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MINOR 5,6-SECOSTEROLS FROM THE MARINE SPONGE *HIPPOSPONGIA COMMUNIS*. ISOLATION AND SYNTHESIS OF (7*Z*,22*E*,24*R*)-24-METHYL-5,6-SECOCHOLESTA-7,22-DIENE-3β,5β,6-TRIOL

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ABSTRACT.—Eight new 5,6-secosterols **2–9** have been isolated from the sponge *Hippospongia communis* and their structures elucidated by analysis of spectral data. A partial synthesis of secosterol **5** confirmed the structure assignment.

During recent years a large number of new sterols has been isolated from marine organisms (1–4), but only a few reports exist describing sterols with bond cleavages in the rings of the tetracyclic nucleus. The only marine sterols of this type reported to date are the 9,11-secosterols isolated from the gorgonian *Pseudopterogorgia americana* (5), the soft coral *Sinularia* sp. (6,7), and the sponge *Dysidea herbacea* (8).

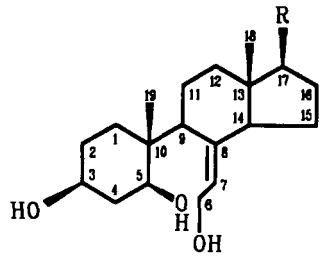
Recently we isolated from the sponge *Hippospongia communis* (Lamarck, 1813) (order Dictyoceratida, family Spongiidae) the novel 5,6-secosterol **1** (9). A further investigation of the lipid extract of the same organism yielded a mixture of the 5,6-secosterols described in this paper.

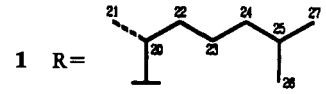
RESULTS AND DISCUSSION

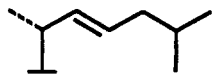
Fresh tissues of the animals were extracted with Me_2CO and $CHCl_3$-MeOH (1:1), the solvent was removed, and the resulting aqueous suspension was extracted with Et_2O. The Et_2O-soluble material was chromatographed over Si gel using $CHCl_3$/MeOH mixtures as the eluent. The fraction eluted with $CHCl_3$-MeOH (97:3) was further separated by hplc on Si gel and reversed-phase hplc to afford secosterols **1–9**.

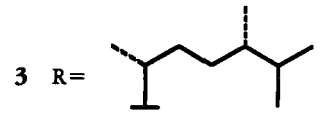
The major component of the sterol mixture was shown to be the previously described 5,6-secosterol **1** (9). Chemical shift values for the H-3α, H-5α, H_2-6, H-7, H_3-18, and H_3-19 protons in the ^{1}H-nmr spectra of secosterols **2–9** were virtually identical with the corresponding values of secosterol **1** (Table 1). In each case, the signal for H_3-19 showed a pyridine-d_5-induced deshielding due to the vicinal interaction with the C-5 hydroxyl group (10). The mass spectra of compounds **1–9** contained common fragment ions at *m/z* 289 ($[M-H_2O-$ side chain$]^+$), 271 ($[M-2H_2O-$ side chain$]^+$), and 253 ($[M-3H_2O-$ side chain$]^+$), suggesting that they all possessed identical nuclei, the differences among the new compounds being confined to the nature of the side chains.

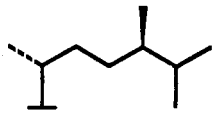
The new secosterol **5** had the molecular formula $C_{28}H_{48}O_3$, established by hrms on the highest mass ion observed in the eims at *m/z* 414 $[M-H_2O]^+$, and significant fragment ions at *m/z* 289 $[M-H_2O-C_9H_{17}]^+$, 271, and 253, indicating the presence of a C_9H_{17} side chain containing a double bond. A ^{1}H-^{1}H nmr COSY-45 experiment allowed the unambiguous determination of the couplings among the vicinal protons in the side chain. The two methyl doublets observed at δ 0.83 and 0.82 (both $J = 6.6$ Hz, H_3-26 and H_3-27) correlated with a diffuse multiplet centered at δ 1.45 (H-25) which, in turn, had a cross peak with a proton at δ 1.82 (H-24). The latter signal showed a correlation with a methyl signal at δ 0.91 ($J = 6.6$ Hz, H_3-28) and with the olefinic double doublet resonating at δ 5.14 ($J = 15.4$ and 7.3 Hz, H-23). The sequence of vicinal protons belonging to the segment C-21/C-23 was secured by the correlation peaks be-

