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Journal of Natural Products
Vol. 55, No. 6, pp. 773-779, June 1992

NEW $\Delta^{8(14)}$-3β,7α-DIHYDROXYSTEROLS FROM THE MARINE SPONGE *PELLINA SEMITUBULOSA*

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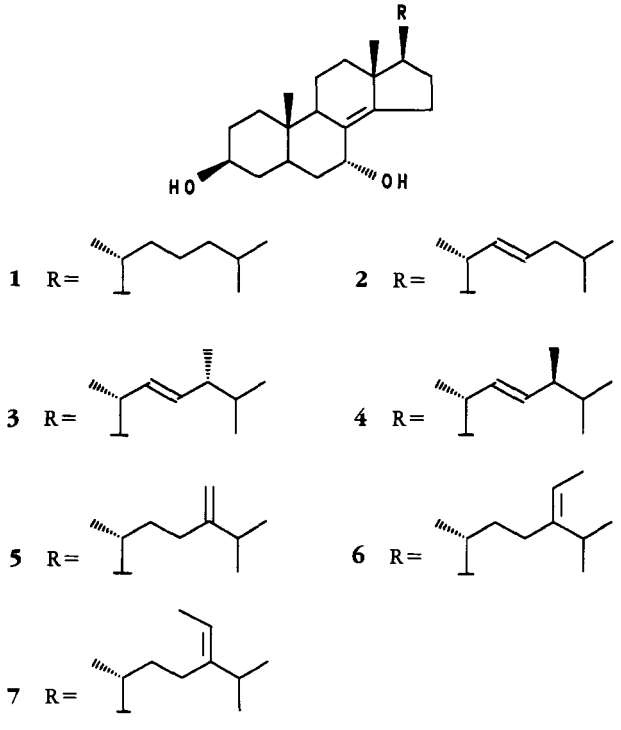
ABSTRACT.—The marine sponge *Pellina semitubulosa* was examined for polyoxygenated sterols. The sponge contained six new 3β,7α-diols, (22*E*)-5α-cholesta-8(14),22-diene-3β,7α-diol [**2**], (22*E*,24*R*)-24-methyl-5α-cholesta-8(14),22-diene-3β,7α-diol [**3**], (22*E*,24*S*)-24-methyl-5α-cholesta-8(14),22-diene-3β,7α-diol [**4**], 24-methyl-5α-cholesta-8(14),24(28)-diene-3β,7α-diol [**5**], (24*Z*)-24-ethyl-5α-cholesta-8(14),24(28)-diene-3β,7α-diol [**6**], and (24*E*)-24-ethyl-5α-cholesta-8(14),24(28)-diene-3β,7α-diol [**7**], along with the previously synthesized 5α-cholest-8(14)-ene-3β,7α-diol [**1**]. The structure of the new natural products was determined by interpretation of spectral data and secured by synthesis of **1** starting from 5α-cholest-7-en-3β-ol.

Sponges have been shown to contain many polyoxygenated sterols in the last decade. In particular species belonging to the Dysideidae family are rich sources of Δ^7-polyhydroxysterols (1–5). As part of our continuing interest in the chemistry of steroids with multiple oxygen functionalities, we report the structural elucidation of seven new $\Delta^{8(14)}$-3β,7α-dihydroxysterols from the sponge *Pellina semitubulosa* (Lamarck) sensu Griessinger (6) and Pulitzer-Finali (7) (order Haplosclerida, family Oceanapiidae), a common sponge in the southern area of the Marsala Lagoon (Sicily) where it lives in shallow waters.



RESULTS AND DISCUSSION

The Et_2O-soluble material from Me_2CO and $CHCl_3$-MeOH (1:1) extracts of the sponge was chromatographed over Si gel using $CHCl_3$/MeOH mixtures as eluent. The fractions eluted with $CHCl_3$-MeOH (9:1) were subjected to hplc separation on Si gel columns. The fraction containing 8(14)-ene-3β,7α-dihydroxysterols was then subjected to reversed-phase hplc [MeOH-H_2O (88:12)] in order to separate the individual compounds.

