BILE ACIDS. LXIV. SYNTHESIS OF 5α-CHOLESTANE-3α,7α,25-TRIOL AND ESTERS OF NEW 5α-BILE ACIDS.[1]

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ABSTRACT

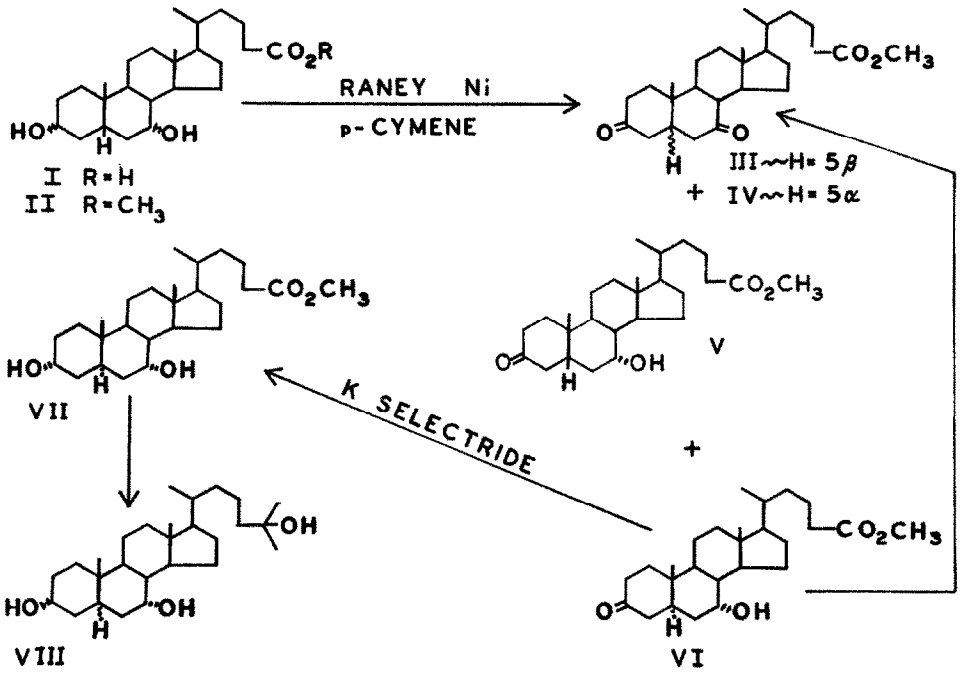
Interest in the structural requirements of a sterol or bile acid for maximal activity by an hepatic microsomal steroid 12α-hydroxylase prompted the preparation of 5α-cholestane-3α,7α,25-triol and 5α-analogs of 3α,7α-dihydroxy-5β-cholane-24-carboxylic acid. Methyl 3α,7α-dihydroxy-5β-cholane-24-carboxylate derived from methyl chenodeoxycholate via the Arndt-Eistert reaction was allomerized with Raney nickel in boiling p-cymene to provide a number of products of which methyl 3,7-dioxo-5β- and 5α-cholane-24-carboxylates, methyl 3-oxo-7α-hydroxy-5β- and 5α-cholane-24-carboxylates, were identified. Reduction with K-Selectride of methyl 3-oxo-7α-hydroxy-5β-cholane-24-carboxylate, provided a high yield of methyl 3α,7α-dihydroxy-5α-cholane-24-carboxylate. Treatment of this ester with an excess of methyl magnesium iodide afforded 5α-cholestane-3α,7α,25-triol. The products were characterized by thin-layer and gas liquid chromatography, proton resonance, infrared and mass spectrometry.

INTRODUCTION

The demonstration of the conversion of allochenodeoxycholic acid to allocholic acid by the rat (4), and by preparations of hepatic microsomal steroid 12α-hydroxylase from several species (5-7) has led to a study of other 5α-bile acids and 5α-sterols as potential substrates for assay of the enzyme. Recent work (8,9) has shown that the sterols, 5α-cholestane-3α,7α-diol and 7α-hydroxy-4-cholesten-3-one (a precursor in the hepatic synthesis of cholic acid), exhibit competitive inhibition toward each other for the microsomal 12α-hydroxylase, and supports the suggestion (6) that the same enzyme is involved in hydroxylation of these sterols at C-12. Since derivatives such as 5α-cholestane-3α,7α,26-triol and 3α,7α-dihydroxy-5α-cholestan-26-oic acid showed greater

activity toward the enzyme (6), it was desirable to prepare additional 5α-bile acids and sterols to assess the structural features necessary for a substrate of optimal activity. This paper delineates the synthesis of several such derivatives; enzymatic studies will be reported elsewhere.

