converted to a *bis-m*-nitrobenzenesulfonate. It is concluded that this substance is closely related to cannabinol in structure. Half the molecule is probably a dihydroxy *n*-amylphenyl, the other half probably an unsaturated alicylic nucleus.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLUMBIA UNIVERSITY]

The Preparation and Properties of 3(α),11-Dihydroxy-12-ketocholanic Acid

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The isolation of naturally occurring steroids in which an oxygen atom is believed to occupy position 11[2,3,4] renders of interest the study of model substances in which ring C carries a functional group in this, and only in this, position.

The original aim of this investigation was to prepare 3,11-dihydroxycholanic acid by elimination of the keto group of 3,11-dihydroxy-12-ketocholanic acid, which has been described recently by Marker and Lawson.[5] Although this object has not been accomplished, we are reporting here the preparation and properties of the starting compound because our results not only extend but in some points fail to confirm the work of these authors. Their procedure is essentially analogous to that of Wieland and Posternak[6] for the preparation of 11-hydroxy-12-ketocholanic acid. They[5] acetylated 3-hydroxy-12-ketocholanic acid, and then brominated it in position 11. The bromo acid was hydrolyzed to 3,11-dihydroxy-12-ketocholanic acid. Neither of the two intermediates was isolated. In our hands this abbreviated procedure failed to yield the end-product in crystalline form. We found it necessary to conduct the reaction in two separate steps. We furthermore had to modify the method of bromination in order to limit the reaction to a substitution with only one bromine atom. Although the bromo acid thus obtained was amorphous and its bromine content still slightly too high, its hydrolysis yielded the desired 3,11-dihydroxy-12-ketocholanic acid without any difficulty. The over-all yield from 3-hydroxy-12-ketocholanic acid was over 50%, whereas Marker and Lawson obtained in 35% yield a product which melted 9° lower than ours.

In order to eliminate any doubt regarding the site of the new hydroxyl group, the monobromo acid was subjected to debromination with anhydrous sodium acetate in analogy to the experiment of Barnett and Reichstein[7] on 11-bromo-12-ketocholanic acid. The ultraviolet absorption spectrum of the resulting compound (m. p. 201°) exhibited a maximum at 241 mμ (ϵ = 9000, solvent alcohol), which is characteristic for α,β-unsaturated ketones. The unsaturated compound is, therefore, $\Delta^{9,11}$-3-acetoxy-12-ketocholenic acid. Furthermore, the acid 3-succinate of methyl 3,11-dihydroxy-12-ketocholanate was oxidized with chromic acid to the corresponding 11,12-diketone. The ultraviolet absorption spectrum of the resulting compound is similar to that reported by Barnett and Reichstein for methyl 11,12-diketocholanate (maximum at 284 mμ, ϵ = 135; minimum at 250 mμ, ϵ = 80, solvent alcohol). Saponification of the diketo ester failed to yield a crystalline compound.

When we tried to repeat the preparation of the 3-monoacetate of 3,11-dihydroxy-12-ketocholanic acid described by Marker and Lawson, we observed that the reaction takes a more complicated course than the report of these authors indicates. Our acetylation product showed the same melting point (268°) as their monoacetyl derivative, but the analysis indicated the loss of a molecule of water as well as the esterification of one hydroxyl group. We are unable to explain the considerable discrepancy between the carbon value of our analyses and that reported by Marker and Lawson, but it must be pointed out that the values assigned in their paper to the monoacetyl acid, $C_{26}H_{40}O_6$, with which their experimental figures agree, are incorrectly calculated (C, 70.5; H, 8.8, instead of C, 69.60; H, 8.99). Furthermore, our substance, in contradistinction to the starting compound, is insoluble in aqueous sodium carbonate. Hydrolysis at room temperature

(1) Commonwealth Fund Fellow, 1938–1939.

(2) Reichstein, *Ergeb. der Vitamin- und Hormonforschung*, **1**, 334 (1938).

(3) Marker, Kamm, Crooks, Oakwood, Wittle and Lawson, THIS JOURNAL, **60**, 210 (1938).

(4) Tschesche and Bohle, *Ber.*, **69**, 793, 2497 (1936).

(5) Marker and Lawson, THIS JOURNAL, **60**, 1334 (1938).

(6) Wieland and Posternak, *Z. physiol. Chem.*, **197**, 17 (1931).

