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MARINE STEROLS. SIDE-CHAIN-OXYGENATED STEROLS, POSSIBLY OF ABIOTIC ORIGIN, FROM THE NEW CALEDONIAN SPONGE *STELODORYX CHLOROPHYLLA*

FRANCESCO DE RICCARDIS, LUIGI MINALE,*

Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli Federico II, Via D. Montesano 49, 80131 Napoli, Italy

MARIA IORIZZI,

Università degli Studi del Molise, Facoltà di Agraria Campobasso,

CÉCIL DEBITUS,

Centre ORSTOM, B.P. A5, Noumèa, Nouvelle Calédonie

and CLAUDE LÉVI

Muséum Nationale d'Histoire Naturelle, 57, rue Cuvier, 75005 Paris, France

ABSTRACT.—The steroidal composition of the sponge *Stelodoryx chlorophylla* was examined, and twenty-two components were identified. The sponge contains "conventional" C_{27}–C_{29}, Δ^5-mono and diunsaturated sterols, sterols with oxygenated side chains, e.g., (22*E*)-3β-hydroxycholesta-5,22-dien-24-one [**5**], and sterols with short oxygenated side chains, e.g., 3β-hydroxy-17β- pregn-5-en-20-one [**6**] and (22*E*)-3β-hydroxy-26,27-bisnorcholesta-5,22-dien-24-one [**7**]. In addition, the extracts of the sponge contain the epimeric steroidal $\Delta^5$3β-7-diols and the steroidal 3β-hydroxy-5-en-7-ones, well recognized autoxidation products of Δ^5- sterols. The origin of the oxidized side chains is discussed.

As a part of our investigation into marine organisms collected in New Caledonia we report the occurrence of sterols with oxidized side chains from the deep-water sponge *Stelodoryx chlorophylla* Lévi sp. nov. (family Myxillidae, order Poecilosclerida). In addition to the "conventional" Δ^5-mono and diunsaturated sterols, this animal has been found to contain (22*E*)-3β-hydroxycholesta-5,22-dien-24-one [**5**], 3β-hydroxy-17β-pregn-5-en-20-one [**6**], and (22*E*)-3β-hydroxy-26,27-bisnorcholesta-5,22-dien-24-one [**7**]. Extracts of this sponge also contain the epimeric steroidal Δ^5-3β-7-diols and the steroidal 3β-hydroxy-Δ^5-7-ones, well recognized as autoxidation products of Δ^5-sterols (1,2). The sponge was collected south of New Caledonia at a depth of 600–540 m in February 1986, and the freeze-dried material, dispatched to our laboratory in Naples in September 1990, was extracted with *n*-hexane in a Soxhlet apparatus. The extract was chromatographed by mplc on Si gel in *n*-hexane/EtOAc followed by reversed-phase hplc on a Whatman Partisil 10 ODS-2 column to give the sterols **1**–**22** (Table 1). The common Δ^5 marine sterols **1**–**4** were identified by comparison of mass spectra with those of standard sterols and confirmed by ^{1}H-nmr spectra. The sterols with side chain oxygenation (**5**, **6**, and **7**), identified by ms, nmr and comparison with published data (3–6), have been previously isolated from a sponge of the genus *Hyrtios* (3). The short side chain steroidal ketones **6** and **7** have also been found in the sponge *Damiriana hawaiiana* (4), whereas the pregnane-derived ketone **6** and its corresponding 20α- and 20β-hydroxy derivatives have also been reported from the sponge *Haliclona rubens* (5) and in trace amounts from *Psammaplysilla purpurea* (6). Before that, the only C_{21} steroid isolated from a marine source was the 3β,6α-dihydroxy-5α-pregn-9(11)-en-20-one, the most widely reported steroid obtained by acid hydrolysis of asterosaponins (7–9) and possibly an artifact generated by retro-aldol cleavage of the genuine

