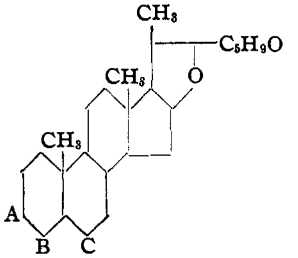
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF STANFORD UNIVERSITY]

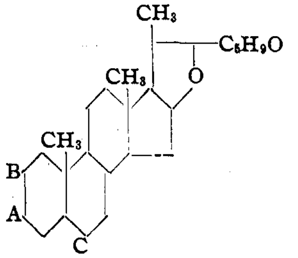
Saponins and Sapogenins. V. Oxidation Products and Structure of Chlorogenin

BY C. R. NOLLER

Chlorogenin, one of the two sapogenins isolated from the hydrolysis products of extracts of *Chlorogalum pomeridianum*[1] has the empirical formula $C_{27}H_{44}O_4$.[2] It is therefore isomeric with gitogenin and like gitogenin contains two hydroxyl groups and two oxygen atoms having non-reactive linkages which are assumed to be oxidic. Since the other sapogenin accompanying chlorogenin is apparently identical with tigogenin, which accompanies gitogenin and digitogenin in the hydrolysis products of extracts of digitalis, it was further assumed that chlorogenin is related structurally to the digitalis sapogenins. Recent work[3] has shown that the latter are identical with the exception of the number of hydroxyl groups in the molecule and that their structures may be represented by formula I or II, preference being given to II.



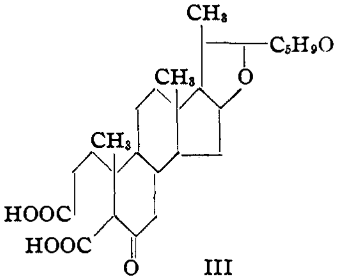
I



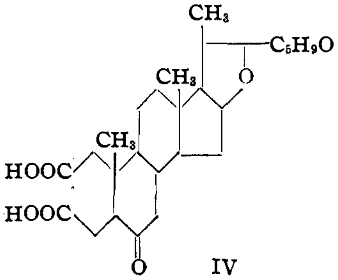
II

Tigogenin has its one hydroxyl group at A, gitogenin's two are at A and B, and digitogenin's three at A, B and C. A third sapogenin of unknown structure but isomeric with gitogenin is also present in the hydrolysis products of digitalis extracts, since Windaus and Shah[4] report the isolation from the oxidation products of crude digitogenin of a diketone, $C_{27}H_{40}O_4$, which is not the diketone corresponding to gitogenin.

It was thought that chlorogenin might have structure I or II with its two hydroxyl groups at A and C. If this were the case one might expect to find digitogenic acid, III or IV, in the oxidation products since opening of ring I might take place on either side of A with simultaneous conversion of the secondary hydroxyl group at C to a carbonyl group.



III



IV

On the other hand, it was conceivable that chlorogenin was identical with the unknown sapogenin of Windaus and Shah, in which case their diketone would be obtained on oxidation.

Oxidation of chlorogenin by the procedure of Kiliani and Merk[5] for the preparation of digitogenic acid showed that neither of these possibilities is actually the case. Both a diketone, $C_{27}H_{40}O_4$, and a ketodibasic acid, $C_{27}H_{40}O_7$, were obtained but they were not identical with the previously reported diketone or with digitogenic acid.

Since a diketone is formed, both hydroxyl groups in chlorogenin must be secondary. The diketone forms a dioxime but only a mono-*o*-phenylenediamine derivative which is not a quinoxaline. This shows that the two ketone groups are not adjacent and indicates that one is

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(1) Liang and Noller, THIS JOURNAL, **57**, 525 (1935).

(2) Fieser and Jacobsen, *ibid.*, **58**, 943 (1936).

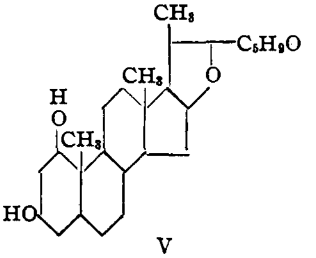
(3) Jacobs and Simpson, *J. Biol. Chem.*, **110**, 429 (1935); Tschesche, *Ber.*, **68**, 1090 (1935); Tschesche and Hagedorn, *ibid.*, **68**, 1412, 2247 (1935); **69**, 797 (1936).

(4) Windaus and Shah, *Z. physiol. Chem.*, **150**, 205 (1925).

