New Steroidal Hydroxyketones and Closely Related Diols from the Marine Sponge Cliona copiosa

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NEW STEROIDAL HYDROXYKETONES AND CLOSELY RELATED DIOLS FROM THE MARINE SPONGE *CLIONA COPIOSA*

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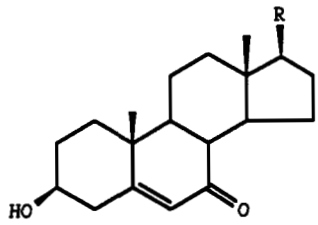
ABSTRACT.—Δ^5-3β-Hydroxy-7-ketosteroids **1–7**, Δ^5-3β,7β-dihydroxysterols **8–13**, and Δ^5-3β,7α-dihydroxysterols **14–17** were isolated from the sponge *Cliona copiosa*, and the structures were elucidated by spectroscopic methods and chemical correlation with known compounds.

In recent years many new polyoxygenated steroids have been isolated from marine sponges (1). Recently, we reported the isolation of six new 3β,5α,6β-trihydroxysterols with a saturated nucleus from the marine sponge *Cliona copiosa* Sarà 1959 (order Hadromerida, family Clionidae) collected in the Bay of Naples (2). We now report the isolation and characterization from the same organism of several dioxygenated sterols: Δ^5-3β-hydroxy-7-ketosteroids **1–7** and the structurally related Δ^5-3β,7β- and Δ^5-3β,7α-dihydroxysterols **8–13** and **14–17**, respectively. Sterols **1**, **3**, **4**, **6**, **9**, **11**, **13**,



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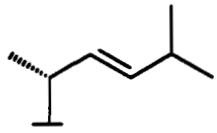

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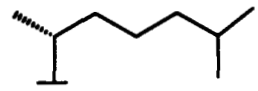

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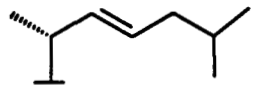

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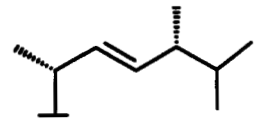

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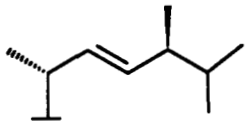

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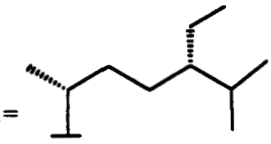

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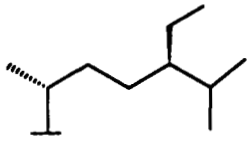
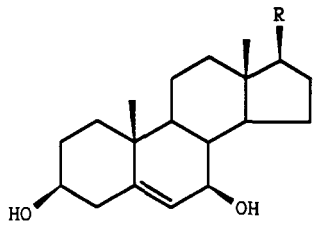
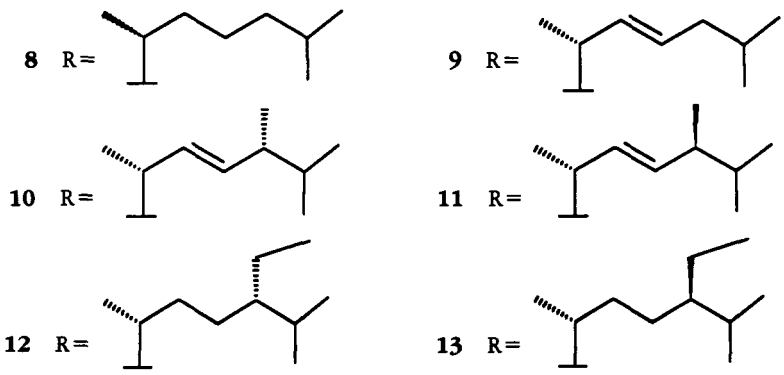




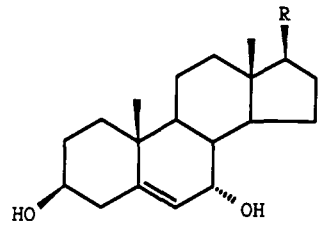


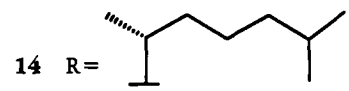
15, and **17** are new compounds, while the remaining have been previously isolated from marine (3) and terrestrial environments (4,5). In recent years attention have been given to Δ^5-3β,7α- and Δ^5-3β,7β-hydroxysterols for their biological activities (4,6).

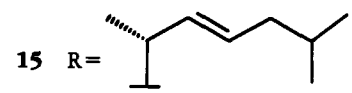
RESULTS AND DISCUSSION

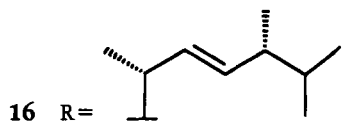
Fresh tissues of the sponge were extracted with Me_2CO and $CHCl_3$-MeOH (1:1), the solvent was removed, and the resulting aqueous suspension was extracted with Et_2O. The Et_2O-soluble material was chromatographed on a Si gel column, using $CHCl_3$ and increasing concentrations of MeOH in $CHCl_3$ as eluent, followed by hplc on a Si gel column to yield the polar sterol fractions. Repeated reversed-phase hplc gave the pure compounds **1–17**.

