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NEW TETRAHYDROXYLATED STEROLS FROM THE MARINE SPONGE *SPONGIA OFFICINALIS*

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ABSTRACT.—Six new tetrahydroxylated sterols **1–6** were isolated from the marine sponge *Spongia officinalis*. The structures of these compounds, including stereochemical details, were deduced by ^1H and ^{13}C nmr, ^1H-^1H COSY, and nOe difference spectroscopy.

In recent years polyhydroxylated sterols have been isolated from marine sponges (1–3). Polyhydroxylated sterol sulfates have been reported in sponges of the Halichondriidae family (4–8), whereas Dictyoceratid sponges contain uncommon Δ^7-polyhydroxylated sterols (9–15). Sponges are also the source of polyhydroxylated 9,11-secosterols (16) and 5,6-secosterols (17,18). As part of our continuing researches on polyol sterols (11,12,14,17,18), we report the isolation and the structural elucidation of six new tetrahydroxylated sterols from the sponge *Spongia officinalis* L. (order Dictyoceratida, family Spongiidae).

RESULTS AND DISCUSSION

Fresh tissues of the sponge collected in the Bay of Napoli were extracted with Me_2CO and $CHCl_3$-MeOH (1:1), and the extracts were partitioned between Et_2O and H_2O. The Et_2O fraction was subjected to repeated Si gel chromatographies using increasing concentration of MeOH in $CHCl_3$ as eluent. The fractions enriched in tetrahydroxysterols were further separated by hplc on Si gel [$CHCl_3$ – MeOH (93:7)]. The final separation of the individual compounds of the polar mixture of sterols was achieved by reversed-phase hplc [MeOH-H_2O (80:20)].

High resolution mass measurement of the most abundant sterol **1** on the ion at *m/z* 416 $[M - H_2O]^+$ established a molecular formula of $C_{27}H_{46}O_4$. In agreement with the elemental composition, the ^{13}C-nmr spectrum in pyridine-d_5 (Table 1) contained signals arising from five methyls, ten methylenes, seven methines, and five non-proton-

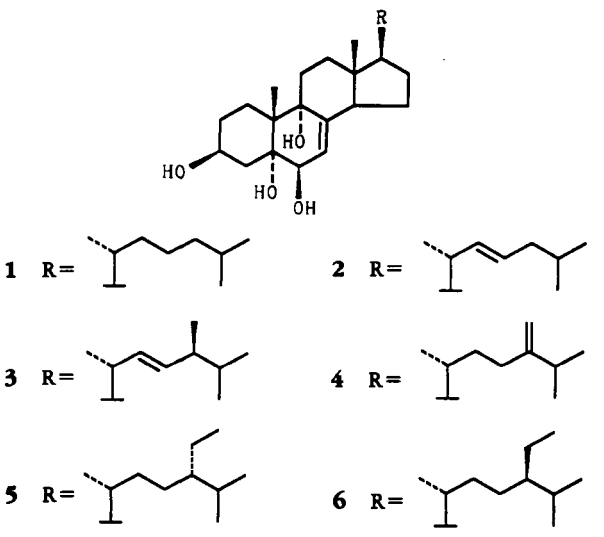

TABLE 1. ^{13}C Chemical Shift Values for Compounds **1–6**.[a]

Carbon	Compound					
	1	**2**	**3**	**4**	**5** and **6**	
C-1	29.05	29.05	29.06	29.05	29.06	
C-2	32.47	32.49	32.46	32.47	32.48	
C-3	67.35	67.35	67.38	67.35	67.35	
C-4	42.07	42.10	42.03	42.08	42.09	
C-5	74.98	75.01	75.06	74.98	74.98	
C-6	73.82	73.82	73.79	73.81	73.92	
C-7	121.34	121.34	121.36	121.36	121.34	
C-8	142.97	142.97	142.97	142.95	142.98	
C-9	78.70	78.70	78.73	78.70	78.70	
C-10	41.29	41.29	41.29	41.27	41.27	
C-11	29.05	29.05	29.06	29.05	29.06	
C-12	36.03	35.93	35.95	36.01	36.03	
C-13	44.27	44.17	44.17	44.29	44.27	
C-14	51.20	51.30	51.32	51.18	51.22	
C-15	23.52	23.49	23.55	23.49	23.53	
C-16	28.27	28.27	28.29	28.27	28.32	
C-17	56.48	56.10	56.13	56.28	56.39	
C-18	11.89	12.07	12.09	11.87	11.89	
C-19	22.48	22.48	22.49	22.48	22.48	
C-20	36.03[b]	40.86	40.99	36.01	37.05	36.91
C-21	19.07	21.29	21.43	19.03	19.16[c]	
C-22	36.51[b]	138.47	136.45	34.96	34.11[d]	34.16[d]
C-23	24.20	126.65	132.22	31.36	26.78	26.50
C-24	39.72	42.20	43.34	156.72	46.32	46.09
C-25	28.27	28.78	33.50	36.01	29.28	29.51
C-26–27	22.48	22.48	19.87	22.00	19.16[c]	20.00
	22.68	22.48	20.39	22.13	19.78	19.27[c]
C-28			18.30	106.64	23.34[e]	23.39[e]
C-29					12.52[f]	12.17[f]

