

NOVEL POLYHYDROXYLATED STEROLS FROM THE
SOFT CORAL *SINULARIA NUMEROSA*

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ABSTRACT.—Two novel polyhydroxylated sterols, numersterols A and B, have been isolated from the South China Sea soft coral *Sinularia numerosa*, and their structures were established as 24-methylenecholestane-1 α ,3 β ,5 α ,6 β -tetrol and 25-methylene-22-homocholestane-1 β ,3 β ,5 α -triol, respectively.

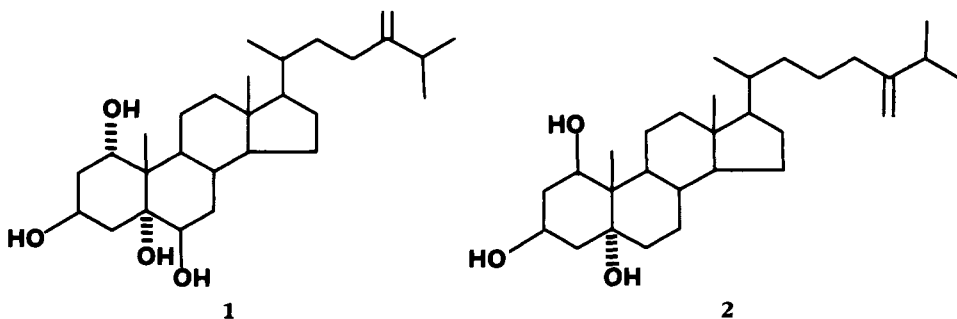
Alcyonarians are known to be a rich source of polyhydroxylated sterols (1–3). Our investigations have led to the isolation of two new polyhydroxylated sterols, numersterols A and B, from *Sinularia numerosa* Tixier-Durivault (Alcyoniidae). In addition, a known sterol, 24-methylenecholesterol, was identified, which is the first isolation from a soft coral.

The EtOH extract obtained from *S. numerosa* was chromatographed to give numersterol A [**1**]. Its mass spectrum ($[M]^+$ m/z 448.3538) and elemental analysis established the molecular formula as $C_{28}H_{48}O_4$. Numersterol A was structurally characterized through a combination of spectral and X-ray diffraction analysis as 24-methylenecholestane-1 α ,3 β ,5 α ,6 β -tetrol [**1**], a new polyhydroxylated sterol.

The ^{13}C -nmr spectrum of **1** showed signals for five methyl groups at 12.51, 17.28, 19.07, 22.16, and 22.26 ppm. The 1H -nmr spectrum at 200 MHz in $CDCl_3$ also indicated five methyl signals at δ 0.70 (3H, s, Me-18), 1.11 (3H, s, Me-19), 1.04 (6H, d, $J = 6.5$ Hz, Me-26 and Me-27), and 0.96 ppm (3H, d, $J = 6$ Hz, Me-21). The signals at δ_C 157.49 (s) and 106.78 (t) and δ_H 4.66 (1H, d, $J = 1$ Hz), 4.73 (1H, d, $J = 1$ Hz, AX pattern) clearly revealed the presence of a terminal double bond (2). Thus, the spectral data corresponded to a methylenecholestane carbon skeleton.

The structure of the side chain was deduced by analysis of the mass spectrum. The fragmentation pattern with peaks at m/z 364 $[M - C_6H_{12}]^+$, 346 $[M - C_6H_{12} - H_2O]^+$, and 321 $[M - C_9H_{17} - H]^+$ corresponded to a C_9 unsaturated side chain, typical of the mass spectra of 24-methylenecholesterol derivatives (4, 5). In addition, the peaks at m/z 282 ($C_{16}H_{26}O_4$), 264 ($C_{16}H_{24}O_3$), 246 ($C_{16}H_{22}O_2$), and 228 ($C_{16}H_{20}O$) were derived by typical ring-D fission with proton migration and stepwise H_2O losses. It was evident that the four oxygen functions in the molecule are located on the A, B, and C rings.

The ir spectrum showed strong hydroxy absorption but no carbonyl absorption.



