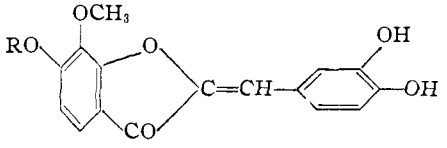
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, LOS ANGELES]

Anthochlor Pigments. VIII. The Pigments of *Coreopsis grandiflora*, Nutt. III.

BY T. A. GEISSMAN AND WILLIAM MOJÉ

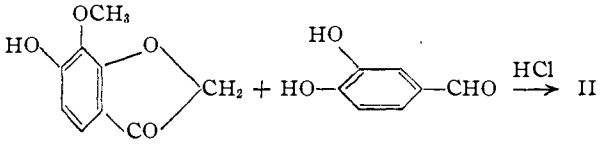
The structures assigned earlier to the benzalcoumaranone pigment leptosidin and its glucoside, leptosin, have been substantiated by the total synthesis of these pigments.

The structures assigned to leptosin (I) and leptosidin (II), isolated from *Coreopsis grandiflora*, Nutt.[1] have to the present time rested upon the synthesis of leptosidin trimethyl ether[2] and upon color reactions by the interpretation of which the locations of the methoxyl and glucosidoxyl groups were chosen. The structures of the naturally-occurring pigments have now been established by the total synthesis of I and II and have been found to be those originally proposed.



I, R = $C_6H_{11}O_5$
II, R = H

The synthesis of leptosidin was readily accomplished by the acid-catalyzed condensation of protocatechuic aldehyde and 6-hydroxy-7-methoxycoumaranone-3



The characterization of the product as II was accomplished by means of its characteristic color reactions, its crystalline triacetate and a comparison of its ultraviolet absorption spectrum with that of the natural pigment (Fig. 1).

Considerable study was devoted to improving the method of synthesis of 6-hydroxy-7-methoxycoumaranone-3 and of 2,6-dihydroxyanisole, from which the coumaranone can be derived. The preparation of 2,6-dihydroxyanisole in low yield has been described by Späth and Schmidt,[3] the procedure involving a lengthy and arduous separation of the mixture of methylation products obtained by treating pyrogallol with a limited quantity of dimethyl sulfate and alkali. In an attempt to devise a more convenient method several alternate syntheses were examined. None of these, however, proved satisfactory; the best method was at length found to be a modification of the procedure of Späth and Schmidt in which a fractional distillation of the methylated mixture replaced the lead acetate separation used by these authors. In this way the yield was increased five-fold, although our yields by the lead acetate route were considerably lower than those reported.[3]

Since the treatment of 3,4,5-trimethoxybenzoic acid with concentrated sulfuric acid gives 3,5-dimethoxy-4-hydroxybenzoic acid in good yield,[4] it was anticipated that the 4-methoxyl group of 2-hydroxy-3,4-dimethoxybenzoic acid would be removed by similar treatment. When this experiment was performed, the product obtained (in 42% yield) was a monomethyl ether of 2,3,4-trihydroxybenzoic acid with a melting point (224–225° dec.) quite different from that reported for 2,3-dihydroxy-4-methoxybenzoic acid (204–206° dec.[5] and 207–208° dec.[6]). That this was, however, not the desired 2,4-dihydroxy-3-methoxybenzoic acid was shown by (a) its smooth decarboxylation to pyrogallol 1-methyl ether and (b) comparison with a sample of 2,4-dihydroxy-3-methoxybenzoic acid prepared by the carbonation[7] of pyrogallol 2-methyl ether. Re-examination of the experimental work of Herzig and Pollock,[5] and Pacsu,[6] disclosed that the compound described by these authors, when prepared according to their methods, was identical in all respects with the product from the sulfuric acid demethylation of 2-hydroxy-3,4-dimethoxybenzoic acid.

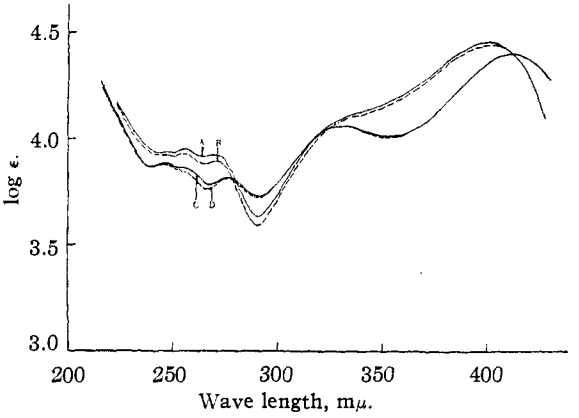


Fig. 1.—Absorption spectra in 95% ethanol: A, leptosidin, synthetic; B, leptosidin, natural; C, leptosin, synthetic; D, leptosin, natural.

