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Structure Elucidation and Synthesis of 3β,6α-Dihydroxy-9-oxo-9,11-seco-5α-cholest-7-en-11-al, a Novel 9,11-Secosterol from the Sponge Spongia Officinalis.

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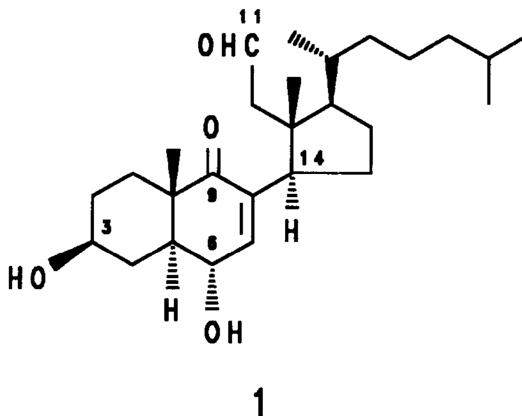
Abstract.- A novel 9,11-secosterol, 3β,6α-dihydroxy-9-oxo-9,11-seco-5α-cholest-7-en-11-al (**1**) , has been isolated from the marine sponge Spongia officinalis and its structure deduced by analysis of spectral data including ^{1}H NMR COSY and ^{1}H-^{13}C correlation NMR experiments. The structure of **1** was confirmed by synthesis starting from 7-dehydrocholesterol.

A number of secosterols have been isolated from marine invertebrates[1-6]. 9,11-Secosterols were reported from a gorgonian[1] and a soft coral[2,3], and more recently 9,11-secosterols[4] and 5,6-secosterols[5,6] have been found among sponges.

As a part of our studies on isolation [5,6,8-12] and synthesis[7] of polyhydroxylated sterols from sponges we now report the isolation, structure elucidation and synthesis of a new ring C secosterol (**1**), from the sponge Spongia officinalis L. (order Dictyoceratida, family Spongiidae).

The sponge was extracted successively with acetone and chloroform-methanol (1:1). The extracts were chromatographed on silica gel using chloroform and increasing concentrations of methanol in chloroform as eluents. The polar fractions eluted with $CHCl_3$-CH_3OH (95:5) were further purified by HPLC on silica gel with $CHCl_3$-CH_3OH (95:5) followed by repeated reversed-phase HPLC using CH_3OH-H_2O (75:25) as eluent.

Structure elucidation of secosterol 1.- The molecular formula of compound 1 was determined as $C_{27}H_{44}O_4$ by high resolution mass and ^{13}C-NMR spectrometry. The IR spectrum showed hydroxyl absorption at 3370 cm^{-1}, and two carbonyl absorptions at 1670 cm^{-1}, typical of a conjugated ketone, and at 1716 cm^{-1} attributed to an aldehyde on the basis of ^1H- and ^{13}C-NMR data (Table) as detailed below. The UV absorption at 241 nm (ϵ=3650) confirmed the presence of the α,β-unsaturated carbonyl functionality. The ^1H-NMR spectrum in pyridine-d_5 contained signals for an olefinic proton at δ 7.07 (bs, H-7), an aldehyde proton at δ 10.26 (bd, J=3.8 Hz, H-11),



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two protons belonging to hydroxyl-bearing methine groups at δ 3.89 (m, H-3) and 4.61 (bd, J=9.8 Hz, H-6), and five methyl resonances that indicated a sterol structure : singlets at δ 0.74 (H_3-18) and 1.20 (H_3-19), a doublet at δ 0.97 (J=6.8 Hz, H_3-21) and a pair of doublets at δ 0.855 and 0.860 (both J= 6.8 Hz, H_3-26 and H_3-27) both coupled to a single proton centred at δ 1.48 (m, H-25). The ^{13}C-NMR spectrum of 1 contained signals for all twenty seven carbon atoms. It confirmed the presence of two methine carbons attached to oxygen (δ 68.83 and 69.79) and showed a pair of sp^2 carbon signals at δ 133.04 (C-8) and 150.96 (C-7) that was attributed to a trisubstituted double bond. Two signals at δ 203.98 (C-11) and 205.10 (C-9) confirmed the presence of two carbonyl carbons in the molecule. The presence of two hydroxyl groups in 1 was also supported by the high resolution mass spectrum which exhibited mass ions for stepwise water loss at m/z 414.3076 (M^+-H_2O, $C_{27}H_{42}O_3$), 399.2879 (M^+-H_2O-CH_3, $C_{26}H_{39}O_3$) and 381.2833 (M^+-$2H_2O$-CH_3, $C_{26}H_{37}O_2$). The fragment ions at m/z 319.1927 (M^+-C_8H_{17}, $C_{19}H_{27}O_4$), 301.1827 (M^+-C_8H_{17}-H_2O, $C_{19}H_{25}O_3$) and 283.1663 (M^+-C_8H_{17}-$2H_2O$, $C_{19}H_{23}O_2$) indicated the presence of a saturated C_8H_{17} side chain[13], while the peak at 241.1259 (M^+-$2H_2O$ and

Table - ^{1}H- and ^{13}C-NMR data for compound 1[a].

