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

Sterols. CX. The Position of the Hydroxyl Groups in Chlorogenin

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Two methods have been published[1,2,3] for the conversion of diosgenin to chlorogenone. One of these,[3] involving the preparation of a 3,6-diketone from a Δ^5-3-hydroxysterol using chromic acid followed by treatment with zinc dust, is a general reaction,[4,5,6] and has been improved in the present paper by using zinc dust both for the decomposition of the excess chromic acid and for the subsequent reduction.

This reaction has now been applied to Δ^5-pregnene-ol-one-20 (IV), a substance readily obtained from stigmasterol by the method of Fernholz.[7] The product is *allo*-pregnanetrione-3,6,20 (VIII), analogous to the diketone from cholesterol.[5,6]

We have recently described[8,9] methods for obtaining pregnane derivatives from sapogenins. These methods have been extended to the preparation of *allo*-pregnanetrione-3,6,20 from chlorogenin and diosgenin through several intermediates. These transformations (see chart) provide additional proof that the double bond of diosgenin is in the 5–6 position and that the hydroxyl groups of chlorogenin are in the 3 and 6 positions.

Experimental Part

Oxidation of Pseudochlorogenin (II) to Δ^{16}-*allo*-Pregnenetrione-3,6,20 (VII).—To a solution of 3 g. of pseudochlorogenin in 1 liter of acetic acid was added a solution of 4.0 g. of chromic anhydride in 50 cc. of 90% acetic acid. The temperature was kept at 25–28° for ninety minutes. It was stirred with zinc dust for fifteen minutes, filtered and vacuum distilled to about 100 cc. Water was added and the product was extracted with ether. The ethereal solution was washed with water and 2% potassium hydroxide. The ether was evaporated and the residue was crystallized from aqueous acetone, m. p. 223–226°.

Anal. Calcd. for $C_{21}H_{28}O_3$: C, 76.8; H, 8.6. Found: C, 76.4; H, 8.8.

Oxidation of Dihydropseudochlorogenin (XII) to Δ^{16}-*allo*-Pregnenetrione-3,6,20 (VII).—A solution of 1.5 g. of dihydropseudochlorogenin in 500 cc. of acetic acid was oxidized similarly to the above, m. p. 224–227°. When mixed with the product obtained by the oxidation of pseudochlorogenin there was no depression in melting point.

Preparation of Chlorogenone (V) from Diosgenin (I).—To a solution of 10 g. of diosgenin in 1 liter of glacial acetic acid at 15–20° was added a solution of 10 g. of chromic anhydride in 100 cc. of 90% acetic acid. It was allowed to stand for one hour at 20°. At the end of this time 20 g. of zinc dust was added in small portions, followed by 20 cc. of water. The mixture was refluxed for five hours, filtered and the acetic acid vacuum distilled. The residue was dissolved in ether and shaken with water and 2% sodium hydroxide. Upon evaporation of the ether to 100 cc. the product crystallized. It was recrystallized from ether; yield, 3.9 g.; m. p. 236–238°. When mixed with chlorogenone (V) prepared by the oxidation of naturally occurring chlorogenin, m. p. 235–237°, there was no depression in melting point.

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

Pseudochlorogenone (VI).—A mixture of 20 g. of chlorogenone and 40 cc. of acetic anhydride was heated in a bomb tube at 200° for ten hours. The acetic anhydride was vacuum distilled and the residue was saponified with 2% alcoholic potassium hydroxide. The product was extracted with ether and washed well with water. Upon evaporation of the ether a solid was obtained which was quite soluble in acetone, alcohol and ethyl acetate. It crystallized poorly from dilute acetone and from ether–pentane it precipitated as an oil. It was used without further purification.

Acid Isomerization of Pseudochlorogenone (V) to Chlorogenone (V).—To 100 mg. of pseudochlorogenone in 20 cc. of ethanol was added 1 cc. of concentrated hydrochloric acid. The product was allowed to stand two hours, water was added and the precipitate filtered. The precipitate was crystallized from ether, m. p. 234–237°. When mixed with chlorogenone, m. p. 234–237°, there was no depression in melting point.

Anal. Calcd. for $C_{27}H_{40}O_4$: C, 75.7; H, 9.4. Found: C, 75.4; H, 9.5.

