[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF GEORGE A. BREON AND COMPANY]

# 3-Keto-6(β)-hydroxycholanic Acid and 3(α)-Hydroxy-6-ketocholanic Acid

BY WILLARD M. HOEHN, JACOB LINSK* AND ROBERT BRUCE MOFFETT

Methyl ester acetates of bile acids provide many examples in which the 3-acetate group is saponified in preference to the 7 or 12-acetate groups. One per cent. hydrogen chloride in methanol[1] or 0.5 *N* potassium hydroxide in alcohol[2] brings about hydrolysis of the 3-acetoxy group without affecting the ester group. Half normal alkali in aqueous-alcoholic solution saponified both the 3-acetoxy group and the ester group. It seemed reasonable to expect that methyl 3(α),6(β)-diacetoxycholanate could be partially saponified in the same manner. Marker and co-workers[3,4] have reported the conversion of 3,6-diacetoxypregnan-20-one to progesterone by a procedure involving preferential saponification of the 3-acetoxy group.

In many experiments under the usual conditions employed for preferential saponification of the 3-acetoxy group a completely saponified material was the main reaction product. This was usually oxidized and the resulting methyl 3,6-diketocholanate isolated in good yield and characterized by melting point and mixed melting point. However, by employing much milder conditions, a procedure was developed for partial saponification of methyl 3(α),6(β)-diacetoxycholanate by which, after oxidation and saponification, 3-keto-6(β)-hydroxycholanic acid was isolated (m. p. 196.5–198°). The acid showed a light positive Zimmermann reaction and the mixed melting point with hyodesoxycholic acid (m.p. 198–200°) was 185–194°. A sample was converted to methyl 3-keto-6(β)-acetoxycholanate. Wolff–Kishner reduction gave 6(β)-hydroxycholanic acid, which was oxidized to 6-ketocholanic acid, and treatment of the latter with sodium hydroxide gave 6-keto-*allo*-cholanic acid, which has been previously reported by Wieland and Dane.[5]

It was shown by Wieland and Dane[5] that low temperature oxidation of hyodesoxycholic acid yields 3(α)-hydroxy-6-ketocholanic acid in 40% yield. Since the methyl ester of this acid was of interest, we carried out the low temperature oxidation directly on the methyl ester and obtained methyl 3(α)-hydroxy-6-ketocholanate in 58% yield. The pure keto ester gave a negative Zimmermann reaction. It was characterized by means of the acetate and the oxime acetate. Hydrogenation of the acetate in acetic acid over platinum oxide gave methyl 3(α)-acetoxy-6(α)-hydroxycholanate, which was crystallized after chromatographing over Fisher alumina. When the hydrogenation was carried out in methanol solution in the presence of Raney nickel and the product was hydrolyzed, the known 3(α),6(α)-dihydroxycholanic acid[6] was obtained in 85% yield. This acid differs from hyodesoxycholic acid only in the opposite configuration of the 6-hydroxyl group. Esterification and acetylation gave methyl 3(α),6(α)-diacetoxycholanate.

Methyl 3(α)-acetoxy-6-keto-cholanate is rearranged by Fisher alumina to give methyl 3(α)-acetoxy-6-keto-*allo*-cholanate. When a sample of methyl 3(α)-acetoxy-6-keto-cholanate was chromatographed over alumina treated to remove alkali, the inversion to the *allo* form took place to a lesser extent.

## Experimental

**Methyl 3(α),6(β)-Diacetoxycholanate.**[7]—This was prepared in 98% yield from methyl hyodesoxycholate. The compound showed a m. p. of 99.5–101.5° and $[\alpha]^{30}D$ + 24.5 ± 3° (methanol). Calcd. for $C_{29}H_{46}O_6$: sapn. equiv., 163.8. Found: sapn. equiv., 165.4.

**Methyl Dehydrohyodesoxycholate.**[7]—Oxidation of methyl hyodesoxycholate in acetic acid solution with chromium trioxide followed by recrystallization from aqueous methanol gave rosets of needles, m. p. 137–139°; $[\alpha]^{25}D$ − 66.7 ± 3° (methanol).

**Dioxime.**—Obtained in 95% yield, the m. p. was 195–198°. Crystallization from ether raised the m. p. to 199–201°.

*Anal.* Calcd. for $C_{25}H_{40}O_4N_2$: N, 6.5; Found[8]: N, 6.1.

