

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

 Δ^3 -Cholenic AcidBY KAZUMI YAMASAKI,¹ VITTORIO ROSNATI,² MARY FIESER AND LOUIS F. FIESER

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It is shown that the main product of pyrolysis of lithocholic acid is Δ^3 -cholenic acid, identical with material prepared previously from 3α - and 3β -hydroxy-4 β -bromocholanic acid. On oxidation with osmium tetroxide the acid is converted chiefly into 3β ,4 β -dihydroxycholanolic acid, which on chromic acid oxidation gives lithobillianic acid. It is found that, because of ester interchange during sodium borohydride reduction, certain esters previously reported as methyl esters are in fact ethyl esters.

In a previous paper³ Δ^3 -cholenic acid was prepared by reduction of either methyl 3α - or 3β -hydroxy-4 β -bromocholanoate with zinc and ethanol or zinc and acetic acid, followed by hydrolysis of the ester group. It was noted that the physical constants of the acid were similar to those of a cholenic acid obtained by Wieland and co-workers⁴ as the major product of the pyrolysis of lithocholic acid. Wieland assigned the Δ^2 -structure to his acid mainly because on selenium dioxide oxidation it gave two isomeric hydroxycholenic acids, assumed to be the 1- and 4-hydroxy- Δ^2 -cholenic acids formed by oxidation of different methylene groups adjacent to the double bond. This evidence seems inconclusive, since the hydroxycholenic acids could also be stereoisomers and since an allylic rearrangement could be involved.

Since Wieland's acid was not available for comparison we prepared a sample according to his procedure and found it identical with the Δ^3 -cholenic acid prepared by reduction of the bromohydrins. Confirmation of the identity and also further evidence that the acid is in fact Δ^3 -cholenic is furnished by the following transformations. Both acids react with osmium tetroxide to form two isomeric *cis*-diols. The major product on oxidation with chromic acid gives lithobillianic acid, rather than the iso acid, and consequently is a 3,4-dihydroxycholanolic acid. This diol forms a sparingly soluble digitonide, whereas the isomer does not. Consequently the major product (85% yield) probably is 3β ,4 β -dihydroxycholanolic acid and the minor product (10% yield) is 3α ,4 α -dihydroxycholanolic acid. This result is unexpected in that it involves front attack, rather than the usual attack from the rear.⁵

The preferential formation of a 3,4-double bond on pyrolysis of lithocholic acid,⁶ contrasts with the behavior of cholestanyl benzoate on pyrolysis. In this case both Δ^2 - and Δ^3 -cholestene are formed, but the Δ^2 -ene predominates.⁷ The difference is probably related to the difference in enolization of 3-ketones of the 5α - and 5β -configuration.

In view of the evidence for preferential formation of a 3,4-double bond on pyrolysis of a 3α -hydroxycholanolic acid, the commonly accepted struc-

tures for products of pyrolysis of other bile acids containing a 3α -hydroxyl should be revised. Thus α -cholatrienic acid, the major product of pyrolysis of cholic acid, is probably $\Delta^{3,6,11}$ -cholatrienic acid, and β -cholatrienic acid is $\Delta^{2,6,11}$ -cholatrienic acid.⁸ Apocholatrienic acid, the product of pyrolysis of apocholic acid, is probably $\Delta^{3,8(14),11}$ -cholatrienic acid.⁸ Choladienic acid-A and -B, obtained by distillation of desoxycholic acid, are probably $\Delta^{3,11}$ -choladienic acid and $\Delta^{2,11}$ -choladienic acid, respectively.⁹ Chenocholadienic acid, obtained from chenodesoxycholic acid⁸ and from ursodesoxycholic acid¹⁰ is probably $\Delta^{3,6}$ -choladienic acid.

