the acid formed the **acetate anhydride,** m. p. 202–203.5°. This gave no depression with a previously reported sample,[5] m. p. 203–204°.

Anal. Calcd. for $C_{21}H_{30}O_5$: C, 69.6; H, 8.3. Found: C, 69.7; H, 8.3.

Conversion of Anhydrotetrahydrosarsasapogenoic Acid to Anhydrosarsasapogenoic Acid.—The methyl ester of anhydrotetrahydrosarsasapogenoic acid was prepared by refluxing the acid (prepared from 3-acetoxydihydrosarsasapogenin) in methanol solution acidified with sulfuric acid. The ester, m. p. 124–126°, was identical with that obtained by the action of diazomethane on the acid.

A mixture of 7 g. of the methyl ester and 60 cc. of acetic anhydride was refluxed for thirty minutes. The acetic anhydride was evaporated *in vacuo* and the residual sirup dissolved in 200 cc. of acetic acid. To this well-stirred solution heated at 55–60° was added 10 g. of chromic anhydride in 80 cc. of 80% acetic acid over a period of two hours. The mixture was heated for an additional two hours at 55–60° and then for one hour at 80°. The excess chromic anhydride was destroyed with 5 cc. of ethanol and the mixture was evaporated *in vacuo* to a volume of approximately 75 cc. The mixture was diluted with water and the solid extracted with ether. The ethereal extract was washed with water and then twice with 3% sodium hydroxide solution.

The ethereal solution containing the neutral material was evaporated to give approximately 2 g. of sirup. This was dissolved in 30 cc. of 95% ethanol and to this solution was added 3 g. of potassium hydroxide and 15 cc. of water. The solution, after refluxing for two hours, was diluted with water and the resulting clear solution acidified with hydrochloric acid. The precipitated acid was taken up in ether. The ethereal solution was evaporated to a volume of about 30 cc. when 575 mg. of compact white crystals separated. These were recrystallized once from methanol–ether to give a product with m. p. 243–245° dec. This gave no depression with a sample of anhydrosarsasapogenoic acid prepared from sarsasapogenoic acid, m. p. 242–244°.

Anal. Calcd. for $C_{27}H_{40}O_4$: C, 75.7; H, 9.4. Found: C, 75.6; H, 9.4.

The acidic fraction from the oxidation yielded the C_{22} keto acid, m. p. 283–285°.

A similar oxidation of the methyl ester acetate of anhydrotetrahydrosarsasapogenoic acid (1.45 g.) prepared from sarsasapogenoic acid by catalytic hydrogenation yielded the C_{22} keto acid, m. p. 284–286° dec.

Anal. Calcd. for $C_{22}H_{34}O_4$: C, 72.9; H, 9.5. Found: C, 72.6; H, 9.4.

The acidic filtrate from this yielded 3-hydroxy-*etio*-bilianic acid, m. p. 219–221°.

Anal. Calcd. for $C_{19}H_{30}O_5$: C, 67.4; H, 8.9. Found: C, 67.2; H, 9.0.

The neutral fraction from the oxidation upon treatment with aqueous ethanolic potassium hydroxide as described previously yielded anhydrosarsasapogenoic acid, m. p. 243–245° dec.

Anal. Calcd. for $C_{27}H_{40}O_4$: C, 75.7; H, 9.4. Found: C, 75.9; H, 9.4.

Summary

The chromic anhydride oxidation products of dihydrosarsasapogenin have been studied.

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[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE]

