New Sulfated Polyhydroxysteroids from the Antarctic Ophiuroid *Astrotoma agassizii*

Alejandro J. Roccatagliata, Marta S. Maier,* and Alicia M. Seldes

Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Pabellón 2, Ciudad Universitaria, 1428 Buenos Aires, Argentina

Received September 19, 1997

Three new and two known sulfated steroidal polyols have been isolated from the Antarctic ophiuroid *Astrotoma agassizii*. All of the new steroids possess a terminal isopropenyl group and a C-21 sulfoxy moiety in the side chain but differ in the steroidal nuclei. The structures of these new steroids were established from spectral and chemical correlations with related steroids as (20*R*)-cholesta-5,24-diene-2β,3α,21-triol 2,21-disulfate (**1**), (20*R*)-5α-cholest-24-ene-2β,3α,21-triol 3,21-disulfate (**3**), and (20*R*)-cholesta-5,24-diene-2α,3α,4β,21-tetrol 3,21-disulfate (**5**). Analysis of the nonsulfated sterol fraction has shown the presence of cholest-5-en-3β-ol, cholesta-5,24-dien-3β-ol and (22E) cholesta-5,22-dien-3β-ol as the major sterols.

Among the echinoderms, starfish and sea cucumbers contain saponins that are responsible for their general toxicity. Starfish also appear as the richest source of polyhydroxysteroids, where they have been found usually as complex mixtures together with minor amounts of their glycosides. On the contrary, ophiuroids are characterized by their content of polar sulfated steroids and the lack of saponins.[1] The presence of two sulfated steroidal monoglycosides has been reported only in the brittle star *Ophioderma longicaudum*.[2] The degree of structural variability of polyhydroxysteroids isolated from ophiuroids is not so large as in starfish. Most of them present C-21 and C-3α sulfate groups and additional hydroxy groups located in rings A and C. Hydroxylation at C-21 was found among starfish metabolites not only from *Euretaster insignis*[3] but also from *Pteraster tesselatus and Pteraster sp.*[4] Sulfated steroidal polyols have shown antiviral properties,[5] cytotoxic action,[6] and inhibition of protein tyrosine kinases.[7] Recently, we have demonstrated the antiviral activity of sulfated steroids isolated from the ophiuroid *Ophioplocus januarii* against four different pathogenic viruses of humans.[8]

In continuation of our studies on echinoderms of cold waters of the South Atlantic[8,9] we have investigated the Antarctic ophiuroid *Astrotoma agassizii* Lyman (family Gorgonocephalidae). We have isolated three new sulfated polyhydroxysteroids (**1**, **3**, and **5**) as well as two known compounds (**2** and **4**) (Chart 1), previously isolated from the Antarctic ophiuroid *Ophiosparte gigas*.[10] Analysis of the nonsulfated sterol fractions has shown the presence of common 3β-hydroxysterols.

The ophiuroid *A. agassizii* was homogenized and extracted with EtOH followed by centrifugation and concentration. The extract was partitioned between *n*-hexane and H_2O. The aqueous phase was then passed through a column of Amberlite XAD-2 resin, which was washed with distilled H_2O and then eluted with MeOH in order to recover the steroidal compounds. Separation and isolation of the individual compounds from the MeOH extract was achieved by vacuum-dry column chromatography on reversed-phase (35−75 μ) Si gel, followed by reversed-phase HPLC.

The new compounds (**1**, **3**, and **5**) contain a terminal isopropenyl group in the side chain. This group has not previously been reported in sulfated polyhydroxysteroids isolated from ophiuroids but has been observed in asterosaponins isolated from starfish.[11] The known compounds **2** and **4** were identified by comparison of their ^{1}H and ^{13}C NMR, FABMS, and optical rotation data with published data.[10]