3α,7α-Dihydroxy-5β-cholane-24-carboxylic acid (I) (10) was converted (11) to the methyl ester (II) (CHART I), which was allomerized by the action of Raney nickel (12-14) to a mixture of 3-oxo-5β- and 5α-derivatives. After the mixture was freed from Raney nickel and solvent, the components of the residue were separated by preparative HPLC on a Pre-Pak 500/Silica Cartridge with a mixture of benzene-ethyl acetate (8:2, v/v).



From early fractions of this chromatography methyl 3,7-dioxo-5β-cholane-24-carboxylate (III, RRT 3.45) and its 5α-analog (IV, RRT 4.44) were separated. The middle fraction from preparative HPLC contained primarily methyl 3-oxo-7α-hydroxy-5β-cholane-24-carboxylate (V) (8.5%) which was identified by comparison with a sample obtained from the ester (II) with the Fetizon reagent (15,16). The desired 3-oxo-7α-hydroxy-5α-derivative (VI) was obtained in 16.7% yield from later fractions of HPLC. Support for the 5α-configuration was found in ir bands at 1077, 1039, 1020, 1002, 963 and 892 cm^{-1} (comparable to those reported (17) for methyl 3-oxo-7α-hydroxy-5α-cholan-24-oate), and in the ratio of RRT of the 5α- and 5β-derivatives (18) (Table 1).

TABLE 1

Ratio of Relative Retention Times of Substituted Methyl 5β- and 5α-Cholan-24-oates (C_{24}) and Cholane-24-carboxylates (C_{25})

	C_{25}/C_{24}		5α/5β	
Substituent	5β	5α	C_{24}	C_{25}
3α,7α-$(HO)_2$	1.21	1.23	1.04	1.05
3=O,7α-(HO)	1.19	1.31	0.97	1.07
3,7-dione	1.13	1.12	1.30	1.29

A comparison of the relative abundances of the ion, m/z 300 (Fig. 1), (14.9 and 3.2%, respectively) derived from the TMSi ether of 3-oxo-7α-hydroxy-5β- and 5α-derivatives (V and VI, respectively) shows a favored cleavage of the A/B cis (5β) steroid. The relative abundances of the ions shown in Fig. 1 bear a striking similarity to those of the corresponding 3-oxo-7α-hydroxy-5β- and 5α-cholan-24-oates (14).

The use of a stereoselective reducing agent, potassium tri-sec-butyl borohydride (K-Selectride) (19,20) for reduction of the 3-oxo-

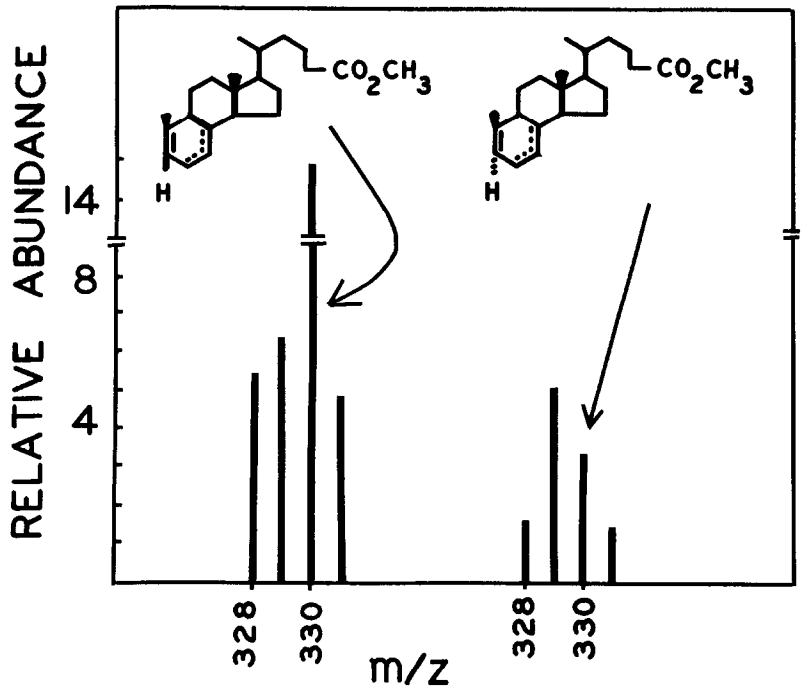


Fig. 1 Comparison of relative abundances of fragment ion m/z 330 derived from the TMSi derivatives of methyl 3-oxo-7α-hydroxy-5β- and 5α-cholane-24-carboxylates with an LKB 9000 mass spectrometer (3.5kV, 70eV).