**3-Keto-6(β)-hydroxcholanic Acid.**—A solution of 108 g. (0.220 mole) of methyl 3(α),6(β)-diacetoxycholanate in 7.5 liters of ethanol was cooled to −2° and 50 ml. of 0.816 *N* potassium hydroxide (0.0408 mole) in alcohol was added. After three days at −4°, 3 ml. of acetic acid was added and the alcohol was distilled off *in vacuo*. The residue was taken up in 500 ml. of benzene, the solution was washed with water, and the solvent was completely removed on a water-bath *in vacuo*.

The viscous residue was dissolved in 300 ml. of acetic acid and oxidized with 35 g. of chromium trioxide in 100 ml. of 70% acetic acid at 25–30° for one hour. The reaction mixture was taken up in benzene, washed with water, and the solvent was distilled off leaving a residue which was dissolved in 200 ml. of 0.5 *N* methanolic sodium methoxide solution. After one hour on a steam-bath the methanol was removed *in vacuo* at 50°. Benzene and water were added, the mixture was filtered, and the benzene solution was separated. After washing with dilute sodium hydroxide solution and then with water, the benzene was distilled off *in vacuo* leaving a viscous residue which weighed 70 g. and consisted of a mixture of methyl esters.

This residue was dissolved in 700 ml. of pyridine containing 150 g. of succinic anhydride. The solution was warmed on the steam-bath for one hour and allowed to stand one day at room temperature. The solution was decanted from crystallized succinic anhydride, concen-

* Present address: Ohio State University, Columbus, Ohio.

(1) Hoehn and Linsk, THIS JOURNAL, **67**, 313 (1945); Reichstein and Sorkin, *Helv. Chim. Acta*, **25**, 802 (1942).

(2) Tai-Shihk Sihn, *J. Biochem.* (Japan) **27**, 425 (1938); Gallagher, *J. Biol. Chem.*, **133**, 34 (1940).

(3) Marker and Krueger, THIS JOURNAL, **62**, 81 (1940).

(4) Marker and Lawson, U. S. Patent 2,366,204.

(5) Wieland and Dane, *Z. physiol. Chem.*, **211**, 4 (1932).

(6) Tukamoto, *J. Biochem.* (Japan), **32**, 451 (1940), reports this acid which he calls 3(α),6(β)-dihydroxycholanic acid, m. p. 208°, $[\alpha]D$ +23.1°; methyl ester, m. p. 114°.

(7) Windaus, *Ann.*, **447**, 244 (1926).

(8) Analysis by J. Buonocore in this Laboratory.

trated *in vacuo* on a steam-bath, and poured into ice cold dilute sulfuric acid. The viscous dark oil which separated was taken up in ether, washed well with ice water, and then extracted with 0.7 liter of ice cold 0.5 *N* sodium hydroxide solution in several portions. The alkaline extracts were washed twice with ether, and made 1 *N* with sodium hydroxide. After refluxing one hour acidification gave a tan, viscous material which showed a light positive Zimmermann test. When boiled with water it became granular giving 40 g. of material, m. p. 177–184°. The solid was esterified by refluxing with methanol and sulfuric acid. The solution was diluted with benzene and washed with dilute sodium hydroxide and then with water. Removal of the solvent gave a crude resinous ester which was subjected to a ketone separation with Girard reagent "P".[9] The ketone containing fraction was hydrolyzed with sodium hydroxide in methanol and water. Acidification of the basic solution gave a gummy acid which hardened on boiling with water; weight 25 g. (29%), m. p. 177–183°. It was recrystallized from ethyl acetate with the aid of decolorizing charcoal (16.7 g. of crude acid gave 8 g. of crystals), m. p. 192–5°. After a second recrystallization from ethyl acetate and drying in a high vacuum at 100°, it melted at 196.5–198°. A mixed melting point with hyodesoxycholic acid (melting at 198–200°) was 185–194°, $[\alpha]^{26}D$ + 15.7 ± 3° (methanol).

*Anal.* Calcd. for $C_{24}H_{38}O_4$: C, 73.80; H, 9.81. Found[10]: C, 73.50; H, 9.86.

**Methyl 3-Keto-6(β)-acetoxy-cholanate.**—One gram of 3-keto-6(β)-hydroxycholanic acid was esterified with methanol and sulfuric acid at room temperature and then acetylated by refluxing with acetic anhydride. It crystallized from a mixture of ether and petroleum solvent (b. p. 30–40°) in needles, m. p. 111–114° (51% yield). Recrystallization from the same solvent raised the m. p. to 114–115°, $[\alpha]^{31}D$ + 15.6 ± 3° (dioxane).

*Anal.* Calcd. for $C_{27}H_{42}O_6$: C, 72.61; H, 9.48. Found[10]: C, 72.57; H, 9.51.

