[Contribution from the Chemical Research Laboratory of the Schering Corporation and the Chemical Laboratory of Northwestern University]

The Preparation of the Homologs of 3-Hydroxy-12-ketocholanic Acid

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Three general methods have been reported for the preparation of 3-hydroxy-12-ketocholanic acid. The first involved the acylation of the 3-hydroxyl group of desoxycholic acid, then chromic acid oxidation of the 12-hydroxyl group followed by saponification.[1,2] The second method employed the selective reduction[1b] of the 3-keto group in dehydrodesoxycholic acid. The third method made use of the preferential oxidation of the 12-hydroxyl group in desoxycholic acid by means of chromic acid in acetic acid.[3]

An attempt to adapt this third method to the preparation of 3-hydroxy-12-keto-*nor* and *bisnor*-cholanic acids resulted in mixtures from which the pure keto acids could not be readily isolated, especially in the case of the *bisnor* acid. These keto acids and the corresponding *etio*-acid have been made by the first method using succinic anhydride for the esterification as outlined. The advantage of this method is that under the conditions as described, succinic anhydride apparently reacts exclusively with the 3-hydroxyl group of the starting acids. The best yields were obtained when the intermediates were not isolated in the pure state To further characterize the products the semicarbazones of 3-hydroxy-12-keto-*nor* and *bisnor*-cholanic acids were prepared. A Wolff–Kishner reduction of the semicarbazone of the *nor*-acid yielded the expected *nor*-lithocholic acid. The acetates of all the keto acids were made. Also, the dimethyl ester of the acid succinate of desoxycholic acid and methyl 3-acetoxy-12-ketocholanate were prepared.

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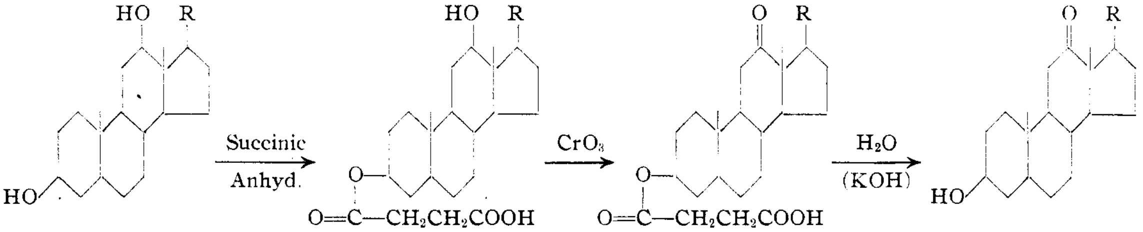
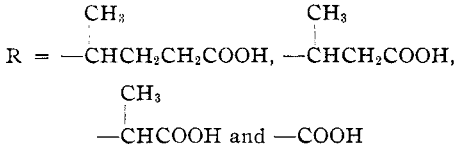
Experimental[4]

3-Acid Succinate of Desoxycholic Acid.—(B) A solution of 13 g. of succinic anhydride in 50 ml. of anhydrous pyridine, made by warming, was cooled to room temperature. To this solution was added 5 g. of desoxycholic acid and the mixture was allowed to stand overnight. It was then dropped slowly into a stirred mixture of 25 ml. of sulfuric acid in ice water. The granular precipitate was washed with water and dried, yielding 5.7 g. of material sufficiently pure for further reactions. It could be purified by crystallization from dilute methanol after treatment with charcoal, m. p. 231–232°, $[\alpha]_D$ +51.5° (150 mg. made up to 15 ml. with dioxane, α + 0.515; l, 1 dm.).





$$R = -\overset{CH_3}{\overset{|}{C}}HCH_2CH_2COOH,\ -\overset{CH_3}{\overset{|}{C}}HCH_2COOH,\ -\overset{CH_3}{\overset{|}{C}}HCOOH \text{ and } -COOH$$

(E) The time of reaction may be shortened by refluxing the pyridine solution for fifteen minutes, then heating for one hour on the steam-bath and working up as before.

Anal. Calcd. for $C_{28}H_{44}O_7$: C, 68.27; H, 9.00; sapon. equiv., 164.2. Found: C, 67.98, 68.54; H, 8.79, 8.83; sapon. equiv., 161.4.

Dimethyl Ester.—(E) The dimethyl ester of the acid succinate was prepared by treatment with diazomethane. When crystallized from dilute methanol and then from a mixture of ether and petroleum ether it melted at 98–100°.

Anal. Calcd. for $C_{30}H_{48}O_7$: C, 69.19; H, 9.29. Found: C, 69.04; H, 9.02.

