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ISOLATION AND SYNTHESIS OF A NEW 9,11-SECOSTEROL FROM THE SPONGE *SPONGIA OFFICINALIS*

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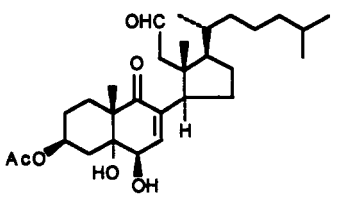
ABSTRACT.—A new 9,11-secosterol [**1**] has been isolated from the sponge *Spongia officinalis*. The structure of the new metabolite has been assigned by interpretation of spectral data and confirmed by synthesis starting from 7-dehydrocholesterol.

The sponge *Spongia officinalis* L. (order Dictyoceratida, family Spongiidae) is a particularly rich source of steroidal metabolites. Previous studies on this organism have led to the isolation of a number of new polyoxygenated steroids, namely, Δ^7-3β,5α,6β-trihydroxysterols (1), Δ^7-3β,5α,6β,9α-tetrahydroxysterols (2), Δ^8- and $\Delta^{8(14)}$-5α,6α-epoxysterols (3), and 9,11-secosterols (4,5). The sponge also produces $\Delta^{5,7}$-sterols (6) which can be seen hypothetically as biogenetic precursors of all of the above-mentioned polyoxysteroids.

A reinvestigation of the polar fractions of the extracts of this sponge has now led to the isolation of a further 9,11-secosterol [**1**], related to the previously isolated 9,11-secosterols from the same source, whose structure elucidation and synthesis are discussed in the present paper.

RESULTS AND DISCUSSION

Compound **1** was obtained as a crystalline, optically active substance that gave no molecular ion peak in the eims spectrum. Nevertheless, a careful examination of the ms and ^{13}C- and ^{1}H-nmr spectra of **1** (the latter two recorded in pyridine-d_5, Table 1) unequivocally established a molecular formula of $C_{29}H_{46}O_6$ for the new metabolite. The high-field region of the ^{1}H-nmr spectrum displayed four methyl resonances (two singlets at δ 1.73 and 0.90, and two doublets at δ 0.97 and 0.85, the latter integrating for six protons), which suggested that the new metabolite was a steroid belonging to the cholestane series. Ir absorptions at 3452, 1725, 1713, 1679, and 1265 cm^{-1} indicated the presence of ester, α,β-unsaturated ketone, and hydroxyl functions in the molecule. The uv absorption at 231 nm (ε 2977) and ^{13}C-nmr ($CDCl_3$) resonances at δ 203.95 (s, C-9), 139.11 (d, C-7) and 136.80 (s, C-8) confirmed the presence of the enone moiety. In the ^{13}C-nmr spectrum recorded in pyridine-d_5 the signal for C-8 is submerged by the solvent signal at δ 135.5. The ester function was part of an acetate group because the ^{1}H-nmr spectrum displayed a three-proton singlet signal at δ 2.00 (acetoxymethyl), while the mass spectrum included an M^+-CH_3COOH ion peak at *m/z* 430. The eims also revealed the presence of two hydroxyl groups in **1** exhibiting fragment ions at *m/z* 472 (M^+-H_2O, highest mass ion observed in the spectrum), 412 ($M^+-H_2O-CH_3COOH$)



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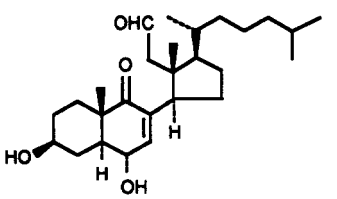

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TABLE 1. ^{1}H- and ^{13}C-nmr Data for Compound **1**.[a]

Position	δ_C[b]	δ_H[c] (m, *J*)	
1	28.29	H_{ax}	2.67 (ddd, 13.9, 13.9, 4.0)
2	27.29		
3	72.31[d]		5.73 (m)
4	36.89	H_{ax}	2.75 (dd, 12.6, 12.6)
		H_{eq}	2.38 (br dd, 12.6, 4.3)
5	76.38		
6	71.49[d]		4.71 (br d, 5.1)
7	142.35		6.94 (d, 5.1)
8	*		
9	205.15		
10	46.54		
11	204.09		10.30 (d, 3.5)
12	50.92	Ha	2.44 (dd, 16.3, 3.5)
		Hb	2.31 (d, 16.3)
13	48.87		
14	43.78		3.94 (dd, 11.8, 8.0)
15	26.58[e]		
16	26.45[e]		
17	51.95		
18	16.57		0.90 (s)
19	21.70[f]		1.73 (s)
20	35.20		
21	19.40		0.97 (d, 6.7)
22	35.73		
23	24.53		
24	39.63		
25	28.15		
26	22.66[g]		0.85 (d, 6.7)
27	22.90[g]		0.85 (d, 6.7)
OH			7.49 (s), 6.69 (s)
CH_3CO-	21.85[f]		2.00 (s)
CH_3CO-	170.40		