**6(β)-Hydroxycholanic Acid.**—A solution of 2 g. of 3-keto-6(β)-hydroxycholanic acid and 2 ml. of hydrazine hydrate in 15 ml. of 10% methanolic sodium methoxide solution was heated in a bomb in an oil-bath at 180–200° for three hours. After cooling the alkaline solution was diluted with water, filtered and acidified. The white flocculent precipitate weighed 1.83 g. (95%), m. p. 213–218°. Recrystallization from acetone gave lustrous platelets which melted at 221–222°, $[\alpha]^{29}D$ + 8.5 ± 3° (dioxane).

*Anal.* Calcd. for $C_{24}H_{40}O_3$: C, 76.55; H, 10.70. Found[10]: C, 76.45; H, 10.70.

**6-Ketocholanic Acid.**—A solution of 0.7 g. of 6-hydroxycholanic acid in 100 ml. of acetic acid and 10 ml. of dioxane was oxidized with 1 g. of chromium trioxide. After one hour at room temperature the solution was diluted with water. The thus precipitated acid was filtered, washed with water and dried. The yield was 0.65 g., m. p. 134–138°. Recrystallization from a mixture of ether and petroleum solvent (b. p. 69°) gave 0.49 g. of soft needles—negative Zimmermann test, m. p. 138–140°, $[\alpha]^{27}D$ − 41.5 ± 3° (dioxane).

*Anal.* Calcd. for $C_{24}H_{38}O_3$: C, 76.96; H, 10.23. Found[10]: C, 77.06; H, 10.30.

**6-Keto-*allo*-cholanic Acid.**[5]—6-Ketocholanic acid (1.6 g. of crude acid, m. p. 134–138°) dissolved in aqueous sodium hydroxide with the development of a yellow color. The solution was warmed for an hour and then allowed to stand overnight. The sodium salt crystallized but without isolation was acidified, and the acid was collected, washed with cold and hot water, and dried. It was recrystallized from a mixture of ether and petroleum solvent (b. p. 30–40°) giving 0.8 g., m. p. 149–152°. A mixed m. p. with 6-ketocholanic acid was 123–135°, $[\alpha]^{20}D$ − 3.5 ± 3° (dioxane).

**Methyl 3(α)-Hydroxy-6-ketocholanate.**—A solution of 101 g. (0.248 mole) of methyl hyodesoxycholate in 1 liter of 70% acetic acid was cooled to −10° in an acetone dry ice bath and 134 ml. of 4.13 *N* chromic acid in 80% acetic acid was added dropwise with stirring during two hours and forty minutes. After an additional two hours, the temperature was allowed to rise to 10° and the flask was stored at that temperature for two days. The chromic acid was completely consumed. The reaction mixture was concentrated at 60°, dissolved in 1.5 liters of benzene, and the solution was washed with dilute hydrochloric acid and then with water until neutral. Removal of the solvent left a yellow resin which crystallized from a mixture of ether and petroleum solvent (b. p. 69°) giving 58.5 g. (58.3%), m. p. 136–139°. Repeated crystallization of a sample from 70–90% methanol raised the m. p. to 144–146°,[11] negative Zimmermann test; $[\alpha]^{25}D$ − 38.6 ± 3° (dioxane).

*Anal.* Calcd. for $C_{25}H_{40}O_4$: C, 74.21; H, 9.97; sap. equiv., 404.6. Found[12]: C, 74.25; H, 9.92; sap. equiv., 397.5.

**Methyl 3(α)-Acetoxy-6-ketocholanate.**—A solution of 1.01 g. of methyl 3(α)-hydroxy-6-ketocholanate in 10 ml. of acetic anhydride was warmed for one hour and diluted with water. The crystalline precipitate weighed 1.1 g., m. p. 147–154°. Recrystallization from absolute ethanol gave crystals melting at 155–157°; $[\alpha]^{25}D$ − 18.8 ± 3° (dioxane).

*Anal.* Calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48; sap. equiv., 223.3. Found[12]: C, 72.42; H, 9.53; sap. equiv., 225.2.

**Oxime.**—A solution of 1 g. of the above ketone, 1 g. of hydroxylammonium chloride, and 1 g. of sodium acetate in 50 ml. of alcohol and 5 ml. of water was refluxed for two hours. The solution was poured into hot water giving 1 g. of granular oxime, m. p. 158–160°. This was recrystallized from aqueous methanol giving needles, m. p. 162–163.5°; $[\alpha]^{25}D$ − 17.6 ± 3° (benzene).