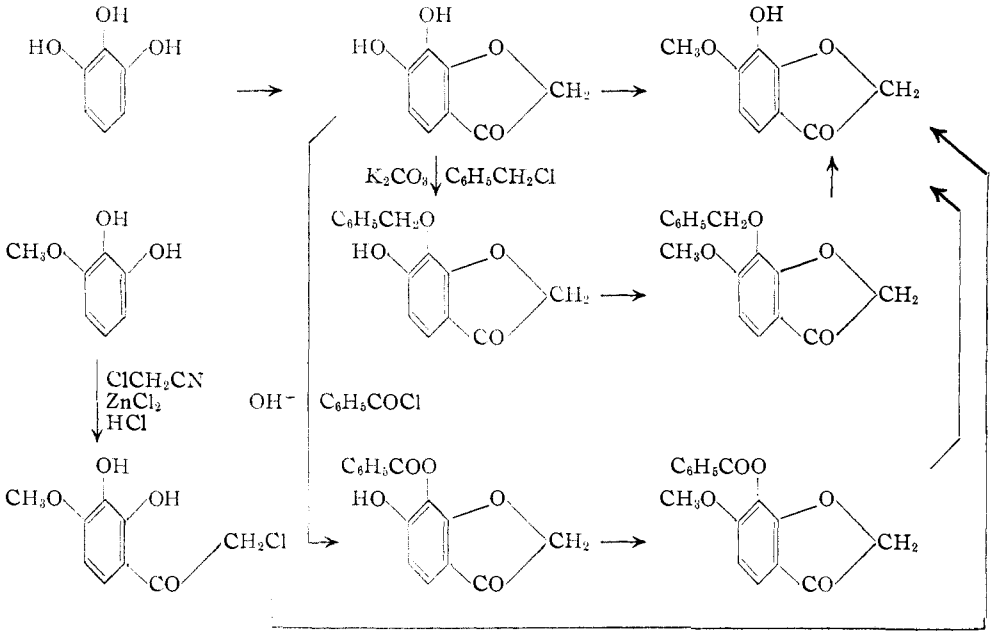
In another study, it was found that the readily accessible 3,5-dihydroxy-4-methoxybenzoic acid[8] was decarboxylated only with difficulty and with concomitant loss of the methyl group to yield pyrogallol, none of the desired pyrogallol 2-methyl ether being obtained.

The ease of preparation of 6,7-dihydroxycoumaranone-3[9] suggested the possibility of preparing the desired 6-hydroxy-7-methoxycoumaranone-3

(1) T. A. Geissman and C. D. Heaton, THIS JOURNAL, **65**, 677 (1943).
(2) T. A. Geissman and C. D. Heaton, *ibid.*, **66**, 486 (1944).
(3) E. Späth and H. Schmidt, *Ber.*, **74**, 193 (1941).
(4) R. L. Alimchandani and A. N. Meldrum, *J. Chem. Soc.*, **117**, 964 (1920).
(5) J. Herzig and J. Pollock, *Monatsh.*, **23**, 700 (1902); **25**, 501 (1904).
(6) E. Pacsu, *Ber.*, **56**, 407 (1923).
(7) W. Baker and H. A. Smith, *J. Chem. Soc.*, 2542 (1931).
(8) E. Fischer, *Ber.*, **51**, 58 (1924).
(9) (a) M. Nencki, *J. Russ. Phys. Chem. Soc.*, **25**, 182 (1883); (b) L. Kesselkaul and S. v. Kostanecki, *Ber.*, **29**, 1886 (1896).

by (a) the preferential methylation of the 7-hydroxyl group; or (b) the successive 6-benzylation, 7-methylation and debenzylation of the dihydroxy coumaranone; or (c) a series such as (b) in which benzoylation instead of benzylation is used.