TABLE 1. Sterol Composition in the Sponge *Stelodoryx chlorophylla.*

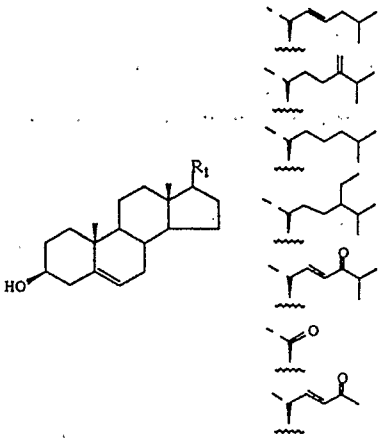
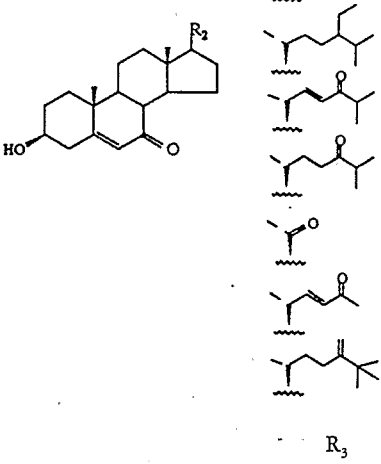
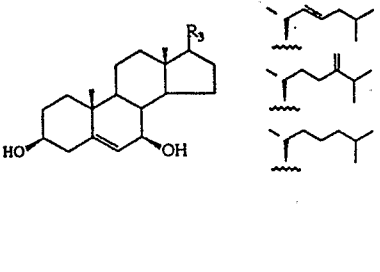
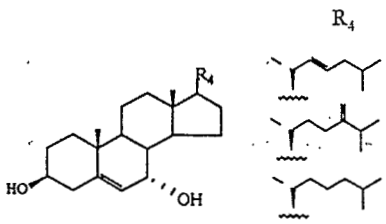
Sterol	Nucleus and side-chain	[M]$^+$	Retention Time hplc[a] (min)	Amount[b] (mg)
	R_1			
1		384	24.0 (A)	5.0
2		398	25.2 (A)	25.0
3		386	30.0 (A)	9.0
4		414	34.0 (A)	4.0
5		398	10.8 (B)	2.9
6		316	6.8 (B)	0.8
7		370	10.0 (B)	1.2
	R_2			
8		398	13.2 (B)	3.0
9		412	14.0 (B)	1.5
10		400	16.0 (B)	3.5
11		428	19.2 (B)	2.0
12		412	7.6 (B)	2.0
13		414	8.8 (B)	1.5
17		330	5.6 (B)	1.7
18		384	11.2 (C)	4.2
19		428	10.8 (C)	3.9
	R_3			
14		400	12.0 (B)	3.8
15		414	12.8 (B)	15.5
16		402	14.4 (B)	4.0

TABLE 1. Continued.

Sterol	Nucleus and side-chain	$[M]^+$	Retention time hplc[a] (min)	Amount[b] (mg)
20		400	20.0 (C)	4.8
21		414	22.0 (C)	22.0
22		402	25.5 (C)	8.0

[a]On a Whatman-Partisil 10 ODS-2 (50 cm×10 mm i.d.) at flow rate 5 ml/min, in A, MeOH-$CHCl_3$ (95:5); B, MeOH; C, MeOH-H_2O (95:5).

[b]From 0.9 kg freeze-dried sponge.

thornasterol A, 3β,6α,20-trihydroxy-5α-cholest-9(11)-en-23-one (10).

3β-Hydroxypregn-5-en-20-one has been reported as an autoxidation product of cholesterol through a biradical oxygen attack resulting in cholesterol 20α-hydroperoxide followed by degradation (11), whereas the enones **5** and **7** have never been described as autoxidation products of cholesterol (1). Indeed, (22*E*)-3β-hydroxycholesta-5,22-dien-24-one could also be an autoxidation product from (22*E*)-5α-cholesta-5,22-dien-3β-ol through the formation of a 24-hydroperoxide intermediate; a point of view supported by the isolation from air-aged cholesterol of cholesterol 24-hydroperoxide which is easily decomposed to the 24-keto derivative (12). (22*E*)-3β-Hydroxy-26,27-bisnorcholesta-5,22-dien-24-one could also be an artifact deriving through the autoxidation of (22*E*)-24-methyl-5α-cholesta-5,22-dien-3β-ol. In view of the fact that the sponge *S. chlorophylla* does not contain 24-methyl-Δ^{22} sterols, we believe that at least the enone **7** is of biological origin rather than an autoxidation product. However the precise origins of marine sterols with oxygenated and/or short side chains and their biological function have not yet been solved.