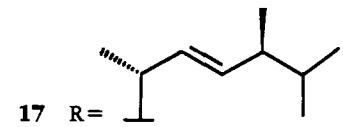
Δ^5-3β-HYDROXY-7-KETOSTEROIDS.—The molecular formula of the most abundant ketosterol **2** was determined as $C_{27}H_{44}O_2$ by high resolution mass measurement of the molecular ion at *m/z* 400.3370 and ^{13}C-nmr data. The uv spectrum showed absorption at λ max 237 nm (ε=9850), while the ir spectrum contained hydroxyl absorption at ν max 3391 and another absorption at 1671 cm^{-1} that indicated the presence of an α,β-unsaturated ketone group. This was confirmed by analysis of ^{13}C-nmr data that showed a carbonyl resonance at δ 202.40, double bond resonances at δ 165.23 and 126.27, and a hydroxymethine signal at δ 70.61. The ^{1}H-nmr spectrum of **2** showed signals for olefinic and hydroxymethine protons at δ 5.69 (d, H-6) and 3.67 (bm, H_{ax}-3), respectively, and resonances for five methyl groups of a cholestane carbon skeleton: singlets at δ 0.68 and 1.19 (H_3-18 and H_3-19, respectively), a doublet at δ 0.92 (H_3-21), and a pair of doublets at δ 0.857 and 0.862 (H_3-26 and H_3-27). The ^{13}C-nmr spectrum of **2** showed that the side chain of this sterol was of the cholesterol type (7). These data suggested that compound **2** was previously synthesized 3β-hydroxycholest-5-en-7-one (8). Data from ^{1}H nmr COSY-45, ^{13}C nmr, and mass spectra (see Experimental) were in good agreement with this structure that was confirmed by comparison of spectral data with those of an authentic sample synthesized according to Parish *et al.* (8).











The mass spectra of compounds **1–7** contained common fragment ions at *m/z* 287 $[M-\text{side chain}]^+$, 269 $[M-H_2O \text{ and side chain}]^+$, and 245, deriving from ring D fission (9), indicating that all components of the sterol mixture possessed identical nuclei and varied only in the side chains. This was supported by ^{1}H-nmr spectra that showed identical chemical shift values for the H-3, H_2-4, H-6, H-8, H_3-18, and H_3-19 protons. Therefore, we only had to establish their side chain structures to complete the structural determination of each compound.

The ketosterol **1** had the molecular formula $C_{26}H_{40}O_2$, deduced by hrms of the molecular ion at *m/z* 384.3051. The mass spectrum contained ion peaks at *m/z* 287 $[M-C_7H_{13}]^+$, 285 $[M-C_7H_{13}-2H]^+$ and 269 $[M-C_7H_{13}-H_2O]^+$ that established the presence of a C_7H_{13} side chain containing a double bond. The ion peak at *m/z* 314 $[M-C_5H_{10}]^+$ indicated that the side chain unsaturation was located at the Δ^{22} position (10). ^{1}H-nmr decoupling experiments confirmed the location of the double bond at Δ^{22}, allowing the entire structural fragment C-21–C-26(C-27) to be built up. These data and the value of the coupling constant (15.4 Hz) between the H-22 and H-23 protons indicated for this sterol the structure of (22*E*)-3β-hydroxy-24-norcholesta-5,22-dien-7-one [**1**].

The ketosterol **3** had the composition $C_{27}H_{42}O_2$by hrms of the molecular ion at *m/z* 398.3168. The mass spectrum contained significant fragment peaks at *m/z* 287 $[M-C_8H_{15}]^+$, 285, and 269 and a peak at 314 $[M-C_6H_{12}]^+$ characteristic of a Δ^{22} sterol possessing a C_8H_{15} side chain (10). Further support for the side chain of **3** was obtained by decoupling experiments and comparison of ^{1}H-nmr data of **3** with those exhibited by a number of other steroids having a trans Δ^{22}-cholesterol-type side chain. The coupling constant between H-22 and H-23 (J=14.7 Hz) agrees with the *E* configuration of the Δ^{22} double bond. Thus the structure of this ketosterol must be (22*E*)-3β-hydroxycholesta-5,22-dien-7-one [**3**].

The ketosterol **4** was found to have a molecular formula of $C_{28}H_{44}O_2$ by hrms of the molecular ion at *m/z* 412.3353. The mass spectrum contained significant fragment peaks at *m/z* 287 $[M-C_9H_{17}]^+$, 285, 269, 369 $[M-C_3H_7]^+$, and 314 $[M-C_7H_{14}]^+$, characteristic of a sterol possessing a C_9H_{17} side chain with an unsaturation at Δ^{22} (10). The ^{1}H-nmr chemical shifts for the side chain protons of this ketosterol were consistent with those of an authentic sample of brassicasterol. Thus, the structure was tentatively

formulated as (22*E*,24*R*)-3β-hydroxy-24-methylcholesta-5,22-dien-7-one [**4**]. This was confirmed by the ^{1}H-nmr spectrum, which showed the expected four doublets of the C-21,C-28,C-26, and C-27 methyl group protons at δ 1.02, 0.91, 0.82, and 0.83, respectively, and by decoupling experiments.