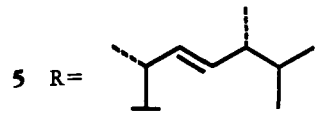


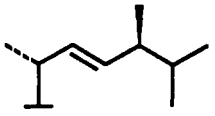


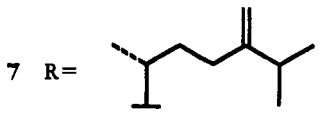


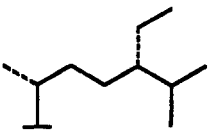


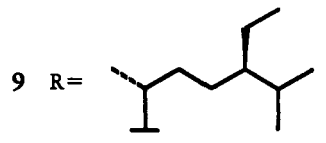












tween the proton at δ 2.00 (m, H-20) and the methyl signal resonating at δ 0.994 (J = 6.6 Hz, H_3-21) and the one-proton double doublet at δ 5.22 (J = 15.4 and 7.3 Hz, H-22) and by the mutual coupling between H-22 and H-23. The large value (15.4 Hz) of the coupling constant between these protons indicated the *E* configuration for the Δ^{22} double bond. Because the ^{1}H-nmr chemical shifts of the observable side chain protons of this sterol were consistent with those of brassicasterol, its structure was formulated as (7*Z*,22*E*,24*R*)-24-methyl-5,6-secocholesta-7,22-diene-3β,5β,6-triol. In order to confirm the structure assignment and determine unambiguously the C-24 configuration of this compound, synthetic **5** was prepared from 5α-ergosta-7,22-diene-3β,5,6α-triol [**10**] (11,12) through the oxidative breakage of the C-5–C-6 bond by treatment with lead tetraacetate in HOAc at room temperature. This reaction afforded (7*Z*)-3β-hydroxy-5-oxo-5,6-secoergosta-7,22-dien-6-al [**11**] which on standing in $CHCl_3$ solution partially transformed into its 7*E* isomer **12**. The mixture of isomers was resolved into the single compounds by reversed-phase hplc. That these compounds were geometric isomers was confirmed by photochemical interconversion of **11** in **12**. Reduction of **11** with $LiAlH_4$ yielded the synthetic compound **5** and its 5α-epimer **13** which were separated by hplc on Si gel [$CHCl_3$-MeOH (95:5)]. ^{1}H-nmr and mass spectral data for synthetic (7*Z*,22*E*,24*R*)-24-methyl-5,6-secocholesta-7,22-diene-3β,5β,6-triol were identical with those of the natural product **5**. When the ^{1}H-nmr

TABLE 1. Selected 400 MHz ^{1}H-nmr chemical shifts ($CDCl_3$) of secosterols **1–9**.[a]

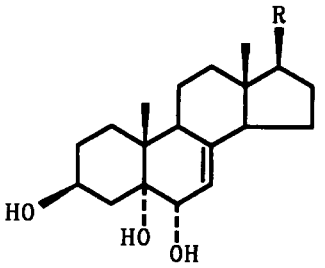
Proton	Compound								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
H-3	3.93 bm	3.93 bm	3.93 bm	3.93 bm	3.93 bm	3.93 bm	3.93 bm	3.93 bm	3.93 bm
H-5	3.93 bm	3.93 bm	3.93 bm	3.93 bm	3.93 bm	3.93 bm	3.93 bm	3.93 bm	3.93 bm
H_a-6	4.23 dd (12.5, 6.5)	4.24 dd (12.8, 6.6)	4.24 dd (12.8, 6.6)	4.24 dd (12.8, 6.6)	4.24 dd (12.7, 6.7)	4.24 dd (12.9, 6.7)	4.23 dd (12.8, 6.6)	4.23 dd (12.6, 6.6)	4.23 dd (12.6, 6.6)
H_b-6	4.36 dd (12.8, 7.3)	4.36 dd (12.8, 7.3)	4.36 dd 12.8, 7.3)	4.36 dd (12.8, 7.3)	4.35 dd (12.8, 7.3)	4.36 dd (12.8, 7.3)	4.36 dd (12.8, 7.3)	4.35 dd (12.8, 7.3)	4.35 dd (12.8, 7.3)
H-7	5.13 bdd (7.2, 6.5)	5.13 bdd (7.3, 6.6)	5.13 bdd (7.1, 6.5)	5.13 bdd (7.1, 6.5)	5.13 bdd (7.3, 6.7)	5.13 bdd (7.1, 6.7)	5.14 bdd (7.2, 6.6)	5.13 bdd (7.3, 6.6)	5.13 bdd (7.3, 6.6)
H-18	0.56 s	0.58 s	0.57 s	0.57 s	0.58 s	0.58 s	0.58 s	0.58 s	0.58 s
H-19	1.03 s	1.04 s	1.04 s	1.04 s	1.03 s	1.04 s	1.04 s	1.04 s	1.04 s
H-20		2.01 m			2.00 m				
H-21	0.89 d (6.3)	0.99 d (6.7)	0.90 d (6.7)	0.89 d (6.7)	0.994 d (6.6)	0.985 d (6.9)	0.93 d (6.7)	0.90 d (6.7)	0.90 d (6.7)
H-22		5.18 dd (15.3, 8.5)			5.14 dd (15.4, 7.3)	5.12 dd (15.3, 7.4)			
H-23		5.30 ddd (15.3, 6.7, 6.7)			5.22 dd (15.4, 7.3)	5.19 dd (15.3, 7.4)			
H-24		1.84 m			1.82 m				
H-25	1.51 m	1.58 m			1.45 m	1.46 m	2.23 b septet		
H-26, -27	0.86 d (6.3)	0.857 d (6.7)	0.851 d (6.7)	0.849 d (6.7)	0.82 d (6.6)	0.82 d (6.9)	1.022 d (6.7)	0.81 d (6.7)	0.81 d (6.7)
		0.860 d (6.7)	0.78 d (6.7)	0.80 d (6.7)	0.83 d (6.6)	0.83 d (6.6)	1.027 d (6.7)	0.83 d (6.7)	0.83 d (6.7)
H-28			0.77 d (6.7)	0.77 d (6.7)	0.91 d (6.6)	0.91 d (6.6)	4.65 and 4.62 bs's		
H-29								0.860 t (6.7)	0.850 t (6.7)