[a] ^{13}C-nmr spectra were recorded at 100.1 MHz. The chemical shift values are given in parts per million (ppm) and referenced to pyridine-d_5 (149.9 ppm). Assignments are based on DEPT experiments and comparison with a model compound (12).

[b-f]Values with identical superscripts may be interchanged.

ated carbons as deduced from DEPT experiments. Four of these signals were assigned to carbon atoms bearing hydroxyl groups, two secondary at δ 67.35 and 73.82, and two tertiary at δ 74.98 and 78.70. Two carbons resonating in the sp^2 region at δ 142.97 (>C=) and 121.34 (=CH−) indicated the presence of a monosubstituted double bond in the molecule, also evident in the ^1H-nmr spectrum (CD_3OD) (Table 2), which included one olefinic proton at δ 5.34. The presence of four hydroxyl groups in **1** was also supported by the mass spectrum, which exhibited ion peaks at *m/z* 416 $[M-H_2O]^+$, 398 $[M-2H_2O]^+$, 380 $[M-3H_2O]^+$, and 362 $(M-4H_2O]^+$, and confirmed by the ^1H-nmr spectrum recorded in pyridine-d_5 (Table 3) that showed the presence of two additional one-proton singlets at δ 6.92 and 6.21 and two one-proton doublets at δ 6.59 and 6.14. The high field region of the ^1H-nmr spectrum in CD_3OD contained signals for five methyl groups of a cholestane structure: singlets at δ 0.65 and 1.11 (H_3-18) and H_3-19), a doublet at δ 0.96 (H_3-21), and a pair of doublets at δ 0.887 and 0.885 (H_3-26 and H_3-27) that were shown to belong to an isopropyl group since they collapsed to two singlets on irradiation at δ 1.55 (H-25). The seven-line multiplet at δ 4.00 had the normal complexity of the 3α-carbinol proton of an A/B trans steroid (19). Its somewhat

TABLE 2. Selected 400 MHz ^{1}H-nmr Chemical Shifts (CD_3OD) of Tetrahydroxysterols **1–6**.[a]

Proton	Compound					
	1	**2**	**3**	**4**	**5**	**6**
H-1ax	2.21 ddd (13.4,3.7,3.7)	2.21 ddd (14.0,3.7,3.7)	2.20 ddd (13.4,3.7,3.7)	2.20 ddd (13.4,3.7,3.7)	2.20 ddd (13.4,3.7,3.7)	2.20 ddd (13.4,3.7,3.7)
H-1eq	1.35[b,c]	1.35[b,c]	1.35[b,c]	1.35[b,c]	1.35[b,c]	1.35[b,c]
H-2ax	1.51[b,c]	1.51[b,c]	1.51[b,c]	1.51[b,c]	1.51[b,c]	1.51[b,c]
H-2eq	1.86[b,c]	1.86[b,c]	1.86[b,c]	1.86[b,c]	1.86[b,c]	1.86[b,c]
H-3	4.00 m	4.00 m	3.99 m	3.99 m	3.99 m	3.99 m
H-4ax	2.12 dd (13.4,13.4)	2.12 dd (13.4,13.4)	2.11 dd (13.4,13.4)	2.12 dd (13.4,13.4)	2.11 dd (13.4,13.4)	2.11 dd (13.4,13.4)
H-4eq	1.66 bdd (13.4,4.9)	1.66 bdd (13.4,4.9)	1.66 bdd (13.4,4.9)	1.66 bdd (13.4,4.9)	1.66 bdd (13.4,4.9)	1.66 bdd (13.4,4.9)
H-6	3.65 dd (5.5,2.4)	3.65 dd (5.5,2.4)	3.65 dd (5.5,2.4)	3.65 dd (5.5,2.4)	3.65 dd (5.5,2.4)	3.65 dd (5.5,2.4)
H-7	5.34 dd (5.5,2.4)	5.34 dd (5.5,2.4)	5.33 dd (5.5,2.4)	5.34 dd (5.5,2.4)	5.33 dd (5.5,2.4)	5.33 dd (5.5,2.4)
H-14	2.49 dddd (9.8,7.3,2.4,2.4)	2.49 dddd (9.8,7.3,2.4,2.4)	2.50 dddd (9.8,7.3,2.4,2.4)	2.50 dddd (9.8,7.3,2.4,2.4)	2.49 dddd (9.8,7.3,2.4,2.4)	2.49 dddd (9.8,7.3,2.4,2.4)
H-15a	1.61[b,c]	1.61[b,c]	1.61[b,c]	1.61[b,c]	1.61[b,c]	1.61[b,c]
H-15b	1.53[b,c]	1.53[b,c]	1.53[b,c]	1.53[b,c]	1,53[b,c]	1.53[b,c]
H-18	0.65 s	0.65 s	0.66 s	0.65 s	0.65 s	0.65 s
H-19	1.11 s	1.11 s	1.11 s	1.11 s	1.11 s	1.11 s
H-20	1.46[b,c]	2.05 m	2.04 m	1.42[c] m	1.40[b,c]	1.40[b,c]
H-21	0.96 d (6.1)	1.06 d (6.7)	1.22 d (6.7)	1.02 d (6.1)	0.97 d (6.1)	0.97 d (6.1)
H-22		5.26 dd (15.3,8.5)	5.18 dd (14.6,7.9)	Ha 1.59[b,c]		
H-23		5.36[d] m	5.23 dd (14.6,6.7)	Hb 1.18[c] m Ha 2.13[c] m Hb 1.92[b,c]		
H-24		Ha and Hb 1.85[c] m	1.62[c] m			
H-25	1.55[c] m	1.58[b,c]	1.47 m	2.28 b septet (6.7)	1.72 m	1.72 m

