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

Configuration of the C_6 Hydroxyl Group in Hyodesoxycholic Acid

BY ROBERT BRUCE MOFFETT AND WILLARD M. HOEHN

The structure of hyodesoxycholic acid (from hog bile) has long been established[1] except for the configuration of the hydroxyl group at C_6. This has been written as both alpha and beta by various workers[2] without adequate proof. In a private communication Mr. James S. Moffatt[3] describes the oxidative degradation of 3(β),6(β)-diacetoxycoprostane[4] (the structure of which has been established[4,5]) to the corresponding 3(β),6(β)-dihydroxycholanic acid. This acid had a melting point of 250° which is in fair agreement with the melting point of 258° reported by Tukamoto[2c] for the acid he obtained by the reduction of dehydrohyodesoxycholic acid. This supports the structure of 3(β),6(β)-dihydroxycholanic acid assigned by Tukamoto to his acid. By elimination this establishes the structure of the other 3(β),6-dihydroxycholanic acid, namely, "β"-hyodesoxycholic acid isolated from hog bile by Kimura[2b] (m. p. 189–190°) and prepared by Tukamoto[2c] (m. p. 190°) by the reduction of dehydrohyodesoxycholic acid.

This leaves in doubt only the configurations of the C_6-hydroxyl-groups in the two 3(α),6-hydroxycholanic acids. In this paper we are reporting the reduction of the 3-keto-6-hydroxycholanic acid[2d,6] in acetic acid containing a little hydrobromic acid with hydrogen and platinum to an acid melting at 191–192°. This acid gives a melting point depression when mixed with hyodesoxycholic acid, and its methyl ester gives a precipitate with digitonin in aqueous methanol. This must therefore be identical with Kimura's "β"-hyodesoxycholic acid which Moffatt has shown to be 3(β),6(α)-dihydroxycholanic acid. Since this acid was obtained from hyodesoxycholic acid without disturbing the configuration of the C_6 hydroxyl group, hyodesoxycholic acid must therefore be assigned the structure 3(α),6(α)-dihydroxycholanic acid, and the other 3(α),6-dihydroxycholanic acid (m. p. 205–208°)[2c,d] must be 3(α),6(β)-dihydroxycholanic acid.

In our previous paper,[2d] this latter acid was obtained by catalytic reduction in alcohol of methyl 3(α)-acetoxy-6-ketocholanate followed by hydrolysis. Since Windaus[1b] has observed a change of configuration at C_5 on reduction of dehydrohyodesoxycholic acid (in acetic acid with platinum), and Wieland and Dane[7] observed a similar change on reduction of 6-ketocholenic acid there might be some possible doubt whether or not we had indeed a cholanic acid derivative and not a 3,6-dihydroxy-*allo*-cholanic acid. This point has been established by the reoxidation of a sample of our methyl 3-acetoxy-6-hydroxycholanate back to the starting methyl 3(α)-acetoxy-6-ketocholanate.

Experimental

Reduction of 3-Keto-6(α)-hydroxycholanic Acid.—To a solution of 548 mg. of 3-keto-6(α)-hydroxycholanic acid[2d] in 10 ml. of acetic acid (redistilled from potassium permanganate) was added 50 mg. of platinum oxide catalyst and a few drops of redistilled 48% hydrobromic acid. This was hydrogenated at room temperature (25°) for a period of two days during which it was shaken for seventeen hours. A total of 33 ml. of hydrogen was absorbed (calcd. about 35 ml.). The solution was filtered, concentrated almost to dryness, and warmed for one-half hour on a steam-bath with 15 ml. of aqueous 3% sodium hydroxide solution. This was diluted with water, extracted with ether and acidified with dilute hydrochloric acid. On shaking with ether the acid only partly dissolved and the remaining crystalline solid was collected and dried, weight 300 mg. Boiling with ethyl acetate gave 200 mg. of crystals melting at 175–185°.

The ether solution from the above was evaporated giving a residue which crystallized on heating with ethyl acetate, weight 103 mg., m. p. 187–189°. This was recrystallized from acetone giving crystals melting at 191–192°. Recrystallization from ethyl acetate did not raise the melting point. A mixed melting point with hyodesoxycholic acid (m. p. 197–198°) gave a definite depression, melting at 184–189°.

