[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF STANFORD UNIVERSITY]

Saponins and Sapogenins. XVII. The Structure of the Side Chain of Chlorogenin

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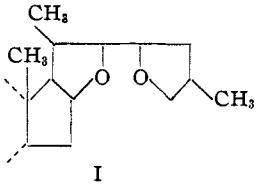
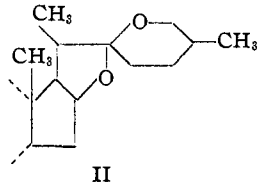
Two formulations have been proposed for the side chain of the steroid sapogenins. That of Tschesche and Hagedorn[2] contains two tetrahydrofuran rings (I) while that of Marker and Rohrmann[3] is a ketone spiroacetal (II).



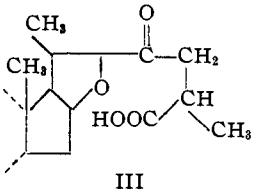
I



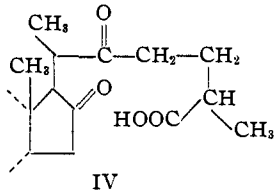
II

In spite of the large number of reactions which the side chain of these compounds has been shown to undergo, a reasonable interpretation of almost all of them can be made using either formulation. An exception is the formation of α-methylglutaric acid on vigorous oxidation of digitogenic acid.[4] Assuming that this fragment comes from the side-chain, it can be explained only by Marker's formulation (II). On the other hand, the structure of anhydrosarsasapogenoic acid that would naturally follow from formulation II would require a shift of a double bond for no apparent reason in order to account for the formation of iodoform from the dibasic acid obtained on oxidation of the anhydro acid.[5]

It appeared to us that it might be easier to distinguish between the two formulations for the sapogenoic acids, structures III and IV being the alternate formulas if the side chains are represented by I and II, respectively. Since the

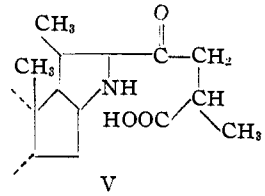


III

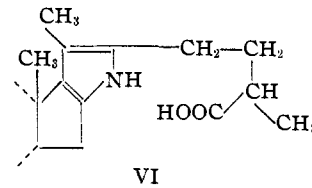


IV

sapogenoic acids do not react with ketone reagents under ordinary conditions, this cannot be used to differentiate between the two possibilities. Formula IV, however, is a 1,4-diketone and might be expected to yield a pyrrole, VI, on treatment with ammonia while Formula III, if it reacted, might be expected to yield an α-ketopyrrolidine, V. Besides an expected difference in properties, V and VI differ in composition by a molecule of water.



V



VI

In studying this reaction, chlorogenoic acid was used because it is more readily obtained than the other sapogenoic acids. The methyl ester diacetate was found to react with ammonia in alcoholic solution even at room temperature to give a product which was soluble in dilute acid and precipitated from an acid solution by alkali.[6] This product is unstable to heat and is altered rapidly in air. No solvents were found from which it could be successfully crystallized and an amorphous chloroplatinate was the only derivative obtained. The rather extensive analyses given in Table I show, however, that the compound must be relatively pure and that in the reaction there is no loss of acetyl groups or of the methoxyl group. Moreover, the reaction takes place with the loss of only one molecule of water. The last fact, together with the ready solubility in dilute acid, seemed to indicate that the compound was not the expected pyrrole. On the other hand, the ready reaction with ammonia indicated that the product was not a pyrrolidine especially since chlorogenin could be recovered unchanged after heating for forty hours with alcoholic ammonia in a sealed tube at 100°.

Since pyrrole gives a weak but characteristic ultraviolet absorption spectrum,[7] it was thought that this might provide a means of identifying a pyrrole nucleus in the product of reaction with ammonia. We believe that the curves shown in Fig. 1 indicate that the pyrrole nucleus is present. Curve 1 is that obtained for methyl chlorogenoate

(1) Research Associate on funds from the Rockefeller Foundation.
(2) Tschesche and Hagedorn, *Ber.*, **68**, 1412 (1935).
(3) Marker and Rohrmann, THIS JOURNAL, **61**, 2072 (1939).
(4) Windaus and Willerding, *Z. physiol. Chem.*, **143**, 33 (1925).
(5) Fieser and Jacobsen, THIS JOURNAL, **60**, 2753 (1938).
(6) This reaction was first carried out in a sealed tube at 150° by Dr. J. H. Hollister at the Chemical Laboratory of Harvard University in the spring of 1939 but the product was a glass and lack of time prevented him from investigating the reaction further.
(7) Menczel, *Z. physik. Chem.*, **125**, 161 (1927).

