[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Δ^3-Cholenic Acid

BY KAZUMI YAMASAKI,[1] VITTORIO ROSNATI,[2] MARY FIESER AND LOUIS F. FIESER

RECEIVED JANUARY 31, 1955

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-4β-bromocholanic acid. On oxidation with osmium tetroxide the acid is converted chiefly into $3\beta,4\beta$-dihydroxycholanic acid, which on chromic acid oxidation gives lithobilianic 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 paper[3] Δ^3-cholenic acid was prepared by reduction of either methyl 3α- or 3β-hydroxy-4β-bromocholanate 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-workers[4] 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 lithobilianic acid, rather than the iso acid, and consequently is a 3,4-dihydroxycholanic acid. This diol forms a sparingly soluble digitonide, whereas the isomer does not. Consequently the major product (85% yield) probably is $3\beta,4\beta$-dihydroxycholanic acid and the minor product (10% yield) is $3\alpha,4\alpha$-dihydroxycholanic 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-hydroxycholanic acid, the commonly accepted structures 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 acid[8] and from ursodesoxycholic acid[10] 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 cholanate prepared by hydrogenation of the latter ester was found to differ from authentic methyl cholanate. 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 4β-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.

Acknowledgment.—This work was supported in part by grants from the National Institutes of Health and the National Science Foundation.

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°, α_D +18° Chf (lit.[4] m.p. 155–156°, α_D +17° Al); m.m.p. with Δ^3-cholenic acid of Fieser and Ettorre[3] 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°, α_D +19° Chf., m.p. depressed on admixture with the "methyl" cholenate of Fieser and Ettorre.[3]

---

(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^3-Cholenic acid and Δ^2-cholenic acid are formed in the approximate ratio of 9:1.[4]

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

(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).

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., α_D −2° Chf. Both the acid and the dibromide corresponded with the acid and dibromide of Fieser and Ettorre[3] (m.p. and m.m.p.). **Methyl 3,4-dibromocholanate** was prepared by esterification with diazomethane, m.p. 145–146°, α_D −2° Chf.

Anal. Calcd. for $C_{25}H_{40}O_2Br$ (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°, α_D + 19° Chf., not depressed on admixture with "methyl" Δ^3-cholenate (Fieser and Ettorre,[3] m.p. 83–84°, α_D +30.5° Di, +19.2° Chf).

Anal. Calcd. for $C_{20}H_{42}O_2$ (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°, α_D −4° 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°, α_D +22° 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 Ettorre[3] 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 Ettorre[3]). After chromatography pure ethyl cholanate was obtained, m.p. 93–94°, α_D +19° Chf. (lit.[11] m.p. 93°, α_D +21° Chf.).

Methyl Δ^3-Cholenate (Fieser and Ettorre Procedure[3]).—Methyl 3-keto-4β-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 3α-acetoxy-4β-bromocholanate** (0.89 g.) was obtained after several crystallizations from methanol–acetone; flat plates, m.p. 150–151°, α_D +34° Chf. This material corresponds to material, m.p. 149–150°, α_D +35° Di, obtained when "methyl" 3β-acetoxy-4β-bromocholanate was refluxed in methanol containing boron fluoride etherate, originally considered an isomeric ester.[8]

Anal. Calcd. for $C_{27}H_{43}O_4Br$ (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 3β-acetoxy-4β-bromocholanate** was obtained as prisms, m.p. 91–92°, α_D +64° (Chf.).

Anal. Calcd. for $C_{27}H_{43}O_4Br$ (511.53): C, 63.39; H, 8.77. Found: C, 63.35; H, 8.45.

The 3α-acetoxy-4β-bromo ester was reduced with zinc and absolute ethanol as before[3]; 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°, α_D +27° (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 3β,4β-dihydroxycholanate**, m.p. 128–129°, α_D +31° Chf., precipitated by a 1% ethanolic solution of digitonin.

Anal. Calcd. for $C_{26}H_{42}O$ (406.34): C, 73.83; H, 10.42. Found: C, 73.56; H, 10.49.

Methyl 3α,4α-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°, α_D +24° Chf.

Anal. Calcd. for $C_{25}H_{42}O_4 \cdot {}^1/_2H_2O$ (367.59): C, 72.25; H, 10.43. Found: C, 72.45; H, 10.49.

Methyl 3β,4β-diacetoxycholanate was prepared by acetylation with acetic anhydride–sodium acetate (4 hr. reflux). Crystallized from acetone–methanol, m.p. 182.5–183°, α_D +24° 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 3α,4α-diacetoxycholanate was prepared similarly; crystallized from aqueous methanol, m.p. 112–113°, α_D +5° 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.

3β,4β-Dihydroxycholanic 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 $C_{24}H_{40}O_4 \cdot {}^1/_2H_2O$ (401.33): C, 71.78; H, 10.29. Found: C, 71.39; H, 10.11.

Oxidation of 3β,4β-Dihydroxycholanic Acid to Lithobilianic 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 lithobilianic 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

---

[11] H. Wieland and E. Boersch, *Z. physiol. Chem.*, **106**, 190 (1919).