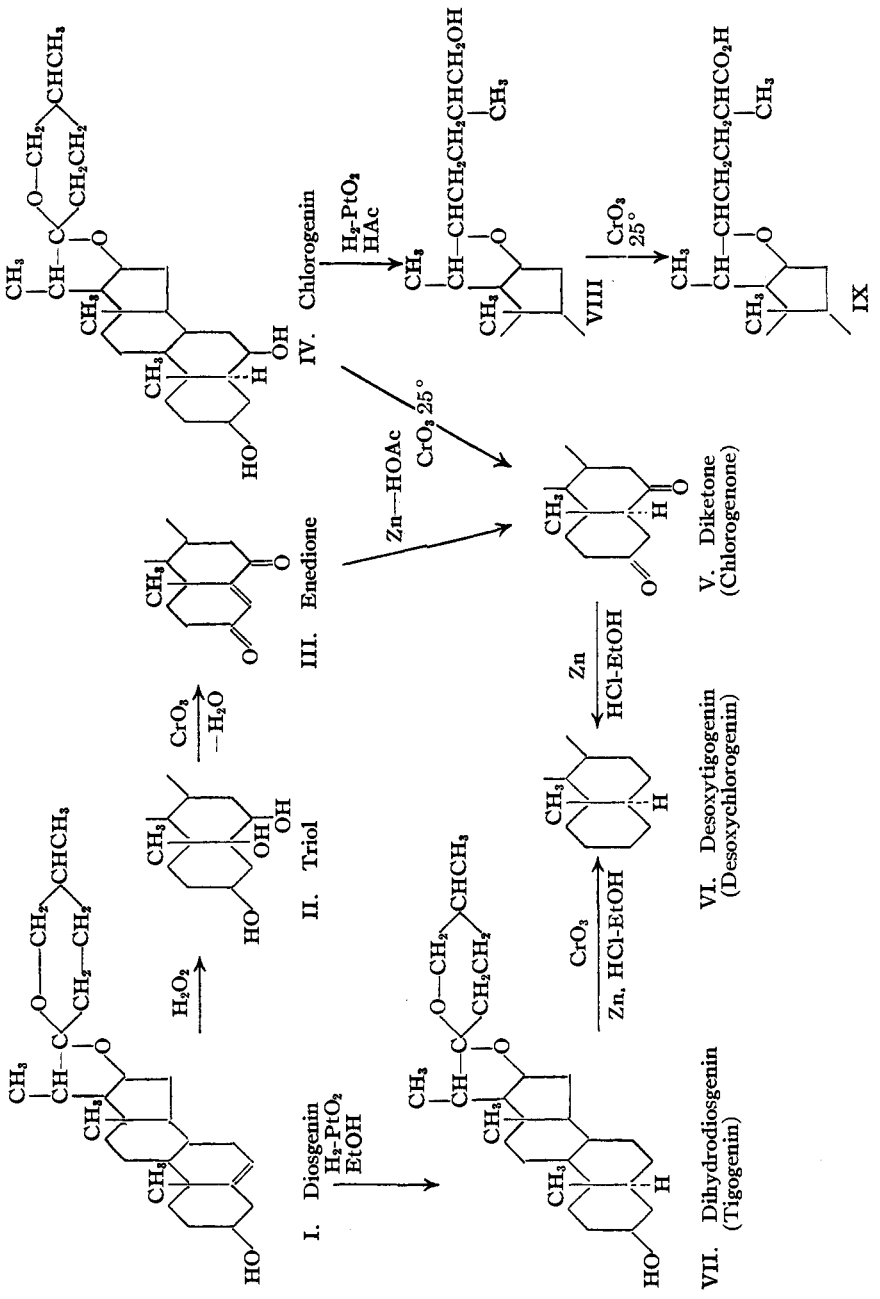
Sterols. LXXX. Reactions of Chlorogenin

BY RUSSELL E. MARKER AND EWALD ROHRMANN

In a previous paper[1] we presented evidence indicating that the nuclear hydroxyl groups of the steroid sapogenin, chlorogenin,[2] were located at C-3 and C-6 and that the hydroxyl group at C-3 was of the β-configuration. Noller[3] had previously postulated that the hydroxyl groups were at C-3 and C-12 and that the group at C-3 was of the α-configuration. We also presented evidence indicating that chlorogenin belongs to the *allo* series in its configuration at C-5. Further evidence concerning the nuclear structure is afforded by the fact that tigogenone and chlorogenone upon Clemmensen reduction yield the same desoxy compound.[4] The catalytic hydrogenation of the desoxy compounds in an acidic medium likewise gives the same dihydrodesoxy compound. This indicates that chlorogenin differs from tigogenin only in the presence of an additional hydroxyl group.