Position	δ_C[b]		δ_H[c] (mult., J)
1	32.82	H_{ax}	2.06[g]
		H_{eq}	1.65[g]
2	31.70	H_{ax}	1.77[g]
		H_{eq}	2.11[g]
3	69.79		3.89 (m)
4	34.25	H_{ax}	1.88 (ddd, 12.4, 12.4, 12.4)
		H_{eq}	2.98 (bddd, 12.4, 3.0, 3.0)
5	49.75		2.17 (m)
6	68.83		4.61 (bd, 9.8)
7	150.96		7.07 (bs)
8	133.04		
9	205.10		
10	45.29[d]		
11	203.98		10.26 (bd, 3.8)
12	50.97	H_a	2.39 (dd, 16.2, 3.8)
		H_b	2.16 (m)
13	46.37[d]		
14	43.50		3.84 (dd, 11.1, 8.1)
15	26.66	H_a	1.73[g]
		H_b	1.55[g]
16	26.38		
17	52.02		
18	16.41		0.74 (s)
19	16.19		1.20 (s)
20	35.27		
21	19.35		0.97 (d, 6.8)
22	35.73		
23	24.48		
24	39.64		
25	28.17		
26	22.69[e]		0.855 (d, 6.8)[f]
27	22.91[e]		0.860 (d, 6.8)[f]

a ^{1}H- and ^{13}C-NMR spectra were recorded in pyridine-d_5 at 400 and 100.1 MHz, respectively.

b Assignment based on DEPT and ^{13}C-^{1}H 2D heterocorrelation experiments and comparison with literature data[14] and model compounds[5,7,8,11,12]. Pyridine-d_5 as internal reference=135.5 ppm.

c Assignment based on COSY-45, proton double quantum 2D-NMR and decoupling experiments. Residual pyridine-d_5 as internal reference (8.71 ppm). Coupling constants are given in Hz.

d-f Assignments may be reversed.

g Submerged by other signals.

ring D fission, $C_{16}H_{17}O_2$) derived from ring D cleavage and suggested that the molecule contained the intact D cycle in the structure. The ^{13}C-NMR spectrum showed that the side chain was of the cholestane type[14] : δ 35.27 (C-20), 19.35 (C-21), 35.73 (C-22), 24.48 (C-23), 39.64 (C-24), 28.17 (C-25), 22.69 and 22.91 (C-26 and C-27). On the basis of the unsaturation count, 1 had to possess three rings and thus a secosterol structure.

Proton NMR COSY-45[15,16], proton double quantum 2D-NMR[17] and decoupling experiments allowed to identify the structural fragment containing the enone moiety as follows. The broad signal at δ 3.89 was suggestive of the axial hydrogen associated with the 3β-hydroxyl group of an A/B trans steroid[18] and was coupled with two one-proton signals at δ 2.98 (bd, J=12.4 Hz) and 1.88 (ddd, J=12.4, 12.4, 12.4 Hz). These signals were also mutually coupled and were assigned, respectively, to the H_{eq}-4 and H_{ax}-4 protons. The H_2-4 protons were further coupled to the methine proton at C-5 (δ 2.17), which was in turn coupled with the hydroxymethine proton at δ 4.61 (H_{ax}-6). The latter proton was vicinally coupled with the olefinic proton at δ 7.07 (bs, H-7) which further long-range coupled with an allylic proton at δ 3.84 (dd, J=11.1, 8.1 Hz, H-14). This proton also correlated with two non-equivalent methylene protons centred at δ 1.73 and 1.55 (H_2-15). In addition, the allylic alcohol methine at δ 4.61 (H-6) was homoallylically coupled with H-14. The value of the coupling constant (9.8 Hz) for H-6 reflects axial-axial coupling with H-5 thus indicating the α-orientation of the 6-OH group. That the C-6 hydroxyl group was equatorially disposed was also indicated by the small downfield shift of the C-19 methyl resonance observed in the pyridine-d_5 ^1H-NMR spectrum, relative to the spectrum recorded in $CDCl_3$ ($CDCl_3$: δ 1.10; C_5D_5N: δ 1.20), and by the more consistent pyridine induced shift of H_{eq}-4 proton ($CDCl_3$: δ 2.30; C_5D_5N: δ 2.98) which implied a spatial proximity of this proton and the 6-OH group. Inspection of the molecular model of 1 revealed that H-6 and H-7 are spatially oriented in such a way to form a dihedral angle near to 90°. This agrees with the small value of the coupling constant (not detectable in the ^1H-NMR spectrum) within this couple of protons, thus accounting for the observed multiplicities of their signals (H-6: bd; H-7: bs).

