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A Method of Synthesis of Allocholanoic Acids

Bile Acids and Steroids 182*

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Methyl 12α-hydroxy-3-keto-5α-cholanoate, methyl 7α-hydroxy-3-keto-5α-cholanoate, and methyl 7α,12α-dihydroxy-3-keto-5α-cholanoate were prepared by reduction of the corresponding Δ^4-3-keto bile acids by lithium in liquid ammonia. Further reduction yielded methyl 3α,12α-dihydroxy-5α-cholanoate, 3α,7α-dihydroxy-5α-cholanoate, and 3α,7α,12α-trihydroxy-5α-cholanoate and the corresponding 3β-epimers.

Since Anderson and Haslewood in 1960 found that tetrahydroxynorstero-cholanic acid was identical with allocholic acid [1] and described the synthesis thereof [2,3] several authors have reported on the synthesis, occurrence and metabolism of bile acids containing the allocholane, *i.e.* the A/B-*trans*, carbon skeleton. Several synthetic routes have been designed in order to prepare these acids. Thus, Anderson and Haslewood obtained a mixture of cholanoic (5β) and allocholanoic (5α) acids on catalytic reduction of methyl 3α,12α-diacetoxy-7-ketochol-5-enoate.[2] Later, stereochemically pure allocholic acid [3] and allodeoxycholic acid [4] were obtained utilizing the observation by Takeda *et al.*[5] that methyl 3α,6α-dihydroxy-7-keto-5β-cholanoate rearranges to 3α,7β-dihydroxy-6-keto-5α-cholanoic acid on alkaline hydrolysis. Allodeoxycholic acid was prepared by Danielsson *et al.*[6] by converting methyl 12α-hydroxy-3-keto-5β-cholanoate to the epimeric 5α-compound by refluxing in isopropyl benzene over Raney Ni. From the mixture of ketones obtained, methyl 12α-hydroxy-3-keto-5α-cholanoate was isolated by chromatography on aluminum oxide.

Stereospecific formation of 5α-steroids is, with few exceptions, obtained by the reduction of 3-keto-4-ene-derivatives of neutral steroids by Li in liquid ammonia.[7] When performed in an inert reaction medium like ethyl ether or dioxane, the reaction stops at the stage of the saturated ketone. In the presence of compounds such as ethanol which have an acid strength comparable to

* The following abbreviations are used: GLC, gas-liquid chromatography; TLC, thin-layer chromatography; MS, mass spectrum.

that of the intermediary enol, the reduction of the conjugated system proceeds to the thermodynamically favoured 3-hydroxy steroid, *i.e.* the equatorial 3β-alcohol in the 5α-series.[8]

When reduced with Li in dry ammonia, methyl 12α-hydroxy-3-ketochol-4-enoate was transformed into a mixture of less polar compounds, the nature of which were not established. Aliphatic esters, however, are known to be attacked by alkali metals in liquid ammonia, forming, among other compounds, primary alcohols and amides.[8] Reduction of 12α-hydroxy-3-ketochol-4-enoic acid with Li in ammonia yielded two main compounds, which, after methylation, proved identical with methyl 12α-hydroxy-3-keto-5α-cholanoate and methyl 3β,12α-dihydroxy-5α-cholanoate.[6] Reduction of 7α,12α-dihydroxy-3-ketochol-4-enoic and 7α-hydroxy-3-ketochol-4-enoic acids also resulted in the formation of two compounds which were found to be the corresponding 3-keto-5α-cholanoic and 3β-hydroxy-5α-cholanoic acids, respectively. No appreciable amounts of the saturated 5β-ketone could be observed by GLC after reduction of the above-mentioned α,β-unsaturated 3-keto bile acids.

