

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE]

Sterols. XCI. Oxidation of the Diacetate of Cholestanediol-3,6

BY RUSSELL E. MARKER, JOHN KRUEGER, JOHN R. ADAMS, JR., AND ELDON M. JONES

Windaus and Hossfeld¹ were unable to obtain *allo*-hyodesoxycholic acid by oxidation of the diacetate of cholestanediol-3,6. The oxidative opening of ring A occurred before the side chain was attacked and the product obtained was a lactone carboxylic acid. Recently we reported² the preparation of cholestanediol-3,6 of m. p. 190° which was epimeric with the cholestanediol-3,6 of m. p. 216° reported by Windaus.³

We have investigated the oxidation of the diacetate of cholestanediol (m. p. of the diol, 190°) and have obtained both *allo*-hyodesoxycholic acid and 6-hydroxyisoandrosterone from the reaction. *allo*-Hyodesoxycholic acid prepared in this way was characterized by direct comparison with a sample of *allo*-hyodesoxycholic acid and its methyl ester prepared from hyodesoxycholic acid by the method of Windaus.⁴

We have also degraded *allo*-hyodesoxycholic acid to *nor-allo*-hyodesoxycholic acid. *bis-nor-allo*-Hyodesoxycholic acid was prepared from *bis-nor*-hyodesoxycholic acid by the method of Windaus.⁴

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Experimental Part

Reduction of 6-Hydroxycholestanone.—6-Hydroxycholestanone (2.2 g.) in 125 cc. of 95% ethanol and 0.2 g. of Adams catalyst was shaken under 3 atmospheres of hydrogen for two hours. The catalyst was filtered off and the filtrate was concentrated by evaporation. The cholestanediol-3,6 which separated on cooling was recrystallized from ethanol. The substance melted at 191° and showed no depression in melting point when mixed with cholestanediol-3,6² prepared by reduction of 6-ketocholestanol; yield 2 g.

Oxidation of the Diacetate of Cholestanediol-3,6.—The cholestanediol used was prepared by reduction of 6-ketocholestanol in acetic acid rather than in ethanol as previously reported.² The diacetyl derivative (200 g.) was mixed with 8 liters of acetic acid and was heated to 90° on a steam-bath. The solution was stirred mechanically and 440 g. of chromic anhydride in 250 cc. of water and 1300 cc. of acetic acid was added dropwise during five hours. The reaction mixture was heated four hours longer. After cooling, excess chromic anhydride was de-

stroyed by cautious addition of 200 cc. of ethanol. The reaction mixture was evaporated *in vacuo* to 25% of its volume. Water and ether were added and the layers separated. The ethereal layer water washed with and was then extracted with 10% sodium hydroxide. The insoluble sodium salt was filtered off and saponified. The free acid was crystallized by extraction from a Soxhlet thimble with boiling ethyl acetate. The *allo*-hyodesoxycholic acid so obtained melted at 280° and gave no depression upon mixing with *allo*-hyodesoxycholic acid prepared from hyodesoxycholic acid.

Anal. Calcd. for C₂₄H₄₀O₄: C, 73.4; H, 10.3. Found: C, 73.4; H, 10.2.

The methyl ester prepared with diazomethane melted at 179°. The mixed melting point with the methyl ester of *allo*-hyodesoxycholic acid (m. p. 181°) prepared from hyodesoxycholic acid was 180°.

Anal. Calcd. for C₂₅H₄₂O₄: C, 73.8; H, 10.4. Found: C, 73.7; H, 10.3.

The ethereal solution from which *allo*-hyodesoxycholic acid had been extracted was washed with water, and then evaporated. The oily residue was dissolved in 500 cc. of ethanol, chilled in ice, and the crystalline unchanged diacetate of cholestanediol was filtered off. The filtrate was evaporated to dryness *in vacuo* and was then steam distilled to remove volatile ketones. The oily suspension was extracted with ether and the ethereal layer was evaporated to dryness. The residue was dissolved in 600 cc. of ethanol, and to this solution was added 15 g. of semicarbazide hydrochloride and 18 g. of sodium acetate. The mixture was refluxed two hours on a steam-bath, then the alcohol was evaporated to half its volume. Addition of 1500 cc. of ether caused a precipitate of sodium chloride which was dissolved in water. The ether layer was washed repeatedly with water in order to remove alcohol. The semicarbazone of the diacetate of 6-hydroxyisoandrosterone was precipitated from the ether layer with pentane. After one recrystallization from methanol-ether-pentane and two recrystallizations from methanol it was obtained as small white needles melting at 222°; yield 3.1 g.

Anal. Calcd. for C₂₄H₃₇O₅N₃: C, 64.4; H, 8.3. Found: C, 64.4; H, 8.3.

One gram of this semicarbazone in 50 cc. of ethanol was refluxed one-half hour with 5 cc. of sulfuric acid in 10 cc. of water. The mixture was poured into water and was extracted with ether. The ether was evaporated and the residue was refluxed one hour with 30 cc. of 2% methanolic potassium hydroxide. The solution was poured into water and was extracted with ether. The ether layer was washed well with water and then pentane was added to precipitate the diol. 6-Hydroxyisoandrosterone, crystallized from methanol-ether-pentane, melted at 205°.