[a]*J* values (Hz) are given in parentheses.

spectra of the isomers **5** and **13** were run in pyridine-d_5, the Me-19 signal of **5** was observed at lower field (δ 1.45) than that of its 5α isomer **13** (δ 1.23). In addition, the significantly downfield shifts of the H-3α and H-5β resonances (see Experimental) in **13** relative to their position in **5** is a result of the pyridine-d_5-induced deshielding due to the interaction of pyridine with the 3-OH and 5-OH (10).

The secosterol **6** has the molecular formula $C_{28}H_{48}O_3$. The ^{1}H-nmr (Table 1) and mass spectral data clearly indicated that it was the C-24 epimer of **5**. The ^{1}H-nmr spectrum of **6** differed from that of **5** in that the H_3-21 doublet was shifted upfield (δ 0.985) when compared to the corresponding signal (δ 0.994) for the secosterol **5** (13,14). Thus **6** must be formulated as (7*Z*,22*E*,24*S*)-24-methyl-5,6-secocholesta-7,22-diene-3β,5β,6-triol.

The molecular formula of compound **2** was determined as $C_{27}H_{46}O_3$ by hrms on the highest peak observed in the mass spectrum at *m/z* 400 $[M-H_2O]^+$. Ions at *m/z* 289 $[M-H_2O-C_8H_{15}]^+$, 271, and 253 indicated for the new compound a side chain having one degree of unsaturation and a C_8H_{15} composition. The high field region of the ^{1}H-nmr spectrum of **2** (Table 1) displayed a doublet at δ 0.99 (J = 6.7 Hz, H_3-21) and two doublets centered at δ 0.860 and 0.857 (both J = 6.7 Hz, H_3-26 and H_3-27), while two olefinic protons were observed at δ 5.30 (ddd, J = 15.3, 6.7, and 6.7 Hz, H-23) and δ 5.18 (dd, J = 15.3 and 8.5 Hz, H-22). These data suggested a Δ^{22}-cholesterol-type side chain for this compound (14). Evidence supporting the above hypothesis was obtained from spin-decoupling experiments. Irradiation of the allylic proton at δ 2.01 (m, H-20) collapsed the double doublet at δ 5.18 (H-22) to a doublet (J = 15.3 Hz) and caused the H_3-21 doublet at δ 0.99 to transform into a singlet. The double



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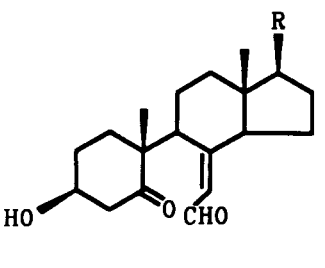

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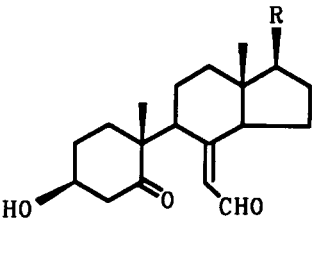

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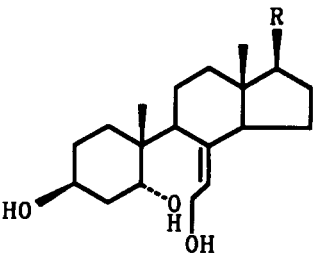

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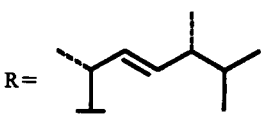

double doublet at δ 5.30 was assigned to the H-23 proton because it collapsed to a double doublet on irradiation at the frequency of the H-22 signal. Irradiation on the H-25 signal at δ 1.58 (m, partially overlapped to other signals) caused the two three-proton doublets due to H_3-26 and H_3-27, at δ 0.857 and 0.860, to collapse to singlets and modified the H_2-24 multiplet centered at δ 1.84. Finally, irradiation at the frequency of this signal modified the H-25 multiplet and collapsed the H-23 signal to a doublet. The configuration of the Δ^{22} double bond was established to be *E* on the basis of the large value (15.3 Hz) of the H-22/H-23 coupling constant. Thus, the structure of this sterol was established as (7*Z*,22*E*)-5,6-secocholesta-7,22-diene-3β,5β,6-triol [**2**].

