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3β,5α,6β-TRIHYDROXYLATED STEROLS WITH A SATURATED NUCLEUS FROM TWO POPULATIONS OF THE MARINE SPONGE *CLIONA COPIOSA*

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ABSTRACT.—Two different populations of the marine sponges *Cliona copiosa*, collected in two different sites of the Mediterranean sea, were examined for polyoxygenated sterols. *C. copiosa* from the bay of Naples contained 5α-cholestane-3β,5,6β-triol [**1**] and the new trihydroxysterols (22*E*)-5α-cholest-22-ene-3β,5,6β-triol [**2**], (22*E*,24*S*)-24-methyl-5α-cholest-22-ene-3β,5,6β-triol [**3**], (22*E*,24*R*)-24-methyl-5α-cholest-22-ene-3β,5,6β-triol [**4**], (24*R*)-24-ethyl-5α-cholestane-3β,5,6β-triol [**5**], and (24*S*)-24-ethyl-5α-cholestane-3β,5,6β-triol [**6**]. The population collected from Marsala lagoon, Sicily, contained only sterols **1**, **5**, and **6**. The structures of these compounds were deduced by analysis of spectral data. Partial synthesis of compounds **1**, **5**, and **6** confirmed the structure assignment.

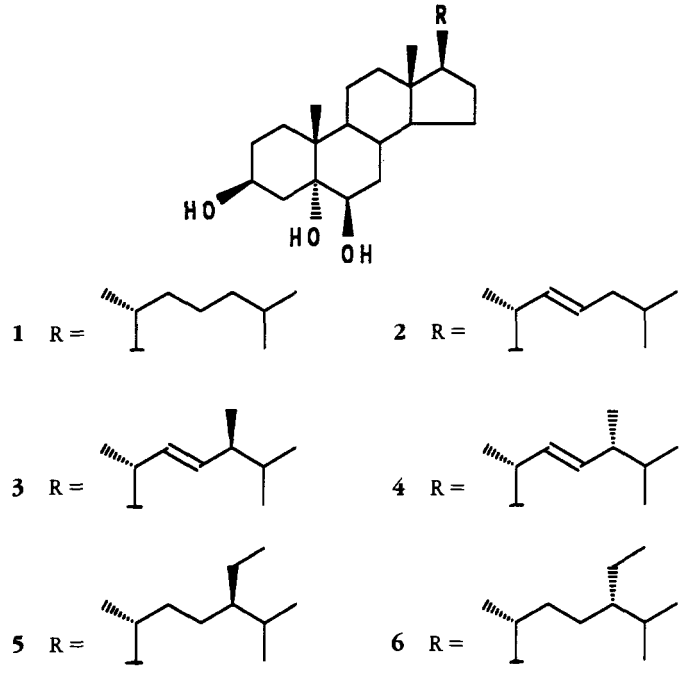
Many new polyhydroxysterols (1–14), polyoxygenated ketosteroids (15–17), 5,6-secosterols (18,19), 9,11-secosterols (20,21), ring A contracted sterols (22), and highly degraded sterols (23) have been reported as constituents of marine sponges.

In continuation of our work on polyhydroxysterols from sponges, we have now found two sources of saturated 3β,5α,6β-trihydroxysterols, two different populations of the marine sponge *Cliona copiosa* Sará 1959 (order Hadromerida, family Clionidae). Both the populations of *C. copiosa* contain 5α-cholestane-3β,5,6β-triol [**1**], (24*R*)-24-ethyl-5α-cholestane-3β,5,6β-triol [**5**], and (24*S*)-24-ethyl-5α-cholestane-3β,5,6β-triol [**6**]. In addition, in the population collected in the bay of Naples, (22*E*)-5α-cholest-22-ene-3β,5,6β-triol [**2**], (22*E*,24*S*)-24-methyl-5α-cholest-22-ene-3β,5,6β-triol [**3**], and (22*E*,24*R*)-24-methyl-5α-cholest-22-ene-3β,5,6β-triol [**4**] are present. 5α-Cholestane-3β,5,6β-triol [**1**] was first isolated from the sponge *Damiriana hawaiiana* (14) and recently from the sea pen *Pteroeides esperi* (24), and was synthesized by Fieser and Rajagopalan (25).