The ^{13}C - and ^1H -nmr spectra taken in CDCl_3 indicated that compound **1** contains one tertiary and three secondary hydroxyl groups: δ_{C} 78.70 (s), 75.95 (d), 75.41 (d), and 64.25 (d) ppm; δ_{H} 3.49 (1H, dd, $J = 1$ and 3.5 Hz), 3.85 (1H, t, $J = 4.0$ Hz), and 4.31–4.50 (1H, m) ppm. Four signals of hydroxyl protons at 1.25 (s), 2.18 (s), 2.61 (d, $J = 5.2$ Hz), and 3.67 (s) ppm observed in CDCl_3 disappeared in CD_3OD .

The signals between δ 1.1 and 2.3, not well resolved in CDCl_3 , became well separable using CD_3OD as solvent. Decoupling experiments clarified the relationship among the C-1 to C-4 protons and confirmed an α -OH located at C-1. Irradiation of the symmetrical multiplet at δ 4.14–4.33, attributable to 3-H, simplified simultaneously the H_2 -2 signals at 1.76–1.87 and the H_2 -4 signals at 2.08–2.18. Both the C-2 and C-4 methylene signals showed large coupling constants 14 and 12.5 Hz, respectively, with the H-3 α , which indicates a vicinal diaxial coupling. Thus, the H-3 is axial, and the presence of a 3β -OH is established. Irradiation of the triplet at δ 3.85 (1H, $J = 4.0$ Hz) caused collapse of the H_2 -2 signals as well. The small value of $J_{1,2}$ (4.0 Hz) suggested the absence of an axial hydrogen at C-1. Therefore, a second secondary hydroxyl group should be located axially at C-1 (α orientation).

In general, the C-3 protons of 3-hydroxy-A/B *trans*-steroids resonate at 3.58 (axial) and 4.15 ppm (equatorial) (6). A downfield multiplet at 4.08 ppm is typical of 3β -hydroxysterols bearing a 5α -hydroxyl group (2). The far downfield signal of numersterol A centered at δ 4.40 revealed that the H-3 α must be subjected to a double 1,3-diaxial interaction with two axial hydroxyl groups at C-1 and C-5, establishing the 1α -OH and 5α -OH configurations.

In the ^1H -nmr spectrum of **1** in pyridine- d_5 , the 18 and 19 angular methyl resonances occurred at δ_{H} 0.75 and 1.57 ppm, respectively. On comparison of the spectra that had been determined in CDCl_3 and pyridine- d_5 , a large pyridine-induced deshielding was observed for Me-19, thus indicating the presence of a hydroxyl group near Me-19 but remote from Me-18. The 6β position is the only position to accord with this demand, and thus a third secondary hydroxyl group must be located at the 6β position. In addition, the small values of coupling constants ($J = 1$ and 3.5 Hz) indicate the presence of an equatorial hydrogen at C-6 (α orientation).

In conclusion, the spectroscopic data revealed the structure of **1** to be 24-methylenecholestane- $1\alpha, 3\beta, 5\alpha, 6\beta$ -tetrol. It differs from a steroid previously isolated from the soft coral *Lobophytum pauciflorum* by the stereochemistry of the hydroxyl groups at C-1 (2).

In order to confirm the stereochemistry of numersterol A, an X-ray analysis was carried out. Figure 1 is a perspective drawing of the structure (less hydrogen atoms).

The second new sterol, numersterol B [**2**], had the molecular formula $\text{C}_{29}\text{H}_{50}\text{O}_3$. Its ^{13}C -nmr and ^1H -nmr spectra showed the following groups: five methyls [δ_{C} 11.9

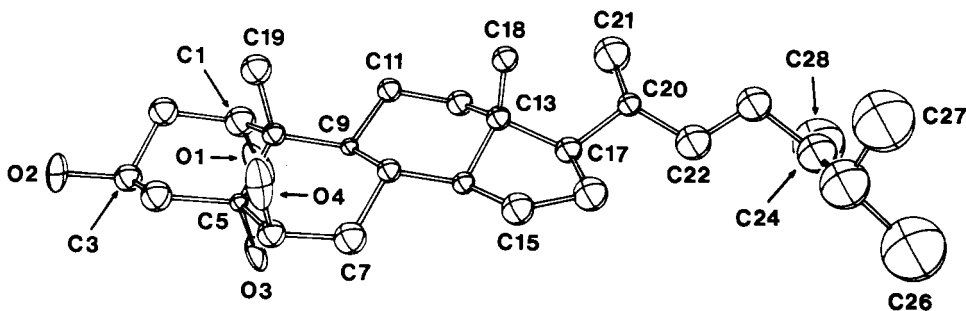


FIGURE 1. Perspective view of the structure of compound **1**.