Compounds **3** and **4** could not be separated by repeated reversed-phase hplc. They each had the molecular formula $C_{28}H_{50}O_3$, as established by hrms on the ion at *m/z* 416 $[M-H_2O]^+$. Fragment ions at *m/z* 289 $[M-H_2O-C_9H_{19}]^+$, 271, and 253 indicated the presence of a saturated C_9H_{19} side chain. The side chain methyl signals for both isomers could clearly be seen in their ^{1}H-nmr spectrum that showed a pair of doublets centered at δ 0.89 ($J=6.7$ Hz) and 0.90 ($J=6.7$ Hz) (Table 1) assigned to the H_3-21 signals of the 24*R* and 24*S* epimers, respectively. The main difference was observed in the chemical shifts of one of the doublets of the isopropyl group. The doublet of the H_3-27 methyl signal resonated at δ 0.78 ($J=6.7$ Hz) for the 24*S* epimer although this doublet occurred at δ 0.80 ($J=6.7$ Hz) for the 24*R* epimer. The assigned configuration at C-24 of the two epimers was supported by spectral comparison of the high field region of their ^{1}H nmr spectrum with those of authentic samples of campesterol and of an epimeric mixture of 24-methylcholestanol, prepared by hydrogenation of 24-methylenecholestanol. Thus the structures of **3** and **4** were formulated as (7*Z*,24*S*)-24-methyl-5,6-secocholest-7-ene-3β,5β,6-triol and (7*Z*,24*R*)-24-methyl-5,6-secocholest-7-ene-3β,5β,6-triol, respectively.

Compound **7** has the molecular formula $C_{28}H_{48}O_3$. The presence of a C_9H_{17} monounsaturated side chain was indicated by the ions at *m/z* 289 $[M-H_2O-C_9H_{17}]^+$, 271, and 253. Its ^{1}H-nmr spectrum (Table 1) contained three methyl doublets at δ 0.93 ($J=6.7$ Hz, H_3-21) and δ 1.027 and 1.022 (both $J=6.9$ Hz, H_3-26 and H_3-27) and two broad singlets at δ 4.72 and 4.65 (H_2-28), which indicated the presence of a terminal methylene group. The location of the side chain double bond between C-24 and C-28 was established by a spin-decoupling experiment. Irradiation of the allylic proton at δ 2.23 (b septet, $J=6.7$ Hz, H-25) collapsed the two methyl doublets at δ 1.027 and 1.022 into singlets and sharpened the two broad olefinic singlets at δ 4.72 and 4.65. Hence, **7** must be formulated as (7*Z*)-24-methyl-5,6-secocholesta-7,24(28)-diene-3β,5β,6-triol.

The last secosterols **8** and **9** could not be separated by repeated reversed-phase hplc. They each had the molecular formula $C_{29}H_{52}O_3$ and a $C_{10}H_{21}$ saturated side chain. The complexity of the methyl region of the ^{1}H-nmr spectrum (Table 1) clearly showed the presence of an epimeric mixture. The side chain chemical shifts for both isomers were assigned by comparison with those of authentic samples of commercial sitosterol and clionasterol isolated from marine phanerogams (13). The H_3-29 resonance of the 24*S* epimer **8** is more deshielded (δ 0.860) than that in the 24*R* epimer **9** (δ 0.850) (14). Consequently, the structures of **8** and **9** were assigned as (7*Z*,24*S*)-24-ethyl-5,6-secocholest-7-ene-3β,5β,6-triol and (7*Z*,24*R*)-24-ethyl-5,6-secocholest-7-ene-3β,6-triol, respectively.

To the best of our knowledge, 5,6-secosterols have never been found as natural products before. From a biosynthetic point of view it seems probable that secosterols **1**–**9** could derive from the Δ^7-3β,5α,6β-trihydroxysterols present in the same organism which are, most likely, biogenetically related to $\Delta^{5,7}$-3β-hydroxysterols present in large amount in the sponge (15).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURS.—^{1}H-nmr spectra were recorded on a Bruker WM-400 spectrometer in either $CDCl_3$ or C_5D_5N solutions. Chemical shift values are referenced to the residual $CHCl_3$ (7.26 ppm) and C_5H_5N (8.71 ppm) solvent signals. Low resolution mass spectra were determined at 70 eV on a Kratos MS 30 mass spectrometer. High resolution mass spectra were recorded on a Kratos MS 50 spectrometer. Fourier transform ir spectra were obtained with a Perkin-Elmer 1760-X FT-Ir. Hplc was performed using a Varian 2510 pump and a Waters dual cell refractometer. Melting points were determined on a Kofler apparatus and are uncorrected.