[a]^{1}H- and ^{13}C-nmr spectra were recorded in pyridine-d_5 at 400 and 100.1 MHz, respectively. The signal indicated with an asterisk overlapped with the solvent signal at δ 135.5. This carbon resonated at δ 136.80 in the spectrum recorded in $CDCl_3$.

[b]Assignment based on DEPT experiments and comparison with literature data (2,3) and 3β,6α-dihydroxy-9-oxo-9,11-seco-5α-cholest-7-en-11-al [**2**] (4) taken as a model compound. Pyridine-d_5 as internal reference (149.9 ppm).

[c]Assignments based on decoupling experiments. Residual pyridine as internal reference (8.71 ppm). Coupling constants are given in Hz.

[d–g]Values with identical superscripts may be interchanged.

and 394 ($M^+ - 2H_2O - CH_3COOH$), for two successive H_2O losses. The presence of two exchangeable protons in the high-field region of the pyridine-d_5 ^{1}H-nmr spectrum of **1** (δ 7.49 and 6.69) confirmed this deduction. On the other hand, ^{13}C- and ^{1}H-nmr resonances at δ 204.09 (d), and 10.30 (d, J=3.5 Hz), respectively, indicated the presence of an aldehyde group in the molecule as well.

The placement of the above-mentioned functionalities (2×OH, 1×CHO, 1×$OCOCH_3$, 1×C=C-C=O) within the steroidal nucleus was established on the basis of the following evidence. A typical seven-line multiplet attributable to the 3α-H ^{1}H-nmr resonance was observed at δ 5.73. Past experience suggested that the unusually high chemical shift value for this proton could not be solely ascribed to acetylation of the C-3 OH group, but also required the presence in the molecule of an α-oriented hydroxyl

group at C-5 (7). That C-5 was indeed a non-protonated carbon was confirmed by the multiplicity of the H_2-4 protons that resonated as two mutually coupled double doublets at δ 2.75 (J=12.6 and 12.6 Hz, H_{ax}-4) and δ 2.38 (J=12.6 and 4.3 Hz, H_{eq}-4). The second splitting observed for each of these signals was due to coupling with the adjacent 3α-H proton, as indicated by decoupling experiments. Evidence corroborating the presence of a C-5, axially disposed OH group was given by the strong pyridine-induced shifts experienced by both the H_{ax}-1 and H_{ax}-3 protons, which resonated downfield at δ 2.67 (ddd, J=13.9, 13.9, and 4.0 Hz) and 5.73, respectively [Δδ (H_{ax}-1)=0.67; Δδ (H_{ax}-3)=0.64 in comparison with the spectrum recorded in $CDCl_3$], due to their 1,3-diaxial relationship, and hence accounted for the vicinity in space of the hydroxyl group in question (5-OH) (7).