*Anal.* Calcd. for $C_{27}H_{43}O_5N$: N, 3.04. Found[13]: N, 3.03.

**Methyl 3(α)-Acetoxy-6(α)-hydroxycholanate.**—A solution of 4.47 g. (0.01 mole) of methyl 3(α)-acetoxy-6-ketocholanate in 120 ml. of C. P. acetic acid was hydrogenated in the presence of 200 mg. of Adams platinum oxide catalyst. After forty-three hours at a hydrogen pressure of 50–55 lb. and room temperature (22–29°) the catalyst was removed by filtration and the solution was concentrated at approximately 50°. The residue was dissolved in benzene and the solution was washed with water, dilute sodium hydroxide and then with water until neutral. The solution was dried and showed $[\alpha]^{20}D$ approximately 37°. It was diluted with petroleum solvent (b. p. 69°) and poured over a column of 100 g. of Fisher adsorption alumina (80–200 mesh). The column was eluted with four 80-ml. portions of petroleum solvent (b. p. 69°) ten portions containing mixtures of petroleum solvent and benzene, five portions containing mixtures of benzene and ether, and five portions containing mixtures of ether and methanol. Nine fractions crystallized on standing and showed melting points within the range 115–123°. These were combined and recrystallized twice from a mixture of ether and petroleum solvent (b. p. 69°), m. p. 122–123°, $[\alpha]^{26}D$ + 44.3 ± 3° (methanol).

*Anal.* Calcd. for $C_{27}H_{44}O_5$: C, 72.28; H, 9.89. Found[10]: C, 72.48; H, 9.89.

**3(α),6(α)-Dihydroxycholanic Acid.**—**A.** Eight fractions of the above crystallized on standing having melting

(9) Girard and Sandulesco, *Helv. Chim. Acta*, **19**, 1095 (1936); Moffett and Hoehn, THIS JOURNAL, **66**, 2098 (1944).

(10) Carbon and hydrogen analysis by Micro-Tech Laboratories, Skokie, Illinois.

(11) Wieland and Kennelly, *Z. physiol. Chem.*, **219**, 146 (1933), prepared the methyl ester from the free acid and report a melting point of 140–142°.

(12) Carbon and hydrogen analysis by the Arlington Laboratories, Fairfax, Virginia.

(13) Analysis by Caroline Roberts in this Laboratory.

points within the range 95–110°. These fractions were combined and hydrolyzed, giving 1.32 g. of 3(α),6(α)-dihydroxycholanic acid, m. p. 203–206°. Recrystallization from aqueous methanol gave fine needles melting sharply at 205°, $[\alpha]^{20}D$ + 37.2 ± 3° (methanol). Mixed melting point with hyodesoxycholic acid 175–180°.

*Anal.* Calcd. for $C_{24}H_{40}O_4$: C, 73.43; H, 10.27. Found[12]: C, 73.88; H, 10.35. The methyl ester[6] crystallized from a mixture of ether and petroleum solvent (b. p. 69°) melted at 112–114° and showed $[\alpha]^{20}D$ + 35.1 ± 3° (methanol).

**B.** To a solution of 1.90 g. of methyl 3(α)-acetoxy-6-ketocholanate in 100 ml. of methanol was added 5 g. of Raney nickel catalyst and the mixture was shaken four hours at a hydrogen pressure of about 50 lb. The solution was filtered from the catalyst and the ester was hydrolyzed with sodium hydroxide. Acidification of the hot solution yielded 1.44 g. (85%) of 3(α),6(α)-dihydroxycholanic acid, m. p. 202.5–204.5°; mixed melting point with hyodesoxycholic acid, 173–182°.

**Methyl 3(α),6(α)-Diacetoxycholanate.**—3(α),6(α)-Dihydroxycholanic acid (1.37 g.) was esterified with diazomethane in ether. The crude ester was acetylated by refluxing for one hour with 10 ml. of acetic anhydride. It was then poured into water, the gum dissolved in benzene, and washed with dilute sodium carbonate and then with water. After removal of the solvent it was crystallized from aqueous methanol giving 1.14 g. (67%) of needles, m. p. 106–108°. A mixture with methyl 3(α),6(β)-diacetoxycholanate (m. p. 98–100°) melted at 88–94°; $[\alpha]^{24}D$ + 26.6 ± 3°.

*Anal.* Calcd. for $C_{29}H_{46}O_6$: C, 70.98; H, 9.45. Found[10]: C, 70.60; H, 9.36.