TABLE 2. Continued.

Proton	Compound					
	1	**2**	**3**	**4**	**5**	**6**
H-26-27	0.887 d (6.7) 0.885 d (6.7)	0.91 d (6.7) 0.91 d (6.7)	0.87 d (6.7) 0.85 d (6.7)	1.039 d (6.7) 1.032 d (6.7)	0.85 d (6.7) 0.87 d (6.7)	0.85 d (6.7) 0.87 d (6.7)
H-28			0.94 d (6.7)	Ha 4.73 bs Hb 4.66 bs		
H-29					0.886 t (7.3)	0.880 t (7.3)

[a]*J* values (Hz) are given in parentheses.
[b]Values deduced from ^{1}H-^{1}H COSY spectrum.
[c]Overlapped to other signals
[d]Overlapped to the H-7 signal.

TABLE 3. Selected 400 MHz ^{1}H-nmr Chemical Shifts (C_5D_5N) of Tetrahydroxysterols **1–6**.[a]

Proton	Compound				
	1	**2**	**3**	**4**	**5** and **6**
H-1ax	2.90 ddd (13.4,13.4,3.7)	2.91 ddd (13.4,13.4,3.5)	2.91 ddd (13.4,13.4,3.5)	2.90 ddd (13.4,13.4,3.7)	2.91 ddd (13.4,13.4,3.7)
H-1eq	1.67 bd (13.4)	1.67 bd (13.4)	1.67 bd (13.4)	1.67 bd (13.4)	1.67 bd (13.4)
H-2ax	2.08[b] m	2.08[b] m	2.08[b] m	2.08[b] m	2.08[b] m
H-2eq	2.35 bd (12.8)	2.35 bd (12.8)	2.35 bd (12.8)	2.35 bd (12.8)	2.35 bd (12.8)
H-3	4.81 m	4.81 m	4.82 m	4.81 m	4.81 m
3-OH	6.14 d (4.9)	6.14 d (4.9)	6.20 d (4.9)	6.13 d (4.9)	6.11 d (4.9)
H-4ax	3.06 dd (12.8,12.8)	3.06 dd (12.8,12.8)	3.06 dd (13.4,13.4)	3.06 dd (13.4,13.4)	3.06 dd (13.4,13.4)
H-4eq	2.48 dd (13.4,4.9)	2.48 dd (13.4,4.9)	2.48 dd (13.4,4.9)	2.48 dd (13.4,4.9)	2.48 dd (13.4,4.9)
5-OH	6.92 s	6.92 s	6.93 s	6.91 s	6.90 s
H-6	4.44 bs ($w_{1/2}$ = 11.6)	4.44 bs ($w_{1/2}$ = 11.6)	4.45 bs ($w_{1/2}$ = 11.6)	4.44 bs ($w_{1/2}$ = 11.6)	4.44 bs ($w_{1/2}$ = 11.6)
6-OH	6.59 d (6.1)	6.59 d (6.1)	6.61 d (6.1)	6.58 d (6.1)	6.57 d (6.1)
H-7	5.82 dd (4.9,1.8)	5.82 dd (4.9,1.9)	5.82 dd (4.9,1.9)	5.81 dd (4.9,1.8)	5.82 dd (4.9,1.8)
9-OH	6.21 s	6.21 s	6.23 s	6.20 s	6.20 s
H-14	2.94 m	2.94 m	2.93 m	2.94 m	2.93 m
H-18	0.68 s	0.69 s	0.69 s	0.67 s	0.68 s
H-19	1.60 s	1.60 s	1.60 s	1.59 s	1.60 s
H-20		2.02 m			
H-21	0.95 d (5.4)	1.22 d (6.7)	1.05 d (6.7)	0.97 d (6.1)	0.98 d (5.5)
H-22		5.23 dd (15.3,8.5)			

TABLE 3. Continued.