Correlations in the ^1H-^1H COSY spectrum, supported by data from the ^1H-^{13}C heterocorrelation, also revealed that the 3α-hydroxymethine proton was adjacent to a couple of contiguous methylenes (H_2-2: δ 2.11 and 1.77; H_2-1: δ 2.06 and 1.65) which terminate on a quaternary carbon atom (C-10) and that the Me-19 protons long-range correlated (W couplings) with H-5 and H_{ax}-1. These evidences, in conjunction with UV data allowed to

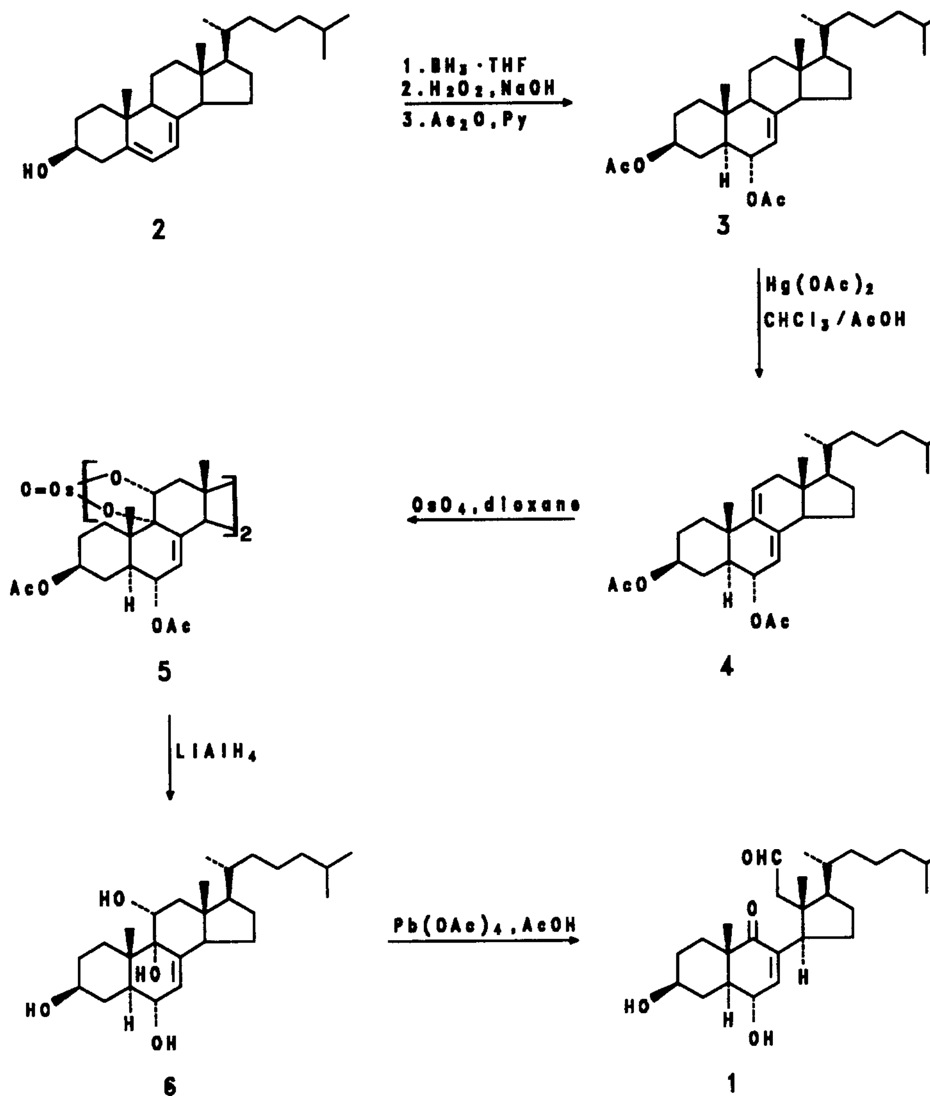
fully elucidate the structural fragment containing rings A and B. Moreover, the proton of the aldehyde group at δ 10.26 was coupled with a couple of one-proton signals resonating at δ 2.39 (dd, J=16.2 and 3.8 Hz, H_a-12) and 2.16 (d, J=16.2 Hz, H_b-12) which also coupled one another and with no other protons thus indicating they to be part of a methylene group next to a blocked position. They were assigned to the CH_2-12 of a 9,11-secosteroidal structure on the basis of further spectral evidences.

The above $-CH_2CHO$ appendage had to be attached to C-13 since a strong nOe effect was induced in both its methylene protons upon irradiation of the Me-18 protons which indicated that the methylene in question and Me-18 are near in the space. Irradiation of the 18-Me protons also produced enhancements of the 19- and 21-Me signals. Furthermore, in the same experiment, a strong nOe effect was seen in the H-7 signal which implied that the ring-D-containing portion of the molecule was slightly rotated around the 8-14 bond. This nOe result strongly supported a ring C secostructure for **1**. In another nOe experiment, irradiation of the Me-19 signal poduced a strong enhancement of the signal at δ 4.61 (H-6) thus confirming that H-6 was axial.

