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The Preparation and Degradation of Lithocholic Acid

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The importance of desoxycorticosterone as the most active of the crystalline substances thus far isolated from the adrenal gland makes it desirable to develop methods for its preparation. Stigmasterol[1] and cholesterol[2] have been used as raw materials for its preparation by several procedures. It is the purpose of this note to describe some improved procedures for the conversion of bile acids into materials suitable for the preparation of desoxycorticosterone and related substances.

TABLE I

DEGRADATION PRODUCTS OF LITHOCHOLIC ACID

Compound	M. p. °C.	$[\alpha]^{25}_{5461}$	Formula	Calcd., % C	Calcd., % H	Found, % C	Found, % H
3-Acetoxy-*bis-nor*-cholanyldiphenylethylene	158–160	+75.5 (in $CHCl_3$)	$C_{38}H_{50}O_2$	84.71	9.36		
3-Acetoxypregnanyldiphenylethylene	150–152	+140 ± 3° (in $CHCl_3$)	$C_{37}H_{48}O_2$	84.61	9.22		
1,1-Diphenyl-methyl-(3-acetoxy-*etio*-cholanyl)-ethylene	158–160	+398 ± 2° (in $CHCl_3$)	$C_{36}H_{46}O_2$	84.65	9.08	84.73	9.31
Pregnanol-3(α)-one-20	148–149	+129 ± 3° (in alcohol)	$C_{21}H_{34}O_2$	79.19	10.76	78.85	10.74
21-Benzalpregnanolone	228–230	+181 ± 3° (in alcohol)	$C_{28}H_{38}O_2$	82.71	9.42	82.34	9.51
3-Acetoxy-*etio*-cholanic acid	226–229	+86.4 ± 3° (in alcohol)	$C_{22}H_{34}O_4$	72.87	9.46	72.70	9.33
etio-Lithocholic acid	270–272		$C_{20}H_{32}O_3$	74.96	10.07	74.51	10.06

The first step was the conversion of cholic and desoxycholic acids to lithocholic acid. This conversion has been accomplished by a number of procedures[3] but the over-all yields have not been encouraging. By the action of benzoyl chloride and pyridine on methyl desoxycholate at 15° the 3-monobenzoate was obtained in good yield. Oxidation to methyl 3-benzoxy-12-ketocholanate with chromic acid proceeded smoothly as did the subsequent preparation of the semicarbazone. The over-all yield of semicarbazone from methyl desoxycholate was more than 80%. Reduction of the semicarbazone by the Wolff–Kishner method yielded an average of 60% of recrystallized lithocholic acid.

The degradation of lithocholic acid by the Wieland–Barbier method was performed as previously described for desoxycholic acid.[4] Reindel and Niederlander[5] and Sawlewicz and Reichstein[6] have described similar degradations. The melting points and specific rotations of the compounds so obtained are given in Table I since some of these values are not recorded in the literature. Oxidation of the acetylated diphenylethylenes was performed at 45–50° because of their insolubility in acetic acid at 15°. The other procedures require no comment.

Ozonolysis of 1,1-diphenyl-methyl-(3-acetoxy-*etio*-cholanyl)-ethylene[7] followed by hydrolysis gave pregnanol-3(α)-one-20.[8] Further degradation proceeded as with the corresponding ketone derived from desoxycholic acid. The benzal ketone was isolated and its properties were found to agree with those given by Marker and Wittle[9] who have recently used this procedure for the preparation of *etio*-cholanic acids from the pregnanolones. The preparation of dehydro-*etio*-lithocholic acid and 4-bromo-3-keto-*etio*-cholanic acid by the usual methods offered no difficulties.

Experimental Part

Methyl 3-Benzoxy-12-hydroxycholanate.—A solution of 32 g. (0.08 mole) of methyl desoxycholate in 150 cc. of dry benzene was cooled to 5°, 10 cc. of dry pyridine was added, and then 9.3 cc. (0.081 mole) of benzoyl chloride in 20 cc.

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(1) Steiger and Reichstein, *Helv. Chim. Acta*, **20**, 1164 (1937).

(2) Serini, Logemann and Hildebrand, *Ber.*, **72**, 391 (1939).

(3) Recent papers are (a) Bergström and Haslewood, *J. Chem. Soc. London*, 540 (1939); (b) Dutcher and Wintersteiner, THIS JOURNAL, **61**, 1992 (1939).

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

(5) Reindel and Niederlander, *Ber.*, **68**, 1969 (1935).

(6) Sawlewicz and Reichstein, *Helv. Chim. Acta*, **20**, 949 (1937).

(7) The Referee has pointed out that we have fallen into error in our previous paper[4] in naming the diphenylethylenes derived from desoxycholic and homologous acids. *3,12-Diacetoxy-bis-nor-cholanyldiphenylethylene* is correct for the compound called 3,12-diacetoxy-*nor*-cholanyldiphenylethylene; *3,12-diacetoxypregnanyl*diphenylethylene (or *3,12-diacetoxy-ter-nor-cholanyldiphenylethylene*) instead of 3,12-diacetoxy-*bis-nor*-cholanyldiphenylethylene; and *1,1-diphenyl-methyl-(3,12-diacetoxy-etio-cholanyl)*-ethylene instead of 3,-12-diacetoxy-*ter-nor*-cholanyldiphenylethylene. The names previously applied included one carbon atom in both the ethylene group and the cholanyl radical.

(8) (a) Marker, Kamm and Jones, THIS JOURNAL, **59**, 1595 (1937); (b) Marker, Kamm and Wittle, *ibid.*, **59**, 1841 (1937); (c) Butenandt and Hildebrandt, U. S. Patent 2,156,275 (1939).