The Δ^5-3β-7β- (**14–16**) and Δ^5-3β-7α-diols (**20–22**) along with the Δ^5-7-ones (**8–13** and **17–19**) appear to be autoxidation products of the corresponding Δ^5 sterols (1,2). Their presence in the steroid mixture could be the consequence of the storage for a long time (three years) of the freeze-dried samples of the sponge *S. chlorophylla*, even if the quantities of some of the apparent autoxidation products (*e.g.*, **15** and **21**) are much higher than expected for autoxidation products. We note that the 24-keto compound **13** has been isolated from *S. chlorophylla* only as the 3β- hydroxy-Δ^5-7-one derivative. The corresponding 24-keto-Δ^5 sterol has previously been reported as a minor component of *Haliclona chilensis* (13). The 25-hydroxy derivative **19**, which equally appears as an autoxidation product (**1**), has also been isolated in relatively high yields only in the form of 3β-hydroxy-Δ^5-7 one. As far as we know, steroids **12**, **13**, **15**, **17**, **18**, **19**, and **21** are new compounds.

EXPERIMENTAL

GENERAL METHODS.—Reversed-phase hplc was performed by using Waters equipments (M 6000 A pump, U6K injector, R 401 refractometer) and a Whatman-Partisil 10 ODS-2 (50 cm×10 mm i.d.), flow rate 5 ml/min. Mass spectra were recorded at 70 eV on a Kratos MS 50 mass spectrometer. Ft-ir spectra were recorded on a Bruker IFS-48 spectrometer in KBr pellet and uv spectra on a Beckman DU 70 spectrometer. ^1H- and ^{13}C-nmr spectra were determined on a Bruker WM-250 in $CDCl_3$. The chemical shifts are given in ppm and referred to the $CHCl_3$ signal observed at 7.27 ppm; the coupling constants are reported

in Hertz. Medium pressure liquid chromatography (mplc) was performed on a Buchi 861 apparatus using an SiO_2 (230–400 mesh) column.

COLLECTION AND EXTRACTION.—The sponge *S. chlorophylla* was collected in the course of the dredging campaigns of the ORSTOM-CNRS Programme Substance Marine d'Interest Biologique (SMIB), on February 1986 south of New Caledonia (23°05′ S, 167°46′ E) at a depth of 600–540 m. A reference sample is kept at the ORSTOM Centre di Nouméa under reference R 1362. The sponge has been identified as a new species and has the following morphological characteristics: it is stipitate, flabellate: 200 to 500 mm in height, 10 to 30 mm in thickness. Color in life: pale green. The skeleton consists of ascending sinuous columns of styles, in bundles. Surface irregular with short conules and small inhalants or exhalant areas. Spicules: Styles 650–780 μm, with mucronate basis, Anchorate isochelae: 55–60 μm; 30–60 μm;13–14 μm. The sponge was freeze-dried (2.7 kg freeze-dried wt, 18% fresh wt) and dispatched to Naples in September 1990. The freeze-dried material (0.9 kg) was extracted in a Soxhlet apparatus with *n*- hexane (5 liters). The *n*-hexane extract was filtered and concentrated under reduced pressure to give 2.3 g of crude material, which was chromatographed by mplc on an $SiCO_2$ column (Merck Kieselgel 60, 230–400 mesh, 200 g) using a solvent step gradient system *n*-hexane–EtOAc (95:5 to 30:70). Fractions of 300 ml were collected and after tlc analysis were combined into six main enriched fractions from which pure compounds were obtained using subsequent reversed-phase hplc. Fractions 12–14 eluted with *n*-hexane–EtOAc (85:15) contained the conventional Δ^5 sterols **1**–**4**, the subsequent fractions 15–17 eluted with *n*-hexane–EtOAc (80:20) mainly contained the enone **5**, whereas fraction 18 eluted with *n*-hexane–EtOAc (78:22) contained the pregnane **6** and (22*E*)-3β-hydroxy-26,27-bisnorcholesta-5,22-dien-24-one [**7**]. The subsequent more polar fractions contained the sterols with nuclear oxygenation. Fractions 24–26 [*n*-hexane–EtOAc (70:30)] yielded the Δ^5-7-enones **8**, **9**, **10**, and **11**; fractions 30–34 [*n*-hexane–EtOAc (60:40)] the Δ^5-7-enones **12** and **13** along with the Δ^5-7β-hydroxy sterols **14**, **15**, and **16**; and fractions 37–42 [*n*-hexane–EtOAc (40:60)] the Δ^5-7-enones **17**, **18**, and **19** along with the Δ^5-7α-hydroxy sterols **20**, **21**, and **22**.

