24ξ-METHYLCHOLESTANE-3β,5α,6β,25-TETROL 25-MONOACETATE

A NOVEL POLYHYDROXYLATED STEROID FROM AN ALCYONARIAN

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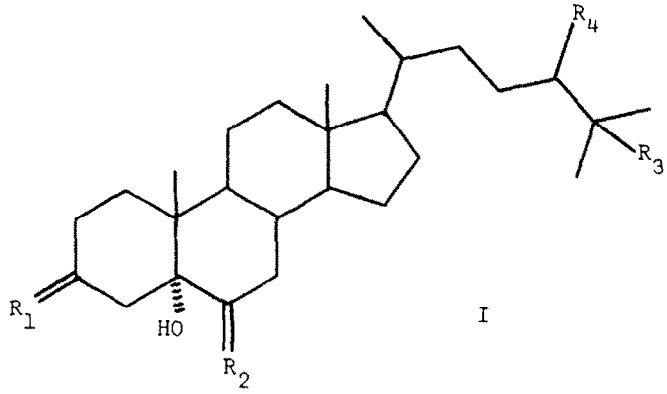
ABSTRACT

24ξ-Methylcholestane-3β,5α,6β,25-tetrol 25-monoacetate has been isolated from an Alcyonarian and its structure elucidated. A synthesis of the corresponding tetrol, 24ξ-methylcholestane-3β,5α,6β,25-tetrol, confirmed the original structure assignment.

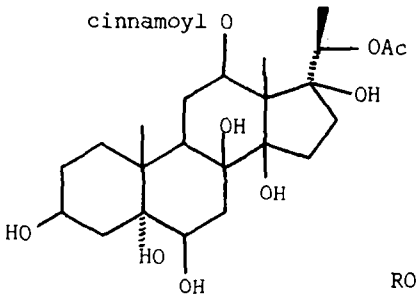
We wish to report the isolation and characterization of a new sterol, 24ξ-methylcholestane-3β,5α,6β,25-tetrol 25-monoacetate (Ia), from the Alcyonarian ("soft coral") Sarcophyton elegans, Moser 1919, in 0.05% yield (dry weight basis). The compound is unusual in its 3β,5α,6β hydroxylation pattern and its naturally occurring 25-acetoxy function is unprecedented in sterols reported thus far.

Although the 3β,5α,6β hydroxylation pattern is readily obtained synthetically [3], only two instances of this pattern in nature have thus far been reported. The structure of cerevisterol (3β,5α,6β-trihydroxyergosta-7,22-diene), a minor yeast and ergot sterol, was reported in 1954 by Alt and Barton [4]. More recently, the heavily oxygenated pregnane, 12-O-cinnamoyl-20-O-acetylglycosarcostin (II), has been isolated from a plant [5]. A number of C-25 hydroxylated sterols have been found in nature, but most of these have been from the specialized class of ecdysones [6]. Other than the ecdysones, very few 25-hydroxy sterols have been synthesized [7] or isolated.

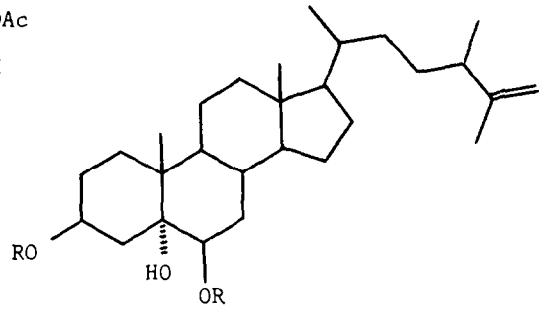


I

Ia	$R_1=R_2=$ 		R_3=OAc	$R_4=CH_3$
Ib	$R_1=R_2=$ 		R_3=OAc	$R_4=CH_3$
Ic	$R_1=R_2=$ 		R_3=OAc	$R_4=CH_3$
Id	$R_1=R_2=O$		R_3=OAc	$R_4=CH_3$
Ie	$R_1=$ 	$R_2=$ 	R_3=OAc	$R_4=CH_3$
If	$R_1=$ 	$R_2=O$	R_3=OAc	$R_4=CH_3$
Ig	$R_1=R_2=$ 		R_3=OH	$R_4=CH_3$
Ih	$R_1=R_2=O$		R_3=H	R_4=H
Ii	$R_1=R_2=$ 		R_3=H	R_4=H
Ij	$R_1=$ 	$R_2=O$	R_3=H	R_4=H



