New Sterol Ester from a Deep Water Marine Sponge, Xestospongia sp.

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NEW STEROL ESTER FROM A DEEP WATER MARINE SPONGE, *XESTOSPONGIA* SP.

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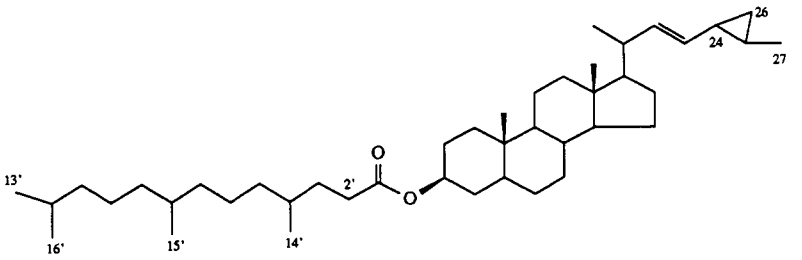
ABSTRACT.—24,26-cyclo-5α-cholest-(22*E*)-en-3β-ol 4′,8′,12′-trimethyltridecanoate, a new sterol ester, has been isolated from a deep water marine sponge, *Xestospongia* sp. The structure was determined from interpretation of spectroscopic and chemical data.

Numerous new sterols have been isolated from marine organisms, and most have novel alkylation patterns in their side chains, but few sterols are known to contain a cyclopropyl ring in the side chain (1–6). In this paper, we report the isolation of a new sterol ester which contains a cyclopropyl ring and an unusual ester group.

The sterol ester was isolated from a deep water sponge, *Xestospongia* sp. (order Haplosclerida, family Petrosiidae), which was collected in the Bahamas, The frozen sponge was homogenized with MeOH-toluene (3:1) and the extract was partitioned between EtOAc and H_2O. From Si gel chromatography and repeated hplc on reversed-phase C-18 and Si gel, the EtOAc-soluble material yielded the sterol ester **1** as a colorless gum. The ester **1** gave molecular formula $C_{43}H_{74}O_2$ by hreims. Its ir spectrum indicated the presence of an ester group (1720 cm^{-1}) and the absence of hydroxyl groups. From a combination of APT (7) and DEPT experiments (8), the 43 resonance lines in the ^{13}C spectrum were assigned to one ester carbonyl, two quaternary carbons, eight methyls, 14 methines, and 18 methylenes. The ^1H-nmr spectrum is consistent with the proposed structure and clearly showed the presence of two methyl singlets, six methyl doublets, two trans-disubstituted olefinic protons, a characteristic H-3 proton α to an ester, and a 1,2-disubstituted cyclopropyl group.

Hydrolysis of **1** with 5% KOH in EtOH at 45° for 1 h followed by methylation of the resulting mixture with CH_2N_2 in Et_2O yielded a mixture containing a methyl ester and the sterol. Si gel chromatography furnished the pure methyl ester **2** and the sterol **3**.

The fatty ester **2** gave a molecular formula $C_{17}H_{34}O_2$ by hreims. Ir data indicated the presence of an ester group (1725 cm^{-1}). The presence of carbon atoms corresponding to an ester carbonyl, OMe, four methyls, three methines, and eight methylenes was determined from APT and DEPT experiments. The ^1H-nmr spectrum confirmed the presence of a methyl ester (δ 3.64, 3H, s) and revealed the presence of a methylene group (δ 2.28, 2H, m) next to the ester carbonyl. The ^1H nmr also



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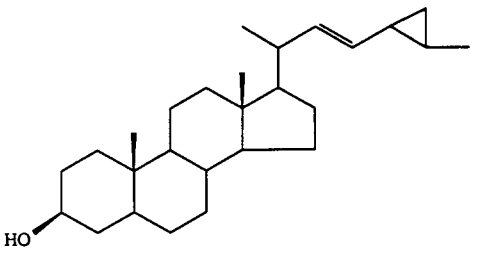