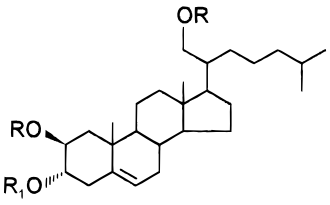
(20*R*)-Cholesta-5,24-diene-2β,3α,21-triol 2,21-disulfate (**1**), the major component of *A. agassizii*, has a sulfoxy group located at the 2β- instead of the common 3α-position. This is the second example of this structural type isolated from a brittle star, the first one being (20*R*)-cholest-5-ene-2β,3α,21-triol 2,21-disulfate (**2**), isolated previously from *Ophiosparte gigas*.[10] The negative-ion FABMS spectrum of **1** showed molecular ion species at *m/z* 597 [M(SO_3Na)(SO_3^-)] and 613 [M(SO_3K)(SO_3^-)] corresponding to a disulfated trihydroxycholestadiene structure. The ^{13}C NMR spectrum was consistent with the presence of 27 carbon atoms (Table 1), and DEPT measurements showed the presence of an oxomethylene signal at δ 69.5 ppm (C-21), two oxomethine resonances at δ 78.3 and 69.5 ppm, as well as two trisubstituted double bonds. The ^{1}H NMR spectrum contained two vinyl methyl signals at δ 1.61 and 1.65 ppm and one olefinic proton signal at δ 5.10 ppm, assigned to the terminal isopropenyl group of the side chain. The ^{13}C NMR spectrum verified the presence of this group: two methyl groups at 25.8 (C-26) and 17.8 ppm (C-27) attached to an olefinic carbon at 132.0 ppm (C-25) and the CH olefinic carbon at δ 125.9 (C-24).

The ^{1}H and ^{13}C NMR data indicated the presence of a sulfate group at C-21 and a trisubstituted double bond (δ_H 5.34 ppm, δ_C 139.5 and 123.5 ppm), which was located at the 5(6) position. ^{1}H NMR assignments were derived from ^{1}H−^{1}H COSY data. Doublet-of-doublet signals at δ 3.97 and 4.18 correlated each other and were assigned to 21-sulfoxymethylene protons. From

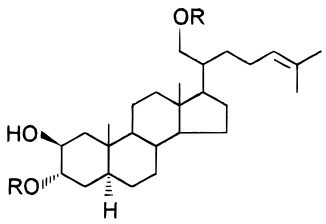
* To whom correspondence should be addressed. Phone and Fax: 0541-782-0529, E-mail: maier@quimor.qo.fcen.uba.ar.

S0163-3864(97)00429-1 CCC: $15.00
Published on Web 02/20/1998

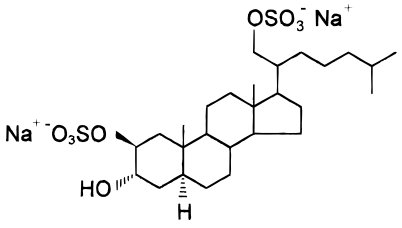
Chart 1



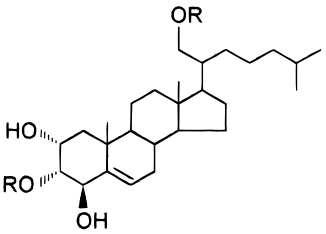
1 R = SO_3^- Na^+, R_1 = H, Δ^{24}
2 R = SO_3^- Na^+, R_1 = H
1a R = SO_3^- Na^+, R_1 = Ac, Δ^{24}
1b R = R_1 = H, Δ^{24}



3 R = SO_3^- Na^+
3a R = H



1c



4 R = SO_3^- Na^+
5 R = SO_3^- Na^+, Δ^{24}
5a R = H, Δ^{24}

Table 1. ^{13}C NMR Chemical Shifts for Compounds **1**, **1c**, **3**, and **5**[a]

