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Sterols in Marine Invertebrates. 51.[1] Isolation and Structure Elucidation of C-18 Functionalized Sterols from the Soft Coral *Sinularia dissecta*

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The isolation and identification of over a dozen C-18 functionalized polyhydroxylated sterols from the soft coral *Sinularia dissecta* are described. All three levels of oxidation (CH_2OH, CHO, and CO_2H) at C-18 were encountered in this rare class of sterols.

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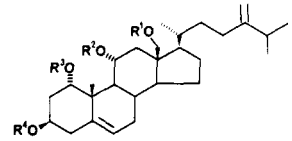
In continuation of our interest in polyhydroxylated sterols from marine sources, we have examined the more polar fractions of soft coral (*Sinularia dissecta*) extracts. In the previous paper[2] was described a group of eight 1α,3β,11α-trihydroxy sterols differing only in the nature of the side chain. Further studies of the same marine organism yielded a group of sterols with the same 1α,3β,11α-hydroxylation pattern as well as an acetylated group located at C-18. To the best of our knowledge this is only the second reported isolation of 18-hydroxylated cholestane derivatives[3] from marine source. In addition, we encountered the analogous 13-formyl and 13-carboxyl derivatives. To our knowledge, no such sterols have been described so far in marine organisms. All sterols were isolated and purified by the earlier described[2] techniques except for the use of HPLC solvent systems containing a higher percentage of water. The spectral data (^1H and ^{13}C NMR and MS) indicated that except for a functionalized C-18 angular methyl group, the sterols possessed the earlier encountered 1α,3β,11α-trihydroxy substitution pattern with the same side chains as described in the previous paper.[2]

Structure Elucidation of the C-18 Acetates. The major group isolated from the polar fractions of the soft coral *Sinularia dissecta* consisted of C-18 acetates **1–5**, the most abundant one being the tetrol monoaceate **1** (Chart I).

The mass spectra of all of the acetates (**1–5**) revealed an intense peak due to the loss of acetic acid from the molecular ion, as well as three successive losses of 18 mass units, indicative of the presence of one acetate and three hydroxyl groups. The C-18 acetate **1** was also characterized by a sharp singlet (CH_3CO) at δ 2.070 in the ^1H NMR spectrum (Table I) and ^{13}C NMR signals at δ 171.19 and 21.09 (Table II), as well as by the presence of strong IR absorption bands at 1740 and 1235 cm^{-1}. The location of the acetoxyl group at C-18 was evidenced by the presence of a singlet (2 H) at δ 3.964, a major mass peak at m/z 397 corresponding to loss of $M^+ - (CH_3CO_2CH_2 + H_2O)$, and a signal due to the methylene carbon atom at δ 63.11, which we have confirmed by the APT technique (attached proton test). Furthermore, the ^1H NMR singlet corresponding to the C-18 methyl group (usually[2] present at δ 0.68) was missing. Additional confirmation for the earlier established[2] Δ⁵-1α,3β,11α-triol pattern was the appearance in the ^1H NMR spectrum of **1** of 1β-,3α- and 11β-carbinol proton signals (δ 4.215, 3.980, and 4.036, respectively) and the presence in the ^{13}C NMR spectrum of signals associated with carbon–carbon double bond (δ 138.75, C-5, and δ 124.18, C-6).

Acetylation of **1** with acetic anhydride in pyridine at room temperature led to the tetraacetate **1a**, which exhibited four strong peaks in the mass spectrum at m/z 554, 494, 434, and 374 (consecutive losses of HOAc) and four acetoxyl singlets (3 H each) in the ^1H NMR spectrum at δ 2.012, 2.036, 2.070, and 2.134.



1 R^1 = Ac; R^2, R^3, R^4 = H
1a R^1, R^2, R^3, R^4 = Ac
1b R^1, R^2, R^4 = Ac; R^3 = H
1c R^1, R^2, R^3, R^4 = H

Alkaline hydrolysis of **1** afforded the tetrol **1c**. Its ^1H NMR spectrum revealed an upfield chemical shift of the C-18 proton signal[4] to δ 3.572 (δ 3.964 in **1**) and the absence of an acetate methyl group resonance. The side chain of sterol **1** was shown to be of the 24-methylenecholesterol type by evaluation of the ^1H and ^{13}C NMR data (Tables I and II). Consequently, the structure of the major component was established as 24-methylenecholest-5-ene-1α,3β,11α,18-tetrol 18-acetate (**1**).

Comparison of the relevant spectroscopic data of sterols **2**, **3**, and **4** indicated that they differ only in the nature of their side chains. On the basis of accumulated data[2,5] they were identified as cholest-5-ene-1α,3β,11α,18-tetrol 18-acetate (**2**), 24(ξ)methylcholest-5-ene-1α,3β,11α,18-tetrol 18-acetate (**3**), and 24(*R*)-methylcholesta-5,22-diene-1α,3β,11α,18-tetrol 18-acetate (**4**).