Additional evidence for structure **1** came from the high resolution mass spectrum which exhibited peaks at *m/z* 389.3035 (M^+-CH_2CHO), due to the loss of the $-CH_2CHO$ grouping, and 177.0903 ($M^+-C_{16}H_{29}O-H_2O$, $C_{11}H_{13}O_2$) and 159.0810 ($M^+-C_{16}H_{29}O-2H_2O$, $C_{11}H_{11}O$) originating from the fission of vinylic 8-14 bond and loss of water and attributable to fragments containing rings A and B. All the above evidences pointed to the 3β,6α-dihydroxy-9,11-seco-5α-cholest-7-en-11-al structure for compound **1**.

In order to confirm the structure assignment, we synthesized compound **1** as outlined in the Scheme.

Synthesis of 1.- The synthesis of **1** was carried out starting from 7-dehydrocholesterol (**2**). Hydroboration of **2** with BH_3-THF complex followed by oxidation with alkaline hydrogen peroxide gave a dihydroxy-compound that was acetylated overnight at room temperature with acetic anhydride in pyridine and then purified to give 5α-cholest-7-ene-3β,6α-diol 3,6-diacetate (**3**) in 60% overall yield from **2** . Mercuric acetate dehydrogenation of diacetate **3**[19] in chloroform/acetic acid for 23 h gave 5α-cholesta-7,9(11)-diene-3β,6α-diol 3,6-diacetate (**4**) in 21 % yield, which, after purification on silica gel column followed by Si-gel HPLC, was treated with osmium tetraoxide in anhydrous 1,4-dioxane. The reaction afforded the dimeric osmium ester **5**[20,21], resulting from the preferential attack of the osmium oxide on the less hindered α-face of the C9-C11 double bond, in good yield (67 %). Cleavage of the osmate ester **5** with


SCHEME
HO
2
1. BH_3·THF
2. H_2O_2, NaOH
3. Ac_2O, Py
AcO
H
OAc
3
$Hg(OAc)_2$
$CHCl_3$/AcOH
AcO
H
OAc
4
OsO_4, dioxane
O=Os
O
O
2
AcO
H
OAc
5
$LiAlH_4$
HO
HO
HO
H
OH
6
Pb(OAc)$_4$, AcOH
OHC
O
HO
H
OH
H
1

lithium aluminum hydride for 75 min. gave the tetrol **6** in almost quantitative yield. When the osmic ester opening was performed with mannitol/KOH[22] no detectable quantity of **6** was obtained also on prolonged treatment (6 days). Scission of the C9-C11 bond was achieved by treatment with lead tetraacetate in CH_3COOH[23] for 1 h. This reaction proceeded almost quantitatively affording the ketoaldehyde **1** whose NMR and mass spectra were virtually identical to those of natural **1**.

The Lemieux-Johnson reaction[24,25] was also attempted on the diene **4**. Although on addition of osmium tetroxide the osmate ester readily precipitated from the dioxane-water mixture as a dark brown material, indicating that the reaction accurred with high rate, the successive treatment with sodium metaperiodate did not give, also on prolonged stirring, the desired 3β,6α-dihydroxy-9-oxo-9,11-seco-5α-cholest-7-en-11-al 3,6-diacetate (**1** 3,6-diacetate).

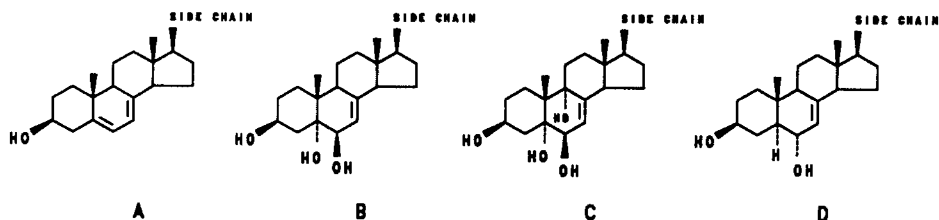
Following another route to the C-ring secostructure **1**, scission of the C9-C11 bond of the steroidal nucleus was attempted _via_ the C9-C11 epoxide intermediate 9α,11α-epoxy-5α-cholest-7-ene-3β,6α-diol 3,6-diacetate easily formed by slow addition at 0°C of m-chloroperbenzoic acid to the diene **4**. Neverthless, treatment of the 9,11-epoxide derivative with aqueous periodic acid[26] was unsuccessful giving only a complex mixture of products.

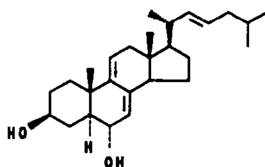
To the best of our knowledge this represents the first synthesis of a natural ring-C secosterol so far reported.

