widely different values for the dipole moment of gliadin. We have preferred to omit the results of such computations because of the uncertainty inherent in them and shall be content to remark that gliadin is much less polar than zein.

Acknowledgment.—We wish to thank J. W. Williams for assistance given in connection with this problem. Grateful acknowledgment is also made to the Wisconsin Alumni Research Foundation and to the University Research Committee for the financial assistance which has made possible the development of these studies.

Summary

1. A combination of the Debye dipole theory and the Perrin study of the hydrodynamics of elongated particles is applied to experimental data involving the protein gliadin in aqueous alcoholic solution.

2. The experimental dispersion of dielectric constant data for dilute gliadin solutions in the frequency range 25,000 to 30,000,000 cycles per second is accounted for quantitatively by the theoretical equations if the molecules are assumed to be ellipsoidal in shape. The values 38,000 for the molecular weight and 8 for the ratio of major to minor axis for the gliadin unit are obtained directly from these data.

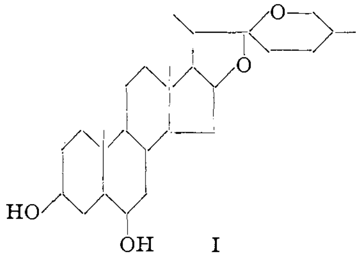
MADISON, WISCONSIN RECEIVED APRIL 24, 1941

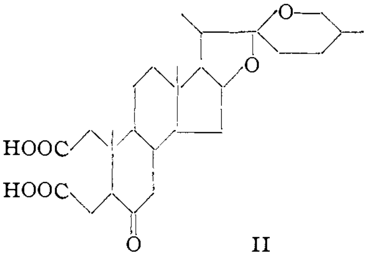
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF STANFORD UNIVERSITY]

Saponins and Sapogenins. XVIII. The Non-Identity of Chlorogenonic, Digitogenic and Digitoic Acids[*]

BY C. R. NOLLER AND SEYMOUR LIEBERMAN[†]

On the basis of extensive work reported by Marker and his co-workers,[1] the two hydroxyl groups of chlorogenin must be located at the 3- and 6-positions of the sterol nucleus (I). Accordingly the ketodibasic acid obtained as one of the products of oxidation of chlorogenin[2] should be identical with digitogenic or digitoic acid (II).


I


II

At the time the original preparation of the ketodibasic acid was reported, it was thought that the 25° difference in the melting points of the acids and the 12° difference for the methyl esters was sufficient evidence of non-identity with digitogenic acid. Moreover, digitogenic acid was reported as being readily isomerized by alkali to digitoic acid[3] whereas the ketodibasic acid obtained from chlorogenin was stable to alkali. That it was not identical with digitoic acid was indicated by the 25° difference in melting points of the acids[3] and the 20° difference for the methyl esters.[4]

Marker and Rohrmann[5] have questioned the purity of the oxidation products of digitogenin and have pointed out that no direct comparison was made of digitogenic or digitoic acid with the ketodibasic acid from chlorogenin. We have now made such a comparison[6] and find that the ketodibasic acid from chlorogenin is indeed different from digitogenic acid and from digitoic acid. Although the depressions in melting points of chlorogenonic acid[7] and its dimethyl ester with digito-

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[†] Research Assistant on funds from the Rockefeller Foundation.

[1] Marker, Tsukamoto and Turner, THIS JOURNAL, **62**, 2525 (1940); Marker, Jones and Turner, *ibid.*, **62**, 2537 (1940); Marker, Jones, Turner and Rohrmann, *ibid.*, **62**, 3006 (1940); Marker and Turner, *ibid.*, **63**, 767 (1941).

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

[3] Kiliani, *Arch. Pharm.*, **231**, 448 (1893).

[4] Windaus and Weil, *Z. physiol. Chem.*, **121**, 68 (1922).

[5] Marker and Rohrmann, THIS JOURNAL, **61**, 3479 (1939).

[6] All of the experimental work on the isolation and comparison of the ketoacids and their methyl esters has been repeated independently by both authors. The work on the conversion of chlorogenonic acid to gitogenic acid is that of the junior author alone.

