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

Sterols. CII. Chlorogenin

BY RUSSELL E. MARKER, ELDON M. JONES AND D. L. TURNER

Marker and Rohrmann[1] assigned the hydroxyl groups of chlorogenin (IV) to positions 3,6 because chlorogenone formed a pyridazine derivative $C_{27}H_{40}O_2N_2$ when treated with hydrazine hydrate. As further proof that the hydroxyl groups of chlorogenin are 3,6 Professor Tsukamoto compared a sample of our chlorogenone with the 3,6 saturated diketone[2] which he obtained from diosgenin and found that they are identical.

It has been shown[3] that the hydrogen atom at C-5 is of the *allo*-configuration since chlorogenone upon refluxing with acids is recovered unchanged, and Clemmensen reduction gave a desoxy compound identical with that obtained from tigogenone.[4] The hydroxyl group at C-3 in chlorogenin is probably of the beta configuration since there is only little epimerization when chlorogenin is boiled with sodium amylate. This is true of all sterols of the *allo*-series in which the 3-OH group is of the beta configuration. Chlorogenin also gives a digitonide which is quite insoluble in 80% ethanol.

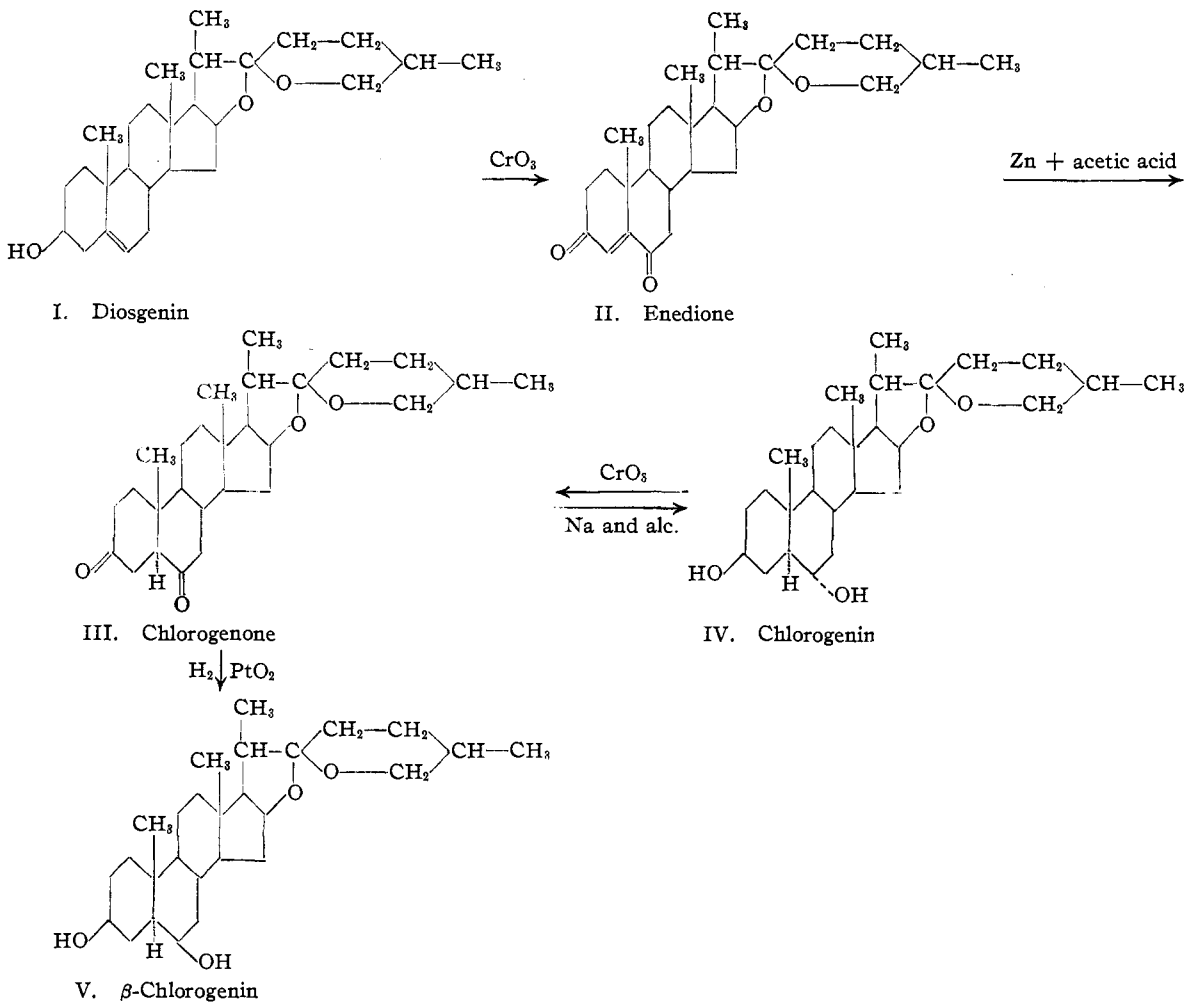
We have now proved conclusively that the structure of chlorogenin proposed by Marker and Rohrmann[5] is correct. A $\Delta^{4,5}$-diosgendione-3,6 can be prepared in poor yield from diosgentriol-3,5,6.[2] We have obtained this substance in good yield directly from diosgenin using a procedure developed by Mauthner[6] and Windaus[7,8] for the preparation of $\Delta^{4,5}$-cholestenedione-3,6 from cholesterol. Reduction with zinc in acetic acid gave 6-keto-tigogenone, which was identical with chlorogenone prepared by the oxidation of naturally occurring chlorogenin. Reduction of the carbonyl groups with sodium in alcohol of both chlorogenone and 6-keto-tigogenone gave chlorogenin which was identical with the naturally occurring chlorogenin; the acetates were also identical. Reduction in alcohol with Adams catalyst gave beta chlorogenin. The reduction products obtained from the chlorogenone of different sources were identical. The identity was confirmed with the acetates.

