2O]^+$ 394 (48), $[394-C_{10}H_{21}]^+$ 253 (18).

SYNTHESIS OF **1**.—Clionasterol (25 mg) was oxidized with OsO_4 (15 mg) in pyridine (0.5 ml) following the process described by Warren *et al.* (4). The product, mp 241–243° (MeOH), yield 17 mg, was found to be identical to naturally occurring **1** in all respects.

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