Proton	Compound				
	1	**2**	**3**	**4**	**5** and **6**
H-23		5.32 ddd (15.3,6.7,6.7)			
H-24		Ha and Hb 1.88 m			
H-25	1.49 m	1.57 m			
H-26–27 . . .	0.86 d (6.8)	0.86 d (6.7)	0.84 d (6.7)	1.04 d (6.7)	0.852,0.846 0.831,0.824
	0.86 d (6.8)	0.86 d (6.7)	0.83 d (6.7)	1.03 d (6.7)	d's (6.7)
H-28			0.93 d (6.7)	Ha 4.84 bs Hb 4.82 bs	
H-29					0.880,0.877 t's (6.7)

[a] ^{1}H-nmr spectra were recorded dissolving 0.6 mg of **1**, 0.7 mg of **2**, 0.8 mg of **3**, 1.4 mg of **4**, and 2.0 mg of the mixture of **5** and **6** separately in 0.5 ml of the solvent. *J* values (Hz) are given in parentheses.

[b] Overlapped to other signals.

low-field chemical shift is typical of 3β-hydroxysterols bearing at 5α-hydroxyl group (9). The proton connectivity pattern was determined by application of two-dimensional homonuclear (^{1}H-^{1}H) correlation spectroscopy using the COSY-45 sequence and by homo-decoupling spectral measurements. Particularly, the two double doublets at δ 2.12 and 1.66, mutually coupled and coupled with the 3α-proton at δ 4.00, were assigned, respectively, to the Hax-4 and Heq-4 protons next to the C-5 substituted position. The 3β-hydroxymethine proton also correlated with two nonequivalent methylene protons at δ 1.51 and 1.86 (Hax-2 and Heq-2, respectively) that, in turn, were coupled to one another and with a couple of protons resonating at 2.21 and 1.35 belonging to an additional methylene group (H_2-1). The olefinic signal at δ 5.34 (H-7) showed vicinal coupling with the hydroxymethine double doublet at δ 3.65 (H-6). Both these signals were coupled (J=2.4 and 2.4 Hz) with only one allylic methine proton at δ 2.51 (H-14), suggesting the presence of a hydroxyl group at the C-9 or C-14 position.

When the ^{1}H-nmr spectrum of **1** was run in pyridine-d_5, a remarkable downfield shift of the signals for Hax-1, Hax-3, Hax-4, and Me-19 was observed in comparison with the spectrum recorded in CD_3OD. The solvent shifts observed were rationalized by placement of the remaining two hydroxyl groups at the 6β and 9α positions, respectively (10,20). The axial disposition of the hydroxyl group at C-9 was also indicated by the slight downfield position of the Hax-1 signal in the ^{1}H-nmr spectrum of **1** (δ 2.21) in CD_3OD. The side chain structure of **1** was established by comparison of its ^{13}C chemical shifts with those of an authentic sample of cholesterol measured in pyridine-d_5. Thus, the structure of **1** was formulated as 5α-cholest-7-ene-3β,5,6β,9-tetraol. The agreement of the chemical shift values observed for the C-18 and C-19 protons in **1** with calculated values (21) for these groups also supported the above structure.

NOe difference experiments (nOeds) performed on compound **1** dissolved in pyridine-d_5 fully confirmed the above stereochemical assumptions on the relative orientation of hydroxyl groups and permitted the assignment of the overall relative stereochemistry of the molecule as the one depicted in Figure 1. Particularly, irradiation of the signal for 9α-OH at δ 6.21 produced significant enhancements of the Hax-1 and H-14 signals. The β disposition of the 6-OH group was confirmed by a positive nOe registered when the C-19 methyl protons at δ 1.60 were irradiated. Furthermore upon irradiation of the H-3α proton, a simultaneous enhancement of the Heq-2 and Heq-4 signals was observed indicating its axial disposition. The chirality at C-17 was