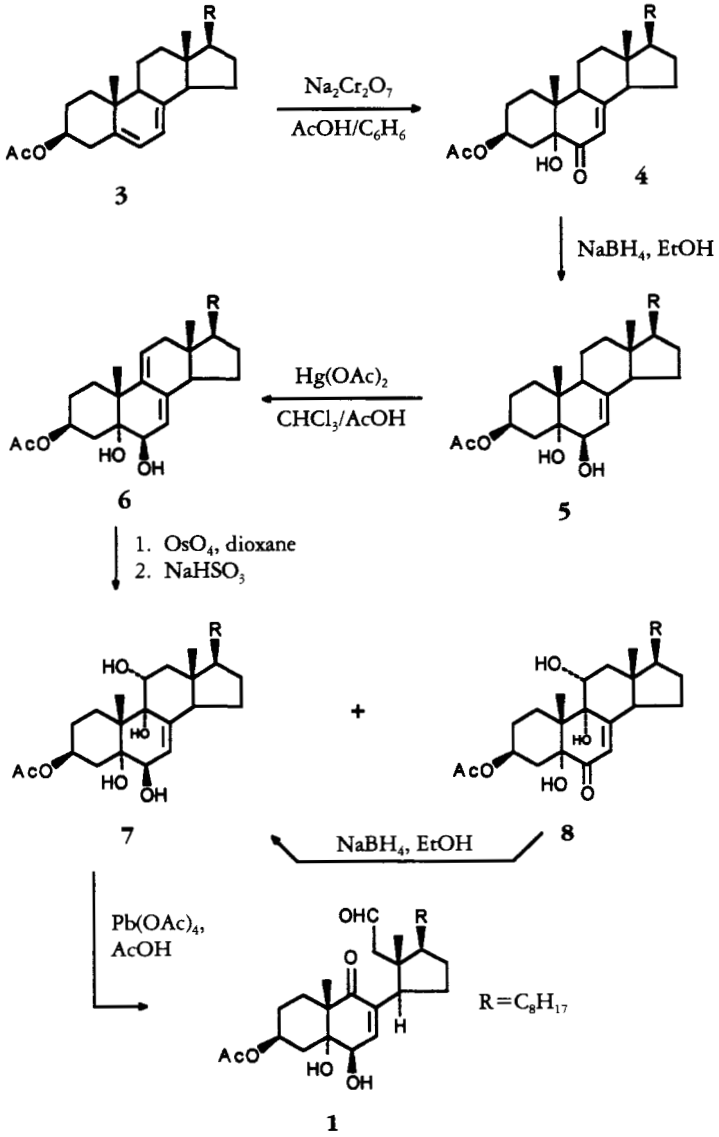
The unsaturation count and consideration of the functionalities present in the molecule indicated a tricyclic skeleton, and therefore a secosterol structure for the metabolite under investigation [**1**]. The similarity of the proton spectrum of **1** with that of compound **2**, a recently isolated 9,11-secosterol from the same sponge (4), strongly suggested that the two steroids were closely related metabolites and that compound **1** was itself a 9,11-secosterol. This suggested the placement of the enone moiety between C-6 and C-10 and the aldehyde group at C-11, as in compound **2**, leaving C-6 as the probable carbon atom carrying the second OH group. These considerations were corroborated by the following spectral data. Decoupling experiments demonstrated that the H-6 proton, resonating at δ 4.71 (br d, J=5.1 Hz), was coupled with the H-7 olefinic proton centered at δ 6.94 (d, J=5.1 Hz) and homoallylically coupled with the H-14 proton at δ 3.94 (dd, J=11.8 and 8.0 Hz). The β orientation of the C-6 OH group was indicated by the strong pyridine-induced shifts experienced by both the Me-19 and H_{ax}-4 protons (Δδ=0.38 and 0.55 ppm, respectively, in comparison with the spectrum recorded in $CDCl_3$). On the contrary, in sterol **2**, these protons experienced only limited downfield shifts due to the inversion in the configuration at C-6 (α-OH), while H_{eq}-4 underwent a more consistent pyridine-induced deshielding for the same reason (4).

The presence in **1** of a -CH_2(12)-CHO(11) moiety was proposed from the following evidence. The H_2-12 protons resonated as a pair of mutually coupled (J=16.3 Hz) signals centered at δ 2.44 (dd) and 2.31 (d) with only the proton resonating at lower field coupled with the aldehydic proton at δ 10.30 (d, J=3.5 Hz), as seen for **2**, while the mass spectrum exhibited intense peaks at *m/z* 447 (M^+-CH_2CHO) and 429 ($M^+-CH_2CHO-H_2O$). That this grouping was linked to C-13 followed from the nOe enhancements exhibited by both the H_2-12 protons when H_3-18 was irradiated. This nOe experiment also revealed the vicinity in space of Me-18 and H-7 (irradiation on H_3-18 also resulted in a strong enhancement of the H-7 signal), a fact that further supported a ring-C seco-structure for **1**. In fact, examination of a Dreiding model of the molecule indicated that the protons in question (H-7 and H_3-18) can be brought near in space only if the right-hand (ring-D-containing) part of the molecule is free to rotate around the 8,14 bond; that is, only if ring C is fragmented at a point of the C-9–C-13 segment (at the C-9–C-11 bond, in our case).