The remaining sterol (**5**) did not show a C-6 vinyl proton signal in its ^1H NMR spectrum. On the basis of that observation and agreement between expected (Δδ 0.249[2]) and measured (Δδ 0.241) upfield shifts of the C-19 methyl signal, as well as other characteristic differences in its spectral properties (Table I and II), we conclude that this compound is 24-methylene-5α-cholestane-1α,3β,11α,18-tetrol 18-acetate (**5**).

Structure Elucidation of 13-Carboxylic Acids. The second group of five sterols (**6–10**) was first characterized by its major component, sterol **6**. Its high-resolution mass spectrum did not show an M^+ peak; however, the low-resolution spectrum did display a weak one (M^+ 460) as well as peaks at m/z 397 ($M^+ - CO_2H - H_2O$), 379 ($M^+ - CO_2H - 2H_2O$), and 361 ($M^+ - CO_2H - 3H_2O$). The

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(1) For preceding paper, see: Proudfoot, J. R.; Li, X.; Djerassi, C. *J. Org. Chem.* **1985**, *50*, 2026.
(2) Jagodzinska, B. M.; Trimmer, J. S.; Fenical, W.; Djerassi, C. *J. Org. Chem.* **1985**, *50*, 1435.
(3) Cimino, G.; De Rosa, S.; De Stefano, S.; Sodano, G. *Tetrahedron Lett.* **1980**, 3303.
(4) (a) Ross, R. A.; Scheuer, P. J. Tetrahedron *Lett.* **1979**, 4701. (b) Kondo, E.; Tori, K. *J. Am. Chem. Soc.* **1964**, *86*, 736.
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Table I. Selected 300-MHz ^{1}H NMR Chemical Shifts ($CDCl_3$) of 1α,3β,11α-Triols[a]

sterol	C-1βH	C-3αH	C-11βH	C-6H	C-18H	C-19H	C-21H	C-26,27H	C-28H	other signals
1	4.215 br s	3.980 m	4.036 m	5.543 m	3.964 s	1.145 s	1.054 d (6.23)	1.024 d (6.84) 1.018 d (6.84)	4.720 s 4.647 s	OAc 2.070 s C-12H 2.620 dd
2	4.222 br s	3.980 m	4.050 m	5.555 m	3.963 s	1.147 s	1.022 d (6.14)	0.866 d (6.59) 0.863 d (6.55)		OAc 2.077 s C-12H 2.620 dd
3	4.230 t (3.00)	3.980 m	4.060 m	5.551 m	3.962 s	1.145 s	1.025 d (6.04)	0.855 d (6.82) 0.783 d (6.80)	0.774 d (6.77)	OAc 2.078 s C-12H 2.614 dd (12.2; 5.45)
4	4.225 t (3.00)	3.980 m	4.060 m	5.553 m	3.977 s 3.961 s	1.148 s	1.117 d (6.42)	0.833 d (6.68) 0.817 d (6.76)	0.907 d (6.83)	OAc 2.100 s C-12H 2.620 dd C-22,23H 5.165 m (7.50; 20.00)
5	4.080 br s	3.980 m	4.000 m		3.943 s	0.904 s	1.037 d (5.85)	1.025 d (6.80) 1.019 d (6.70)	4.721 s 4.645 s	OAc 2.076 s C-12H 2.580 dd
6	4.148 br s	3.974 m	4.252 m	5.540 m		1.030 s	1.027 d (6.60)	1.018 d (6.80) 1.017 d (6.80)	4.719 s 4.644 s	C-12H 2.885 dd (12.35; 5.80)
7	4.141 br s	3.974 m	4.266 m	5.532 m		1.021 s	0.995 d (6.53)	0.849 d (6.81) 0.778 d (6.79)	0.769 d (6.83)	C-12H 2.875 dd (12.15; 5.90)
8	4.160 br s	3.980 m	4.279 m	5.540 m		1.040 s	1.074 br s	0.942 d (6.59) 0.937 d (6.94)	0.855 d (6.60)	C-23H 0.876 s C-12H 2.900 dd cyclopropyl: −0.123, 1 H, m; 0.208, 2 H, m; 0.489, 1 H, m
9	4.134 br s	3.976 m	4.280 m	5.532 m		1.019 s	0.983 d (6.38)	0.904 d (6.32) 0.882 d (6.93)	0.850 d (6.82)	C-12H 2.880 dd cyclopropyl: 0.135, 2 H, m; 0.310, 1 H, m; 0.530, 3 H, m
10	4.061 br s	3.985 m	4.200 m			1.008 s	1.009 d (6.14)	1.023 d (6.83) 1.019 d (7.02)	4.740 s 4.660 s	
11	4.172 t (3.00)	3.960 m	4.225 m	5.507 m	9.756 s	1.007 s	0.923 d (6.45)	1.017 d (6.23) 1.012 d (6.69)	4.726 s 4.640 s	C-12H 2.800 dd
12	4.167 t (3.00)	3.960 m	4.225 m	5.507 m	9.750 s	1.005 s	0.895 d (6.48)	0.845 d (6.80) 0.773 d (6.68)	0.767 d (6.69)	C-12H 2.781 dd (12.20; 5.85)
13	4.180 t (3.00)	3.960 m	4.230 m	5.510 m	9.745 s	1.010 s	0.978 br s	0.949 d (6.97) 0.944 d (6.80)	0.861 d (6.64)	C-23H 0.886 s C-12H 2.800 dd cyclopropyl: −0.107, 1 H, dd; 0.230, 2 H, m; 0.497, 1 H, m
14	4.027 br s	3.950 m	4.110 m		9.745 s	0.775 s	0.903 d (6.50)	1.017 d (6.59) 1.013 d (6.71)	4.726 s 4.638 s	C-12H 2.750 dd