(q), 13.0 (q), 18.6 (q), 21.8 (q), and 21.8 (q) ppm; δ_{H} 0.65 (3H, s, Me-18), 1.06 (3H, s, Me-19), 0.99 (6H, d, $J = 6.5$ Hz, Me-27 and Me-28), 0.91 (3H, d, $J = 7$ Hz, Me-21) ppm], a terminal double bond [δ_{C} 156.8 (s) and 106.2 (r) ppm, δ_{H} 4.69 (1H) and 4.72 (1H) ppm], and one tertiary and two secondary hydroxyls [δ_{C} 75.2 (s), 73.2 (d), 66.4 (d) ppm; δ_{H} 4.03 (1H, m, br), 3.95 (1H, br) ppm].

The signal of numersterol B at δ 4.03 corresponds to an H-3 α in the cholestane-3 β ,5 α -diols (2,8). We suggest that the third hydroxyl group of **2** is located at position 1 β . The signal of the H-1 α appears at a somewhat lower field (δ 3.95 ppm) than the H-3 α of the unsubstituted cholestane-3 β -ol and is due to the deshielding of the 5 α -OH. In addition, the ^{13}C chemical shifts for cholestane-1 α -OH, -1 β -OH, -6 α -OH, -6 β -OH, -11 α -OH, and -11 β -OH were calculated using the reference substance cholestane-3 β ,5 α -diol (9) by applying the hydroxyl substituent effects reported by Eggert *et al.* (10). In the case of 1 β -OH, the calculated values are in accord with the observed values (Table 1).

Comparison of the ^1H - and ^{13}C -nmr spectra of **2** with those of **1** indicated that the compounds might have similar side chains. Thus a C_{10} unsaturated side chain would be

TABLE 1. ^{13}C -nmr Chemical Shifts of Numersterol A [**1**], Numersterol B [**2**], and Two Referent Compounds, and Some Calculated Values (in parentheses).

Carbon	Compound			
	1 (CD_3OD)	2 (CDCl_3)	24 ξ -methylcholestane-1 β ,3 β ,5 α ,6 β -tetrol ^a ($\text{C}_5\text{H}_5\text{D}$)	24-methylene-5 α -cholest-7-ene-3 β ,6 α -diol ^b (CDCl_3)
C-1	75.91	73.2(72.0)	73.7	
C-2	39.19	41.6(43.0)	44.1	
C-3	64.83	66.4(64.9)	65.3	
C-4	41.93	42.2	43.2	
C-5	79.16	75.2(73.5)	76.9	
C-6	76.42	34.8	77.0	
C-7	35.34	27.7	35.7	
C-8	31.49	35.7	31.7	
C-9	41.51	45.0(46.2)	47.1	
C-10	43.99	44.0(45.8)	44.9	
C-11	21.65	23.6	25.0	
C-12	41.26	39.8	41.3	
C-13	41.74	42.5	42.6	
C-14	57.55	56.3	56.7	
C-15	25.25	24.4	24.9	
C-16	29.30	29.6	28.5	
C-17	57.55	56.9	56.7	
C-18	12.60	13.0	12.5	
C-19	17.28	11.9(11.0)	10.7	
C-20	34.91	37.8	34.0	36.1
C-21	19.10	18.6	19.0	18.9
C-22	36.01	38.9	36.6	34.8
C-23	32.12	26.0		31.3
C-24	157.48	31.5		156.8
C-25	36.99	156.8		36.1
C-26	22.45	33.9		21.9
C-27	22.28	21.8		22.0
C-28	106.66	21.8		106.1
C-29		106.2		

^aData from Yamada *et al.* (2).