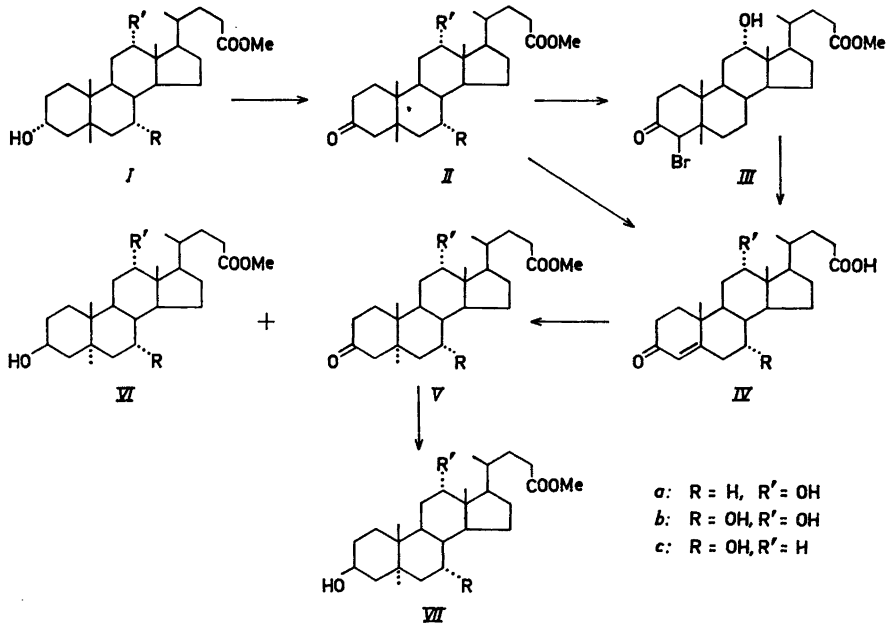

Fig. 1.

In analogy with the reduction of α,β-unsaturated steroidal ketones a stereospecific reduction of Δ^4-3-keto bile acids was thus achieved. Reduction of these acids also yielded the equatorial alcohols to some extent, which might be attributed to the presence of an ammonium salt of the carboxyl group supplying the necessary protons for further reduction of the enolic intermediate.

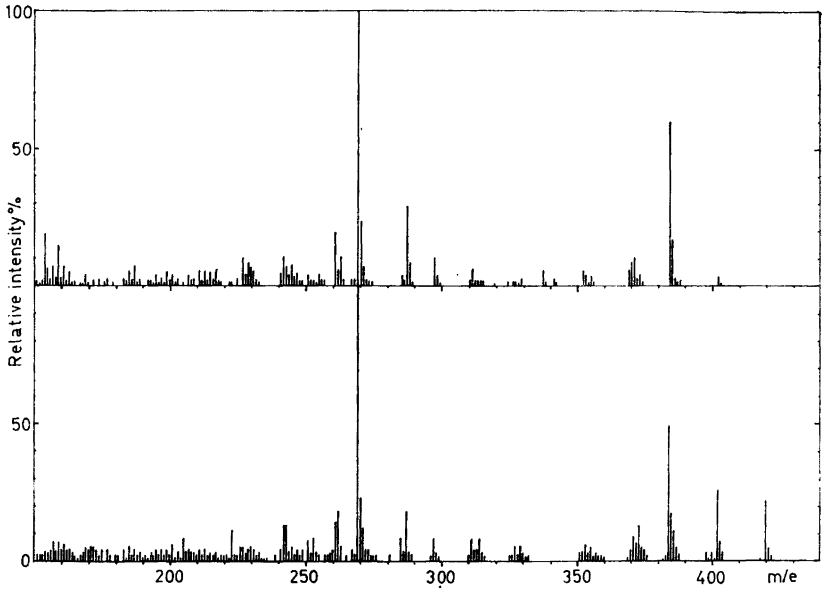

Fig. 2. Mass spectra of methyl 7α,12α-dihydroxy-3-keto-5α-cholanoate (top) and methyl 7α,12α-dihydroxy-3-keto-5β-cholanoate (bottom). Conditions: Energy of bombarding electrons: 70 eV, sample temperature 20°.

As the 7α-hydroxyl group of methyl 7α-hydroxy-3-ketochol-4-enoate and methyl 7α,12α-dihydroxy-3-ketochol-4-enoate is acid and alkali labile, the corresponding free acids could not be obtained by hydrolysis of the esters. These Δ^4-3-keto acids were therefore prepared by oxidation of the corresponding 3-keto acids with SeO_2 in ethanol.[9]

The A/B *trans* configuration of the saturated 3-keto bile acids prepared by reduction with Li in ammonia could be confirmed by mass spectrometry as the cleavage of ring A is more favoured in the 5β-series than in the 5α-series.[10] Thus, the relative sizes of the peaks corresponding to *m/e* 316 in the mass spectrum of methyl monohydroxy-monoketo- and to *m/e* 314 in the mass spectrum of methyl dihydroxy-monoketo-cholanoates are larger in the ketones belonging to the normal series than in those belonging to the allo series (Figs. 2 and 3). It is also noteworthy that the peaks corresponding to $M-H_2O$ and $M-2H_2O$, respectively, are larger in the mass spectra of methyl 7α-hydroxy-3-keto-5α-cholanoate and methyl 7α,12α-dihydroxy-3-keto-5α-cholanoate than in the spectra of their 5β-epimers. This difference was not observed in the spectra of methyl 12α-hydroxy-3-keto-5α-cholanoate and its 5β-epimer and might therefore be attributed to a facilitated loss of the 7α-hydroxyl group in the 5α-series.