The molecular formula of the ketosterol **5** was determined as $C_{28}H_{44}O_2$ on the basis of hrms of the molecular ion at *m/z* 412.3347. The ^{1}H-nmr and mass spectral data clearly indicated that compound **5** was the C-24 epimer of the ketosterol **4**. In fact, in the ^{1}H-nmr spectrum of **5** the H_3-21 doublet appeared upfield at δ 1.01 (J=6.6 Hz) when compared to the corresponding H_3-21 signal (1.02) for the sterol **4** (11). Hence, **5** must be formulated as (22*E*,24*S*)-3β-hydroxy-24-methylcholesta-5,22-dien-7-one.

The ketosterols **6** and **7** could not be separated by reversed-phase hplc. They each had the molecular formula $C_{29}H_{48}O_2$ established by hrms on the molecular ion at *m/z* 428.3638. In their mass spectrum the fragment ions at *m/z* 287 $[M-C_{10}H_{21}]^+$ and 269 indicated the presence of a saturated $C_{10}H_{21}$ side chain. The side chain methyl signals for both isomers were assigned by comparison of the ^{1}H-nmr spectrum of the isolated ketosterols with the ^{1}H-nmr spectra of authentic samples of sitosterol and clionasterol. The main difference in the ^{1}H-nmr spectrum of the two epimers **6** and **7** was observed in the chemical shift of the Me-29 triplet which is more deshielded in the 24*S* epimer **6** (δ 0.85) than in the 24*R* epimer **7** (δ 0.84) (12). Thus, the structures of **6** and **7** were formulated as (24*S*)-3β-hydroxy-24-ethylcholest-5-en-7-one and (24*R*)-3β-hydroxy-24-ethylcholest-5-en-7-one, respectively.

Δ^5-3β,7β-DIHYDROXYSTEROLS AND Δ^5-3β,7α-DIHYDROXYSTEROLS.—The sponge *C. copiosa* also contained the 3β,7β-diols **8–13** and 3β,7α-diols **14–17**. Compound **8** and its 7α epimer **14** have not been found as naturally occurring sterols but have been previously synthesized (6,13). The structures of **8** and **14** were confirmed by comparison of their ^{1}H-nmr and mass spectral data (see Experimental) with those of authentic samples prepared by $LiAlH_4$ reduction of **2** (13). The stereochemistry of the 7-hydroxymethine group of compounds **8** and **14** was proven by comparison of the chemical shift value and shape of the olefinic proton at C-6 that in the 400 MHz ^{1}H-nmr spectrum appeared at δ 5.29 (dd, J=2.2 and 2.2 Hz) in the 7β epimer **8** and at δ 5.60 (d, J=5.5 Hz) in 7α epimer **14** (6,13).

Sterols **9–13** showed spectral properties similar to those of cholest-5-ene-3β,7β-diol **8** (see Experimental). This established an identical sterol nucleus in all six metabolites, which differed only in the side chain structures. These were determined based on reference to data of model compounds **2–7**.

Spectral data (see Experimental) also indicated that compounds **14–17** possessed an identical Δ^5-3β,7α-diol nucleus but differed in their side chain structures that were determined in the same way as those of **8–13**. Sterols **14**, **15**, **16**, and **17** had the side chains identical to those found in sterols **2**, **3**, **4**, and **5**, respectively.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ^{1}H-nmr spectra were recorded on a Bruker WM-400 spectrometer, and ^{13}C-nmr spectra were obtained on a Varian 200 spectrometer operating at 50.3 MHz. The ^{1}H chemical shifts were referenced to the residual $CHCl_3$ signal (7.26 ppm). J values are given in Hertz. The ^{13}C chemical shifts were referenced to the solvent ($CDCl_3$, 77.0 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.

EXTRACTION AND ISOLATION.—The sponge *C. copiosa*, identified by Dr. G. Corriero, University of Genova, was collected in the Bay of Naples at a depth of 15 m. A voucher specimen is on file at our

laboratories. Fresh collected specimens (213 g dry wt after extraction) were extracted once with Me_2CO and twice with $CHCl_3$-MeOH (1:1) at room temperature. The extracts were combined and concentrated under reduced pressure to obtain an aqueous suspension which was extracted with Et_2O. The organic layer was dried over Na_2SO_4 and the solvent removed to obtain an oily residue (9.4 g) that was chromatographed on an open Si gel column (400 g, 72×5.5 cm) using $CHCl_3$ and increasing amounts of MeOH in $CHCl_3$ as eluent. Fractions (200 ml) were collected and checked by ^1H nmr for sterol content.

The fractions 58–66 (166.8 mg), eluted with $CHCl_3$-MeOH (97:3), that contained 3β-hydroxy-5-en-6-one steroids **1–7** were purified by hplc on a Si gel column (Hibar LiChrosorb Si-60, 250×10 mm), using $CHCl_3$-MeOH (99:1) as the mobile phase, and rechromatographed on reversed-phase hplc on a Hibar Superspher RP-18 (250×4 mm) column eluted with MeOH-H_2O (92:8) to give pure steroids **1** (1.3 mg), **2** (31 mg), **3** (5.1 mg), **4** (1.6 mg), **5** (3.9 mg), and **6** and **7** together (2.1 mg).

