from 200 gal. of cows' pregnancy urine was sublimed in a high vacuum and a fraction (900 mg.) collected at 100–200°. This sublimate was treated in the usual manner with digitonin to give 230 mg. of digitonide. The digitonide was decomposed with pyridine but attempts to isolate pure dehydroisoandrosterone benzoate were unsuccessful, for only a few milligrams of the benzoate, m. p. 218–240°, was obtained. This impure benzoate gave no depression with authentic dehydroisoandrosterone benzoate.

The filtrate from the digitonide was worked up as described for the isolation of androsterone oxime from bulls' urine and yielded androsterone oxime (17 mg.), m. p. and mixed m. p. 212–214°.

Anal. Calcd. for $C_{19}H_{31}O_2N$: C, 74.7; H, 10.2. Found: C, 74.5; H, 10.2.

Hydrolysis of Androsterone Oxime from Cows' Pregnancy Urine and Bulls' Urine.—Since the amounts of androsterone oxime from cows' pregnancy urine and bulls' urine were very small, the two specimens were combined.

The combined androsterone oxime in 10 cc. of alcohol and 5 cc. of 4 *N* sulfuric acid was refluxed for four hours. After sublimation in a high vacuum and crystallization from 80% methanol the product (10 mg.) melted at 177–180° and gave no depression with androsterone, m. p. 183°.

Anal. Calcd. for $C_{19}H_{30}O_2$: C, 78.6; H, 10.4. Found: C, 78.3; H, 10.4.

Estrone from Bulls' Urine.—The alkaline hydrolysate obtained in the course of the treatment of 100 gal. (380 liters) of bulls' urine was saturated with carbon dioxide and the precipitated tar removed with ether. After evaporation of the ether on a steam-bath the residual tar (350 g.) was dissolved in 1 liter of alcohol and heated with 30 g. of Girard's reagent for thirty minutes. The solution was diluted with water and ether, the water layer extracted with ether, and then hydrolyzed with hydrochloric acid. The tar (3.2 g.) thus obtained still contained much non-ketonic material; so it was treated again with Girard's reagent (3 g.) to give 820 mg. of ketones. This was freed of non-phenolic impurities by dissolving it in alkali, extracting the alkaline solution with ether, and removing the phenolic ketones by ether extraction of the acidified solution.

The phenolic ketone mixture was then distilled in a high vacuum and the fraction collecting at 140–180° crystallized from alcohol–water. Since the product thus obtained was somewhat oily even after treatment with Norite, it was sublimed in a high vacuum, and a fraction collected at 150–160°. After crystallization from 50% alcohol this yielded 9 mg. of estrone, m. p. 257–258°, which gave no depression with an authentic sample.

Anal. Calcd. for $C_{18}H_{22}O_2$: C, 79.9; H, 8.3. Found: C, 79.8; H, 8.4.

The product (5 mg.) was benzoylated by the Schotten–Baumann method to give a benzoate, m. p. 203–205°, which gave no depression with estrone benzoate.

Summary

Androsterone, dehydroisoandrosterone, and estrone have been isolated from bulls' urine. Androsterone has been isolated from cows' pregnancy urine, and indications of the presence of dehydroisoandrosterone in the same urine also have been obtained. These results are discussed in the light of the author's theory of the biogenesis of the steroidal hormones.