group of the ester (VI) provided the axial 3α-hydroxy derivative (VII) in 86.4% yield. The ir spectrum showed bands at 3367 (OH), 1748 and 1169 (methyl ester) and 1081, 1061, 1034, 1005, 961 and 887 cm^{-1}, comparable to data reported for methyl allochenodeoxycholate (17). Additional support for the 5α-configuration was obtained from the mass spectrum of the free triol and of its TMSi derivative, and from the PMR spectrum: δ = 0.66 (s) (18-H), 0.77 (s) (19-H) of 5α-saturated steroids (21,22), 0.92 (d) (21-H), 3.79 (m) (7-H) (22), and 4.01 (m) (3-H) (22).

The ratios of RRT of the methyl esters of the C_{24} and C_{25} series for the 3α,7α-dihydroxy, 3-oxo-7α-hydroxy, and 3,7-dioxo-derivatives of the 5β- and 5α-series are compared in Table 1. Excellent agreement was found between the ratios of the C_{25}/C_{24} esters of the 5β- and 5α-series, and the 5α/5β ratios for the C_{24} and C_{25} cholanates for the diol and the dione. The generalization (18) that esters of the 5α-series are retained longer on selective phases (e.g., QF-1 and OV-17) is violated by the isomers, methyl 3-oxo-7α hydroxy-5β- and 5α-cholan-24-oate but not for those derivatives in the 24-carboxylate series. The data compared in Table 1, however, are derived from use of columns packed with 3% QF-1 (18) and with 3% OV-210. The stationary phase in the latter is said to be chemically identical with that of QF-1, but of better defined parameters. Our experience with the two phases shows that RRT are generally interchangeable between the columns until the numbers become large (i.e., long retention times), wherein significant differences may be seen. In general, the data of Table 1 support the 5α-configuration of the C_{25}-derivatives reported here.

Reduction of the 3-oxo-group of compound (VI) with $NaBH_4$ and analysis of the TMSi ether on OV-17, showed the presence of 17.1% of the 3α-ol (VII) (RRT 1.21) and 82.9% of the 3β-ol (RRT 1.35). Reduction of the 3-oxo-7α-hydroxy-5β-derivative (V) with K-Selectride and analysis of the products by TLC showed the presence of very small amounts of unreacted material (V), minor amounts of the 3α-ol (II) and the major product, the 3β-ol.

Treatment of the ester (VII) with an excess of methyl magnesium iodide provided the C_{27} sterol (VIII), 5α-cholestane-3α,7α,25-triol. Bands in the ir (1078, 1034, 1020, 1005, 957 and 904 cm^{-1}) (14), and

the ratios of RRT of the TMSi ethers of the 5α- and 5β-C_{27} sterols on OV-210 (0.914) and on OV-17 (0.911) support the 5α-configuration. The mass spectrum of the TMSi derivative was quite similar to that of the 5β-analog (10), particularly with regard to the base peak, m/z 131, characteristic of the TMSi ether of 25-hydroxy sterols (23,24). Although few major fragment ions appeared in the spectrum [comparable to that of the 5β-derivative (10)], the more abundant identifiable ions were m/z 456, [M-(2x90)]; 336, [M-(3x90)]; 251, [M-(3x90+15)]; 345, [M-(2x90+111)]; 343, [M-(2x90+111+2H)]; 255, [M-(3x90+111)]; 253, [M-(3x90+111+2H)]; and 243. The ion m/z 243 is a characteristic fragment of bis TMSi esters of 3α,7α-diols (24,25). The greater abundance of the ion m/z 343 for the 5α-derivative (20%) compared to the 5β-derivative [<5% (10)] is consistent with the behavior of 5α-sterols and 5α-bile acids (24). The fragmentation pattern of the free sterol at 20eV (Fig. 2) failed to provide a molecular ion (m/z 420), but did show the ions m/z 402, 384 and 366 which represent loss of 1, 2 and 3 molecules of water. Dehydration and loss of the side chain is indicated by the ions m/z 273, [M-(111+2x18)] and 255, [M-(111+3x18)] (similar to the ions m/z 345 and 255 derived from the tris TMSi ether). In a spectrum obtained at ionizing energy of 70eV the abundances of the ions m/z 271 and 253 corresponding to 2 and 3 nuclear double bonds, respectively, are greater than those of m/z 273 and 255, and indicate generation of a third nuclear double bond. Wyllie and Djerassi (26) have shown that sterols with unsaturated side chains fragment with migration of nuclear hydrogen (e.g., C-17 and C-16) (see also 27) to provide a very abundant ion representative of the steroid nucleus with an "extra double bond", illustrated formally as a Δ^{16} derivative (26). Accordingly, the frag-