The fractions 68–69 (170 mg) were further separated by hplc on the above Si gel column eluted with $CHCl_3$-MeOH (99.5:0.5) into the 3β,7β- (**8–13**) and 3β,7α-dihydroxysterol (**14–17**) fractions. Final separation of the above fractions was achieved by reversed-phase hplc on the above column eluted with MeOH-H_2O (88:12) to give **8** (2.5 mg), **9** (0.9 mg), **10** (0.7 mg), **11** (1.4 mg), **12** and **13** together (1.5 mg), **14** (2.1 mg), **15** (0.5 mg), **16** (0.5 mg), and **17** (1.6 mg).

(22E)-3β-Hydroxy-24-norcholesta-5,22-dien-7-one [**1**].—Ft-ir (film) ν max 3390, 1670 cm^{-1}; uv λ max ($CHCl_3$) 237 nm (ε=9800); ^1H nmr ($CDCl_3$) δ 5.69 (1H, d, J=1.5 Hz, H-6), 5.27 (1H, dd, J=15.4 and 5.9 Hz, H-23), 5.18 (1H, dd, J=15.4 and 8.1 Hz, H-22), 3.67 (1H, bm, H_{ax}-3), 2.51 (1H, ddd, J=14.7, 5.1 and 2.2 Hz, H_{eq}-4), 2.39 (1H, ddd, J=14.7, 14.7, and 1.5 Hz, H_{ax}-4), 2.24 (1H, dd, J=11.0 and 11.0 Hz, H-8), 2.19 (1H, m, H-25), 1.99 (m, overlapped, H-20), 1.20 (3H, s, H_3-19), 1.00 (3H, d, J=6.6 Hz, H_3-21), 0.94 (6H, d, J=6.6 Hz, H_3-26 and H_3-27) and 0.69 (3H, s, H_3-18); hrms *m/z* (rel. int.) $[M]^+$ 384.3051 (calcd for $C_{26}H_{40}O_2$ 384.3029) (81), $[M-H_2O]^+$ 366.2925 ($C_{26}H_{38}O$) (3), $[M-C_5H_{10}]^+$ 314.2239 ($C_{21}H_{30}O_2$) (100), [M−side chain]$^+$ 287.2029 ($C_{19}H_{27}O_2$) (92), [M−side chain−2H]$^+$ 285.1865 ($C_{19}H_{25}O_2$) (36), [M−side chain−H_2O]$^+$ 269.1895 ($C_{19}H_{25}O$) (17), [M−ring D]$^+$ 245.1543 ($C_{16}H_{21}O_2$) (2).

3β-Hydroxycholest-5-en-7-one [**2**].—Mp 170–171° [petroleum ether-$CHCl_3$ (1:1)] [lit. (14) mp 171–172°]; Ft-ir (film) ν max 3391, 1671 cm^{-1}; uv λ max ($CHCl_3$) 237 nm (ε=9850); [α]D −78 (c=0.29, $CHCl_3$); ^1H nmr ($CDCl_3$) δ 5.69 (1H, d, J=1.5 Hz, H-6), 3.67 (1H, bm, H_{ax}-3), 2.50 (1H, ddd, J=14.7, 5.1 and 2.2 Hz, H_{eq}-4), 2.39 (1H, ddd, J=14.7, 14.7 and 1.5 Hz, H_{ax}-4), 2.24 (1H, dd, J=11.0 and 11.0 Hz, H-8), 1.19 (3H, s, H_3-19), 0.92 (3H, d, J=6.7 Hz, H_3-21), 0.862 (3H, d, J=6.7 Hz, H_3-26 or H_3-27), 0.857 (3H, d, J=6.7 Hz, H_3-27 or H_3-26), 0.68 (3H, s, H_3-18); ^{13}C nmr ($CDCl_3$) [assignments were made by comparison with published values (7)] δ 36.46 (C-1), 31.29 (C-2), 70.61 (C-3), 41.90 (C-4), 165.23 (C-5), 126.27 (C-6), 202.40 (C-7), 45.52 (C-8), 50.07 (C-9), 38.38 (C-10), 21.34 (C-11), 38.82 or 39.58 (C-12), 41.90 (C-13), 50.07 (C-14), 26.41 (C-15), 28.60 (C-16), 54.91 (C-17), 12.07 (C-18), 17.40 (C-19), 35.80 (C-20), 18.96 (C-21), 36.28 (C-22), 23.92 (C-23), 39.58 or 38.82 (C-24), 28.09 (C-25), 22.64 (C-26), 22.88 (C-27); hrms *m/z* (rel. int.) $[M]^+$ 400.3370 (calcd for $C_{27}H_{44}O_2$ 400.3341) (100), $[M-Me]^+$ 385.3087 ($C_{26}H_{41}O_2$) (4), $[M-H_2O]^+$ 382.3220 ($C_{27}H_{42}O$) (5), $[M-H_2O-Me]^+$ 367.2950 ($C_{26}H_{39}O$) (13), 287.2025 ($C_{19}H_{27}O_2$) (11), 269.1919 ($C_{19}H_{25}O$) (2), 245.1550 ($C_{16}H_{21}O_2$) (4).