[a] The chemical shifts values are given in parts per million (ppm) and were referenced to $CDCl_3$ (7.260 ppm). The coupling constants are given in hertz and are enclosed in parentheses.

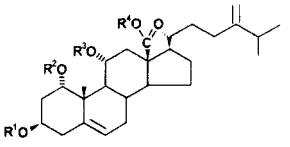
Table II. ^{13}C NMR Assignments of Selected Compounds[a]

carbon number	sterol					
	1	3	6	7	1a	6b
1	74.44	74.48	74.45	74.4	76.87	76.76
2	38.22	38.27	38.18	38.2	38.54	38.57
3	66.10	66.17	66.31	66.3	69.29	69.25
4	42.03	42.08	42.08	42.0	41.58	43.24
5	138.15	138.81	138.95	138.7	136.51	136.71
6	124.18	124.23	123.96	123.7	125.30	125.54
7	32.57	32.63	32.77	32.5	32.06	31.90
8	31.87	31.93	32.22	32.2	32.12	32.95
9	48.22	48.29	48.35	48.3	45.52	45.68
10	42.77	42.82	42.83	42.6	41.48	42.00
11	67.45	67.54	68.02	68.0	71.05	71.56
12	46.16	46.22	47.25	47.4	31.82	31.76
13	45.90	45.91	55.80	55.7	46.08	55.77
14	56.12	56.14	56.00*	55.9*	55.08	55.71
15	24.10	24.15	24.82	24.7	24.07	24.98
16	28.12	28.13	29.46	29.5	28.17	29.69
17	55.07	55.12	56.65*	56.5*	56.09	56.70
18	63.11	63.19	178.20	178.3	61.82	179.06
19	19.29	19.34	19.48	19.3	19.36	19.35
20	35.53	36.08	36.60	36.6	35.99	36.96
21	18.93	19.18	18.48	18.5	19.01	18.81
22	34.42	33.68	34.71	33.1	34.98	34.99
23	30.60	30.19	30.97	30.3	31.13	31.25
24	156.39	39.06	156.32	39.0	156.39	156.40
25	33.71	31.48[5a,b]	33.92	31.2[5a,b]	34.42	34.39
26	21.81	17.59	21.83	17.4	21.64	21.80
27	21.97	20.52	21.92	20.3	22.20	22.21
28	106.15	15.42	106.30	15.2	106.69	106.72
CH_3[c]	21.09	21.16			22.30	22.30
C=O	171.19	171.25			170.67	170.33
CH_3[c]					20.98	20.98
C=O					170.10	169.85
CH_3[c]					20.91	20.90
C=O					169.59	169.75
CH_3[c]					20.89	
C=O					169.41	

[a] Assignment of chemical shifts for close-lying peaks are marked with an asterisk and may be reversed. Chemical shifts in ppm. [b] C_6D_6 was used as a solvent. [c] Acetoxy methyl group.

presence of a carboxyl group was confirmed by the ^{13}C NMR signal at δ 178.20 (COOH). Both ^1H and ^{13}C NMR spectra of compound **6** lacked signals due to the C-18 angular methyl group.

Treatment of **6** with ethereal diazomethane yielded the methyl ester **6a**, which possessed a sharp ^1H NMR singlet (3 H) at δ 3.656. Acetylation of acid **6** afforded the triacetate **6b**, which was characterized by its ^1H NMR

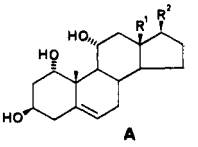


6 R^1,R^2,R^3,R^4 = H
6a R^1,R^2,R^3 = H; R^4 = Me
6b R^1,R^2,R^3 = Ac; R^4 = H

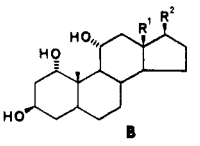
spectral data: the presence of three singlets (3 H each) at δ 2.010, 2.043, and 2.070 and the characteristic downfield shifts of three carbinol proton signals ($\Delta\delta$ 0.726 to 4.874, $\Delta\delta$ 0.856 to δ 4.830, and $\Delta\delta$ 1.128 to δ 5.380) respectively for the 1β-, 3α- and 11β-protons. Similar downfield shifts of the carbinol proton signals ($\Delta\delta$ 0.731, 0.846, and 1.164, respectively) were noticed in our previous studies[2] for the 1α,3β,11α-triol system and its triacetate. The side chain structure of sterol **6** was deduced, from the ^1H and ^{13}C NMR data (Tables I and II), as that of the 24-methylenecholestane type, thus confirming the final structure of compound **6** as 24-methylene-1α,3β,11α-trihydroxycholest-5-en-18-oic acid.

