PARTIAL OXIDATION OF CHOLIC ACID*

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The behavior of polyhydroxy steroids toward oxidizing agents has been studied extensively in the bile acids. Wieland and Dane (1) originally suggested that the order in which the hydroxyl groups of cholic acid are dehydrogenated is 7–3–12 (older literature, 12–3–7). This was based upon their claim that oxidation of desoxycholic acid yielded 3-keto-12-hydroxycholanic acid, while 7,12-dihydroxycholanic acid (probably a mixture of isomers) was oxidized by CrO₃ to 7-keto-12-hydroxycholanic acid. The same investigators (2) proved that the 6-hydroxyl of hyodesoxycholic acid was more readily attacked by CrO₃, since low temperature oxidation gave the 6-keto-3-hydroxy compound. Iwasaki (3) prepared 3-hydroxy-7-ketocholanic acid from both chenodesoxycholic acid and ursodesoxycholic acid by chromic acid oxidation at low temperature in agreement with the Wieland and Dane suggestion.

In 1937 Kaziro and Shimada (4) proved that the hydroxyl groups of the bile acids were not oxidized in the order 7–3–12, since partial oxidation of desoxycholic acid by CrO₃ yielded 3-hydroxy-12-ketocholanic acid rather than the expected 3-keto-12-hydroxy acid. When these authors investigated the partial oxidation of cholic acid, they obtained in 65 per cent yield 3-hydroxy-7,12-diketocholanic acid ("reductodehydrocholic acid"). Under a variety of conditions they were unable to obtain any monoketo acid; when 3-keto-7,12-dihydroxycholanic acid was oxidized, the sole product obtained was dehydrocholic acid. They confirmed the considerable difference in the oxidation of the 3 OH compared with those at C₇ and C₁₂ and concluded that these latter were almost identical in the ease with which they were oxidized by chromic acid.

It seemed to us that there must be a significant difference in the ease of oxidation of the C₇ and C₁₂ hydroxyl groups of cholic acid and that the results of Kaziro and Shimada were to be explained by their failure to isolate the 7-ketocholic acid. This view is probable, since the reductodehydrocholic acid which they obtained exhibits an unsatisfactory melting

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point behavior, so that an impurity might not easily be recognized. We therefore studied the low temperature oxidation of cholic acid and the methyl ester; we separated the reaction products by conversion to the acetates of the methyl esters and found that these could be readily purified by chromatographic adsorption on aluminum oxide. We obtained the hitherto undescribed 7-keto-3,12-dihydroxycholanic acid in 40 per cent yield. These experiments then demonstrate that in cholic acid the hydroxyl group at C7 is most readily attacked by CrO3 and at the same time offer a method by which desoxycholic acid may be prepared from the much more abundant cholic acid. Haslewood (5) has independently reported a similar conclusion.

**EXPERIMENTAL**

*Oxidation of Methyl Cholate*—40 gm. of methyl cholate (m.p. 145–147°) were dissolved in 300 cc. of stable glacial acetic acid and 50 cc. of water. The solution was cooled to −7° and 81.5 cc. of 3.5 N CrO3 in 25 per cent acetic acid (3 equivalents) were added dropwise over 4 hours, so that the temperature never rose over 0°. The reaction mixture was stored overnight at 0°, sodium bisulfite was added in excess, and the mixture evaporated on the steam bath under reduced pressure. The residue was dissolved in ethyl acetate, washed with water, dilute acid, sodium bicarbonate solution, and again with water. After drying over anhydrous sodium sulfate the solution was evaporated and the residue weighed 38.7 gm.