3

contained signals for four methyl doublets [δ 0.820 (3H, d, J = 6.5 Hz), 0.846 (6H, d, J = 6.5 Hz), 0.847 (3H, d, J = 6.5 Hz)]. These four methyl doublets together with three methine carbons in the ^{13}C spectrum confirmed the presence of a terminal isopropyl group. In the COSY spectrum, the methylene protons adjacent to the ester carbonyl observed at δ 2.28 revealed coupling to a diastereotopic methylene group observed at δ 1.45 (1H) and 1.65 (1H). The latter proton was further coupled to a methine proton observed at δ 1.39 which in turn was coupled to the methyl group observed at δ 0.847 (3H, d, J = 6.5 Hz). These data require the fragment -CH(Me)CH_2-CH_2-COOMe. The position of the remaining methyl group was established from high resolution mass spectral data (9) (Figure 1); thus **2** was identified as 4,8,12-trimethyltridecanoic aid methyl ester. The free acid was isolated previously from the phospholipids of the sponge *Petrosia fuciformis* (10).

The sterol **3** gave the molecular formula $C_{27}H_{44}O$ by hreims. Ir data indicated the presence of a hydroxyl group (3500–3300 cm^{-1}). From a combination of APT and DEPT experiments the 27 resonance lines in the carbon spectrum were assigned to two tertiary methyl carbons, two secondary methyl carbons, ten methylene carbons, nine methine carbons, two quaternary carbons, and two olefinic carbons. One of the methine carbons appeared at δ 71.39 and was assigned to the usual C-3 position of the sterol. The ^1H-nmr spectrum confirmed the presence of an axial hydroxymethine (δ 3.56, W½ = 23 Hz) at C-3 (2) and two disubstituted trans-coupled olefinic protons [δ 4.88 (dd, J = 15.2, 8.2 Hz), 5.24 (dd, J = 15.2, 8.4 Hz)]. The ^1H nmr also contained signals for four of the five methyl groups typical of a sterol: δ 0.62 (s, C-18), 0.78 (s, C-19), and two doublets [δ 0.95 (d, J = 6.5 Hz), 1.01 (d, J = 6.5 Hz)].

The mass spectrum of **3** gave a base peak at m/z 273.2217 (Δ 0.1 mmu for $C_{19}H_{29}O$) indicating the cleavage of the side chain. This fragment established the presence of a monohydroxylated saturated steroid nucleus and also the possible Δ^{22} side chain (11). The fragment peak at m/z 255.2105 (Δ 0.4 mmu for $C_{19}H_{27}$) confirmed the presence of a hydroxy group in the sterol nucleus. Comparison of the ^{13}C data for positions C-5 (δ 44.9) and C-10 (δ 35.5) with other known compounds confirmed an A/B trans ring junction (12). The side chain was established by interpreting the data from a COSY experiment and several difference double resonance experiments. In the COSY experiment of **1** in $CDCl_3$, the allylic proton observed at δ 1.94 (H-20) revealed couplings to the olefinic proton observed at δ 5.26 (H-

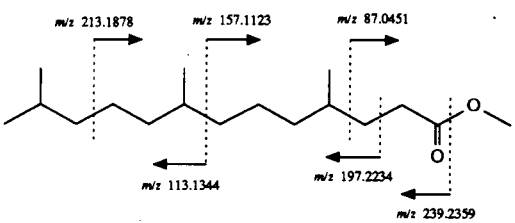

FIGURE 1. Fragmentation pattern of **2**.