Biogenetic considerations. The sponge _S. officinalis_ contains, along with the secosterol **1**, $\Delta^{5,7}$-sterols, Δ^{7}-3β,5α,6β-tri-[10] and Δ^{7}-3β,5α,6β,9α-tetra-hydroxysterols[12] (type-A, -B and -C sterols, respectively, in the Figure) which, presumably, are related biogenetically to each other. It is interesting to note that _Spongionella gracilis_ (order Dictyoceratida, family Dysideidae), a sponge closely related to _S. officinalis_, contains, in addition to type-A, -B and -C sterols, Δ^{7}-3β,6α-dihydroxysterols (type-D sterols in the Figure) and the triene sterol **7**. Standing the phylogenetic relationship between the two sponges, it seems probable that sterol **1** could derive from a sterol having the same nucleus as that of sterol **7** through the oxidative cleavage of the 9,11 double bond.

Studies aimed to the isolation from _S. officinalis_ of the $\Delta^{7,9(11)}$-sterol in question as well as to the synthesis of labeled deacetyl **4** for feedeng experiments are currently in progress.

FIGURE





EXPERIMENTAL

^{1}H- and ^{13}C-NMR spectra were recorded on Bruker WM 270 and 400, and Varian 200 spectrometers in $CDCl_3$ or C_5D_5N solutions. Proton chemical shifts were referenced to the residual $CHCl_3$ and C_5D_5N signals (7.26 and 8.71 ppm, respectively). ^{13}C-NMR chemical shifts were referenced to the solvents ($CDCl_3$: 77.0 ppm; C_5D_5N: 149.9 ppm). Nuclear Overhauser enhancement spectra were obtained at 400 MHz in a degassed pyridine solution. High resolution electron impact mass spectra were recorded on a Kratos MS 50 spectrometer. FABMS was determined with a V. G. ZAB 2SE mass spectrometer on a sample dissolved in a glycerol matrix . The sample was bombarded with Xe atoms at 25 kV energy and the spectra were recorded on UV paper. Fourier transform IR spectra were obtained with a Perkin-Elmer 1760-X FT-ir spectrophotometer. High performance liquid chromatographies were performed using a Varian 2510 pump equipped with a Waters dual cell refractometer. Melting points were determined on a Reichert Termovar type 300429 Kofler hot stage melting apparatus and are uncorrected. Optical rotations were meausured on a Perkin-Elmer Model 141 polarimeter. Column chromatography was carried out on Merck Silica gel 40 (70-230 mesh) and 60 (230-400 mesh).

Extraction of the sponge and isolation of 1.- S. 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'Universita' di Napoli". Freshly collected animals (460 g dry weight after extraction) were extracted two times with acetone and then with $CHCl_3$-CH_3OH (1:1) for a period of three days at 20°C. The combined extracts were concentrated under reduced pressure and the aqueous residue was extracted several times with diethyl ether. The combined ethereal extracts were evaporated and the oily residue (25.6 g) was chromatographed on a silica gel column (600 g, 4 cm diameter) eluted with solvents of increasing polarity from light petroleum through $CHCl_3$ and increasing amounts of CH_3OH in $CHCl_3$. 200 mL fractions were collected and checked by ^1H-NMR for sterol content. Fractions 40-51 (420 mg), eluted slightly before Δ^7-3β,5α,6β,9α-tetrahydroxylated sterols[12] with $CHCl_3$-CH_3OH (95:5), contained secosterol 1. These fractions were purified by HPLC on a Hibar LiChrosorb Si-60 column (250 x 10 mm) using various $CHCl_3$-CH_3OH mixtures as the mobile phase. The fraction emerging from the Si-gel HPLC (25 mg) was further purified by reversed-phase HPLC on a Hibar Superspher RP-18 column (250 x 4 mm) eluted with CH_3OH/H_2O (75:25) to give 1 (14 mg), still contaminated by other steroidal material. The final purification of 1 was achieved on a Wathman PartiSphere RP-18 (110 x 4.7 mm) column (CH_3OH-H_2O, 75:25). A resonable separation of 1 was achieved by cutting the broad hplc peak into three slices. The slower eluted fraction from this separation contained compound 1 sufficiently pure for spectral studies. Reinjection of the remaining two fractions in the same conditions allowed to recover an overall quantity of 1 of 10 mg.

3β,6α-Dihydroxy-9-oxo-9,11-seco-5α-cholest-7-en-11-al (1). Amorphous powder; $[\alpha]_D$=+5.0 (c=0.2, $CHCl_3$); FTIR (neat) ν_{max} 3370, 1670, 1716 cm^{-1}; UV (CH_3OH) λ_{max} 241 (ϵ=3650) nm; ^1H- and ^{13}C-NMR (see Table); HREIMS m/z (assignement, relative intensies) 432.3272 (M^+, $C_{27}H_{44}O_4$ requires 432.3240, 12), 414.3076 (M^+-H_2O, $C_{27}H_{42}O_3$, 3), 404.3326 (M^+-CO, $C_{26}H_{44}O_3$, 8), 399.2879 (M^+-H_2O-CH_3, $C_{26}H_{39}O_3$, 26), 389.3035 (M^+-CH_2CHO, $C_{25}H_{41}O_3$, 100), 381.2833 (M^+-$2H_2O$-CH_3, $C_{26}H_{37}O_2$, 9), 319.1927 (M^+-C_8H_{17}, $C_{19}H_{27}O_4$, 18), 301.1827 (M^+-C_8H_{17}-H_2O, $C_{19}H_{25}O_3$, 22), 283.1663 (M^+-C_8H_{17}-$2H_2O$, $C_{19}H_{23}O_2$, 9), 241.1259 (M^+-$2H_2O$ and ring D fission, $C_{16}H_{17}O_2$, 3), 177.0903 (fragment comprising rings A and B-H_2O, $C_{11}H_{13}O_2$, 13), 159.0810 (fragment comprising rings A and B-$2H_2O$, $C_{11}H_{11}O$, 9).

