which had formed were collected. The product (0.11 g.) melted at 250° (dec.); mixed with natural leptosidin (m.p. 252–254° dec.) the melting point was 250–254° dec. The absorption spectrum of the synthetic material was identical with that of the natural pigment (Fig. 1), and the deep red colors of the solutions of the two specimens in alkali were identical.

Anal. Calcd. for $C_{16}H_{12}O_6$: C, 64.00; H, 4.03. Found: C, 63.91; H, 4.26.

The acetate, prepared by heating 70 mg. of synthetic leptosidin with 1 g. of anhydrous sodium acetate and 5 ml. of acetic anhydride, formed pale yellow needles, m.p. 166.5–167° (from aqueous ethanol), not depressed by admixture with the acetate of the natural pigment (m.p. 166–166.5°).

Anal. Calcd. for $C_{22}H_{18}O_9$: C, 61.97; H, 4.24. Found (synthetic material): C, 62.02; H, 4.51.

3′,4′-Dibenzoyloxy-6-hydroxy-7-methoxycoumaranone-3.—To a solution of 1.8 g. of 6-hydroxy-7-methoxycoumaranone-3 and 4 g. of 3,4-dibenzoyloxybenzaldehyde[16] in 100 ml. of glacial acetic acid was added 3 ml. of concentrated hydrochloric acid. The solution was shaken for 20 hours, during which time a brick-red solid separated. The mixture was poured into 200 ml. of water and the solid collected, washed and dried. Recrystallization of the product from nitromethane gave 4.4 g. of golden-yellow needles which turned orange upon exposure to laboratory air. The orange crystals melted at about 206° (dec.), the m.p. depending somewhat upon the heating rate.

Anal. Calcd. for $C_{30}H_{20}O_8$: C, 70.86; H, 3.97. Calcd. for $C_{30}H_{20}O_8 \cdot {}^1/_2H_2O$: C, 69.63; H, 4.19. Found (dried to yellow form): C, 70.44, 70.44; H, 4.21, 4.20. Found (yellow material allowed to hydrate to orange form): C, 69.65; H, 4.24.

A weighed sample (130.6 mg.) of the yellow material gained 2.2 mg. after 2 hours, 4.2 mg. after 24 hours and 4.5 mg. after 36 hours of exposure to air. These figures correspond to water contents of 1.66, 3.12 and 3.34%, respectively.

Calcd. for $C_{30}H_{20}O_8 \cdot {}^1/_2H_2O$: H_2O, 1.74; for $C_{30}H_{20}O_8 \cdot H_2O$: H_2O, 3.42.

Leptosin (A) 3′,4′-Dibenzoyloxy-6-tetraacetylglucosidoxy-7-methoxybenzalcoumaranone-3.—To 1.02 g. of 3′,4′-dibenzoyloxy-6-hydroxy-7-methoxybenzalcoumaranone-3 dissolved in a mixture of 35 ml. of acetone and 1.2 g. of potassium hydroxide in 20 ml. of water, was slowly added a solution of 0.83 g. of acetobromoglucose in 25 ml. of acetone. The bright orange solution was allowed to stand at room temperature for 14 hours. The acetone was removed under reduced pressure and room temperature and the residual material poured into water. The brown-orange solid which separated was collected, dried, dissolved in benzene (20 ml.) and filtered from a small amount of brown amorphous material. The warm filtrate was diluted with 25 ml. of petroleum ether (b.p. 30–60°); on cooling 0.99 g. of a yellow solid separated. This was recrystallized from methanol, giving tiny yellow needles, m.p. 106–109° (varying with heating rate). Further purification was not carried out, the product described being used in the next step.

Anal. Calcd. for $C_{44}H_{38}O_{17}$: C, 63.00; H, 4.57. Found: C, 64.08, 64.27; H, 4.70, 4.87.

(B) **3′,4′-Dihydroxy-6-β-D-glucosidoxy-7-methoxy-benzalcoumaranone-3 (Leptosin).**—A solution of 0.75 g. of the acetylated glucoside in 150 ml. of methanol was saturated at 0° with ammonia. The blood-red solution was allowed to stand at 0° for 24 hours and then evaporated *in vacuo*. The residual red, oily material was dried over phosphorus pentoxide and triturated with 500 ml. of anhydrous ether and the residual 0.154 g. of red-brown solid extracted (Soxhlet) with ether until the solvent no longer became colored. Crystallization of the residual, undissolved solid from aqueous methanol (Nuchar) yielded 73 mg. of golden-orange needles, m.p. 218–221 dec. On drying at 100° (1 mm.), the orange needles changed to a bright yellow. A solution of the material in dilute aqueous sodium hydroxide was a deep blue-violet in color. These properties are all identical with those of the natural glucoside. A mixture of the synthetic material with a sample of the natural glucoside (m.p. 218–224° dec.) melted at 218–224° dec. The absorption spectra of the synthetic and natural pigments were identical (Fig. 1).