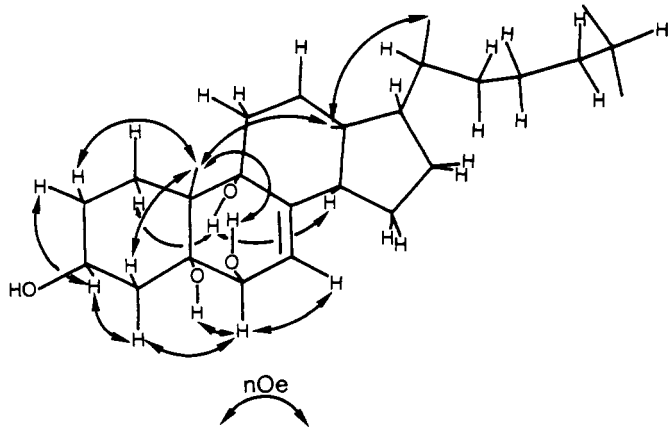

FIGURE 1. Summary of the nOe experiments for compound **1**.

also determined by nOeds experiments that proved that the Me-18 and Me-21 groups are in the nOe proximity.

The mass spectra of compounds **1–6** contained common fragment ions at *m/z* 285 $[M-2H_2O-$ side chain$]^+$, 267 $[M-3H_2O-$ side chain$]^+$, and 249 $[M-4H_2O-$ side chain$]^+$, indicating that all components of the sterol mixture possessed identical nuclei and varied only in the side chains. This was supported by ^1H- and ^{13}C-nmr data. Therefore, we only had to establish their side chain structures to complete the structural determination of each compound.

Compound **2** had the molecular formula $C_{27}H_{44}O_4$ deduced from the high resolution mass measurement of the highest peak observed in the mass spectrum at *m/z* 414 $[M-H_2O]^+$ and ^{13}C-nmr data. The ion peaks at *m/z* 285 $[M-2H_2O-C_8H_{15}]^+$, 267 $[M-3H_2O-$ side chain$]^+$, and 249 $[M-4H_2O-$ side chain$]^+$ indicated for this compound a side chain having one degree of unsaturation and a C_8H_{15} composition. ^1H- and ^{13}C-nmr spectra suggested a Δ^{22}-cholesterol-type side chain (22,23). Evidence supporting the above hypothesis was gained by decoupling experiments, performed both in CD_3OD and pyridine-d_5 solutions, and from the COSY-45 spectrum (CD_3OD). Pyridine-d_5 was chosen in order to resolve the overlapping of the H-7 and H-23 signals that occurred when the ^1H spectrum of **2** was run in CD_3OD. Referring to the experiments carried out in CD_3OD solution, the whole side-chain proton connectivity was deduced as follows: The proton resonating at δ 5.26 (H-22) showed a correlation with the protons at δ 5.36 (H-23) and 2.05 (H-20); the latter was, in turn, coupled with the methyl signal at δ 1.06 (H_3-21). Similarly , a signal at δ 1.58 (H-25) was shown to be coupled with two methyl doublets at δ 0.91 (H_3-26 and H_3-27), as well as to a signal at δ 1.85 (H_2-24). This one had cross peaks with both the olefinic signals at δ 5.36 and 5.26 (H-23 and H-22, respectively). Thus, the structure of this sterol was established as (22*E*)-5α-cholesta-7,22-diene-3β,5,6β,9-tetraol [**2**]. 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. The side chain structure was supported by ^{13}C-nmr data (23) (Table 1).

Compound **3** had the composition $C_{28}H_{46}O_4$ established on the basis of the accurate mass measurement on the highest peak observed in the mass spectrum at *m/z* 428 $[M-H_2O]^+$ and ^{13}C-nmr data. The significant fragment ions at *m/z* 285 $[M-2H_2O-C_9H_{17}]^+$, 267, and 249 indicated the presence of a C_9H_{17} side chain containing one double bond. A ^1H-^1H COSY-45 experiment in CD_3OD delineated the connectivities among the vicinal protons in the side chain. The two methyl doublets observed at δ 0.87 and 0.85 (H_3-26 and H_3-27) correlated with a diffuse multiplet centered at δ 1.47 (H-25) which, in turn, had a cross peak with a proton at δ 1.62 (H-24). The latter signal showed a correlation with a methyl signal at δ 0.94 (H_3-28) and with the olefinic double doublet resonating at δ 5.23 (H-23). The proton at δ 2.04 (H-20) correlated with the methyl signal resonating at δ 1.22 (H_3-21) and with the one-proton double doublet at δ 5.18 (H-22) which, in turn, correlated with the olefinic signal at δ 5.23 (H-23). The large value (14.6 Hz) of the coupling constant between these protons was indicative of the *E* configuration of the Δ^{22} double bond. The structure of **3** was, therefore, formulated as (22*E*,24*S*)-24-methyl-5α-cholesta-7,22-diene-3β,5,6β,9-tetraol. The assigned configuration at C-24 of this epimer followed from the spectral comparison of the high field region of the ^1H spectrum and ^{13}C-nmr spectrum with those of an authentic sample of an epimeric mixture of brassicasterol (24*R*) and 24-*epi*-brassicasterol (24*S*), measured in the same solvent (23).