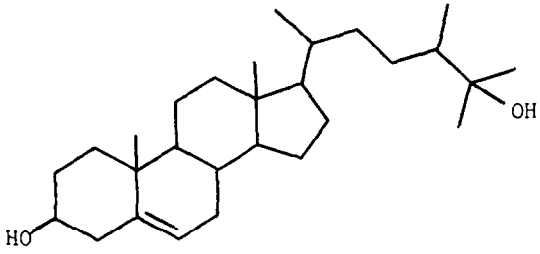
II



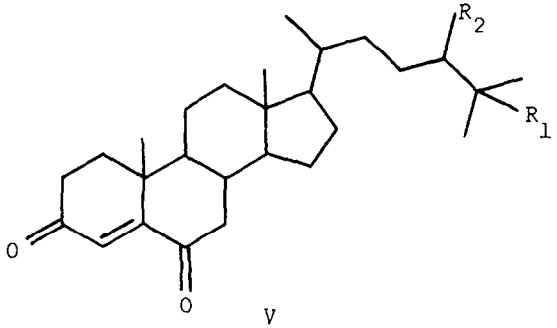
IV

IVa R=H

IVb R=Si$(CH_3)_3$



III



V

Va R_1=OAc R_2=CH_3

Vb R_1=R_2=H

A noteworthy instance is 5-cholestene-3β,25-diol which is found in human aorta [8]. The closest naturally occurring sterol, 24ξ-methyl-5-cholestene-3β, 25-diol (III), was also found in an Alcyonarian [9] and may well be the precursor of Ia.

24ξ-Methylcholestane-3β,5α,6β,25-tetrol 25-monoacetate (Ia) could be separated from the other polar steroids by trimethyl silylation using chlorotrimethyl silane and bis-(trimethylsilyl)-acetamide in pyridine producing Ib whose 100 MHz nmr spectrum featured 9-proton singlets at δ 0.06 and 0.11 and 1-proton multiplets centered at δ 3.45 and 3.98 establishing the presence of two secondary hydroxyls in Ia. The extremely broad nature (a range of 0.4 ppm) of the signal at δ 3.98 suggested [10] a 3α proton. A sharp methyl singlet at δ 1.96 and a strong ir absorption at 1725 cm^{-1} indicated the presence of an acetate functionality. The spectrum of Ib showed angular methyl singlets at δ 0.67 (3,s), 1.12 (3,s) and 1.38 (6,s) and methyl doublets centered at δ 0.86 and δ 0.91 consistent with the 24-methylcholestane carbon skeleton.

The bis-trimethylsilyl acetate Ib upon gas chromatography at 256° underwent thermal loss of acetic acid to give the 25-methylene sterol IVb, a compound with a broad methylene singlet in the 100 MHz nmr spectrum at δ 4.64 (2,s). The disappearance of the 2-methyl singlet at δ 1.38 and the appearance of a 3-proton methyl singlet at δ 1.61 indicated the presence of a 25-methylene group and located the acetoxy function in Ia at C-25. Acid catalyzed hydrolysis of the bis-trimethylsilyl derivative IVb produced the free sterol IVa. High-resolution mass spectrometry of IVa (Fig. 1) showed ions of elemental composition $C_{28}H_{48}O_3$, $C_{28}H_{46}O_2$, $C_{28}H_{44}O$, and $C_{28}H_{42}$