In a previous paper[4] we alluded to the highly interesting studies of Tsukamoto, Ueno and Ota[5] on diosgenin (I), an unsaturated steroid sapogenin which yields tigogenin upon reduction. Of especial interest are the experiments of Tsukamoto, Ueno, Ota and Tschesche[6] on the oxidation of dios-

(1) Marker and Rohrmann, THIS JOURNAL, **61**, 946 (1939).
(2) Liang and Noller, *ibid.*, **57**, 525 (1935).
(3) Noller, *ibid.*, **59**, 1092 (1937).
(4) Marker and Rohrmann, *ibid.*, **61**, 1516 (1939).
(5) Tsukamoto, Ueno and Ota, *C. A.*, **31**, 3493 (1937); *ibid.*, **32**, 2537 (1938); Tsukamoto and Ueno, *ibid.*, **32**, 7470 (1938).
(6) Tsukamoto, Ueno, Ota and Tschesche, *J. Pharm. Soc., Japan*, **57**, 283 (1937).



genin with hydrogen peroxide. The product of this reaction is a triol (II) which upon oxidation with chromic anhydride and subsequent dehydration yielded an enedione (III). Reduction of this product with zinc and acetic acid yielded a diketone (V), $C_{27}H_{40}O_4$, m. p. 234°. This diketone yielded a crystalline pyridazine derivative, $C_{27}H_{40}O_2N_2$, dec. 308°.

Professor T. Tsukamoto has kindly compared a sample of chlorogenone, m. p. 234°, with the saturated diketone (V) obtained from diosgenin. The substances appeared to be identical in properties and the mixed melting point showed no depression.

It now appears that the only objection which can be advanced against the formulation of chlorogenin as a 3,6-dihydroxy compound is the fact that on mild oxidation with chromic anhydride, Noller[3] obtained a keto dibasic acid which he stated to be different from digitogenic acid, the chromic anhydride oxidation product of digitogenin. However, no direct comparison of the acids was reported. We have pointed out previously[7] that the chemistry of the oxidation of digitogenin is in a rather confused state. In nearly all of the work on digitogenic acid and its alkali isomerization product, digitoic acid, there appears to be a lack of adequate data on the purity of the acids.

The relationships existing between diosgenin, tigogenin and chlorogenin raise many interesting questions in regard to the biogenesis of the steroidal saponins.

In the present work chlorogenin has been subjected to the reactions characteristic of sarsasapogenin.[8] As would be expected, chlorogenin resembles tigogenin more closely than it does sarsasapogenin in its various reactions involving the side chain. It shows little tendency to undergo isomerization with hydrochloric acid in ethanol solution. Attempts to reduce the substance by the Clemmensen method were unsuccessful.

(7) Marker and Rohrmann, THIS JOURNAL, **61**, 2724 (1939).
(8) Marker and Rohrmann, *ibid.*, **61**, 746 (1939).

Chlorogenin upon catalytic hydrogenation in acetic acid yielded dihydrochlorogenin (VIII), a substance analogous to dihydrosarsasapogenin. Dihydrochlorogenin formed a tris-3,5-dinitrobenzoate, indicating the presence of a third hydroxyl group. Mild oxidation of dihydrochlorogenin with chromic anhydride readily yielded an acid, $C_{27}H_{40}O_6$, which formed a disemicarbazone and a methyl ester. This acid (IX) has been designated tentatively as 3,6-dehydroanhydrotetrahydrochlorogenoic acid[9] because of its analogy to 3-dehydroanhydrotetrahydrosarsasapogenoic acid.[9]

Chlorogenin diacetate reacted readily with one mole of bromine in acetic acid to yield bromochlorogenin diacetate. Reduction of the bromo compound with sodium and ethanol yielded chlorogenin.[8,10]

McMillan and Noller,[11] in their studies on the oxidation of chlorogenin diacetate with chromic anhydride under relatively mild conditions, obtained the chlorogenin lactone diacetate as one of the products. Both their lactone diacetate and the hydroxy lactone were crystallized from methanol and their analyses indicated the presence of methanol of crystallization. We have oxidized chlorogenin diacetate with chromic anhydride under somewhat more vigorous conditions to obtain the lactone diacetate in yields comparable to the lactone yields obtained in an analogous oxidation of sarsasapogenin acetate. Analyses of our lactone diacetate, dihydroxy lactone, lactone dibenzoate and diketo lactone, none of which were crystallized from methanol, indicated the absence of any solvent of crystallization. From the acidic products of the oxidation there was obtained a small amount of an acid which analyses indicated as analogous to the C_{22} keto acid obtained by a similar oxidation of sarsasapogenin acetate.[12] Unfortunately, the yield of this acid was too low to permit further characterization.