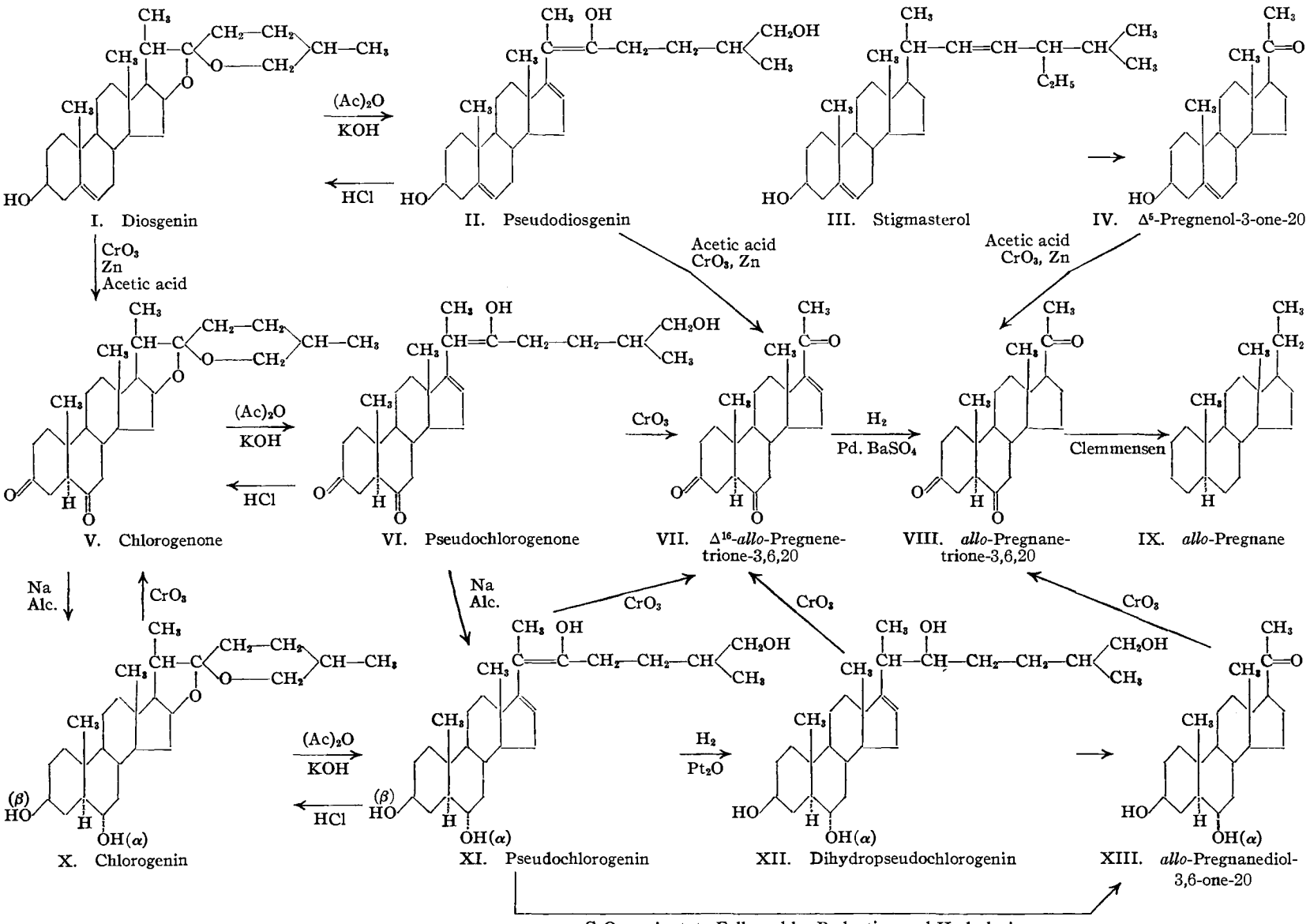
Sodium Reduction of Pseudochlorogenone (VI) to Pseudochlorogenin (XI).—To a solution of 200 mg. of pseudochlorogenone in 25 cc. of absolute ethanol was added 2 g. of sodium. It was refluxed until the sodium was in solution. Water was added and the solid was filtered. It was recrystallized from acetone, m. p. 267–270°. When mixed with pseudochlorogenin (m. p. 270°) it gave no depression in melting point. When mixed with chlorogenin (m. p. 270°) it melted at 245–255°.