3-Hydroxy-12-ketocholanic Acid.—(B) To a cold solution of 5.7 g. of the crude acid succinate, described above,

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[1] (a) H. Wieland, E. Dane and E. Scholz, *Z. physiol. Chem.*, **211**, 261 (1932); (b) K. Kyogoku, *ibid.*, **246**, 99 (1937); (c) R. E. Marker and E. J. Lawson, This Journal, **60**, 1334 (1938); (d) W. M. Hoehn and H. L. Mason, *ibid.*, **62**, 569 (1940).

[2] In a discussion of the best method for the preparation of 3-hydroxy-12-ketocholanic acid Dr. E. Schwenk gave me the directions for its preparation, using succinic anhydride for the esterification. Later, when correspondence revealed that we had independently prepared the *nor* and *bisnor* keto acids by this method, the manuscripts were combined for publication.—B. R.

[3] (a) K. Kajiro and T. Shimada, *Z. physiol. Chem.*, **249**, 220 (1937); (b) S. Bergstrøm and G. A. D. Haslewood, *J. Chem. Soc.*, 540 (1939).

[4] All melting points are corrected. Work done in Bloomfield will be marked with (B), and that in Evanston with (E).

in acetic acid was added a solution of 1.13 g. of chromium trioxide in 5 ml. of water. After standing at room temperature for five hours it was poured into 1250 ml. of ice-water containing a little bisulfite. The mixture was chilled overnight and then filtered. The crude product was saponified by heating on a steam-bath in aqueous alkali for two to three hours. The alkaline solution was filtered through supercel and the filtrate upon acidifying with hydrochloric acid gave 4.7 g. of 3-hydroxy-12-ketocholanic acid, m. p. 163–164°. Crystallization from dilute methanol gave material melting at 164–165°, $[\alpha]_D$ +86.6° (150 mg. made up to 15 ml. with dioxane, α + 0.866; *l*, 1 dm.); reported[1b] m. p. 164–165°, $[\alpha]^{20}_D$ +110°.

Acid Succinate of 3-Hydroxy-12-ketocholanic Acid.—(E) A solution of 0.5 g. of 3-hydroxy-12-ketocholanic acid and 1 g. of succinic anhydride in 10 ml. of dry pyridine was refluxed for one hour. The mixture was poured into water and acidified with hydrochloric acid, giving 0.518 g. (82.5%) of crude acid melting at 234–236°. Two crystallizations from a mixture of benzene and methanol gave a product melting at 242–244°.

Anal. Calcd. for $C_{28}H_{42}O_7$: C, 68.55; H, 8.63; neut. equiv., 245.3. Found: C, 69.03; H, 8.28; neut. equiv., 246.7.

Methyl 3-Acetoxy-12-ketocholanate.—(E) A 1-g. sample of 3-hydroxy-12-ketocholanic acid was converted to the methyl ester by treatment with diazomethane in dry ether. The residue, after removal of the ether, was acetylated by refluxing its acetic acid acetic anhydride solution. Water and ether were added, and the ether layer was washed with 5% sodium carbonate, water, and dried. The ether solution was concentrated until crystallization started, and then cooled. About 0.9 g. (80%) of the ester, m. p. 148.5–150°, was obtained.

Anal. Calcd. for $C_{27}H_{42}O_5 \cdot H_2O$: C, 72.94; H, 9.12. Found: C, 72.69, 73.02; H, 9.07, 9.08.

3-Acid Succinate of *nor*-Desoxycholic Acid.—(B) This acid succinate was prepared from *nor*-desoxycholic acid by a procedure similar to that for the acid succinate of desoxycholic acid. The yield was 5.8 g. (90%). Crystallization from ether–petroleum ether gave material melting at 241–242°, $[\alpha]_D$ +54.8° (150 mg. made up to 15 ml. with dioxane, α +0.548; *l*, 1 dm.).

Anal. Calcd. for $C_{27}H_{42}O_7$: C, 67.76; H, 8.85. Found: C, 67.54, 67.72; H, 9.06, 8.83.

Acid Succinate of 3-Hydroxy-12-keto-*nor*-cholanic Acid.—(E) A solution of 0.5 g. of the acid succinate of *nor*-desoxycholic acid in 15 ml. of glacial acetic acid was treated with a solution of 0.1 g. of chromium trioxide in 0.5 ml. of water. After standing at room temperature for sixteen hours, the mixture was poured into ice-water and extracted with ether. The ether solution was washed with water, dried and concentrated to a small volume. On cooling, 0.384 g. (77.3%) of crystalline keto-acid was obtained melting at 257–258°.