STATE COLLEGE, PENNA. RECEIVED FEBRUARY 13, 1939

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

Sterols. LVIII. The Position of the Nuclear Hydroxyl Groups in Chlorogenin

BY RUSSELL E. MARKER AND EWALD ROHRMANN

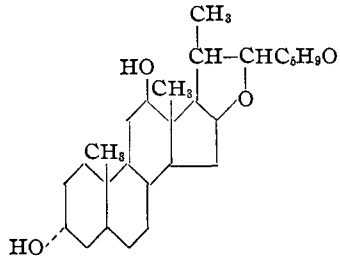
Chlorogenin, a steroid sapogenin having the composition $C_{27}H_{44}O_4$, was isolated and described by Liang and Noller,[1] the substance being obtained along with tigogenin from the acid hydrolysis products of extracts of *Chlorogalum pomeridianum*. The substance is isomeric with gitogenin, obtained from digitalis plants, but differs in the position of the two nuclear hydroxyl groups since the compound yields a diketone (II) of the composition $C_{27}H_{42}O_4$ on mild oxidation. Noller[2] assigned one of the hydroxyl groups to the favored C-3 position while the other was assigned tentatively to the sterically hindered C-12 position since the diketone formed only a mono-*o*-phenylenediamine derivative. Chlorogenin was reported to give no precipitate with digitonin and from this it was inferred that the hydroxyl group at C-3 had the α-configuration. In still more recent work Noller[3] gave surface film measurements which indicated that the two hydroxyl groups were in different rings and on the basis of previous evidence preference was given to structure (I).

Noller[2] first suspected that chlorogenin was related structurally to the digitalis sapogenins since the substance was found together with tigogenin. That such a relationship is probable

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

(2) Noller, *ibid.*, **59**, 1092 (1937).

(3) Noller, *ibid.*, **60**, 1629 (1938).



I. Chlorogenin (Noller)

is further supported by the fact that we have isolated from the acid hydrolysis extracts of *Chlorogalum pomeridianum* in addition to chlorogenin and tigogenin a substance which appears to be identical with gitogenin.[4]

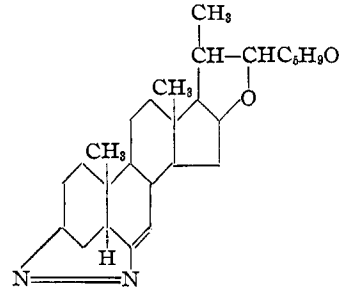
Contrary to the findings of Noller,[2] we have found that chlorogenin quite readily gives an insoluble digitonide with digitonin, this being especially so in 80% ethanol. This suggests that one of the hydroxyl groups is situated at C-3 as suggested by Noller but that it possesses the β- rather than the α-configuration. Since chlorogenin gives a diketone on mild oxidation, the second hydroxyl group cannot be in either the C-4 or C-2 position as these positions would give rise to acidic products upon oxidation. Position C-1 also can be eliminated since the resulting β-diketone would be soluble in aqueous alkali. Moreover, the surface film measurements of Noller[3] indicate that only one hydroxyl group is present in the first ring. Since chlorogenone forms a dioxime and a disemicarbazone the position at C-11 cannot be considered as a possibility. The only remaining logical positions for the second hydroxyl group are at C-12 as suggested by Noller or at C-6 or C-7.

The assignment of the second hydroxyl group to the sterically hindered C-12 position on the basis that chlorogenone only formed a mono-*o*-phenylenediamine derivative is no longer tenable since we have found that cholestanedione-3,6 forms only a mono-*o*-phenylenediamine derivative. The fact that chlorogenone is unstable when treated with alcoholic potassium hydroxide solution indicates that one of the carbonyl groups is situated adjacent to a labile asymmetric center. From the fact that cholestanone-7 appears to be unaffected by alcoholic potassium hydroxide even on heating, it appears that the carbonyl group in question must be located at C-6. This is further indicated by the fact that cholestanedione-3,6 is unstable with alcoholic alkali.

(4) Results to be published later.

Chlorogenone upon Clemmensen reduction in ethanolic solution with unamalgamated zinc yields a compound of the composition $C_{27}H_{44}O_2$, m. p. 178°. From the work of Borsche,[5] who found that dehydrocholic acid could be reduced to 7,12-diketo-cholanic acid by the Clemmensen method, it might be inferred that chlorogenin cannot have a hydroxyl group at either C-7 or C-12. However, this cannot be taken as conclusive evidence since we have found that cholestanone-7 is reduced easily to cholestane by a Clemmensen reduction in ethanolic solution with unamalgamated zinc. Cholestanedione-3,6 has been reduced to cholestane by the Clemmensen method by Windaus.[6]

Chlorogenone on treatment with hydrazine hydrate[7] readily forms a crystalline substance of the composition $C_{27}H_{40}O_2N_2$. The substance is undoubtedly a pyridazine derivative (II) which indicates that chlorogenone is either a 1,3- or a 1,4-diketone. Since chlorogenone is insoluble in aqueous alkali it can only be a 1,4-diketone and the 3,6-structure for chlorogenin seems inevitable.