**Rearrangement of Methyl 3(α)-Acetoxy-6-ketocholanate on Fisher Alumina.**—A sample of methyl 3(α)-acetoxy-6-ketocholanate (m. p. 155–157°) was dissolved in a small volume of benzene and the solution was poured over a column of Fisher adsorption alumina (80–200 mesh). Elution with benzene and then with benzene-ether, gave solutions which left, when the solvent was evaporated, crystalline residues melting in the range of 168–174°. Recrystallization from aqueous methanol gave needles, m. p. 179–182°. The rotation was negative and the mixed m. p. with methyl 3(α)-acetoxy-6-keto-*allo*-cholanate[14] (m. p. 179–181°) prepared from 3-hydroxy-6-keto-*allo*-cholanic acid[5,14] showed no depression. Methyl 3-acetoxy-6-keto-*allo*-cholanate showed $[\alpha]^{20}D$ − 18.3 ± 3° (benzene).

A sample of the alumina was washed with 10% methanolic acetic acid, then repeatedly with water and reactivated at 200–230°. A sample of methyl 3(α)-acetoxy-6-ketocholanate chromatographed on it as described above gave several fractions; the first to come off proved to be unchanged and 3(α)-acetoxy-6-ketocholanate and the last was the *allo* form.

## Summary

1. 3-Keto-6(β)-hydroxycholanic acid has been prepared by the partial hydrolysis of methyl 3(α),6(β)-diacetoxycholanate, followed by oxidation and saponification.

2. This was characterized by converting it to the known 6-keto-*allo*-cholanic acid by a Wolff–Kishner reduction, followed by oxidation and rearrangement.

3. Methyl 3(α)-hydroxy-6-ketocholanate was prepared by partial oxidation of methyl hyodesoxycholate and several derivatives were made.

4. Methyl 3(α)-acetoxy-6(α)-hydroxycholanate was obtained by the hydrogenation of methyl 3(α)-acetoxy-6-ketocholanate and several derivatives were prepared.

(14) Fernholz, *Z. physiol. Chem.*, **232**, 202 (1935).

KANSAS CITY, MISSOURI RECEIVED MAY 23, 1946

---

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF GEORGE A. BREON & CO.]

# Degradation of Hyodesoxycholic Acid

BY ROBERT BRUCE MOFFETT, JAMES E. STAFFORD, JACOB LINSK* AND WILLARD M. HOEHN

Several workers[1] have reported the degradation of hyodesoxycholic acid by the Barbier–Wieland method. In this work we have repeated the degradation by the procedure of Hoehn and Mason.[2] Several intermediates, hitherto unreported, were isolated and we have found melting points and rotations of several other compounds at variance with those reported previously.

We have also applied to the degradation of hyodesoxycholic acid the recently reported method which Miescher and co-workers[3] used for the degradation of several bile acids. By this method the diphenylethylene derivative of a bile acid is brominated with N-bromosuccinimide followed by dehydrobromination with dimethylaniline and oxidation with chromic acid to the corresponding pregnan-20-one.

By both methods of degradation the same compound, 3(α),6(β)-diacetoxypregnan-20-one, was obtained, m. p. 131–133°, $[\alpha]^{24}D$ + 53 ± 3° (in dioxane). Marker and Krueger[1b] report a compound melting at 100° (no rotation given) to have this structure. Hydrolysis of this acetate gave pregnan-3(α),6(β)-diol-20-one; m. p. 187–190°, $[\alpha]^{26}D$ + 61.8 ±3° (in methanol). Kimura and Sugiyama[1a] report a compound melting at 198° with $[\alpha]D$ + 6.52° (in alcohol) to have this structure. It seems certain that our pregnan-3,6-diol-20-one and acetate are not identical with the compounds previously reported; and, in view of the fact that we obtained the same acetate by two different methods, great doubt is cast on the structure of the previously reported compounds.

Numerous attempts to remove one of the ace-

* Present address: Ohio State University, Columbus, Ohio.

(1) (a) Kimura and Sugiyama, *J. Biochem.* (Japan), **29**, 409 (1939); (b) Marker and Krueger, THIS JOURNAL, **62**, 79 (1940); (c) Marker and Lawson, U. S. Patents: 2,337,563; 2,337,564; and 2,366,204.

(2) Hoehn and Mason, THIS JOURNAL, **60**, 1493 (1938).

(3) (a) Meystre, Frey, Wettstein and Miescher, *Helv. Chim. Acta*, **27**, 1815 (1944); (b) Meystre, Ehmann, Neher and Miescher, *ibid.*, **28**, 1252 (1945); (c) Meystre and Miescher, *ibid.*, **28**, 1497 (1945); (d) Meystre and Miescher, *ibid.*, **29**, 33 (1946).