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

Sterols. CXXXI. Sapogenins. LIII. The Configuration of the Hydroxyl Groups in Chlorogenin*

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The configuration of the hydroxyl group in tigogenin was established conclusively as "β" by its conversion to *allo*-pregnanol-3(β)-one-20. The method employed excluded the possibility of an alteration in configuration.[1]

Chlorogenin, like tigogenin, is of the *allo*-series[2,3] and has been shown to have hydroxyl groups at C-3 and C-6.[2,3,4] Part of the evidence was dependent on the position of the double bond in diosgenin which has recently[5,6] been definitely located at 5,6. The evidence for the assignment of the β-configuration to the C-3 hydroxyl group of chlorogenin has rested on analogy with the behavior of other 3(β)-hydroxy-*allo* steroids. Thus the reduction of cholestanedione-3,6 with sodium and alcohol gave cholestanediol-3(β),6(α), while similar treatment of chlorogenone gave natural chlorogenin.[2] Chlorogenin, like cholestanediol-3(β),6[7] was precipitated with digitonin. It also failed to epimerize with sodium amylate.

We have now obtained final proof of the configuration assigned to the C-3 hydroxyl group by relating chlorogenin to tigogenin. Wieland and Dane[8] have shown that hyodesoxycholic acid can be partially oxidized with chromic acid to 3-hydroxy-6-keto-cholanic acid. Using a similar procedure, chlorogenin was oxidized. The 3(β)-hydroxy compounds were separated with digitonin; the residue was chlorogenone. The digitonin precipitable fraction was separated with Girard's reagent into chlorogenin and 6-keto-tigogenin. The latter was converted to tigogenin by the Clemmensen method.

Noller[9] had favored the α-configuration for the C-3 hydroxyl group of chlorogenin because he was unable to precipitate chlorogenin with digitonin. In a later paper[10] he has discussed the relation between the "solubility" of digitonides and the configuration of the C-3 hydroxyl group in steroids. His "solubility product" cannot have the meaning assigned to this term in the theory of dilute solutions since the formation of a digitonide is not necessarily an equilibrium reaction. However, he concludes that since the digitonide of *epi*-dihydrocholesterol appears to be "less soluble" than that of sarsasapogenin it is necessary to compare the epimeric carbinols in order to make conclusions concerning configuration.

We have now investigated the epimer of chlorogenin. Chlorogenone was reduced by the Meerwein method which has been shown to give a mixture of the epimeric carbinols in every steroidal ketone to which it has been applied. The resulting mixture was treated in alcohol with digitonin, the latter in a concentration of about 2%. From the insoluble digitonide, chlorogenin and β-chlorogenin[2] were isolated. The β-chlorogenin was obtained in larger quantity. From the fraction which did not precipitate with digitonin, an isomeric carbinol differing from chlorogenin and β-chlorogenin was isolated. That this was *epi*-chlorogenin was shown by treatment with sodium and amyl alcohol. *epi*-Tigogenin was converted to tigogenin by this method and similarly the epimeric chlorogenin fraction gave natural chlorogenin and a smaller quantity of β-chlorogenin.

Thus the digitonin method has been shown to be applicable to distinguish and separate the epimeric chlorogenins and the behavior of chlorogenin and its epimer is fully analogous to that of other pairs of steroid carbinols epimeric at C-3. This is the case also with sarsasapogenin, tigogenin, smilagenin and diosgenin, all of which have been shown to have 3(β)-hydroxy groups by conversion to substances of known configuration. Thus there is no reason to assume that steroidal sapogenins are exceptional in their precipitation with digitonin.

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Experimental Part

Reduction of Chlorogenone with Aluminum Isopropylate.—A mixture of 10 g. of chlorogenone, 30 g. of aluminum

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(1) Marker and Turner, THIS JOURNAL, **62**, 3003 (1940).
(2) Marker, Jones and Turner, *ibid.*, **62**, 2537 (1940).
(3) Marker and Rohrmann, *ibid.*, **61**, 946, 3479 (1939).
(4) Marker, Jones, Turner and Rohrmann, *ibid.*, **62**, 3006 (1940).
(5) Marker, Tsukamoto and Turner, *ibid.*, **62**, 2525 (1940).
(6) Marker and Turner, *ibid.*, **63**, 767 (1941).
(7) Fernholz, *Z. physiol. Chem.*, **232**, 97 (1935).
(8) Wieland and Dane, *ibid.*, **212**, 41 (1932).
(9) Noller, THIS JOURNAL, **59**, 1092 (1937).
(10) Noller, *ibid.*, **61**, 2717 (1939).

isopropylate and 300 cc. of dry isopropyl alcohol was refluxed for eight hours. At the end of this time the solvent was removed over a period of four hours. The residue was shaken with dilute hydrochloric acid and ether. The ether was removed and the residue was dissolved in a small amount of ethyl alcohol. To this was added a hot solution of 30 g. of digitonin in 1.5 l. of 90% alcohol. It was allowed to stand at 15° overnight. The precipitate was filtered, washed well with alcohol and dried. The digitonin complex was decomposed by warming with pyridine. Ether was added and the digitonin was filtered. The filtrate was freed of pyridine and then vacuum distilled. The residue weighed 5.4 g. This was crystallized repeatedly from acetone to give a product, m. p. 249–251°, which gave no depression in melting point when mixed with β-chlorogenin, m. p. 249–251°. Mixed with chlorogenin, it melted at 236–244°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.9; H, 10.2. Found: C, 74.7; H, 10.3.