carbon	1	1c	3	5
1	39.0	38.9	40.5	39.7
2	78.3	78.8	70.0	70.7
3	69.5	69.5	78.4	82.6
4	35.8	32.5	30.3	79.3
5	139.5	41.4	41.0	142.0
6	123.5	29.4	29.2	128.9
7	32.8	33.3	33.2	32.8
8	32.7	36.4	36.3	32.8
9	51.8	56.7	56.6	51.8
10	37.7	36.4	36.6	36.7
11	21.8	22.0	21.9	21.8
12	40.1	40.8	41.1	40.2
13	43.3	43.6	43.6	43.4
14	57.9	57.8	57.8	58.2
15	25.1	25.1	25.0	25.0
16	28.5	28.4	28.8	28.6
17	51.8	52.0	51.9	51.8
18	12.5	12.8	12.7	12.6
19	22.1	14.6	14.6	25.0
20	40.8	41.3	40.6	40.9
21	69.5	69.5	69.3	69.3
22	30.8	30.8	30.8	30.8
23	25.4	24.5	25.4	25.4
24	125.9	40.5	126.1	126.0
25	132.0	29.1	132.0	132.0
26	25.8	23.1	25.8	25.9
27	17.8	23.2	17.8	17.8

[a] Values are recorded at 50.3 MHz in CD_3OD.

the $^1H-^1H$ COSY spectrum it was determined that the signal at δ 4.03 was coupled to the protons at δ 4.42 (H-2) and 2.81 (H-4_{ax}) and was assigned on this basis to H-3. The relative stereochemistry of the protons on C-2 and C-3 was defined by the coupling constants for H-2 (br d, J = 2.5 Hz) and H-3 (br d, J = 2.5 Hz) and assigned as equatorial due to the absence of any large vicinal couplings. The sulfate group was located at C-2 by comparison of the chemical shifts of C-1 (39.0 ppm) and C-4 (35.8 ppm) with those of (20*R*)-cholest-5-en-2β,3α,21-triol 2,21-disulfate (C-1 39.1 ppm and C-4 35.8 ppm)[10] and the isomeric (20*R*)-cholest-5-ene-2β,3α,21-triol 3,21-disulfate (C-1 40.1 ppm and C-4 33.5 ppm),[12] by its chemical shift value (δ 4.42), and by the downfield shift observed for the C-3 proton of the monoacetylated derivative **1a** (see the Experimental Section). The axial orientation of the hydroxyl group at C-2 was also supported by a pyridine-induced solvent shift of the 19-methyl protons (Δ +0.58) of the desulfated derivative **1b** (see the Experimental Section), which indicated a 1,3-diaxial interaction between H-19 and the hydroxyl group at C-2.[13] The 20*R*-configuration assigned to **1** was further supported by comparison of the ^1H NMR chemical shifts of the 21-hydroxymethylene protons of the desulfated derivative **1b** with those of the reference compounds (20*R*)- and (20*S*)-cholest-5-ene-3β,21-diol.[14]

The negative-ion FABMS of **3** showed molecular ion species at *m/z* 599 [M(SO_3Na)(SO_3^-)] and 615 [M(SO_3K)(SO_3^-)] corresponding to a disulfated trihydroxycholestene structure. The ^{13}C NMR spectrum of **3** showed 27 carbon atoms (Table 1), and DEPT measurements revealed the presence of an oxomethylene signal at δ 69.3 ppm (C-21), two oxomethine resonances at δ 78.4 and 70.0 ppm, two methyl groups at δ 25.8 (C-26) and 17.8 (C-27) attached to a quaternary olefinic carbon at 132.0 ppm (C-25), and a CH olefinic carbon at δ 126.1 (C-24). The ^1H NMR spectrum showed two vinyl methyl signals at δ 1.60 and 1.65 ppm and an olefinic proton signal at δ 5.09 ppm. In the $^1H-^1H$ COSY spectrum, these three signals were correlated to each other, establishing the presence of a terminal isopropenyl