The major component **5** of the sterol mixture gave a molecular ion peak in the hrms at *m/z* 414.3486, corresponding to a molecular formula of $C_{28}H_{46}O_2$ requiring six sites of unsaturation, and other significant peaks at *m/z* 396.3358 $[M-H_2O]^+$, 363.3028 $[M-2H_2O-Me]^+$, 289.2143 $[M-C_9H_{17}]^+$, 271.2068 $[M-C_9H_{17}-H_2O]^+$, and 253.1954 $[M-C_9H_{17}-2H_2O]^+$ that indicated the presence of two hydroxyl groups and a C_9H_{17} monounsaturated side chain. The ir spectrum showed hydroxyl absorption at 3365 cm^{-1}. The ^{1}H-nmr spectrum (Table 1) contained signals for five methyl groups consistent with the sterol carbon skeleton: singlets at δ 0.67 and 0.85 (H_3-18 and H_3-19), a doublet at δ 0.96 (H_3-21) and a pair of doublets at δ 1.026 and 1.021 (H_3-26 and H_3-27).

^{13}C- and ^{1}H-nmr spectra (Table 1) contained, respectively, resonances at δ 66.73 (C-7) and 71.09 (C-3) and 4.52 (1H, bdd, H-7) and 3.65 (1H, dddd, H-3) for two hydroxymethine functions, and signals for terminal and tetrasubstituted double bonds [^{13}C nmr: δ 156.74, 148.24, 128.51, 106.00; ^{1}H nmr: δ 4.62 and 4.65 (1H each, bs's, H_2-28)].

Comparison of the carbon side-chain resonances of 24-methylene cholesterol and other steroids of our collection with those exhibited by **5** suggested a 24-methylene cholesterol-type side chain for this sterol. This was confirmed by the presence in the mass spectrum of **5** of fragments at *m/z* 330.2593 $[M-C_6H_{12}]^+$ and 315.2389 $[M-C_6H_{12}-Me]^+$, deriving from the McLafferty rearrangement, and by the chemical shifts of the exo-methylene group (H_2-28 δ 4.65 and 4.62) and the isopropyl moiety (H-25 δ 2.23,septet; H_3-26 and H_3-27 δ 1.021 and 1.026) in the proton spectrum. Hence, we were left with two secondary hydroxyl groups and a fully substituted double bond to be arranged within the nucleus of this sterol to complete the structure determination.

The nuclear double bond could only be located at the $\Delta^{8(9)}$ or $\Delta^{8(14)}$ position. The complexity (seven-line multiplet) and chemical shift of the carbinol methine signal resonating at δ 3.65 were those normally seen for a 3α-carbinol proton of an A/B trans-steroid, while the proton resonance at δ 4.53 suggested that the second OH group was allylic.

Definitive structural information was obtained from ^{1}H-^{1}H COSY-45 (8) and proton double quantum (9) 2D nmr spectra, which allowed the identification in the molecule of the two independent scalar coupled spin systems pertaining to the C-1 to C-7 and C-9 to C-12 structural fragments. Particularly useful for the identification of H_{ax}-1 and H_{ax}-12 resonances, which we took as starting points for tracing out the proton connections within the above fragments, were the correlation peaks observed between H_{ax}-1 and Me-19 and H_{ax}-12 and Me-18 (W-type couplings) in the COSY spectrum. The COSY spectrum showed that H_{ax}-1 was connected with three other resonances, two of which, at δ 1.35 and 1.82, were coupled to each other. These latter two must be due to the geminal pair at C-2, the remaining one being ascribable to the H_{eq}-1 which in the 1D spectrum resonated as a clean double double doublet (J = 12.5, 2.9 and 2.9 Hz) at δ 1.68. The digital resolution (3 Hz/point) used for acquiring the COSY spectrum was adequate to resolve large couplings such as axial-axial or geminal couplings

TABLE 1. Selected ^{1}H- and ^{13}C-nmr Data for Compound **5**[a].

Position	δH[b] (m, *J*)	δC[c]
1	H_{ax} 1.16 (m)[e]	36.27
	H_{eq} 1.68 (ddd, 12.5, 2.9, 2.9)	
2	H_{ax} 1.35 (m)[d]	31.45
	H_{eq} 1.82 (m)[d]	
3	3.65 (dddd, 11.0, 11.0, 5.0, 5.0)	71.09
4	H_{ax} 1.23 (m)[e]	37.79
	H_{eq} 1.60 (m)[e]	
5	1.76 (dddd, 12.5, 12.5, 2.9, 2.9)	37.17
6	H_{ax} 1.42 (ddd, 12.5, 12.5, 2.9)[d]	35.54
	H_{eq} 1.54 (ddd, 12.5, 2.9, 2.9)[d]	
7	H_{eq} 4.52 (bdd, 2.4, 2.4)	66.73
8		128.51
9	1.15 (m)[e]	44.18
10		36.77
11	H_{ax} 1.46 (m)[d]	19.37
	H_{eq} 1.63 (m)[e]	
12	H_{ax} 1.15 (m)[e]	36.77
	H_{eq} 1.96 (ddd, 12.5, 3.7, 3.7)	
13		42.98
14		148.24
15		25.08
16		26.86
17		56.27
18	0.85 (s)	17.94
19	0.67 (s)	11.88
20	1.50 (m)[e]	34.33
21	0.96 (d, 6.7)	19.03
22		34.33
23	H_a 2.08 (m)[d]	30.71
	H_b 1.90 (m)[e]	
24		156.74
25	2.23 (septet, 6.6)	36.27
26	1.026 (d, 6.7)[f]	21.85[f]
27	1.021 (d, 6.7)[f]	21.98[f]
28	H_a 4.65 (bs)	106.00
	H_b 4.62 (bs)	