Anal. Calcd. for $C_{22}H_{22}O_{11}$: C, 57.14; H, 4.80. Found: C, 56.76; H, 4.99.

Leptosin Hexaacetate.—A mixture of 10 mg. of (synthetic) leptosin, 23 mg. of anhydrous sodium acetate and 0.60 ml. of acetic anhydride was heated to boiling. When the color of the solution had faded to a pale yellow, water was added and the precipitate collected and recrystallized from aqueous dioxane and then from ethyl acetate–petroleum ether. The pale yellow needles melted at 235.5–236° dec., and a mixture with a sample of the acetate (m.p. 234–235° dec.) prepared from natural leptosin melted at 234–236° dec. The natural and synthetic materials showed identical absorption spectra (max. 375 mμ, log ε 4.30; 328 mμ, log ε 4.35; min. 276 mμ, log ε 3.57; max. 241 mμ, log ε 4.07).[17]

Anal. Calcd. for $C_{34}H_{34}O_{17}$: C, 57.14; H, 4.80. Found: C, 56.79; H, 4.96.

(16) F. Hayduck, *Ber.*, **36**, 2930 (1903).

(17) M. K. Seikel and T. A. Geissman, THIS JOURNAL, **72**, 5720 (1950).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, FACKENTHAL LABORATORIES, FRANKLIN AND MARSHALL COLLEGE]

The Structure of the Cholestane-3-carboxylic Acids[1]

BY EDWARD N. SQUIRE

The carboxylic acid group may be introduced into the 3α- or 3β-positions of the cholestane nucleus. Treatment of cholesterylmagnesium chloride with carbon dioxide leads to the formation of 5-cholestene-3-carboxylic acid. Hydrogenation of this acid produces cholestane-3α-carboxylic acid. Carbonation of the cholestanylmagnesium chloride reagent gives cholestane-3β-carboxylic acid. A proof of structure for these two acids is presented.

In order to clarify a series of C_8 substituted cholestane derivatives under investigation, it was necessary to examine the structure of cholestane-3-carboxylic acid. Marker[2] first reported the synthesis of this compound by the catalytic hydrogenation of 5-cholestene-3-carboxylic acid which had been prepared by treatment of the cholesteryl Grignard reagent with carbon dioxide.

The α configuration was assigned to the hydrogen atom at C_5. There exists substantial evidence to warrant this, since hydrogenations of the 5-cholestene nucleus in acid media lead to a *trans* A/B ring fusion (*cf.* cholesterol to cholestanol,[3] *epi*cholesterol to *epi*cholestanol,[2] 3β-chloro-5-cholestene to 3β-chlorocholestane,[4] 3,5-cholestadiene to cholestane,[5]

(1) A grant from the Research Corporation, N. Y., supported this work.

(2) R. E. Marker, T. S. Oakwood and H. M. Crooks, THIS JOURNAL, **58**, 481 (1936).

(3) L. Ruzicka and E. Eichenberger, *Helv. Chim. Acta*, **18**, 430 (1935).

(4) R. E. Marker, THIS JOURNAL, **57**, 1755 (1935); R. E. Marker, F. C. Whitmore and O. Kamm, *ibid.*, **57**, 2358 (1935).

(5) H. E. Stavely and W. Bergmann, *J. Org. Chem.*, **1**, 567, 575 (1937).

etc.). Physical measurements[6] of cholestane substantiate the *trans* A/B ring system.

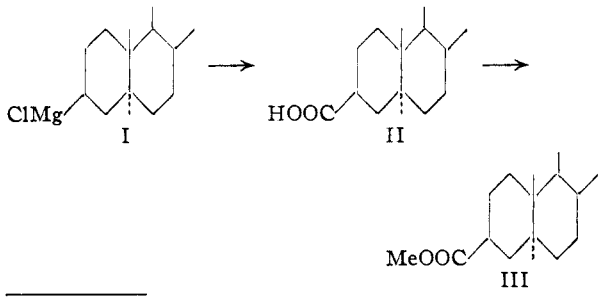
The configuration of the C_3 carboxylic acid group of 5-cholestene-3-carboxylic acid was not denoted. Because Marker[2] had oxygenated the cholesteryl Grignard reagent and found the product to be a mixture of *epi*cholesterol and cholesterol, the carbon dioxide product was assumed to be an equimolar mixture of *cis*- and *trans*-carboxylic acids. Later work indicated[7] the 5-cholestene-3-carboxylic acid to be at least 90% isomerically pure. The present report substantiates this finding and, furthermore, verifies the configuration of the carboxylic acid group at C_3.[8] Conversions establishing the structures of the cholestane-3-carboxylic acids follow.