By a comparison of the reduction products of chlorogenone and cholestanedione-3,6 we have obtained additional evidence indicating that the hydroxyl group at C-3 in chlorogenin is of the beta configuration. Windaus[9] reduced cholestanol-3-one-6 with sodium and ethanol and obtained a diol which melted at 216°. We have reduced cholestanedione-3,6 with sodium and ethanol and obtained the diol melting at 216°. When chlorogenone was reduced with sodium in ethanol in the same manner as cholestanedione-3,6 we obtained a product in good yield which was identical with the original naturally occurring chlorogenin. Since the method of reduction of chlorogenone was identical with that of the reduction of cholestanedione, the configuration of the hydroxyl group at C-3 is of the beta type. This was to be expected, for in all known cases the reduction by sodium in alcohol of a 3-keto sterol in which the configuration at C-5 is *allo* gives rise to a 3OH of the beta configuration.

Reduction of cholestanol-3-one-6[10] and cholestanone-3-ol-6[11] with hydrogen in the presence of Adams catalyst gave a cholestanediol, melting at 190°, which is isomeric with that obtained by Windaus, differing only in the configuration of the hydroxyl groups at C-6. In both of these diols the hydroxyl groups at C-3 have the beta configuration. Upon reduction of chlorogenone with hydrogen and platinum oxide catalyst we obtained a compound, melting at 246–248°, which is isomeric with chlorogenin, differing only in the configuration of the hydroxyl group at C-6. We call this beta-chlorogenin.

A further confirmation of the conclusions from these reductions is the fact that *allo*-hyodesoxycholic acid was formed by the reduction of 3,6-diketoallocholanic acid with hydrogen and platinum oxide catalyst.[12] The same acid also was formed by the oxidation of the diacetate[11] of cholestanediol-3,6 of m. p. 190°, showing that the hydroxyl groups in cholestanediol, m. p.

(1) Marker and Rohrmann, THIS JOURNAL, **61**, 946 (1939).
(2) Tsukamoto, Ueno, Ota and Tschesche, *J. Pharm. Soc. Japan*, **57**, 283 (1937).
(3) Marker and Rohrmann, THIS JOURNAL, **61**, 1516 (1939)
(4) Tschesche and Hagedorn, *Ber.*, **68**, 1412 (1935).
(5) Marker and Rohrmann, THIS JOURNAL, **61**, 3479 (1939).
(6) Mauthner and Suida, *Monatsh.*, **17**, 579 (1896).
(7) Windaus, *Ber.*, **39**, 2249 (1906).
(8) Windaus, *ibid.*, **40**, 257 (1907).
(9) Windaus, *ibid.*, **50**, 133 (1917).
(10) Marker and Krueger, THIS JOURNAL, **62**, 79 (1940).
(11) Marker, Krueger, Adams and Jones, *ibid.*, **62**, 645 (1940).
(12) Windaus, *Ann.*, **447**, 233 (1926).