[a]The ^{1}H-nmr spectrum was recorded in $CDCl_3$ at 400 MHz. The ^{13}C-nmr spectrum was recorded in $CDCl_3$ at 100.1 MHz.

[b]Assignments are based on a ^{1}H-^{1}H COSY-45 experiment.

[c]Assignments are based on reported data for synthetic 5α-cholest-8(14)-ene-3β,7α-diol (16) and comparison with 24-methyl-5α-cholesta-7,24(28)-diene-3β,6α-diol (17) and 24-methyl-5α-cholesta-7,24(28)-diene-3β,5,6β,9-tetraol (18).

[d]Overlapped with other signals.

[e]Submerged by other signals.

[f]Assignments may be reversed.

between protons in a cyclohexane ring. Thus, we could observe that the signal at δ 1.35 was a quartet (only considering the large couplings) and that at δ 1.35 a doublet as expected for H_{ax}-2 and H_{eq}-2, respectively, in the saturated A ring of a 3β-hydroxysterol. These two protons both showed coupling with the H-3 hydroxymethine proton at δ 3.65 (dddd), which in turn had cross peaks with two other methylene protons resonating, overlapped to other signals, at δ 1.23 and 1.60. The multiplicity (ddd) extracted from the COSY spectrum of the signal at δ 1.23 indicated it to be the H_{ax}-4 proton next

to a C-5 unsubstituted position. Also observed in the COSY spectrum were the couplings between H_{ax}-4 and H_{eq}-4 and H-5, H-5, and H_2-6, and those of the latter ones with H-7. The C-9–C-12 structural fragment was built up starting from H_{ax}-12, which resonated almost at the same frequency as H_{ax}-1 (Table 1), as follows. H_{ax}-12 exhibited a coupling pattern similar to that observed for H_{ax}-1, having cross peaks with signals at δ 1.46, 1.63, and 1.96. The latter proton was a double double doublet (J = 12.5, 3.7, and 3.7 Hz) in the proton spectrum and was assigned to H_{eq}-12. The other two signals were attributable to H_2-11. These were both connected to a proton at δ 1.15 (H-9), which showed no further couplings. These observations were rationalized by locating the nuclear double bond at the $\Delta^{8(14)}$ position and, of course, the two -OH groups at C-3 and C-7. The proton double quantum 2D nmr spectrum was less informative, only confirming some proton connectivities within the C-1–C-7 and C-9–C-12 segments.

The α orientation of 7-OH followed from the value of the coupling constants (J = 2.4 and 2.4 Hz) of H-7, which required that this proton be spatially oriented in such a way as to form with the H_2-6 protons two dihedral angles near 60° (as judged from the examination of the Drieding model of **5**). The multiplicity of the H_2-6 signals, though hardly discernible in the crowded upfield region of the $CDCl_3$ proton spectrum, was clearly observable in the spectrum recorded in pyridine-d_5 (H_{eq}-6 δ 1.85, ddd, J = 13.2, 2.9 and 2.9 Hz; H_{ax}-6 δ 1.55, ddd, J = 13.2, 13.2 and 2.9 Hz).

Further corroboration for the α configuration of the 7-OH group arose from the observation of the pyridine-induced shift (10) of the H_2-6 protons in comparison with the values they exhibited in the spectrum recorded in $CDCl_3$. While H_{ax}-6 experienced a rather reduced downfield shift (Δδ 0.13 ppm), H_{eq}-6 was downshifted to a larger extent (Δδ 0.31 ppm). This observation was consistent with the spatial vicinity of the latter proton with 7-OH.

All the above data indicated a 24-methyl-5α-cholesta-8(14),24(28)-diene-3β,7α-diol structure for compound **5**.