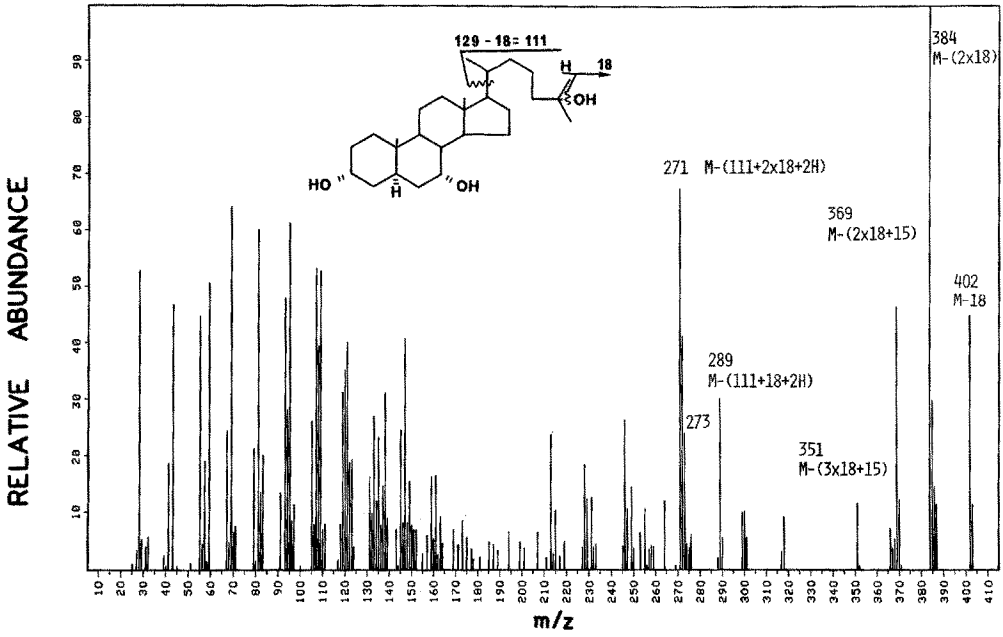


Fig. 2 Mass spectrum of 5α-cholestane-3α,7α,25-triol. LKB Model 9000: 3.5kV; 20eV; ion source, 160°; direct inlet probe.

ment ions m/z 271 and 253 of the triol (and m/z 343 and 253 from the TMSi ether) indicate the generation of a third nuclear double bond due to transfer of hydrogen from the nucleus to a newly generated double bond in the side chain, probably at positions 24-25. A similar argument applies (26) to the cluster of ions at m/z 299-301 and 317, 218, 319 in the spectrum of the triol.

EXPERIMENTAL

Materials and Methods: All solvents were analytical grade or were redistilled. Raney nickel powder was a product of W.R. Grace and Co. (So. Pittsburgh, Tenn.). Potassium tri-sec-butylborohydride (K-Selectride) was obtained from Aldrich Chemical Co. (Milwaukee, WI). Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Infra-

red spectra were recorded on a Model 21 Perkin-Elmer double beam spectrophotometer as nujol mulls. PMR spectra were recorded either on a Varian Model A-60 or an HA-100 spectrometer with $CDCl_3$ as solvent and tetramethylsilane as internal standard. Mass spectrometry was carried out with an LKB Model 9000 (11) at 70eV, except for the spectrum of Fig. 2 which was obtained at 20eV. Gas chromatography (18) was carried out with columns packed with 3% OV-210 as follows: column, 258°C, detector and flash heater at 275°C, or column 233°C and detector and flash heater at 250°C. Columns with 3% OV-17 were operated at 258°C with flash heater and detector at 268°C. TMSi derivatives were prepared as reported (18). Analytical tlc and plc were carried out on glass plates coated with 0.25 mm and 0.4 mm, respectively, of silica gel $HF_{254+266}$ (Brinkmann Instruments, Inc., Westbury, NY). Unless otherwise stated, R_f's were measured in the solvent system acetone-benzene (40:60) and are relative to methyl deoxycholate. Preparative HPLC (22) was carried out with a Waters Associates Prep LC/System 500. 3α,7α-Dihydroxy-5β-cholane-24-carboxylic acid (I), m.p. 215-217° (reported (10) m.p. 210-212°), was esterfied with a mixture of methanol, 2,2-dimethoxypropane and concentrated hydrochloric acid to provide the methyl ester (II), m.p. 88-90° (reported (10) m.p. 94-95°); by glc of the TMSi ether on OV-17 the purity of the product was 99.7%.