We wish to thank Parke, Davis and Company for their generous help and assistance in the various phases of this work. We are especially grateful to Professor T. Tsukamoto of Kanazawa University, Kanazawa, Japan, for comparing a sample of chlorogenone with the saturated diketone derived from diosgenin. We also wish to thank Mr. Paul H. Williams, and Drs. Elmer J. Lawson and H. C. Benedict for their assistance in obtaining the bulbs of *Chlorogalum pomeridianum*.

(9) Marker and Rohrmann, THIS JOURNAL, **61**, 2072 (1939).
(10) Marker and Rohrmann, *ibid.*, **61**, 1921 (1939).
(11) McMillan and Noller, *ibid.*, **60**, 1630 (1938).
(12) Marker and Rohrmann, *ibid.*, **61**, 1285 (1939).

Experimental Part

The chlorogenin was isolated from *Chlorogalum pomeridianum* by the procedure described by Liang and Noller.[2] The material was crystallized from methanol as white needles, m. p. 265–268°.

A sample of chlorogenin (1 g.), m. p. 265–268°, was refluxed for fifty hours with 150 cc. of 95% ethanol and 30 cc. of concentrated hydrochloric acid. The product (800 mg.) recovered from the reaction melted at 258–265°. A mixture with chlorogenin melted at 260–267°.

Chlorogenin was recovered essentially unchanged after treatment with amalgamated zinc in ethanol–hydrochloric acid solution. When heated at 80° with an acetic acid solution of selenium dioxide a red precipitate formed almost at once. No product was isolated.

Dihydrochlorogenin.—A mixture of 7 g. of chlorogenin, 1 g. of Adams catalyst and 160 cc. of glacial acetic acid was shaken with hydrogen at 70° and 3 atmospheres for sixteen hours. The mixture was filtered and the filtrate evaporated *in vacuo*. The residual sirup, which would not crystallize, was refluxed on the steam-bath for thirty minutes with an excess of alcoholic potassium hydroxide. The resulting solution was diluted with water. The precipitate was collected, washed with water and dried. The white residue was crystallized from 95% ethanol to give 5 g. of white needles, m. p. 233–235°, sparingly soluble in ether.

Anal. Calcd. for $C_{27}H_{46}O_4$: C, 74.6; H, 10.7. Found: C, 74.8; H, 10.6.

It gave a precipitate with a 2% ethanolic solution of digitonin but did not react with bromine or selenium dioxide.

Tris-3,5-dinitrobenzoate of Dihydrochlorogenin.—To a solution of 400 mg. of dihydrochlorogenin in 15 cc. of dry pyridine was added 800 mg. of 3,5-dinitrobenzoyl chloride. The resulting solution was allowed to stand at room temperature for eight hours. It was then heated at 60° for three hours and the material poured into an excess of dilute hydrochloric acid. The resulting precipitated mixture, after standing overnight, was collected, washed with water, dried, and crystallized from acetone to give white crystals, m. p. 210–212°.

Anal. Calcd. for $C_{41}H_{50}O_{14}N_4$: C, 68.1; H, 7.0. Calcd. for $C_{48}H_{52}O_{19}N_6$: C, 56.7; H, 5.2. Found: C, 57.1; H, 5.1.

Oxidation of Dihydrochlorogenin.—To a solution of 1.5 g. of dihydrochlorogenin in 60 cc. of glacial acetic acid was added a solution of 1.5 g. of chromic anhydride in 30 cc. of 80% acetic acid. After standing at room temperature for two hours the solution was diluted with water and the mixture extracted with ether. The ethereal extract was washed first with water and then with dilute sodium carbonate solution. The sodium carbonate washings were acidified with hydrochloric acid and the white precipitate which separated was collected and washed with water. The dried material was crystallized from acetone as white plates, m. p. 202–204°.

Anal. Calcd. for $C_{27}H_{40}O_6$: C, 72.9; H, 9.1; neut. equiv., 454. Found: C, 73.1; H, 9.1; neut. equiv., 460.

The acid reacted with an ethanolic solution of semicarbazide acetate under the usual conditions to yield a **disemicarbazone** which was crystallized from ethanol to give a product, with m. p. 240° dec.

Anal. Calcd. for $C_{29}H_{46}O_5N_6$: C, 62.3; H, 8.3. Found: C, 62.5; H, 8.4.

With diazomethane the acid yielded a **methyl ester** which was crystallized from ether–pentane as white needles, m. p. 156.5–158°.

Anal. Calcd. for $C_{28}H_{42}O_5$: C, 73.3; H, 9.2. Found: C, 73.5; H, 9.4.