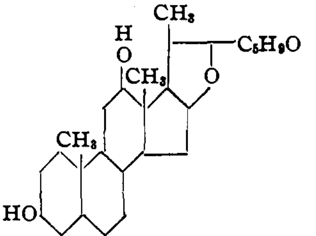
(5) Kiliani and Merk, *Ber.*, **34**, 3564 (1901).

sterically hindered. The pure diketone on further oxidation gives the same ketodibasic acid obtained by the direct oxidation of chlorogenin so that the diketone is probably an intermediate in the formation of the acid. The dibasic acid gives a dimethyl ester which is readily saponified to the original acid indicating that neither carbomethoxy group is sterically hindered. Chlorogenin is not precipitated from alcoholic solutions by digitonin but if the diketone is reduced with sodium and alcohol, a small amount of material is obtained which is slowly precipitated by digitonin.[6] This indicates that one of the hydroxyl groups in chlorogenin is at A but that the configuration is the opposite of that present in cholesterol.[7]

Assuming that the side chain has the same structure as that in the digitalis sapogenins, a consideration of the above data leads to formulas V and VI as being the most likely for chlorogenin. Because of the small amount of the ketodibasic acid that has been available it has not been possible to determine its structure but it does not appear to be either an α- or β-keto acid so that formula VI is favored.



V



VI

Experimental

Oxidation of Chlorogenin.—In a typical run 5 g. of chlorogenin, m. p. 277–279°, was covered with 50 cc. of glacial acetic acid, the flask placed in a pan of water and 40 g. of the sodium dichromate-sulfuric acid oxidizing mixture of Kiliani and Merk[6] added in small portions with shaking during the course of two or three minutes. The chlorogenin goes into solution almost completely and then the product begins to precipitate. After standing overnight the product was filtered, washed with 30% acetic acid until the filtrate was colorless, and then thoroughly with water.

Diketone, $C_{27}H_{40}O_4$.—The moist oxidation product was taken up in 200 cc. of benzene and extracted twice with 5% sodium carbonate solution and once with water. The cloudy benzene solution settled on standing overnight and was decanted. Concentration on the steam-bath to 30 cc., filtering hot and cooling gave 1.7 g., m. p. 233–235°. Evaporation of the mother liquor to dryness gave 1.1 g., m. p. 230–235°. The product is too soluble in benzene or dioxane for good crystallization, but crystallizes well from methyl alcohol, ethyl alcohol and acetone. After repeated recrystallization from all three solvents the fine needles shrink at 236° and melt at 247–248°; $[\alpha]^{22}_{\mathrm{D}}$ -69.6, $[\alpha]^{22}_{546.1}$ -81.4 in dioxane. The diketone appears to be dimorphous as it sometimes melts sharply at 236–238° and the melting point is not raised by further recrystallization.

Anal.[8] Calcd. for $C_{27}H_{40}O_4$: C, 75.67; H, 9.40. Found: C, 75.37; H, 9.45.

Dioxime, $C_{27}H_{42}O_4N_2$.—A mixture of 0.1 g. of diketone and 0.14 g. of crystalline sodim acetate was dissolved in boiling alcohol, and 0.07 g. of hydroxylamine hydrochloride added. After refluxing for four hours, the solution was poured into water, washed and dried in air. After several recrystallizations from absolute ethyl acetate, it melted at 242–243°. An alcoholic solution of the dioxime does not react with solutions of nickel salts.

Anal. Calcd. for $C_{27}H_{42}O_4N_2$: C, 70.72; H, 9.22; N, 6.11. Found: C, 70.70; H, 9.15; N. 5.80.

o-Phenylenediamine Derivative, $C_{33}H_{46}O_3N_2$.—A solution of 0.08 g. of diketone and 0.04 g. of *o*-phenylenediamine in 5 cc. of alcohol was refluxed for one hour. On cooling, tan colored crystals separated which melted at 254–261° with decomposition to a red oil. Repeated crystallization from alcohol gave a pale yellow product which melted at 255–261° when heated slowly but melted at 265–267° when the capillary tube was placed in a bath preheated to 255°.

Anal. Calcd. for $C_{33}H_{44}O_2N_2$: C, 79.16; H, 8.85; N, 5.59. Calcd. for $C_{33}H_{46}O_3N_2$: C, 76.40; H, 8.95; N, 5.40. Found: C, 76.30; H, 8.90; N, 5.26.

Ketodibasic Acid, $C_{27}H_{40}O_7$.—The sodium carbonate extracts of the crude oxidation product were acidified with 15% hydrochloric acid. Only the first extract gave a precipitate, which was filtered, washed and dried in air. There was obtained 0.109 g., m. p. 196–200°. This acid is insoluble in benzene, very soluble in dioxane and soluble in hot acetone, ethyl acetate and chloroform. It does not crystallize well from any of these solvents or their mixtures with benzene. It is very soluble in acetic acid but crystallizes well from hot concentrated solutions. The combined acid fractions from several oxidations after repeated crystallization from acetic acid melted at 235–237° with decomposition and preliminary shrinking; $[\alpha]^{22}_{\mathrm{D}}$ -42.8, $[\alpha]^{22}_{546.1}$ -49.0 in dioxane. As the analyses show, this product, which was dried by allowing it to stand for several weeks in a vacuum desiccator over potassium hydroxide, contains one molecule of acetic acid of crystallization.

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(6) We are indebted to Mr. F. M. McMillan for this information.

(7) Fernholz, *Z. physiol. Chem.*, **232**, 97 (1935).