The methylation of 6,7-dihydroxycoumaranone-3 with dimethyl sulfate, methyl iodide and diazomethane yielded the 6-methyl ether. Benzylation with benzyl chloride and potassium carbonate alkali in acetone afforded a poor yield of the 7-benzyl ether. Benzoylation of the dihydroxycoumaranone gave the 7-benzoyl derivative. The structures of the 7-benzyl and 7-benzoyl derivatives were demonstrated by methylation with diazomethane and debenzylation (or debenzoylation) to yield 6-methoxy-7-hydroxycoumaranone-3, which was prepared from 2,3-dihydroxyanisole *via* the chloroketone formed in the Hoesch acylation of this phenol by means of chloroacetonitrile.



From these observations it was possible to devise a route to the desired 6-hydroxy-7-methoxycoumaranone-3 by an appropriate series of benzoylation, benzylation, debenzoylation, methylation and debenzylation, but there appeared to be no advantage in such a complex procedure and this course was not examined. The required 6-hydroxy-7-methoxycoumaranone-3 was prepared by way of the pyrogallol 2-methyl ether obtained by the partial methylation of pyrogallol and separation of the resulting mixed ethers by fractional distillation.

It should be noted that Felix and Friedlander[10] described a methoxyhydroxycoumaranone which they prepared by the reaction of α-chlorogallacetophenone with a limited amount of dimethyl sulfate in the presence of alkali. The melting point they report for the resulting coumaranone corresponds best with that found in the present work for the 6-methoxy-7-hydroxy isomer, but the piperonal derivative described by Felix and Friedlander differs markedly from the piperonal derivatives prepared from both isomers in the present work.

The synthesis of the glucoside, leptosin, was first attempted by the most direct route: the glycosylation of 6-hydroxy-7-methoxycoumaranone-3 with acetobromoglucose, followed by the condensation of the tetraacetylglucoside with protocatechuic aldehyde. The reaction between acetobromoglucose and the coumaranone yielded only oily products by methods involving the use of alkali or the use of silver oxide in quinoline. Attempts to condense the impure tetraacetylglucosides with protocatechuic aldehyde led to inhomogeneous, gummy materials from which no leptosin or leptosin derivatives (*e.g.*, acetates) could be isolated. Model experiments in which resacetophenone was used afforded good yields of the known 4-tetraacetylglucosidoxy-2-hydroxyacetophenone.[11]

The synthesis of leptosin was eventually achieved by the route shown in the following diagram. The ultraviolet absorption spectra of natural and synthetic leptosin are shown in Fig. 1. The synthetic material was identical in its melting point and that of its hexaacetate and in its characteristic color changes with increasing *p*H, with the naturally-occurring pigment.

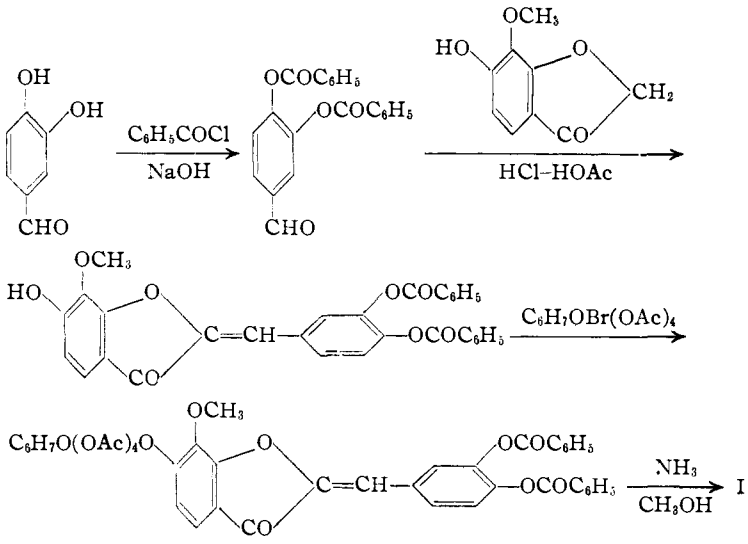
Experimental[12]

Pyrogallol 2-Methyl Ether.—Eight hundred grams of pyrogallol was dissolved in 1280 ml. of water in a 5-l. flask equipped with a stirrer, dropping funnel and nitrogen inlet. After the addition of 600 ml. of dimethyl sulfate, 2800 ml. of 10% aqueous sodium hydroxide was added over 90 minutes, with vigorous stirring and under nitrogen. The mixture was then heated on the steam-bath for 30 min., cooled and acidified with hydrochloric acid, saturated with sodium chloride and extracted with ether. The material remaining after removal of the ether was fractionated at 16 mm. and the fractions boiling at 135–165° (16 mm.) redistilled through a column packed with glass helices. The material

(10) A. Felix and P. Friedlander, *Monatsh.*, **31**, 55 (1910).