The filtrate was crystallized from acetone and a mixture of acetone–methyl alcohol to give a product melting at 272–274°, which gave no depression in melting point when mixed with naturally occurring chlorogenin.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.9; H, 10.2. Found: C, 74.9; H, 10.0.

Upon refluxing with acetic anhydride it gave a diacetate which melted at 155–157° and gave no depression in melting point when mixed with a sample of the diacetate of naturally occurring chlorogenin.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.0; H, 9.4. Found: C, 72.2; H, 9.2.

The fraction not precipitated by digitonin was freed of excess digitonin by evaporation to 100 cc. and precipitated by the addition of ether. The residue after removal of the solvent was crystallized from acetone to give a product melting at 270–274°, which gave a depression of 16° when mixed with natural chlorogenin.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.9; H, 10.2. Found: C, 74.8; H, 10.1.

A test portion of the total fraction which did not precipitate with digitonin was again treated with excess digitonin in alcohol, but no precipitation occurred. The total product of epimeric sapogenins was refluxed for six hours with 15 g. of sodium in 200 cc. of *n*-amyl alcohol. Water was added and the product was extracted with ether. The solvent was removed *in vacuo* and the residue was dissolved in a small amount of alcohol and added to a hot solution of 15 g. of digitonin in 750 cc. of 90% alcohol. Upon standing overnight a heavy precipitate formed. This was filtered, washed well with alcohol and dried. It was decomposed by pyridine as previously described; yield of crude product, 2.4 g. Crystallization from acetone gave a product melting at 272–274°, which gave no depression in melting point when mixed with naturally occurring chlorogenin, m. p. 272–274°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.9; H, 10.2. Found: C, 74.8; H, 10.1.

It gave a diacetate, m. p. 156–158°, which gave no depression when mixed with the diacetate of naturally occurring chlorogenin.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.0; H, 9.4. Found: C, 71.9; H, 9.3.

The mother liquors from the chlorogenin yielded β-chlorogenin, m. p. 250–251°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.9; H, 10.2. Found: C, 74.6; H, 10.0.

Isomerization of *epi*-Tigogenin.—To a solution of 100 mg. of *epi*-tigogenin in 50 cc. of *n*-amyl alcohol was added 5 g. of sodium. The product was refluxed for eight hours, water was added and it was extracted with ether. After removal of the solvent, the residue was dissolved in a small amount of ethanol and to it was added a solution of 1 g. of digitonin in 50 cc. of 95% alcohol. There was an immediate precipitation. After standing overnight this was filtered and dried, yield 410 mg. It was decomposed by pyridine and the product was crystallized from methanol; m. p. 202–204°. Mixed with tigogenin, it gave no depression in melting point; mixed with *epi*-tigogenin, it melted at 182–193°.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.6. Found: C, 77.7; H, 10.5.

epi-Tigogenin did not give a precipitate with digitonin under the conditions used above.

Conversion of Chlorogenin to Tigogenin.—To a solution of 2.2 g. of chlorogenin dissolved in 400 cc. of acetic acid and cooled to 15° was added a solution of 335 mg. of chromic anhydride in 20 cc. of 90% acetic acid. After standing for three hours at 15° a small amount of zinc dust was added and the product was filtered. The filtrate was vacuum distilled to 100 cc. and the residue was extracted with a large volume of ether, freed of acetic acid by washing with sodium carbonate solution, and the ether was removed. The residue was dissolved in a small amount of ethanol and a solution of 8 g. of digitonin in 400 cc. of 90% alcohol was added. It was allowed to stand overnight and the digitonide was filtered and washed with alcohol. The product which did not form a digitonide was crystallized from acetone to give chlorogenone, m. p. 235–237°; yield 180 mg.

The digitonide was dried and decomposed by heating on a steam-bath with pyridine. The solution was poured into ether and filtered. The filtrate was washed well with dilute hydrochloric acid and concentrated. The residue was dissolved in 50 cc. of ethanol and heated on a steam-bath for thirty minutes with Girard reagent. The non-ketonic fraction was crystallized from acetone to give a product, m. p. 270–273°, which gave no depression with chlorogenin.