^bData from Piccialli and Sica (7).

present in **2** instead of a C₉ one in **1**. The nmr spectra of **2** showed three methyl groups on the side chain. In comparing the ¹³C-nmr data of **2** and 24-methylene-5 α -cholest-7-ene-3 β ,6 α -diol (**7**) (Table 1), it was noted that the side chain of compound **2** was of the unsaturated cholesterol type with one more methylene group extending the side chain. The mass spectrum of **2** containing the fragments at *m/z* 362 [M - C₆H₁₂]⁺, 344 [M - C₆H₁₂ - H₂O]⁺, 326 [M - C₆H₁₂ - 2H₂O]⁺, and 83 [C₆H₁₁]⁺ were consistent with the suggested unsaturated side chain structure. Compound **2** is assigned the structure 25-methylene-22-homocholestane-1 β ,3 β ,5 α -triol.

EXPERIMENTAL

INSTRUMENTATION.—¹H- and ¹³C-nmr spectra were recorded on a Bruker WH 200 and 300 instrument and a JEOL FX 90Q instrument. Chemical shift values are in δ , calibrated with internal TMS standard in the solvents stated. A VG Analytical ZAB (hrms) Mass Spectrometer and a Nicolet 5DX FTIR Spectrometer were used. Optical rotation was measured with a Perkin-Elmer 241 polarimeter.

ANIMAL MATERIAL.—The fresh soft coral *S. numerosa* (wet wt 4.5 kg) was collected from the South China Sea near the Port San-ya of Hainan Island. A voucher specimen is deposited in the Research Center of Organic Natural Products, Zhongshan University, Guangzhou, China.

EXTRACTION AND ISOLATION.—The specimens were chopped and immersed in EtOH. The concentrated EtOH extract was extracted with EtOAc. The EtOAc extract was chromatographed over a column of Sil gel, eluting with petroleum ether stepwise increasing the concentration of Me₂CO. The fractions eluted with 8% Me₂CO gave the known sterol, 24-methylenecholesterol. The 40% Me₂CO eluate was chromatographed again to give numersterol B [**2**]. The more polar fractions eluted with 60% Me₂CO were further chromatographed and crystallized from MeOH to give numersterol A [**1**].

NUMERSTEROL A [1].—White prisms, mp 297–299°, [α]_D²⁵ +4.51 (*c* = 0.332, MeOH); ir (KBr) 3330, 1070, 1050 cm⁻¹ (OH); ¹H nmr (CDCl₃, 200 MHz), δ 0.70 (3H, s, Me-18), 0.96 (3H, d, *J* = 6 Hz, Me-21), 1.04 (6H, d, *J* = 6.5 Hz, Me-26 and Me-27), 1.11 (3H, s, Me-19), 1.25 (1H, s, OH), 2.18 (1H, s, OH), 2.61 (1H, d, OH), 3.49 (1H, dd, *J* = 1 and 3.5 Hz, H-6 α), 3.67 (1H, s, OH), 3.85 (1H, t, *J* = 4 Hz, H-1 β), 4.31–4.50 (1H, m, H-3 α), 4.66 and 4.73 (2H, d, *J* = 1 Hz, =CH₂, C-28); ¹H nmr (CD₃OD, 300 MHz) δ 0.74 (3H, s, Me-18), 1.00 (3H, d, *J* = 6 Hz, Me-21), 1.04 (6H, dd, *J* = 1 and 6.5 Hz, Me-26 and Me-27), 1.11 (3H, s, Me-19), 1.76–1.87 (2H, dt, H₂-2), 2.08–2.18 (2H, dd, H₂-4), 3.25 (1H, t, H-6 α), 3.69 (1H, t, H-1 β), 4.14–4.50 (1H, W_{1/2} = 17.5 Hz, H-3 α), 4.56 and 4.63 (2H, d, =CH₂, C-28). *Anal.* found C 75.10, H 10.95; calcd for C₂₈H₄₈O₄, C 74.95, H 10.78. *Hrms m/z* (% rel. int.) [M]⁺ 448.3538 (C₂₈H₄₈O₄) (18), [M - Me]⁺ 433.3349, [M - H₂O]⁺ 430.3452 (15), [M - 2H₂O]⁺ 412.3368 (36), [M - 2H₂ - Me]⁺ 397.3108 (23), 346.2496 [C₂₂H₃₄O₃]⁺ (50), (M - side chain - 2H)⁺ 321.2089 (62), [M - side chain - 2H - H₂O]⁺ 303.1971 (20), [321 - 2H₂O]⁺ 285.1881 (30), [ring D fission - H₂O]⁺ 264.1765 (15), [ring D fission - 2H₂O]⁺ 246 (17), [ring D fission - 3H₂O]⁺ 228 (16), 55 (100).