22) and to the methyl group observed at δ 0.95 (H-21). The proton H-22 was observed to be further coupled to the olefinic proton at δ 4.88 (H-23). The 15.2 Hz coupling constant between H-22 and H-23 is consistent with *E* geometry of the olefinic bond. The proton H-23 revealed a cross peak to the upfield allylic proton at δ 0.95 (H-24) which in turn showed couplings to the proton at δ 0.60 (H-25) and to the methylene protons at δ 0.42 (H-26) and 0.34 (H-26). The upfield chemical shift values for H-25 and 2H-26 established a cyclopropyl ring system and also accounted for the remaining degree of unsaturation. In the COSY spectrum, the H-25 revealed cross peaks to the remaining methyl doublet at δ 1.01 (H-27), methylene group 2H-26 methine H-24, and thus confirmed the presence of a 1,2-disubstituted cyclopropyl ring system in **1**. Irradiation of Me-27, two H-26, H-24, and H-25 did not yield detectable nOe's on the adjacent protons; the relative stereochemistry of C-24 and C-25 could not be established with these data. Similarly the chemical shift differences 0.95 (H-21) and 5.26 (H-22) found in sterol **3** compared to 0.992 (H-21) and 5.284 (H-22) for synthetic (24*S*,25*S*)-glaucasterol and 0.998 (H-21) and 5.269 (H-22) for synthetic (24*R*,25*R*)-glaucasterol (13) did not give conclusive evidence for the relative stereochemistry in positions 24 and 25. From these data we concluded that the structure of **3** is 24,26-cyclo-5α-cholest-(22*E*)-en-3β-ol, which was previously reported from a soft coral *Sarcophyton glaucum* (4). Compound **1** was not found active against P-388 murine leukemia, *Candida albicans*, or *Herpes simplex* virus type 1 and was inactive in the two-way mixed lymphocyte reaction assay.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded on a Perkin-Elmer 1310 spectrophotometer. Nmr spectra were recorded on a Bruker instrument operating at 360 MHz for ^{1}H and 90.5 MHz for ^{13}C. The high resolution mass spectra were obtained on a VG ZAB-2SE mass spectrometer at the University of Illinois at Urbana-Champaign. Optical rotations were measured with a Jasco DIP 360 digital polarimeter.

COLLECTION.—The sponge *Xestospongia* sp. (sample number 14-III-87-1-13), was collected in the Bahamas at a depth of 170 feet using the Johnson-Sea-Link manned submersible. A voucher specimen is on deposit at the Harbor Branch Oceanographic Museum (catalog number 003:00054). It is an amorphous, thickly encrusting sponge, light brown to reddish brown externally and light tan internally. In EtOH, the sponge is light tan. Symbiotic zoanthids (*Parazoanthus* sp.) occur on the surface. The consistency of the sponge is slightly brittle and very fragile. The surface was smooth, the ectosome not easily detachable. The choanosome consists of a confused reticulation of thin strongyles in various sizes. It is similar in morphology and its confused-reticulate architecture to *Xestospongia wiedenmayeri* Van Soest (14) but differs from it in having strongyles of various sizes instead of oxea in one size category. Several specimens of *Xestospongia* sp. have been examined. All have the same spicule type and architecture, so it is a stable characteristic, sufficent to keep this species distinct from *Xestospongia wiedenmayeri* at the present time.

EXTRACTION AND ISOLATION.—Freshly thawed sponge (229 g wet wt) was extracted three times with MeOH-toluene (3:1). The concentrated extract was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction (0.340 g) was chromatographed on Si gel (Kieselgel 60H) using a hexane/CH_2Cl_2 step gradient followed by a CH_2Cl_2/MeOH step gradient. Rechromatography of the sterol fraction (12 mg) that eluted with 25% CH_2Cl_2/hexane on hplc (Altech Adsorbosphere Si, 5μ, 250 × 10 mm) using 20% CH_2Cl_2/hexane followed by reversed-phase hplc (Altech Adsorbosphere C-18, 5μ, 250 × 10 mm) with 25% CH_2Cl_2/MeOH gave the sterol ester **1** (0.003% yield, wet wt) as a colorless gum.

ESTER **1**.—$[\alpha]^{25}$D 6.0° (*c* = 0.02, $CHCl_3$); ir ($CHCl_3$) 1720 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 0.34 (1H, ddd, *J* = 8.3, 5.4, 2.7 Hz, H-26), 0.42 (1H, ddd, *J* = 8.3, 8.3, 4.5 Hz, H-26), 0.60 (1H, m, H-25), 0.62 (3H, s, H-18), 0.79 (3H, s, H-19), 0.82 (3H, d, *J* = 6.6 Hz, H-14′ or 15′), 0.84 (9H, d, *J* = 6.5 Hz, H-13′, -16′, and -14′ or -15′), 0.95 (3H, d, *J* = 7.2 Hz, H-21), 0.95 (1H, m, H-24), 1.01 (3H, d, *J* = 6.0 Hz, H-27), 1.94 (1H, m, H-20), 2.23 (2H, m, H-2′), 4.67 (1H, m, H-3), 4.88 (1H, dd, *J* = 15.2, 8.3 Hz, H-23), 5.26 (1H, dd, *J* = 15.2, 8.2 Hz, H-22); ^{13}C nmr ($CDCl_3$) δ 12.23 (2q, C-18 and -19), 14.62 (d, C-25), 14.80 (t, C-26), 18.60 (q, C-21), 19.30 (q, C-14′ or -15′), 19.64 (q, C-14′ or -15′), 20.56 (q, C-27), 21.19 (t), 22.31 (d),