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

Oxidation of Pseudochlorogenone (VI) from Diosgenin to Δ^{16}-*allo*-Pregnenetrione-3,6,20 (VII).—To a solution of

(1) Tsukamoto, Ueno, Ota and Tschesche, *J. Pharm. Soc., Japan*, **57**, 283 (1937).
(2) Marker and Rohrmann, THIS JOURNAL, **61**, 3479 (1939).
(3) Marker, Jones and Turner, *ibid.*, **62**, 2537 (1940).
(4) Mauthner and Suida, *Monatsh.*, **17**, 579 (1896).
(5) Windaus, *Ber.*, **39**, 2249 (1906).
(6) Windaus, *ibid.*, **40**, 257 (1907).
(7) Fernholz, *ibid.*, **67**, 2027 (1934).
(8) Marker and Rohrmann, THIS JOURNAL, **61**, 3593 (1939); **62**, 518, 521, 896, 898 (1940).
(9) Marker, Rohrmann and Jones, *ibid.*, **62**, 648 (1940).

CHART[a]



[a] Reactions not appearing in the experimental part of this paper will be found in the previous papers on sterols in THIS JOURNAL by Marker, *et al.*

5 g. of crude pseudochlorogenone (from diosgenin) in 500 cc. of glacial acetic acid was added a solution of 5 g. of chromic anhydride in 50 cc. of 90% acetic acid. It was allowed to stand at 25–28° for ninety minutes. Zinc dust was added and the product was vacuum distilled to about 100 cc. It was then extracted with ether and washed with water and 2% sodium hydroxide. The solvent was removed and the residue was crystallized from aqueous acetone, m. p. 223–226°. Mixed with Δ^{16}-*allo*-pregnenetrione-3,6,20, m. p. 224–227°, prepared by the oxidation of dihydropseudochlorogenin, it gave no depression in melting point.

Anal. Calcd. for $C_{21}H_{28}O_3$: C, 76.8; H, 8.6. Found: C, 76.5; H, 8.4.

Reduction of Δ^{16}-*allo*-Pregnenetrione-3,6,20 (VII) to *allo*-Pregnanetrione-3,6,20 (VIII).—(a) To a solution of 500 mg. of Δ^{16}-*allo*-pregnenetrione-3,6,20, from natural pseudochlorogenin, in 120 cc. of absolute ethyl alcohol was added 500 mg. of platinum oxide catalyst. The mixture was shaken under an atmosphere of hydrogen at 45 pounds pressure for two hours. The catalyst was filtered and the filtrate was evaporated. The residue was dissolved in 15 cc. of acetic acid and a solution of 300 mg. of chromic anhydride in 10 cc. of 80% acetic acid was added. It was allowed to stand for thirty minutes at room temperature. Water was added and the product was extracted with ether. The ethereal solution was washed well with water and dilute potassium hydroxide solution. Upon evaporation of the ether to 5 cc. the product crystallized. This was filtered and the solid was recrystallized from ether–acetone, m. p. 232–235°.

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.3; H. 9.2. Found: C 76.1; H, 9.4.

(b) To a solution of 100 mg. of Δ^{16}-*allo*-pregnenetrione-3,6,20 (VII), from chlorogenone prepared from diosgenin in 100 cc. of absolute alcohol was added 200 mg. of palladium–barium sulfate catalyst. The product was shaken under hydrogen at a pressure of 15 pounds for three hours. The catalyst was filtered and the solvent was removed. The residue was crystallized from ether–acetone, m. p. 232–235°. When mixed with the product obtained above there was no depression in melting point.

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.3; H, 9.2. Found: C, 76.0; H, 9.4.

Oxidation of the Diacetate of Dihydropseudochlorogenin (XII).—To a solution of 3 g. of the diacetate of dihydropseudochlorogenin in 100 cc. of glacial acetic acid was added a solution of 3 g. of chromic anhydride in 30 cc. of 90% acetic acid. It was allowed to stand at room temperature for ninety minutes and then was diluted with water. The product was extracted with ether and the ethereal solution was washed well with water and a 2% solution of potassium hydroxide. The ether was evaporated but the residue failed to crystallize. This was dissolved in 100 cc. of absolute ethanol and shaken with 500 mg. of palladium–barium sulfate catalyst under a pressure of 1 atm. of hydrogen for three hours. The catalyst was filtered and the filtrate was refluxed with a small amount of potassium hydroxide solution for thirty minutes. The alcohol was evaporated to about 20 cc. and water was added. The product was extracted with ether, washed with water and crystallized from ether–acetone, m. p. 206–209°, yield 520 mg.