A few milligrams of this acid was converted to its methyl ester with diazomethane in ether. The solvent was removed and the amorphous residue was taken up in 90% methanol and a few drops of a saturated solution of digitonin in 90% methanol was added. On standing in an open flask a flocculent precipitate separated. Methyl hyodesoxycholate did not give a precipitate with digitonin under similar conditions.

Oxidation of Methyl 3(α)-Acetoxy-6(β)-hydroxycholanate.—A solution of 24 mg. of methyl 3(α)-acetoxy-6(β)-hydroxycholanate[2d] in 2 ml. of acetic acid was treated with 0.295 ml. (20% excess) of 4.34 *N* chromic acid solution. After standing overnight at 25° a few drops of methanol was added and the solution was poured into ice water and extracted with ether. The ether solution was washed with water, then with sodium bicarbonate solution and dried over sodium sulfate. On concentrating the ether solution the product crystallized, giving 15 mg. of crystals melting at 156–157°. A mixed melting point with methyl 3(α)-acetoxy-6-ketocholanate gave no depression, but a mixed melting point with 3(α)-acetoxy-6-keto-allocholanate (m. p. 182–184°) gave a definite depression, melting at 145–152°.

Summary

1. 3-Keto-6-hydroxycholanic acid, derived from hyodesoxycholic acid, has been reduced to

(1) (a) Windaus and Bohne, *Ann.*, **433**, 278 (1923); (b) Windaus, *ibid.*, **447**, 233 (1926); (c) Wieland, Dane and Martius, *Z. physiol. Chem.*, **215**, 18 (1933).

(2) (a) Sugiyama, *J. Biochem. Japan*, **25**, 157 (1937); (b) Kimura, *Z. physiol. Chem.*, **248**, 280 (1937); (c) Tukamoto, *J. Biochem. Japan*, **32**, 451, 467 (1940); (d) Hoehn, Linsk and Moffett, THIS JOURNAL, **68**, 1855 (1946). (e) Moffett, Stafford, Linsk and Hoehn, *ibid.*, **68**, 1857 (1946).

(3) James S. Moffatt (Chem. Department, University of Glasgow, Glasgow, W. 2, Scotland), "3(β),6(β)-Dihydroxycholanic acid, an Epimeride of Hyodesoxycholic Acid," submitted for publication October, 1946.

(4) Prelog and Tagmann, *Helv. Chim. Acta*, **27**, 1880 (1944).

(5) Reichstein and Reich, *Ann. Rev. Biochem.*, **15**, 155 (1946).

(6) Gallagher and Xenos, *J. Biol. Chem.*, **165**, 365 (1946).

(7) Wieland and Dane, *Z. physiol. Chem.*, **212**, 41 (1932).

3(β)-6(α)-dihydroxycholic acid (m. p. 191–192°). In conjunction with work soon to be published by James S. Moffatt this establishes the configuration of the C_6-hydroxyl group in hyodesoxycholic acid.

2. Methyl 3(α)-acetoxy-6(β)-hydroxycholanate previously reported as obtained by reduction of methyl 3(α)-acetoxy-6-ketocholanate has been reoxidized to the starting ketone thus confirming the cholanic acid structure of this compound.

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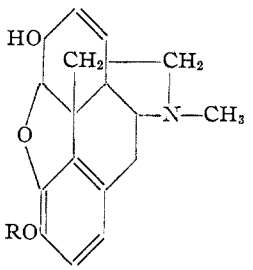
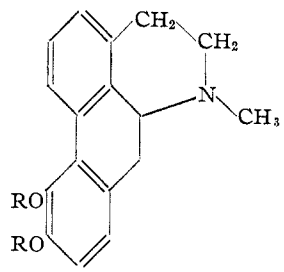
A Possible Route to the Location of the Nitrogen Atom in Morphine. I

BY H. L. HOLMES[1] AND C. C. LEE[2]