Bromochlorogenin Diacetate.—To a solution of 500 mg. of chlorogenin diacetate in 40 cc. of glacial acetic acid containing two drops of 48% hydrobromic acid was added 1.1 cc. of 1.05 *M* bromine in glacial acetic acid as rapidly as the bromine was absorbed. Hydrogen bromide was liberated. The solution was poured into water and the precipitate collected and washed with water. The dried white residue was crystallized from methanol to give white crystals, m. p. 200° with slight decomposition.

Anal. Calcd. for $C_{31}H_{47}O_6Br$: C, 62.5; H, 7.9. Found: C, 62.5; H, 7.8.

Dihydrodesoxychlorogenin (Dihydrodesoxytigogenin).—A mixture of 100 mg. of desoxychlorogenin, m. p. 173–175°, prepared by Clemmensen reduction of chlorogenone with amalgamated zinc, 120 cc. of acetic acid and 500 mg. of Adams catalyst was shaken with hydrogen at 3 atmospheres pressure for sixteen hours at 25°. The product was worked up as described for dihydrochlorogenin. Crystallization from ether–pentane gave white needles, m. p. 92.5–93.5°. This gave no depression with a sample of dihydrodesoxytigogenin,[4] m. p. 92.5°.

Anal. Calcd. for $C_{27}H_{46}O_2$: C, 80.5; H, 11.5. Found: C, 80.4, 80.5; H, 11.6, 11.6.

Oxidation of Chlorogenin Diacetate.—To a solution of 4 g. of chlorogenin diacetate in 175 cc. of glacial acetic acid heated on the steam-bath at 90–95° was added 5.5 g. of chromic anhydride in 50 cc. of 80% acetic acid over a period of one hour. The mixture was heated for an additional ninety minutes. After evaporating to a volume of approximately 50 cc. *in vacuo* the mixture was diluted with water and the precipitated solids taken up in ether. The ethereal extract was washed with water and then with 3% sodium hydroxide solution to remove acidic products.

The sodium hydroxide solution was heated for fifteen minutes on the steam-bath, cooled and acidified with hydrochloric acid. The precipitated acids were taken up in ether and crystallized from acetone to give a small amount of white crystals, m. p. 224–226°.

Anal. Calcd. for $C_{22}H_{34}O_5$: C, 69.8; H, 9.0. Found: C, 69.6, 69.4; H, 9.1, 9.0.

The ethereal solution containing the neutral fraction was evaporated and the residue crystallized from ether–pentane–acetone to give white needles, m. p. 247–250°, of the **lactone diacetate.**

Anal. Calcd. for $C_{26}H_{38}O_6$: C, 69.9; H, 8.6. Found: C, 69.9; H, 8.6.

Hydrolysis of the lactone diacetate with ethanolic potassium hydroxide gave **chlorogenin lactone** which crystallized from ether–acetone as white needles, m. p. 250–251.5°.

Anal. Calcd. for $C_{22}H_{34}O_4$: C, 72.9; H, 9.5. Found: C, 72.6; H, 9.7.

Oxidation of chlorogenin lactone with chromic anhydride in acetic acid at 25° for one hour gave the **diketo lactone** which crystallized from ether–pentane as compact white crystals, m. p. 243–245°.

Anal. Calcd. for $C_{22}H_{30}O_4$: C, 73.7; H, 8.4. Found: C, 73.6; H, 8.2.

Treatment of chlorogenin lactone with benzoyl chloride in pyridine at 25° for twenty hours gave the **lactone dibenzoate** which crystallized from aqueous acetone as white needles, m. p. 278–280°.

Anal. Calcd. for $C_{36}H_{42}O_6$: C, 75.75; H, 7.4. Found: C, 75.4; H, 7.4.

Summary

Chlorogenone prepared by the oxidation of chlorogenin appears to be identical with the 3,6-diketone of diosgenin.

Chlorogenin was reduced catalytically to give dihydrochlorogenin, mild oxidation of which gave an acid, $C_{27}H_{40}O_5$, which formed a disemicarbazone and a methyl ester.

Chlorogenin formed a monobromo derivative with bromine in acetic acid. Reduction of this gave back chlorogenin.

The lactone diacetate, dihydroxy lactone, lactone dibenzoate and diketo lactone were prepared from chlorogenin.

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