Final confirmation for structure **5** came from the synthesis of 5α-cholest-8(14)-ene-3β,7α-diol, a product also found in the natural steroidal mixture, following a described procedure (11).

The ^{1}H-nmr spectra of compounds **1–6** all showed the presence of the double double doublet at δ 3.65, associated with the presence of H_{ax}-3, the deshielded resonance at δ 4.53, assigned to H_{eq}-7, and methyl singlets at δ 0.67 and 0.85 (H_3-18 and H_3-19), indicating that all the isolated sterols possessed identical nuclear structure and differed only in the side chain. This was supported by the mass spectra of all the sterols that contained common fragment ions at m/z 289 $[M - \text{side chain}]^+$, 271 $[M - \text{side chain} - H_2O]^+$, and 253 $[M - \text{side chain} - 2H_2O]^+$.

Compound **1** has not previously been found as a naturally occurring sterol but has been synthesized (11). An authentic specimen prepared as described in the literature (11) was identical in all respects with the naturally occurring sterol **1**.

The molecular formula $C_{27}H_{44}O_2$ for **2** was established by hrms. The presence of an unsaturated C_8H_{15} side chain was indicated by the ion peaks at m/z 289 $[M - C_8H_{15}]^+$, 271, and 253. The ^{1}H-nmr spectrum of **2** displayed two one-proton resonances at δ 5.31 (ddd, J = 15.4, 6.6, and 6.6 Hz, H-23) and 5.23 (dd, J = 15.4 and 8.1 Hz, H-22) and methyl doublets at δ 1.03 (3H, J = 6.6 Hz, H_3-21) and 0.865 (6H, J = 6.6 Hz, H_3-26 and H_3-27). The above ^{1}H-nmr data, when compared with those exhibited by a number of other steroids having a trans Δ^{22}-cholesterol-type side chain (12–14), strongly suggested a side chain of this type for our compound. Confirmatory evidence for the above structure was gained by spin decoupling experiments, which allowed the

entire structural fragment C-21–C-26 (C-27) to be built up. Thus, the structure of this sterol was established as (22*E*)-5α-cholesta-8(14),22-diene-3β,7α-diol [**2**].

Sterols **3** and **4** showed very similar hplc retention times on an RP-18 column. They could be obtained as pure compounds only by cutting the fronts and tails of their hplc peaks. The molecular formula, $C_{28}H_{46}O_2$ for both sterols, was established by hreims on the molecular ion peak at *m/z* 414. Their mass spectra exhibited the same fragmentation pattern, suggesting that a close structural relationship existed between these compounds; they contained mass peaks at *m/z* 289 $[M - C_9H_{17}]^+$, 271, and 253 for C_{28} sterols with a C_9H_{17} side chain containing a double bond. The ^{1}H-nmr spectra of **3** and **4** were very similar to each other and showed virtually identical chemical shift values for methyl doublets resonating at δ 0.92 (H_3-28), 0.83 and 0.84 (H_3-26 and H_3-27). Both contained a further coupled AB system attributable to a trans disubstituted double bond (J_{AB} = 15.4 Hz) centered at δ 5.20. The only noticeable difference in their ^{1}H-nmr spectra was confined to the chemical shift value of a fourth methyl group (H_3-21), which in one of the isomers (**3**) resonated at δ 1.032 and in the other one (**4**) at δ 1.023. These data, in conjunction with evidence arising from decoupling studies, unequivocally indicated that the compounds under investigation differed from sterol **2** only by the presence of an additional methyl group at C-24 with either an *R* or an *S* configuration. The configuration at the C-24 chiral center in the two isomers was assigned on the basis of the chemical shift value of the H_3-21 methyl group, which in the (24*R*)-epimer is upfield (δ 1.023) when compared with the resonance pertinent to the (24*S*)-epimer (1.032) (15). Therefore, **3** must be formulated as (22*E*,24*R*)-24-methyl-5α-cholest-8(14),22-diene-3β,7α-diol and **4** as (22*E*,24*S*)-24-methyl-5α-cholest-8(14),22-diene-3β,7α-diol.