(22E)-3β-Hydroxycholesta-5,22-dien-24-one [**5**].—Ms *m/z* $[M]^+$ 398 (100%) 380 (60%), 255 (50.6%); ^{1}H nmr ($CDCl_3$) δ 0.73 (3H, s, H-18), 1.02 (3H, s, H-19), 1.10 (9H, d, *J*=7 Hz, H-21, -26, -27), 2.84 (1H, septet, H- 25), 3.53 (1H, m, H-3), 5.36 (1H, m, H-6), 6.08 (1H, d, *J*=16.0 Hz, H-23), 6.73 (1H, dd, *J*=16.0, 8.7 Hz, H-22); ^{13}C nmr ($CDCl_3$) δ 37.1 (C-1), 31.6 (C-2), 71.6 (C-3), 42.1 (C-4), 140.6 (C-5), 121.4 (C-6), 31.7 (C-7), 31.4 (C-8), 49.9 (C-9), 36.3 (C-10), 20.9 (C-11), 39.8 (C-12), 42.5 (C-13), 56.5 (C-14), 24.1 (C-15), 28.0 (C-16), 54.8 (C-17), 12.0 (C-18), 19.2 (C-19), 39.4 (C-20), 19.1 (C-21), 152.4 (C-22), 125.9 (C-23), 204.5 (C-24), 38.1 (C-25), 18.3 (C-26), 18.4 (C-27).

3β-Hydroxy-17β-pregn-5-en-20-one [**6**].—Ms *m/z* $[M]^+$ 316 (100%) 298 (60%), 255 (25.6%), 231 (70%), 213 (30%); ^{1}H nmr ($CDCl_3$) δ 0.64 (3H, s, H-18), 1.02 (3H, s, H-19), 2.13 (3H, s, CO*Me*), 3.53 (1H, m, H-3), 5.36 (1H, m, 6-H); ir (KBr) ν max 1703 (C=O st).

(22E)-3β-Hydroxy-26,27-bisnorcholesta-5,22-dien-24-one [**7**].—[α]D −41.0 ($CHCl_3$, *c*=0.2); ms *m/z* $[M]^+$ 370 (60%), 352 (56%), 273 (24%), 255 (100%); ^{1}H nmr ($CDCl_3$) δ 0.73 (3H, s, H-18), 0.99 (3H, s, H-19), 1.12 (3H, d, *J*=6.2 Hz, H-21), 2.24 (3H, s, CO*Me*), 3.53 (1H, m, H-3), 5.36 (1H, m, H-6), 6.00 (1H, d, *J*=16.2 Hz, H-23), 6.66 (1H, dd, *J*=16.2, 8.7 Hz, H-22); uv ($CHCl_3$) λ max 235 nm (ε=10.000); ir (KBr) ν max 1670 (C=O st), 1632 (*C*=*C*- C=O st).

(22E)-3β-Hydroxycholesta-5,22-dien-7-one [**8**].—^{1}H nmr ($CDCl_3$) δ 0.70 (3H, s, H-18), 0.87 (6H, d, *J*=7 Hz, H-26, -27), 1.02 (3H, d, *J*=7 Hz, H-21), 1.20 (3H, s, H-19), 3.68 (1H, m, H-3), 5.27 (2H, m, H-22, -23), 5.70 (1H, bs, H-6).

3β-Hydroxyergosta-5,24(28)-dien-7-one [**9**].—^{1}H nmr ($CDCl_3$) δ 0.69 (3H, s, H-18), 0.96 (3H, d, *J*=7 Hz, H-21), 1.03 (6H, d, *J*=7 Hz, H-26, -27), 1.20 (3H, s, H-19), 3.68 (1H, m, H-3), 4.66–4.72 (2H, bs, H-28), 5.70 (1H, bs, H-6).

3β-Hydroxycholest-5-en-7-one [**10**].—^{1}H nmr ($CDCl_3$) δ 0.69 (3H, s, H-18), 0.86 (6H, d, *J*=7 Hz, H-26, -27), 0.93 (3H, d, *J*=7 Hz, H-21), 1.20 (3H, s, H-19), 3.68 (1H, m, H-3), 5.70 (1H, bs, H-6).

3β-Hydroxystigmast-5-en-7-one [**11**].—^{1}H nmr ($CDCl_3$) 0.69 (3H, s, H-18), 0.81 (3H, d, *J*=7 Hz, H-26), 0.84 (3H, d, *J*=7 Hz, H-27), 0.93 (3H, d, *J*=7 Hz, H-21), 1.21 (3H, s, H-19), 3.69 (1H, m, H-3), 5.70 (1H, bs, H-6).