(7) Barnett and Reichstein, *Helv. Chim. Acta*, **21**, 926 (1938).

with standard alkali resulted in the neutralization of two equivalents of base and recovery of the original acid. The neutral compound is, therefore, either an 11-(or 12-enol)-lactone or a dimolecular anhydride, more likely the latter.[8] When the compound was boiled for an hour with 33% acetic acid, an acid extractable with aqueous sodium carbonate and melting at 106° was obtained in good yield. According to the analysis, which conforms with $C_{26}H_{40}O_6 \cdot 1/2H_2O$, this acid represents, in all probability, the true monoacetate of 3,11-dihydroxy-12-ketocholanic acid.

Besides the anhydride, which can be obtained in 20–30% yield by direct crystallization of the acetylated material, an acidic product, which would not crystallize, was formed in the acetylation reaction. Analyses and titration data indicated that this product is a diacetyl derivative of the starting acid. When the acetylation reaction was conducted in pyridine, this acidic substance was the only reaction product.

Both the anhydride and the amorphous diacetoxy acid yielded the starting acid on alkaline hydrolysis. Careful comparison of the specific rotations of the two hydrolysis products and of the original preparation revealed no significant differences. This finding precludes the possibility that the simultaneous formation of neutral and acidic reaction products on treatment with acetic anhydride is due to the existence of two 11-epimers in the original preparation, one of which might be amenable to acetylation at C_{11} under the conditions employed.

All our attempts to prepare keto derivatives of 3,11-dihydroxy-12-ketocholanic acid have been unsuccessful. The preparations of the semicarbazone, the hydrazone, and the oxime were essayed, but in each case the starting material was recovered unchanged. Marker and Lawson claimed to have prepared a semicarbazone melting at 232° in 18% yield, but no analysis of the product is reported. We have experienced consistent failures in our attempts to prepare this semicarbazone by the method of Marker and Lawson. We also tried, without success, to let the acid react with semicarbazide acetate in cold pyridine solution, in boiling butyl alcohol, and in alcohol at 190° in a bomb tube. Apparently the steric hindrance exerted by the groups on the adjoining carbon atoms is too great to permit reaction with the 12-keto group.

Treatment of the acid with hydrazine hydrate and sodium ethylate at 200° resulted in the formation of a compound, m. p. 162°, with two oxygen atoms less than the starting product. Obviously the keto group at C_{12} had been eliminated, but at the same time the hydroxyl at C_{11} was lost by a dehydration reaction.

(8) Professor Reichstein (private communication) has observed the formation of dimolecular anhydrides, which could be hydrolyzed with acetic acid, on acetylation of hydroxy acids of a similar type. We are highly indebted to him for this information.

Experimental

3-Hydroxy-12-ketocholanic acid was obtained in good yield from desoxycholic acid by the method of Kaziro and Shimada[9] as modified by Cortese,[10] m. p. 162–163°. The 3-acetyl derivative was prepared by treatment with acetic anhydride in pyridine, m. p. 197–198°. The sample for analysis was dried *in vacuo* at 110°.

Anal. Calcd. for $C_{26}H_{40}O_5$: C, 72.18; H, 9.32. Found: C, 72.01; H, 9.18.

3(α)-Acetoxy-11-bromo-12-ketocholanic Acid.—Five grams of 3(α)-hydroxy-12-ketocholanic acid was refluxed for one hour in a mixture of 15 cc. of glacial acetic acid and 2.5 cc. of acetic anhydride. The reaction mixture, after dilution to 50 cc. with glacial acetic acid, was treated with 0.7 cc. of bromine in 40 cc. of glacial acetic acid. The solution was stirred mechanically during the dropwise addition of the bromine, which required three to four hours. The temperature was maintained at 50–60° during this period and thereafter for four to five hours, while stirring was continued. After cooling to room temperature, 250 cc. of water was added slowly with stirring. The reddish-yellow gum which separated was dissolved in 350 cc. of 95% ethanol, and this solution was poured slowly into 2450 cc. of water. The resulting milky suspension formed a flocculent precipitate upon the addition of 16 cc. of glacial acetic acid. On filtering and washing once with a solution of ethanol, water and acetic acid (3.5, 24.5, and 0.16 parts, respectively), and twice with water, 5.82 g. of the amorphous bromo acid was obtained, which melted with decomposition at 159°. The sample for analysis was dried *in vacuo* at 110°.

Anal. Calcd. for $C_{26}H_{39}O_5Br$: Br, 15.62. Found: Br, 16.1, 16.3, 16.86.