EXTRACTION AND ISOLATION.—The sponge *H. communis* was collected in the Bay of Naples and supplied by the Zoological Station of Naples. Voucher specimens are on file at our laboratories. The freshly collected sponge (439 g dry wt after extraction) was cut into pieces and extracted at room temperature with Me_2CO and $CHCl_3$-MeOH (1:1). The combined lipid extracts were concentrated under reduced pressure to obtain an aqueous suspension, which was extracted with Et_2O. The organic extract was dried over Na_2SO_4 and the solvent removed to obtain an oily residue (20.65 g) that was chromatographed on a Si gel column eluted with solvents of increasing polarity from petroleum ether through $CHCl_3$ and increasing amounts of MeOH in $CHCl_3$. The fractions eluted with $CHCl_3$-MeOH (97:3) were further purified by hplc on a Hibar LiChrosorb Si-60 column (250 × 10 mm) eluted with $CHCl_3$-MeOH (95:5). The 5,6-secosterol mixture (25 mg) was fractionated by reversed-phase hplc on a Hibar RP-18 column (250 × 4 mm) eluted with MeOH-H_2O (85:15) to give the following compounds in order of elution: **2** (1.0 mg), **7** (1.0 mg), **6** (1.8 mg), **5** (0.5 mg), **1** (3.8 mg), **3** and **4** (0.6 mg together), **8** and **9** (1.5 mg together).

SPECTRAL DATA.—*(7Z,22E)-5,6-Secocholesta-7,22-diene-3β,5β,6-triol* [**2**].—Ir ($CHCl_3$) ν max 3400 cm^{-1}; ^{1}H nmr ($CDCl_3$) see Table 1; ^{1}H nmr (pyridine-d_5, 400 MHz) δ 5.64 (1H, bdd, J = 6.1 and 6.1 Hz, H-7), 5.33 (1H, ddd, J = 14.6, 7.9, and 7.9 Hz, H-23), 5.25 (1H, dd, J = 14.6 and 7.9 Hz, H-22), 4.82 (1H, dd, J = 13.4 and 6.7 Hz, H_a-6), 4.71 (1H, dd, J = 13.4 and 6.1 Hz, H_b-6), 4.16 (1H, bm, H-3α or H-5α), 4.04 (1H, bm, H-5α or H-3α), 1.57 (1H, m, H-25), 1.45 (3H, s, H_3-19), 1.03 (3H, d, J = 6.7 Hz, H_3-21), 0.87 (6H, d, J = 6.7 Hz, H_3-26 and H_3-27), 0.69 (3H, s, H_3-18); hreims *m/z* (rel. int.) $[M-H_2O]^+$ 400.3314 (25.6) (calcd for $C_{27}H_{44}O_2$, 400.3341), $[M-H_2O-Me]^+$ 385.3151 ($C_{26}H_{41}O_2$) (17.6), $[M-2H_2O]^+$ 382.3272 ($C_{27}H_{42}O$) (16.8), $[M-2H_2O-Me]^+$ 367.2988 ($C_{26}H_{39}O$) (12.0), $[M-3H_2O]^+$ 364.3078 ($C_{27}H_{40}$) (7.2), $[M-3H_2O-Me]^+$ 349.2861 ($C_{26}H_{37}$) (8.0), $[M-$ side chain $-H_2O]^+$ 289.2094 ($C_{19}H_{29}O_2$) (40.0), $[M-$ side chain $-2H_2O]^+$ 271.2123 ($C_{19}H_{27}O$) (92.8), $[M-$ side chain $-3H_2O]^+$ 253.1957 ($C_{19}H_{25}$) (47.2), [ring A]$^+$ 129.0882 ($C_7H_{13}O_2$) (78.4), [ring A − H]$^+$ 128.0838 ($C_7H_{12}O_2$) (100).

(7Z,24S)-24-Methyl-5,6-secocholest-7-ene-3β,5β,6-triol [**3**] *and (7Z,24R)-24-methyl-5,6-secocholest-7-ene-3β,5β,6-triol* [**4**].—Ir ($CHCl_3$) ν max 3400 cm^{-1}; ^{1}H nmr ($CDCl_3$) see Table 1; ^{1}H nmr (pyridine-d_5, 400 MHz) δ 5.64 (bdd, J = 6.7 and 6.7 Hz, H-7), 4.83 (dd, J = 13.5 and 6.7 Hz, H_a-6), 4.71 (dd, J = 13.5 and 6.0 Hz, H_b-6), 4.17 (bm, H-3α or H-5α), 4.05 (bm, H-5α or H-3α), 1.45 (s, H_3-19), 0.68 (s, H_3-18); hreims *m/z* $[M-H_2O]^+$ 416.3602 (9.5) (calcd for $C_{28}H_{48}O_2$, 416.3654), 401.3437 $[C_{27}H_{45}O_2]^+$ (10.2), 398.3507 $[C_{28}H_{46}O]^+$ (14.3), 365.3212 $[C_{27}H_{41}]^+$ (10.3), 289.2180 $[C_{19}H_{29}O_2]^+$ (17.7), 271.2107 $[C_{19}H_{27}O]^+$ (17.6), 253.1975 $[C_{19}H_{25}]^+$ (13.6), 129.0915 $[C_7H_{13}O_2]^+$ (100), 128.0837 $[C_7H_{12}O_2]^+$ (71.4).