5α-Cholest-7-ene-3β,6α-diol 3,6-diacetate (3). To a cooled solution (ice-

bath) of cholesta-5,7-dien-3β-ol (**2**) (1.010 g, 2.63 mmol), in anhydrous THF (400 mL) under nitrogen, 115 mL of a 1M solution of BH_3-THF complex (Aldrich) was added. The mixture was stirred in the dark for 1 h at 0°C and 1 h at room temperature. Then 200 mL of H_2O was slowly added followed by 70 mL of 3N sodium hydroxide and the slow addition of 170 mL of 35% hydrogen peroxide. After 75 min. the mixture was concentrated *in vacuo* and extracted with $CHCl_3$. The chloroform extract was washed with water, dried over Na_2SO_4 and evaporated. The crude residue (1.3 g) was acetylated overnight at room temperature with pyridine/acetic anhydride (1:1 v/v). Purification on silica gel column with a step gradient from petroleum ether to petroleum ether/ethyl ether 9:1 gave 567 mg of **3** as a crystalline product, which was indistinguishable from a sample of **3** prepared by acetylation of natural 5α-cholest-7-ene-3β,6α-diol previously isolated from *S. gracilis*[8]. **3**: oil; $[\alpha]_D$=+7.6 (c=0.3, $CHCl_3$); FTIR (neat) ν_{max} 1736, 1245 cm^{-1}; ^1H-NMR and MS (see ref.8).

5α-Cholesta-7,9(11)-diene-3β,6α-diol 3,6-diacetate (4). To a solution of 567 mg (1.16 mmol) of 5α-cholest-7-ene-3β,6α diol 3,6-diacetate (**3**) in 3.2 mL of $CHCl_3$, 797 mg (2.5 mmol) of mercuric acetate in 4.1 mL CH_3COOH was added and the suspension was stirred at room temperature (approximately 25°C) for 23 h and then filtered. The filtrate was kept for 2 h at 75°C and 30 min. under reflux and filtered again . The filtrate was treated with saturated aqueous $NaHCO_3$ solution and extracted with $CHCl_3$. The extract was washed with water, dried over Na_2SO_4 and concentrated *in vacuo*. The residue (557 mg) was chromatographed over a silica gel column eluted with increasing concentrations of ethyl ether in petroleum ether. 9:1 Ethyl ether/petroleum ether eluted 206 mg of crude **4** which was further purified by HPLC on a Hibar LiChrosorb RP-18 (250 x 10 mm) column using CH_3OH as the eluent to give pure 5α-cholesta-7,9(11)-diene-3β,6α-diol 3,6-diacetate (**4**) (120 mg) which showed nuclear ^1H-NMR resonances virtually identical to those exhibited by the product prepared by acetylation of natural (22E)-5α-cholesta-7,9(11),22-triene-3β,6α-diol, previously isolated from *S. gracilis*[10]. **4**: oil; $[\alpha]_D$=+95.4 (c=1.2, $CHCl_3$); FTIR (neat) ν_{max} 1737, 1245 cm^{-1}; UV (CH_3OH) λ_{max} 251 (ε=12500), 243 (ε=18500), 237 (ε=16580) nm; ^1H-NMR ($CDCl_3$, 200 MHz) δ 5.56 (1H, bd, J=5.8 Hz, H-11), 5.19 (1H, bs, H-7), 5.14 (1H, bd, J=10.8 Hz, H-6), 4.67 (1H, m, H-3), 2.09 and 2.03 (3H each, s's, acetates), 1.02 (3H, s, $H_3$19), 0.95 (3H,d, J=6.6 Hz, H_3-21), 0.87 (6H, d, J=6.6 Hz, H_3-26 and H_3-27), 0.51 (3H, s, H_3-18); ^{13}C-NMR ($CDCl_3$, 67.9 MHz) δ 11.27, 18.37, 20.66, 21.29, 21.37, 22.50, 22.78, 22.95, 23.85, 27.38, 27.95, 28.29, 29.31, 34.54, 35.62, 35.84, 35.95, 39.41, 42.14, 42.44, 43.61, 51.29, 56.20,

72.26, 72.51, 119.94, 121.36, 138.15, 141.91, 170.50, 171.15; EIMS: m/z (assignment, relative intensities) 484 (M^+, 2), 469 (M^+-CH_3, 1), 442 (M^+-CH_2CO, 3), 424 (M^+-CH_3COOH, 30), 409 ($M^+CH_3COOH-CH_3$, 4), 382 ($M^+-CH_3COOH-CH_2CO$, 59), 364 (M^+-2CH_3COOH, 100), 349 ($M^+-2CH_3COOH-CH_3$, 42), 311 ($M^+-C_8H_{17}-CH_3COOH$, 1), 251 ($M^+-C_8H_{17}-2CH_3COOH$, 41), 209 ($M^+-C_8H_{17}-2CH_3COOH-42$, 51).