(22E)-3β-Hydroxycholesta-5,22-diene-7,24-dione [**12**].—[α]D −40.0 ($CHCl_3$, *c*=0.2); ms *m/z* $[M]^+$ 412 (10%), 394 (37%), 287 (53%), 269 (100%); ^{1}H nmr ($CDCl_3$) δ 0.73 (3H, s, H-18), 1.11 (9H, d, *J*=7 Hz, H-21, -26, -27), 1.21 (3H, s, H-19), 2.84 (1H, septet, *J*=6.7 Hz), 3.69 (1H, m, H-3), 5.71 (1H, bs, H-6), 6.08 (1H, d, *J*=15.6 Hz, H-23), 6.73 (1H, dd, *J*=15.6, 9.4 Hz, H-22); uv ($CHCl_3$) λ max 242.5 nm (ε=9800); ir (KBr) ν max 1670 (C=O st), 1628 (*C*=*C*- C=O st).

3β-Hydroxycholest-5-ene-7,24-dione [**13**].—[α]D −27.5 ($CHCl_3$, *c*=0.1); ms *m/z* $[M]^+$ 414 (100%), 396 (50%); ^{1}H nmr ($CDCl_3$) δ 0.69 (3H, s, H-18), 0.93 (3H, d, *J*=7 Hz, H-21), 1.10 (6H,

d, J=7 Hz, H-26, -27), 1.20 (3H, s, H-19), 2.61 (1H, septet, J=6.7 Hz, H-25), 3.69 (1H, m, H-3), 5.70 (1H, bs, H-6).

(22E)-3β,7β-Dihydroxycholesta-5,22-diene [**14**].—^{1}H nmr ($CDCl_3$) δ 0.72 (3H, s, H-18), 0.86 (6H, d, J=7 Hz, H-26, -27), 1.02 (3H, d, J=7 Hz, H-21), 1.06 (3H, s, H-19), 3.56 (1H, m, H-3), 3.85 (1H, dt, J=7.8, 1.5 Hz, H-7α), 5.28 (2H, m, H-22, -23), 5.30 (1H, t, J=1.5 Hz, H-6).

3β,7β-Dihydroxyergosta-5,24(28)-diene [**15**].—^{1}H nmr ($CDCl_3$) δ 0.70 (3H, s, H-18), 0.96 (3H, d, J=7 Hz, H-21), 1.02 (6H, d, J=7 Hz, H-26, -27), 1.06 (3H, s, H-19), 3.56 (1H, m, H-3), 3.85 (1H, dt, J=7.8, 1.5 Hz, H-7α), 4.66–4.72 (2H, bs, H-28), 5.30 (1H, t, J=1.5 Hz, H-6); ^{13}C nmr see Table 2.

3β,7β-Dihydroxycholest-5-ene [**16**].—^{1}H nmr ($CDCl_3$) δ 0.70 (3H, s, H-18), 0.87 (6H, d, J=7 Hz, H-26, -27), 0.92 (3H, d, J=7 Hz, H-21), 1.06 (3H, s, H-19), 3.56 (1H, m, H-3), 3.85 (1H, dt, J=7.8, 1.5 Hz, H-7α), 5.30 (1H, t, =1.5 Hz, H-6).

3β-Hydroxy-17β-pregn-5-ene-7,20-dione [**17**].—[α]D −45.8 ($CHCl_3$, c=0.4); ms *m/z* $[M]^+$ 330 (39%), 287 (15%), 245 (100%), 227 (30%); ^{1}H nmr ($CDCl_3$) δ 0.67 (3H, s, H-18), 1.21 (3H, s, H-19), 2.14 (3H, s, H-21), 3.69 (1H, m, H-3), 5.72 (1H, bs, H-6); ^{13}C nmr see Table 2; ir (KBr) ν max 1700, 1670 (C=O st), 1632 (C=C-C=O st); uv ($CHCl_3$) λ max 244 (ε=6000).

(22E)-3β-Hydroxy-26,27-bisnorcholesta-5,22-diene-7,24-dione [**18**].—[α]D −60 ($CHCl_3$, c=0.3); ms *m/z* $[M]^+$ 384 (27%), 287 (100%), 269 (16%); ^{1}H nmr ($CDCl_3$) δ 0.72 (3H, s, H-18), 1.12 (3H, d, J=7 Hz, H-21), 1.21 (3H, s, H-19), 2.24 (3H, s, H-25), 3.69 (1H, m, H-3), 5.70 (1H, bs, H-6), 6.00 (1H, d, J=15.6 Hz, H-23), 6.67 (1H, dd, J=15.6, 8.7 Hz, H-22); ^{13}C nmr see Table 2; uv ($CHCl_3$) λ max 245 nm (ε=7100); ir (KBr) ν max 1668 (C=O st), 1662 (C=C-C=O st).