Anal. Calcd. for C₁₉H₃₀O₃: C, 74.4; H, 9.9. Found: C, 74.4; H, 9.9.

(1) Windaus and Hossfeld, *Z. physiol. Chem.*, **145**, 177 (1925).

(2) Marker and Krueger, *THIS JOURNAL*, **62**, 79 (1940).

(3) Windaus, *Ber.*, **50**, 133 (1917).

(4) Windaus, *Ann.*, **447**, 233 (1926).

nor-allo-Hydesoxycholic Acid.—Fifty grams of *allo-hydesoxycholic acid* was refluxed for two hours with 1 l. of 1% methanolic hydrogen chloride. The solution was concentrated by evaporation *in vacuo*. On cooling, the methyl ester which crystallized out was filtered, dried and dissolved in hot toluene. The toluene solution was added to 16 moles of ethereal phenylmagnesium bromide. The suspension was refluxed for two hours, then the ether was distilled off and was replaced with dry toluene. The mixture was heated overnight on a steam-bath. The carbinol was isolated, acetylated and dehydrated in the usual way. The ethylenic compound so obtained was oxidized with chromic oxide and *nor-allo-hydesoxycholic acid* was thus obtained.² The acid, recrystallized from methanol melted at 225°.

Anal. Calcd. for $C_{23}H_{38}O_4$: C, 73.0; H, 10.1. Found: C, 73.2; H, 10.1.

3,6-Diketo-bis-nor-cholanic Acid.—Fifteen grams of *bis-nor-hydesoxycholic acid*² dissolved in 300 cc. of acetic acid was treated at 12–15° during fifteen minutes with 15 g. of chromic oxide dissolved in 15 cc. of water and 35 cc. of acetic acid. The mixture was stirred a further ten minutes and then was diluted with water. The suspension was extracted with ether and the ethereal layer after washing with water was evaporated to dryness. The residue was crystallized from ether–pentane. *3,6-Diketo-bis-nor-cholanic acid* melted at 185°; yield 12 g.

Anal. Calcd. for $C_{22}H_{32}O_4$: C, 73.3; H, 8.9. Found: C, 72.7; H, 8.9.

The methyl ester of *3,6-diketo-bis-nor-cholanic acid* was prepared with ethereal diazomethane. It melted at 170° after crystallization from ether–pentane. The mixed melting point with the acid (m. p. 185°) was 160°.

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.7; H, 9.1. Found: C, 73.9; H, 8.9.

3,6-Diketo-bis-nor-allo-cholanic Acid.—The diketo acid of m. p. 185° prepared above was refluxed for forty-five minutes with 150 cc. of acetic acid containing 1.5 cc. of c. p. hydrochloric acid. The mixture was concentrated to incipient crystallization by evaporation *in vacuo*. Ether was added and the gray precipitate was filtered off. The acid crystallized from ethanol as leaflets of m. p. 244°.

Anal. Calcd. for $C_{22}H_{32}O_4$: C, 73.3; H, 8.9. Found: C, 73.0; H, 8.9.

The methyl ester prepared with ethereal diazomethane after recrystallization from ether–pentane melted at 211°. The mixed melting point with acid of m. p. 244° was 200°.

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.7; H, 9.1. Found: C, 73.8; H, 9.3.

bis-nor-allo-Hydesoxycholic Acid.—Five and one-half grams of *3,6-diketo-bis-nor-allo-cholanic acid* in 100 cc. of acetic acid was shaken with Adams catalyst under 3 atm. of hydrogen for three hours. The catalyst was filtered off and the filtrate was concentrated by evaporation *in vacuo*. The acid crystallized from methanol as white needles, of m. p. 259°; yield 5 g.

Anal. Calcd. for $C_{22}H_{36}O_4$: C, 72.5; H, 10.0. Found: C, 72.1; H, 9.9.

The methyl ester was prepared with ethereal diazomethane. It crystallized from ether–pentane as white leaflets of m. p. 233°. The mixed melting point with acid of m. p. 256° was 233°.

Anal. Calcd. for $C_{23}H_{38}O_4$: C, 72.9; H, 10.1. Found: C, 73.0; H, 10.1.

Diacetate of bis-nor-allo-hydesoxycholic Acid.—One-half a gram of *bis-nor-allo-hydesoxycholic acid* was refluxed for ten minutes with 20 cc. of acetic anhydride. The solvent was evaporated *in vacuo* and the residue was dissolved in methyl alcohol. The crystals obtained melted at 115°.

Anal. Calcd. for $C_{26}H_{40}O_8 \cdot 0.5CH_3OH$: C, 68.5; H, 9.1. Found: C, 68.2; H, 9.0.

The methyl ester was prepared with ethereal diazomethane. The substance crystallized by rubbing with pentane melted at 135°. The mixed melting point with acid (m. p. 115°) was 111°.

Anal. Calcd. for $C_{27}H_{42}O_8$: C, 70.1; H, 9.2. Found: C, 70.1; H, 9.0.

Summary

allo-Hydesoxycholic acid and 6-hydroxyisoandrosterone have been prepared by oxidation of the diacetate of cholestanediol-3,6. The preparation of *nor-allo-hydesoxycholic acid* and of *bis-nor-allo-hydesoxycholic acid* has been described.

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