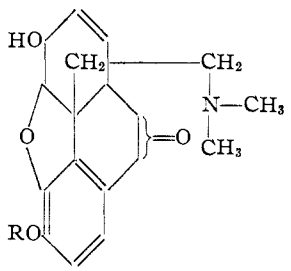
Although twenty years have elapsed since the proposal of an adequate structure for morphine I (R = H)[3] no evidence has been presented to definitely locate both the carbon and nitrogen ends of the ethanamine chain. The conversion of morphine to apomorphine, II (R = H), and the synthesis of apomorphine dimethyl ether, II (R = CH_3),[4,5] might be construed as evidence for the location of the nitrogen atom at C_9 of the hydrophenanthrene nucleus. However, in the light of the conversion of thebaine to thebenine, there is no reason to suppose that the nitrogen end of the ethanamine chain, like the carbon end, has not suffered a change. It was the oxidation of codeine, I (R = CH_3) to hydroxycodeine[6] followed by characterization of its methine (III) as a ketone[7,8] and identification of its acetolysis product as a 9- or 10- acetoxy derivative of acetylmethylmorphol (IV) that unequivocally located the nitrogen at C_9 or C_{10}.



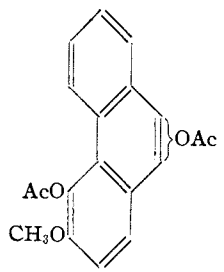
R = H or CH_3
I



R = H or CH_3
II



III



IV

It is to be seen that if the nitrogen atom were related to the contiguous hydroxyl of hydroxycodeine then location of the hydroxyl group would definitely establish the position of the nitrogen atom in the morphine molecule. In relating the nitrogen atom to the hydroxyl group only three possibilities need be considered: (a) are the nitrogen atom and the hydroxyl attached to the same carbon atom, (b) are the nitrogen atom and hydroxyl attached to different carbon atoms and (c) is hydroxycodeine an intermediate product (an amine oxide) that is readily isomerized to a product falling into one of the above categories.[9] In the event that hydroxycodeine is of type "a" then like hydroxystrychnine and cotarnine it should exhibit properties diagnostic for a carbinolamine. Furthermore, since the structure about the nitrogen atom of codeine exhibits a close similarity to that about N^b of strychnine[10] then it might be expected that hydroxycodeine would result by a method similar to that for the preparation of hydroxystrychnine.[11] This has not been realized. The yield of hydroxycodeine has been improved to 15% by the controlled oxidation of codeine with chromic acid.[12] The melting point of this base is largely dependent upon the type of apparatus used and upon the rate of heating and it has been found more satisfactory to characterize this base by its rotation in chloroform solution ($[\alpha]^{22}D$ −115 ± 1°). The chloroplatinate, methiodide and picrate have also been prepared.

A control experiment with strychnine, run simultaneously with the attempted oxidation of codeine, gave hydroxystrychnine which was characterized by conversion to its methyl and ethyl ethers by solution in the respective alcohols. Hydroxycodeine, under similar conditions, failed to give an ether. Extension of the analogy with hydroxystrychnine failed, for unlike this base,[13]

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(2) This work, which has been sponsored by a grant from the National Research Council of Canada, has been presented in a thesis to the College of Engineering in partial fulfilment of the requirements for the degree of Bachelor of Science in Chemical Engineering.

(3) Gulland and Robinson, *Mem. Proc. Manchester Lit. & Phil. Soc.*, **69**, 79 (1925).

(4) Pschorr and Avenarius, *Ber.*, **62**, 321 (1929).

(5) Späth and Hromatka, *ibid.*, **62**, 325 (1929).

(6) Ach and Knorr, *ibid.*, **36**, 3067 (1903).

(7) Pschorr and Einbeck, *ibid.*, **40**, 1980 (1907).

(8) Knorr and Hörlein, 2042 (1907).

(9) Pinner and Wolffenstein, *ibid.*, **25**, 1428 (1892).

(10) Holmes, Openshaw and Robinson, *J. Chem. Soc.*, 908 (1946).

(11) Leuchs, *Ber.*, **70**, 1543 (1937).

(12) Knorr and Schneider, *ibid.*, **39**, 1414 (1906).

(13) Leuchs, Flammersfeld, Villain and Schöne, *ibid.*, **76**, 1065 (1943).