(7Z,22E,24R)-24-Methyl-5,6-secocholesta-7,22-diene-3β,5β,6-triol [**5**].—Ir ($CHCl_3$) ν max 3400 cm^{-1}; ^{1}H nmr ($CDCl_3$) see Table 1; ^{1}H nmr (pyridine-d_5, 400 MHz) δ 5.65 (1H, bdd, J = 6.1 and 6.1 Hz, H-7), 4.82 (1H, dd, J = 12.8 and 6.7 Hz, H_a-6), 4.71 (1H, dd, J = 12.8 and 6.0 Hz, H_b-6), 4.16 (1H, bm, H-3α or H-5α), 4.04 (1H, bm, H-5α or H-3α), 1.45 (3H, s, H_3-19), 0.68 (3H, s, H_3-18); hreims *m/z* $[M-H_2O]^+$ 414.3550 (30.8) (calcd for $C_{28}H_{46}O_2$, 414.3498), 399.3137 $[C_{27}H_{43}O_2]^+$ (41.4), 396.3365 $[C_{28}H_{44}O]^+$ (32.5), 381.3102 $[C_{27}H_{41}O]^+$ (23.7), 378.3242 $[C_{28}H_{42}]^+$ (5.9), 363.3070 $[C_{27}H_{39}]^+$ (13.6), 289.2079 $[C_{19}H_{29}O_2]^+$ (61.5), 271.2053 $[C_{19}H_{27}O]^+$ (100), 253.1957 $[C_{19}H_{25}]^+$ (58.0), 129.0887 $[C_7H_{13}O_2]^+$ (58.6), 128.0830 $[C_7H_{12}O_2]^+$ (71.0).

(7Z,22E,24S)-24-Methyl-5,6-secocholesta-7,22-diene-3β,5β,6-triol [**6**].—Ir ($CHCl_3$) ν max 3400 cm^{-1}; ^{1}H nmr ($CDCl_3$) see Table 1; ^{1}H nmr (pyridine-d_5, 400 MHz) δ 5.65 (1H, dd, J = 6.7 and 6.7 Hz, H-7), 4.82 (1H, dd, J = 13.5 and 6.7 Hz, H_a-6), 4.71 (1H, dd, J = 13.5 and 6.0 Hz, H_b-6), 4.16 (1H, bm, H-3α or H-5α), 4.04 (1H, bm, H-5α or H-3α), 1.45 (3H, s, H_3-19), 0.68 (3H, s, H_3-18); eims *m/z* $[M-H_2O]^+$ 414.3561 (53.3) (calcd for $C_{28}H_{46}O_2$, 414.3498), 399.3342 $[C_{27}H_{43}O_2]^+$ (47.5), 396.3421 $[C_{28}H_{44}O]^+$ (50.0), 363.3076 $[C_{27}H_{39}]^+$ (20.8), 289.2213 $[C_{19}H_{29}O_2]^+$ (65.8), 271.2057 $[C_{19}H_{27}O]^+$ (100), 253.1936 $[C_{19}H_{25}]^+$ (56.6), 129.0890 $[C_7H_{13}O_2]^+$(80.0), 128.0840 $[C_7H_{12}O_2]^+$ (79.2).

(7Z)-24-Methyl-5,6-secocholesta-7,24(28)-diene-3β,5β,6-triol [**7**].—Ir ($CHCl_3$) ν max 3400 cm^{-1}; ^{1}H nmr ($CDCl_3$) see Table 1; ^{1}H nmr (pyridine-d_5, 400 MHz) δ 5.63 (1H, bdd, J = 6.7 and 6.7 Hz, H-7),

4.82 (1H, dd, J = 13.5 and 6.7 Hz, H_a-6), 4.71 (1H, dd, J = 13.5 and 6.1 Hz, H_b-6), 4.16 (1H, bm, H-3α or H-5α), 4.04 (1H, bm, H-5α or H-3α), 1.45 (3H, s, H_3-19), 0.67 (3H, s, H_3-18); hreims *m/z* $[M-H_2O]^+$ 414.3558 (41.4) (calcd for $C_{28}H_{46}O_2$, 414.3498), 399.3186 $[C_{27}H_{43}O_2]^+$ (39.6), 396.3380 $[C_{28}H_{44}O]^+$ (31.0), 363.3021 $[C_{27}H_{39}]^+$ (18.0), 289.2075 $[C_{19}H_{29}O_2]^+$ (50.9), 271.2060 $[C_{19}H_{27}O]^+$ (59.5), 253.1957 $[C_{19}H_{25}]^+$ (32.8), 129.0880 $[C_7H_{13}O_2]^+$ (87.1), 128.0836 $[C_7H_{12}O_2]^+$ (100).