22.61, 22.70 (2q, C-13′ and -16′), 24.14 (t), 24.36 (t), 24.79 (t), 27.52 (t), 27.97 (d), 28.49 (t), 28.62 (t), 31.99 (t), 32.11 (t), 32.45 (d), 32.58 (t, C-2′), 32.75 (d), 34.10 (t), 35.51 (d), 36.78 (t), 37.01 (t), 37.32 (t), 37.35 (s, C-10), 37.38 (t), 39.37 (t), 39.71 (d), 39.86 (t), 42.52 (s, C-13), 44.70 (d), 54.27 (d), 56.22 (d), 56.50 (d), 73.47 (d, C-3), 130.61 (d, C-23), 134.15 (d, C-22), 173.72 (s, C-1′); hreims *m/z* 622.5706 (Δ 1.4 mmu for $C_{43}H_{74}O_2$); lreims *m/z* (rel. int.) 622 (5%), 540 (16), 512 (32), 511 (65), 285 (10), 269 (8), 257 (18), 255 (15), 163 (5), 147 (6), 135 (6), 110 (6), 109 (54), 81 (15), 69 (100).

HYDROLYSIS OF **1**.—A solution of **1** (4 mg) in ethanolic KOH (5%, 2 ml) was heated at 45° for 1 h. The mixture was evaporated to dryness, dissolved in MeOH and methylated with excess of CH_2N_2 in Et_2O. Chromatography of the residue on a column of Si gel with hexane-CH_2Cl_2 (1:3) furnished the ester **2** (1.2 mg) and the sterol **3** (2.4 mg).

ESTER **2**.—$[\alpha]^{26}D$ 0° (*c* = 0.004, $CHCl_3$); ir ($CHCl_3$) 1727 cm^{-1}; ^1H nmr ($CDCl_3$) δ 0.820 (3H, d, *J* = 6.5 Hz), 0.846 (6H, d, *J* = 6.5 Hz), 0.847 (3H, d, *J* = 6.5 Hz), 1.39 (1H, m, H-4′), 1.45, 1.65 (2H, m, H-3′), 3.64 (3H, s, OMe); ^{13}C nmr ($CDCl_3$) δ 19.25 (q), 19.66 (q), 22.61 (q), 22.70 (q), 24.33 (t), 24.79 (t), 27.97 (d), 31.93 (t), 31.99 (t), 32.44 (d), 32.76 (d), 36.96 (t), 37.35 (t), 39.37 (t), 51.44 (q), 174.57 (s); hreims *m/z* $[M]^+$ 270.2577 ($C_{17}H_{34}O_2$, Δ 1.9 mmu), $[M-OMe]^+$ 239.2359 ($C_{16}H_{31}O$, Δ 1.5 mmu), $[M-CH_2-CH(CH_3)_2]^+$ 213.1878 ($C_{13}H_{25}O_2$, Δ 2.4 mmu), $[M-CH_2COOCH_3]^+$ 197.2234 ($C_{14}H_{29}$, Δ 3.5 mmu), $[M-C_8H_{17}]^+$ 157.1123 ($C_9H_{17}O_2$, Δ 0.5 mmu), $[M-C_9H_{17}O_2]^+$ 113.1344 ($C_8H_{17}O$, Δ 1.4 mmu), $[M-C_{13}H_{27}]^+$ 87.0451 ($C_4H_7O_2$, Δ 0.5 mmu).