RESULTS AND DISCUSSION

Fresh tissues of the sponges were extracted with Me_2CO and $CHCl_3$-MeOH (1:1), the solvent was removed, and the resulting aqueous suspension was extracted with Et_2O. The Et_2O-soluble material was chromatographed over Si gel using $CHCl_3$ and increasing concentrations of MeOH in $CHCl_3$ as eluent. The fractions eluted with $CHCl_3$-MeOH (95:5) were further separated by hplc on Si gel [$CHCl_3$-MeOH (96:4)] followed by reversed-phase hplc [MeOH-H_2O (92:8)] to afford the 3β,5α,6β-trihydroxysterols.

The molecular formula $C_{27}H_{48}O_3$ for **1** was established by hrms. The Ft-ir spectrum showed hydroxyl absorption at 3400 cm^{-1}. The ^{1}H-nmr spectrum contained signals for five methyl groups of a sterol carbon skeleton, namely singlets at δ 0.67 (H_3-18) and 1.18 (H_3-19), a doublet at δ 0.90 (H_3-21), and a pair of doublets at δ 0.862 and 0.856 (H_3-26 and H_3-27). The presence of three hydroxyl groups in **1** was indicated by the mass spectrum, which exhibited mass ions for stepwise H_2O loss at *m/z* 402 $[M-H_2O]^+$, 384 $[M-2H_2O]^+$, and 366 $[M-3H_2O]^+$. The prominent fragment peaks at *m/z* 289 $[M-H_2O-C_8H_{17}]^+$, 271 $[M-2H_2O-C_8H_{17}]^+$ and 253 [M –



$3H_2O - C_8H_{17}]^+$ indicated the presence of a C_8H_{17} side chain. Inspection of the ^{1}H-nmr spectrum of **1** and the downfield shift observed for the H-3, H_{ax}-4, H_{eq}-4, H_{eq}-6, and H_3-19 signals in the spectrum recorded in pyridine-d_5 indicated hydroxylation at 3β,5α,6β. Thus, the structure of **1** was formulated as 5α-cholestane-3β,5,6β-triol. This structure was confirmed by comparison of its ^{1}H-nmr and mass spectra with those of a synthetic sample prepared as described in literature (25).

Comparison of ^{1}H-nmr spectra of the new trihydroxysterols **2**, **3**, **4**, **5**, and **6** in $CDCl_3$ with that of **1** showed essentially identical chemical shift values for the H-3, H_2-4, H-6, H_3-18, and H_3-19 resonances, indicating that all sterols possessed an identical ring skeleton but differed in their side chain structure. In addition, the signals of H_{ax}-3, H_{ax}-4, and H_3-19 showed the typical pyridine-d_5-induced deshielding due to the 1,3-diaxial interactions with the C-5 and C-6 hydroxyl groups. The structures of the side chains of sterols **2–6** were deduced from mass spectral and ^{1}H-nmr data. The structures of **5** and **6** were confirmed by synthesis.

The molecular formula of **2** was established as $C_{27}H_{46}O_3$ by hreims on the mass peak at *m/z* 400 $[M - H_2O]^+$. The presence of an unsaturated C_8H_{15} side chain was indicated by the ion peaks at *m/z* 289 $[M - H_2O - C_8H_{15}]^+$, 287 $[M - H_2O - C_8H_{15} + 2H]^+$, 271, 269, and 253. The ^{1}H-nmr spectrum included two olefinic protons at δ 5.19 (dd, J = 15.3 and 7.9 Hz, H-22) and δ 5.29 (ddd, J = 15.3, 6.7 and 6.7 Hz, H-23). Irradiation of the H-20 signal at δ 2.01 (m) decoupled the H_3-21 doublet at δ 1.00 (J = 6.7 Hz) and the H-22 olefinic proton at δ 5.19 (dd, J = 15.3 and 7.9 Hz) that was in turn coupled to the H-23 olefinic signal. Irradiation of the H-25 signal at δ 1.58 transformed the two doublets corresponding to H_3-26 and H_3-27 at δ 0.855 and 0.859 (both J = 6.1 Hz) to two singlets and modified the H_2-24 multiplet centered at δ 1.84. Finally, irradiation at the frequency of the H_2-24 protons sharpened the H-25 multiplet and collapsed the H-23 signal to a doublet. The coupling constant of 15.3 Hz between H-22 and H-23 olefinic signals indicated the configuration of the Δ^{22} double bond to be *E*. These data indicated that the structure of this trihydroxysterol must be (22*E*)-5α-cholest-22-ene-3β,5,6β-triol [**2**].