II. Pyridazine Derivative of Chlorogenone

The question of the configuration at C-5 is still uncertain although there are some indications that chlorogenin belongs to the *allo* series. The stability of chlorogenone to hot alcoholic hydrochloric acid indicates that the C-5 configuration is *allo*. If the substance had the normal configuration inversion would certainly have occurred since Windaus[8] observed that 3,6-diketo-cholanic acid-24 (α-dehydro-hyo-desoxycholic acid) readily underwent such an inversion both with acids and alkali to give a compound of the *allo* series. Chlorogenin upon treatment with sodium and amyl alcohol gave only slight evidence of isomerization having taken place, which is consistent with a compound of the *allo* series.

(5) Borsche, *Ber.*, **52**, 1363 (1919).

(6) Windaus, *ibid.*, **50**, 133 (1917).

(7) Windaus, *ibid.*, **39**, 2249 (1908); Fernholz, *Ann.*, **508**, 215 (1934).

(8) Windaus, *ibid.*, **447**, 233 (1926).

The catalytic hydrogenation of chlorogenone in neutral medium proceeded very rapidly to give a mixture of isomers which could not be separated by crystallization. Oxidation of the mixture readily yielded chlorogenone

The fact that Noller[2] obtained by the chromic anhydride oxidation of chlorogenin a keto dibasic acid which was different from digitogenic acid suggests that chlorogenin differs from the other digitalic sapogenins in its nuclear configuration. This suggests the desirability of further study of the oxidation products of 3,6-diols.

We wish to thank Dr. Oliver Kamm and Parke, Davis and Company for their generous help and assistance in the various phases of this work. We also wish to thank Dr. Elmer J. Lawson and Mr. Paul H. Williams for their generous assistance in obtaining the bulbs of *Chlorogalum pomeridianum* from which the chlorogenin was obtained.

Experimental Part

Treatment of Chlorogenone with Alcoholic Potassium Hydroxide and with Hydrochloric Acid.—To a solution of 200 mg. of chlorogenone in 75 cc. of 95% ethanol was added a solution of 1 g. of potassium hydroxide in 25 cc. of 95% ethanol. The resulting solution became pale greenish-yellow colored at once and the color became quite intense after standing for a few minutes. The solution was allowed to stand at room temperature for sixteen hours when it was poured into water. The resulting mixture was extracted with ether and the ethereal extract washed with dilute hydrochloric acid and water. The ether was evaporated on the steam-bath. The yellow colored residue appeared to be a complex mixture and no crystalline product could be isolated.

Cholestanedione-3,6 behaved in a similar manner to yield a yellow colored solution from which no crystalline product could be isolated.

A solution of 50 mg. of chlorogenone in 10 cc. of 95% ethanol and 2 cc. of concentrated hydrochloric acid was refluxed on the steam-bath for one hour. The colorless solution was diluted with water and the mixture extracted with ether. The ethereal extract was washed with water and the ether evaporated on the steam-bath. The residue was crystallized from acetone to give an excellent yield of white needles, m. p. 233–236°. This gave no depression with the original chlorogenone.

Treatment of Chlorogenin with Sodium and Amyl Alcohol.—To a boiling solution of 1 g. of chlorogenin in 70 cc. of *n*-amyl alcohol was added 4 g. of sodium over a period of thirty minutes. The resulting mixture was refluxed for a period of eight hours. The solution was then cooled, diluted with ether and washed first with water, then with dilute hydrochloric acid and finally with water. The solvents were evaporated on the steam-bath and the residue crystallized from methanol to give a good yield of white needles, m. p. 265°. These gave no depression with a sample of chlorogenin, m. p. 265–268°.