[7] Since the name "chlorogenic acid" is in common use for a well-known depside, the name "chlorogenonic acid" is proposed for the ketodibasic acid from chlorogenin.

genic acid and its dimethyl ester, respectively, and the differences in the optical rotations are not great, other properties conclusively prove that they are not identical. Outstanding is the behavior toward alkali, the chlorogenonic acid being entirely stable whereas digitogenic acid is very easily isomerized. Moreover, the solubility of digitogenic dimethyl ester in methyl alcohol is almost three times as large and in ligroin approximately twice as large as that of chlorogenonic dimethyl ester in the same solvents. The solubility of the magnesium salt of chlorogenonic acid is greater than that of digitogenic acid and it cannot be crystallized readily from 95% ethyl alcohol as is the case with the magnesium salt of digitogenic acid.[12]

The evidence for non-identity of chlorogenonic acid with digitoic acid is just as conclusive. The depression in melting point of chlorogenonic acid with digitoic acid is quite marked and the optical rotation of digitoic acid is about twice that of chlorogenonic acid. Chlorogenonic acid crystallizes readily from glacial acetic acid with one molecule of acid of crystallization whereas digitoic acid crystallizes well only from aqueous acetic acid solutions and the product is free of acid of crystallization. As is the case with digitogenic acid and in contrast to chlorogenonic acid, we have found that the magnesium salt of digitoic acid[10] is only slightly soluble in water and crystallizes well from hot 95% ethyl alcohol. The dimethyl ester of chlorogenonic acid crystallizes readily from methyl alcohol or ligroin whereas the dimethyl ester of digitoic acid crystallized only with difficulty and in most instances could not be obtained crystalline.

Having proved that chlorogenonic acid was not identical with either digitogenic acid or digitoic acid, we considered the possibility that oxidation of chlorogenin had not taken place between C-2 and C-3 as expected but that some other position had been attacked. That this is not the case has been shown by a Wolff–Kishner reduction of chlorogenonic acid. The product and its dimethyl ester are identical with gitogenic acid and its dimethyl ester, respectively, as shown by melting points, mixed melting points, optical rotation and crystal form. The stability of both chlorogenin and digitogenin to aqueous alcoholic hydrochloric acid[8] indicates that the difference between chlorogenonic acid and digitoic acid is not due to a difference in the configuration of the side chain. Thus we have not been able so far to reconcile the properties of chlorogenonic acid with the 3,6-location of the hydroxyl groups in chlorogenin.

Experimental

Chlorogenonic Acid.—A product having the previously recorded melting point of 235–237°[2] is difficult to obtain, the melting point of most pure samples being 229–230° with shrinkage at 225°. Melting always takes place with the evolution of bubbles and is probably accompanied by decomposition; $[\alpha]^{25}_{D}$ −40.6°, $[\alpha]^{25}_{546}$ −51.3° in dioxane compared with the previously recorded values of $[\alpha]^{22}_{D}$ −42.8 and $[\alpha]^{22}_{546}$ −49.0.

Anal. Calcd. for $C_{27}H_{40}O_7 \cdot C_2H_4O_2$: C, 64.91; H, 8.26. Found: C, 64.78; H, 8.44.

On adding a solution of magnesium nitrate to a neutral aqueous solution of the sodium salt, a gelatinous precipitate was formed which was difficultly soluble in hot 95% alcohol and did not crystallize on cooling. The mother liquors from the precipitation of the magnesium salt on acidification gave a precipitate which after washing and drying amounted to half of the original sample. This material was identical with that regenerated from the magnesium salt as shown by melting point and mixed melting point, indicating the marked solubility of the magnesium salt in water.

The melting point of the **dimethyl ester** has been raised to 159.5–160.5°; $[\alpha]^{23}_{D}$ −45.1, $[\alpha]^{23}_{546}$ −54.0 in dioxane compared with the previously recorded values of $[\alpha]^{22}_{D}$ −39.1, $[\alpha]^{22}_{546}$ −46.5; solubility in 60–70° ligroin at 25.8°, 0.0063 g. per gram of solvent; in methyl alcohol at 24.6°, 0.0100 g. per gram of solvent.

Anal. Calcd. for $C_{29}H_{44}O_7$: C, 69.02; H, 8.78. Found: C, 68.86; H, 8.94.

When 0.1 g. of the dimethyl ester was refluxed for one hour with 10 cc. of 0.1 *N* potassium hydroxide in methyl alcohol, and the acid recovered by acidification and washing, it melted at 229–230° with shrinking at 225°.