(8) All carbon, hydrogen and nitrogen analyses are by Dr. A. Schoeller, Berlin.

Anal. Calcd. for $C_{25}H_{38}O_3(COOH)_2 \cdot CH_3COOH$: C, 64.91; H, 8.26; neut. equiv., 178.7. Found: C, 65.89; H, 8.53; neut. equiv., 176.1.

The solutions from the above determinations of neutralization equivalent were evaporated to a small volume on the steam-bath, diluted to 25 cc. with water, filtered and acidified with 0.05 *N* hydrochloric acid. The precipitate was filtered on a hardened paper and washed with hot water until free of chlorides. After drying it melted at 234–236° and analysis showed it to be free of acetic acid.

Anal. Calcd. for $C_{25}H_{38}O_3(COOH)_2$: C, 68.05; H, 8.46; neut. equiv., 238.3. Found: C, 68.03; H, 8.30; neut. equiv., 240.5.

In order to determine whether this dibasic acid was derived from the diketone, 3.5 g. of pure diketone was covered with 35 cc. of acetic acid and treated with 28 g. of the oxidizing mixture of Kiliani and Merck. After fifty-two hours the product was filtered and washed, and amounted to 0.8 g. The filtrate was diluted to 1 liter and filtered. The light green precipitate was dissolved in 15 cc. of hot glacial acetic acid and poured into 500 cc. of water. The precipitate after drying weighed 0.5 g. The total product of 1.3 g. was dissolved in benzene, extracted with sodium carbonate solution and the extract acidified. After filtering, washing and drying, the acid fraction weighed 0.3 g. and melted at 210–215°. After recrystallization from acetic acid it melted at 235–237° with decomposition and did not depress the melting point of the acid obtained by the direct oxidation of chlorogenin.

Dimethyl Ester of Ketodibasic Acid.—A solution of diazomethane in ether was added in excess to 0.3 g. of the dibasic acid containing acetic acid of crystallization. On evaporation of the ether an oil remained which soon solidified. It was soluble in methyl alcohol and acetone and only slightly soluble in petroleum ether, but crystallized well from 60–70° ligroin. After two crystallizations it melted at 158–159°; $[\alpha]^{22}_{D} - 39.1$, $[\alpha]^{22}_{546.1} - 46.5$. These rotations were microdeterminations which are not as accurate as the macro determinations made on the diketone and ketodibasic acid. Titration showed the product to contain no free carboxyl groups.

Anal. Calcd. for $C_{25}H_{38}O_3(COOCH_3)_2$: C, 69.02; H, 8.78; sap. equiv., 252.2. Found: C, 68.76; H, 8.55; sap. equiv., 256.5.

The acid recovered from the determinations of saponification equivalent did not depress the melting point of the original ketodibasic acid.

Summary

A tentative structural formula for chlorogenin is proposed which is based on the products of oxidation with dichromic acid.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF SOUTH CAROLINA]

Identification of Alcohols by 3-Nitrophthalic Anhydride[1]

BY G. M. DICKINSON, LEO H. CROSSON AND J. E. COPENHAVER

The use of 3-nitrophthalic anhydride as a reagent for the identification of alcohols was suggested by Nicolet and Sachs,[2] who prepared esters from this acid with nine of the lower alcohols. Ashdown and Monier[3] gave an extensive discussion of the use of this reagent at the Atlanta Meeting of the American Chemical Society in 1930. There has been much study of the higher alcohols within the last few years and it was thought that an extension of this series would be of interest.

The general procedure of the above workers was followed for the preparation of the acid anhydride reagent and the lower esters, but it was found that certain modifications were necessary for the higher derivatives. Their work and that of others showed that the 2-mono-alkyl esters were the principal product and that the 3-mono-alkyl esters were eliminated by crystallization.

Esters of the lower, less expensive alcohols were prepared by heating 3 g. of 3-nitrophthalic anhydride with approximately 2.5 g. of the alcohol and refluxed in a 50-cc. Erlenmeyer flask for one hour. The same quantities were used up to nonyl alcohol but with the increase in the boiling point of the alcohols, they were heated to 100° for two hours to ensure better esterification. From *n*-decyl to *n*-octadecyl, 3 g. of 3-nitrophthalic anhydride and one of alcohol were dissolved in purified toluene and refluxed for three hours.

Recrystallization of the Esters

As shown by Nicolet and Sachs, the esters from methyl to *n*-butyl were recrystallized from water without hydrolysis.

Upon the addition of water and warming, the esters above propyl separated as oils, which, up to and including *n*-hexyl, crystallized on cooling.

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[1] This is an extract from the theses presented by Messrs. Dickinson and Crosson in partial fulfilment of the requirements for the Master of Science degree at the University of South Carolina.

[2] Nicolet and Sachs, THIS JOURNAL, **47**, 2348 (1925).

[3] Ashdown and Monier, *Ind. Eng. Chem., News Ed.*, **8**, No. 6, 6 (1930).