The pure sterols **3** and **4** have the molecular formula $C_{28}H_{48}O_3$ deduced by hreims on the highest mass peak at *m/z* 414 $[M-H_2O]^+$. The mass spectra showed the same fragmentation pattern, suggesting that they are epimers, and contained the typical peaks at *m/z* 289 $[M-H_2O-C_9H_{17}]^+$, 287 $[M-H_2O$ and loss of side chain plus $2H]^+$, 271 and 253 for a C_{28} sterol with a C_9H_{17} side chain containing a double bond. ^{1}H-nmr decoupling experiments established the presence of a trans Δ^{22} double bond and a methyl group at C-24 (δ 0.91) in **3** and **4**. Their ^{1}H-nmr spectra showed differences for the H_3-21 doublets. The H_3-21 doublet of **3** was shifted upfield at δ 0.991 ($J=6.7$ Hz) when compared to the corresponding H_3-21 signal (δ 1.001, $J=6.7$ Hz) for the sterol **4** (26). Thus **3** must be formulated as (22*E*,24*S*)-24-methyl-5α-cholest-22-ene-3β,5,6β-triol and **4** as (22*E*,24*R*)-24-methyl-5α-cholest-22-ene-3β,5,6β-triol.

The new compounds **5** and **6** could not be separated by reversed-phase hplc. They each have the molecular formula $C_{29}H_{52}O_3$ deduced by hreims on the highest mass peak at *m/z* 430 $[M-H_2O]^+$. Significant fragment ions at *m/z* 289 $[M-H_2O-C_{10}H_{21}]^+$, 271, and 253 indicated the presence of a $C_{10}H_{21}$-saturated side chain. The complexity of the methyl region of the ^{1}H-nmr spectra of **5** and **6** strongly suggested (27) the presence of an epimeric mixture of (24*R*)-24-ethyl-5α-cholestane-3β,5,6β-triol [**5**] and (24*S*)-24-ethyl-5α-cholestane-3β,5,6β-triol [**6**]. The complete structure and the stereochemistry at C-24 of these sterols was established by comparison of the ^{1}H-nmr and mass spectra of the isolated material with those of synthetic sterols prepared from commercial sitosterol and clionasterol isolated from the alga *Caulerpa prolifera*. The ^{1}H-nmr spectrum of **5** and **6** revealed that the natural material consists of a 4:1 mixture of the C-24 epimers (28). Owing to the scarcity of the isolated material, the ^{13}C-nmr data of compounds **5** and **6** were secured with synthetic materials. The assignments were made by DEPT experiments and comparison of values with the published ^{13}C-nmr data for sterol **1** (29) and sterols having the same side chain (12).

Our results show some differences between the two populations of *Cl. copiosa*. Diversity in both skeleton morphology and sterol composition may be considered as an aspect of the phenotypical and physiological variability of the species due to the ecological features of the habitat where the two populations live in geographically separated areas and at a different depth.