Allomerization of Methyl 3α,7α-dihydroxy-5β-cholane-24-carboxylate (II) with Raney Nickel: Methyl 3α,7α-dihydroxy-5β-cholane-24-carboxylate (II) (15 g) was refluxed for two hours with freshly prepared W-2 Raney nickel (14) and 225 ml of freshly distilled p-cymene as reported for methyl chenodeoxycholate (17). After filtration of the catalyst and removal of the solvents, the residue was taken up in ether and dried. Removal of the solvent provided 11.27 g of white solid which was separated into 33 fractions by preparative HPLC on a Pre-Pak 500/Silica cartridge with a mixture of benzene-ethyl acetate (8:2) (total volume, 5.4L).

Fractions 1-3 (total volume 600 ml) furnished 2.4 g of white solid which was mainly a mixture of two compounds with RRT 1.23 and 1.33 (OV-210, 258°C). Identification of these less polar materials and several minor components will be reported later.

Fractions 4-13 (total volume 750 ml) were evaporated separately. Residue from fractions 4 and 5 was combined (886 mg) and crystallized from acetone to provide 353 mg of white crystalline compound, mp 180-181°; R_f 1.76; RRT 3.45 (OV-210, 258°C). This product was identified as methyl 3,7-dioxo-5β-cholane-24-carboxylate (III) by comparison with an authentic sample obtained by oxidation of compound V. Fractions 6-13 (1.1 g) were pooled and shown to contain a mixture of the isomeric diones, (III) and (IV) by comparison of their RRTs, (3.45 and 4.44, respectively, on OV-210, 258) with those of authentic samples.

Fractions 14-18 (total volume 330 ml) furnished 1.28 g of white crystalline material, m.p. 129-130°C, (RRT 2.85, OV-210, 258°) which was identified as methyl 3-oxo-7α-hydroxy-5β-cholane-24-carboxylate (V) by mass spectrometry and by comparison with a sample derived from methyl 3α,7α-dihydroxy-5β-cholane-24-carboxylate (II) by the Fetizon procedure (15).

Fractions 19-33 (total volume 3.45L) were combined, and consisted mainly of methyl 3-oxo-7α-hydroxy-5α-cholane-24-carboxylate (VI) as indicated by tlc and glc. After evaporation of the solvent 4.6 g of white crystalline material remained which was further purified by preparative HPLC and crystallization from acetone to provide 2.5 g (16.7%) of pure product (VI); m.p. 189-91°C; R_f 1.60; RRT 3.04 (OV-210, 258°C); RRT (TMSi) 2.09 (OV-17); ir 3533 (OH), 1745 (CO_2CH_3), 1709 (C=O), 1278, 1231, 1197, 1162 (CO_2CH_3), 1077 (OH), 1039, 1020, 1005, 963, 952 and 892 cm^{-1}. The mass spectrum of the TMSi ether of the ester (VI) provided major fragment ions with relative abundances and structural assignments as follows: m/z 490, 4.4, M^+; 475, 23 [M-15]; 400, 100 [M-90]; 385, 36.9 [M-(90+15)]; 369, 30.9 [M-(90+31)]; 367, 16.1 [M-(90+33)]; 334, 14.2 [M-(129+27)]; 330, 14.9 (Fig. 1); 319, 37.6 [M-(129+42)]; 271, 89.4 [M-(129+90)]; 244, 37.5 [M-(129+90+27)].

Anal. Calcd. for $C_{26}H_{42}O_4$; C, 74.60; H, 10.03. Found: C, 74.60; H, 10.15.