X-RAY STRUCTURAL ANALYSIS OF 1.—Crystals grown slowly from EtOH were poor in quality, and after several unsuccessful trials a thin plate of dimensions 0.42 × 0.38 × 0.07 mm was selected. Diffraction measurements were made on a Nichollet R3m four-circle diffractometer (graphite-monochromatized MoK α radiation, λ = 0.71069 Å), and determination of the crystal class, orientation matrix, and accurate unit-cell parameters were performed according to established procedures (11).

CRYSTAL DATA. ¹—C₂₈H₄₈O₄, MW = 448.68, monoclinic, space group C2 (No. 5), *a* = 11.66(1), *b* = 7.525(3), *c* = 30.39(2) Å, β = 93.13(7)°, *V* = 2662(3) Å³, *D_m* (floatation in KI/H₂O) = 1.112 g·cm⁻³, *Z* = 4, *D_{calcd}* = 1.120 g·cm⁻³, *F*(000) = 992, μ (MoK α) = 0.68 cm⁻¹.

The reflections declined rapidly in intensity with increasing Bragg angle, and data were recorded only to 2 θ max = 40° at 22°. The raw data were processed with the learnt-profile procedure (12) to yield 1181 unique reflections, and absorption corrections (μ r = 0.01, transmission factors 0.285 to 1.000) were based on a pseudoellipsoidal fit to azimuthal scans of selected strong reflections over a range of 2 θ values (13, 14).

The structure was solved by direct phase determination guided by negative quartets (15). Owing to the unfavorable data-to-parameter ratio, the nonhydrogen atoms in the asymmetric unit were subjected to

¹Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

isotropic refinement, except O-1, O-2, and O-4, which were varied anisotropically. The methylene, terminal ethylene, and methine hydrogen atoms were generated geometrically (C-H fixed at 0.96 Å) and allowed to ride on their respective parent carbon atoms, and the methyl groups were treated as rigid groups. These hydrogen atoms were included in structure-factor calculations with assigned isotropic thermal parameters.

All computations were performed on a Data General Nova 3/12 minicomputer with the SHELXTL system (16). Analytic expressions of neutral-atom scattering factors incorporating the real and imaginary components of anomalous dispersion were employed (17). Blocked-cascade least-squares refinement (18) converged to $R = 0.145$ for 152 parameters and 830 observed reflections [$|F_o| > 3\sigma(F_o)$], the weighting function being $w = \{\sigma^2(|F_o|) + 0.0035|F_o|^2\}^{-1}$.

The final atomic coordinates are listed in Table 2. Selected bond distances and bond angles are given in Table 3. Hydrogen coordinates, anisotropic thermal parameters, and structure factors are available as supplementary material upon request from the authors.

NUMERSTEROL B [2].—Colorless crystals from Me₂CO petroleum ether, mp 121–122°, ν max (KBr) 3380, 1052 cm⁻¹ (OH); ¹H nmr (90 MHz, CDCl₃) δ 0.65 (3H, s, Me-18), 0.99 (6H, d, $J = 6.5$ Hz, Me-27 and Me-28), 0.91 (3H, d, $J = 7$ Hz, Me-21), 1.06 (3H, s, Me-19), 1.65 (1H, s, OH), 3.95 (1H, br m, H-1 α), 4.03 (1H, m, H-3 α), 4.05 (2H, s, OH), 4.69 and 4.72 (2H, =CH₂, C-29); m/z [M]⁺ 446 (C₂₉H₅₀O₃), [M - H₂O]⁺ 428, [M - 2H₂O]⁺ 410, [M - 2H₂O - Me]⁺ 395, [M - C₆H₁₂]⁺ 362, [M - C₆H₁₂ - H₂O]⁺ 344, [M - C₆H₁₂ - 2H₂O]⁺ 326, 319, 301, 283, [ring D fis-

TABLE 2. Atomic Coordinates ($\times 10^3$ for x and y ; $\times 10^4$ for z) and Thermal Parameters* ($\text{\AA}^2 \times 10^3$) for Compound 1.