The presence of a cholesterol-type side-chain was indicated by ^{1}H-nmr and ms evidence. As previously noted, the proton spectrum of **1** included resonances for an isopropyl group (H_3-26 and H_3-27) at δ 0.85 and for a methyl linked to a methine group at δ 0.97 (H_3-21), while the ms spectrum exhibited fragment ions at *m/z* 377 ($M^+-C_8H_{17}$), 341 ($M^+-C_8H_{17}-2H_2O$), 299 ($M^+-C_8H_{17}-CH_3COOH-H_2O$) and 281 ($M^+-C_8H_{17}-CH_3COOH-2H_2O$), that indicated the presence of a C_8H_{17} saturated side-chain.

Final confirmation of the stereostructure of **1** was obtained by synthesis starting from 7-dehydrocholesteryl acetate [**3**] following a synthetic protocol similar to that

previously used for the synthesis of compound **2** (Scheme 1). Thus, 7-dehydrocholesteryl acetate [**3**] was oxidized with $Na_2Cr_2O_7$ in CH_3COOH/C_6H_6, as described by Fieser *et al.* for its Δ^{22} C-24 methyl homologue (ergosterol) (8), to give α-ketol **4**, which was reduced with $NaBH_4$ in EtOH to afford 5α-cholest-7-ene-3β,5,6β-triol 3-acetate [**5**]. Reaction of **5** with $Hg(OAc)_2/CH_3COOH$ in $CHCl_3$ (9) yielded the 7,9(11)-diene **6**, a key intermediate when the scission of the 9(11)-double bond is to be accomplished. Dihydroxylation at the C-9–C-11 positions was obtained by reacting **6** with OsO_4 in 1,4-dioxane (10) for 1 h followed by treatment with $NaHSO_3$, which gave the tetrahydroxysterol **7**, along with a small amount of the α,β-unsaturated ketone **8** derived from further oxidation at the C-6 carbon atom. Compound **8** was easily converted into **7** by $NaBH_4$ reduction in EtOH for 30 min. Finally, compound **7** was reacted with crystalline $Pb(OAc)_4$ in CH_3COOH (11) for 5 min. This reaction led to the



SCHEME 1

exclusive scission of the C-9–C-11 bond leaving the C-5–C-6 trans-diol system unaffected, to give a product which had spectral (^{1}H-nmr, ^{13}C-nmr, ir, uv, ms) and chromatographic properties identical to those exhibited by natural **1**. In addition, the optical rotations of the synthetic and natural materials were identical, thus establishing that the absolute configuration of the new compound is the one indicated.

From a biogenetic point of view it seems reasonable that both the 9,11-secosterols **1** and **2** could be derived from a common 5,7,9(11)-triene sterol through oxidation at the C-5 and C-6 carbons, in the case of **1**, or only at C-6, in the case of **2**, with concomitant oxidative cleavage of the 9,11 double bond.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were recorded on Bruker WM 270 and 400 spectrometers in pyridine-d_5 or $CDCl_3$. ^{1}H-Nmr chemical shifts were referenced to the residual $CHCl_3$ and pyridine signals (7.26 and 8.71 ppm, respectively). ^{13}C-Nmr chemical shifts were referenced to the solvent signals ($CDCl_3$: 77.0 ppm; C_5D_5N: 149.9 ppm). The multiplicity of the ^{13}C-nmr resonances was determined by DEPT experiments (12). NOe nmr spectra were obtained at 400 MHz in a degassed pyridine solution. Hreims were recorded on a Kratos AEI-MS mass spectrometer. Lreims were recorded on a TRIO 2000 mass spectrometer. Ft-ir spectra were obtained with a Perkin-Elmer 1760-X Ft-ir spectrophotometer. Uv spectra were recorded with a Perkin-Elmer model 550-S spectrophotometer. Hplc separations were performed using a Varian 2510 pump equipped with a Waters R403 differential refractometer. Mps were determined on a Reichert Termovar type 300429 Kofler hot-stage melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer model 141 polarimeter. Cc was performed on Merck Si gel 40 (70–230 mesh). Tlc analyses were performed on Merck precoated Si gel F_{254} plates (0.25 mm thick).