Anal. Calcd. for $C_{27}H_{40}O_7$: C, 68.05; H, 8.46. Found: C, 68.41; H, 8.50.

3-Hydroxy-12-keto-*nor*-cholanic Acid.—(E) A solution of 0.2 g. of the acid succinate of 3-hydroxy-12-keto-*nor*-cholanic acid in 10 ml. of 10% potassium hydroxide solution was refluxed for two hours. After cooling and acidifying 0.15 g. (95%) of crude acid was obtained, which on crystallizing from acetone gave a product melting at 250–251°.

(B) When 5.8 g. of the 3-acid succinate of *nor*-desoxycholic acid was oxidized and saponified, by a procedure similar to that described for the preparation of 3-hydroxy-12-ketocholanic acid, a yield of 4.4 g. of 3-hydroxy-12-keto-*nor*-cholanic acid was obtained. Crystallization from dilute methanol gave material melting at 248–250°, $[\alpha]_D$ +69.7° (150 mg. made up to 15 ml. with dioxane, α +0.697; *l*, 1 dm.).

Anal. Calcd. for $C_{23}H_{36}O_4$: C, 73.36; H, 9.65. Found: C, 73.47, 73.54; H, 9.70, 9.64.

Acetate.—(B) A solution of 3 g. of 3-hydroxy-12-keto-*nor*-cholanic acid in 15 ml. of pyridine and 3 ml. of acetic anhydride was allowed to stand overnight. The solution was poured into ice and sulfuric acid and filtered. The precipitate crystallized from dilute methanol giving 3.1 g. of 3-acetoxy-12-keto-*nor*-cholanic acid melting at 207.8–209.5°, $[\alpha]_D$ +99.7° (150 mg. made up to 15 ml. with dioxane, α +0.997; *l*, 1 dm.).

Anal. Calcd. for $C_{25}H_{38}O_5$: C, 71.73; H, 9.15. Found: C, 71.56; H, 9.06.

Semicarbazone.—(E) A solution of 0.77 g. of 3-hydroxy-12-keto-*nor*-cholanic acid in 25 ml. of alcohol was mixed with a solution of 0.77 g. of semicarbazide hydrochloride and 0.77 g. of sodium acetate in 7 ml. of water. The solution was refluxed for two and three-quarters hours and then cooled in the refrigerator. A gelatinous precipitate separated, which after washing with water and drying yielded 0.8 g. (90%) of a white powder. From alcohol it again separated as a gelatinous precipitate. It did not melt, but decomposed at about 250–275°.

Anal. Calcd. for $C_{24}H_{39}O_4N_3$: N, 9.69. Found: N, 9.79.

***nor*-Lithocholic Acid.**—(E) A mixture of 0.89 g. of the crude semicarbazone of 3-hydroxy-12-keto-*nor*-cholanic acid and 12 ml. of a saturated alcoholic solution of sodium ethoxide was heated in a sealed tube at 180–200° for twelve hours. After cooling the product was dissolved in 500 ml. of water, filtered and acidified. The acid was collected and recrystallized from alcohol, giving 0.328 g. (44.0%) of needles melting at 177–179°. An additional amount was obtained from the alcoholic filtrate. After two more crystallizations from alcohol (once with decolorizing charcoal) a pure product melting sharply at 183–183.5° was obtained. Two values for the melting point,[5] 181–182° and 186–187°, for this compound have been reported.

3-Acid Succinate of *bisnor*-Desoxycholic Acid.—(B) By a procedure similar to the one used for the *nor*-acid a yield of 12.6 g. (99%) of the 3-acid succinate of *bisnor*-desoxycholic acid was obtained from 10 g. of *bisnor*-desoxycholic acid. When crystallized from ether–petroleum ether it melted at 234–235°, $[\alpha]_D$ +33.9° (150 mg. made up to 15 ml. with dioxane, α +0.339; *l*, 1 dm.).

Anal. Calcd. for $C_{26}H_{40}O_7$: C, 67.22; H, 8.68. Found: C, 67.00, 67.51; H, 9.00, 8.81.

Acid Succinate of 3-Hydroxy-12-keto-*bisnor*-cholanic Acid.—(E) By a chromic acid oxidation similar to that used for the *nor*-acid a yield of 0.233 g. (66.8%) of the acid suc-

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[5] F. Reindel and K. Niederlander, *Ber.*, **68**, 1969 (1935); J. Sawlewicz and T. Reichstein, *Helv. Chim. Acta*, **20**, 949 (1937).

cinate of 3-hydroxy-12-keto-*bisnor*-cholanic acid melting at 252–254° was obtained from 0.35 g. of the 3-acid succinate of *bisnor*-desoxycholic acid. A sample recrystallized from alcohol gave the same melting point.