group in the side chain. The trans A/B ring fusion followed from the chemical shift of the C-19 (δ 14.6 ppm).[16] The presence of a 21-sulfoxy group was confirmed by two one-proton doublet-of-doublet signals at δ 3.94 and 4.17 ppm coupled to each other by 9.5 Hz, in the ^{1}H NMR spectrum, while signals for two protons at δ 4.06 and 4.39 indicated the presence of two methine protons on oxygen-bearing carbons. The positions of the hydroxyl group at C-2 and the sulfate group at C-3 were established from the correlations observed between the H-2 methine (δ 4.06) and the vicinal H-3 methine (δ 4.39) and the H-1 methylene pair (δ 1.33, 1.75). A W-type long-range coupling between H-1a (δ 1.33) and the C-19 methyl protons (δ 0.99) was also evident in the ^{1}H$-^1$H COSY spectrum. The relative stereochemistry of protons on C-2 and C-3 was assigned as equatorial due to the absence of any large vicinal couplings. The position and axial disposition of the hydroxyl group at C-2 were also supported by a pyridine-induced solvent shift of the C-19 methyl protons ($\Delta = +0.35$). The sulfate group was placed at C-3 based on the downfield shift of H-3 to δ 4.39 and by comparison with the ^{1}H NMR of the desulfated derivative **3a** at δ 3.75 ppm. An independent confirmation of the sulfate position at C-3 was provided by a simple chemical transformation on compound **1**; hydrogenation over palladium hydroxide afforded (20*R*)-5α-cholestane-2β,3α,21-triol 2,21-disulfate (**1c**). The ^{13}C NMR spectra of compounds **1c** and **3** differed in the chemical shifts of C-1 (δ 38.9 in **1c**, 40.5 in **3**) and C-4 (δ 32.5 in **1c**, 30.3 in **3**) (Table 1). The upfield shifts of 1.6 ppm for C-1 in **1c** and 2.2 ppm for C-4 in **3** due to the presence of a sulfate group at C-2 and C-3, respectively, are in accordance with the chemical shifts reported for C-1 (δ 39.2) and C-4 (δ 30.6) in 5α-cholestane-2β,3α,26-triol 2,3,26-trisulfate.[15] (20*R*)-5α-Cholest-24-ene-2β,3α,21-triol 3,21-disulfate (**3**) is one of the few examples of a polyhydroxysteroid with a trans A/B ring fusion isolated from ophiuroids.[12,15]

The most polar sulfated sterol of *A. agassizii* was characterized as (20*R*)-cholesta-5,24-diene-2α,3α,4β,21-tetrol 3,21-disulfate (**5**). Its 24-saturated analogue **4** has previously been isolated from the Antarctic ophiuroid *Ophiosparte gigas*.[10] The negative FABMS of **5** showed molecular ion species at *m/z* 613 and 629 corresponding to [M(SO_3Na)(SO_3^-)] and [M(SO_3K)(SO_3^-)], respectively. The ^{1}H and ^{13}C NMR spectra confirmed the presence of the terminal isopropenyl group (δ_{H-26} 1.61, δ_{C-26} 25.9; δ_{H-27} 1.67, δ_{C-27} 17.8; δ_{H-24} 5.10, δ_{C-24} 126.0, δ_{C-25} 132.0). The ^{13}C NMR spectrum was consistent with the presence of 27 carbon atoms (Table 1), including three oxomethine resonances at δ 70.7, 79.3, and 82.6 ppm and the $-CH_2OSO_3^-$ signal at δ 69.3 ppm (C-21). The presence of the sulfate group at C-21 was confirmed in the ^{1}H NMR spectrum by the two signals at δ 3.97 and 4.18 ppm coupled to each other by 9.5 Hz. The ^{1}H NMR spectrum also contained signals for the methine protons at δ 3.88, 4.27, and 4.42 ppm (see the Experimental Section), and double-resonance experiments proved that they were located on carbons 2, 4, and 3, respectively. Irradiation of signal at δ 4.42 (H-3) sharpened the H-4 proton at δ 4.27 to a broad singulet and transformed the multiplet at δ 3.88 (H-2) into a double doublet (J = 6.2, 4.0 Hz). Coupling constant analysis allowed us to assign protons on C-3 and C-4 as equatorial (J = 5.1, 2.2 Hz) and the proton on C-2 as axial due to its large coupling (J = 6.2 Hz) with the vicinal 1-H_{ax}. The α-configuration of the C-2 hydroxyl group was confirmed by the NOESY cross-peaks δ 4.42/3.88 (H-3/H-2) and δ 3.88/1.26 (H-2/Me-10), indicating that all of these groups are on the β face of the molecule.