EXTRACTION OF THE SPONGE AND ISOLATION OF **1**.—*Spongia officinalis* was collected in the Bay of Naples in March 1990. Reference specimens are on file at the Dipartimento di Chimica Organica e Biologica dell' Università di Napoli. Freshly collected animals (433 g dry wt after extraction) were extracted twice with Me_2CO and twice with $CHCl_3$-MeOH (1:1). The combined extracts were concentrated under reduced pressure and the aqueous residue was extracted with Et_2O. The combined Et_2O extracts were evaporated and the oily residue (29.64 g) was chromatographed on a Si gel column (600 g, 4 cm diameter) eluted with $CHCl_3$ and increasing amounts of CH_3OH in $CHCl_3$, with 200-ml fractions collected. Fractions 23–26 (345 mg), eluted with $CHCl_3$-CH_3OH (99:1), were combined and subjected to hplc separation on a Hibar LiChrosorb Si-60 column (250×10 mm; flow 2.5 ml/min) using hexane-EtOAc (3:7) as the mobile phase. The first eluted fraction from this separation (R_t 8.8 min; 9.7 mg) was further purified by normal-phase hplc on a Hibar LiChrosorb Si-60 column (250×4 mm) eluted with hexane-EtOAc (6:4) to give pure **1** (3.3 mg).

3β-Acetoxy-5,6β-dihydroxy-9-oxo-9,11-seco-5α-cholest-7-en-11-al [**1**].—Mp 164–165° [petroleum ether (80–100°)-$CHCl_3$, 8:2]; $[\alpha]_D$ −52.5° (*c*=0.3, $CHCl_3$); ir (neat) ν max 3452, 1725, 1713, 1679, 1265 cm^{-1}; uv (MeOH) λ max 231 nm (ε 2977); ^{1}H nmr (pyridine-d_5) and ^{13}C nmr (pyridine-d_5), see Table 1; ^{1}H nmr ($CDCl_3$, 400 MHz) δ 9.89 (1H, d, *J*=3.7 Hz, H-12), 6.48 (1H, d, *J*=4.9 Hz, H-6), 5.09 (1H, m, H_α-3), 4.06 (1H, d, *J*=4.9 Hz, H_α-6), 3.58 (1H, dd, *J*=11.0 and 11.0 Hz, H-14), 2.27 (1H, dd, *J*=15.9 and 3.7 Hz, H_a-12), 2.20 (1H, dd, *J*=12.8 and 12.8 Hz, H_{ax}-4), 2.06 (m, H_{eq}-2, overlapped with other signals), 2.05 (3H, s, acetate), 2.01 (1H, d, *J*=15.9 Hz, H_b-12), 2.00 (m, H_{ax}-1, overlapped with other signals), 1.89 (1H, dd, *J*=12.8 and 4.9 Hz, H_{eq}-4), 1.63 (1H, dddd, *J*=12.8, 12.8, 12.8, and 4.3 Hz, H_{ax}-2), 1.35 (3H, s, H_3-19), 0.92 (3H, d, *J*=6.7 Hz, H_3-21), 0.86 (6H, d, *J*=6.7 Hz, H_3-26 and H_3-27), 0.79 (3H, s, H_3-18); eims *m/z* 472 (M^+-H_2O, 10), 448 (M^+-CH_2CO, 5), 447 (M^+-CH_2CHO, 12), 430 (M^+-CH_3COOH, 8), 429 ($M^+-CH_2CHO-H_2O$, 15), 412 ($M^+-H_2O-CH_3COOH$, 18), 394 ($M^+-2H_2O-CH_3COOH$, 20), 377 (M^+−side chain, 12), 369 ($M^+-CH_2CHO-H_2O-CH_3COOH$, 35), 351 ($M^+-CH_2CHO-2H_2O-CH_3COOH$, 20), 341 ($M^+$−side chain−$2H_2O$, 10), 299 ($M^+$−side chain−$CH_3COOH-H_2O$, 15), 281 ($M^+$−side chain−$CH_3COOH-2H_2O$, 20); hreims *m/z* 472.3175 (M^+-H_2O), $C_{29}H_{44}O_5$ requires 472.3189.

SYNTHESIS OF **1** (SCHEME 1).—*5α-Cholest-7-ene-3β,5,6β-triol 3-acetate* [**5**].—5α-Cholesta-5,7-diene-3β-yl acetate (5.0 g, 12 mmol) was oxidized with $Na_2Cr_2O_7$ dihydrate (1.5 g, 5 mmol) according to Fieser's procedure (8). The reaction mixture was chromatographed on a Si gel column (350 g, 4.5 cm i.d.) eluted with increasing amounts of Et_2O in petroleum ether. The fractions eluted with petroleum ether-Et_2O (72:28) contained 1.54 g of crude ketone **4** which was subjected to $NaBH_4$ reduction without further purification as follows. To a solution of crude **4** (950 mg) in EtOH (30 ml), excess $NaBH_4$ was added and the suspension stirred at room temperature for 2 h, then excess reagent was destroyed by dropwise addition of CH_3COOH. The reaction mixture was treated with brine (20 ml) and extracted with Et_2O (3×20 ml).