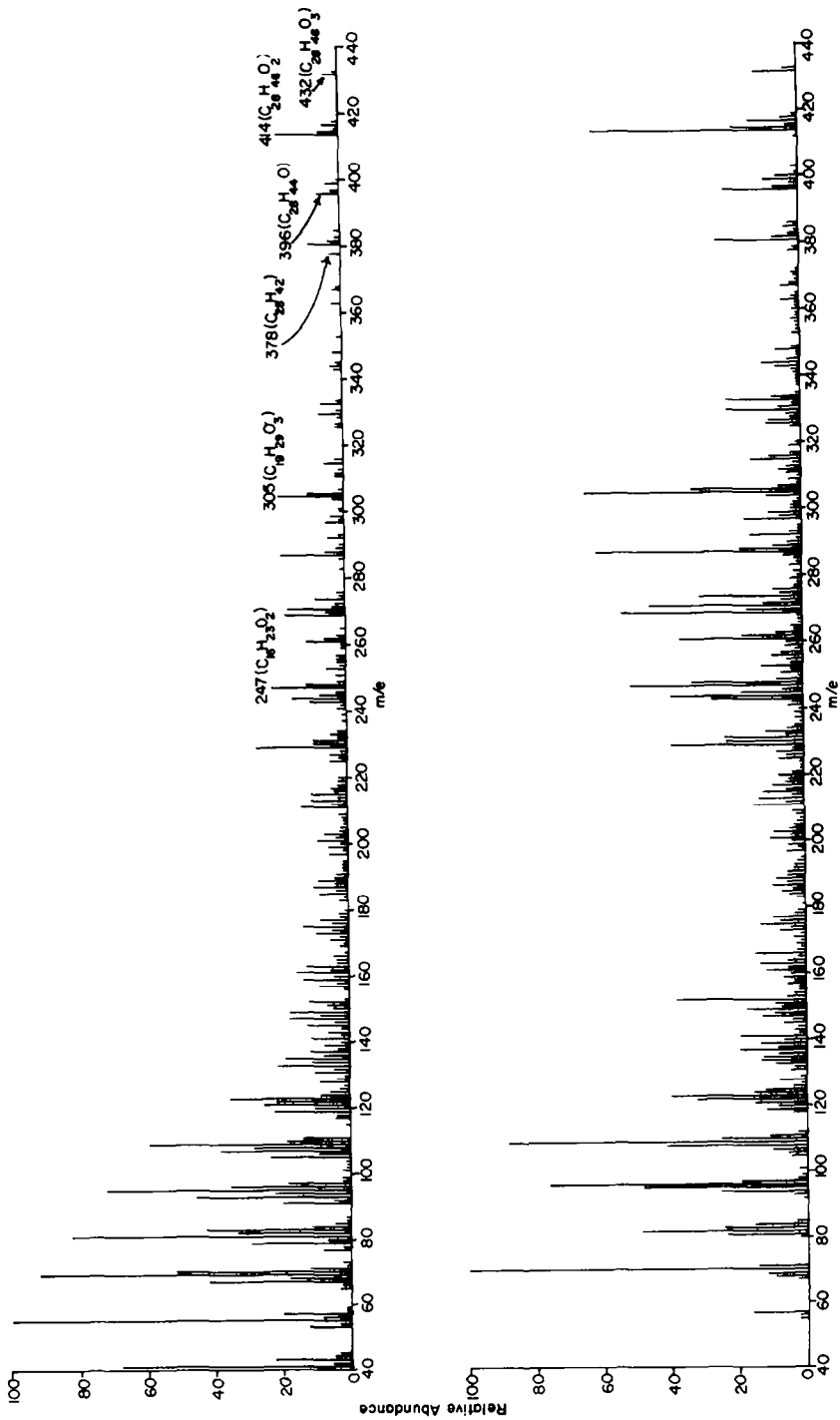


Figure 1. The 15 eV (lower) and 70 eV (upper) mass spectra of 24ξ-methyl-4-cholestene-3β,5α,6β-triol (IVa) showing the elemental composition of selected ions.

establishing the empirical formula of IVa and showing it to be a triol by stepwise water losses. A peak for $C_{16}H_{23}O_2$ corresponds to the typical ring D fission [11] with loss of a hydrogen atom plus loss of water, thus locating the three hydroxyl groups in rings A, B, and C. A peak for $C_{19}H_{29}O_3$ correspor to loss of the side chain together with two hydrogen atoms; such a fragmentati is typical [11] of a nine carbon side chain containing a double bond. Treatment of Ia with excess acetic anhydride in pyridine produced a triacetate Ic showing three acetate methyl singlets in the nmr. The protons α to the aceto: groups were centered at δ 4.66 and 5.14, a >1 ppm shift, consistent with secondary acetates [10].