5-Cholestene-3-carboxylic acid[2,7] was converted to only one pure methyl ester,[2,7] m.p. 101.5–102.5°, $[\alpha]^{25}D$ −17.6°, (*c* 1.48 in chloroform). Catalytic hydrogenation of the methyl ester in acid media afforded 3-carbomethoxycholestane,[2,7] m.p. 71.5–73°, $[\alpha]^{25}D$ 30.1°, (*c* 1.13 in chloroform). Hydrogenation of the 5-cholestene-3-carboxylic acid over platinum dioxide catalyst in acid media gave the cholestane-3-carboxylic acid,[2] m.p. 209–211°, $[\alpha]^{25}D$ 40.5° (*c*, 0.592 in chloroform).

Another series of steroids epimeric at C_3 to those just described was prepared in the following manner. Catalytic hydrogenation of cholesteryl chloride gave cholestanyl chloride. By means of the Grignard reagent (I) the chloride was converted into the carboxylic acid (II), m.p. 206–207°, $[\alpha]^{25}D$ 28.7°. Conversion of this acid to its methyl ester resulted in (III), m.p. 66–67.5°, $[\alpha]^{25}D$ 17°.

TABLE I

Steroid	M_D	$(M_D^\beta - M_D^\alpha)$
Cholestane-3α-carboxylic acid	169	−49
Cholestane-3β-carboxylic acid	120	
3α-Carbomethoxycholestane	129	−56
3β-Carbomethoxycholestane	73	



By means of the differences in optical rotation[9,10] those C_3 substituted compounds arising from the treatment of cholesterylmagnesium chloride with carbon dioxide were assigned the α configuration, while those resulting from the action of CO_2 on the cholestanylmagnesium chloride reagent I were denoted β. These designations are illustrated in Table I and clearly exemplify the rule that the 3β-epimers are less dextrorotatory than those of a 3α-configuration.

Experimental[11]

Cholestane-3β-carboxylic Acid (II).—To 2.0 g. of magnesium powder and 4.6 g. of methyl iodide contained in a 500-ml. flask there was added 10 ml. of dry ether, and the methylmagnesium iodide immediately formed. Ten milliliters of dry ether containing 4.0 g. of cholestanyl chloride was then added to the Grignard reagent mixture. The mixture was heated at reflux temperature for 24 hours and then exposed to 1 atm. of carbon dioxide gas at room temperature for two hours. The Grignard adduct was decomposed by the addition of 100 ml. of a saturated ammonium chloride solution followed by the addition of 100 ml. of 20% hydrochloric acid. The resulting suspension was extracted with 200 ml. of ethyl ether; the ether suspension was filtered from some bicholestanyl[12] which decomposed at 260–270°, m.p. >300°. One hundred milliliters of acetone was added to the ether filtrate; the solution was evaporated to about 20 ml. and chilled to 0°. The solid which was filtered from this was taken up in 75 ml. of hot benzene and upon slow evaporation fine, colorless needles separated and were removed at the pump; wt. of cholestane-3-β-carboxylic acid, 2.5 g., m.p. 206–207°, $[\alpha]^{25}D$ 28.7° (*c* 0.836 in chloroform).

Anal. Calcd. for $C_{28}H_{48}O_2$: C, 80.71; H, 11.61. Found: C, 80.29, 79.98; H, 11.47, 11.30; ash, 0.61, 0.53.

3β-Carbomethoxycholestane (III).—To 0.200 g. of cholestane-3-β-carboxylic acid was added 100 ml. of dry methanol containing 0.5 ml. of concentrated sulfuric acid and the mixture was heated at reflux temperature for three hours and then allowed to stand overnight at room temperature. The mixture was then cooled and poured into 150 ml. of cold water. After coagulation of the product was complete, the precipitate was filtered off at the pump, washed well with distilled water, and dried. The white solid was taken into solution with 10 ml. of ether; 10 ml. of methanol was added, and after standing at room temperature for 48 hours, the long (25–40 mm.) needles were filtered from the solution to yield 0.191 g. of 3 β-carbomethoxycholestane, m.p. 66–67.5°, $[\alpha]^{25}D$ 17° (*c* 2.00 in chloroform); mixed m.p. with 3-α-carbomethoxycholestane, 59–64°.

Anal. Calcd. for $C_{29}H_{50}O_2$: C, 80.87; H, 11.70. Found: C, 80.89, 80.72; H, 11.87, 11.69.

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(7) R. H. Baker and E. N. Squire, THIS JOURNAL, **70**, 1487 (1948); **70**, 4134 (1948).

(8) R. H. Baker and Q. R. Petersen, *ibid.*, **73**, 4080 (1951). While this work was in progress, a communication from Professor Baker announced the finding that carbonation of the cholesteryl Grignard reagent led to a 3α-COOH configuration.

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(10) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," Reinhold Publishing Corp., New York, N. Y., 3rd ed., 1949. p. 216.

(11) Microanalyses by Dr. Carl Tiedke. Melting points were taken using a Fisher melting point apparatus.

(12) E. N. Squire, THIS JOURNAL, **73**, 2586 (1951).