(11) L. Reichel and J. Steudel, *Ann.*, **553**, 83 (1942).

(12) Melting points and boiling points are uncorrected.



(92.9 g.) boiling at 146–147.5° (17 mm.) solidified on cooling and was recrystallized from carbon tetrachloride, yielding 67 g. of white needles, m.p. 84.5–85°. An additional 8.5 g. was obtained from a 55-g. fraction boiling at 144–146° (16 mm.). (It is probable that the yield could be increased considerably by refractionation of neighboring fractions.)

The product gave no precipitate with aqueous lead acetate. It formed a bis-*p*-nitrobenzoate (in pyridine), m.p. 222–223°.

Anal. Calcd. for $C_{21}H_{14}O_9N_2$: C, 57.54; H, 3.22. Found: C, 57.56; H, 3.24.

2-Hydroxy-3,4-dimethoxybenzoic acid was prepared according to Baker and Savage.[13] The product melted at 168–169° (lit.,[13] 169–172°).

2,3-Dihydroxy-4-methoxybenzoic Acid.—A solution of 10.0 g. of 2-hydroxy-3,4-dimethoxybenzoic acid in 50 ml. of concentrated sulfuric acid was kept at 75° for four hours and then allowed to stand for 20 hours at room temperature. The orange solution was poured onto ice and the grayish precipitate collected and washed with water. Recrystallization from aqueous alcohol gave 3.8 g. of white needles, m.p. 224–225° (dec.). The compound gave a blue color, changing to brown, with alcoholic ferric chloride.

Anal. Calcd. for $C_8H_8O_5$: C, 52.18; H, 4.38; OCH_3, 16.85; neut. equiv., 184. Found: C, 52.43; H, 4.52; OCH_3, 16.91; neut. equiv., 183.

The colorless **diacetate** had m.p. 160–161°.

Anal. Calcd. for $C_{12}H_{12}O_7$: C, 53.73; H, 4.51. Found: C, 54.14; H, 4.76.

The compound was shown to be identical with 2,3-dihydroxy-4-methoxybenzoic acid when a sample of the latter was prepared by the method of Pacsu,[6] who reported its m.p. as 207–208°.

2,4-Dihydroxy-3-methoxybenzoic Acid.—A mixture of 2.52 g. of 2,6-dihydroxyanisole, 10 g. of potassium bicarbonate and 20 ml. of water was heated on the steam-bath for four hours, then heated to reflux under a stream of carbon dioxide for another hour, cooled and acidified with concentrated hydrochloric acid. The precipitate was collected, washed with water, dried (1.47 g.) and recrystallized from nitromethane. The long, flat needles (1.25 g.) melted at 196.5–197° (dec.). The acid gave a purple color with ferric chloride which did not change on standing.

Anal. Calcd. for $C_8H_8O_5$: C, 52.16; H, 4.38. Found: C, 52.05; H, 4.42.

Decarboxylation of 2,4-dihydroxy-3-methoxybenzoic acid by heating (220–240°, one hour) proceeded normally, yielding 2,6-dihydroxyanisole, m.p. 83–84°.

Decarboxylation of 2,3-dihydroxy-4-methoxybenzoic acid by heating at 230–240° for 1.75 hours, followed by distillation of the product, yielded 2,3-dihydroxyanisole, which formed a bis-*p*-nitrobenzoate, m.p. 179–180°, identical with a sample prepared from authentic 2,3-dihydroxyanisole.

(13) W. Baker and R. I. Savage, *J. Chem. Soc.*, 1602 (1938).

Anal. Calcd. for $C_{21}H_{14}O_9N_2$: C, 57.54; H, 3.22. Found: C, 57.45; H, 3.27.

ω-Chlorogallacetophenone was prepared by a modification of the boron trifluoride method described by Killelea and Lindwall[14] for the preparation of resacetophenone. The conversion of the chloroketone into 6,7-dihydroxycoumaranone-3 was accomplished in the usual manner by the use of sodium acetate in ethanol. The product melted at 230–232° (lit.[15] 229°).