Solvolysis of **5** in a dioxane−pyridine mixture afforded the desulfated compound **5a**. In the ^{1}H NMR spectrum of **5a** in CD_3OD, the resonance frequencies of H-2 and H-4 were shifted only by 0.32 and 0.31 ppm relative to **5**, while the H-3 signal moved upfield to δ 3.72 ppm, supporting the location of the sulfate group at C-3.

Separation of the crude sterol mixture from the *n*-hexane extract was achieved by vacuum-dry column chromatography on silica gel using *n*-hexane, 25% stepwise gradient elution from *n*-hexane to EtOAc, and MeOH. Analysis of the sterol mixture by GC−MS revealed the presence of cholest-5-en-3β-ol (58.0%), cholesta-5,24-dien-3β-ol (29.4%), and (22*E*)-cholesta-5,22-dien-3β-ol (12.0%). The sterol mixture was partitioned by reversed-phase HPLC into two fractions, which were each analyzed by GC−MS and ^{1}H NMR spectroscopy. Both fractions showed signals at δ 5.35 (H-5) and 3.50 (H-3α) ppm, typical for 3β-hydroxy-Δ^5-sterols. Fraction 1 contained cholesterol, the major sterol of the mixture. The ^{1}H NMR spectrum of fraction 2 contained two singlets at δ 1.60 (Me-26) and 1.68 (Me-27) ppm and a triplet at δ 5.09 (H-24), ascribable to the side chain of desmosterol as well as the C-21 methyl doublet at δ 1.01 due to the Δ^{22}-trans bond of (22*E*)-cholesta-5,22-dien-3β-ol.[17]

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ACE-200 instrument. FABMS were obtained on a VG-ZAB mass spectrometer. Preparative HPLC was carried out on a SP liquid chromatograph equipped with a Spectra Series P100 solvent delivery system, a Rheodyne manual injector, and a refractive index detector using a C_{18} Bondclone 10 μ column (30 cm × 7.8 mm i.d) and YMC-Pack ODS-A 5 μ column (25 cm × 20 mm i.d.). TLC was performed on precoated Si gel F254 and C_{18} reversed-phase plates. GC was performed on a Hewlett-Packard 5890A chromatograph equipped with a flame-ionization detector and an HP-5 column (25 m × 0.2 mm i.d.) containing cross-linked 5% PhMe silicone (carrier gas N_2, temperatures between 150 and 280 °C at a rate of 15 °C/min). The identities of the sterols were assigned by GC−MS using a TRIO-2 VG mass spectrometer coupled to a Hewlett-Packard 5890A gas chromatograph.

Animal Material. Specimens of *A. agassizii* (9.5 kg) were collected at different locations around the South Georgia Islands. The organisms were identified by Dr. Alejandro Tablado of the Museo de Ciencias Naturales "Bernardino Rivadavia", where a voucher specimen is preserved (MACN no. 33857).

Extraction and Isolation. The animals, frozen prior to storage, were homogenized in EtOH (10 L) and centrifuged. The EtOH was evaporated, and the residue was partitioned between H_2O and *n*-hexane. The aqueous extract was purified through an Amberlite XAD-2

column (1 kg) and eluted with distilled water (until a negative chloride reaction was observed) followed by MeOH. The MeOH eluate was evaporated to give a glassy material that was subjected to vacuum-dry column chromatography on Davisil C_{18} reversed-phase (35−75 μ) using H_2O, H_2O−MeOH mixtures with increasing amounts of MeOH and finally MeOH. Fractions of 400 mL were collected and analyzed by TLC on SiO_2 in *n*-BuOH−HOAc−H_2O (4:5:1) (upper layer) and by C_{18} reversed-phase TLC [MeOH−H_2O (65:35)] and detected by spraying with H_2SO_4. Final purification of the fractions containing the sulfated compounds was accomplished by HPLC on a C_{18} Bondclone column with MeOH−H_2O (50:50) to give the pure compounds **1** (63.2 mg), **2** (8.3 mg), **3** (18.5 mg), **4** (34.3 mg), and **5** (23.1 mg).