The combined Et_2O extracts were washed with a saturated $NaHCO_3$ solution, dried ($MgSO_4$), and concentrated. The residue was chromatographed on a Si gel column eluting with petroleum ether/Et_2O mixtures of increasing polarity. Petroleum ether-Et_2O (1:1) was used to elute 620 mg of a material which was further separated by hplc on a Hibar LiChrosorb Si-60 (250×10 mm) column using hexane-EtOAc (65:35) as eluent to give 410 mg of pure 5α-cholest-7-ene-3β,5,6β-triol 3-acetate [**5**].

Compound **5** exhibited: Mp 236–238° [petroleum ether (80–100°)-$CHCl_3$, 8:2]; $[\alpha]_D$ −31.6° (c=1.5, $CHCl_3$); ir (film) ν max 3414, 1728, 1714, 1255 cm^{-1}; ^1H nmr ($CDCl_3$, 270 MHz) δ 5.33 (1H, br ddd, J=5.4, 2.4, and 2.4 Hz, H-7), 5.13 (1H, m, H_α-3), 3.60 (1H, br dd, J=5.4 and 5.4 Hz, H_α-6), 2.23 (1H, dd, J=12.2 and 12.2 Hz, H_{ax}-4), 2.02 (3H, s, acetate), 1.08 (3H, s, H_3-19), 0.92 (3H, d, J=6.7 Hz, H_3-21), 0.86 (6H, d, J=6.1 Hz, H_3-26 and H_3-27), 0.57 (3H, s, H_3-18); ^{13}C nmr ($CDCl_3$, 100.1 MHz) δ 170.67 (s), 143.94 (s), 117.46 (d), 75.67 (s), 73.71 (d), 71.15 (d), 56.26 (d), 54.64 (d), 43.80 (d), 43.23 (s), 39.48 (t), 39.34 (t), 37.16 (s), 36.15 (d), 36.06 (t), 35.81 (t), 32.64 (t), 27.99 (d), 27.76 (t), 26.95 (t), 23.95 (t), 22.94 (t), 22.79 (q), 22.54 (q), 21.95 (t), 21.41 (q), 18.83 (q), 18.60 (q), 12.08 (q); eims *m/z* 442 (M^+-H_2O, 12), 382 ($M^+-H_2O-CH_3COOH$, 96), 367 ($M^+-H_2O-CH_3COOH-CH_3$, 77), 353 (100), 329 (M^+-H_2O−side-chain, 7), 311 (M^+−side-chain−$2H_2O$, 5), 269 (M^+−side-chain−H_2O-CH_3COOH, 35), 251 (M^+−side-chain-$2H_2O-CH_3COOH$, 27).