The remaining members of the 13-carboxylic acid group (**7–9**, but not **10**), were identical in their nuclear structure, as evidenced by their spectral data (Table I), and differed

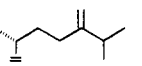
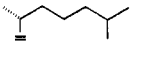
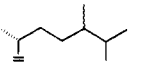
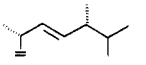
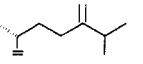
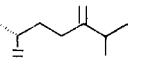
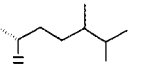
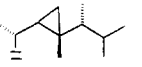
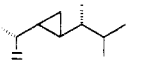
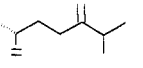
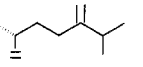
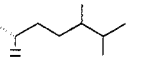
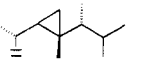
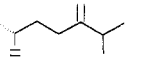
Chart I



A



B

Sterol	Nucleus	R^1	R^2
1	A	CH_2-OAc	
2	A	CH_2-OAc	
3	A	CH_2-OAc	
4	A	CH_2-OAc	
5	B	CH_2-OAc	
6	A	COOH	
7	A	COOH	
8	A	COOH	
9	A	COOH	
10	B	COOH	
11	A	CHO	
12	A	CHO	
13	A	CHO	
14	B	CHO	

only in the nature of their side chains. They were identified as 24(ξ)methyl-1α,3β,11α-trihydroxycholest-5-ene-18-oic acid (**7**), 1α,11α-dihydroxygorgosterol-13-carboxylic acid (**8**), and 1α,11α-dihydroxy-23-demethylgorgosterol-13-carboxylic acid (**9**) on the basis of previously published data for similar side chain containing structures.[2,6]

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The ^1H NMR spectrum of acid **10** did not show the presence of a C-6 vinyl proton. Otherwise, the NMR data were similar to those of the 18-acetate **5** (for chemical shifts of 1β,3α,11β-carbinol protons, see Table I) and thus led to its tentative identification as 24-methylene-1α,3β,11α-trihydroxy-5α-cholestan-18-oic acid.

Structure Elucidation of 13-Formyl Compounds. The remaining four sterols **11–14** all exhibited ^1H NMR singlets (1 H) at δ 9.75, characteristic of an aldehyde proton (CHO). All other NMR data were similar to those of the respective 13-carboxylic acids, including signals due to the carbinol protons and the C-19 methyl group (see Table I). It follows that sterols **11–13** have structures analogous to those of acids **6–8** (including side chains), except for the nature of the C-13 substituent. Aldehyde **14** was shown to be the analogue of acid **9** (lack of Δ^5-unsaturation). As shown in the Experimental Section, in both the high- and low-resolution mass spectra there are peaks corresponding to the loss of the formyl group, usually in conjunction with losses of water ($M^+ - CHO - H_2O$, $M^+ - CHO - 2H_2O$, $M^+ - CHO - 3H_2O$) and/or fragments of the side chain.

Summary. The isolation of three groups of C-18 functionalized sterols (18-acetates, 13-carboxylic acids, and 13-aldehydes) from the same marine organism is, so far, unprecedented. There are a few scattered reports[7] of intramolecular hemiketal esters derived from the 13-carboxylic group in the pregnane series; 13-formyl[8] as well as C-18-hydroxyl derivatives[4,7,9] are known in the pregnane and androstane series, but only one has been reported in marine organisms.[3]

It is premature to speculate upon the biological role of these highly oxygenated sterols in this soft coral until something is learned about their biogenesis and further fate. As indicated earlier[2], the fact that similar nuclei possessing diverse side chains are encountered suggests the existence of enzyme systems—either in the coral or in some symbiont—that introduce the 1α- and 11α-hydroxyl groups into a dietary precursor and that may also functionalize the C-18 angular methyl group. Eventual isolation of the enzyme systems may provide a possible practical route to otherwise rare steroids—notably those with oxygenated C-11 and C-18 positions.