(22E)-3β-Hydroxycholesta-5,22-dien-7-one [**3**].—Ft-ir (film) ν max 3390, 1671 cm^{-1}; uv λ max ($CHCl_3$) 238 nm (ε=9900); [α]D −93 (c=0.19, $CHCl_3$); ^1H nmr ($CDCl_3$) δ 5.68 (1H, d, J=1.5 Hz, H-6), 5.30 (1H, dt, J=14.7 and 6.6 Hz, H-23), 5.20 (1H, dd, J=14.7 and 7.3 Hz, H-22), 3.67 (1H, bm, H_{ax}-3), 2.51 (1H, ddd, J=14.7, 5.1 and 2.2 Hz, H_{eq}-4), 2.39 (1H, ddd, J=14.7, 14.7 and 1.5 Hz, H_{ax}-4), 2.24 (1H, dd, J=11.0 and 11.0 Hz, H-8), 2.00 (1H, m, H-20), 1.83 (2H, dd, J=6.6 and 6.6 Hz, H_2-24), 1.58 (m, overlapped, H-25), 1.20 (3H, s, H_3-19), 1.01 (3H, d, J=6.6 Hz, H_3-21), 0.86 (6H, d, J=6.6 Hz, H_3-26 and H_3-27), 0.69 (3H, s, H_3-18); hrms *m/z* (rel. int.) $[M]^+$ 398.3168 (calcd for $C_{27}H_{42}O_2$, 398.3174) (80), $[M-H_2O]^+$ 380.3060 ($C_{27}H_{40}O$) (12), $[M-C_6H_{12}]^+$ 314.2241 ($C_{21}H_{30}O_2$) (70), 287.2014 ($C_{19}H_{27}O_2$) (100), 285.1870 ($C_{19}H_{25}O_2$) (51), 269.1889 ($C_{19}H_{25}O$) (36), 245.1530 ($C_{16}H_{21}O_2$) (10).

(22E,24R)-3β-Hydroxy-24-methylcholesta-5,22-dien-7-one [**4**].—Ft-ir (film) ν max 3392, 1672 cm^{-1}; uv λ max ($CHCl_3$) 237 nm (ε=9800); ^1H nmr ($CDCl_3$) δ 5.69 (1H, d, J=1.5 Hz, H-6), 5.19 (2H, m, H-22 and H-23), 3.68 (1H, bm, H_{ax}-3), 2.51 (1H, ddd, J=14.7, 5.1, and 2.2 Hz, H_{eq}-4), 2.39 (1H, ddd, J=14.7, 14.7, and 1.5 Hz, H_{ax}-4), 2.24 (1H, dd, J=11.0 and 11.0 Hz, H-8), 2.01 (1H, m, H-20), 1.84 (1H, sextet, J=6.1 Hz, H-24), 1.46 (1H, septet, J=6.6 Hz, H-25), 1.20 (3H, s, H_3-19), 1.02 (3H, d, J=6.6 Hz, H_3-21), 0.91 (3H, d, J=6.6 Hz, H_3-28), 0.83 (3H, d, J=6.6 Hz, H_3-26 or H_3-27), 0.82 (3H, d, J=6.6 Hz, H_3-27 or H_3-26), 0.69 (3H, s, H_3-18); hrms *m/z* (rel. int.) $[M]^+$ 412.3353 (calcd for $C_{28}H_{44}O_2$, 412.3341) (71), $[M-Me]^+$ 397.3117 ($C_{27}H_{41}O_2$) (5), $[M-H_2O]^+$ 394.3245 ($C_{28}H_{42}O$) (18), $[M-H_2O-Me]^+$ 379.3023 ($C_{27}H_{39}O$) (5), $[M-C_7H_{14}]^+$ 314.2216 ($C_{21}H_{30}O_2$) (68), $[M-C_3H_7]^+$ 369.2800 ($C_{25}H_{37}O_2$) (24), 351.2667 ($C_{25}H_{35}O$) (6), 287.2025 ($C_{19}H_{27}O_2$) (100), 285.1875 ($C_{19}H_{25}O_2$) (47), 269.1888 ($C_{19}H_{25}O$) (40), 245.1530 ($C_{16}H_{21}O_2$) (13), 227.1415 ($C_{19}H_{19}O$) (8).

(22E,24S)-3β-Hydroxy-24-methylcholesta-5,22-dien-7-one [**5**].—Ft-ir (film) ν max 3391, 1672 cm^{-1}; uv λ max ($CHCl_3$) 238 nm (ε=9850); [α]D −62 (*c*=0.19, $CHCl_3$); ^1H nmr ($CDCl_3$) δ 5.68 (1H, d, *J*=1.5 Hz, H-6), 5.17 (2H, m, H-22 and H-23), 3.67 (1H, bm, H_{ax}-3), 2.50 (1H, ddd, *J*=14.7, 5.1 and 2.2 Hz, H_{eq}-4), 2.39 (1H, ddd, *J*=14.7, 14.7, and 1.5 Hz, H_{ax}-4), 2.24 (1H, dd, *J*=11.0 and 11.0 Hz, H-8), 2.01 (1H, m, H-20), 1.84 (1H, sextet, *J*=6.1 Hz, H-24), 1.46 (1H, septet, *J*=6.6 Hz, H-25), 1.20 (3H, s, H_3-19), 1.01 (3H, d, *J*=6.6 Hz, H_3-21), 0.91 (3H, d, *J*=6.6 Hz, H_3-28), 0.84 (3H, d, *J*=6.6 Hz, H_3-26 or H_3-27), 0.82 (3H, d, *J*=6.6 Hz, H_3-27 or H_3-26), 0.69 (3H, s, H_3-18); hrms *m/z* (rel. int.) $[M]^+$ 412.3347 (calcd for $C_{28}H_{44}O_2$, 412.3341) (60), $[M-Me]^+$ 397.3130 ($C_{27}H_{41}O_2$) (13), $[M-H_2O]^+$ 394.3230 ($C_{28}H_{42}O$) (16), $[M-H_2O-Me]^+$ 379.3015 ($C_{27}H_{39}O$) (7), $[M-C_7H_{14}]^+$ 314.2246 ($C_{21}H_{30}O_2$) (78), $[M-C_3H_7]^+$ 369.2813 ($C_{25}H_{37}O_2$) (19), 287.2020 ($C_{19}H_{27}O_2$) (100), 285.1860 ($C_{19}H_{25}O_2$) (45), 269.1884 ($C_{19}H_{25}O$) (36), 245.1553 ($C_{16}H_{21}O_2$) (16), 227.1447 ($C_{19}H_{19}O$) (12).