In the course of this work it was noted that although Wieland's cholenic acid is identical with Δ^3 -cholenic acid prepared from the bromohydrins, the methyl ester of the Wieland acid (m.p. 74.5–75.5°) differs from the ester of Δ^3 -cholenic acid prepared by Fieser and Ettorre (m.p. 83–84°); mixed melting points of the two showed depressions. Moreover the methyl cholanoate prepared by hydrogenation of the latter ester was found to differ from authentic methyl cholanoate. The discrepancy was eventually cleared up when it was found that the Fieser and Ettorre esters are actually ethyl esters and that ester interchange had taken place in the reduction of methyl 4 β -bromodehydrolithocholate with sodium borohydride in absolute ethanol. When the reduction was carried out in methanol the true methyl esters of the two bromohydrins were obtained.

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Experimental

Methyl Δ^3 -Cholenate (Wieland Procedure).⁴—Lithocholic acid (3 g., m.p. 180–187°) was heated in a sublimation apparatus in metal-bath under a vacuum of 15 mm. until melted (190°) and then at 320–330° for 1.5–2 hr. The sublimate, m.p. 136–154°, 2.7–2.8 g., was purified by chromatography. Eluates melting in the range 147–154.5° were combined (1.5 g.) and after several crystallizations from methanol Δ^3 -cholenic was obtained, m.p. 154–156°, $\alpha_D +18^\circ$ Chf (lit.⁴ m.p. 155–156°, $\alpha_D +17^\circ$ Al); m.m.p. with Δ^3 -cholenic acid of Fieser and Ettorre³ 154–156°.

Purification through the methyl ester is also convenient. Esterification of the sublimate (5 g.) by the Fischer-Speier method gave 3.45 g. of methyl ester, m.p. 70–71°. Pure methyl Δ^3 -cholenate was obtained after several recrystallizations from methanol, m.p. 73–74°, $\alpha_D +19^\circ$ Chf., m.p. depressed on admixture with the "methyl" cholenate of Fieser and Ettorre.³

(8) Contrast D. H. R. Barton, *ibid.*, 2174 (1949).

(9) Contrast Elsevier's "Encyclopaedia of Organic Chemistry," Vol. 14, Series III, 1940, p. 169.

(10) T. Iwasaka, *Z. physiol. Chem.*, **244**, 181 (1936).

(1) On leave from Tottori University School of Medicine, Yonago, Japan.

(2) Of the Istituto Superiore di Sanita, Rome; research fellow under sponsorship of the Institute of International Education.

(3) L. F. Fieser and R. Ettorre, *THIS JOURNAL*, **75**, 1700 (1953).

(4) H. Wieland, K. Kraus, H. Keller and H. Ottawa, *Z. physiol. Chem.*, **241**, 47 (1936).

(5) L. F. Fieser, *Experientia*, **6**, 312 (1950).

(6) Δ^2 -Cholenic acid and Δ^3 -cholenic acid are formed in the approximate ratio of 9:1.⁴

(7) D. H. R. Barton and W. J. Roseufelder, *J. Chem. Soc.*, 1048 (1951).

Anal. Calcd. for $C_{25}H_{40}O_2$ (372.57): C, 80.57; H, 10.82. Found: C, 80.59, 80.42; H, 10.90, 10.92.

The free acid obtained on hydrolysis melted at 155–156°. It was converted into the less soluble dibromide of Wieland,⁴ m.p. 237–238° dec., $\alpha_D -2^\circ$ Chf. Both the acid and the dibromide corresponded with the acid and dibromide of Fieser and Ettore³ (m.p. and m.m.p.). **Methyl 3,4-dibromocholanate** was prepared by esterification with diazomethane, m.p. 145–146°, $\alpha_D -2^\circ$ Chf.

Anal. Calcd. for $C_{25}H_{40}O_2Br_2$ (532.40): C, 56.40; H, 7.57; Br, 30.02. Found: C, 56.14; H, 7.69; Br, 29.67.