Experimental Section

General Methods. Reverse-phase HPLC was performed by using Waters equipment (M6000 A pump, U6K injector, R401 refractometer), Whatman Partisil M9 10/50 ODS-2 column (9 mm i.d. × 50 cm), and Altex Ultrasphere ODS (10 mm i.d. × 30 cm) column. The eluent was 10–30% aqueous acetonitrile. Mass spectra were recorded at 70 eV on MAT-711-Data Center (low resolution), Varian MAT-711 (high-resolution, double-focusing spectrometer equipped with a PDP-11/45 computer for data acquisition and reduction), or Kratos MS-50S (double-focusing) mass spectrometers using a direct inlet system. The 300-MHz ^1H and some of the ^{13}C NMR spectra were recorded on a Nicolet NMC 300-MHz wide-bore spectrometer. The remaining ^{13}C NMR spectra were run on a Varian XL 400 spectrometer. The chemical shifts were given in ppm with $CDCl_3$ as internal standard, and the coupling constants are reported in hertz. IR spectra were run on a Nicolet MX-1 FT-IR instrument. Silica gel 60 (E. Merck, 70–230 mesh) was used for column chromatography.

Collection and Extraction of *Sinularia dissecta*. *Sinularia dissecta* Tixier-Durivault (1.6 kg wet) was collected at −12 m depth in Sept, 1979, in Palau, Western Caroline Islands. The sample was stored frozen, next freeze-dried, pulverized, and extracted 3× with $CDCl_3$. Removal of solvent under reduced pressure left a dark, viscous residue (22 g), which by TLC analysis (silica gel, Et_2O) was found to contain relatively polar metabolites (the polyhydroxysterols; R_f 0.1–0.2) as major constituents.

Chromatography. The extract (20 g) was initially chromatographed over TLC-grade (Merck) silica gel with isooctane/EtOAc mixtures by using rapid-elution methods. Twelve fractions were obtained with the major polar compounds dispersed between fractions 8–12 (eluted with 80–100% EtOAc). Fractions 9–12 were combined to yield 2 g of a complex mixture.

Separation of the Polyhydroxylated Sterol Mixture. The polar fractions of the polyhydroxy sterols (homogeneous by TLC) were dissolved in acetonitrile and subjected to preparative reverse-phase HPLC on an ODS-2 column with acetonitrile/water (8.5:1.5) as the mobile phase. Further purification of the polyhydroxy sterols **1–14** was achieved by repeated HPLC with Altex ODS column with 10% aqueous acetonitrile (sterols **1–5** and **11–14**) and 25% aqueous acetonitrile (sterols **6–10**) as the mobile phases.

24-Methylenecholest-5-ene-1α,3β,11α,18-tetrol 18-acetate (1): IR 3365, 1740, 1235, 1040 cm^{-1}; for 300-MHz ^1H NMR, see Table I; for ^{13}C NMR, see Table II; high-resolution EI-MS, m/z (assignment, relative intensity) 470.3394 ($M^+ - H_2O$, $C_{30}H_{46}O_4$, 22), 452.3282 ($C_{30}H_{44}O_3$, 27), 410.3214 ($C_{28}H_{42}O_2$, 19), 397.3084 ($C_{27}H_{41}O_2$, 20), 392.3050 ($C_{28}H_{40}O$, 14), 379.2971 ($C_{27}H_{39}O$, 8), 374.2945 ($C_{28}H_{38}$, 5), 361.2903 ($C_{27}H_{37}$, 16), 283.1715 ($C_{19}H_{23}O_2$, 7), 267.1753 ($C_{19}H_{23}O$, 27), 227.1445 ($C_{16}H_{19}O$, 6), 209.1321 ($C_{16}H_{17}$, 20), 201.1265 ($C_{14}H_{17}O$, 9).

Cholest-5-ene-1α,3β,11α,18-tetrol 18-acetate (2): for 300-MHz ^1H NMR, see Table I; low-resolution MS, m/z (relative intensity) 476 (M^+, 1), 458 (69), 440 (42), 422 (6), 388 (10), 386 (44), 370 (4), 365 (11), 324 (59), 157 (50), 91 (70), 69 (71), 55 (80), 43 (100).

24ξ-Methylcholest-5-ene-1α,3β,11α,18-tetrol 18-acetate (3): for 300-MHz ^1H NMR, see Table I; for ^{13}C NMR see Table II; high-resolution EI-MS, m/z (assignment, relative intensity) 472.3555 ($M^+ - H_2O$, $C_{30}H_{48}O_4$, 7), 454.3425 ($C_{30}H_{46}O_3$, 8), 412.3360 ($C_{28}H_{44}O_2$, 5), 399.3287 ($C_{27}H_{43}O_2$, 7), 394.3229 ($C_{28}H_{42}O$, 11), 381.3156 ($C_{27}H_{41}O$, 3), 376.3127 ($C_{28}H_{40}$, 5), 363.3038 ($C_{27}H_{39}$, 5), 201.1281 ($C_{14}H_{17}O$, 4), 157.1015 ($C_{12}H_{13}$, 13).