According to the octant rule, 3-ketones of the 5α-steroid series are expected to show a positive Cotton effect in their RD curves whereas the corresponding 5β-epimers would show a negative Cotton effect.[11] This difference was previ-

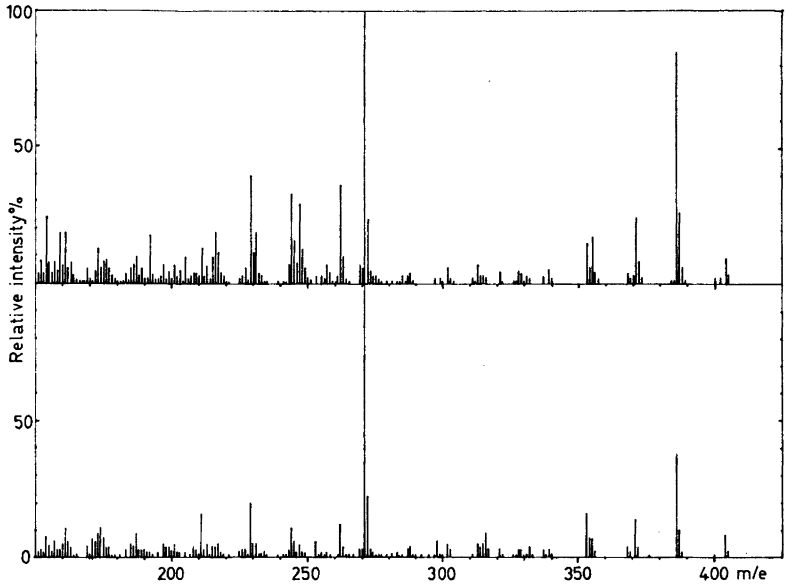

Fig. 3. Mass spectra of methyl 7α-hydroxy-3-keto-5α-cholanoate (top) and methyl 7α-hydroxy-3-keto-5β-cholanoate (bottom). Conditions as in Fig. 2.

ously shown to exist between the RD curve of 12α-hydroxy-3-keto-5α-cholanoate and that of its 5β-epimer.[6] The RD curves of methyl 7α-hydroxy-3-keto-5α-cholanoate and methyl 7α,12-dihydroxy-3-keto-5α-cholanoate both showed a positive Cotton effect with a ketone amplitude [12] of + 48 and + 46, respectively, and rotational maxima at 308 mμ. The ketone amplitude of methyl 7α-hydroxy-3-keto-5β-cholanoate and methyl 7α,12α-dihydroxy-3-keto-5β-cholanoate was −3 and −4, respectively.

Generally, reductions of steroidal ketones with metal hydrides give predominantly the equatorial alcohol and so do catalytic reductions.[7] The ratio of axial to equatorial alcohol formed by catalytic reduction can be raised by addition of an acid. Catalytic reduction of methyl 12α-hydroxy-3-keto-5α-cholanoate in the presence of HCl gave methyl 3α,12α-dihydroxy-5α-cholanoate and methyl 3β,12α-dihydroxy-5α-cholanoate in a ratio of about 1:2.[6] Haddad *et al.*[13] showed that the reduction of substituted cyclohexanones and of 5α-cholestan-3-one with trimethylphosphite and iridium(IV) chloride in an aqueous solution of isopropanol mainly led to the axial alcohols. Applying this reaction to 3-keto bile acids of the 5β-series, high and stereospecific yields of the axial alcohol, *i.e.* the 3β-hydroxy bile acids, were obtained. On reduction according to this method methyl 12α-hydroxy-3-keto-5α-cholanoate yielded methyl 3α,12α-dihydroxy-5α-cholanoate [6] as the main epimer. Similarly, the 3α-hydroxy epimers were obtained on reduction of methyl 7α-hydroxy-3-keto-5α-cholanoate and 7α,12α-dihydroxy-3-keto-5α-cholanoate.