The biosynthetic side chain transformations in marine 3β-monohydroxysterols have been studied only very recently (30), while until now attention has not been paid to the biosynthetic origin of polyhydroxylated sterols from marine sources (31). It is interesting to note that the 3β-monohydroxysterol fractions of the two different populations of sponge *C. copiosa* are mainly constituted of Δ^5-unsaturated sterols. Recently we isolated Δ^7-3β,5α,6β-trihydroxysterols from sponges containing $\Delta^{5,7}$-3β-hydroxysterols (6,11) and hypothesized that the former could originate from the latter. Analogously, it seems reasonable to suppose that saturated trihydroxysterols **1–6** may arise biogenetically from the corresponding Δ^5-sterols.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker WM-400 spectrometer. The ^{1}H chemical shifts were referenced to the residual $CHCl_3$ and C_5H_5N signals (7.26, 8.71 ppm, respectively). The ^{13}C chemical shifts were referenced to the solvent ($CDCl_3$ 77.0 ppm, C_5D_5N 135.5 ppm). Low resolution mass spectra were determined at 70 eV with an AEI MS 30 mass spectrometer. High resolution mass spectra were recorded on a Kratos MS 50 spectrometer. Ir spectra were obtained with a Perkin-Elmer 1760-X Ft-ir. Hplc was carried out with a Varian 2510 pump and a Waters Associates R403 differential refractometer. Melting points were determined on a Kofler apparatus and are uncorrected.

BIOLOGICAL MATERIALS.—Several specimens of two populations of *C. copiosa* were collected from

two different sites of the Mediterranean Sea: the Marsala Lagoon (Sicily, depth 1 m) and the Bay of Naples (depth 15 m). The two populations show some differences in their spicular size and morphology. In particular, the specimens collected from Naples show a greater length of tylostyles and a marked variability in their head shape (32). Voucher specimens of both sponges are on file at our laboratories.

EXTRACTION AND ISOLATION.—*Trihydroxysterols from* Cl. copiosa *collected in the Marsala lagoon.*—Fresh tissues (287 g, dry wt after extraction) were extracted once with Me_2CO and twice with $CHCl_3$-MeOH (1:1). Removal of the solvents under reduced pressure left an aqueous suspension which was extracted with Et_2O. The Et_2O layer was evaporated to give an oily residue (24.56 g), which was fractionated on an open Si gel column (700 g) using $CHCl_3$ and increasing amounts of MeOH in $CHCl_3$ as eluent. $CHCl_3$-eluted fractions of the Si gel column contained 3β-hydroxysterols (0.95 g) that were crystallized from MeOH to give the sterol mixture that was in part further purified by hplc on a Si gel column [Hibar LiChrosorb Si-60, 250 × 4 mm, hexane-EtOAc (7:3)]. The more polar compounds eluted with $CHCl_3$-MeOH (9:1) (204 mg) were purified by flash chromatography on a Si gel column eluted under a slight N_2 pressure with a solvent gradient system from $CHCl_3$ to $CHCl_3$-MeOH (8:2). The early eluted fractions [$CHCl_3$-MeOH (95:5)] containing polyhydroxylated sterols were combined and further separated by hplc on a Si gel column (Hibar LiChrosorb Si-60, 250 × 4 mm) using $CHCl_3$-MeOH (96:4) as the mobile phase. The more polar fractions obtained from this separation, containing 3β,5α,6β-trihydroxysterols (3.8 mg), were fractionated by reversed-phase hplc on a Hibar Supersphere RP-18 (250 × 4 mm) column eluted with MeOH-H_2O (92:8) to give pure **1** (1.1 mg) and **5** and **6** together (0.4 mg).

Trihydroxysterols from C. copiosa *collected in the Bay of Naples.*—Extraction with Me_2CO and $CHCl_3$/MeOH of fresh material (212.7 g dry wt after extraction) and chromatography of crude extract (9.4 g) as above gave 3β-hydroxysterols (0.63 g) and the trihydroxylated sterol fraction (8 mg) which was fractionated by reversed-phase hplc as described above to give pure **1** (2.9 mg), **2** (0.9 mg), **3** (0.5 mg), **4** (0.5 mg), and **5** and **6** together (1.5 mg).