24(*R*)-Methylcholesta-5,22-diene-1α,3β,11α,18-tetrol 18-acetate (4): for 300-MHz ^1H NMR, see Table I; low-resolution MS, m/z (relative intensity) 488 (M^+, 1), 470 (17), 452 (7), 398 (10), 386 (3), 336 (7), 157 (19), 91 (42), 69 (71), 55 (79), 43 (100).

24-Methylene-5α-cholestane-1α,3β,11α,18-tetrol 18-acetate (5): for 300-MHz ^1H NMR, see Table I; high-resolution EI-MS, m/z (assignment, relative intensity) 472.3544 ($M^+ - H_2O$, $C_{30}H_{48}O_4$, 3), 454.3462 ($C_{30}H_{46}O_3$, 12), 436.3332 ($C_{30}H_{44}O_2$, 2), 430.3446 ($C_{28}H_{46}O_3$, 1), 417.3373 ($C_{27}H_{45}O_3$, 6), 412.3361 ($C_{28}H_{44}O_2$, 13), 399.3282 ($C_{27}H_{43}O_2$, 32), 394.3241 ($C_{28}H_{42}O$, 10), 381.3158 ($C_{27}H_{41}O$, 29), 376.3132 ($C_{28}H_{40}$, 3), 370.2672 ($C_{28}H_{34}$, 4), 363.3068 ($C_{27}H_{39}$, 7), 209.1323 ($C_{16}H_{17}$, 10), 159.1173 ($C_{12}H_{15}$, 25), 140.0829 ($C_8H_{12}O_2$, 15), 109.0652 (C_7H_9O, 16).

24-Methylene-1α,3β,11α-trihydroxycholest-5-en-18-oic acid (6): IR 3365, 1735 cm^{-1}; for 300-MHz ^1H NMR, see Table I; for ^{13}C NMR, see Table II; high-resolution EI-MS, m/z (assignment, relative intensity) 442.3083 ($M^+ - H_2O$, $C_{28}H_{42}O_4$, 100), 424.2999 ($C_{28}H_{40}O_3$, 56), 406.2863 ($C_{28}H_{38}O_2$, 30), 379.2980 ($C_{27}H_{39}O$, 22), 361.2897 ($C_{27}H_{37}$, 56), 358.2152 ($C_{22}H_{30}O_4$, 55), 340.2045 ($C_{22}H_{28}O_3$, 49), 322.1954 ($C_{22}H_{26}O_2$, 29), 271.1686 ($C_{18}H_{23}O_2$, 22).

24ξ-Methyl-1α,3β,11α-trihydroxycholest-5-en-18-oic acid (7): for 300-MHz ^1H NMR, see Table I; for ^{13}C NMR, see Table II; high-resolution EI-MS, m/z (assignment, relative intensity) 444.3243 ($M^+ - H_2O$, $C_{28}H_{44}O_4$, 84), 426.3118 ($C_{28}H_{42}O_3$, 100), 408.3033 ($C_{28}H_{40}O_2$, 30), 372.3009 ($C_{25}H_{40}O_2$, 56), 326.2964 ($C_{24}H_{38}$, 35), 253.1605 ($C_{18}H_{21}O$, 17), 157.1015 ($C_{12}H_{13}$, 25).

1α,11α-Dihydroxygorgosterol-13-carboxylic acid (8): for 300 MHz ^1H NMR, see Table I; high-resolution EI-MS, m/z (assignment, relative intensity) 470.3398 ($M^+ - H_2O$, $C_{30}H_{46}O_4$, 12), 452.3283 ($C_{30}H_{44}O_3$, 22), 434.3169 ($C_{30}H_{42}O_2$, 12), 406.3216

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($C_{29}H_{42}O$, 6), 391.2659 ($C_{27}H_{35}O_2$, 5), 363.2345 ($C_{25}H_{31}O_2$, 10), 335.2391 ($C_{24}H_{31}O$, 7), 243.1388 ($C_{16}H_{19}O_2$, 12), 157.1016 ($C_{12}H_{13}$, 35), 91.0550 (C_7H_7, 72), 83.0870 (C_6H_{11}, 100).

1α,11α-Dihydroxy-23-demethylgorgosterol-13-carboxylic acid (9): for 300-MHz ^1H NMR, see Table I; high-resolution EI-MS, m/z (assignment, relative intensity) 456.3251 ($M^+ - H_2O$, $C_{29}H_{44}O_4$, 11), 438.3152 ($C_{29}H_{42}O_3$, 5), 420.3027 ($C_{29}H_{40}O_2$, 7), 413.2673 ($C_{26}H_{37}O_4$, 12), 375.3058 ($C_{28}H_{39}$, 9), 358.2163 ($C_{22}H_{30}O_4$, 12), 189.0907 ($C_{12}H_{13}O_2$, 4), 121.0656 (C_8H_9O, 13), 83.0866 (C_6H_{11}, 44).

24-Methylene-1α,3β,11α-trihydroxy-5α-cholestan-18-oic acid (10): for 300-MHz ^1H NMR, see Table I; low-resolution MS, m/z (relative intensity) 444 ($M^+ - H_2O$, 2), 426 (3), 408 (5), 399 (12), 381 (7), 360 (25), 342 (12), 324 (20).