The synthesis of allocholanoic acids is summarized in the scheme of Fig. 1.

EXPERIMENTAL

All melting points are uncorrected.

TLC of dihydroxycholanoic acid derivatives was performed in phase system S 12.[14] Monohydroxy-monoketones were run in a mixture of benzene, dioxane, and acetic acid, 80:20:4.5. Trihydroxycholanoic acid and dihydroxy-monoketocholanoic acid derivatives were separated in phase system S 6.[14] GLC was carried out on a 6 foot × 5 mm column packed with 3 % QF-1 on Gas-Chrom P. Column temperature was 245° and argon pressure 1.8 kg/cm^2.[15,16]

Methyl 12α-hydroxy-3-keto-5β-cholanoate (IIa), methyl 7α,12α-dihydroxy-5β-cholanoate (IIb), and methyl 7α-hydroxy-3-keto-5β-cholanoate (IIc) were prepared as described by Jones *et al.*[17] and Danielsson *et al.*[18]

12α-Hydroxy-3-ketochol-4-enoic acid (IVa) was prepared by alkaline hydrolysis of methyl 12α-hydroxy-3-ketochol-4-enoate obtained by the method described by Riegel and McIntosh.[19]

Methyl 12α-hydroxy-3-keto-5α-cholanoate (Va). 12α-Hydroxy-3-ketochol-4-enoic acid, 850 mg, was stirred in 75 ml of liquid ammonia in the apparatus shown in Fig. 4. A solution of 86 mg of Li in 50 ml of liquid ammonia was added dropwise until the blue color of the reaction mixture remained constant for 2 min. Then 1.5 g of ammonium chloride was added and the ammonia evaporated. The bile salts obtained were dissolved in water, precipitated by dilute hydrochloric acid, and extracted with ether. The ether extract was washed with water until neutral, the ether evaporated, and the residue was methylated by refluxing for 2 h in 2 % H_2SO_4 in methanol. The oily residue obtained after working up the reaction mixture in the usual manner was purified by chromatography on a column of 50 g of aluminium oxide, activity grade III. The column was eluted with increasing amounts of ethyl acetate in benzene. Ethyl acetate, 5—7 %, in benzene, eluted 440 mg of methyl 12α-hydroxy-3-keto-5α-cholanoate. After crystallization from ether-petroleum ether 260 mg was obtained. M.p. 134—136° (reported 134—146°).[6] The m.p. was not depressed on admixture of authentic methyl 12α-hydroxy-3-keto-5α-cholanoate.

Ethyl acetate, 15 % in benzene, eluted 170 mg of a compound with the same gas-chromatographic behaviour as methyl 3β,12α-dihydroxy-5α-cholanoate.

Methyl 3α,12α-dihydroxy-5α-cholanoate (VIIa). Methyl 12α-hydroxy-3-keto-5α-cholanoate, 380 mg, was refluxed for 72 h in a mixture of 210 ml of isopropanol, 21 ml of water, and 21 ml of freshly distilled trimethyl phosphite, to which 150 mg of iridium(IV) chloride was added. The reaction mixture was extracted with ether and hydrolyzed with 2 % potassium hydroxide in methanol. After esterification with 2 % H_2SO_4 in methanol, the product was purified on a column of 50 g of aluminium oxide, activity grade III. Ethyl acetate, 17—20 % in benzene, eluted 262 mg of methyl 3α,12α-dihydroxy-5α-cholanoate, which after crystallization from methanol-water yielded 215 mg with m.p. 174—175° (reported 174—176°).[6] The m.p. was not depressed on admixture of authentic methyl 3α,12α-dihydroxy-5α-cholanoate.