SPECTRAL DATA.—*5α-Cholestane-3β,5,6β-triol* [**1**].—Mp 235–237° (hexane) [lit. (25) 237–239°]; Ft-ir (film) ν max 3400 cm^{-1}; ^1H nmr ($CDCl_3$, 400 MHz) δ 4.10 (1H, m, H-3), 3.54 (1H, bs, $w_{1/2}$ = 8 Hz, H-6), 2.08 (1H, dd, J = 12.5 and 12.5 Hz, H_{ax}-4), 1.61 (m, overlapped to other signals, H_{eq}-4), 1.51 (m, H-25), 1.18 (3H, s, H_3-19), 0.90 (3H, d, J = 6.6 Hz, H_3-21), 0.862 and 0.856 (3H each, d's, both J = 6.6 Hz, H_3-26 and H_3-27), 0.67 (3H, s, H_3-18); ^1H nmr (pyridine-d_5, 400 MHz) δ 4.81 (1H, m, H-3), 4.13 (1H, bs, H-6), 2.90 (1H, dd, J = 12.5 and 12.5 Hz, H_{ax}-4), 2.32 (1H, dd, J = 12.5 and 4.3 Hz, H_{eq}-4), 1.62 (3H, s, H_3-19), 0.76 (3H, s, H_3-18); eims *m/z* (rel. int.) 402 (100), 387 (10), 384 (81), 369 (56), 366 (7), 351 (10), 289 (6), 271 (15), 253 (7), 262 (49), 244 (61), 226 (9), 247 (68), 229 (65), 211 (35).

(22E)-5α-Cholest-22-ene-3β,5,6β-triol [**2**].—Ft-ir (film) ν max 3400 cm^{-1}; ^1H nmr ($CDCl_3$, 400 MHz) δ 5.29 (1H, ddd, J = 15.3, 6.7 and 6.7 Hz, H-23), 5.19 (1H, dd, J = 15.3 and 7.9 Hz, H-22), 4.09 (1H, m, H-3), 3.54 (1H, bs, $w_{1/2}$ = 8 Hz, H-6), 2.08 (1H, dd, J = 12.5 and 12.5 Hz, H_{ax}-4), 2.01 (m, overlapped, H-20), 1.84 (m, overlapped, H_2-24), 1.61 (m, overlapped, H_{eq}-4), 1.58 (m, overlapped, H-25), 1.18 (3H, s, H_3-19), 1.00 (3H, d, J = 6.7 Hz, H_3-21), 0.859 and 0.855 (3H each, d's, both J = 6.1 Hz, H_3-26 and H_3-27), 0.68 (3H, s, H_3-18); eims *m/z* (rel. int.) 400 (13), 385 (2), 382 (4), 367 (7), 364 (3), 289 (6), 271 (100), 253 (69), 262 (5), 244 (9), 226 (3), 247 (12), 229 (21), 211 (18); hreims *m/z* 400.3342 ($C_{27}H_{44}O_2$ requires 400.3339).

(22E,24S)-24-Methyl-5α-cholest-22-ene-3β,5,6β-triol [**3**].—Ft-ir (film) ν max 3400 cm^{-1}; ^1H nmr ($CDCl_3$, 400 MHz) δ 5.18 (1H, dd, J = 15.3 and 5.5 Hz, H-23), 5.13 (1H, dd, J = 15.3 and 4.9 Hz, H-22), 4.09 (1H, m, H-3), 3.54 (1H, bs, $w_{1/2}$ = 8 Hz, H-6), 2.08 (1H, dd, J = 12.5 and 12.5 Hz, H_{ax}-4), 1.97 (m, overlapped, H-20), 1.84 (m, overlapped, H-24), 1.61 (m, overlapped, H_{eq}-4), 1.45 (m, overlapped, H-25), 1.18 (3H, s, H_3-19), 0.991 (3H, d, J = 6.7 Hz, H_3-21), 0.91 (3H, d, J = 6.7 Hz, H_3-28), 0.833 and 0.816 (3H each, d's, both J = 6.7 Hz, H_3-26 and H_3-27), 0.68 (3H, s, H_3-18); eims *m/z* (rel. int.) 414 (12), 396 (3), 381 (5), 378 (2), 289 (5), 271 (100), 262 (5), 244 (9), 226 (5), 247 (12), 229 (25), 211 (24); hreims *m/z* 414.3495 ($C_{28}H_{46}O_2$ requires 414.3486).