Atom	x	y	z	U_{eq}/U
O-1	406(2)	678(2)	791(4)	97(2)*
O-2	111(1)	599(2)	-83(4)	72(2)*
O-3	358(1)	341(2)	756(4)	64(3)
O-4	109(1)	210(2)	1350(4)	88(2)*
C-1	297(2)	707(3)	933(8)	79(4)
C-2	206(2)	739(3)	565(7)	67(4)
C-3	194(2)	569(3)	256(6)	53(4)
C-4	157(2)	413(3)	524(7)	62(4)
C-5	244(1)	378(2)	916(5)	23(3)
C-6	222(2)	209(3)	1148(7)	59(4)
C-7	304(2)	173(3)	1517(7)	75(4)
C-8	322(2)	327(2)	1849(6)	41(3)
C-9	350(1)	500	1590(5)	26(3)
C-10	259(2)	546(3)	1235(6)	42(3)
C-11	381(2)	653(3)	1925(6)	50(4)
C-12	471(2)	603(3)	2270(6)	53(4)
C-13	437(2)	445(2)	2542(6)	43(4)
C-14	421(2)	299(2)	2194(6)	40(3)
C-15	408(2)	128(3)	2499(7)	72(4)
C-16	496(2)	166(3)	2889(8)	83(4)
C-17	533(2)	358(3)	2829(7)	53(4)
C-18	334(2)	483(3)	2803(6)	52(4)
C-19	140(2)	598(3)	1427(7)	68(4)
C-20	568(2)	436(2)	3292(7)	47(4)
C-21	597(2)	646(3)	3301(10)	118(4)
C-22	669(2)	333(3)	3548(9)	107(4)
C-23	691(2)	393(3)	4012(8)	101(4)
C-24	791(3)	268(4)	4206(11)	145(4)
C-25	754(2)	83(3)	4306(14)	198(5)
C-26	859(3)	-42(4)	4422(21)	307(5)
C-27	673(3)	117(4)	4670(21)	309(5)
C-28	903(2)	342(4)	4274(15)	250(5)

*Asterisk indicates equivalent isotropic temperature factor U_{eq} defined as $1/3$ of the trace of the orthogonalized U matrix. The exponent of the isotropic temperature factor takes the form $-8\pi^2 U \sin^2 \theta / \lambda^2$.

TABLE 3. Bond Lengths (Å) and Bond Angles (degrees) for Compound 1.