Ethyl Δ^3 -cholenate was prepared by treatment of the acid with absolute ethanol containing a trace of concd. sulfuric acid and crystallized from ethanol; m.p. 80–81°, $\alpha_D +19^\circ$ Chf., not depressed on admixture with "methyl" Δ^3 -cholenate (Fieser and Ettore,³ m.p. 83–84°, $\alpha_D +30.5^\circ$ Di, $+19.2^\circ$ Chf.).

Anal. Calcd. for $C_{27}H_{44}O_2$ (382.36): C, 80.75; H, 11.22. Found: C, 81.02; H, 11.17.

The ethyl ester (2 g.) on treatment with bromine (8 g.) in acetic acid was converted into two dibromo esters, separable by chromatography. One (0.76 g.) after crystallization from methanol melted at 120.5–121.5°, $\alpha_D -4^\circ$ Chf.

Anal. Calcd. for $C_{26}H_{42}O_2Br_2$ (546.40): C, 57.10; H, 7.75; Br, 29.25. Found: C, 57.14; H, 7.66; Br, 30.10.

When refluxed in methanol containing concd. hydrochloric acid this ester was converted into the methyl 3,4-dibromocholanate, m.p. 145–146°, described above.

The other product of bromination was purified by rechromatography and crystallization from methanol; m.p. 102.5–103.5°, $\alpha_D +22^\circ$ Chf.

Anal. Calcd. for $C_{26}H_{40}O_2Br_2$ (546.40): C, 57.10; H, 7.75; Br, 29.25. Found: C, 57.34; H, 7.76; Br, 28.75.

Catalytic hydrogenation of methyl Δ^3 -cholenate in acetic acid (Adams catalyst) gave methyl cholanate, m.p. 87–88°, m.m.p. with "methyl" cholanate of Fieser and Ettore³ distinctly depressed. When the methyl ester was refluxed in absolute ethanol containing a trace of hydrogen chloride, the ethyl ester was obtained, m.p. 90–91°, not depressed on admixture with "methyl" cholanate (Fieser and Ettore³). After chromatography pure ethyl cholanate was obtained, m.p. 93–94°, $\alpha_D +19^\circ$ Chf. (lit.¹¹ m.p. 93°, $\alpha_D +21^\circ$ Chf.).

Methyl Δ^3 -Cholenate (Fieser and Ettore Procedure³).—Methyl 3-keto-4 β -bromocholanate was reduced with sodium borohydride as in the earlier procedure except that methanol was employed as solvent. As before the product was acetylated and separated into two fractions by treatment with methanol. From the methanol-insoluble fraction **methyl 3 α -acetoxy-4 β -bromocholanate** (0.89 g.) was obtained after several crystallizations from methanol-acetone; flat plates, m.p. 150–151°, $\alpha_D +34^\circ$ Chf. This material corresponds to material, m.p. 149–150°, $\alpha_D +35^\circ$ Di, obtained when "methyl" 3 β -acetoxy-4 β -bromocholanate was refluxed in methanol containing boron fluoride etherate, originally considered an isomeric ester.³

Anal. Calcd. for $C_{27}H_{44}O_4Br$ (511.53): C, 63.39; H, 8.47. Found: C, 63.32; H, 8.25.

Material from the mother liquors of the above crystallizations were combined with the methanol-soluble fraction (1.04 g. crude); after four crystallizations from methanol **methyl 3 β -acetoxy-4 β -bromocholanate** was obtained as prisms, m.p. 91–92°, $\alpha_D +64^\circ$ (Chf.).

Anal. Calcd. for $C_{27}H_{44}O_4Br$ (511.53): C, 63.39; H, 8.77. Found: C, 63.35; H, 8.45.

The 3 α -acetoxy-4 β -bromo ester was reduced with zinc and absolute ethanol as before³; the product after crystallization from methanol was identical with methyl Δ^3 -cholenate prepared by the Wieland method; m.p. 73–74°, m.m.p. 73–74°, $\alpha_D +27^\circ$ (Chf.).