Anal. Calcd. for $C_{26}H_{38}O_7$: C, 67.51; H, 8.28. Found: C, 67.65; H, 8.56.

3-Hydroxy-12-keto-*bisnor*-cholanic Acid.—(E) Hydrolysis of 0.2 g. of the acid succinate of 3-hydroxy-12-keto-*bisnor*-cholanic acid in a manner similar to that described for the *nor*-acid gave 0.11 g. (40%) of 3-hydroxy-12-keto-*bisnor*-cholanic acid after it had been crystallized from ethanol. It melted at 295–297°.

(B) By the oxidation and saponification of 6.2 g. of the 3-acid succinate of *bisnor*-desoxycholic acid, as in the preparation of 3-hydroxy-12-ketocholanic acid, 4.8 g. of 3-hydroxy-12-keto-*bisnor*-cholanic acid was obtained. When crystallized from dilute methanol it melted at 298–299°, $[\alpha]_D$ +84.6° (150 mg. made up to 15 ml. with dioxane, α + 0.846; *l*, 1 dm.).

Anal. Calcd. for $C_{22}H_{34}O_4$: C, 72.90; H, 9.46. Found: C, 72.73, 73.00; H, 9.30, 9.62.

Acetate.—(B) When 2 g. of 3-hydroxy-12-keto-*bisnor*-cholanic acid was acetylated in the usual way 2.1 g. (94%) of 3-acetoxy-12-keto-*bisnor*-cholanic acid was obtained. After crystallizing from methanol it melted at 246–247°, $[\alpha]_D$ +65.9° (150 mg. made up to 15 ml. with dioxane, α + 0.659; *l*, 1 dm.).

Anal. Calcd. for $C_{24}H_{36}O_5$: C, 71.41; H, 8.97. Found: C, 71.36; H, 9.10.

Semicarbazone.—(E) By a procedure similar to that used with the *nor*-acid, 0.63 g. of 3-hydroxy-12-keto-*bisnor*-cholanic acid was converted to 0.512 g. (72.8%) of the semicarbazone in the form of silky needles. An additional yield of 0.213 g. of less pure material was obtained by diluting the filtrate. The semicarbazone did not melt, but decomposed with foaming at about 210–230°.

Anal. Calcd. for $C_{23}H_{37}O_4N_3$: N, 10.01. Found: N, 10.04.

3-Hydroxy-12-keto-*etio*-cholanic Acid.—(B) To a cold solution of 525 mg. of succinic anhydride in 2.5 ml. of anhydrous pyridine was added 200 mg. of *etio*-desoxycholic acid. After standing at room temperature overnight the solution was heated for one hour on a steam-bath, and then poured into a mixture of ice and sulfuric acid. The mixture was extracted with ether which was dried and evaporated. The residue melted at 161–169°.

This crude acid succinate was dissolved in 5 ml. of acetic acid, cooled and treated with an aqueous solution of 57.5 mg. of chromium trioxide. After standing for five hours at room temperature, the reaction mixture was poured into ice-water containing bisulfite, and chilled. The mixture was extracted with ether, the ether was evaporated, and the residue was saponified with aqueous alkali. A yield of 120 mg. of crude 3-hydroxy-12-keto-*etio*-cholanic acid was obtained which melted at 210–212°. This material was dissolved in acetone and water was added until turbid. By allowing the solution to evaporate slowly the material crystallized in long needles, and was recrystallized from acetone and ligroin, m. p. 213–215°, $[\alpha]_D$ +127.2° (150 mg. made up to 15 ml. with dioxane, α + 1.272; *l*, 1 dm.).

Anal. Calcd. for $C_{20}H_{30}O_4$: C, 71.83; H, 9.04. Found: C, 71.60; H, 9.23.

Acetate.—(B) 3-Acetoxy-12-keto-*etio*-cholanic acid was prepared, and melted at 205–206°.

Anal. Calcd. for $C_{22}H_{32}O_5$: C, 70.17; H, 8.57. Found: C, 70.00; H, 8.75.

Summary

1. 3-Hydroxy-12-ketocholanic acid and its *nor*, *bisnor* and *etio* homologs were prepared by oxidizing and saponifying the 3-acid succinates of desoxycholic acid and its homologs.

2. A Wolff–Kishner reduction of the semicarbazone of 3-hydroxy-12-keto-*nor*-cholanic acid yielded *nor*-lithocholic acid.

3. Several derivatives of these compounds are reported.

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