Dimeric osmate ester (5). To 28 mg (0.058 mmol) of **4** dissolved in 750 μL of freshly distilled 1,4-dioxane[27], excess osmium tetraoxide was added at room temperature under stirring. The mixture, which became dark brown within a few minutes, was left at room temperature for 4 h. After removal of the solvent, the residue was purified by Si-gel TLC (eluent $CHCl_3$-CH_3OH, 99:1) followed by HPLC on a LiChrosorb Si-60 (250 x 4 mm) column using n-hexane-EtOAc (7:3) as the eluent giving 24 mg of pure osmate ester **5**: brown oil; $[\alpha]_D$=-290 (c=0.2 , $CHCl_3$); FTIR (neat) ν_{max} 1737, 1244, 1031, 995, 757, 632 cm^{-1}; ^1H-NMR (pyridine-d_5, 270 MHz) (assignments based on a ^1H-^1H COSY-45 experiment) δ 5.40 (1H, bd, J=2.1 Hz, H-7), 5.39 (1H, dd, J=7.7 and 7.7 Hz, H-11), 5.09 (1H, ddd, J=10.2, 2.1 and 2.1 Hz, H-6), 4.48 (1H, m, H-3), 2.61 (1H, dd, J=14.1 and 7.7 Hz, H_{eq}12), 2.55 (1H, ddd, J=10.2, 10.2 and 3.8 Hz, H-5), 2.17 (1H, bdd, J=9.4 and 9.4 Hz, H-14), 2.05 and 1.99 (3H each, s's, acetates), 1.99 (m, overlapped, H_{eq}-4), 1.68 (1H, bd, J=13.2 Hz, H_{eq}-2), 1.59 (m, long-range coupled with H_3-18, H_{ax}-12), 1.57 (m, overlapped, H_a-15), 1.51 (m, overlapped, H-25), 1.41 (m, overlapped, H_b-15), 1.48 (m, overlapped, H_{ax}-2), 1.35 (m, overlapped, H-20), 1.32 (m, overlapped, H_{ax}-4), 1.24 (3H, s, H_3-19), 0.90 (3H, d, J=6.0 Hz, H_3-21), 0.86 (6H, d, J=6.4 Hz, H_3-26 and H_3-27), 0.67 (3H, s, H_3-18), 0.48 (1H, bddd, J=13.2, 13.2 and 3.4 Hz, H_{ax}-1); ^{13}C-NMR ($CDCl_3$, 67.9 MHz), δ 12.60, 13.36, 18.61, 21.21, 21.33, 22.54, 22.84, 22.94, 23.79, 27.08, 27.96, 30.06, 32.84, 35.82, 35.82, 37.51, 39.43, 42.37, 42.61, 46.08, 51.42, 55.55, 71.55, 72.23, 89.21, 98.99, 125.65, 140.52, 170.28, 171.43; FABMS gave a significant ion cluster at m/z 1135, 1136, 1137, 1138, 1139 ($MH^+-CH_3COOH-CH_2CO$); EIMS m/z (assignment, relative intensities) 424 ($M^+-C_{31}H_{48}O_9Os-CH_3COOH$, 11), 409 ($M^+-C_{31}H_{48}O_9Os-CH_3COOH-CH_3$, 1), 382 ($M^+-C_{31}H_{48}O_9Os-CH_3COOH-CH_2CO$, 4), 364 ($M^+-C_{31}H_{48}O_9Os-2CH_3COOH$, 100), 349 ($M^+-C_{31}H_{48}O_9Os-2CH_3COOH-CH_3$, 64), 311 ($M^+-C_{31}H_{48}O_9Os-C_8H_{17}-CH_3COOH$, 13), 251 ($M^+-C_{31}H_{48}O_9Os-C_8H_{17}-2CH_3COOH$, 36), 209 ($M^+-C_{31}H_{48}O_9Os-C_8H_{17}-2CH_3COOH-42$, 41).