3(α),11-Dihydroxy-12-ketocholanic Acid.—The bromo acid (5.82 g.) was refluxed for twenty minutes in 115 cc. of 20% methyl alcoholic potassium hydroxide solution. The hydrolysate was poured into 1150 cc. of ice water. On acidification with hydrochloric acid a flocculent precipitate formed, which was separated by filtration and washed with water; yield 4.4 g. It was dissolved in 325 cc. of hot 95% ethanol and 825 cc. of water was added to the hot solution. On slow cooling a crystalline precipitate formed; yield 2.94 g. Several recrystallizations from aqueous ethanol yielded plates, m. p. 202°, $[\alpha]^{24}D$ +65.7° (2.5% in 95% alcohol). Further recrystallization from 95% ethanol produced blunt rods, m. p. 205°, $[\alpha]^{27}D$

(9) Kaziro and Shimada, *Z. physiol. Chem.*, **249**, 220 (1937).

(10) Cortese, unpublished experiments. The coöperation of Dr. Cortese in permitting us the use of his method prior to publication is gratefully acknowledged.

+67.1° (1.3% in 95% alcohol). The sample for analysis was dried *in vacuo* at 110°.

Anal. Calcd. for $C_{24}H_{38}O_5$: C, 70.9; H, 9.42; neut. equiv., 406. Found: C, 70.8; H, 9.41; neut. equiv. (115.4 mg.), 412.

Methyl 3(α),11-Dihydroxy-12-ketocholanate.—An ether solution of 20 mg. of the acid was treated with diazomethane in ether. After two hours in the refrigerator, the excess diazomethane was removed by gentle heating and the ether was washed with 5% sodium carbonate solution and with water. Evaporation of the ether yielded 18.7 mg. The ester crystallized from methanol in triangular plates, m. p. 157°. The sample for analysis was dried *in vacuo* at 110°.

Anal. Calcd. for $C_{25}H_{40}O_5$: C, 71.39; H, 9.59. Found: C, 70.97; H, 9.36.

3(α),11-Diformoxy-12-ketocholanic Acid.—A solution of 69.8 mg. of the acid, m. p. 202°, in 0.5 cc. of 98–100% formic acid, was allowed to stand at room temperature for twenty-four hours. When 0.4 cc. of 95% ethanol and 1.1 cc. of water were added, an oil separated out which was extracted quickly with ether. The residue of the washed and dried ether solution was freed from traces of formic acid *in vacuo*. After several recrystallizations from aqueous ethanol, needles were obtained, m. p. 146–148°. The sample for analysis was dried *in vacuo* at 110°.

Anal. Calcd. for $C_{26}H_{38}O_7$: C, 67.51; H, 8.28. Found: C, 67.41; H, 8.43.

Acetylation of 3(α),11-Dihydroxy-12-ketocholanic Acid.—A solution of 678 mg. of the acid (m. p. 199–201°) in 21 cc. of acetic anhydride was boiled under reflux for one and one-half hours. The acetic anhydride was decomposed with ice water. The gummy, white precipitate was centrifuged, washed, and dissolved in a minimal amount of hot 95% ethanol. A crystalline precipitate formed on standing overnight in the refrigerator. This was removed by centrifuging and washed with 95% ethanol and ether (116 mg.). The supernatant and washings were poured into 50 cc. of water and thoroughly extracted with ether. The ether solution was then extracted exhaustively with 5% sodium carbonate solution, then with water and brought to dryness (257 mg.).

The combined sodium carbonate extracts were acidified with hydrochloric acid and extracted with ether. The residue from the washed and dried ether yielded 431 mg.

The yields of alkali insoluble and alkali soluble fractions represent approximately a 50% partition, a finding which was uniform in several experiments. In this experiment 17% of the starting material was obtained as the crystalline anhydride. In other experiments yields of 27, 32, and 26% were obtained.

The Crystalline Anhydride.—The crystalline product from the acetylation reaction was purified by recrystallization from 95% ethanol, m. p. 268°. It crystallized in fine, white needles, soluble in hot 95% alcohol and insoluble in ether and aqueous alkali. The sample for analysis was dried *in vacuo* at 110°.

Anal. Calcd. for $C_{26}H_{38}O_5$: C, 72.52; H, 8.90. Found: C, 72.18; H, 8.78. C, 72.11; H, 8.84. C, 72.14; H, 8.89. *Titration.* A solution of 14.5 mg. (0.0337 millimole) in absolute alcohol reacted neutral to phenolphthalein. Standard alkali was added and the excess titrated back after forty-four hours. Alkali neutralized: calculated, 0.067 milliequiv.; found, 0.0668 milliequiv. The titration mixture was acidified and extracted with ether. The ether was washed with water and evaporated, and the residue was crystallized from aqueous ethanol, m. p. 203–204°. $[\alpha]^{24}D$ +67.2° (0.33% in 95% alcohol).