Methyl 3α,7α-dihydroxy-5α-cholane-24-carboxylate (VII): To a solution of the ketone (VI) (418 mg; 1 mmol) in 10 ml of dry tetrahydrofuran at -78°C, 5 ml of 0.5 M K-Selectride (potassium tri-sec-butylborohydride) was added dropwise over a period of 5 min. After stirring the solution at -78°C for 5 hr, it was brought to room temperature, treated with 0.4 ml of 3N NaOH followed by 0.5 ml of 30% H_2O_2. The solution was diluted with water and the product was extracted with ether from which was obtained 408 mg of a low melting solid. Analysis of a portion of the product by glc of the TMSi derivative (OV-17) showed the presence of unreacted material (VI) (13.6%) and the desired product (VII) (86.4%), respectively (28). Purification of the remaining product by plc (acetone-benzene, 15:85) followed by crystallization from hexane-acetone provided an analytical sample of compound (VII) (195 mg); m.p. 122°C, R_f 0.87; RRT 1.50 (OV-210) and RRT (TMSi) 1.21 (OV-17); ir 3367, 1748, 1270, 1239, 1209, 1169, 1034, 1005, 961 and 887 cm^{-1}; PMR (δ) (vide ante). The mass spectrum of the bis TMSi derivative showed important fragment ions with relative abundances, and structural assignments as followed: m/z 564, 2.0, M^+; 549, 5.4 [M-15]; 474, 90.1 [M-90]; 459, 16, [M-(90+15)]; 384, 100, [M-(2x90)]; 369, 34.1 [M-(3x90+15)]; 345, 14 [M-(129+90)]; 225, 47.2, [M-(129+2x90)]; 243, 11.8; 228, 24.6; 213, 39.6 (24).

5α-Cholestane-3α,7α,25-triol (VIII): A solution of 209 mg of methyl 3α,7α-dihydroxy-5α-cholane-24-carboxylate (VII) in 10 ml of dry ether was added slowly to a solution of methyl magnesium iodide (prepared from 150 mg of Mg and 0.4 ml of redistilled methyl iodide) in ether at 0°C. After refluxing the reaction mixture for 24 hr, the Grignard complex was decomposed by addition of a saturated solution of ammonium chloride, and the product was extracted with ether. The crude reaction product obtained after evaporation of the ether was purified by plc (acetone-benzene, 40:60), and crystallization from methanol. A white crystalline product (120 mg) was obtained which exhibited these properties: m.p. 197-8°C, R_f 0.59; RRT (TMSi) 0.81 on OV-210 (233°C) and 1.15 on OV-17; ir 3333, 1230, 1158, 1095, 1078, 1063, 1034, 1020, 1005, 957 and 904 cm^{-1}. The mass spectrum of the triol (VIII) at 20eV showed (Fig. 2) the characteristic fragment ions m/z 402, 45.1 [M-18]; 384, 100 [M-(2x18)];

369, 46.4 [M-(2x18+15)]; 366, 7.2, [M-(3x18)]; 351, 11.6, [M-(3x18+115)]; 289, 30.1, [M-(129+2H)]; 273, 23.9, [M-(111+2x18)]; 271, 67.6, [M-(111+2x18+2H)]; 264, 12.1, [M-(111+18+27)]; 255, 10.6, [M-(111+3x18)]; 253, 6.6, [M-(111+3x18+2H)]; 249, 14.3, [M-(111+18+42)]; 246, 26.6, [M-(111+2x18+27)]; 231, 12.6, [M-(111+2x18+42)]. At 70 eV, base peak was the ion m/z 55 and the abundances of other relevant ions were: m/z 289, 63.7; 273, 69.3; 271, 88.7; 255, 55.9; 253, 63.7.

Anal. Calcd. for $C_{27}H_{48}O_3$; C, 77.09; H, 11.50. Found: C, 76.78; H, 11.39.

Methyl 3,7-dioxo-5α-cholane-24-carboxylate (IV): To a solution of 20 mg of compound (VI) in 10 ml of acetone, Jones reagent (29) was added dropwise until a brown color persisted. After 5 min the solution was diluted with water, extracted with ether, and the ether layer was washed successively with water, aqueous bicarbonate and water, and was dried. Evaporation of the solvent furnished 16 mg of a white crystalline product; m.p. 188-9°, R_f 1.69; RRT 4.44 (OV-210).

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1. The following abbreviations have been used: tlc, thin layer chromatography; plc, preparative layer chromatography; HPLC, high performance liquid chromatography; glc, gas liquid chromatography; TMSi, trimethylsilyl derivatives; RRT, relative retention time (relative to methyl deoxycholate); RRT (TMSi), relative retention time (relative to bis trimethylsilyl ether of methyl deoxycholate). The IUPAC names for the substances discussed are: allochenodeoxycholic acid, 3α,7α-dihydroxy-5α-cholan-24-oic acid; allocholic acid, 3α,7α,12α-trihydroxy-5α-cholan-24-oic acid; all allo compounds are 5α-derivatives.
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