Digitogenin.—Crude digitogenin obtained by the hydrolysis of digitonin was recrystallized from benzene and a small amount of methyl alcohol until the melting point was above 272°. It was then converted to the triacetate by refluxing with acetic anhydride and sodium acetate and this crystallized from methyl alcohol until the melting point reached 185–190°. The digitogenin obtained on hydrolysis was recrystallized several times from benzene and methyl alcohol to a constant melting point of 289–293° with shrinking at 285°. The highest melting point previously recorded[9] is 280–283°.

Digitogenic Acid.—Digitogenin was oxidized by the procedure of Kiliani and Merk[10] and also by means of chromium trioxide in glacial acetic acid. In both cases the acidic fraction after repeated crystallizations from glacial acetic acid showed shrinkage at 170–175° and melted at 215.5–217.5°. When the capillary tube containing a sample was immersed in a preheated bath, complete melting took place as low as 183°. A sample which had stood for a year

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[8] Marker and Rohrmann, THIS JOURNAL, **61**, 2724 (1939); **62**, 647 (1940).

[9] Tschesche, *Ber.*, **68**, 1090 (1935).

[10] Kiliani and Merk, *ibid.*, **34**, 3564 (1901).

showed shrinkage at 170° and melted at 202–208°; $[\alpha]^{23}_{D}$ $-41.2°$, $[\alpha]^{23}_{546}$ $-47.6°$ in dioxane. As shown by the analysis, digitogenic acid, like chlorogenonic acid, contains one molecule of acetic acid of crystallization.

Anal. Calcd. for $C_{27}H_{40}O_7 \cdot C_2H_4O_2$: C, 64.91; H, 8.26. Found: C, 64.87; H, 8.41.

When digitogenic acid, shrunk 170°, m. p. 215.5–217.5°, was mixed with chlorogenonic acid, shrunk 225°, m. p. 229–230°, the mixture shrunk at 170°, wet the capillary at 193°, was about half melted at 205° and completely clear at 211°.[11]

Digitogenic acid was converted to the **dimethyl ester** with diazomethane and after repeated crystallization from 60–70° ligroin melted at 154.4–159.5° with preliminary shrinking at 153°. A melting point of 146° has been recorded previously.[4] After this sample had stood for a year, it melted at 148–151° with shrinking at 145°; $[\alpha]^{23}_{D}$ $-49.4°$, $[\alpha]^{23}_{546}$ $-54.8°$ in dioxane; solubility in 60–70° ligroin at 25.8°, 0.0101 g. per gram of solvent; in methyl alcohol at 24.6°, 0.0280 g. per gram of solvent.

Anal. Calcd. for $C_{29}H_{44}O_7$: C, 69.02; H, 8.78. Found: C, 68.79; H, 8.79.

When digitogenic dimethyl ester, shrunk 153°, m. p. 154.5–159.5°, was mixed with an equal part of chlorogenonic dimethyl ester, shrunk 159°, m. p. 159.5–160.5°, the mixture melted at 148–156° with shrinking at 147°.

Digitoic Acid.—When digitogenic acid was heated on the steam-bath in aqueous alkaline solution or when the dimethyl ester was saponified with alcoholic alkali, the recovered acid fraction was a solid melting at 70–100°. All attempts to crystallize this material from common solvents and mixed solvents failed. When the acid fraction was dissolved in dilute alkali, acidified until just neutral to phenolphthalein and a solution of magnesium nitrate added, a copious precipitate formed which soon became granular. It was crystallized twice from 95% ethyl alcohol and decomposed into the free acid by shaking with 1 *N* nitric acid and ether. After washing and evaporation of the ether the residue melted at 130–150°. This acid could not be crystallized from glacial acetic acid. After one crystallization from aqueous ethyl alcohol, it melted at 200–207° with shrinking at 195°. Dilute acetic acid proved to be a better solvent for crystallization and after several recrystallizations the melting point remained constant at 207–209° with shrinking at 205°. It was dried for analysis in a vacuum desiccator over solid potassium hydroxide. Unlike chlorogenonic and digitogenic acids the product was free of acetic acid of crystallization; $[\alpha]^{25}_{D}$ $-85.7°$, $[\alpha]^{25}_{546}$ $-103.9°$ in dioxane.