Anal. Calcd. for $C_{21}H_{34}O_3$: C, 75.4; H, 10.2. Found: C, 75.7; H, 10.2.

Oxidation of Acetylated Pseudochlorogenin (XI).—A mixture of 3 g. of pseudochlorogenin, from natural chlorogenin and 10 cc. of acetic anhydride was refluxed for thirty minutes. The excess acetic anhydride was distilled *in vacuo* and the residual sirup was dissolved in 100 cc. of acetic acid. To this was added a solution of 3 g. of chromic anhydride in 30 cc. of 80% acetic acid. It was allowed to stand at 25–28° for ninety minutes. Water was added and the product was extracted with ether. The ethereal solution was washed well with water and sodium hydroxide solution. The ether was evaporated but the residue did not crystallize from the ordinary solvents. This was dissolved in 120 cc. of absolute ethyl alcohol and 500 mg. of platinum oxide catalyst was added. It was shaken with hydrogen at room temperature and a pressure of 40 pounds for twenty hours. The catalyst was filtered and the solvent was removed *in vacuo*. The residue was dissolved in 30 cc. of acetic acid and to this was added a solution of 1 g. of chromic anhydride in 10 cc. of 80% acetic acid. It was allowed to stand at room temperature for twenty-five minutes, water was added and the product was extracted with ether. The ethereal solution was washed with water and dilute sodium hydroxide solution. The ether was removed and the residue was hydrolyzed by refluxing with alcoholic potassium hydroxide. The product thus obtained was crystallized from ether–acetone and from dilute acetone, m. p. 208–210°. When mixed with the product obtained on the oxidation of the diacetate of dihydropseudochlorogenin it gave no depression in melting point.

Anal. Calcd. for $C_{21}H_{34}O_3$: C, 75.4; H, 10.2. Found: C, 75.8; H, 10.2.

Oxidation of *allo*-Pregnanediol-3,6-one-20 (XIII) to *allo*-Pregnanetrione-3,6,20 (VIII).—To a solution of 100 mg. of *allo*-pregnanediol-3,6-one-20 in 20 cc. of glacial acetic acid was added a solution of 100 mg. of chromic acid in 10 cc. of 90% acetic acid. After standing at room temperature for thirty minutes water was added and the product was extracted with ether. The ethereal solution was washed well with water and potassium hydroxide solution. The solvent was removed and the residue was crystallized from ether–acetone, m. p. 232–235°. It gave no depression in melting point when mixed with *allo*-pregnanetrione-3,6,20 prepared by the reduction of Δ^{16}-*allo*-pregnenetrione-3,6,20.

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.3; H, 9.2. Found: C, 76.0; H, 9.2.

Oxidation of Pseudodiosgenin (II) to Δ^{16}-*allo*-Pregnenetrione-3,6,20 (VII).—To a solution of 1 g. of pseudodiosgenin in 100 cc. of acetic acid at 20° was added a solution of 1.5 g. of chromic anhydride in 20 cc. of 90% acetic acid. It was allowed to stand at 20° for thirty minutes after which time the temperature was raised to 25–28° for one hour. At the end of this time 5 g. of zinc dust was added, followed by 5 cc. of water. It was refluxed for four hours. The product was filtered and the solvent removed *in vacuo*. The residue was extracted with ether and washed well with 2% sodium hydroxide. The ether was removed and the residue crystallized from dilute acetone, m. p. 226–228°.

When mixed with Δ^{16}-*allo*-pregnenetrione-3,6,20 prepared from naturally occurring chlorogenin there was no depression in melting point.

Anal. Calcd. for $C_{21}H_{28}O_3$: C, 76.8; H, 8.6. Found: C, 76.4; H, 8.9.