(7Z,24S)-24-Ethyl-5,6-secocholest-7-ene-3β,5β,6-triol [**8**] *and (7Z,24R)-24-ethyl-5,6-secocholest-7-ene-3β,5β,6-triol* [**9**].—Ir ($CHCl_3$) ν max 3400 cm^{-1}; ^1H nmr ($CDCl_3$) see Table 1; ^1H nmr (pyridine-d_5, 400 MHz) δ 5.64 (bdd, J = 6.7 and 6.7 Hz, H-7), 4.83 (dd, J = 13.5 and 6.7 Hz, H_a-6), 4.71 (dd, J = 13.5 and 6.0 Hz, H_b-6), 4.17 (bm, H-3α or H-5α), 4.04 (bm, H-5α or H-3α), 1.45 (s, H_3-19), 0.68 (s, H_3-18); hreims *m/z* $[M-H_2O]^+$ 430.3803 (19.7) (calcd for $C_{29}H_{50}O_2$, 430.3811), 415.3621 $[C_{28}H_{47}O_2]^+$ (11.3), 412.3635 $[C_{29}H_{48}O]^+$ (54.9), 289.2160 $[C_{19}H_{29}O_2]^+$ (9.1), 271.2087 $[C_{19}H_{27}O]^+$ (11.3), 253.1940 $[C_{19}H_{25}]^+$ (10.6), 129.0872 $[C_7H_{13}O_2]^+$ (100), 128.0830 $[C_7H_{12}O_2]^+$ (28.1).

SYNTHESIS OF (7Z,22E,24R)-3β-HYDROXY-5-OXO-24-METHYL-5,6-SECOCHOLESTA-7,22-DIEN-6-AL [**11**].—A solution of (22E,24R)-24-methyl-5α-cholesta-7,22-diene-3β,5,6α-triol [**10**] (80 mg) (11,12) in HOAc (15 ml) was treated with 200 mg of lead tetraacetate at room temperature. After 10 min the mixture was quenched with ethylene glycol (0.5 ml), and the solution was diluted with H_2O and extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ solution, dried, and evaporated. The residue was chromatographed on a Hibar Superspher RP 18 column (250 × 4 mm) using MeOH-H_2O (9:1) as eluent to yield (7Z,22E,24R)-3β-hydroxy-5-oxo-24-methyl-5,6-secocholesta-7,22-dien-6-al [**11**]. This product on standing in $CHCl_3$ solution partially interconverted into its 7E isomer **12**. Compounds **11** and **12** were separated by reversed-phase hplc [MeOH-H_2O (9:1)], and 5.5 mg of **12** and 10 mg of **11** were obtained.

The 7Z isomer **11**: mp 160–161°; $[\alpha]_D$ + 105.2 (c = 0.9, $CHCl_3$); ir ($CHCl_3$) ν max 3416, 2873, 1705, 1656, 1616 cm^{-1}; ir (film) ν max 3441, 2870, 1691, 1652, 1618 cm^{-1}; ^1H nmr ($CDCl_3$, 400 MHz) δ 9.60 (1H, d, J = 8.3 Hz, H-6), 5.64 (1H, d, J = 8.3 Hz, H-7), 5.24 (1H, dd, J = 15.3 and 7.0 Hz, H-22 or H-23), 5.14 (1H, dd, J = 15.3 and 8.3 Hz, H-23 or H-22), 4.22 (1H, m, H-3α), 2.67 (1H, dd, J = 12.7 and 4.4 Hz, Ha-4), 2.58 (1H, dd, J = 12.7 and 7.0 Hz, Hb-4), 1.36 (3H, s, H_3-19), 1.01 (3H, d, J = 6.4 Hz, H_3-21), 0.91 (3H, d, J = 6.9 Hz, H_3-28), 0.84 (3H, d, J = 6.9 Hz, H_3-26 or H_3-27), 0.82 (3H, d, J = 6.9 Hz, H_3-27 or H_3-26), 0.61 (3H, s, H_3-18); eims *m/z* (rel. int.) $[M]^+$ 428 (16.5), $[M-H_2O]^+$ 410 (98.0), $[M-CHO]^+$ 399 (19.5), $[M-H_2O-Me]^+$ 395 (30.0), $[M-2H_2O]^+$ 392 (25.5), $[M-H_2O-CO]^+$ 382 (72.0), $[M-H_2O-CHO]^+$ 381 (37.5), $[M-C_5H_9O_2]^+$ 327 (100), $[M-C_7H_{12}O_2]^+$ 300 (30.1), $[M-H_2O-$ side chain$]^+$ 285 (29.5); hreims *m/z* 428.3327 ($C_{28}H_{44}O_3$ requires 428.3290).

The 7E isomer **12**: mp 147–148°; $[\alpha]_D$ −12.7 (c = 0.4, $CHCl_3$); ir ($CHCl_3$) ν max 3416, 2873, 1705, 1656, 1616; ir (film) ν max 3410, 2872, 1701, 1654, 1617 cm^{-1}; ^1H nmr ($CDCl_3$, 400 MHz) δ 10.03 (1H, d, J = 7.9 Hz, H-6), 5.87 (1H, m, H-7), 5.86 (1H, dd, J = 15.3 and 7.3 Hz, H-22 or H-23), 5.15 (1H, dd, J = 15.3 and 7.9 Hz, H-23 or H-22), 4.33 (1H, m, H-3α), 1.21 (3H, s, H_3-19), 1.04 (3H, d, J = 6.7 Hz, H_3-21), 0.92 (3H, d, J = 6.7 Hz, H_3-28), 0.84 (3H, d, J = 6.7 Hz, H_3-26 or H_3-27), 0.82 (3H, d, J = 6.7 Hz, H_3-27 or H_3-26), 0.69 (3H, s, H_3-18); eims *m/z* $[M]^+$ 428 (17.7), 410 (98.9), 399 (18.7), 395 (28.1), 392 (23.9), 382 (70.8), 381 (38.5), 327 (100), 300 (32.2), 285 (32.2); hreims *m/z* 428.3305 ($C_{28}H_{44}O_3$ requires 428.3290).