(22E,24R)-24-Methyl-5α-cholest-22-ene-3β,5,6β-triol [**4**].—Ft-ir (film) ν max 3400 cm^{-1}; ^1H nmr ($CDCl_3$, 400 MHz) δ 5.18 (1H, dd, J = 15.3 and 5.5 Hz, H-23), 5.13 (1H, dd, J = 15.3 and 4.9 Hz, H-22), 4.09 (1H, m, H-3), 3.54 (1H, bs, $w_{1/2}$ = 8 Hz, H-6), 2.08 (1H, dd, J = 12.5 and 12.5 Hz, H_{ax}-4), 1.97 (m, overlapped, H-20), 1.84 (m, overlapped, H-24), 1.61 (m, overlapped, H_{eq}-4), 1.45 (m, overlapped, H-25), 1.18 (3H, s, H_3-19), 1.001 (3H, d, J = 6.7 Hz, H_3-21), 0.91 (3H, d, J = 6.7 Hz, H_3-28), 0.833 and 0.816 (3H each, d's, both J = 6.7 Hz, H_3-26 and H_3-27), 0.68 (3H, s, H_3-18); eims *m/z* (rel. int.) 414 (15), 396 (6), 381 (7), 378 (3), 289 (8), 271 (100), 262 (6), 244 (9), 226 (7), 247 (13), 229 (27), 211 (25); hreims *m/z* 414.3489 ($C_{28}H_{46}O_2$ requires 414.3486).

(24R)-24-Ethyl-5α-cholestane-3β,5,6β-triol [**5**] *and (24S)-24-ethyl-5α-cholestane-3β,5,6β-triol* [**6**].—

Ft-ir (film) ν max 3400 cm^{-1}; ^1H nmr ($CDCl_3$, 400 MHz) δ 4.09 (m, H-3), 3.54 (bs, $w_{1/2}$ = 8 Hz, H-6), 2.08 (dd, J = 12.5 and 12.5 Hz, H_{ax}-4), 1.61 (m, overlapped, H_{eq}-4), 1.18 (s, H_3-19), 0.91 (d, J = 6.6 Hz, H_3-21), 0.852 (t, J = 7.3 Hz, H_3-29 of **6**), 0.843 (t, J = 7.3 Hz, H_3-29 of **5**), 0.834 and 0.812 (d's, both J = 6.6 Hz, H_3-26 and H_3-27 of **5**), 0.69 (s, H_3-18); eims *m/z* (rel. int.) 430 (14), 412 (4), 397 (3), 394 (6), 289 (7), 271 (100), 262 (5), 244 (10), 226 (8), 247 (12), 229 (25).

GENERAL PROCEDURE FOR THE SYNTHESIS OF **1**, **5**, AND **6**.—The sterol (cholesterol 260 mg, sitosterol 100 mg, clionasterol 6.7 mg) was dissolved in THF (15 ml, 6 ml, 0.6 ml), and 99% HCO_2H (2.9 ml, 1 ml, 0.1 ml) was added. The solution was heated to 80° for 5 min, cooled at room temperature, and treated with 30% H_2O_2 (0.5 ml, 0.2 ml, 0.02 ml), following the procedure of Fieser and Rajagopalan (25). After 16 h, the mixture was diluted with H_2O and extracted with EtOAc. The extract was treated with 25% NaOH in MeOH under reflux for 15 min. Usual workup and purification over Si gel column [$CHCl_3$-MeOH (98:2)] give the corresponding 3β,5α,6β-trihydroxysterol (**1**, 160 mg; **5**, 53 mg; **6**, 3.5 mg).