(20*R*)-Cholesta-5,24-diene-2*β*,3α,21-triol 2,21-disulfate (1): obtained as a white powder; $[\alpha]^{25}_D$ −13.0° (*c* 0.92, MeOH); ^{1}H NMR (CD_3OD, 200.1 MHz) δ 0.73 (3H, s, Me-18), 1.16 (3H, s, Me-19), 1.61 (3H, s, Me-26), 1.65 (3H, s, Me-27), 2.81 (1H, bd, J = 14.7 Hz, H-4_{ax}), 3.97 (1H, dd, J = 9.5, 6.2 Hz, H-21), 4.03 (1H, bd, J = 2.5 Hz, H-3β), 4.18 (1H, dd, J = 9.5, 3.7 Hz, H-21), 4.42 (1H, bd, J = 2.5 Hz, H-2α), 5.10 (1H, t, J = 6.7 Hz, H-24), 5.34 (1H, m, H-6).

(20*R*)-5α-Cholest-24-ene-2*β*,3α,21-triol 3,21-disulfate (3): obtained as a white powder; $[\alpha]^{25}_D$ +20.4° (*c* 0.25, MeOH); ^{1}H NMR (CD_3OD, 200.1 MHz) δ 0.70 (3H, s, Me-18), 0.99 (3H, s, Me-19), 1.60 (3H, s, Me-26), 1.65 (3H, s, Me-27), 3.94 (1H, dd, J = 9.5, 6.2 Hz, H-21), 4.06 (1H, bd, J = 2.5 Hz, H-2α), 4.17 (1H, dd, J = 9.5, 3.7 Hz, H-21), 4.39 (1H, bd, J = 2.5 Hz, H-3β), 5.09 (1H, t, J = 6.7 Hz, H-24).

(20*R*)-Cholesta-5,24-diene-2α,3α,*4β*,21-tetrol 3,21-disulfate (5): obtained as a white powder; $[\alpha]^{25}_D$ +1.4° (*c* 0.14, MeOH); ^{1}H NMR (CD_3OD, 200.1 MHz) δ 0.75 (3H, s, Me-18), 1.26 (3H, s, Me-19), 1.61 (3H, s, Me-26), 1.67 (3H, s, Me-27), 3.88 (1H, m, H-2β), 3.97 (1H, dd, J = 9.5, 5.9 Hz, H-21), 4.18 (1H, dd, J = 9.5, 3.7 Hz, H-21), 4.27 (1H, bd, J = 2.2 Hz, H-4α), 4.42 (1H, dd, J = 5.1, 2.2 Hz, H-3β), 5.10 (1H, t, J = 6.7 Hz, H-24), 5.65 (1H, m, H-6).

(20*R*)-Cholesta-5,24-diene-2*β*,3α,21-triol 3-Acetate, 2,21-Disulfate (1a). Compound **1** (13.3 mg) was acetylated with pyridine (1 mL) and acetic anhydride (1 mL) by stirring for 3 h at room temperature. The reaction mixture was treated with 10 mL of ice−water and extracted with CH_2Cl_2 (2 × 5 mL). The CH_2Cl_2 solution was washed with H_2O (5 mL), dried with Na_2SO_4, and then concentrated in vacuo to yield a white powder. The acetate was purified by RP-18 TLC [MeOH:H_2O (90:10)] to obtain 8.6 mg of **1a**: ^{1}H NMR (CD_3OD, 200.1 MHz) δ 0.73 (3H, s, Me-18), 1.17 (3H, s, Me-19), 1.61 (3H, s, Me-26), 1.66 (3H, s, Me-27), 2.06 (3H, s, OAc), 2.80 (1H, bd, J = 14.6 Hz, H-4_{ax}), 3.96 (1H, dd, J = 9.5, 6.2 Hz, H-21), 4.17 (1H, dd, J = 9.5, 3.7 Hz, H-21), 4.48 (1H, bd, J = 2.5 Hz, H-2α), 5.09 (2H, bs, H-3β, H-24), 5.29 (1H, m, H-6).