I. Diosgenin II. Enedione

III. Chlorogenone IV. Chlorogenin

V. β-Chlorogenin

190°, have the same configuration as those of *allo*-hyodesoxycholic acid, in which both are beta. Correspondingly, the hydroxyl groups of beta-chlorogenin are both of the beta configuration. Since chlorogenin and beta-chlorogenin differ only in the configuration of the hydroxyl groups at C-6, the hydroxyl groups of the naturally occurring chlorogenin are β at C-3 and α at C-6.

Upon the oxidation of crude digitogenin, Windaus[13] isolated a neutral fraction of ketones which were not derived from gitogenin or digitogenin, one of which is a diketo sapogenin melting at 227–228°. We have oxidized the accumulated residues from the purification of digitogenin and have obtained the diketone which Windaus reported. By repeated crystallization we obtained a melting point of 233–236°. This is identical with chlorogenone.

Windaus also isolated a diketone from the oxidation melting at 199°, which upon treatment with 0.1 *N* alcoholic potassium hydroxide gave the higher melting diketone which we identified as chlorogenone. Because of this isomerization it appears that this diketone is derived from a sapogenin which is identical with chlorogenin except that the configuration at C-5 is of the coprostane type rather than of the *allo* configuration as found in chlorogenin. This is consistent with the ease of isomerization by acid or alkali of compounds having the coprostane configuration at C-5 and a keto group at C-6,[14] whereas those with the *allo* configuration remain unchanged under this treatment.

We wish to thank Parke, Davis and Company for their generous assistance. We also thank Dr. Takeo Tsukamoto for supplying us with the diosgenin, Dr. John Krueger for supplying choles-

(13) Windaus, *Z. physiol. Chem.*, **150**, 205 (1925).

(14) Windaus, *Ann.*, **447**, 233 (1926); Wieland and Dane, *Z. physiol. Chem.*, **212**, 263 (1932).

tanedione-3,6 and Dr. H. C. Benediet for his assistance in obtaining bulbs of *Chlorogalum pomeridianum.*

Experimental Part

Reduction of Chlorogenone with Sodium and Ethyl Alcohol.—To a boiling solution of 1 g. of chlorogenone, m. p. 237°, in 200 cc. of absolute ethyl alcohol was added 10 g. of sodium during one hour. The mixture was diluted with water and extracted with ether. The ether extract was washed with water and the ether was evaporated. The residue, after treatment in acetone with Norit, was crystallized from acetone to give 600 mg. of fine, white needles, m. p. 271°. A mixture with chlorogenin, m. p. 271–272°, melted at 271–272°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.9; H, 10.2. Found: C, 75.2; H, 10.4.

With hot acetic anhydride, the reduction product gave a **diacetate** which was crystallized from aqueous methanol, m. p. 151°. A mixture with chlorogenin diacetate, m. p. 152°, melted at 151–152°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.05; H, 9.4. Found: C, 72.1; H, 9.5.

Catalytic Reduction of Chlorogenone.—A mixture of 1.5 g. of chlorogenone, 300 mg. of Adams catalyst, and 200 cc. of ethanol was shaken with hydrogen at room temperature and three atmospheres pressure for four hours. The mixture was filtered and the filtrate was evaporated *in vacuo.* The remaining solid was crystallized from aqueous ethanol and then from acetone to give 1.2 g. of fine, white needles melting at 246–248°. When mixed with naturally occurring chlorogenin, m. p. 271–272°, it melted at 233–250°. This product will be referred to as beta-chlorogenin.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.9; H, 10.2. Found: C, 74.5; H, 10.3.

With hot acetic anhydride this material gave a diacetate which was crystallized from aqueous methanol, m. p. 120°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.05; H, 9.4. Found: C, 72.4; H, 9.4.

When treated with benzoyl chloride in pyridine, it gave a dibenzoate, m. p. 198–200°, when crystallized from aqueous acetone.

Anal. Calcd. for $C_{41}H_{52}O_6$: C, 76.9; H, 8.1. Found: C, 77.1; H, 8.2.

Dihydro-β-chlorogenin.—A mixture of 800 mg. of β-chlorogenin, 200 mg. of Adams catalyst and 75 cc. of glacial acetic acid was shaken with hydrogen at room temperature and three atmospheres pressure for fifteen hours. The mixture was filtered and the solvent was evaporated *in vacuo.* The resulting residue was hydrolyzed with boiling methanolic potassium hydroxide and poured into water. The mixture was extracted with ether and the extract was washed with water. The residue from evaporation of the ether crystallized from aqueous acetone to give compact white crystals of dihydro-β-chlorogenin, m. p. 209–210°. A mixture with dihydrochlorogenin, m. p. 228–231°, melted at 193–215°.