5α-Cholesta-7,9(11)-diene-3β,5,6β-triol 3-acetate [**6**].—To a solution of 232 mg (0.5 mmol) of 5α-cholest-7-ene-3β,5,6β-triol 3-acetate [**5**] in 6.5 ml of $CHCl_3$, 368 mg (1.2 mmol) of $Hg(OAc)_2$ in 12.7 ml of CH_3COOH was added and the suspension was stirred at room temperature (approximately 25°) for 20 h and then filtered. The filtrate was treated with saturated aqueous $NaHCO_3$ solution and extracted with $CHCl_3$. The extract was washed with H_2O, dried over $MgSO_4$, and concentrated *in vacuo*. The residue (215 mg) was chromatographed over a Si gel column eluted with petroleum ether-Et_2O (4:6) to give 163 mg of a mixture which, on tlc analysis ($CHCl_3$-MeOH, 95:5), was shown to be mainly composed of two uv-visible spots. Hplc separation by reversed-phase hplc on a Hibar LiChrosorb RP-18 (250×10 mm) column (eluent MeOH-H_2O, 96:4) gave 40 mg of 5α-cholesta-7,9(11)-diene-3β,5,6β-triol 3-acetate [**6**] still contaminated by another product. Final purification of **6** was achieved by analytical tlc ($CHCl_3$-EtOAc, 85:15) thus affording 25 mg of pure **6**: mp 184–185° [petroleum ether (80–100°)-$CHCl_3$, 8:2]; $[\alpha]_D$ +11.1° (c=0.8, $CHCl_3$); ir (neat) ν max 3597, 3446, 1713, 1278 cm^{-1}; uv (CH_3OH) λ max 245 nm (ε 10086); ^1H nmr ($CDCl_3$, 270 MHz) δ 5.72 (1H, br d, J=6.8 Hz, H-11), 5.42 (1H, br d, J=5.9 Hz, H-7), 5.17 (1H, m, H_α-3), 3.81 (1H, br d, J=5.9 Hz, H_α-6), 2.38 (1H, dd, J=17.6 and 6.8 Hz, Ha-12), 2.14 (1H, br d, J=17.6 Hz, Hb-12), 2.03 (3H, s, acetate), 1.28 (3H, s, H_3-19), 0.91 (3H, d, J=6.3 Hz, H_3-21), 0.86 (6H, d, J=6.8 Hz, H_3-26 and H_3-27), 0.57 (3H, s, H_3-18); ^{13}C nmr ($CDCl_3$, 67.9 MHz) δ 170.57 (s), 139.91 (s), 138.92 (s), 126.37 (d), 118.10 (d), 75.04 (s), 73.89 (d), 71.08 (d), 56.32 (d), 51.29 (d), 42.65 (s), 42.37 (t), 40.60 (s), 39.47 (t), 35.98 (t), 34.17 (t), 31.06 (t), 28.24 (t), 27.99 (d), 26.91 (t), 26.18 (q), 23.91 (t), 23.10 (t), 22.79 (q), 22.53 (q), 21.40 (q), 18.42 (q), 11.35 (q); eims *m/z* 458 (M^+, 4), 440 (M^+-H_2O, 5), 425 ($M^+-H_2O-CH_3$, 4), 398 (M^+-CH_3COOH, 20), 380 ($M^+-CH_3COOH-H_2O$, 65), 365 ($M^+-CH_3COOH-H_2O-CH_3$, 28), 327 (M^+−side-chain−H_2O, 8), 285 (M^+−side-chain−CH_3COOH, 25), 267 (M^+−side-chain−$CH_3COOH-H_2O$, 38), 95 (100).

5α-Cholest-7-ene-3β,5,6β,9α,11α-pentol 3-acetate [**7**] *and 3β-acetoxy-5,9α,11α-trihydroxy-5α-cholest-7-en-6-one* [**8**].—To 14.6 mg (0.03 mmol) of **6** dissolved in 4 ml of freshly distilled 1,4-dioxane, excess osmium tetroxide was added, and the mixture stirred at room temperature for 90 min. Removal of the solvent under reduced pressure gave the crude osmate ester as a dark brown material which was hydrolyzed as follows. The osmate ester was dissolved in 2 ml of 1,4-dioxane and 2 ml of a saturated $NaHSO_3$ solution were added under stirring. After 15 min the mixture was filtered and the filtrate was washed two times with a 2 N HCl solution, dried ($MgSO_4$), and taken to dryness. The residue (15.0 mg) was separated by hplc on a Hibar LiChrosorb Si-60 (250×4 mm) column using hexane-EtOAc (85:15) as eluent to afford 10.6 mg of pure tetrol **7** and 2.5 mg of ketone **8**. $NaBH_4$ reduction of compound **8** in the same conditions used for the reduction of **4** gave, after hplc purification in the above conditions (hexane-EtOAc, 85:15), 2.2 mg of **7**. Compound **7** exhibited mp 210–211° [petroleum ether (80–100°)-$CHCl_3$, 8:2]; $[\alpha]_D$ −4.1° (c=0.5, $CHCl_3$); ir (neat) ν max 3401, 1714, 1265 cm^{-1}; ^1H nmr (pyridine-d_5, 400 MHz) δ 5.87 (1H, dd, J=5.4 and 1.6 Hz, H-7), 5.77 (1H, m, H_α-3), 4.50 (1H, dd, J=11.4 and 5.1 Hz, H_β-11), 4.37 (1H, dd, J=5.4 and 2.2 Hz, H_α-6), 2.88 (1H, dd, J=12.4 and 12.4 Hz, H_{ax}-4), 2.38 (1H, dd, J=12.1 and 5.1 Hz, H_{eq}-12), 2.33 (1H, dd, J=12.4 and 5.1 Hz, H_{eq}-4), 2.00 (3H, s, acetate), 1.95 (1H, dd, J=12.1 and 12.1 Hz, H_{ax}-12), 1.73 (3H, s, H_3-19), 0.94 (3H, d, J=5.7 Hz, H_3-21), 0.87 (6H, d, J=6.4 Hz, H_3-26 and H_3-27), 0.68 (3H, s, H_3-18); ^{13}C nmr (pyridine-d_5, 67.9 MHz) δ 170.47 (s), 141.58 (s), 123.38 (d), 78.65 (s), 76.96 (s), 73.36 (d), 71.95 (d), 70.04 (d), 56.32 (d), 51.02 (d), 47.10 (t), 42.87 (s), 42.42 (s), 39.71 (t), 37.82 (t), 36.44 (d), 36.29 (t), 29.38 (t), 28.23 (t), 28.23 (d), 28.02 (t), 24.20 (t), 23.51 (t), 22.96 (q), 22.69 (q), 21.40 (q), 21.40 (q), 18.92 (q), 12.78 (q); eims *m/z* 492 (M^+, 2), 474 (M^+-H_2O, 20), 456 (M^+-2H_2O, 18), 414 ($M^+-H_2O-CH_3COOH$, 7), 396 ($M^+-2H_2O-CH_3COOH$, 33), 283 (M^+−side-chain−$2H_2O-CH_3COOH$, 37), 265 (M^+−side-chain−$3H_2O-CH_3COOH$, 24), 93 (100), 81 (100), 69 (100).