24-Methylene-1α,3β,11α-trihydroxycholest-5-en-18-al (11): IR 3365, 1738 cm^{-1}; for 300-MHz ^1H NMR, see Table I; low-resolution MS, m/z (relative intensity) 444 (M^+, 3), 426 (15), 415 (2), 408 (9), 397 (13), 390 (3), 379 (5), 342 (3), 324 (5), 306 (2), 155 (19), 69 (70), 55 (95), 41 (100).

24ξ-Methyl-1α,3β,11α-trihydroxycholest-5-en-18-al (12): for 300-MHz ^1H NMR, see Table I; low-resolution MS, m/z (relative intensity) 446 (M^+, 1), 428 (12), 410 (7), 400 (13), 399 (6), 392 (4), 381 (6), 363 (5), 255 (6), 157 (14), 91 (38), 69 (29), 55 (52), 43 (100).

1α,11α-Dihydroxygorgosterol-13-carbaldehyde (13): for 300-MHz ^1H NMR, see Table I; high-resolution EI-MS, m/z (assignment, relative intensity) 472.3553 (M^+, $C_{30}H_{48}O_4$, 1), 454.3428 ($C_{30}H_{46}O_3$, 7), 436.3313 ($C_{30}H_{44}O_2$, 7), 418.3216 ($C_{30}H_{42}O$, 4), 393.2978 ($C_{24}H_{41}O_4$, 3), 383.2576 ($C_{25}H_{35}O_3$, 6), 364.3140 ($C_{27}H_{40}$, 3), 361.2382 ($C_{22}H_{33}O_4$, 6), 342.2199 ($C_{22}H_{30}O_3$, 6), 324.2103 ($C_{22}H_{28}O_2$, 4), 319.2077 ($C_{23}H_{27}O$, 7), 306.1973 ($C_{22}H_{26}O$, 5), 295.2056 ($C_{21}H_{27}O$, 4), 271.1696 ($C_{18}H_{23}O_2$, 19), 254.1641 ($C_{18}H_{22}O$, 11), 157.1010 ($C_{12}H_{13}$, 30), 109.0653 (C_7H_9O, 12), 83.0857 (C_6H_{11}, 66).

24-Methylene-1α,3β,11α-trihydroxy-5α-cholestan-18-al (14): for 300-MHz ^1H NMR, see Table I; low-resolution MS, m/z (relative intensity) 446 (M^+, 2), 428 (3), 410 (3), 392 (7), 362 (2), 326 (3), 55 (79), 41 (100).

24-Methylenecholest-5-ene-1α,3β,11α,18-tetrol Tetraacetate (1a). The triol **1** (10 mg) was acetylated in pyridine (0.2 mL) by treatment with acetic anhydride (0.1 mL) at room temperature overnight. The pale yellow oil (13 mg), obtained after standard workup, was separated by reverse-phase HPLC using 10% H_2O/acetonitrile to give first a colorless oil (4 mg), which was identified as the triacetylated derivative **1b**: ^1H NMR δ 1.020 (d, J = 6.75 Hz, 3 H, 21-CH_3), 1.024 (d, J = 6.80 Hz, 3 H, 27-CH_3), 1.025 (d, J = 6.80 Hz, 3 H, 26-CH_3), 1.116 (s, 3 H, 19-CH_3), 2.024, 2.041, and 2.132 (3 s, 9 H, OAc), 2.71 (dd, 1 H), 3.760 (br s, 1 H, 1β-H), 3.830 and 4.208 (AB q, J = 11.90 Hz, 2 H, 18-CH_2OAc),[7,9c,d] 4.646 and 4.721 (2 s, 2 H, 28-CH_2), 5.000 (m, 1 H, 3α-H), 5.155 (m, 1 H, 11β-H), 5.630 (m, 1 H, 6-H); high-resolution EI-MS, m/z (assignment, relative intensity) 512.3521 (M^+ – AcOH, $C_{32}H_{48}O_5$, 9), 452.3277 ($C_{30}H_{44}O_3$, 57), 434.3203 ($C_{30}H_{42}O_2$, 15), 392.3058 ($C_{28}H_{40}O$, 45), 379.3015 ($C_{27}H_{36}O$, 17), 374.2981 ($C_{28}H_{38}$, 17), 361.2888 ($C_{27}H_{37}$, 22), 308.2153 ($C_{22}H_{28}O$, 5), 159.0820 ($C_{11}H_{11}O$, 5), 155.0862 ($C_{12}H_{11}$, 32). The next compound (9 mg) was identified as the tetraacetate **1a**: ^1H NMR δ 1.018 (d, J = 6.60 Hz, 3 H, 27-CH_3), 1.019 (d, J = 6.60 Hz, 3 H, 26-CH_3), 1.022 (d, J = 6.30 Hz, 3 H, 21-CH_3), 1.141 (s, 3 H, 19-CH_3), 2.012, 2.036, 2.070, and 2.134 (4 s, 12 H, OAc), 2.63 (dd, 1 H), 3.822 and 4.210 (AB q, J = 11.90 Hz, 2 H, 18-CH_2OAc),[7,9c,d] 4.643 and 4.719 (2 s, 2 H, 28-CH_2), 4.829 (m, 1 H, 3α-H), 4.981 (br s, 1 H, 1β-H), 5.103 (m, 1 H, 11β-H), 5.626 (m, 1 H, 6-H); for ^{13}C NMR, see Table II; high-resolution EI-MS, m/z (assignment, relative intensity) 554.3586 (M^+ – AcOH, $C_{34}H_{50}O_6$, 1), 494.3403 ($C_{32}H_{46}O_4$, 1), 434.3201 ($C_{30}H_{42}O_2$, 55), 421.3082 ($C_{29}H_{41}O_2$, 2), 374.2976 ($C_{28}H_{38}$, 38), 361.2908 ($C_{27}H_{37}$, 100), 290.2042 ($C_{22}H_{26}$, 8), 173.0974 ($C_{12}H_{13}O$, 4).