Anal. Calcd. for $C_{27}H_{46}O_4$: C, 74.6; H, 10.7. Found: C, 74.4; H, 10.7.

Reduction of Cholestanedione-3,6 with Sodium and Ethyl Alcohol.—To a boiling solution of 1.3 g. of cholestanedione-3,6 in 200 cc. of absolute ethanol was added 12 g. of sodium during one hour. The reaction mixture was worked up in the manner described for the similar reduction of chlorogenone. After crystallization from aqueous acetone, the product melted at 215–216°.

Anal. Calcd. for $C_{27}H_{48}O_2$: C, 80.1; H, 12.0. Found: C, 79.8; H, 11.9.

$\Delta^{4,5}$-Diosgendione-3,6.—Diosgenin (2 g.) was dissolved in 100 cc. of hot acetic acid. The solution was cooled to 20° and a solution of chromium trioxide (2 g.) in 40 cc. of 80% acetic acid was added. The mixture was left at 18–20° for one hour. The excess chromic acid was decomposed with ethanol and the mixture was poured into water. The product was taken up in ether. The ether extract was washed with dilute sodium carbonate after washing out the acetic acid with water. Concentration of the ether gave 1.0 g. of $\Delta^{4,5}$-diosgendione-3,6 which was washed with pentane and recrystallized from ethanol giving pale yellow needles, m. p. 192–195°.

Anal. Calcd. for $C_{27}H_{38}O_4$: C, 76.02; H, 8.98. Found: C, 75.87; H, 9.14.

6-Keto-tigogenone (Chlorogenone).—The total crude $\Delta^{4,5}$-diosgendione-3,6 obtained from the oxidation of diosgenin (2 g.) was dissolved in 150 cc. of glacial acetic acid. After the addition of 5 cc. of water and 4 g. of zinc dust the mixture was refluxed for four hours. It was filtered and the product was taken up in ether. The ethereal solution was washed well with dilute potassium hydroxide solution. The ether was removed and the product crystallized from acetone, m. p. 235–237°. When mixed with chlorogenone, m. p. 235–237°, it melted at 235–237°; yield, 1.1 g.

Anal. Calcd. for $C_{27}H_{40}O_4$: C, 75.66; H, 9.41. Found: C, 75.54; H, 9.44.

Catalytic Reduction of 6-Keto-tigogenone to β-Chlorogenin.—A mixture of 1 g. of 6-keto-tigogenone, 500 mg. of Adams catalyst and 100 cc. of ethanol was shaken with hydrogen at 3 atm. at room temperature for two hours. After filtering the catalyst, the solvent was removed and the residue crystallized from acetone, m. p. 243–246°. When mixed with β-chlorogenin, m. p. 245–247°, prepared by the catalytic reduction of an authentic sample of chlorogenone it melted at 244–247°.

When refluxed with acetic anhydride it gave a diacetate which was crystallized from methanol, m. p. 118–120°. When mixed with an authentic sample of the diacetate of β-chlorogenin, m. p. 120°, it melted at 119–120°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.0; H, 9.4. Found: C, 71.86; H, 9.67.

Reduction of 6-Keto-tigogenone with Sodium and Ethanol.—To a solution of 200 mg. of 6-keto-tigogenone in 50 cc. of absolute ethanol 4 g. of sodium was added in small pieces. After the sodium had dissolved the product was poured into water and extracted with ether. The solvent was removed and the residue crystallized from methanol; yield, 70 mg., m. p. 271–274°. When mixed with chlorogenin, m. p. 272–275°, it melted at 272–275°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.9; H, 10.2. Found: C, 74.9; H, 10.4.

When refluxed with acetic anhydride it gave a diacetate which was crystallized from methanol and melted at 150–152°. When mixed with chlorogenin acetate (from natural chlorogenin), m. p. 151–153°, it melted at 151–153°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.0; H, 9.4. Found: C, 72.2; H, 9.7.

Oxidation of Digitogenin Residues.—The residues from the crystallization of 100 g. of digitogenin as the acetate were hydrolyzed and extracted with ether. These weighed 38 g. This was dissolved in 1 liter of acetic acid. Chromic acid (50 g.) in 500 cc. of 90% acetic acid was added slowly, while keeping the temperature at 20°. It was allowed to stand at this temperature for twenty minutes, water was added and the product extracted with ether. The acids were removed by shaking with water and sodium carbonate solution, and the ether was evaporated. The residue was refluxed for one hour with 1% potassium hydroxide in methanol. The solution was then diluted with water, extracted with ether and the product sublimed in a high vacuum at 130–160°. The sublimate was crystallized from methanol to give a product melting at 233–236°. When mixed with chlorogenone, m. p. 235–237°, it melted at 234–237°.