Oxidation of the parent sterol Ia using Jones reagent produced the diketone Id which displayed no intense ultraviolet absorption, but when Id wa: treated with dry hydrogen chloride in dichloromethane the isolated product Va had λ_{max} at 250 nm in close agreement with the model 4-cholestene-3,6-dione (Vb) (λ_{max} 252 nm, lit. [12] 253 nm). Furthermore, the substance exhibited an intense mass spectral peak at m/e 137, typical [13] of Δ^4-3,6-diketones. Partial acetylation of Ia led to the 3,25-diacetate Ie. Oxidation with Jones reagent yielded the keto diacetate If, which was stable to dilute KOH/MeOH and showed no uv spectrum before or after basifying. The spectr data for Ia and its products correspond well to those for synthetic cholestane-3β,5α,6β-triol (Ii) which was put as a model compound through analogous transformations according to standard procedures [14]. For example, the 100 MHz nmr of cholestane-3β,5α,6β-triol (Ii) features angular methyl singlets at δ 0.70 and 1.17 compared with δ 0.68 and 1.17 for Ia. The chemical shift

and peak shape of protons α to hydroxyl in the nmr of Ia are identical to those of Ii and are centered at δ 3.47 (1,m) and 4.12 (1,m). The CD Cotton effects for various ketones (If vs. Ij; Id vs. Ih; Va vs. Vb) derived from Ia and their model counterparts from the cholestane analog Ii were quite similar to each other and in good agreement with values given by Crabbé [15] for simple 3- and 6-keto steroids.

24ξ-Methylcholestane-3β,5α,6β,25-tetrol (Ig), prepared by basic hydrolysis of Ia, was also synthesized from 24ξ-methyl-5-cholestene-3β,25-diol (III)[9] by the procedure of Fieser and Rajagopalan [3]. The two specimens of Ig were identical by nmr, mass spectrometry, and thin layer chromatography thus confirming the original structure assignment of Ia.

EXPERIMENTAL

Melting points are reported uncorrected. Ultraviolet spectra were measured in methanol on a Cary 14 spectrometer. Optical rotations were taken on a Perkin-Elmer 141 polarimeter in absolute ethanol. Infrared spectra were measured on a Perkin-Elmer 421 grating spectrometer. Nuclear magnetic resonance (nmr) spectra were recorded using a Varian XL-100 spectrometer equipped with Fourier transform capability. Deuterio chloroform was used as the nmr solvent, unless otherwise stated, and values are in δ^{TMS} calibrated either with internal TMS or by setting $CHCl_3$ at δ 7.25. Low resolution mass spectra were taken on AEI MS-9, Varian MAT 711, or Atlas CH-4 mass spectrometers while peak element-mapping high-resolution spectra utilized the Varian MAT 711 instrument.

Vapor phase chromatography (vpc) was carried out with a Hewlett-Packard 402 high efficiency instrument using glass columns packed with 3% U.C.W.-98 on Chromosorb W.H.P. (80-100 mesh) obtained from Hewlett-Packard. Column chromatography was carried out using E. Merck, neutral, activity grade II aluminum oxide. Thin layer chromatography (tlc) was performed using 5x20 cm., 250-μ silica gel HF_{254} plates. Visualization was made possible using a 2% ceric sulfate solution in 1M sulfuric acid spray followed by heating on a hot plate.

Isolation of 24ξ-methylcholestane-3β,5α,6β,25-tetrol 25-monoacetate from Sarcophyton elegans. (a) 3β,6β-bis-(Trimethylsilyloxy)-24ξ-methylcholestane-