The dihydroxysterols **6** and **7** could not be separated by reversed-phase hplc. The molecular formula of the two sterols was established as $C_{29}H_{48}O_2$ by accurate mass measurement on the molecular ion peak at *m/z* 428. Fragment ions at *m/z* 289, 271, and 253 indicated the presence of a $C_{10}H_{19}$ side chain containing one double bond, while the intense ion peak at *m/z* 297.2207 $[M - H_2O - CH_3 - C_7H_{14}]^+$, derived from a McLafferty rearrangement, suggested that a $\Delta^{24(28)}$-unsaturation was present in the side chain. ^{1}H-nmr decoupling experiments established the presence of ethylidene groups attached to C-24 for both compounds. Since for both epimers the chemical shifts of the side chain signals were consistent with those of authentic samples of fucosterol and 28-isofucosterol, the structures of **6** and **7** were formulated as (24*Z*)-24-ethyl-5α-cholesta-8(14),24(28)-diene-3β,7α-diol and (24*E*)-24-ethyl-5α-cholesta-8(14),24(28)-diene-3β,7α-diol, respectively. The stereochemistry of the $\Delta^{24(28)}$ double bond was deduced from the chemical shift of the H-25 protons, which were characterized by a septet centered at δ 2.83 in **6** (*Z*-isomer) and at δ 2.20 in **7** (*E*-isomer). The ^{1}H-nmr spectrum also indicated that compounds **6** and **7** were in the approximate ratio of 1:2.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—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. The ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker WM-400 spectrometer in $CDCl_3$ solutions. The ^{1}H spectrum of **5** was also recorded in pyridine-d_5. 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). Ir spectra were obtained with a Perkin-Elmer 1760-X Ft-ir. Hplc was carried out on a Varian 2510 pump and a Waters Associates R 403 differential refractometer. Melting point for synthetic **1** was determined on a Kofler apparatus and is uncorrected.

BIOLOGICAL MATERIALS.—*P. semitubulosa* is a common sponge in the southern areas of the Marsala Lagoon where it lives in shallow waters (0.5–1.5 m depth), free on soft bottoms, or epibiotic on algae. About 100 specimens have been collected; they were massive with several fistulae. The massive body was

about 10 cm in diameter; fistulae were from 0.5 to 2 cm in length. The color varied from whitish to the typical mauve. A voucher specimen is on file at our laboratories.

EXTRACTION AND ISOLATION.—The fresh sponge (440 g dry wt after extraction) was extracted twice with Me_2CO (24 h for each extraction) and twice with $CHCl_3$-MeOH (1:1). The extracts were combined and concentrated in vacuo to give an aqueous suspension which was extracted with Et_2O. The Et_2O solution was evaporated to give an oily residue (29.3 g), which was fractionated on an open column of SiO_2 (600 g, 4 cm diameter) using $CHCl_3$ and increasing amounts of MeOH in $CHCl_3$ as eluent. Fractions of 200 ml were collected and analyzed by tlc on SiO_2. Fractions 21–22 (461 mg), eluted with $CHCl_3$-MeOH (9:1), were combined and subjected to flash chromatography eluting with the same solvent system; 50-ml fractions were collected. Fractions 17–18 from this separation contained the crude dihydroxysterol material, which was further purified by two successive normal phase hplc steps on Hibar LiChrosorb Si-60 columns [250 × 10 mm, eluent *n*-hexane–EtOAc (6:4); 250 × 4 mm, eluent $CHCl_3$-MeOH (95:5)]. The mixture of dihydroxysterols (7 mg), pure by tlc, was fractionated by reversed-phase hplc on a Hibar Superspher RP-18 (250 × 4 mm) column eluted with MeOH-H_2O (88:12) to obtain the following fractions in order of elution: fractions 1 and 2 contained **2** (1 mg) and **5** (3.4 mg), respectively; fraction 3 (2.1 mg) was reinjected and contained **1** (0.6 mg), **3** (0.7 mg), and **4** (0.8 mg). Fraction 4 (1.7 mg) contained **6** and **7**.

SPECTRAL DATA.—*5α-Cholest-8(14)-ene-3β,7α-diol* [**1**].—Ft-ir (film) ν max 3365 cm^{-1}; ^1H nmr ($CDCl_3$) δ 4.53 (1H, dd, J = 2.4 and 2.4 Hz, H_{eq}-7), 3.65 (1H, dddd, J = 11.0, 11.0, 5.0, 5.0 Hz, H-3), 0.94 (3H, d, J = 6.1 Hz, H_3-21), 0.87 (6H, d, J = 6.7 Hz, H_3-26 and H_3-27), 0.85 (3H, s, H_3-19), 0.67 (3H, s, H_3-18); hrms *m/z* (rel. int.) $[M]^+$ 402.3509 (calcd for $C_{27}H_{46}O_2$, 402.3497) (15), $[M-H_2O]^+$ 384.3378 ($C_{27}H_{44}O$) (39), $[M-H_2O-Me]^+$ 369.3150 ($C_{26}H_{41}O$) (28), $[M-2H_2O-Me]^+$ 351.3067 ($C_{26}H_{39}$) (7), [M − side chain]$^+$ 289.2186 ($C_{19}H_{29}O_2$) (5), [M − side chain − H_2O]$^+$ 271.2073 ($C_{19}H_{27}O$) (100), [M − side chain − H_2O − 2H]$^+$ 269.1901 ($C_{19}H_{25}O$) (6), [M − side chain − $2H_2O$]$^+$ 253.1945 ($C_{19}H_{25}$) (31), [M − side chain − $2H_2O$ − 2H]$^+$ 251.1787 ($C_{19}H_{23}$) (2), [M − side chain − $2H_2O$ and ring D fission]$^+$ 211.1505 ($C_{16}H_{19}$) (14).