Solvolysis of Compounds 1, 3, and 5. A solution of each compound (4.5 mg) in dioxane (0.1 mL) and pyridine (0.1 mL) was heated at 120 °C for 2 h in a stoppered reaction vial. After the solution had cooled, H_2O (1 mL) was added, and the solution was extracted with *n*-BuOH (3 × 0.5 mL). The combined organic extracts were washed with H_2O and evaporated to dryness under reduced pressure. The residue (3.0 mg) was analyzed by TLC and by 200 MHz ^{1}H NMR spectroscopy without purification.

(20*R*)-Cholesta-5,24-diene-2*β*,3α,21-triol (1b): ^{1}H NMR (Cl_3CD, 200.1 MHz) δ 0.68 (3H, s, Me-18), 1.15 (3H, s, Me-19), 1.59 (3H, s, Me-26), 1.66 (3H, s, Me-27), 2.93 (1H, bd, J = 14.7 Hz, H-4_{ax}), 3.66 (3H, m, H-2α, H-3β, H-21), 3.79 (1H, dd, J = 9.5, 3.7 Hz, H-21), 5.08 (1H, t, J = 6.7 Hz, H-24), 5.42 (1H, m, H-6); ^{1}H NMR (Py-d_5, 200.1 MHz) δ 0.83 (3H, s, Me-18), 1.74 (3H, s, Me-19), 1.63 (3H, s, Me-26), 1.69 (3H, s, Me-27), 3.45 (1H, bd, J = 13.9 Hz, H-4_{ax}), 3.93 (1H, bd, J = 10.4 Hz, H-21), 4.49 (2H, bd, J = 7.0 Hz, H-2α, H-3β), 4.11 (1H, bd, J = 10.4 Hz, H-21), 5.10 (1H, t, J = 6.7 Hz, H-24), 5.38 (1H, m, H-6).

(20*R*)-5α-Cholest-24-ene-2*β*,3α,21-triol (3a): ^{1}H NMR (CD_3OD, 200.1 MHz) δ 0.69 (3H, s, Me-18), 0.98 (3H, s, Me-19), 1.60 (3H, s, Me-26), 1.66 (3H, s, Me-27), 3.50 (1H, dd, J = 9.5, 6.2 Hz, H-21), 3.66 (1H, m, H-2α), 3.75 (2H, m, H-21, H-3β), 5.10 (1H, t, J = 6.7 Hz, H-24).

(20*R*)-Cholesta-5,24-diene-2α,3α,*4β*,21-tetrol (5a): ^{1}H NMR (CD_3OD, 200.1 MHz) δ 0.73 (3H, s, Me-18), 1.29 (3H, s, Me-19), 1.61 (3H, s, Me-26), 1.67 (3H, s, Me-27), 3.51 (1H, dd, J = 10.0, 5.9 Hz, H-21), 3.56 (1H, m, H-2β), 3.72 (2H, m, H-3β, H-21), 3.96 (1H, bd, J = 2.3 Hz, H-4α), 5.10 (1H, t, J = 6.7 Hz, H-24), 5.65 (1H, m, H-6).

Hydrogenation of Compound 1. Compound **1** (14.2 mg) was dissolved in MeOH, 4.5 mg of palladium hydroxide was added, and the resulting mixture was hydrogenated for 26 h at 40 psig. After filtration of the catalyst and evaporation of the solvent, the residue was purified by reversed-phase TLC [MeOH:H_2O (90:10)] to obtain 6.6 mg of compound **1c**.

(20*R*)-5α-Cholestane-2*β*,3α,21-triol 2,21-disulfate (1c): ^{1}H NMR (CD_3OD, 200.1 MHz) δ 0.71 (3H, s, Me-18), 0.87 (6H, d, J = 6.6 Hz, Me-26 and Me-27), 0.99 (3H, s, Me-19), 3.94 (1H, dd, J = 9.5, 6.2 Hz, H-21), 4.03 (1H, bd, J = 2.5 Hz, H-3β), 4.19 (1H, dd, J = 9.5, 3.7 Hz, H-21), 4.40 (1H, bd, H-2α).