(24S)-3β-Hydroxy-24-ethylcholest-5-en-7-one [**6**] *and (24R)-3β-hydroxy-24-ethylcholest-5-en-7-one* [**7**].—Ft-ir (film) ν max 3390, 1671 cm^{-1}; uv λ max ($CHCl_3$) 237 nm (ε=9800); ^1H nmr ($CDCl_3$) δ 5.69 (d, *J*=1.5 Hz, H-6), 3.67 (bm, H_{ax}-3), 2.50 (ddd, *J*=14.7, 5.1, and 2.2 Hz, H_{eq}-4), 2.39 (ddd, *J*=14.7, 14.7, and 1.5 Hz, H_{ax}-4), 2.24 (dd, *J*=11.0 and 11.0 Hz, H-8), 1.19 (s, H_3-19), 0.93 (d, *J*=6.7 Hz, H_3-21 of **6**), 0.92 (d, *J*=6.7 Hz, H_3-21 of **7**), 0.85 (t, *J*=7.3 Hz, H_3-29 of **6**), 0.84 (t, *J*=6.6 Hz, H_3-29 of **7**), 0.83 (d, *J*=6.6 Hz, H_3-26 or H_3-27), 0.81 (d, *J*=6.6 Hz, H_3-27 or H_3-26), 0.68 (s, H_3-18); hrms *m/z* (rel. int.) $[M]^+$ 428.3638 (calcd for $C_{29}H_{48}O_2$, 428.3654) (100), $[M-Me]^+$ 413.3432 ($C_{28}H_{45}O_2$) (2), $[M-H_2O]^+$ 410.3559 ($C_{29}H_{46}O$) (3), $[M-H_2O-Me]^+$ 395.3300 ($C_{28}H_{43}O$) (11), 287.2016 ($C_{19}H_{27}O_2$) (12), 269.1882 ($C_{19}H_{25}O$) (3), 245.1550 ($C_{16}H_{21}O_2$) (4).

Cholest-5-ene-3β,7β-diol [**8**].—Ft-ir (film) ν max 3421 cm^{-1}; [α]D +3 (*c*=0.12, $CHCl_3$) [lit. (13) [α]D +3]; ^1H nmr ($CDCl_3$) δ 5.29 (1H, dd, *J*=2.2 and 2.2 Hz, H-6), 3.85 (1H, bdd, *J*=7.7 and 2.2 Hz, H_α-7), 3.54 (1H, bm, H_{ax}-3), 2.34 (1H, ddd, *J*=13.7, 4.9 and 1.6 Hz, H_{eq}-4), 2.25 (1H, bdd, *J*=13.7 and 13.7 Hz, H_{ax}-4), 1.05 (3H, s, H_3-19), 0.92 (3H, d, *J*=6.0 Hz, H_3-21), 0.86 (6H, d, *J*=6.7 Hz, H_3-26 and H_3-27), 0.69 (3H, s, H_3-18); hrms *m/z* (rel. int.) $[M]^+$ 402.3534 (calcd for $C_{27}H_{46}O_2$, 402.3397) (8), $[M-H_2O]^+$ 384.3396 ($C_{27}H_{44}O$) (100), $[M-H_2O-15]^+$ 369.3165 ($C_{26}H_{41}O$) (4), $[M-2H_2O]^+$ 366.3280 ($C_{27}H_{42}$) (48), $[M-2H_2O-15]^+$ 351.3029 ($C_{26}H_{39}$) (8), $[M-$side chain$-H_2O]^+$ 271.2066 ($C_{19}H_{27}O$) (4), $[M-$side chain$-2H_2O]^+$ 253.1962 ($C_{19}H_{25}$) (9), $[M-H_2O$ and ring D fission$]^+$ 229.1582 ($C_{16}H_{21}$) (1) $[M-2H_2O$ and ring D fission$]^+$ 211.1486 ($C_{16}H_{19}$) (7).