Mild Clemmensen Reduction of Chlorogenone.—Four hundred milligrams of chlorogenone was dissolved in 100 cc. of 95% ethanol. To this solution was added 20 g. of 20-mesh zinc and the mixture heated to boiling. A total of 15 cc. of concentrated hydrochloric acid was then added over a period of eight hours. The solution was poured into water and the mixture extracted with ether. The ethereal extract was washed with water and the ether evaporated on the steam-bath. The residue was sublimed in high vacuum at 150°. The sublimed solid was crystallized from acetone to give white plates, m. p. 177–178°. This was found to be identical with a sample obtained by the reduction of chlorogenone under similar conditions using amalgamated zinc.

Anal. Calcd. for $C_{27}H_{44}O_2$: C, 80.9; H, 11.1. Found: C, 80.7, 80.8; H, 11.2, 10.9.

A similar reduction of cholestanedione-3,6 and of cholestanone-7 gave cholestane in good yields.

Chlorogenin Digitonide.—To a boiling solution of 100 mg. of chlorogenin in 10 cc. of 95% ethanol was added a hot solution of 600 mg. of digitonin in 20 cc. of 90% ethanol. A crystalline precipitate started to separate after a few minutes. The mixture was allowed to stand at room temperature for two hours when the precipitate was collected, washed with 95% ethanol and dried. The dried material weighed 275 mg.

The digitonide was dissolved in 100 cc. of dry pyridine and the resulting solution warmed on the steam-bath for twenty minutes. The solution was then poured into 400 cc. of ether and the resulting mixture washed with water, dilute hydrochloric acid and water. The ether was evaporated on the steam-bath and the residue crystallized from acetone to give 50 mg. of white needles, m. p. 265°. This gave no depression with chlorogenin.

The digitonide of chlorogenin is somewhat more soluble in ethanol than other digitonides such as cholesterol digitonide. The digitonide separates somewhat more readily from 80% ethanol.

***o*-Phenylenediamine Derivative of Cholestanedione-3,6.**—A mixture of 80 mg. of cholestanedione-3,6, 50 mg. of *o*-phenylenediamine and 5 cc. of absolute ethanol was refluxed for one hour. Crystalline material separated soon after the refluxing was started. The mixture finally was cooled and the crystals collected and recrystallized several times from absolute ethanol. The product was obtained as pale yellow needles, m. p. 207–210°, dec., to a red oil.

Anal. Calcd. for $C_{33}H_{50}ON_2$: C, 80.8; H, 10.3; N, 5.7. Calcd. for $C_{33}H_{58}N_4$: C, 80.3; H, 10.0; N, 9.6. Found: C, 80.5; H, 10.5; N, 5.6.

Pyrazidine Derivative of Chlorogenone.—A solution of 200 mg. of chlorogenone in 10 cc. of 95% ethanol and 0.2 cc. of hydrazine hydrate (42% aqueous solution) was refluxed on the steam-bath for five hours. The solution was then poured into 50 cc. of water and the resulting colloidal solution acidified with hydrochloric acid. The precipitated white solid was collected, washed with water and dried. The product was crystallized from benzene–methanol to give fine white needles. The product did not melt below 290° but began to decompose at about 270°. The substance was very soluble in benzene but almost insoluble in methanol.

Anal. Calcd. for $C_{27}H_{40}O_2N_2$: C, 76.4; H, 9.5; N, 6.6. Found: C, 76.5; H, 9.5; N, 6.8.

Disemicarbazone of Chlorogenone.—A mixture of 500 mg. of chlorogenone, 1 g. of semicarbazide hydrochloride, 1.2 g. of sodium acetate, 5 cc. of 95% ethanol and 5 cc. of water was refluxed on the steam-bath for one hour. The solution was then cooled and the white solid collected and crystallized from ethanol to give a product which darkened at 250° but did not melt at 290°.

Anal. Calcd. for $C_{29}H_{46}O_4N_6$: C, 64.2; H, 8.5. Found: C, 64.4; H, 8.7.