5α-Cholestane-3β,5,6β-triol [**1**].—Mp 236–238° (hexane); Ft-ir (film) ν max 3400 cm^{-1}; ^1H nmr data virtually identical to those of natural **1**; ^{13}C nmr (pyridine-d_5, 100.1 MHz) δ 32.55 (C-1), 33.47 (C-2), 67.61 (C-3), 42.79 (C-4), 76.03 (C-5), 76.36 (C-6), 35.80 (C-7), 31.36 (C-8), 46.04 (C-9), 39.25 (C-10), 21.99 (C-11), 40.91 (C-12), 43.28 (C-13), 56.78 (C-14), 24.83 (C-15), 28.86 (C-16), 56.89 (C-17), 12.65 (C-18), 17.42 (C-19), 36.39 (C-20), 19.21 (C-21), 36.76 (C-22), 24.45 (C-23), 40.01 (C-24), 29.46 (C-25), 22.98 (C-26), 23.22 (C-27).

(24R)-24-Ethyl-5α-cholestane-3β,5,6β-triol [**5**].—Mp 246–248° (hexane); Ft-ir (film) ν max 3400 cm^{-1}; ^1H nmr ($CDCl_3$, 400 MHz) δ 4.09 (1H, m, H-3), 3.54 (1H, bs, $w_{1/2}$ = 8 Hz, H-6), 2.08 (1H, dd, J = 12.5 and 12.5 Hz, H_{ax}-4), 1.61 (m, overlapped, H_{eq}-4), 1.18 (3H, s, H_3-19), 0.91 (3H, d, J = 6.6 Hz, H_3-21), 0.842 (3H, t, J = 7.3 Hz, H_3-29), 0.833 and 0.811 (3H each, d's, both J = 6.6 Hz, H_3-26 and H_3-27), 0.69 (3H, s, H_3-18); ^{13}C nmr (pyridine-d_5, 100.1 MHz) δ 32.56 (C-1), 33.36 (C-2), 67.43 (C-3), 42.90 (C-4), 75.92 (C-5), 76.32 (C-6), 35.77 (C-7), 31.26 (C-8), 45.95 (C-9), 39.19 (C-10), 21.82 (C-11), 40.71 (C-12), 43.10 (C-13), 56.63 (C-14 and C-17), 24.68 (C-15), 28.71 (C-16), 12.43 (C-18), 17.28 (C-19), 36.57 (C-20), 19.05 (C-21), 34.27 (C-22), 26.50 (C-23), 46.10 (C-24), 29.50 (C-25), 19.28 (C-26), 20.02 (C-27), 23.42 (C-28), 12.19 (C-29); hreims *m/z* 430.3800 ($C_{29}H_{50}O_2$ requires 430.3798).

(24S)-24-Ethyl-5α-cholestane-3β,5,6β-triol [**6**].—Mp 247–249° (hexane); Ft-ir ν max 3400 cm^{-1}; ^1H nmr ($CDCl_3$, 400 MHz) δ 4.09 (1H, m, H-3), 3.54 (1H, bs, $w_{1/2}$ = 8 Hz, H-6), 2.08 (1H, dd, J = 12.5 and 12.5 Hz, H_{ax}-4), 1.61 (m, overlapped, H_{eq}-4), 1.18 (3H, s, H_3-19), 0.91 (3H, d, J = 6.6 Hz, H_3-21), 0.850 (3H, t, J = 7.3 Hz, H_3-29), 0.826 and 0.807 (3H each, d's, both J = 6.6 Hz, H_3-26 and H_3-27), 0.69 (3H, s, H_3-18); ^{13}C nmr (pyridine-d_5, 100.1 MHz) δ 32.57 (C-1), 33.36 (C-2), 67.41 (C-3), 42.93 (C-4), 75.90 (C-5), 76.30 (C-6), 35.76 (C-7), 31.25 (C-8), 45.94 (C-9), 39.18 (C-10), 21.81 (C-11), 40.69 (C-12), 43.08 (C-13), 56.62 (C-14 or C-17), 24.68 (C-15), 28.69 (C-16), 56.55 (C-17 or C-14), 12.42 (C-18), 17.28 (C-19), 36.71 (C-20), 19.08 (C-21), 34.21 (C-22), 26.77 (C-23), 46.33 (C-24), 29.28 (C-25), 19.19 (C-26), 19.78 (C-27), 23.35 (C-28), 12.53 (C-29); hreims *m/z* 430.3796 ($C_{29}H_{50}O_2$ requires 430.3798).

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