5α-Cholest-7-ene-3β,6α,9α,11α-tetrol (6). The osmate ester **5** (10 mg, 0.0081 mmol) in anhydrous ether (3 mL) was treated with excess lithium aluminum hydride and the mixture was stirred at room temperature for 75

min. Excess reagent was destroyed by dropwise addition of water and the ether layer was decanted. The white precipitate was washed with Et_2O and then with $CHCl_3$. The organic phases were separately taken to dryness, dried (Na_2SO_4), evaporated under reduced pressure and checked by TLC. Since no appreciable difference was observed on TLC analysis the organic materials were combined. HPLC purification on a Hibar LiChrosorb Si-60 (250 x 4 mm) column , using $CHCl_3$-CH_3OH (9:1) as eluent, afforded 6.5 mg of pure tetrol **6** : m.p 232-234 °C (CH_3OH); $[\alpha]_D$=+17.6 (c=0.4, CH_3OH); FTIR (neat) 3400 cm^{-1}; ^1H-NMR (pyridine-d_5, 270 MHz) (assignments based on a ^1H-^1H COSY-45 experiment) δ 6.26 (1H, d, J=6.8 Hz, OH-6), 5.96 (1H, d, J=5.5 Hz, OH-11), 5.86 (1H, dd, J=7.7 and 7.7 Hz, H-7), 5.52 (1H, d, J=6.8 Hz, OH-3), 5.19 (1H, s, OH-9), 4.47 (1H, ddd, J=11.9, 5.5 and 5.5 Hz, H-11), 4.18 (1H, dddd, J=9.8, 6.8, 1.7 and 1.7 Hz, H-6), 3.97 (1H, m, H-3), 3.15 (1H, bddd, J=12.4, 4.3, 2.1 and 2.1 Hz, H_{eq}-4), 2.77 (1H, dddd, J=9.4, 9.4, 1.7 and 1.7 Hz, H-14), 2.68 (1H, ddd, J=14.1, 3.8 and 3.8 Hz, H_{eq}-1), 2.52 (1H, ddd, J=12.4, 9.8 and 4.3 Hz, H-5), 2.40 (1H, dd, J=11.9 and 5.5 Hz, H_{eq}-12), 2.31 (1H, ddd, J=14.1, 14.1 and 4.7 Hz, H_{ax}-1), 2.18 (1H, bd, 12.4 Hz, H_{eq}-2), 1.95 (1H, dd, J=11.9 and 11.9 Hz, H_{ax}-12), 1.85 (1H, m, overlapped, H_{ax}-2), 1.82 (1H, ddd, J=12.4, 12.4 and 12.4 Hz, H_{ax}-4), 1.80 (1H, m, overlapped, H_a-16), 1.47 (3H, overlapping multiplets, H-25 and H_2-15), 1.30 (3H, s, H_3-19), 1.30 (1H, m, overlapped, H-20), 1.22 (1H, m, H_b-16), 1.18 (1H, m, H-17), 1.11 (2H, m, H_2-24), 0.95 (3H, d, J=6.4 Hz, H_3-21), 0.85 (6H, d, J=6.4 Hz, H_3-26 and H_3-27), 0.69 (3H, s, H_3-18); ^{13}C-NMR (pyridine-d_5, 67.9 MHz), δ 12.65, 16.23, 18.94, 22.66, 22.93, 23.32, 24.13, 28.22, 28.29, 32.71, 33.07, 36.01, 36.30, 36.35, 39.68, 41.25, 43.32, 43.52, 47.57, 51.01, 56.28, 69.56, 69.56, 70.24, 75.15, 129.45, 139.77; EIMS m/z (assignment, relative intensities) 416 (M^+-H_2O, 33), 411 (M^+-H_2O-CH_3, 17), 398 (M^+-$2H_2O$, 13), 383 (M^+-$2H_2O$-CH_3, 19), 380 (M^+-$3H_2O$, 7), 365 (M^+-$3H_2O$-CH_3, 17), 347 (M^+-$4H_2O$-CH_3, 5), 303 (M^+-C_8H_{17}-H_2O, 100), 285 (M^+-C_8H_{17}-$2H_2O$, 24), 267 (M^+-C_8H_{17}-$3H_2O$, 15), 249 (M^+-C_8H_{17}-$4H_2O$, 13).

Reaction of 5α-cholest-7-ene-3β,6α,9α,11α-tetrol (6) with Lead Tetraacetate. Synthetic 1. To a solution of 5α-cholest-7-ene-3β,6α,9α,11α-tetrol (**6**) (2 mg, 0.0046 mmol) in CH_3COOH (750 μL), crystalline lead tetraacetate (2.2 mg) was added in little portions during 30 min. at room temperature. After each addition the evolution of the reaction was monitored by TLC ($CHCl_3$-CH_3OH, 9:1). When no starting material remained, the reaction was quenched by adding two drops of ethylene glycol, the mixture diluted with ice-water and then extracted with $CHCl_3$. The organic layer was washed with aqueous $NaHCO_3$, dried

(Na_2SO_4) and evaporated . The residue was chromatagraphed on a Hibar LiChrosorb Si-60 (250 x 4 mm) column using $CHCl_3$-CH_3OH (96:4) as eluent to yield 1.8 mg of ketoaldehyde **1** which had spectral (^{1}H-NMR, ^{13}C-NMR, IR, UV, MS) and chromatographic properties identical to those exhibited by the natural product **1** .

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