Alkaline Hydrolysis of 1. A solution of **1** (3 mg) in EtOH (0.5 mL) was treated with 5% aqueous NaOH solution (0.3 mL), and the mixture was stirred at room temperature for 24 h. Water (1 mL) was added, and the mixture was extracted twice with AcOEt (5 mL). Usual workup gave 2.5 mg of tetrol **1c**: ^1H NMR δ 1.024 (d, J = 6.83 Hz, 3 H, 27-CH_3), 1.027 (d, J = 6.83 Hz, 3 H, 26-CH_3), 1.088 (d, J = 6.36 Hz, 3 H, 21-CH_3), 1.143 (s, 3 H, 19-CH_3), 2.70 (dd, 1 H), 3.572 (AB q, J = 11.15 Hz, 2 H, CH_2OH, lit.[4a] δ 3.64), 3.979 (m, 1 H, 3α-H), 4.164 (m, 1 H, 11β-H), 4.233 (t, J = 3.00 Hz, 1 H, 1β-H), 4.657 and 4.724 (2 s, 2 H, 28-CH_2), 5.556 (m, 1 H, 6-H); low-resolution MS, m/z (relative intensity) 428 ($M^+ - H_2O$, 4), 410 (8), 497 (4), 492 (2), 379 (2), 362 (3), 361 (7), 263 (25), 256 (19), 149 (60), 43 (100).

Methyl 24-Methylene-1α,3β,11α-trihydroxycholest-5-en-18-oate (6a). The carboxylic acid **6** (2 mg) was treated with ethereal diazomethane solution (1 mL) for 20 min, and the solvent was evaporated under reduced pressure leaving the crude ester **6a** (2.1 mg) sufficiently pure for analytical purposes: ^1H NMR δ 1.003 (d, J = 6.59 Hz, 3 H, 21-CH_3), 1.011 (d, J = 6.81 Hz, 3 H, 27-CH_3), 1.012 (d, J = 6.80 Hz, 3 H, 26-CH_3), 1.023 (s, 3 H, 19-CH_3), 2.90 (dd, 1 H), 3.974 (m, 1 H, 3α-H), 4.140 (br s, 1 H, 1β-H), 4.195 (m, 1 H, 11β-H), 4.642 and 4.718 (2 s, 2 H, 28-CH_2), 5.535 (m, 1 H, 6-H).

24-Methylene-1α,3β,11α-trihydroxycholest-5-en-18-oic Acid Triacetate (6b). The triol **6** (4 mg) was acetylated in pyridine (0.2 mL) with acetic anhydride (0.1 mL) in the presence of 4-(dimethylamino)pyridine for 12 h at room temperature. The reaction mixture was poured into water and extracted with ether. The ether extract was washed with water, diluted HCl, water, and saturated $NaHCO_3$, dried, and evaporated to afford the triacetate **6b**: ^1H NMR δ 0.999 (d, J = 6.10 Hz, 3 H, 21-CH_3), 1.008 (d, J = 6.84 Hz, 3 H, 27-CH_3), 1.009 (d, J = 6.84 Hz, 3 H, 26-CH_3), 1.099 (s, 3 H, 19-CH_3), 2.010, 2.043, and 2.070 (3 s, 9 H, OAc), 2.90 (dd, 1 H), 4.631 and 4.711 (2 s, 2 H, 28-CH_2), 4.830 (m, 1 H, 3α-H), 4.874 (br s, 1 H, 1β-H), 5.380 (m, 1 H, 11β-H), 5.610 (m, 1 H, 6-H); low-resolution MS, m/z (relative intensity) 586 (M^+, 1), 526 (8), 481 (4), 466 (6), 421 (3), 406 (80), 361 (14), 263 (26).

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