PHOTOCHEMICAL ISOMERIZATION OF (7Z,22E,24R)-3β-HYDROXY-5-OXO-24-METHYL-5,6-SECOCHOLESTA-7,22-DIEN-6-AL [**11**] INTO ITS 7E ISOMER **12**.—A solution of **11** (3 mg) in $CDCl_3$ (1.5 ml) was irradiated at room temperature for 1 h with a simple 200 W sun lamp. The ^1H-nmr spectrum analysis of the $CDCl_3$ solution showed a complete interconversion of compound **11** into its 7E isomer.

SYNTHESIS OF (7Z,22E,24R)-24-METHYL-5,6-SECOCHOLESTA-7,22-DIENE-3β,5β,6-TRIOL [**5**] AND ITS 5α ISOMER **13**.—A solution of 10 mg of (7Z,22E,24R)-3β-hydroxy-5-oxo-24-methyl-5,6-secocholesta-7,22-dien-6-al [**11**] in 5 ml of anhydrous Et_2O was added to a suspension of 50 mg of $LiAlH_4$ in 6 ml of anhydrous Et_2O. After stirring at room temperature for 20 min, H_2O was added cautiously until a white precipitate was formed. Et_2O was decanted and the precipitate washed with Et_2O. Evaporation of the solvent afforded a 1:1 mixture of (7Z,22E,24R)-24-methyl-5,6-secocholesta-7,22-diene-3β,5β,6-triol [**5**] and its 5α isomer **13**, which were separated by hplc on a Hibar LiChrosorb Si-60 (250 × 4 mm) column using $CHCl_3$-MeOH (95:5) as eluent. The ^1H-nmr and mass spectra of synthetic (7Z,22E,24R)-24-methyl-5,6-secocholesta-7,22-diene-3β,5β,6-triol were identical in all respects with those of the natural product **5**.

The 5α isomer **13**: mp 145–147° [$CHCl_3$-petroleum ether (8:2)]; $[\alpha]_D$ +50.0 (c = 0.5, $CHCl_3$); ir (film) ν max 3400 cm^{-1}; ^1H nmr ($CDCl_3$, 400 MHz) δ 5.22 (1H, dd, J = 15.4 and 7.3 Hz, H-22 or H-

23), 5.17 (1H, t, J = 7.3 Hz, H-7), 5.14 (1H, dd, J = 15.4 and 7.3 Hz, H-23 or H-22), 4.27 (2H, bd, J = 7.3 Hz, H_2-6), 4.07 (1H, bs, $W_{1/2}$ = 8.1 Hz, H-5β), 3.96 (1H, m, H-3α), 1.04 (3H, s, H_3-19), 0.99 (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), 0.57 (3H, s, H_3-18); ^{1}H nmr (pyridine-d_5, 400 MHz) δ 5.57 (1H, dd, J = 6.7 and 6.7 Hz, H-7), 5.25 (1H, dd, J = 15.3 and 6.1 Hz, H-22 or H-23), 5.19 (1H, dd, J = 15.3 and 7.3 Hz, H-23 or H-22), 4.73 (1H, dd, J = 12.8 and 6.1 Hz, H_a-6), 4.68–4.54 (3H, overlapping multiplets, H_b-6, H-3α, H-5β), 1.23 (3H, s, H_3-19), 1.05 (3H, d, J = 6.7 Hz, H_3-21), 0.93 (3H, d, J = 6.7 Hz, H_3-28), 0.850 (3H, d, J = 6.7 Hz, H_3-26 or H_3-27), 0.846 (3H, d, J = 6.7 Hz, H_3-27 or H_3-26), 0.69 (3H, s, H_3-18); eims *m/z* (rel. int.) $[M-H_2O]^+$ 414 (86.1), $[M-H_2O-Me]^+$ 399 (53.9), $[M-2H_2O]^+$ 396 (20.0), $[M-2H_2O-Me]^+$ 381 (20.9), $[M-3H_2O]^+$ 378 (3.5), $[M-3H_2O-Me]^+$ 363 (14.8), $[M-\text{side chain}-H_2O]^+$ 289 (45.2), $[M-\text{side chain}-2H_2O]^+$ 271 (57.4), $[M-\text{side chain}-3H_2O]^+$ 253 (36.5), $[\text{ring A}]^+$ 129 (100), $[\text{ring A}-H]^+$ 128 (81.7); hreims *m/z* 414.3437 ($C_{28}H_{46}O_2$ requires 414.3498).

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