The *n*-hexane extract was evaporated, and the residue (24.4 g) was subjected to vacuum-dry column chromatography on silica gel 60 using *n*-hexane, *n*-hexane−AcOEt mixtures with increasing amounts of AcOEt, AcOEt, and finally MeOH. Fractions (500 mL) were collected and analyzed by TLC on SiO_2 in Cl_2CH_2−MeOH (98:2) and detected by spraying with H_2SO_4. Fractions corresponding to *n*-hexane-AcOEt (50:50) and (40:60) provided the crude sterol mixture, which was analyzed by GC and fractionated by HPLC on a YMN-Pack ODS-A column with MeOH to give two fractions. Each fraction was analyzed by GC−MS and ^{1}H NMR spectroscopy.

Acknowledgment. This work was supported by the International Foundation for Science (IFS), CONICET, and the Universidad de Buenos Aires. We wish to thank UMYMFOR (CONICET-FCEyN) for spectroscopic analysis.

References and Notes

(1) D'Auria, M. V.; Minale, L.; Riccio, R. *Chem. Rev. (Washington, D.C.)* **1993**, *93*, 1839−1895.

(2) Riccio, R.; D'Auria, M. V.; Minale, L. *J. Org. Chem.* **1986**, *51*, 533−536.
(3) D'Auria, M. V.; Finamore, E.; Minale, L.; Pizza, C.; Riccio, R.; Zollo, M.; Pusset, M.; Tirard, P. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2277−2282.
(4) Levina, E. V.; Andriyaschenko, P. V.; Stonik, V. A.; Kalinovsky, A. I. *Comp. Biochem. Physiol.* **1996**, *114B*, 49−52.
(5) McKee, T. C.; Cardellina, J. H.; Riccio, R.; D'Auria, M. V.; Iorizzi, M.; Minale, L.; Moran, R. A.; Gulakowski, R. J.; McMahon, J. B.; Buckheit, R. W., Jr.; Snader, K. M., Boyd, M. R. *J. Med. Chem.* **1994**, *37*, 793−797.
(6) Anderson, L.; Bohlin, L.; Iorizzi, M.; Riccio, R.; Minale, L.; Moreno-López, W. *Toxicon* **1989**, *27*, 179−188.
(7) Fu, X.; Schmitz, F. J.; Lee, R. H.; Papkoff, J. S.; Slate, D. L. *J. Nat. Prod.* **1994**, *57*, 1591−1594.
(8) Roccatagliata, A. J.; Maier, M. S.; Seldes, A. M.; Pujol, C. A.; Damonte, E. B. *J. Nat. Prod.* **1996**, *59*, 887−889.
(9) Roccatagliata, A. J.; Maier, M. S.; Seldes, A. M. *J. Nat. Prod.* **1995**, *58*, 1941−1944.
(10) D'Auria, M. V.; Gomez Paloma, L.; Minale, L.; Riccio, R.; Zampella, A.; Morbidoni, M. *Nat. Prod. Lett.* **1993**, *3*, 197−201.
(11) D'Auria, M. V.; Fontana, A.; Minale, L.; Riccio, R. *Gazz. Chim. Ital.* **1990**, *120*, 155−163.
(12) D'Auria, M. V.; Gómez Paloma, L.; Minale, L.; Riccio, R.; Zampella, A. *J. Nat. Prod.* **1995**, *58*, 189−196.
(13) Maier, M. S.; Seldes, A. M.; Gros, E. G. *Magn. Reson. Chem.* **1991**, *29*, 137−142.
(14) Byon, C.; Buyuktur, P.; Choay, P.; Gut, M. *J. Org. Chem.* **1977**, *42*, 3619−3621.
(15) D'Auria, M. V.; Riccio, R.; Minale, L.; La Barre, S.; Pusset, J. *J. Org. Chem.* **1987**, *52*, 3947−3952.
(16) Patil, A. D.; Freyer, A. J.; Breen, A.;, Carte, B.; Johnson, R. K. *J. Nat. Prod.* **1996**, *59*, 606−608.
(17) Rubinstein, I.; Goad, L. J.; Clague, D. H.; Mulheirn, L. J. *Phytochemistry* **1976**, *15*, 195−200.

NP970429C