7α,12α-Dihydroxy-3-ketochol-4-enoic acid (IVb). Methyl 7α,12α-dihydroxy-3-keto-5β-cholanoate, 38 g, was hydrolyzed with 2 M methanolic potassium hydroxide at room

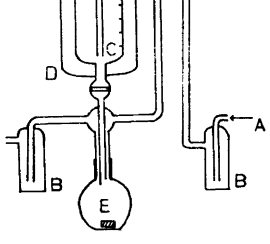

Fig. 4. A, NH_3(g) inlet; B, wash bottle charged with NaOH; C, calibrated cylinder; D, dry ice condenser; E, reaction vessel with magnetic stirrer.

temperature. The crude acid was refluxed in 1500 ml of 96 % ethanol with 25 g of SeO_2 for 4 h. The reaction mixture was filtered and the solvent evaporated. The residue was dissolved in 1000 ml of chloroform and treated with active carbon. After filtration the solvent was evaporated under reduced pressure and the residue was purified by chromatography on a column of 750 g of silicic acid (Mallinkrodt, Analytical Reagent, New York, U.S.A.). The column was eluted with increasing amounts of acetone in benzene. Acetone, 25 % in benzene, eluted a material which, after crystallization from methanol-water, afforded 11 g of 7α,12α-dihydroxy-3-ketochol-4-enoic acid. M.p. 231—233°; $[\alpha]_D^{22}$ + 61° (c = 0.86 in methanol). λ_{max} 244 mμ; ε = 15 300. (Found: C 70.8; H 8.9. Calc. for $C_{24}H_{36}O_5$: C 71.3; H 9.0).

Methyl 7α,12α-dihydroxy-3-keto-5α-cholanoate (Vb), and methyl 3β,7α,12α-trihydroxy-5α-cholanoate (VIb). 7α,12α-Dihydroxy-3-ketochol-4-enoic acid, 50 mg, was stirred in 20 ml of liquid ammonia as above. Li, 9 mg, dissolved in 25 ml of liquid ammonia, was added dropwise until persistent blue color. Ammonium chloride, 100 mg, was then added and the ammonia was evaporated. The bile acids were isolated as described above and purified, after methylation, by chromatography on a column of 5 g of aluminium oxide, activity grade IV. Ethyl acetate, 30 % in benzene, eluted a material, which after crystallization from acetone-water afforded 15 mg of methyl 7α,12α-dihydroxy-3-keto-5α-cholanoate. M.p. 152—154°; $[\alpha]_D^{22}$ + 45° (c = 0.88 in methanol). (Found: C 71.4; H 9.6. Calc. for $C_{22}H_{40}O_5$: C 71.4; H 9.6). RD in methanol (c = 0.1) $[\varphi]_{435}$ + 435°, $[\varphi]_{308}$ + 2530°, $[\varphi]_{267}$ −2070°, $[\varphi]_{239}$ −1225°, $[\varphi]_{218}$ −2430°.

The MS of this compound is shown in Fig. 2.

Ethyl acetate, 75 % in benzene, eluted a material which after crystallization from acetone-water yielded 9 mg of methyl 3β,7α,12α-trihydroxy-5α-cholanoate. The same compound was obtained in a yield of about 50 % by reduction of 7α,12α-dihydroxy-3-ketochol-4-enoic acid (50 mg) in the presence of ethanol (0.1 ml). M.p. 186—187°. $[\alpha]_D^{22}$ + 58° (c = 0.65 in methanol). (Found: C 69.5; H 9.8. Calc. for $C_{25}H_{43}O_5 \cdot C_3H_6O$ (acetone): C 69.9; H 10.1).

Methyl 3α,7α,12α-trihydroxy-5α-cholanoate (VIIb). Methyl 7α,12α-dihydroxy-3-keto-5α-cholanoate, 100 mg, was refluxed for 72 h in a mixture of 50 ml of isopropanol, 5 ml of water, and 5 ml of trimethylphosphite to which 15 mg of iridium(IV) chloride was added. After hydrolysis and re-esterification, the product was purified by chromatography on a column of 5 g of aluminum oxide, grade IV. The column was eluted with increasing amounts of ethyl acetate in benzene, ethyl acetate, and increasing amounts of methanol in ethyl acetate. Ethyl acetate, 30 % in benzene, eluted 15 mg of starting material, and methanol, 5 % in ethyl acetate, eluted 31 mg of material with the same TLC and GLC properties as methyl 3α,7α,12α-trihydroxy-5α-cholanoate.* This material crystallized slowly from methanol on evaporation of the solvent. M.p. 225—226° (reported 225°).[3] $[\alpha]_D^{22}$ + 28° (c = 1.02 in methanol).