3(α)-Acetoxy-11-hydroxy-12-ketocholanic Acid.—A solution of 18 mg. of the anhydride (m. p. 268°) in 6 cc. of 33% acetic acid was boiled under reflux for one hour. The reaction mixture was extracted with ether and the ether in turn extracted with 5% sodium carbonate solution. The alkaline extract was worked up in the usual way; yield, 14.4 mg. This product crystallized from aqueous ethanol in needles, m. p. 106°. The sample for analysis was dried *in vacuo* at 61°.

Anal. Calcd. for $C_{26}H_{40}O_6 \cdot {}^1/_2H_2O$: C, 68.24; H, 9.03. Found: C, 68.35; H, 9.14.

The Acidic Fraction from the Acetylation Reaction.—Efforts to crystallize the acidic fraction from the original separation of the acetylation mixture were unsuccessful. The sample for analysis was dried *in vacuo* at 110°.

Anal. Calcd. for $C_{28}H_{42}O_7$: C, 68.54; H, 8.63. Found: C, 68.47; H, 9.41.

In the hope that the ester might be obtained in crystalline form the compound was esterified with diazomethane. An oily product which could not be crystallized was obtained.

Titration. A solution of 41.8 mg. of the ester (0.0828 millimol.) in absolute methanol was neutral to phenolphthalein. Standard alkali was added and the excess titrated back after sixty hours. Alkali neutralized: calculated, 0.2485 milliequiv.; found, 0.2247 milliequiv. A solution of 55.3 mg. (0.1096 millimol.) was treated similarly. Alkali neutralized: calculated, 0.3288 milliequiv.; found, 0.352 milliequiv.

The acid was recovered from the titration mixture by extraction with ether, and was crystallized from aqueous ethanol, m. p. 203–204°; $[\alpha]^{23}D$ +63.4° (0.68% in 95% alcohol). All later runs yielded preparations with the higher value (+67°).

Acetylation in Pyridine.—A solution of 54 mg. of the starting acid in 2 cc. of pyridine was treated with 1.8 cc. of acetic anhydride. After standing overnight at room temperature, the acetic anhydride was decomposed with water, and the reaction products taken up in ether. The ether solution was washed with 5% hydrochloric acid, and then extracted with 5% sodium carbonate solution. Upon acidification and extraction with ether the alkaline extract yielded 63 mg. of a colorless oil. The sample for analysis was dried *in vacuo* at 110°.

Anal. Calcd. for $C_{28}H_{42}O_7$: C, 68.55; H, 8.62. Found: C, 68.55; H, 8.50.

$\Delta^{9,11}$-3(α)-Acetoxy-12-ketocholenic Acid.—A solution of 400 mg. of the amorphous bromo acid and 200 mg. of fused sodium acetate in 2.5 cc. of glacial acetic acid was heated in a bomb tube at 185–190° for seventeen hours. The acetic acid of the reaction mixture was partially neutralized with sodium carbonate, and the reaction products were extracted with ether. The ether solution was filtered to remove tar, and then extracted with 5% sodium car-

bonate solution. A precipitate separated in the alkaline solution, which was removed by centrifuging. The aqueous solution of the precipitate was acidified with hydrochloric acid, and extracted with ether. The ether extract, after washing and drying, yielded 98.5 mg. Since the residue did not crystallize well, it was subjected to three additional precipitations as the sodium salt. The acid finally obtained on decomposition of the latter was crystallized from aqueous ethanol until the melting point became constant at 201°. The sample for analysis was dried *in vacuo* at 110°.

Anal. Calcd. for $C_{26}H_{38}O_5$: C, 72.52; H, 8.90. Found: C, 72.63; H, 9.00.

Acid Succinate of Methyl 3(α)-Hydroxy-11,12-diketocholanate.—A solution of 275 mg. of crystalline methyl 3,11-dihydroxy-12-ketocholanate and 655 mg. of succinic anhydride in 10 cc. of dry pyridine was allowed to stand at room temperature for twenty hours. It was then heated under reflux at 135° (bath temperature) for one-half hour. The reaction mixture was poured into water and extracted with ether. After removal of the pyridine by repeated washing with 5% hydrochloric acid, the ether was evaporated. The residue was dissolved in 7.5 cc. of glacial acetic acid, 0.5 cc. of water and 100 mg. of chromic acid added. After standing at room temperature for twenty hours, the reaction mixture was diluted with water and extracted with ether. The ether solution was then extracted with 5% sodium carbonate solution, which, after removal of the acetic acid, resulted in the precipitation of the reaction product as the sodium salt. This was converted into the free acid in the usual way (76 mg.). Crystallization from 80% methanol yielded plates, m. p. 194–196°. The sample for analysis was dried *in vacuo* at 110°.