Compound **4** had a molecular formula $C_{28}H_{46}O_4$ deduced by hrms on the ion at *m/z* 428 $[M-H_2O]^+$ and ^{13}C-nmr data. The presence of a C_9H_{17} monounsaturated side chain was indicated by the ion peaks at *m/z* 285 $[M-2H_2O-C_9H_{17}]^+$, 267, and 249.

The ^{1}H-^{1}H COSY-45 experiment (CD_3OD) showed that the allylic proton at δ 2.28 (H-25) was vicinally coupled with the two methyl doublets observed at δ 1.039 and 1.032 (H_3-26 and H_3-27) and long-range-coupled to one of the H_2-28 protons at δ 4.73 (Ha-28). The other olefinic signal at δ 4.66 (Hb-28) was long-range-coupled to two allylic protons observed at δ 2.13 (Ha-23) and 1.92 (Hb-23) that were correlated with a pair of protons centered at δ 1.59 and 1.18 (Ha-22 and Hb-22). These protons showed correlation with each other and with a diffuse multiplet at δ 1.42 (H-20). The latter signal had a cross peak with a methyl signal at δ 1.02 (H_3-21). The protons at δ 2.13 and 1.92 were shown to be coupled with each other as well. Assignment of the chemical shifts for the side chain carbons in the ^{13}C-nmr spectrum of **4** was based on comparison with the known values for 24-methylene-5α-cholest-7-ene-3β,6α-diol (11). Thus, the structure of the new sterol could be formulated as 24-methylene-5α-cholest-7-ene-3β,5,6β,9-tetraol [**4**].

Compounds **5** and **6** could not be separated by repeated reversed-phase hplc. They each had the molecular formula $C_{29}H_{50}O_4$ (hrms and ^{13}C-nmr data) and a $C_{10}H_{21}$ saturated side chain indicated by the ion peaks at *m/z* 285, 267, and 249. Unfortunately, the relative position of the side chain protons could not be unequivocally established either through spin decoupling work or by COSY experiments owing to the overlapping of their signals. However, the complexity of the methyl region of the ^{1}H-nmr spectrum and ^{13}C-nmr data clearly showed the presence of an epimeric mixture of (24*S*)-24-ethyl-5α-cholest-7-ene-3β,5,6β,9-tetraol [**5**] and (24*R*)-24-ethyl-5α-cholest-7-ene-3β,5,6β,9-tetraol [**6**]. The chemical shifts of the side chain carbons and methyl protons for both epimers, as well as the absolute configuration at C-24, were established by comparison of the pertinent ^{1}H- and ^{13}C-nmr signals with those of commercial sitosterol and clionasterol isolated from marine phanerogames (24), all measured in pyridine-d_5. Diagnostic differences are noted in the chemical shift values of the C-20, C-22, C-23, C-24, C-25, C-26, C-27, C-28, and C-29 signals (23). Moreover, in the ^{1}H-nmr spectrum of the mixture, the H_3-29 resonance of the 24*S* epimer **5** is typically more deshielded than that in the 24*R* epimer **6** (22).

Although labeling work is required to prove the bioorigin of the tetrahydroxylated sterols **1–6**, the co-occurrence in the sponge *S. officinalis* of these sterols, Δ^7-3β,5α,6β-trihydroxysterols (18), and the corresponding $\Delta^{5,7}$-3β-hydroxysterols (25) indicates that the latter sterols may be biosynthetic precursors of both the above-mentioned Δ^7-tri- and Δ^7-tetrahydroxysterols present in the same organism, as was earlier pointed out for di- and trihydroxylated sterols found in the sponge *Spongionella gracilis* (11,12). It seems also reasonable to assume that all polyhydroxylated sterols with a double bond in the 7 position with a common 3β,5α,6β-hydroxylation pattern, found in sponges (9,11–15) or in other organisms (26–29), may be formed from the corresponding $\Delta^{5,7}$-3β-hydroxysterols. In this connection, noteworthy is the recent finding of 9α,11α-epoxycholest-7-ene-3β,5α,6β-triol from *Planaxis sulcatus* (29), a member of the gastropod molluscs that are known to contain $\Delta^{5,7}$-sterols (30,31). This epoxy compound may be an intermediate in the formation of the tetrahydroxysterol **1**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker WM-400 spectrometer in either CD_3OD or pyridine-d_5 solutions. ^{1}H-nmr chemical shift values were referenced to the residual MeOH (3.31 ppm) and C_5H_5N (8.71 ppm) signals; ^{13}C-nmr chemical shifts were referenced to C_5D_5N (135.5 ppm). Low resolution mass spectra were determined at 70 eV on an AEI MS 30 mass spectrometer. High resolution mass spectra were recorded on a Kratos MS 50 spectrometer. Ft-ir spectra were recorded on a Perkin-Elmer 1760-X Ft-ir. High performance liquid chromatographies were carried out using a Varian 2510 pump and a Waters dual cell refractometer. Optical rotations were mea-

sured on a Perkin-Elmer Model 141 polarimeter. Melting points were determined on a Reichert Thermovar apparatus and are uncorrected.