6-Hydroxy-7-methoxycoumaranone-3.—A stirred mixture of 39.0 g. of 2,6-dihydroxyanisole, 18 ml. of chloroacetonitrile, 20 g. of freshly fused zinc chloride and 400 ml. of dry ether was saturated with dry hydrogen chloride for 30 min. at 0° and then for 3 hours at 20°. After the mixture had been kept at 0° overnight the solid was collected, washed with 100 ml. of dry ether and dissolved in 500 ml. of water. The solution was refluxed for 90 minutes, cooled and the solid precipitate collected, washed with water and dried. The crude chloroketone (42 g.) was heated under reflux for 90 min. with a solution of 52 g. of anhydrous sodium acetate in 150 ml. of absolute ethanol. The solid (25.5 g.) which separated when the solution was poured into 500 ml. of a cold saturated aqueous solution of sodium chloride was recrystallized from methanol. The coumaranone formed nearly colorless needles, m.p. 156–157°. It gave a light pink color with ferric chloride.

Anal. Calcd. for $C_9H_8O_4$: C, 60.00; H, 4.47. Found: C, 59.93; H, 4.57.

6-Methoxy-7-hydroxycoumaranone-3 was prepared by the Hoesch chloroacetylation of 2,3-dihydroxyanisole, followed by the ring closure of the intermediate chloroketone with sodium acetate in alcohol. The coumaranone formed pale yellow needles, m.p. 211–212° (dec.), and gave a deep red color with ferric chloride.

Anal. Calcd. for $C_9H_8O_4$: C, 60.00; H, 4.47. Found: C, 60.03; H, 4.63.

Methylation of 6,7-dihydroxyketone by means of (a) diazomethane in methanol–ether, (b) dimethyl sulfate in acetone with dry potassium carbonate, and (c) methyl iodide in acetone with dry potassium carbonate, yielded 6-methoxy-7-hydroxycoumaranone-3 along with, in some experiments, a small amount of the dimethyl ether, m.p. 122–123°.

Benzylation of 6,7-dihydroxycoumaranone-3 (0.05 mole) with benzyl chloride (0.075 mole) in the presence of sodium iodide and potassium carbonate in acetone gave a 47% yield of 6-hydroxy-7-benzyloxycoumaranone-3, m.p. 166–167°. It gave no color with ferric chloride.

Anal. Calcd. for $C_{15}H_{12}O_4$: C, 70.30; H, 4.71. Found: C, 70.47; H, 4.85.

The structure of the monobenzyl ether was established (a) by methylating it with diazomethane to 6-methoxy-7-benzyloxycoumaranone-3, m.p. 108.5–109.5°.

Anal. Calcd. for $C_{16}H_{14}O_4$: C, 71.10; H, 5.22. Found: C, 71.23; H, 4.88.

(b) The methyl benzyl ether was debenzylated by heating with glacial acetic acid–concentrated hydrochloric acid to yield the known 6-methoxy-7-hydroxycoumaranone-3, identified by comparison with the authentic material. An attempt to prepare 6-methoxy-7-benzyloxycoumaranone by the benzylation of 6-methoxy-7-hydroxycoumaranone led to a colorless compound which contained two benzyl groups and had evidently resulted from nuclear benzylation.

Anal. Calcd. for $(C_{16}H_{14}O_4 + 2C_7H_6)$: C, 79.98; H, 5.82. Found: C, 79.94, 80.02; H, 5.76, 6.19.

Leptosidin (3′,4′,6-Trihydroxy-7-methoxybenzalcoumaranone-3).—To a solution of 0.115 g. of 6-hydroxy-7-methoxycoumaranone-3 and 0.156 g. of protocatechuic aldehyde in 10 ml. of absolute ethanol was added 0.2 ml. of concentrated hydrochloric acid. The red solution was kept at 75° for 3 hours and poured into 100 ml. of water. After the solution had been kept at 0° overnight the yellow needles

(14) J. R. Killelea and H. G. Lindwall, THIS JOURNAL, **70**, 428 (1948).

(15) W. Feuerstein and K. Brass, *Ber.*, **37**, 817 (1904).

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).

LOS ANGELES, CALIFORNIA RECEIVED JUNE 29, 1951

[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).