(22E)-5α-Cholesta-8(14),22-diene-3β,7α-diol [**2**].—Ft-ir (film) ν max 3365 cm^{-1}; ^1H nmr ($CDCl_3$) δ 5.31 (1H, ddd, J = 15.4, 6.6, and 6.6 Hz, H-23), 5.23 (1H, dd, J = 15.4 and 8.1 Hz, H-22), 4.52 (1H, bdd, J = 2.4 and 2.4 Hz, H_{eq}-7), 3.65 (1H, dddd, J = 11.0, 11.0, 5.0, 5.0 Hz, H-3), 2.08 (1H, m, overlapped to other signals, H-20), 1.85 (2H, m, overlapped, H_2-24), 1.58 (1H, m, overlapped, H-25), 1.03 (3H, d, J = 6.7 Hz, H_3-21), 0.860 (3H, s, H_3-19), 0.865 (6H, d, J = 6.6 Hz, H_3-26 and H_3-27), 0.67 (3H, s, H_3-18); hrms *m/z* (rel. int.) $[M]^+$ 400.3369 (calcd for $C_{27}H_{44}O_2$, 400.3341) (58), $[M-H_2O]^+$ 382.3231 ($C_{27}H_{42}O$) (74.2), $[M-H_2O-Me]^+$ 367.2999 ($C_{26}H_{39}O$) (46), $[M-2H_2O-Me]^+$ 349.2882 ($C_{26}H_{37}$) (5), 289.2190 ($C_{19}H_{29}O_2$) (30), 271.2042 ($C_{19}H_{27}O$) (100), 269.1872 ($C_{19}H_{25}O$) (13), 253.1952 ($C_{19}H_{25}$) (51), 251.1804 ($C_{19}H_{23}$) (4), 211.1494 ($C_{16}H_{19}$) (13).

(22E,24R)-24-Methyl-5α-cholesta-8(14),22-diene-3β,7α-diol [**3**].—Ft-ir (film) ν max 3365 cm^{-1}; ^1H nmr ($CDCl_3$) δ 5.20 (2H, AB system further coupled, J_{AB} = 15.3 Hz, H-22 and H-23), 4.53 (1H, bdd, J = 2.4 and 2.4 Hz, H_{eq}-7), 3.65 (1H, dddd, J = 11.0, 11.0, 5.0, 5.0 Hz, H-3), 2.08 (1H, m, overlapped, H-20), 1.62 (1H, m, overlapped, H-24), 1.58 (1H, m, overlapped, H-25), 1.032 (3H, d, J = 6.7 Hz, H_3-21), 0.86 (3H, s, H_3-19), 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), 0.83 (3H, d, J = 6.7 Hz, H_3-27 or H_3-26), 0.67 (3H, s, H_3-18); hrms *m/z* (rel. int.) $[M]^+$ 414.3452 (calcd for $C_{28}H_{46}O_2$, 414.3472) (7), $[M-H_2O]^+$ 396.3412 ($C_{28}H_{44}O$) (33), $[M-H_2O-Me]^+$ 381.3187 ($C_{27}H_{41}O$) (22), $[M-2H_2O-Me]^+$ 363.3056 ($C_{27}H_{39}$) (5), 289.2208 ($C_{19}H_{29}O_2$) (8), 271.2056 ($C_{19}H_{27}O$) (100), 269.1887 ($C_{19}H_{25}O$) (20), 253.1972 ($C_{19}H_{25}$) (45), 251.1752 ($C_{19}H_{23}$) (9), 211.1462 ($C_{16}H_{19}$) (14).