Summary

The nuclear hydroxyl groups of chlorogenin are shown to be at the 3,6-positions rather than at the 3,12-positions as postulated by Noller.

Evidence is given in support of the *allo*-configuration at C-5.

STATE COLLEGE, PENNA. RECEIVED DECEMBER 3, 1939

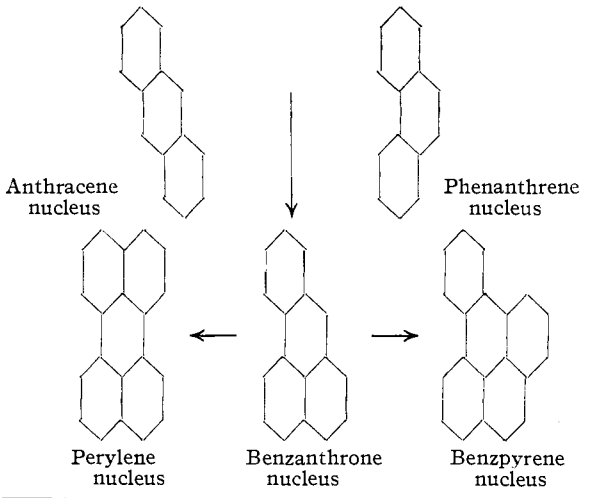
[CONTRIBUTION FROM THE JACKSON LABORATORY, E. I. DU PONT DE NEMOURS AND COMPANY]

Hydrofluoric Acid as a Condensing Agent. I

BY WILLIAM S. CALCOTT, JOHN M. TINKER AND VIKTOR WEINMAYR

The preparation of benzanthrone from anthraquinone and its reduction products and from phenanthrene by a "*peri*" synthesis is well known.[1] The condensation of these compounds with acrolein (formed from glycerol in the course of the reaction) takes place in the presence of acid condensing agents, especially sulfuric acid.

In the literature there is no record of the use of a *peri* synthesis to add two additional benz rings to anthraquinone, reduced anthraquinone or phenanthrene; nor that a further *peri* synthesis could be effected using benzanthrone or a reduction product of benzanthrone. If such a *peri* condensation would take place, one should obtain a perylene or benzpyrene or both from anthraquinone, benzanthrone, or phenanthrene. The following formulas serve to illustrate the possible condensations schematically.



(1) O. Bally, *Ber.*, **38**, 194 (1905); O. Bally and R. Scholl, *ibid.*, **44**, 1660 (1911); R. Scholl and H. K. Meyer, *ibid.*, **69**, 154 (1936).

We have found that these reactions outlined above could be performed if essentially anhydrous hydrofluoric acid were used as the condensing agent. Thus we obtained perylene from phenanthrene and from 1,10-trimethylene-9-hydroxyphenanthrene, which is a reduction product of benzanthrone, and 4,5-benzpyrene from 9,10-dihydroanthracene. Acenaphthene gave a product showing a very characteristic brilliant green color in sulfuric acid. *peri*-Naphthindone was obtained from α- and β-naphthol. Acrolein was used in all cases instead of glycerol.

High or non-fusible products were usually obtained as by-products. These substances could not be purified readily for identification and were probably higher condensation products. Undoubtedly conditions could be worked out under which these side reactions could be retarded and higher yields of pure products could be obtained.

We do not know what intermediate steps are involved in these condensations. The final products of the condensation are partially hydrogenated compounds from which perylene and benzpyrene are obtained by dehydrogenation. Presumably a nuclear alkylation takes place first, because we have found anhydrous hydrofluoric acid to be an excellent alkylating agent. Thus, 1,10-trimethylene-9-hydroxyphenanthrene (I) may first yield compound (II), which by loss of water is transformed by ring closure to a hydrogenated perylene (III). Perylene (IV) is finally obtained by dehydrogenation.

It appears, therefore, that hydrofluoric acid is a more suitable condensing agent than sulfuric acid. When preparing benzanthrone from anthraquinone with glycerol in sulfuric acid, it is