Anal. Calcd. for $C_{27}H_{40}O_4$: C, 75.7; H, 9.4. Found: C, 75.5; H, 9.4.

Summary

Evidence has been presented indicating that the hydroxyl groups of chlorogenin are 3-beta and 6-alpha.

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Sterols. CIII. The Oxidation of Pregnanetriols

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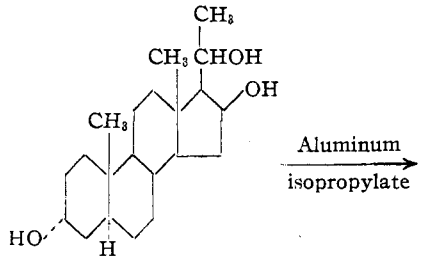
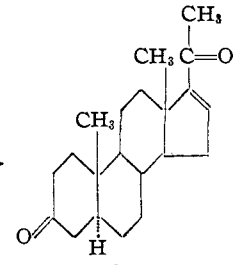
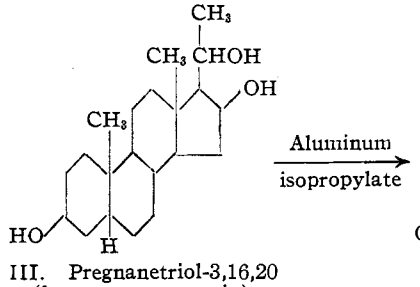
An *allo*-pregnanetriol isolated from the urine of pregnant mares[1] was believed by Odell and Marrian[2] to be pregnanetriol-3(α),6,20. Marker and Wittle[3] obtained much evidence indicating that the compound was *allo*-pregnanetriol-3,16,20.



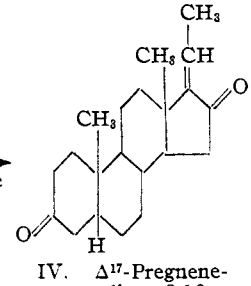
I. *allo*-Pregnanetriol-3,16,20 (from mares' urine)



II. Δ^{16}-*allo*-Pregnenedione-3,20



III. Pregnanetriol-3,16,20 (from sarsasapogenin)



IV. Δ^{17}-Pregnenedione-3,16

Recently pseudo-tigogenin was converted to Δ^{16}-*allo*-pregnenedione-3,20 by oxidation with chromic acid under mild conditions. This was reduced with hydrogen using a barium sulfate–palladium catalyst to *allo*-pregnanedione-3,20.[4] Oxidation using aluminum isopropylate and a large excess of cyclohexanone[5] has now been applied to the *allo*-pregnanetriol-3,16,20 from mares' pregnancy urine. The product obtained was Δ^{16}-*allo*-pregnenedione-3,20, identical with that obtained from tigogenin. This compound has also been obtained by Butenandt, Mamoli and Heusner[6] from androsterone. The oxidation evidently occurs first at the 3 and 20 positions and the product loses water under the conditions used to give the unsaturated diketone. Reduction with palladium–barium sulfate catalyst and hydrogen gives *allo*-pregnanedione-3,20, a compound previously prepared from *allo*-pregnanetriol-3,16,20 in poor yield.[7]

The preparation of Δ^{16}-*allo*-pregnenedione-3,20 from the triol of Marrian proves conclusively that the structure proposed by Marker and Wittle[3] is correct.

Sarsasapogenin has been oxidized to pregnanetriol-3,16,20 of the coprostane series.[8] By using the dry persulfate reagent of von Baeyer[9] the yield of triol in this oxidation

(1) Haslewood, Marrian and Smith, *Biochem. J.*, **28**, 1316 (1934).
(2) Odell and Marrian, *J. Biol. Chem.*, **125**, 333 (1938).
(3) Marker and Wittle, THIS JOURNAL, **61**, 855 (1939).
(4) Marker and Rohrmann, *ibid.*, **62**, 898 (1940).
(5) *Cf.* Oppenauer, *Rec. trav. chim.*, **56**, 137 (1937).
(6) Butenandt, Mamoli and Heusner, *Ber.*, **72**, 1614 (1939).
(7) Marker, Kamm, Wittle, Oakwood and Lawson, THIS JOURNAL, **60**, 1067 (1938).
(8) Marker, Rohrmann, Crooks, Wittle, Jones and Turner, *ibid.*, **62**, 525 (1940).
(9) Von Baeyer and Villiger, *Ber.*, **32**, 3625 (1899).