O-1-C-1	1.39(3)	O-2-C-3	1.39(2)
O-3-C-5	1.47(2)	O-4-C-6	1.49(2)
C-1-C-2	1.51(3)	C-1-C-10	1.60(3)
C-2-C-3	1.59(3)	C-3-C-4	1.50(3)
C-4-C-5	1.54(3)	C-5-C-6	1.48(3)
C-5-C-10	1.60(2)	C-6-C-7	1.46(3)
C-7-C-8	1.54(3)	C-8-C-9	1.56(2)
C-8-C-14	1.53(2)	C-9-C-10	1.51(2)
C-9-C-11	1.57(2)	C-10-C-19	1.59(3)
C-11-C-12	1.49(3)	C-12-C-13	1.51(3)
C-13-C-14	1.53(3)	C-13-C-17	1.53(3)
C-13-C-18	1.50(3)	C-14-C-15	1.60(3)
C-15-C-16	1.55(3)	C-16-C-17	1.52(3)
C-17-C-20	1.56(3)	C-20-C-22	1.58(3)
C-20-C-21	1.61(3)	C-22-C-23	1.49(4)
C-23-C-24	1.59(4)	C-24-C-25	1.49(4)
C-24-C-28	1.43(4)	C-25-C-27	1.52(7)
C-25-C-26	1.56(4)		
O-1-C-1-C-2	114(2)	O-1-C-1-C-10	110(2)
C-2-C-1-C-10	110(2)	C-1-C-2-C-3	110(2)
O-2-C-3-C-2	110(2)	O-2-C-3-C-4	109(2)
C-2-C-3-C-4	109(2)	C-3-C-4-C-5	112(2)
O-3-C-5-C-4	110(2)	O-3-C-5-C-6	100(1)
C-4-C-5-C-6	113(1)	O-3-C-5-C-10	106(1)
C-4-C-5-C-10	112(1)	C-6-C-5-C-10	114(1)
O-4-C-6-C-5	112(2)	O-4-C-6-C-7	104(2)
C-5-C-6-C-7	114(2)	C-6-C-7-C-8	115(2)
C-7-C-8-C-9	109(2)	C-7-C-8-C-14	115(2)
C-9-C-8-C-14	107(1)	C-8-C-9-C-10	113(1)
C-8-C-9-C-11	109(1)	C-10-C-9-C-11	115(1)
C-1-C-10-C-5	106(2)	C-1-C-10-C-9	113(1)
C-5-C-10-C-9	108(1)	C-1-C-10-C-19	107(2)
C-5-C-10-C-19	110(1)	C-9-C-10-C-19	113(2)
C-9-C-11-C-12	113(1)	C-11-C-12-C-13	113(2)
C-12-C-13-C-14	102(2)	C-12-C-13-C-17	116(2)
C-14-C-13-C-17	98(1)	C-12-C-13-C-18	113(2)
C-14-C-13-C-18	116(2)	C-17-C-13-C-18	111(2)
C-8-C-14-C-13	115(1)	C-8-C-14-C-15	115(1)
C-13-C-14-C-15	101(1)	C-14-C-15-C-16	102(2)
C-15-C-16-C-17	105(2)	C-13-C-17-C-16	106(2)
C-13-C-17-C-20	120(2)	C-16-C-17-C-20	108(2)
C-17-C-20-C-22	114(2)	C-17-C-20-C-21	116(2)
C-22-C-20-C-21	109(2)	C-20-C-22-C-23	114(2)
C-22-C-23-C-24	105(2)	C-23-C-24-C-25	115(2)
C-23-C-24-C-28	118(2)	C-25-C-24-C-28	127(3)
C-24-C-25-C-27	101(2)	C-24-C-25-C-26	112(2)
C-27-C-25-C-26	117(4)		

sion)⁺ 265, [ring D fission - H₂O]⁺ 247, [ring D fission - 2H₂O]⁺ 229, [ring D fission - 3H₂O]⁺ 211, 83, 55.

24-METHYLENE CHOLESTEROL.—Colorless needles from Me₂CO, C₂₈H₄₆O, mp 130–131°. Its identity was confirmed by the physical and spectral analysis (19): ir ν max (KBr) 3440, 1668, 1647, 1060, 888, 776 cm⁻¹; ¹H nmr (CDCl₃, 90 MHz) δ 0.69 (3H, s, Me-18), 1.01 (6H, d, *J* = 7 Hz, Me-26 and Me-27), 1.03 (3H, s, Me-19), 1.08 (3H, d, *J* = 6 Hz, Me-21), 3.53 (1H, br, H-3 α), 4.67 and 4.71 (2H, =CH₂, C-28), 5.35 (1H, d, *J* = 4 Hz, 6-H); ¹³C nmr (CDCl₃) δ 157.0 (s), 141.1 (s), 121.9 (d), 106.3 (t), 72.1 (d), 57.1 (d), 56.3 (d), 50.4 (d), 42.6 (s), 42.5 (t), 40.1 (t), 37.6 (t), 36.8 (s), 36.1 (d), 35.0 (t), 34.1

(d), 32.3 (d), 32.2 (t), 31.9 (t), 31.3 (t), 28.5 (t), 24.6 (t), 22.3 (q), 22.2 (q), 21.4 (t), 19.7 (q), 19.0 (q), 12.1 (q); ms m/z 398 ($C_{28}H_{46}O$), $[M - \text{side chain} - 2H]^+$ 271, $[\text{ring D fission} - H]^+$ 231, $[\text{ring D fission} - H_2O - H]^+$ 213, 55.

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