EXTRACTION AND ISOLATION.—*S. officinalis* was collected in the Bay of Napoli and supplied by Stazione Zoologica di Napoli. Voucher specimens are on file at our laboratories. The freshly collected sponge (460 g, dry wt after extraction) was extracted with Me_2CO and $CHCl_3$-MeOH (1:1) for 3 days. The extracts were concentrated under reduced pressure to obtain an aqueous suspension that was extracted with Et_2O. The Et_2O solution was dried over Na_2SO_4 and the solvent removed to obtain an oily residue (25.6 g) that was chromatographed on a gravity-flow column (600 g, 4 cm diameter) of Si gel eluted with solvent of increasing polarity from petroleum ether through $CHCl_3$ and increasing amounts of MeOH in $CHCl_3$. Fractions of 200 ml were collected. Fractions 50–60 eluted 82 mg of previously isolated 3β,5α,6β-trihydroxysterols (14); fractions 66–80 (461 mg), eluted after the trihydroxylated sterols with $CHCl_3$-MeOH (94:6), gave a mixture of the tetrahydroxylated sterols contaminated by other products. These fractions were further chromatographed on Si gel, eluting with increasing concentrations of MeOH in $CHCl_3$ under a slight N_2 pressure. Fractions of 30 ml were taken. Tetrahydroxysterols, dispersed among fractions 47–55 [eluent $CHCl_3$-MeOH (96:4)], were further purified by hplc on a Hibar Lichrosorb Si-60 column (250 × 4 mm) using $CHCl_3$MeOH (93:7) as eluent. The mixture of tetrahydroxylated sterols, which showed one spot on tlc [$CHCl_3$-MeOH (85:15)], was fractionated by reversed-phase hplc on a Hibar RP-18 column (250 × 4 mm) eluted with MeOH-H_2O (80:20) to obtain the following compounds in order of elution: **2** (1.5 mg), **4** (1.7 mg), **3** (1.9 mg), **1** (2.8 mg), **5** and **6** (together 2.0 mg). The hplc retention times for compounds **2–6** relative to **1** were: **2** (0.73), **4** (0.81), **3** (0.94), **5** and **6** (1.63).

SPECTRAL DATA.—*5α-Cholest-7-ene-3β,5,6β,9-tetraol* [**1**].—Mp 218–220° [from MeOH-H_2O (9:1)]; $[\alpha]^{25}D$ −33.3° (*c* = 0.06, MeOH); ir ($CHCl_3$) ν max 3390 cm^{-1}; ^{13}C nmr see Table 1; ^1H nmr (CD_3OD) see Table 2; ^1H nmr (pyridine-d_5) see Table 3; hrms *m/z* (composition, assignment, rel. int.) $[M-H_2O]^+$ 416.3289 (calcd for $C_{27}H_{44}O_3$, 416.3291) (66), $[M-2H_2O]^+$ 398.3185 ($C_{27}H_{42}O_2$) (100), $[M-2H_2O-Me]^+$ 383.2952 ($C_{26}H_{39}O_2$) (64), $[M-3H_2O]^+$ 380.3052 ($C_{27}H_{40}O$) (56), $[M-3H_2O-Me]^+$ 365.2896 ($C_{26}H_{37}O$) (34), $[M-4H_2O]^+$ 362.2951 ($C_{27}H_{38}$) (6), [M − side chain − $2H_2O]^+$ 285.1842 ($C_{19}H_{25}O_2$) (64), [M − side chain − $3H_2O]^+$ 267.1768 ($C_{19}H_{23}O$) (52), [M − side chain − $4H_2O]^+$ 249.1636 ($C_{19}H_{21}$) (18).