Anal. Calcd. for $C_{27}H_{40}O_7$: C, 68.05; H, 8.46. Found: C, 67.84; H, 8.63.

A mixture of the above acid with chlorogenonic acid, shrunk 225°, m. p. 229–230°, showed shrinkage at 195° and melted at 199–210°.

That the above acid is identical with Kiliani's digitoic acid, there can be no reasonable doubt. The melting point always has been reported[3,10,12] as 210° and Kiliani and Merk[10] found that their digitoic acid gave a water insoluble magnesium salt. Moreover digitoic acid has been crystallized from aqueous alcohol[3,12] indicating that, like our product, its solubility in anhydrous solvents was too great for convenient crystallization.

On the other hand, we have had great difficulty in obtaining a crystalline dimethyl ester, even from our purest samples of digitoic acid, whereas Windaus and Weil[4] apparently had no difficulty in crystallizing their product from methyl alcohol. That no reaction other than esterification took place in our product on reaction with diazomethane was shown by the fact that our non-crystalline ester on saponification gave an acid which was completely precipitated as the magnesium salt. The latter on decomposition gave an acid, shrunk 203°, m. p. 206–209° without further purification. Only on one occasion was it possible to isolate a few milligrams of crystalline ester of m. p. 141–143°. This when mixed with digitogenic dimethyl ester melted at 135–137°, while a mixture with chlorogenonic dimethyl ester melted at 138–140°.

We also have attempted to prepare digitoic acid dimethyl ester by the isomerization of digitogenic dimethyl ester with acetic anhydride and sodium acetate according to the procedure of Windaus and Weil[4] but the product again could not be crystallized.

That digitoic acid is not the sole product of the action of alkali on digitogenic acid is shown by the fact that the mother liquors from the precipitation of the magnesium salt give a copious precipitate on acidification. This is not due to the solubility of the magnesium salt of digitoic acid since the recovered acid could not be precipitated from more concentrated neutral solutions. Although the crude acid was a solid, shrunk 150°, m. p. 160–180°, it could not be crystallized from any common solvents or mixtures of solvents. Conversion to the methyl ester likewise gave a non-crystallizable product.

Reduction of Chlorogenonic Acid.—A sealed bomb tube containing 0.15 g. of chlorogenonic acid which had been rigidly purified through its dimethyl ester, 10 cc. of absolute ethyl alcohol, 0.3 g. of sodium and 0.8 cc. of 95–100% hydrazine hydrate was heated at 200° for twelve hours. The contents of the tube were acidified and the precipitate extracted with hot methyl alcohol to separate the organic from the inorganic material. Evaporation of the alcohol gave a residue which was taken up in ether and extracted with 5% aqueous potassium hydroxide. Acidification gave 0.11 g. of acid fraction which, after crystallization from glacial acetic acid, melted at 244–247° with shrinking at 242°. Further recrystallization from dilute acetone gave 40 mg., shrunk 243°, m. p. 244–247°; $[\alpha]^{27}_{D}$ $-56.8°$ in acetone, compared with the recorded value of $[\alpha]^{20}_{D}$ $-60.0°$ in acetone for gitogenic acid.[13] A mixture with a sample of gitogenic acid, shrunk 246°, m. p. 248–250°, melted at 243.5–247° with shrinking at 243°.

Conversion to the methyl ester with diazomethane and crystallization from methyl alcohol gave long needles melting sharply at 132–133°. After standing about two months the crystal form changed and the sample melted at 145–146° with shrinkage at 131°. Recrystallization from ligroin (60–70°) gave rectangular plates, m. p. 145.5–146°, identical in appearance with those of gitogenic dimethyl ester from the same solvent.

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(11) All mixed melting points reported in this paper were taken simultaneously in the same bath with samples of both pure components.

(12) Kiliani and Windaus, *Ber.*, **32**, 2204 (1899).

(13) Jacobs and Simpson, *J. Biol. Chem.*, **110**, 435 (1935).

Anal. Calcd. for $C_{29}H_{46}O_6$: C, 70.96; H, 9.46. Found: C, 71.08; H, 9.56.

A mixture with gitogenic acid dimethyl ester, m. p. 146–147°, melted at 146–147°.