Compound **8** exhibited: $[\alpha]_D$ −6.0° (*c*=0.2, $CHCl_3$); ir (neat) ν max 3385, 1735, 1683, 1242 cm^{-1}; uv (CH_3OH) λ max 232 nm (ε 5820); ^1H nmr (pyridine-d_5, 400 MHz) δ 5.97 (1H, br s, H-7), 5.62 (1H, m, H_α-3), 4.40 (1H, dd, *J*=12.1 and 4.4 Hz, H_β-11), 2.95 (1H, br dd, *J*=8.3 and 8.3 Hz, H-14), 2.70 (1H, br dd, *J*=12.1 and 4.4 Hz, H_{eq}-4), 2.40 (1H, dd, *J*=12.1 and 5.1 Hz, H_{eq}-12), 2.17 (1H, dd, *J*=12.1 and 12.1 Hz, H_{ax}-4), 2.06 (1H, d, *J*=12.1 and 12.1 Hz, H_{ax}-12), 1.98 (3H, s, acetate), 1.31 (3H, s, H_3-19), 0.92 (3H, d, *J*=5.7 Hz, H_3-21), 0.87 (6H, d, *J*=7.0 Hz, H_3-26 and H_3-27), 0.64 (3H, s, H_3-18); eims *m/z* 490 (M^+, 1), 472 (M^+-H_2O, 12), 430 (M^+-CH_3COOH, 5), 412 ($M^+-CH_3COOH-H_2O$, 48), 394 ($M^+-CH_3COOH-2H_2O$, 16), 359 (M^+−side-chain−H_2O, 27), 299 (M^+−side-chain−H_2O-CH_3COOH, 10), 281 (M^+−side-chain−$2H_2O-CH_3COOH$, 20), 181 (100), 121 (100), 93 (100).

Reaction of 5α-cholest-7-ene-3β,5,6β,9α,11α-pentol 3-acetate [**7**] *with lead tetraacetate to produce synthetic* **1**.—To a solution of 5α-cholest-7-ene-3β,5,6β,9α,11α-pentol 3-acetate [**7**] (6.0 mg, 0.012 mmol) in CH_3COOH (1 ml), crystalline lead tetraacetate (6.0 mg) was added portionwise over a 5 min period at room temperature. When the reaction was complete [10 min, tlc analysis ($CHCl_3$-CH_3OH, 9:1)], two drops of ethylene glycol were added and the mixture diluted with ice-H_2O and extracted with $CHCl_3$. The organic layer was washed with aqueous $NaHCO_3$, dried ($MgSO_4$), and evaporated. The residue was chromatographed on a Hibar LiChrosorb Si-60 (250×4 mm) column using $CHCl_3$-CH_3OH (96:4) as eluent to give 4.0 mg of ketoaldehyde **1** which had spectral (^1H-nmr, ^{13}C-nmr, ir, uv, ms) and chromatographic properties identical to those exhibited by the natural product, **1**. Furthermore, synthetic **1** had $[\alpha]_D$ −50.8° (*c*=0.4, $CHCl_3$).

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