(22E)-5α-Cholesta-7,22-diene-3β,5,6β,9-tetraol [**2**].—Mp 220–222° [from MeOH-H_2O (9:1)]; $[\alpha]^{25}D$ −16.6° (*c* = 0.06, MeOH); ir ($CHCl_3$) ν max 3390 cm^{-1}; ^{13}C nmr see Table 1; ^1H nmr (CD_3OD) see Table 2; ^1H nmr (pyridine-d_5) see Table 3; hrms *m/z* $[M-H_2O]^+$ 414.3151 (calcd for $C_{27}H_{42}O_3$, 414.3133) (68), $[M-2H_2O]^+$ 396.3027 ($C_{27}H_{40}O_2$) (100), $[M-2H_2O-Me]^+$ 381.2768 ($C_{26}H_{37}O_2$) (36), $[M-3H_2O]^+$ 378.2930 ($C_{27}H_{38}O$) (32), $[M-3H_2O-Me]^+$ 363.2665 ($C_{26}H_{35}O$) (18), $[M-4H_2O]^+$ 360.2838 ($C_{27}H_{36}$) (4), 285.1875 ($C_{19}H_{25}O_2$) (50), 267.1731 ($C_{19}H_{23}O$) (45), 249.1629 ($C_{19}H_{21}$) (14).

(22E,24S)-24-Methyl-5α-cholesta-7,22-diene-3β,5,6β,9-tetraol [**3**].—Mp 252–254° [from MeOH-H_2O (9:1)]; $[\alpha]^{25}D$ −25.0° (*c* = 0.08, MeOH); ir ($CHCl_3$) ν max 3390 cm^{-1}; ^{13}C nmr see Table 1; ^1H nmr (CD_3OD) see Table 2; ^1H nmr (pyridine-d_5) see Table 3; hrms *m/z* $[M-H_2O]^+$ 428.3300 (calcd for $C_{28}H_{44}O_3$, 428.3291) (45), $[M-2H_2O]^+$ 410.3203 ($C_{28}H_{42}O_2$) (100), $[M-2H_2O-Me]^+$ 395.2969 ($C_{27}H_{39}O_2$) (33), $[M-3H_2O]^+$ 392.3056 ($C_{28}H_{40}O$) (30), $[M-3H_2O-Me]^+$ 377.2858 ($C_{27}H_{37}O$) (19), $[M-4H_2O]^+$ 374.2960 ($C_{28}H_{38}$) (5), 285.1836 ($C_{19}H_{25}O_2$) (62), 267.1773 ($C_{19}H_{23}O$) (61), 249.1631 ($C_{19}H_{21}$) (19).

24-Methylene-5α-cholest-7-ene-3β,5,6β,9-tetraol [**4**].—Mp 221–223° [from MeOH-H_2O (9:1)]; $[\alpha]^{25}D$ −57.1° (*c* = 0.14, MeOH); ir ($CHCl_3$) ν max 3390 cm^{-1}; ^{13}C nmr see Table 1; ^1H nmr (CD_3OD) see Table 2; ^1H nmr (pyridine-d_5) see Table 3; hrms *m/z* $[M-H_2O]^+$ 428.3293 (calcd for $C_{28}H_{44}O_3$, 428.3291) (47), $[M-2H_2O]^+$ 410.3205 ($C_{28}H_{42}O_2$) (100), $[M-2H_2O-Me]^+$ 395.2968 ($C_{27}H_{39}O_2$) (60), $[M-3H_2O]^+$ 392.3101 ($C_{28}H_{40}O$) (29), $[M-3H_2O-Me]^+$ 377.2870 ($C_{27}H_{37}O$) (18), $[M-4H_2O]^+$ 374.2969 ($C_{28}H_{38}$) (5), 285.1832 ($C_{19}H_{25}O_2$) (52), 267.1748 ($C_{19}H_{23}O$) (50), 249.1639 ($C_{19}H_{21}$) (24).

(24S)-24-Ethyl-5α-cholest-7-ene-3β,5,6β,9-tetraol [**5**] *and (24R)-24-ethyl-5α-cholest-7-ene-3β,5,6β,9-tetraol* [**6**].—Ir ($CHCl_3$) ν max 3390 cm^{-1}; ^{13}C nmr see Table 1; ^1H nmr (CD_3OD) see Table 2; ^1H nmr (pyridine-d_5) see Table 3; hrms *m/z* $[M-H_2O]^+$ 444.3584 (calcd for $C_{29}H_{48}O_3$, 444.3603) (43), $[M-2H_2O]^+$ 426.3472 ($C_{29}H_{46}O_2$) (100), $[M-2H_2O-Me]^+$ 411.3251 ($C_{28}H_{43}O_2$) (40), $[M-3H_2O]^+$ 408.3381 ($C_{29}H_{44}O$) (37), $[M-3H_2O-Me]^+$ 393.3185 ($C_{28}H_{41}O$) (24), $[M-4H_2O]^+$ 390.3295 ($C_{29}H_{42}$) (6), 285.1980 ($C_{19}H_{25}O_2$) (80), 267.1743 ($C_{19}H_{23}O$) (70), 249.1625 ($C_{19}H_{21}$) (26).

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