*Separation of Oxidation Mixture*—The oily residue was acetylated by heating with acetic anhydride in pyridine for 22 hours. The acetates were dissolved in 3.5 liters of 90–100° petroleum ether and poured over a column of 350 gm. of aluminum oxide (Aloco brand, C-30 grade) which had been activated by heating for 30 minutes at about 450°. The percolating fluid was collected in liter portions and the washing continued with 90–100° petroleum ether until no significant amount of solid was eluted. This required 12 liters and 20 gm. of solid were eluted. This material was crystallized by rubbing with a small amount of 90–100° petroleum ether. After three crystallizations from the same solvent, the product was obtained as long prisms melting at 114–117° corrected, [α]D = +64° (absolute ethanol), and proved to be 3,12-diacetoxy-7-ketocholestanic methyl ester.1

\[ \text{CaH}_{34} \text{O}_7 \]
Calculated, C 69.00, H 8.79; found, C 69.08, H 8.69

The oxime was prepared by boiling a solution of 490 mg. in ethanol with 600 mg. of sodium acetate and 500 mg. of hydroxylamine hydrochloride

1 All analyses were made by Dr. T. S. Ma of the Department of Chemistry, University of Chicago.
for 3 hours. The product crystallized from methanol in long needles, m.p. 155.5–157° corrected.

\[ \text{C}_{26}\text{H}_{46}\text{O}_7\text{N} \]

Calculated. C 67.01, H 8.51, N 2.70

Found. C 66.99, H 8.73, N 3.00

The 3-acetoxy-7,12-diketocholanic methyl ester was removed from the column by elution with 90–100° petroleum ether containing 2 per cent ethyl acetate. The methyl ester of dehydrocholic acid could finally be removed with ethyl acetate alone. In general about 40 per cent of the monoketo, 40 per cent of the diketo, and 20 per cent of the triketo compounds were obtained.

7-Keto-3,12-dihydroxycholanic Acid—10.2 gm. of 7-keto-3,12-diacetoxy-methyl cholanate (m.p. 112–114°) were dissolved in 50 cc. of 95 per cent ethanol, 75 cc. of 1.7 N aqueous NaOH added, and the mixture heated under a reflux for 4 hours. After neutralization to phenolphthalein, 66.8 cc. of N base were neutralized; calculated for 3 equivalents 66.5 cc. of N base. Most of the alcohol was distilled and the aqueous solution added dropwise with stirring to dilute H\(_2\)SO\(_4\) at 50°. The acid crystallized on cooling and was recrystallized from ethyl acetate as well formed prisms, m.p. 197–199° (corrected), with a negligible rotation in absolute ethanol.

\[ \text{C}_{24}\text{H}_{40}\text{O}_6 \]

Calculated, C 70.88, H 9.43; found, C 70.54, H 9.36

The methyl ester prepared with diazomethane crystallized from ethyl acetate–petroleum ether in small needles, m.p. 152–154°.

\[ \text{C}_{25}\text{H}_{44}\text{O}_6 \]

Calculated, C 71.37, H 9.59; found, C 71.35, H 9.75

Preparation of Desoxycholic Acid—The semicarbazone of the 7-keto-3,12-diacetoxyethyl cholanate was prepared by heating 1.0 gm. of ester with 1.27 gm. of semicarbazide acetate in a mixture of 40 cc. of absolute methanol, 20 cc. of pyridine, and 5 cc. of water under a reflux for 6 hours. The product was well washed with boiling water and crystallized from ethanol. Needles, m.p. 175–177° (corrected).

\[ \text{C}_{19}\text{H}_{17}\text{O}_7\text{N}_3 \]

Calculated, N 7.48; found, N 7.78

440 mg. of the semicarbazone were heated with 6 cc. of absolute alcohol containing 350 mg. of sodium and 0.6 cc. of hydrazine hydrate in a sealed tube at 200° for 8 hours. The reaction mixture was acidified and a white semicrystalline product separated; weight 280 mg. The material crystallized well in clumps of narrow prisms from dilute acetic acid; weight 216 mg., m.p. 139–143° (corrected). The melting point of a mixture with desoxycholic-acetocholeic acid was 139–142°. The diformyl derivative was prepared by warming at 55° for 5 hours with 88.5 per cent formic acid
and crystallized from dilute ethanol, m.p. 192.5–194°. Cortese and Bauman (6) record a melting point of 193–194°.

**SUMMARY**

1. The hydroxyl groups of cholic acid are oxidized by CrO₃ in acetic acid in the order, 7–12–3.
2. A method for the separation of 7-keto-3,12-dihydroxycholanic acid from the oxidation mixture is described.
3. This compound can be reduced to desoxycholic acid in good over-all yield from cholic acid.

**BIBLIOGRAPHY**

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