(22E)-Cholesta-5,22-diene-3β,7β-diol [**9**].—Ft-ir (film) ν max 3421 cm^{-1}; [α]D 34 (*c*=0.09, $CHCl_3$); ^1H nmr ($CDCl_3$) δ 5.30 (1H, ddd, *J*=15.4, 6.0, and 6.0 Hz, H-23), 5.29 (1H, dd, *J*=2.2 and 2.2 Hz, H-6), 5.20 (1H, dd, *J*=15.4 and 7.1 Hz, H-22), 3.84 (1H, bdd, *J*=7.7 and 2.2 Hz, H_α-7), 3.54 (1H, bm, H_{ax}-3), 2.34 (1H, ddd, *J*=13.7, 4.9, and 1.6 Hz, H_{eq}-4), 2.25 (1H, bdd, *J*=13.7 and 13.7 Hz, H_{ax}-4), 1.05 (3H, s, H_3-19), 1.01 (3H, d, *J*=6.6 Hz, H_3-21), 0.86 (6H, d, *J*=6.7 Hz, H_3-26 and H_3-27), 0.70 (3H, s, H_3-18); ms *m/z* $[M]^+$ 400, $[M-H_2O]^+$ 382, $[M-2H_2O]^+$ 364, 289, 271, 253.

(22E,24R)-24-Methylcholesta-5,22-diene-3β,7β-diol [**10**].—Ft-ir (film) ν max 3420 cm^{-1}; ^1H nmr ($CDCl_3$) δ 5.29 (1H, dd, *J*=2.2 and 2.2 Hz, H-6), 5.18 (2H, m, H-22 and H-23), 3.85 (1H, bdd, *J*=7.7 and 2.2 Hz, H_α-7), 3.54 (1H, bm, H_{ax}-3), 2.34 (1H, ddd, *J*=13.7, 4.9, and 1.6 Hz, H_{eq}-4), 2.25 (1H, bdd, *J*=13.7 and 13.7 Hz, H_{ax}-4), 1.05 (3H, s, H_3-19), 1.02 (3H, d, *J*=6.6 Hz, H_3-21), 0.91 (3H, d, *J*=6.6 Hz, H_3-28), 0.83 (3H, d, *J*=6.7 Hz, H_3-26 or H_3-27), 0.82 (3H, d, *J*=6.6 Hz, H_3-27 or H_3-26), 0.70 (3H, s, H_3-18); ms *m/z* $[M]^+$ 414, $[M-H_2O]^+$ 396, $[M-2H_2O]^+$ 378, $[M-$side chain$]^+$ 289, 287, 271, 253.

(22E,24S)-24-Methylcholesta-5,22-diene-3β,7β-diol [**11**].—Ft-ir (film) ν max 3422 cm^{-1}; [α]D −6 (*c*=0.14, $CHCl_3$); ^1H nmr ($CDCl_3$) δ 5.29 (1H, dd, *J*=2.2 and 2.2 Hz, H-6), 5.18 (2H, m, H-22 and H-23), 3.85 (1H, bdd, *J*=7.7 and 2.2 Hz, H_α-7), 3.54 (1H, bm, H_{ax}-3), 2.34 (1H, ddd, *J*=13.7, 4.9, and 1.6 Hz, H_{eq}-4), 2.25 (1H, bdd, *J*=13.7, and 13.7 Hz, H_{ax}-4), 1.05 (3H, s, H_3-19), 1.01 (3H, d, *J*=6.6 Hz, H_3-21), 0.91 (3H, d, *J*=6.6 Hz, H_3-28), 0.83 (3H, d, *J*=6.7 Hz, H_3-26 or H_3-27), 0.82 (3H, d, *J*=6.6 Hz, H_3-27 or H_3-26), 0.70 (3H, s, H_3-18); mass spectral data are identical with those of **10**.

(24S)-24-Ethylcholest-5-ene-3β,7β-diol [**12**] *and (24R)-24-ethylcholest-5-ene-3β,7β-diol* [**13**].—Ft-ir (film) ν max 3421 cm^{-1}; ^1H nmr ($CDCl_3$) δ 5.29 (dd, *J*=2.2 and 2.2 Hz, H-6), 3.84 (bdd, *J*=7.7 and 2.2 Hz, H_α-7), 3.54 (bm, H_{ax}-3), 2.34 (ddd, *J*=13.7, 4.9, and 1.6 Hz, H_{eq}-4), 2.25 (bdd, *J*=13.7 and 13.7 Hz, H_{ax}-4), 1.05 (s, H_3-19), 0.93 (d, *J*=6.6 Hz, H_3-21), 0.85 (t, *J*=7.3 Hz, H_3-29 of **12**), 0.84 (t, *J*=6.6 Hz, H_3-29 of **13**), 0.83 (d, *J*=6.6 Hz, H_3-26 or H_3-27), 0.81 (d, *J*=6.6 Hz, H_3-27 or H_3-26), 0.70 (s, H_3-18); ms *m/z* $[M]^+$ 430, $[M-H_2O]^+$ 412, $[M-2H_2O]^+$ 394, 289, 271, 253.