Summary

Chlorogenonic acid is not identical with either digitogenic or digitoic acid. Wolff–Kishner reduction yields gitogenic acid showing that oxidation of chlorogenin has taken place between C-2 and C-3. On the basis of our present knowledge, these facts are in conflict with the location of the hydroxyl groups of chlorogenin at the 3- and 6-positions.

STANFORD UNIV., CALIF.

RECEIVED MAY 23, 1941

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

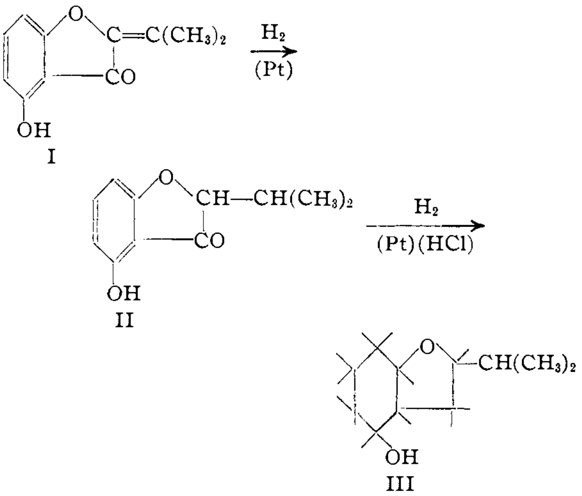
Derivatives of Coumaran. VIII. Reductions in the Coumaranone Series. A New Synthesis of Dihydrotubanol

BY R. L. SHRINER AND MICHAEL WITTE

Although most hydroxycoumaranones have been readily reduced by catalytic methods,[1] difficulty has been encountered in the reduction of the carbonyl group in the 4-hydroxycoumaran-3-one series. Since the physical properties of 4-hydroxycoumaranones differ markedly from those of other hydroxycoumaranones, it has been suggested that, in these compounds, the hydroxyl group is chelated with the carbonyl.[2] This would explain the unusual stability which 4-hydroxycoumaranones exhibit toward catalytic hydrogenation.

Although 4-hydroxycoumaranones are stable to hydrogen, 4-hydroxycoumarans are not. Thus LaForge and Haller found that tubaic acid was cleaved by hydrogen to give tetrahydrotubaic acid.[3] Goodhue and Haller[4] reduced rotenone in butyl acetate solution in the presence of Raney nickel and found that considerable amounts of rotenic acid and tetrahydrorotenone were formed. The present communication describes the catalytic reduction products of 2-isopropylidene-4-hydroxycoumaran-3-one (I) and its benzoate. Although a considerable number of reductions were carried out with platinum and nickel at various temperatures and pressures only those procedures leading to definite compounds with the heterocyclic ring intact will be described.

Catalytic reduction of I in ethanol with platinum and hydrogen at 3 atmospheres yielded the dihydro derivative (II). In the presence of a trace of hydrochloric acid further reduction occurred to give chiefly 2-isopropyl-4-hydroxyhexahydrocoumaran or octahydrotubanol (III). Compound II was characterized by the preparation and analyses of its ketazine, monoacetate, diacetate, dibenzoate and a *bis*-phenylurethan. The latter derivatives are enol esters similar to that previously established for the enol acetate[5] of 6-methoxycoumaran-3-one.



A high pressure reduction of I in absolute ethanol at 60° with Raney nickel yielded a compound whose analysis indicated that it was the diethyl ether of 2-isopropyl-3,4-dihydroxydihydrobenzofuran. With dioxane as the solvent high pressure hydrogenation yielded 2-isopropyl-3,4-dihydroxyoctahydrobenzofuran characterized by its analysis, non-phenolic properties and formation of a diphenylurethan.

In the presence of a trace of hydrochloric acid an absolute ethanol solution of the benzoate (IV) absorbed six moles of hydrogen in the presence of

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[1] Shriner and Anderson, THIS JOURNAL, **61**, 2705 (1939).

[2] Shriner and Witte, *ibid.*, **61**, 2328 (1939).

[3] Haller and LaForge, *ibid.*, **53**, 4460 (1931).

[4] Goodhue and Haller, *Ind. Eng. Chem., Anal. Ed.*, **12**, 652 (1940).

[5] Shriner and Anderson, THIS JOURNAL, **60**, 1418 (1938).