5α-25-diol 25-monoacetate (Ib). The dried soft coral (1 kg) was broken into small chunks (1-5 g each) and blended with ethyl acetate in a Waring blender. The solid material was filtered off, placed in a Soxhlet extractor, and continuously extracted with ethyl acetate for 48 hrs. The combined filtrate and extraction liquors were dried ($MgSO_4$) and evaporated at reduced pressure to give a 110 g brown oily residue. The product sought had a tlc Rf of 0.15 using chloroform/ethanol (9:1) as eluent and appeared brown with ceric sulfate visualization. The oily mixture was chromatographed using 1100 g of alumina II and starting with chloroform eluent to remove most unwanted natural products, and gradually increasing the ethanol content to 3-4% when the desired material appeared. This material (about 2 g) was trimethylsilylated by adding 5 g of dry pyridine and 1 g each of chlorotrimethyl silane and bis-(trimethylsilyl)-acetamide. The pyridine solution stood for 24 hrs after which volatile materials were removed by pumping at 0.1 mm for one hr. The major component had a tlc Rf of 0.28 using hexane/ethyl acetate (10:1) and appeared deep purple with ceric sulfate spray. The residue was chromatographed on alumina II using hexane and slowly increasing polarity with ethyl acetate until 4% when the silylated steroid was obtained as a clear glassy solid: ir (CCl_4) 1725 cm^{-1} (acetate C=O); nmr ($CDCl_3$) δ 0.06 (9,s), 0.11 (9,s), 0.67 (3,s), 0.86 (3,d,J=6Hz), 0.91 (3,d,J=6Hz), 1.12 (3,s), 1.38 (6,s), 1.55 (s), 2.96 (3,s), 3.45 (1,m), 3.98 (1,m); mass spectrum at 70 eV, m/e (relative intensity) 618 (5,M-H_2O), 576 (2,M-CH_3COOH), 558 (30,M-H_2O+CH_3COOH), 544 (8), 529 (5), 475 (6), 471 (11), 468 (26,M-CH_3COOH+H_2O+$HOSi(CH_3)_3$), 454 (7), 415 (29,M-CH_3COOH+2Si$(CH_3)_3$+CH_3), 401 (8), 379 (5), 333 (22), 319 (5), 129 (40), 95 (33), 81 (36), 75 (54), 73 (100), 69 (50), 55 (40), 43 (36). (b) 24ξ-Methylcholestane-3β,5α,6β,25-tetrol 25-monoacetate (Ia). The trimethylsilyl ether Ib (695 mg) was added to 20 ml of methanol and stirred with 3 drops of 5% HCl for 5 mins. The resulting mixture was treated with a slight excess of solid Na_2CO_3 and evaporated to dryness. The residue was taken up into chloroform and water and the water layer was extracted three times with chloroform. The chloroform solution was dried ($MgSO_4$), filtered, and flash evaporated to give an oily solid. The solid was chromatographed on an alumina II column by first washing with chloroform and then with chloroform/ethanol (10:1). The pure sterol obtained in the chloroform/ethanol fractions had the following characteristics: m.p. 233-236°; $[\alpha]_D$ -11.0 (c, 0.583, ethanol); ir ($CHCl_3$) 1718 cm^{-1} (acetate C=O); nmr ($CDCl_3$) δ 0.68 (3,s), 0.87 (3,d,J=6Hz), 0.93 (3,d,J=6Hz), 1.17 (3,s), 1.38 (6,s), 1.96 (3,s), 3.47 (1,m), 4.12 (1,m); mass spectrum at 70 eV, m/e (relative intensity) [592 present at 15 eV only (1,M^+)], 474 (1,M-H_2O), 456 (2,M-2H_2O), 432 (11,M-CH_3COOH), 417 (4,M-CH_3COOH+CH_3), 414 (23,M-CH_3COOH+H_2O), 400 (6), 399 (4,M-CH_3COOH+H_2O+CH_3), 396 (12,M-CH_3COOH+2H_2O), 381 (5,M-CH_3COOH+2H_2O+CH_3), 378 (3,M-CH_3COOH+3H_2O), 348 (4), 344 (3), 333 (3), 330 (9), 305 (23,M-side chain+2H), 287 (13,M-side chain+2H+H_2O), 271 (11), 269 (12,M-side chain+2H+2H_2O), 123 (25), 109 (32), 107 (25), 95 (47), 93 (28), 83 (36), 81 (51), 69 (52), 67 (23), 60 (33), 55 (63), 43 (100).

3β,6β-bis-(Trimethylsilyloxy)-24ξ-methyl-25-cholestene-5α-ol (IVb). Compound Ib was vpc chromatographed at 256° to give a glass-like solid as the major

product. This product (IVb) was evidently formed by pyrolysis on the hot glass of the vpc column: ir (CCl_4) no C=O band; nmr ($CDCl_3$) δ 0.06 (9,s), 0.10 (9,s), 0.64 (3,s), 0.87 (3,d,J=6Hz), 0.96 (3,d,J=6Hz), 1.10 (3,s), 1.61 (3,s), 3.42 (1,m), 4.00 (1,m), 4.64 (2,s, broad); mass spectrum at 70 eV, m/e (relative intensity) 576 (6,M^+), 558 (75,M-H_2O), 543 (9,M-H_2O+CH_3), 529 (7), 468 (48,M-H_2O+HOSi(CH_3)$_2$), 415 (53,M-2Si(CH_3)$_2$+CH_3), 333 (38), 129 (41), 95 (36), 81 (37), 75 (40), 73 (100), 69 (52), 55 (37).