3β,25-Dihydroxyergosta-5,24(28)-dien-7-one [**19**].—[α]D −31.9 ($CHCl_3$, c=0.3); ms *m/z* $[M]^+$ 428 (81%), 410 (30%), 329 (100%); ^{1}H nmr ($CDCl_3$) δ 0.70 (3H, s, H-18), 0.98 (3H, d, J=7

TABLE 2. ^{13}C-nmr Data ($CDCl_3$) of the New Compounds **15**, **17**, **18**, **19**, and **21**.

Carbon	Compound				
	15	**17**	**18**	**19**	**21**
C-1	36.9	36.2	36.2	36.2	37.1
C-2	31.5	30.9	31.0	31.0	31.4
C-3	71.4	70.3	70.3	70.4	71.4
C-4	41.7	41.6	41.6	41.6	42.1
C-5	143.4	165.2	165.1	164.9	146.3
C-6	125.4	125.8	125.9	126.0	123.9
C-7	73.3	201.3	201.7	202.0	65.4
C-8	40.8	45.1	45.1	45.1	37.6
C-9	48.2	49.6	49.6	49.7	42.3
C-10	36.4	38.2	38.1	38.1	37.4
C-11	21.0	20.9	21.0	21.1	20.8
C-12	39.5	37.5	38.3	38.5	39.2
C-13	42.9	44.2	43.3	43.0	42.2
C-14	55.2	49.8	49.7	49.8	49.5
C-15	26.3	26.3	26.4	26.1	24.3
C-16	28.5	23.4	28.1	28.4	28.3
C-17	55.9	62.1	53.7	54.4	55.7
C-18	11.8	13.1	12.1	11.8	11.7
		17.1	17.1	17.4	18.8
		209.6	39.6	35.7	35.8
		31.5	19.3	18.8	18.3
		—	153.5	35.3	34.7
		—	129.0	27.4	30.9
		—	199.0	156.5	157.0
		—	26.7	73.4	33.9
		—	—	29.1	22.1
		—	—	29.2	22.1
		—	—	106.6	106.0

Hz, H-21), 1.21 (3H, s, H-19), 1.36 (6H, s, H-26, -27), 3.69 (1H, m, H-3), 4.78–5.10 (2H, bs, H-28). 5.70 (1H, bs, H-6): ^{13}C nmr see Table 2; uv ($CHCl_3$) λ max 242 nm (ε=10000).

(22E)-3β,7α-Dihydroxycholesta-5,22-diene [**20**].—^{1}H nmr ($CDCl_3$) δ 0.77 (3H, s, H-18), 0.91 (6H, d, *J*=7 Hz, H-26, -27), 1.03 (3H, s, H-19), 1.05 (3H, d, *J*=7 Hz, H-21), 3.52 (1H, m, H-3), 3.80 (1H, m, H-7β), 5.30 (2H, m, H-22, -23), 5.59 (1H, d, *J*=5.2 Hz, H-6).

3β,7α-Dihydroxyergosta-5,24(28)-diene [**21**].—^{1}H nmr ($CDCl_3$) δ 0.76 (3H, s, H-18), 1.01 (3H, d, *J*=7 Hz, H-21), 1.04 (3H, d, *J*=7 Hz, H-26), 1.04 (3H, s, H-19), 1.06 (3H, d, *J*=7 Hz, H-27), 3.51 (1H, m, H-3), 3.79 (1H, m, H-7β), 4.68–4.74 (2H, bs, H-28), 5.58 (1H, d, *J*=5.2 Hz, H-6); ^{13}C nmr see Table 2.

3β,7α-Dihydroxycholest-5-ene [**22**].—^{1}H nmr ($CDCl_3$) δ 0.76 (3H, s, H-18), 0.92 (6H, d, *J*=7 Hz, H-26, -27), 0.98 (3H, d, *J*=7 Hz, H-21), 1.04 (3H, s, H-19), 3.51 (1H, m, H-3), 3.81 (1H, m, H-7β), 5.57 (1H, d, *J*=5.2 Hz, H-6).

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