Anal. Calcd. for $C_{29}H_{42}O_8$: C, 67.16; H, 8.16. Found: C, 66.65; H, 8.39.

Saponification with alkali did not yield any crystallizable products.

Attempted Preparation of the Semicarbazone.—A solution of 100 mg. of 3(α),11-dihydroxy-12-ketocholanic acid, 110 mg. of semicarbazide hydrochloride and 110 mg. of potassium acetate in 2.4 cc. of 83% ethanol was refluxed for fifteen hours, and then worked up in the usual way. After recrystallization, 86.5 mg. of the starting acid was recovered, m. p. 200–202°. Attempts to prepare the semicarbazone in pyridine solution, by refluxing in butyl alcohol, and by heating at 190° in a bomb tube, were similarly unsuccessful. Furthermore, all efforts to prepare the hydrazone and the oxime met with failure.

Treatment of 3(α),11-Dihydroxy-12-ketocholanic Acid with Sodium Ethylate and Hydrazine Hydrate.—A mixture of 500 mg. of the acid, 500 mg. of sodium in 17.5 cc. of absolute alcohol, and 2.5 cc. of hydrazine hydrate was heated in a bomb tube at 197–200° for eight hours. The reaction mixture was poured into water, acidified with hydrochloric acid, and extracted with ether. The ether was washed with water, dried over sodium sulfate, and evaporated to yield an oil which could not be crystallized. The entire product was dissolved in 17 cc. of pyridine together with 1.275 g. of succinic anhydride, and was heated under reflux at 130–135° (bath temperature) for one hour. After cooling, the reaction mixture was poured into water and extracted with ether. The ether solution was extracted with 5% sodium carbonate solution, and the alkali soluble material recovered in the usual way (600 mg.). Crystallization from aqueous ethanol yielded 78 mg. of plates melting at 227° with decomposition. The sample for analysis was dried *in vacuo* at 110°.

Anal. Calcd. for $C_{28}H_{42}O_6$: C, 70.85; H, 8.92. Found: C, 70.80; H, 8.75.

Titration. 17.93 mg. (0.0378 millimol.); neutralization equivalent, calculated 237; found 241. After subsequent saponification with excess alkali, calculated 0.0377 milliequiv.; found 0.0370 milliequiv. of base neutralized.

Saponification of the acid succinate with 0.1 *N* sodium hydroxide produced an acid which softened at 135° and melted at 162–163°. The sample for analysis was dried *in vacuo* at 110°.

Anal. Calcd. for $C_{24}H_{38}O_8$: C, 76.96; H, 10.25. Calcd. for $C_{24}H_{38}O_8 \cdot {}^1/_2H_2O$: C, 75.15; H, 10.35. Found: C, 75.63; H, 10.48.

The analyses reported in this paper were performed by Mr. William Saschek.

Summary

The procedure of Marker and Lawson for the preparation of 3,11-dihydroxy-12-ketocholanic acid has been modified so as to give better yields. The intermediate 11-bromo acid was isolated in amorphous form. The position of the new substituent group was confirmed by conversion of the bromo acid into $\Delta^{9,11}$-3-hydroxy-12-ketocholenic acid, and of the dihydroxy acid into the acid 3-succinate of methyl 3-hydroxy-11,12-diketocholanate. The acetyl derivative of 3,11-dihydroxy-12-ketocholanic acid described by Marker and Lawson is actually a neutral compound, that is, either a 3-acetoxy lactone or a dimolecular anhydride. On hydrolysis with acetic acid this substance yields the true 3-acetoxy-11-hydroxy-12-keto-cholanic acid. The 11-hydroxy group is also capable of acylation as evidenced by the preparation of a crystalline diformyl and of an amorphous diacetyl derivative.

All attempts to prepare the semicarbazone of 3,11-dihydroxy-12-ketocholanic acid, described by Marker and Lawson, as well as other ketone derivatives, failed. Treatment of the acid with sodium ethylate and hydrazine hydrate at 200° resulted in elimination of both oxygen atoms in ring C.

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