(9) Marker and Wittle, THIS JOURNAL, **61**, 1329 (1939).

of dry benzene was added at such a rate that no rise in temperature occurred. The mixture was allowed to come to room temperature and to stand for three hours. The benzene solution was washed with three 100 cc. portions of 0.5 *N* hydrochloric acid, then with 0.2 *N* sodium hydroxide and finally with water. After removal of water with anhydrous sodium sulfate, the benzene was distilled in a vacuum. The glassy residue was dissolved by warming with 200 cc. of dry ether. The etherate of methyl 3-benzoxy-12-hydroxycholanate crystallized from the solution on standing. It was air-dried and then melted at 78–80° with frothing; yield, 87–89%.

Anal. Calcd. for $C_{32}H_{46}O_5 \cdot {}^1/_2C_4H_{10}O$: C, 74.55; H, 9.39. Found: C, 74.35; H, 9.36.

When two molar equivalents of benzoyl chloride and pyridine were used, the dibenzoate of methyl desoxycholate was obtained, m. p. 145–146°.

Methyl 3-Benzoxy-12-ketocholanate.—A solution of 44 g. (0.08 mole) of methyl 3-benzoxy-12-hydroxycholanate etherate in 70 cc. of acetic acid was evaporated in a vacuum to remove the ether, and the residue was dissolved in 400 cc. of glacial acetic acid. To this solution, cooled to 15°, was added 8 g. of chromic acid in 100 cc. of 90% acetic acid. The mixture stood overnight in the refrigerator and was worked up in the usual manner for the neutral product. This substance usually was not isolated. It can be crystallized in the icebox from a large volume of absolute alcohol and then melts at 94–95°.

Semicarbazone.—The crude keto ester was dissolved in 250 cc. of alcohol and a solution of 19 g. (0.17 mole) of semicarbazide hydrochloride and 15 g. of sodium acetate in 100 cc. of 80% alcohol was added. This mixture was refluxed for fourteen hours. The bulk of the semicarbazone separated during the refluxing and was removed by filtration of the hot solution. A second crop, obtained by cooling, was purified by boiling with water. The total yield, after drying at 110° for eight hours, was 43 g. (95% based on the etherate). It melted at 160–162° after recrystallization from alcohol.

Lithocholic Acid (1).—A mixture containing 45 g. of sodium and 43 g. (0.076 mole) of the semicarbazone in 400 cc. of absolute alcohol was heated in an autoclave at about 200° for seven hours. The reaction product was dissolved in hot water and acidified to congo red with dilute hydrochloric acid. The precipitated acid was washed on the filter with hot water, dried and crystallized from acetone. The yield of lithocholic acid (m. p. 183–185°; $[\alpha]^{25}_{D}$ +34°; $[\alpha]^{25}_{5461}$ +39°) was 18.4 g. (64.6%).

Anal. Calcd. for $C_{24}H_{40}O_3$: C, 76.55; H, 10.71. Found: C, 76.36; H, 10.68.

Lithocholic Acid (2).—Seventy grams (0.110 mole) of the disemicarbazone of methyl 3-benzoxy-7,12-diketocholanate (prepared by procedures similar to those used for the preparation of the semicarbazone of methyl 3-benzoxy-12-ketocholanate) was added to a solution of 70 g. of sodium in 700 cc. of methyl alcohol and heated in an autoclave at 174 ± 5° for six hours. After solution of the reaction products in warm water the methyl alcohol was removed in a vacuum. The aqueous fraction was warmed until all solids were dissolved and was then poured with stirring into an excess of dilute hydrochloric acid. The precipitated acid, which was often somewhat gummy, was suspended in 0.8 *N* sodium hydroxide solution and heated to 40–50° on the water-bath. The sodium lithocholate which separated under these conditions was filtered out, resuspended in 1 *N* sodium hydroxide and again isolated. It was then dissolved in hot water and the solution was poured into a slight excess of dilute hydrochloric acid. The lithocholic acid was filtered off and washed well with water. Dried at 110° and crystallized from dry acetone, the yield was 25.9 g. (62.5%) of lithocholic acid (m. p. 183.5–185°).

Dehydro-*etio*-lithocholic Acid.—A solution of 390 mg. (0.00124 mole) of *etio*-lithocholic acid in 20 cc. of glacial acetic acid was cooled to 15° and 100 mg. of chromic acid in 1 cc. of 90% acetic acid was added. After one hour the excess of chromic acid was reduced with alcohol. The solvents were removed in a vacuum and the residue was extracted with 200 cc. of ether and 100 cc. of water. The ether layer was separated, washed with water until colorless and dried over anhydrous sodium sulfate. The acid, after crystallization from acetone, weighed 330 mg. (84%). Crystallization from benzene gave a product which melted at 246–248°. Marker and Wittle[9] report a melting point of 246–249° but no analytical figures are given.

Anal. Calcd. for $C_{20}H_{30}O_3$: C, 75.43; H, 9.49. Found: C, 75.46; H, 9.70.

4-Bromo-dehydro-*etio*-lithocholic Acid.—To a solution of 784 mg. (0.00246 mole) of dehydro-*etio*-lithocholic acid in 30 cc. of glacial acetic acid at 18° was added 6.3 cc. of 0.82 *N* bromine in glacial acetic acid solution. After standing ten minutes the solvents were removed in a vacuum. The residue was taken up in ethyl acetate from which crystallized 620 mg. (63.5%); m. p. 190–192°.

Anal. Calcd. for $C_{20}H_{29}O_3Br$: C, 60.45; H, 7.19. Found: C, 60.30; H, 7.40.

Summary

Lithocholic acid has been prepared from cholic and desoxycholic acids with an over-all yield of 50%. Some of the intermediate compounds of the Wolff–Kishner degradation of lithocholic acid have been described.

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