24ξ-Methyl-25-cholestene-3β,5α,6β-triol (IVa). The unsaturated trimethylsilyl ether IVb was cleaved with acid just like its saturated counterpart Ib to yield the unsaturated triol IVa which was subjected to high-resolution mass spectrometry with the following results: m/e (relative intensity, composition, interpretation, millimass error) 432.36182 (8.1, $C_{28}H_{48}O_3$, M^+, 1.528), 417.33740 (3.7, $C_{27}H_{45}O_3$, M-CH_3, 0.456), 414.34937 (25.0, $C_{28}H_{46}O_2$, M-H_2O, -0.304), 396.33813 (11.3, $C_{28}H_{44}O$, M-2H_2O, -1.177), 381.31738 (8.2, $C_{27}H_{41}O$, M-2H_2O+CH_3, 1.692), 378.32935 (3.3, $C_{28}H_{42}$, M-3H_2O, 0.607), 333.24072 (8.2, $C_{21}H_{33}O_3$, -2.311), 305.21118 (19.9, $C_{19}H_{29}O_3$, M-side chain+2H, -0.489), 287.20020 (13.8, $C_{19}H_{27}O_2$, M-side chain+2H+H_2O, -0.896), 269.18945 (14.4, $C_{19}H_{25}O$, M-side chain+2H+2H_2O, -1.198), 247.16988 (11.8, $C_{16}H_{23}O_2$, ring D fission+H+H_2O, -0.194), 229.15904 (17.0, $C_{16}H_{21}O$, ring D fission+H+2H_2O, -0.194), 211.14735 (7.9, $C_{16}H_{19}$, ring D fission+H+3H_2O, -1.313), 109.10156 (36.2, C_8H_{13}, -0.168), 95.08572 (46.2, C_7H_{11}, -0.343), 81.07030 (56.0, C_6H_9, -0.125), 69.07025 (74.7, C_5H_9, -0.174), 55.05499 (100.0, C_4H_7, 0.218), 41.03934 (98.9, C_3H_5, 0.213).

24ξ-Methylcholestane-3β,5α,6β,25-tetrol 3,25-diacetate (Ie) and 24ξ-methylcholestane-3β,5α,6β,25-tetrol 3,6,25-triacetate (Ic). 24ξ-methylcholestane-3β,5α,6β,25-tetrol 25-monoacetate (Ia, 15 mg) was dissolved in 0.5 ml of pyridine, treated with one drop of acetic anhydride and let stand for 24 hrs. The solution was mixed with 10 ml of chloroform and extracted with 5% HCl, 5% $NaHCO_3$, dried ($MgSO_4$), filtered, and evaporated to an oil. The oil was shown to contain two major and one minor components by tlc analysis. The Rf's were 0.26, 0.19, and 0.11 (minor), with chloroform/ethanol (60:1) eluent, visualized purple with ceric sulfate. The two major components were purified by column chromatography using 1 g of alumina II and eluting with hexane/chloroform (1:1) with a gradual increase in the percentage of chloroform. The triacetate Ic was eluted first as a clear oil with the following spectral properties: nmr ($CDCl_3$) δ 0.66 (3,s), 0.87 (6,two unresolved overlapping doublets), 1.14 (3,s), 1.37 (6,s), 1.95 (3,s), 2.00 (3,s), 2.05 (3,s), 4.68 (1,m, a 1 ppm downfield shift from Ia), 5.20 (1,m, a 1 ppm downfield shift from Ia); mass spectrum at 15 eV, m/e (relative intensity) [516, 501, 498, 487 not seen at 70 eV] 516 (5, M-CH_3COOH), 501 (7,M-CH_3COOH+CH_3), 498 (2, M-CH_3COOH+H_2O), 487 (2), 456 (22,M-2CH_3COOH), 438 (27,M-2CH_3COOH+H_2O), 428 (19), 412 (11), 396 (81, M-3CH_3COOH), 389 (35,M-CH_3COOH+side chain+2H), 382 (18), 381 (16,M-3CH_3COOH+CH_3), 380 (29), 379 (20), 378 (58,M-3CH_3COOH+H_2O), 371 (18, M-CH_3COOH+side chain+2H+H_2O), 363 (14,M-3CH_3COOH+H_2O+CH_3), 354 (10), 329 (21,M-2CH_3

COOH+side chain+2H), 327 (17), 312 (19), 309 (18), 253 (25), 167 (23), 152 (36), 149 (36), 123 (34), 120 (27), 109 (36), 96 (43), 82 (28), 70 (56), 60 (100).