7α-Hydroxy-3-ketochol-4-enoic acid (IVc). Methyl 7α-hydroxy-3-keto-5β-cholanoate, 500 mg, was hydrolyzed in 2 M KOH. The crude acid was refluxed in 400 ml of 96 % ethanol with 300 mg of SeO_2 for 48 h. The reaction mixture was acidified by addition of 2 M HCl and the mixture was extracted with ether. The solvent was evaporated, the residue was dissolved in 100 ml of chloroform and treated with active carbon. After filtration the solvent was evaporated and the residue was purified by reversed phase chromatography with phase system F 1.[20] Those fractions which exhibited an absorption at 244 mμ and appeared homogeneous on TLC were combined. After evaporation of the solvent 105 mg of crystalline material was obtained by crystallization from methanol. M.p. 231—233°; $[\alpha]_D^{22}$ + 61° (c = 0.86 in methanol) λ_{max} = 244 mμ, ε = 14 600. (Found: C 70.9; H 9.0. Calc. for $C_{24}H_{36}O_4 \cdot CH_3OH$: C 71.4; H 9.6).

A small sample was methylated with diazomethane and analyzed by MS. The base peak occurred at m/e = 384, equal to M−18. A large peak (36 % of the base peak) occurred at m/e = 269. This ion corresponds presumably to the steroid nucleus with one keto group and two double bond, one of which has been formed by the loss of water.

Methyl 7α-hydroxy-3-keto-5α-cholanoate (Vc) and methyl 3β,7α-dihydroxy-5α-cholanoate (VIc). 7α-Hydroxy-3-ketochol-4-enoic acid, 50 mg, was reduced by Li in liquid ammonia as above. GLC of the methylated reaction mixture indicated a yield of about 50 % of the saturated ketone. In addition, material with GLC properties typical of a dihydroxy-

* Generously supplied by prof. G. A. D. Haslewood, Guy's Hospital Medical School, London.

cholanoic acid methyl ester was obtained in a yield of about 15 %. This material was the main product obtained when reducing the unsaturated acid in the presence of ethanol and was assumed to be methyl 3β,7α-dihydroxy-5α-cholanoate. Material obtained by reduction of 7α-hydroxy-3-ketochol-4-enoic acid in the absence of ethanol was methylated with 2 % H_2SO_4 in methanol and purified by chromatography on a column of aluminium oxide, grade III. Ethyl acetate, 8 % in benzene, eluted methyl 7α-hydroxy-3-keto-5α-cholanoate which was crystallized from acetone-water. M.p. 137–138°; $[\alpha]_D^{22}$ + 16° (c = 1.1 in methanol). (Found: C 73.9; H 9.9. Calc. for $C_{25}H_{40}O_4$: C 74.2; H 10.0). RD in methanol (c = 0.1) $[\varphi]_{400}$ + 195°, $[\varphi]_{308}$ + 2270°, $[\varphi]_{265}$ −2550°, $[\varphi]_{245}$ −1800°, $[\varphi]_{217}$ −2670°.

The MS of this substance is shown in Fig. 3.

Ethyl acetate, 15–20 % in benzene, eluted methyl 3β,7α-dihydroxy-5α-cholanoate which was crystallized from acetone-water. M.p. 159–160°, $[\alpha]_D^{22}$ + 14° (c = 1.1 in methanol). (Found: C 73.5; H 10.4. Calc. for $C_{25}H_{42}O_4$; C 73.9; H 10.3).

Methyl 3α,7α-dihydroxy-5α-cholanoate (VIIc). Methyl 7α-hydroxy-3-keto-5α-cholanoate, 100 mg, was refluxed for 72 h in a mixture of 50 ml of isopropanol, 5 ml of water, and 5 ml of trimethylphosphite to which 15 mg of iridium(IV) chloride was added. After hydrolysis and re-esterification as above, the product was purified by chromatography on a column of 5 g of aluminium oxide, grade III. Ethyl acetate, 8 % in benzene. eluted 43 mg of starting material and ethyl acetate, 20–25 % in benzene, eluted 29 mg of methyl 3α,7α-dihydroxy-5α-cholanoate. Crystallization from acetone-water yielded 21 mg. M.p. 116–118°;* $[\alpha]_D^{22}$ + 7° (c = 0.9 in methanol). (Found: C 73.4; H 10.3. Calc. for $C_{25}H_{42}O_4$: C 73.9; H 10.3).

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* *Note added in proof.* In a private communication, prof. W. Elliott (St. Louis Univ. Miss.) has reported a m.p. of 125–126° of this compound prepared in a different way. However, GLC of trimethylsilyl ethers of the methyl esters showed that the preparations were of comparable purity.