The ketonic fraction, 320 mg., was dissolved in 50 cc. of ethyl alcohol to which was added 10 g. of 20-mesh zinc. Over a period of three hours, 50 cc. of concentrated hydrochloric acid was added. The product was extracted with ether and crystallized from methanol, m. p. 200–203°. Mixed with tigogenin, m. p. 202–204°, it melted at 201–203°.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.8; H, 10.6. Found: C, 77.9; H, 10.7.

It gave an acetate when refluxed with acetic anhydride which melted at 205–208°. When mixed with an authentic sample of tigogenin acetate there was no depression in melting point.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.9; H, 10.1. Found: C, 75.7; H, 10.2.

Summary

1. Chlorogenin has been converted to tigogenin without altering the C-3 hydroxyl group. This proves that the C-3 hydroxyl has the β-configuration.

2. *epi*-Chlorogenin has been prepared.

3. Chlorogenin and its epimer are analogous to other *allo*-steroid carbinols epimeric at C-3 in behavior toward digitonin and sodium amylate.

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Acetylenic Ethers. II. Ethoxy- and Butoxy-acetylene

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In the first paper of this series,[2] the synthesis and properties of phenoxyacetylene and substituted phenoxyacetylenes were described. It seemed likely that simple alkoxyacetylenes would show quite different properties. The following reactions have been employed to synthesize two such compounds

$$CH_2BrCH(OR)_2 \longrightarrow CHBr_2CH(OR)_2 \xrightarrow[C_2H_5OH]{Zn}$$

$$BrCH{=}CHOR \xrightarrow{KOH} H{-}C{\equiv}C{-}OR$$

Bromoalkoxyethylenes were prepared by the action of zinc and alcohol on dibromoacetals in the same way that alkenes are obtained from β-bromoethers.[3] When the starting material was the dibutyl acetal, a mixed acetal of monobromoacetaldehyde, $BrCH_2CH(OC_2H_5)OC_4H_9$, was obtained as a by-product, apparently by the addition of a molecule of alcohol to bromobutoxyethylene. When a large run was attempted this mixed acetal was the principal product. No diethyl acetal was isolated in the preparation of bromoethoxyethylene, where the reaction with zinc was more rapid. The elimination of hydrogen bromide from the bromoalkoxyethylene by distillation from powdered potassium hydroxide corresponds to the final step in the synthesis of phenoxyacetylene.

Ethoxy- and butoxy-acetylene are colorless, mobile, evil-smelling liquids which must be distilled under reduced pressure. Their structures were proved by analysis, molecular weight determinations and hydrogenation to the corresponding saturated ethers. The observed molecular refractions showed only slight exaltations as compared with the values calculated from constants derived from saturated ethers and 1-alkynes.[4] Chalmers[5] found that the molecular refractions of aliphatic vinyl ethers were also normal.

While phenoxyacetylene gave a white silver salt which was sufficiently stable to permit analysis,[6] the alkoxyacetylenes gave only black precipitates probably containing metallic silver. These burned with a puff when dry, but showed no tendency to explode. When the freshly precipitated derivative was treated with dilute nitric or sulfuric acid, the odor of ethyl or butyl acetate was at once apparent, and these esters could be isolated in low yield. Ethoxyacetylene gave a white mercury derivative which rapidly turned brown. While no attempt has yet been made to synthesize such substituted acetylenic ethers as RC≡CO-Alkyl by the use of sodium or bromomagnesium derivatives of alkoxyacetylenes, ethoxyacetylene gave one mole of methane in the Grignard "machine"[7] and none of the reagent was used in other ways.

Although ethoxy- and butoxy-acetylene polymerize much less rapidly than phenoxyacetylene, they remained completely colorless only when sealed in glass and stored at minus eighty degrees. A yellow color developed in a few days at zero degrees or in a few hours at room temperature. When tubes containing the most carefully purified samples were opened at room temperature a yellow color appeared in a few minutes. As in the case of phenoxyacetylene, the moderately rapid heating of a small sample in a sealed tube to around 100° resulted in an explosion with the production of a black solid.

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(2) Jacobs, Cramer and Weiss, THIS JOURNAL, **62**, 1849 (1940).

(3) Dykstra, Lewis and Boord, *ibid.*, **52**, 3401 (1930).

(4) Eisenlohr values of the atomic refractivities from Landolt-Börnstein, "Physikalische Tabellen," 5th ed., Vol. II, 985. The revised constant for the triple bond was used, *ibid.*, Supp. Vol. IIIb, 1696.

(5) Chalmers, *Can. J. Research*, **7**, 464 (1932).

(6) Slimmer, *Ber.*, **36**, 289 (1909).

(7) Kohler, Stone and Fuson, THIS JOURNAL, **49**, 3181 (1927); Kohler and Richtmyer, *ibid.*, **52**, 3736 (1930).