Anal. Calcd. for $C_{25}H_{40}O_2$ (372.57): C, 80.59; H, 10.82. Found: C, 80.23; H, 10.72.

The same product was obtained by zinc-ethanol reduction of the isomeric bromohydrin acetate. On catalytic hydrogenation methyl cholanate was obtained (m.p., m.m.p., rotation, analysis).

Oxidation of Methyl Δ^3 -Cholenate with Osmium Tetroxide.—The ester (m.p. 71–72°, 360 mg.) was dissolved in ether (30 ml.) and treated with a solution of osmium tetroxide (300 mg.) in ether (30 ml.). The following day the solvent was evaporated and sodium hydrosulfite (4 g.) in aqueous methanol was added and the reaction refluxed for 4 hr., when crystals were deposited. The solvent was removed and the residue extracted with hot methanol and then caused to crystallize by addition of hot water to turbidity; 330 mg. (84% yield), m.p. 121–122°. Further recrystallizations gave pure **methyl 3 β ,4 β -dihydroxycholanate**, m.p. 128–129°, $\alpha_D +31^\circ$ Chf., precipitated by a 1% ethanolic solution of digitonin.

Anal. Calcd. for $C_{25}H_{42}O$ (406.34): C, 73.83; H, 10.42. Found: C, 73.56; H, 10.49.

Methyl 3 α ,4 α -dihydroxycholanate, m.p. 95–96°, 40 mg. (10% yield), was obtained from the filtrate. It crystallized from aqueous methanol as a solvate, m.p. 153–154°, $\alpha_D +24^\circ$ Chf.

Anal. Calcd. for $C_{25}H_{42}O_4 \cdot \frac{1}{2}H_2O$ (367.59): C, 72.25; H, 10.43. Found: C, 72.45; H, 10.49.

Methyl 3 β ,4 β -diacetoxycholanate was prepared by acetylation with acetic anhydride-sodium acetate (4 hr. reflux). Crystallized from acetone-methanol, m.p. 182.5–183°, $\alpha_D +24^\circ$ Chf.

Anal. Calcd. for $C_{29}H_{46}O_6$ (466.37): C, 71.34; H, 9.34. Found: C, 71.03; H, 9.48.

Methyl 3 α ,4 α -diacetoxycholanate was prepared similarly; crystallized from aqueous methanol, m.p. 112–113°, $\alpha_D +5^\circ$ Chf.

Anal. Calcd. for $C_{29}H_{46}O_6$ (466.37): C, 71.34; H, 9.34. Found: C, 71.45; H, 9.45.

3 β ,4 β -Dihydroxycholanolic acid, obtained by hydrolysis with ethanolic potassium hydroxide in the usual way, crystallized from aqueous ethanol as leaflets, m.p. 191–192°.

Anal. Calcd. for $C_{24}H_{40}O_4 \cdot \frac{1}{2}H_2O$ (401.33): C, 71.78; H, 10.29. Found: C, 71.39; H, 10.11.

Oxidation of 3 β ,4 β -Dihydroxycholanolic Acid to Lithobilanic Acid.—The dihydroxy acid (150 mg.) in 3 ml. of acetic acid was oxidized with chromic acid (100 mg.) dissolved in 1 ml. of acetic acid and 0.2 ml. of water. A slight rise in temperature was noted. Crystalline material gradually separated and after 2 hr. water was added carefully. Acidic material was extracted from the crude product and crystallized from acetic acid, 70% yield, m.p. 284–285°, not depressed on admixture with lithobilanic acid prepared by oxidation of lithocholic acid.

Equivalent for $C_{24}H_{38}O_6$ (422/3): calcd., 140. Found, 139.

The trimethyl ester was prepared with diazomethane; needles from methanol, m.p. and m.m.p. 111–112°.

Anal. Calcd. for $C_{27}H_{44}O_6$ (464.35): C, 69.78; H, 9.55. Found: C, 69.71; H, 9.58.

CAMBRIDGE, MASSACHUSETTS

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