(22E,24S)-24-Methyl-5α-cholesta-8(14),22-diene-3β,7α-diol [**4**].—Ft-ir (film) ν max 3365 cm^{-1}; ^1H nmr ($CDCl_3$) δ 5.20 (2H, AB system further coupled, J_{AB} = 15.3 Hz, H-22 and H-23), 4.52 (1H, dd, J = 2.4 and 2.4 Hz, H_{eq}-7), 3.65 (1H, ddd, J = 11.0, 11.0, 5.0, 5.0 Hz, H-3), 2.08 (1H, m, overlapped, H-20), 1.62 (1H, m, overlapped, H-24), 1.58 (1H, m, overlapped, H-25), 1.023 (3H, d, J = 6.7 Hz, H_3-21), 0.86 (3H, s, H_3-19), 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), 0.83 (3H, d, J = 6.7 Hz, H_3-27 or H_3-26), 0.67 (3H, s, H_3-18); hrms *m/z* (rel. int.) $[M]^+$ 414.3502 (calcd for $C_{28}H_{46}O_2$, 414.3497) (10), $[M-H_2O]^+$ 396.3412 ($C_{28}H_{44}O$) (28), $[M-H_2O-Me]^+$ 381.3187 ($C_{27}H_{41}O$) (20), $[M-2H_2O-Me]^+$ 363.3056 ($C_{27}H_{39}$) (5), 289.2208 ($C_{19}H_{29}O_2$) (7), 271.2056 ($C_{19}H_{27}O$) (100), 269.1887 ($C_{19}H_{25}O$) (21), 253.1972 ($C_{19}H_{25}$) (47), 251.1752 ($C_{19}H_{23}$) (13), 211.1462 ($C_{16}H_{19}$) (11).

24-Methyl-5α-cholesta-8(14),24(28)-diene-3β,7α-diol [**5**].—Ft-ir (film) ν max 3365 cm^{-1}; ^1H nmr ($CDCl_3$) see Table 1; ^1H nmr (pyridine-d_5) δ 6.07 (1H, d, J = 4.9 Hz, OH-3), 5.76 (1H, bs, OH-7), 4.86 and 4.82 (2H, bs's, H_2-28), 4.78 (1H, bs, H-7), 3.93 (1H, m, H-3), 1.85 (1H, ddd, J = 13.2, 2.9 and

2.9 Hz, H_{eq}-6), 1.55 (1H, ddd, J = 13.2, 13.2 and 2.9 Hz, H_{ax}-6), 1.05 (3H, d, J = 6.7 Hz, H_3-26 or H_3-27), 1.04 (3H, d, J = 6.7 Hz, H_3-27 or H_3-26), 0.94 (3H, d, J = 5.5 Hz, H_3-21), 0.88 (3H, s, H_3-19), 0.77 (3H, s, H_3-18); ^{13}C nmr see Table 1; hrms *m/z* (rel. int.) $[M]^+$ 414.3486 (calcd for $C_{28}H_{46}O_2$, 414.3497) (69), $[M-H_2O]^+$ 396.3358 ($C_{28}H_{44}O$) (100), $[M-H_2O-Me]^+$ 381.3142 ($C_{27}H_{41}O$) (67), $[M-2H_2O-Me]^+$ 363.3028 ($C_{27}H_{39}$) (5), $[M-C_6H_{12}]^+$ 330.2593 ($C_{22}H_{34}O_2$) (3), $[M-84-Me]^+$ 315.2389 ($C_{21}H_{31}O_2$) (2), $[M-84-H_2O-Me]^+$ 297.2216 ($C_{21}H_{29}O$) (52), 289.2143 ($C_{19}H_{29}O_2$) (4), 271.2068 ($C_{19}H_{27}O$) (68), 269.1899 ($C_{19}H_{25}O$) (16), 253.1954 ($C_{19}H_{25}$) (23), 251.1811 ($C_{19}H_{23}$) (6), 211.1496 ($C_{16}H_{19}$) (8).