The second compound eluted, the diacetate Ie, was a white solid with the following characteristics: m.p. 184-191°; nmr ($CDCl_3$) δ 0.67 (3,s), 0.89 (6, overlapping doublets, J=6Hz for each), 1.18 (3,s), 1.38 (6,s), 193 (3,s), 2.01 (3,s), 3.53 (1,m), 5.18 (1,m, a 1 ppm shift downfield from Ia); mass spectrum at 15 eV, m/e (relative intensity) 474 (11,M-CH_3COOH), 459 (19,M-CH_3COOH+CH_3), 456 (32,M-CH_3COOH+H_2O), 396 (100, M-2CH_3COOH), 347 (92,M-CH_3COOH(at C-25)+side chain+2H), 329 (66,M-CH_3COOH (at C-25)+side chain+2H+H_2O), 312 (40), 271 (35), 269 (47,M-2CH_3COOH+side chain+2H), 253 (34), 244 (43), 243 (44), 229 (57,M-CH_3COOH+ring D fission+H), 226 (34), 152 (45), 123 (47), 109 (66), 96 (71), 82 (50), 73 (70), 70 (65), 60 (47), 43 (42).

24ξ-Methyl-6-oxocholestane-3β,5α,25-triol 3,25-diacetate (If). The 3,25-diacetate Ie (3 mg) was dissolved in 0.5 ml of acetone and treated with one drop of Jones reagent and stirred for one hr. The mixture was dissolved in chloroform, washed with water, dried ($MgSO_4$), filtered and evaporated to give an oily product (2.5 mg) which was chromatographed using 1 g of alumina II and eluted with hexane/chloroform (1:1). The resulting solid ketone If (0.8 mg) was homogeneous by tlc with an Rf of 0.14 in chloroform/ethanol (60:1): m.p. 226-229°; nmr ($CDCl_3$) δ 0.63 (3,s), 0.81 (3,s), 0.88 (6,m), 1.25 (3,s), 1.38 (3,s), 1.95 (3,s), 2.01 (3,s), 5.00 (1,m); mass spectrum at 70 eV, m/e (relative intensity) [532 present at 15 eV only (1,M^+)], 472 (5,M-CH_3COOH), 458 (2), 457 (1), 443 (1), 412 (10,M-2CH_3COOH), 398 (3), 394 (4,M-2CH_3COOH+H_2O), 388 (4), 373 (6), 345 (12,M-CH_3COOH(at C-25)+side chain+2H), 330 (5), 310 (4), 297 (6), 285 (4), 269 (7), 241 (5), 227 (6), 175 (9), 133 (14), 123 (18), 121 (19), 109 (31), 95 (38), 83 (36), 81 (42), 69 (51), 55 (62), 43 (100).

3,6-Dioxo-24ξ-methylcholestane-5α,25-diol 25-monoacetate (Id). A solution of 12 mg of CrO_3 in 0.2 ml of water and 0.5 ml of acetic acid was slowly dripped into a stirred solution of 30 mg of the triol Ia in 2 ml of glacial acetic acid. After 72 hrs of stirring the reaction mixture was extracted with chloroform and the crude product isolated as in the preparation of If (see above). Recrystallization from methanol gave a white crystalline product m.p. 258-259° (dec.); nmr ($CDCl_3$) δ 0.67 (3,s), 0.90 (6, overlapping doublets, J=5), 1.00 (3,s), 1.39 (6,s), 1.97 (3,s); mass spectrum at 70 eV (relative intensity) 470 (1,M-H_2O), 410 (30,M-CH_3COOH), 396 (9), 381 (4), 341 (32), 327 (23), 326 (23), 311 (29), 296 (6), 283 (33,M-side chain+2H), 270 (17), 259 (16), 257 (25), 243 (18, ring D fission+H), 137 (74), 123 (22), 109 (32), 95 (42), 83 (62), 70 (100), 60 (72), 55 (97), 43 (80).