diacetate. This shows the typical absorption maximum at 2800 Å. for an isolated carbonyl group and duplicates almost exactly the curves for sarsasapogenoic acid acetate and chlorogenoic acid diacetate reported by Fieser, Fry and Jones.[8] The extinction coefficient is considerably higher and the band broader than has been reported for dipropionylethane[9] but this may be caused by several factors. In the first place the general absorption of the sapogenoate may be raised by the presence of one carbomethoxy and two acetoxy groups and secondly the spectrum is for a methyl alcoholic solution whereas that for dipropionylethane is in hexane. Curve II is the absorption spectrum of the product of reaction with ammonia. This shows two bands with maxima at 2790 Å. and 2970 Å. Curve III given by Menczel[7] for pyrrole likewise shows two bands but the maxima are at shorter wave lengths (2620 and 2680 Å.) and the intensity of the absorption is very much weaker. It appears that here again there is the superposition of the effects of other absorbing groups in the molecule, of a change in solvent and also the possibility of absorbing impurities since the unknown compound could not be crystallized and is so readily altered by exposure to air. The fact, however, that the absorption spectrum shows some structure is definitely in favor of the pyrrole formulation and against the formulation as an α-ketopyrrolidine.

In the light of this conclusion reasonable explanations of the behavior of the compound can be given. While pyrrole has only weakly basic properties, the alkylated pyrroles are considerably more basic and more stable to acids. Thus 0.2 cc. of 2,4-dimethylpyrrole will dissolve completely in 1 cc. of 6 *N* hydrochloric acid and can be reprecipitated, before darkening takes place, by the addition of alkali. Other alkylated pyrroles such as 3-methyl-4-ethylpyrrole, 2,3-dimethyl-4-ethylpyrrole, 2,4-dimethyl-4-ethylpyrrole and 2,3,5-trimethyl-4-ethylpyrrole have been reported to be soluble in dilute acids.[10] Hence the solubility of the reaction product in question, which can be considered as a tetra alkyl substituted pyrrole, is understandable although it is surprising that a compound of such high molecular weight with no other highly solubilizing groups should be so readily soluble. The analytical discrepancy must be accounted for by assuming that the compound contains a molecule of water of crystallization. This is entirely possible since the compound was isolated from an aqueous medium and dried at room temperature. At this point it should be noted that the carbon analyses are lower than the permitted error. Since the same values were obtained by two different laboratories and five other analyses are satisfactory, it can be concluded that for this particular compound the standard micro procedure does not give complete combustion.

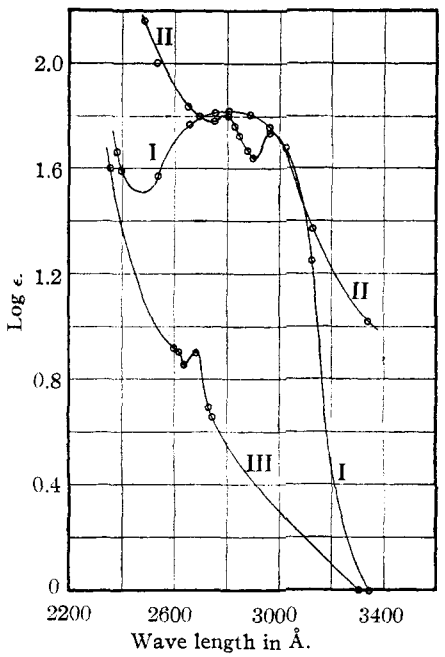


Fig. 1.—Curve I, methyl chlorogenoate diacetate in methyl alcohol; Curve II, ammonia reaction product in methyl alcohol; Curve III, pyrrole in hexane (Menczel[7]).

One other noteworthy fact is that in spite of the ease of reaction with ammonia (and also with methylamine), methyl chlorogenoate diacetate could not be made to react with aniline even on heating for ten hours in a sealed tube at 160°. The ester was recovered unchanged even to the extent that the acetyl and ester groups remained intact.

The authors gratefully acknowledge the assistance of Professor P. A. Leighton and Dr. D. H. Volman in the determination of the absorption spectra.

Experimental

Reaction of Methyl Chlorogenoate Diacetate with Ammonia.—A solution of 0.5 g. of the ester[11] in 25 cc. of

(8) Fieser, Fry and Jones, THIS JOURNAL, **61**, 1849 (1939).

(9) Ramart-Lucas, "Traité de Chimie Organique," Paris, Vol. II, part I, p. 127, 1936.

(10) Willstaetter and Asahina, *Ann.*, **385**, 203 (1911); Piloty and Stock, *ibid.*, **392**, 234, 239 (1912); Piloty, Stock and Dorman, *ibid.*, **406**, 362 (1914).