(24Z)-24-Ethyl-5α-cholesta-8(14),24(28)-diene-3β,7α-diol [**6**] *and (24E)-24-ethyl-5α-cholesta-8(14),24(28)-diene-3β,7α-diol* [**7**].—Ft-ir (film) ν max 3365 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 5.19 (q, J = 6.8 Hz, H-28 of **7**), 5.11 (q, J = 6.8 Hz, H-28 of **6**), 4.53 (bdd, J = 2.4 and 2.4 Hz, H_{eq}-7), 3.65 (dddd, J = 11.0, 11.0, 5.0, 5.0 Hz, H-3), 2.83 (septet, J = 6.6 Hz, H-25 of **6**), 2.20 (septet, J = 6.6 Hz, H-25 of **7**), 1.58 (d, J = 6.8 Hz, H_3-29 of **6**), 1.56 (d, J = 6.8 Hz, H_3-29 of **7**), 1.01 (d, J = 6.6 Hz, H_3-21 of **7**), 0.98 (d, J = 6.7 Hz, H_3-27 and H_3-26), 0.95 (d, J = 6.6 Hz, H_3-21 of **6**), 0.86 (s, H_3-19), 0.67 (s, H_3-18); hrms *m/z* (rel. int.) $[M]^+$ 428.3672 (calcd for $C_{29}H_{48}O_2$, 428.3654) (22), $[M-H_2O]^+$ 410.3619 ($C_{29}H_{48}O_2$) (100), $[M-H_2O-Me]^+$ 395.3297 ($C_{28}H_{43}O$) (33), $[M-2H_2O-Me]^+$ 377.3210 ($C_{28}H_{41}$) (2), 297.2207 ($C_{21}H_{29}O$) (32), 289.2172 ($C_{19}H_{29}O_2$) (3), 271.2078 ($C_{19}H_{27}O$) (80), 269.1901 ($C_{19}H_{25}O$) (5), 253.1963 ($C_{19}H_{25}$) (20), 251.1809 ($C_{19}H_{23}$) (4), 211.1496 ($C_{16}H_{19}$) (2).

SYNTHESIS OF 5α-CHOLEST-8(14)-ENE-3β,7α-DIOL [**1**].—Compound **1** prepared as described by Fieser and Ourrison (11). Synthetic **1** had: mp 153–155° [*n*-hexane–$CHCl_3$ (7:3)] [lit. (11) 157–158°]; Ft-ir (film) ν max 3365 cm^{-1}; ^{1}H nmr data ($CDCl_3$) virtually identical to those of natural **1**; eims *m/z* (rel. int.) 402 (17), 384 (41), 369 (29), 351 (9), 289 (5), 271 (78), 269 (6), 253 (29), 251 (2), 211 (13).

ACKNOWLEDGMENTS

This research was supported by "Ministero della Pubblica Istruzione." Mass spectral data were provided by the "Servizio di Spettrometria di Massa del CNR e dell'Universita' di Napoli." The assistance of the staff is gratefully acknowledged.

LITERATURE CITED

1. D.J. Faulkner, *Nat. Prod. Rep.*, **1**, 551 (1984).
2. D.J. Faulkner, *Nat. Prod. Rep.*, **4**, 539 (1987).
3. D.J. Faulkner, *Nat. Prod. Rep.*, **5**, 613 (1988).
4. D.J. Faulkner, *Nat. Prod. Rep.*, **7**, 269 (1990).
5. G. Notaro, V. Piccialli, and D. Sica, *J. Nat. Prod.*, **54**, 1570 (1991), and references cited therein.
6. J.M. Griessinger, *Bull. Mus. Natl. Hist. Nat.*, **3** (3), 97 (1971).
7. G. Pulitzer-Finali, *Bull. Mus. Ist. Biol. Univ. Genova*, **45**, 7 (1977).
8. A. Bax, R. Freeman, and G.A. Morris, *J. Magn. Reson.*, **42**, 164 (1981).
9. T.H. Mareci and R. Freeman, *J. Magn. Reson.*, **51**, 531 (1983).
10. P.V. Demarco, E. Farkas, D. Doddrell, B.L. Mylari, and E. Wenkert, *J. Am. Chem. Soc.*, **90**, 5480 (1968).
11. L.F. Fieser and G. Ourrison, *J. Am. Chem. Soc.*, **75**, 4404 (1953).
12. A. Madaio, V. Piccialli, D. Sica, and G. Corriero, *J. Nat. Prod.*, **52**, 952 (1989).
13. A. Madaio, G. Notaro, V. Piccialli, and D. Sica, *J. Nat. Prod.*, **53**, 565 (1990).
14. A. Migliuolo, G. Notaro, V. Piccialli, and D. Sica, *J. Nat. Prod.*, **53**, 1414 (1990).
15. T.B. Tam Ha, W.C.M.C. Kokke, and C. Djerassi, *Steroids*, **40**, 433 (1982).
16. M. Tsuda, E.J. Parish, and G.J. Schroepfer Jr., *J. Org. Chem.*, **44**, 1282 (1979).
17. V. Piccialli and D. Sica, *J. Nat. Prod.*, **49**, 779 (1986).
18. A. Madaio, G. Notaro, V. Piccialli, and D. Sica, *J. Nat. Prod.*, **53**, 565 (1990).

Received 27 September 1991