Clemmensen Reduction of *allo*-Pregnanetrione-3,6,20 (VIII) to *allo*-Pregnane (IX).—To a solution of 400 mg. of *allo*-pregnanetrione-3,6,20 in 200 cc. of 95% ethanol was added 25 g. of amalgamated zinc (20-mesh). The mixture was heated to boiling and 60 cc. of concentrated hydrochloric acid was added over a four-hour period. The product was diluted with water and extracted with ether. It was crystallized from methanol, m. p. 80–82°. Mixed with pregnane (m. p. 80°), it melted at 55–60°. When mixed with *allo*-pregnane (82–83°) it gave no depression in melting point.

Anal. Calcd. for $C_{21}H_{36}$: C, 87.4; H, 12.6. Found: C, 87.2; H, 12.5.

Oxidation of Δ^5-Pregnenol-3-one-20 (IV) to *allo*-Pregnanetrione-3,6,20 (VIII).—To a solution of 100 mg. of Δ^5-*allo*-pregnenol-3-one-20 (from stigmasterol, III) in 20 cc. of glacial acetic acid was added a solution of 100 mg. of chromic anhydride in 3 cc. of 90% acetic acid. It was allowed to stand at 20° for one hour. At the end of this time 3 g. of zinc dust and 3 cc. of water was added and the product was refluxed for four hours. The product was separated from the zinc and extracted well with ether. The ethereal solution was washed with water and dilute alkali. The residue after removal of the solvent was crystallized from ether–acetone, m. p. 232–235°. When mixed with *allo*-pregnanetrione-3,6,20, m. p. 232–235° prepared from naturally occurring chlorogenin, it gave no depression in melting point.

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.3; H, 9.2. Found: C, 76.1; H, 9.4.

We wish to thank Parke, Davis and Company for their assistance.

Summary

1. Transformations have been carried out which indicate conclusively that the hydroxyl groups of chlorogenin occupy positions 3 and 6.

2. In the course of the work a method has been developed for converting 3-hydroxy-Δ^5 sterols into the corresponding 3,6-diketones.

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Sterols. CXI. Sapogenins. XL. The Conversion of Chlorogenin to Tigogenin

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When the semicarbazones of steroidal ketones with the carbonyl group in the 3 position are heated with sodium ethylate, the 3-hydroxyl compounds are formed. Thus Marker and Lawson[1] observed that pregnanol-20(α)-one-3 semicarbazone gave pregnanediol-3(α),20(α) in 85% yield and Marker and Rohrmann[2] obtained sarsasapogenin from the semicarbazone of sarsasapogenone. Dutcher and Wintersteiner[3] studied a number of other sterol semicarbazones and found that the semicarbazone group at the 3 position "yields mainly the corresponding C-3 epimeric carbinols," but semicarbazones at the 7 and 12 positions give the usual reduction to the methylene stage.

We have now taken advantage of the sodium ethylate reaction to convert the disemicarbazone of chlorogenone to tigogenin. As a model experiment the disemicarbazone of cholestanedione-3,6 was first heated with sodium ethylate. Cholestanol-3(β) was separated from the other reaction products by precipitation with digitonin. The disemicarbazone of chlorogenone[4] was then similarly treated. The digitonin precipitable fraction gave material which was identified in its original form and as the acetate with tigogenin and tigogenin acetate.

We wish to thank Parke, Davis and Company for their generous help.

Experimental Part

Treatment of Cholestanedione-3,6 Disemicarbazone with Sodium Ethylate.—The disemicarbazone of cholestanedione-3,6 was prepared in the usual way.

Sodium (2 g.) was dissolved in 25 cc. of absolute ethanol and 3 g. of the disemicarbazone of cholestanedione-3,6 was added. The mixture was heated in a bomb tube at 180° for seven hours. The product was poured into water and extracted with ether. The ether was removed and the residue was dissolved in 25 cc. of ethanol and treated with a solution of 5 g. of digitonin in 250 cc. of ethanol. The precipitated digitonide was decomposed with pyridine in the usual manner and gave material which when recrystallized from acetone melted at 138–139°. When mixed with an authentic sample of cholestanol-3(β) there was no depression in melting point.

(1) Marker and Lawson, THIS JOURNAL, **61**, 852 (1939).
(2) Marker and Rohrmann, *ibid.*, **61**, 1284 (1939).
(3) Dutcher and Wintersteiner, *ibid.*, **61**, 1992 (1939).
(4) Marker and Rohrmann, *ibid.*, **61**, 946 (1939).