3,6-Dioxo-24ξ-methyl-4-cholestene-25-ol acetate (Va). The hydroxydione Id (6 mg) was dissolved in 5 ml of chloroform in an ice bath and dry HCl was

bubbled through for 5 mins. The chloroform was removed under reduced pressure and the residue was dissolved in a micro test tube in 2 drops of dichloromethane. After the addition of 6 drops of methanol, light yellow crystals formed, m.p. 167-168°. A tlc analysis of the product using chloroform as the eluent showed one product with a Rf of 0.25 (bright red color with ceric sulfate). The Δ^4-3,6-diketone Va was unstable and spectral data were recorded on freshly prepared material: nmr ($CDCl_3$) 0.73 (3,s), 0.88 (3,d,J=7), 1.00 (3,d,J=6), 1.17 (3,s), 1.26 (2,s), 1.39 (6,s), 1.56 (2,d,J=4), 1.93 (3,s), 6.18 (1,s); mass spectrum at 70 eV, m/e (relative intensity) [470 present at 15 eV (.2,M^+)], 424 (4), 410 (22,M-CH_3COOH), 395 (3,M-CH_3COOH+CH_2), 341 (29), 327 (12), 326 (12), 313 (10), 312 (14), 311 (18), 297 (4), 283 (20,M-side chain+2H+CH_3COOH), 270 (10), 257 (15), 243 (12,M-CH_3COOH+ring D fission+H), 137 (48), 136 (15) [strong peaks at m/e 137 and 136 are diagnostic for steroidal Δ^4-3,6-diketones [13]], 109 (20), 95 (28), 83 (34), 81 (35), 70 (100), 69 (55), 55 (80), 41 (62).

24ξ-Methylcholestane-3β,5α,6β,25-tetrol (Ig). (a) By hydrolysis of Ia. 24ξ-Methylcholestane-3β,5α,6β,25-tetrol 25-acetate (Ia, 20 mg) was placed in 1 ml of diethylene glycol with a small lump of potassium hydroxide in a 15 ml flask. The mixture was heated in a 200° silicone oil bath and stirred until homogeneous. Heating was continued for 30 mins. The brown solution was taken up in 20 ml of water and extracted twice with chloroform/methanol (60:40) and once with chloroform. The combined extracts were dried ($MgSO_4$), filtered, and evaporated to leave a slightly brown solid. An analytical tlc showed one spot with chloroform/ethanol (7:1) eluent at Rf 0.16 while starting material was at 0.24 (both brown with ceric sulfate spray); yield 17 mg. Recrystallization from methanol-water gave a white crystalline product m.p. 251.5-252°; nmr (CD_3OD) δ 0.72 (3,s), 0.90 (3,d,J=6Hz), 0.96 (3,d,J=6Hz), 1.13 and 1.17 (sharp overlapping singlets, relative intensities not apparent), 4.0 (1,m), other peak for proton α to OH obscured by solvent; mass spectrum at 70 eV, m/e (relative intensity) 432 (14,M-H_2O), 414 (18,M-2H_2O), 400 (7), 399 (5), 396 (5,M-3H_2O), 378 (1,M-4H_2O), 381 (5), 374 (13), 359 (7), 356 (11), 305 (14,M-H_2O+side chain+2H), 289 (32,M-2H_2O+side chain), 271 (28,M-3H_2O+side chain), 253 (7,M-4H_2O+side chain), 247 (11), 244 (11), 229 (13), 109 (18), 107 (18), 95 (30), 81 (34), 69 (28), 59 (100), 55 (38), 43 (24).

24ξ-Methylcholestane-3β,5α,6β,25-tetrol (Ig). (b) From III by oxidation. 24ξ-Methyl-5-cholestene-3β,25-diol (III, 6 mg) was subjected to the standard procedure [3] of treatment with formic acid, then oxidation with 30% hydrogen peroxide, followed by heating with 25% sodium hydroxide. The product (5 mg) was a mixture of two components by tlc analysis, with Rf's of 0.17 and 0.11 (brown and black with ceric sulfate spray) using chloroform/ethanol (7:1) as eluent. These two components were separated using a 0.5 g column of alumina II and eluting with chloroform/ethanol (20:1). The first compound to emerge from the column had identical tlc characteristics to Ig prepared from Ia by hydrolysis (see above). Overlay of the 100 Hz nmr spectra and the mass spectra for the two preparations of Ig showed the products to be identical. Unfortunately, insufficient material was available for recrystallization in order to compare melting points.

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