Cholest-5-ene-3β,7α-diol [**14**].—Ft-ir (film) ν max 3420 cm^{-1}; [α]D −98 (*c*=0.17, $CHCl_3$) [lit. (13) [α]D −91]; ^1H nmr ($CDCl_3$) δ 5.60 (1H, d, *J*=5.6 Hz, H-6), 3.84 (1H, bs, $W_{1/2}$=11 Hz, H_β-7), 3.61 (1H, bm, H_{ax}-3), 2.36 (1H, dd, *J*=13.7 and 8.2 Hz, H_{eq}-4), 2.28 (1H, dd, *J*=13.7 and 13.7 Hz, H_{ax}-4), 0.92 (3H, d, *J*=6.0, H_3-21), 1.00 (3H, s, H_3-19), 0.86 (6H, d, *J*=6.7, H_3-26 and H_3-27), 0.70 (3H, s, H_3-18); mass spectral data are identical with those of **8**.

(22E)-Cholesta-5,22-diene-3β,7α-diol [**15**].—Ft-ir (film) ν max 3421 cm^{-1}; [α]D −76.7 (*c*=0.05, $CHCl_3$); ^1H nmr ($CDCl_3$) δ 5.60 (1H, d, *J*=5.5 Hz, H-6), 5.29 (1H, ddd, *J*=15.4, 6.0 and 6.0 Hz, H-23), 5.20 (1H, dd, *J*=15.4 and 7.1 Hz, H-22), 3.84 (1H, bs, $W_{1/2}$=11 Hz, H_β-7), 3.61 (1H, bm, H_{ax}-3), 2.36 (1H, dd, *J*=13.7 and 8.2 Hz, H_{eq}-4), 2.28 (1H, dd, *J*=13.7 and 13.7 Hz, H_{ax}-4), 1.03(3H, d, *J*=6.7 Hz, H_3-21), 1.00 (3H, s, H_3-19), 0.86 (6H, d, *J*=6.7 Hz, H_3-26 and H_3-27), 0.70 (3H, s, H_3-18); mass spectral data are identical with those of **9**.

(22E,24R)-24-Methylcholesta-5,22-diene-3β,7α-diol [**16**].—Ft-ir (film) ν max 3421 cm^{-1}; ^1H nmr ($CDCl_3$) δ 5.60 (1H, d, *J*=5.5 Hz, H-6), 5.19 (1H, dd, *J*=14.8 and 6.6 Hz, H-23), 5.15 (1H, dd, *J*=14.8 and 6.0 Hz, H-22), 3.84 (1H, bs, $W_{1/2}$=11 Hz, H_β-7), 3.61 (1H, bm, H_{ax}-3), 2.36 (1H, dd, *J*=13.7 and 8.2 Hz, H_{eq}-4), 2.28 (1H, dd, *J*=13.7 and 13.7 Hz, H_{ax}-4), 1.02 (3H, d, *J*=6.6 Hz, H_3-21), 1.00 (3H, s, H_3-19), 0.91 (3H, d, *J*=6.6 Hz, H_3-28), 0.83 (3H, d, *J*=6.7 Hz, H_3-26 or H_3-27), 0.82 (3H, d, *J*=6.6 Hz, H_3-27 or H_3-26), 0.70 (3H, s, H_3-18); mass spectral data are identical with those of **10**.

(22E,24S)-24-Methylcholesta-5,22-diene-3β,7α-diol [**17**].—Ft-ir (film) ν max 3423 cm^{-1}; [α]D −73.3 (*c*=0.08, $CHCl_3$); ^1H nmr ($CDCl_3$) δ 5.60 (1H, d, *J*=5.5 Hz, H-6), 5.19 (1H, dd, *J*=14.8 and 6.6 Hz, H-23), 5.15 (1H, dd, *J*=14.8 and 6.0 Hz, H-22), 3.84 (1H, bs, $W_{1/2}$=11 Hz, H_β-7), 3.61 (1H, bm, H_{eq}-3), 2.36 (1H, dd, *J*=13.7 and 8.2 Hz, H_{eq}-4), 2.28 (1H, dd, *J*=13.7 and 13.7 Hz, H_{ax}-4), 1.01 (3H, d, *J*=6.6 Hz, H_3-21), 1.00 (3H, s, H_3-19), 0.91 (3H, d, *J*=6.6 Hz, H_3-28), 0.83 (3H, d, *J*=6.7 Hz, H_3-26 or H_3-27), 0.82 (3H, d, *J*=6.6 Hz, H_3-27 or H_3-26), 0.70 (3H, s, H_3-18); mass spectral data are identical with those of **11**.

SYNTHESIS OF 3β-HYDROXYCHOLEST-5-EN-7-ONE [**2**], CHOLEST-5-ENE-3β,7β-DIOL [**8**], AND ITS 7α EPIMER **14**.—3β-Hydroxycholest-5-en-7-one [**2**] was synthesized starting from cholest-5-en-3β-ol 3-acetate and PCC following the procedure described by Parish *et al.* (8) for cholest-5-en-3β-ol 3-benzoate. Reduction of compound **2** with $LiAlH_4$ in Et_2O at 35° for 1.5 h as described by Shoppee and Newman (13) afforded a 5:1 mixture of the 7β and 7α epimers of cholest-5-ene-3β,7-diol, which were separated by hplc on a Hibar LiChrosorb Si-60 column (250×10 mm) using $CHCl_3$-MeOH (99.5:0.5) as eluent. All spectral data of compounds **8** and **14** are in agreement with the literature values (6,13). The ^1H-nmr and mass spectra of synthetic products **2**, **8**, and **14** were identical in all respects with those of the natural products.

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