STEROL **3**.—$[\alpha]^{26}D$ 19° (*c* = 0.01, $CHCl_3$); ir ($CHCl_3$) 3400 cm^{-1}; ^1H nmr ($CDCl_3$) δ 0.34 (1H, ddd, *J* = 8.3, 5.4, 2.7 Hz, H-26), 0.42 (1H, ddd, *J* = 8.3, 8.3, 4.5 Hz, H-26), 0.60 (1H, m, H-25), 0.62 (3H, s, H-18), 0.79 (3H, s, H-19), 0.95 (3H, d, *J* = 7.2 Hz, H-21), 0.95 (1H, m, H-24), 1.01 (3H, d, *J* = 6.5 Hz, H-27), 1.94 (1H, m, H-20), 3.57 (1H, m, H-3), 4.88 (1H, dd, *J* = 15.2, 8.3 Hz, H-23), 5.26 (1H, dd, *J* = 15.2, 8.2 Hz, H-22); ^{13}C nmr ($CDCl_3$) δ 12.2 (q), 12.3 (q), 14.6 (d), 14.8 (t), 18.5 (q), 20.5 (q), 21.2 (t), 22.3 (d), 24.1 (t), 28.4 (t), 28.7 (t), 31.5 (t), 32.0 (t), 35.5 (d), 35.5 (s), 37.0 (t), 38.2 (t), 39.6 (d), 39.9 (t), 42.5 (s), 44.9 (d), 54.4 (d), 56.2 (d), 56.5 (d), 71.3 (d), 130.6 (d), 134.1 (d); hreims *m/z* $[M]^+$ 384.3412 ($C_{27}H_{44}O_1$, Δ 2.1 mmu), $[M-Me]^+$ 369.3147 ($C_{26}H_{41}O$, Δ 1.0 mmu), $[M-C_6H_{10}]^+$ 302.2601 ($C_{21}H_{34}O$, Δ 0.8 mmu), $[M-C_8H_{15}]^+$ 273.2217 ($C_{19}H_{29}O$, Δ 0.1 mmu), $[M-C_8H_{15}-H_2O]^+$ 255.2108 ($C_{19}H_{27}$, Δ 0.4 mmu); lreims *m/z* 384 (9%), 369 (4), 327 (2), 302 (22), 287 (15), 285 (10), 273 (100), 257 (15), 255 (14), 161 (27), 109 (72), 93 (37), 81 (48), 67 (50).

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LITERATURE CITED

1. P.J. Scheuer, Ed., "Marine Natural Products: Chemical and Biological Perspectives," Academic Press, New York, Vol. 1, 1978 and Vol. 5, 1983.
2. B.N. Ravi, W.C.M.C. Kokke, C. Delseth, and C. Djerassi, *Tetrahedron Lett.*, 4379 (1978).
3. M. Kobayashi and H. Mitsuhashi, *Steroids*, **40**, 665 (1982).
4. M. Kobayashi, T. Ishizaka, and H. Mitsuhashi, *Chem. Pharm. Bull.*, **31**, 1803 (1983).
5. C. Bonini, R.B. Kinnel, M. Li, P.J. Scheuer, and C. Djerassi, *Tetrahedron Lett.*, **24**, 277 (1983).
6. G.A. Doss and C. Djerassi, *J. Am. Chem. Soc.*, **110**, 8124 (1988).
7. S.L. Patt and J.N. Schoolery, *J. Magn. Reson.*, **46**, 535 (1982).
8. D.M. Doddrell, D.T. Pegg, and M.R. Bendall, *J. Magn. Reson.*, **48**, 323 (1982).
9. R.M. Silverstein, G.C. Bassler, and T.C. Morrill, "Spectrometric Identification of Organic Compounds," 4th ed., John Wiley and Sons, New York, 1981, p. 17.
10. E. Ayanoglu, R.D. Walkup, D. Sica, and C. Djerassi, *Lipids*, **17**, 617 (1982).
11. S.G. Wyllie and C. Djerassi, *J. Org. Chem.*, **33**, 305 (1968).
12. H. Eggert, C.L. VanAntwerp, N.S. Bhacca, and C. Djerassi, *J. Org. Chem.*, **41**, 71 (1976).
13. Y. Fujimoto, M. Kimura, and T. Terasawa, *Tetrahedron Lett.*, **25**, 1805 (1984).
14. R.W.M. Van Soest, *Studies on the Fauna of Curacao and other Carribbean Islands*, **62**, 1 (1980).

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