(11) McMillan and Noller, THIS JOURNAL, **60**, 1630 (1938).

absolute alcohol was saturated with dry ammonia during which time the flask was kept in an ice-bath. The solution then was allowed to stand at room temperature for twenty-four hours while passing a stream of ammonia through it at the rate of about ten bubbles per minute to exclude air. At the end of this time the solution was still colorless. After transferring to a distilling flask, the solvent was removed by passing a stream of nitrogen through the solution and applying suction. A colorless oil remained which was free of the odor of acetamide. The oil was dissolved in 6 *N* hydrochloric acid and filtered. Addition of potassium hydroxide solution to the filtrate gave a white flocculent precipitate which was filtered, washed with water and dried in a vacuum desiccator over calcium chloride. This product was soluble in methyl and ethyl alcohols, acetone and benzene, sparingly soluble in ether and dioxane and insoluble in cyclohexane and petroleum ether. All attempts to crystallize the compound failed since the crystallizates were always more oily and darker than the original material. The same was true of the product precipitated from benzene solution with petroleum ether. The product decomposed on heating on the steam-bath and on standing in solution when exposed to air.

For analyses and spectroscopic work a freshly prepared sample was redissolved in acid and reprecipitated in two fractions with alkali, rejecting the first small fraction that precipitated. The colorless precipitate after washing was dried in a vacuum desiccator over phosphorus pentoxide.

All attempts to prepare crystalline derivatives of this compound were unsuccessful but an amorphous **chloroplatinate** was obtained as follows: To a solution of 0.2 g. of the compound in 6 *N* hydrochloric acid was added a solution of 0.2 g. of platinum chloride in 2 cc. of 6 *N* hydrochloric acid. The yellow precipitate was filtered, washed and dried in a vacuum desiccator. It was soluble in acetone and in ethyl, *n*-propyl and *n*-butyl alcohols and insoluble in ether. It was purified by dissolving in *n*-propyl alcohol and precipitating with ether. For analysis the sample, which was hygroscopic, was dried in a vacuum at 60° and the platinum content determined by ignition. Analyses of the compound and of the chloroplatinate are given in Table I.

TABLE I

ANALYSES OF REACTION PRODUCT AND CHLOROPLATINATE

Detn.	Calcd. for $C_{22}H_{47}O_6N$	Calcd. for $C_{22}H_{49}O_7N$	Found			Average
Carbon	70.94	68.66	67.85[a]	67.45[a]	67.46[b]	67.59
Hydrogen	8.75	8.83	9.15[a]	9.00[a]	8.94[b]	9.03
Nitrogen	2.58	2.50	2.67[a]	2.68[a]	2.72[b]	2.69
Acetyl	15.88	15.37	15.25[a]	14.93[a]		15.09
Methoxyl	5.74	5.54	5.38	5.65		5.51
Platinum	13.07	12.76	12.77	12.85		12.81

[a] Analyses by Dr. Carl Tiedcke, New York. [b] Analyses by Arlington Laboratories, Chagrin Falls, Ohio.

An acid soluble product was obtained on heating methyl chlorogenoate diacetate with an alcoholic solution of methylamine but nothing further was done with this material. A solution of 1 g. of the ester and 1 g. of aniline in 30 cc. of absolute alcohol was heated in a sealed tube at 160° for ten hours, the solvent evaporated and the aniline removed by steam distillation. The residue solidified and after crystallization from methyl alcohol, melted at 159–160° and did not depress the melting point of the original ester. Evaporation of the filtrates from the crystallization did not yield any acetanilide. When chlorogenin was heated in a sealed tube with alcoholic ammonia at 100° for forty hours, it was recovered unchanged. Similarly when chlorogenin diacetate was heated with alcoholic ammonia for fifteen hours at 150°, deacetylation was the only reaction observed.

Absorption Spectra.—A Cornu prism spectrograph with wide dispersion was used with a water-cooled hydrogen discharge tube as a source of light. On each plate was recorded the spectrum of a mercury arc together with exposures through the pure solvent, and through screens having transmissions of 69.7, 45.5, 26.6, 11.2 and 3.5%. Tracings of the plates were made using the recording microphotometer described by Leighton, Smith and Henson[12] and the extinction coefficients for the unknown calculated. As a check on the accuracy of the method, the absorption spectrum of potassium chromate in the same region was determined and excellent agreement obtained with the results of others.[13]

Summary

Methyl chlorogenoate diacetate reacts readily with ammonia to give a compound whose ultraviolet absorption spectrum indicates that it contains a pyrrole nucleus. This is evidence that the original ester contains the 1,4-diketone grouping and favors the ketone spiroacetal formulation for the side chains of steroid sapogenins.

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(12) Leighton, Smith and Henson, *Rev. Sci. Instruments*, **5**, 431 (1934).

(13) Hogness, Zscheile and Sidwell, *J. Phys. Chem.*, **41**, 402 (1937); Stuecklen, *J. Optical Soc. Am.*, **29**, 38 (1939).