[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Saponins and Sapogenins. XIII. The Precipitability of Steroid Sapogenins by Digitonin

BY C. R. NOLLER

Marker and Rohrmann[1] have reported that chlorogenin is precipitated by digitonin, a finding contrary to our previously published experience.[2] They added to a boiling solution of 100 mg. of chlorogenin in 10 cc. of 95% alcohol, a hot solution of 600 mg. of digitonin in 20 cc. of 90% alcohol. We have repeated their experiment and have found that about half of the chlorogenin is indeed precipitated. However, assuming the addition complex to be a 1:1 molecular compound and digitonin to have the formula $C_{56}H_{92}O_{29}$, a 110% excess of digitonin was used and the concentrations were such that the solubility of digitonin at room temperature was exceeded greatly. Thus if 600 mg. of digitonin is dissolved in a hot solution of 10 cc. of 95% alcohol and 20 cc. of 90% alcohol and the solution cooled and inoculated with a trace of digitonin, 455 mg. will crystallize out on standing at room temperature. It is conceivable that this heavy precipitation would bring down a considerable quantity of chlorogenin along with it. In the usual procedure for the preparation of digitonides, one adds a solution of the compound being tested to an equal volume of a 1% solution of digitonin (1 g. in 100 cc. of 90% alcohol) so that there is no danger of digitonin crystallizing since the solubility of digitonin at room temperature is approximately 0.5 g. per 100 cc. of 90% alcohol. Under these conditions chlorogenin does not precipitate.

In view of the extensive use of the test it seemed desirable to attempt to establish a more definite criterion of compound formation with digitonin. The relative solubility of the compound and its components is the only property that can be used conveniently in this series and it would seem that to show compound formation it is necessary to show that the solubility of the digitonide is less than that of either of its components. If a solution of cholesterol or of β-cholestanol in 90% alcohol, saturated at room temperature, is mixed with an equal volume of a saturated solution of digitonin in the same solvent, a precipitate forms almost immediately. Under the same conditions tigogenin yields a precipitate after about twenty minutes, gitogenin after fifteen hours, while sarsasapogenin, chlorogenin, *epi*-coprosterol and *epi*-dihydrocholesterol give no precipitate after standing for five days. If, however, the solutions of the last four compounds are concentrated to half volume to bring the concentration of the compound and of digitonin to that of saturated solutions, all except *epi*-coprosterol give some precipitate after standing for five hours. It is seen therefore that neither condition gives a satisfactory test since before concentration sarsasapogenin, which is known to give a less soluble digitonide than its epimer,[3] did not precipitate whereas after concentration *epi*-dihydrocholesterol, which has the α-configuration, also was precipitated.

One obvious inaccuracy in the use of saturated solutions of the compounds for qualitative tests is that the solubility, and hence the concentration, is different for each compound. A semi-quantitative approach to the problem was made as follows. Five cubic centimeters of a saturated solution of the compound was evaporated to dryness and the residue weighed. This residue was then dissolved by warming with 5 cc. of a saturated solution of digitonin, tightly stoppered and allowed to stand overnight. The precipitate that had formed was filtered the next morning by suction through tared funnels, dried at 80° and weighed. If no precipitate had formed, which was the case with *epi*-dihydrocholesterol, sarsasapogenin and chlorogenin the solutions were inoculated with traces of precipitates that had been formed previously in more concentrated solutions, and allowed to stand for another twenty-four hours and the precipitates filtered. The filtrates were returned to the flasks and allowed to stand an additional three days to be certain that no more precipitate formed. In the case of those compounds that precipitated on mixing 5 cc. of a saturated solution with 5 cc. of digitonin solution, these precipitates after standing overnight were filtered and weighed. Assuming a molecular ratio of 1:1 and a molecular weight for digitonin

(1) Marker and Rohrmann, THIS JOURNAL, **61**, 946 (1939).
(2) Noller and McMillan, *ibid.*, **60**, 1092 (1937).
(3) Askew, Farmer and Kon, *J. Chem. Soc.*, 1399 (1936).

TABLE I

SOLUBILITY PRODUCTS OF STEROID DIGITONIDES

Compound[a]	Soly.[b] in 90% ethyl alcohol, g. per 5 cc.	Digitonide[b] pptd., g.	Compd. pptd., g.	Digitonin pptd., g.	Concn. of compd. in soln., g. per l.	Concn. of digitonin in soln., g. per l.	Soly. prod., mole per l.	Concn. product on mixing equal volumes of satd. solns., mole per l.
Cholesterol	0.0412	0.0285	0.0068	0.0217	3.44		Small	
β-Cholestanol	.0174	.0302	.0072	.0230	1.02		Small	
Tigogenin	.0051	.0165	.0042	.0123	0.09	0.90	1.6×10^{-7}	2.1×10^{-6}
Gitogenin	.0181	.0220	.0057	.0163	2.48	1.00	4.7×10^{-6}	7.2×10^{-6}
epi-Dihydrocholesterol	.0120	.0119	.0029	.0090	1.82	2.46	9.4×10^{-6}	5.3×10^{-6}
Sarsasapogenin	.0170	.0158	.0040	.0118	2.60	1.90	9.7×10^{-6}	7.1×10^{-6}
Chlorogenin	.0404	.0135	.0035	.0100	7.38	2.26	3.1×10^{-5}	1.6×10^{-5}
epi-Coprosterol	.0524	None					Large	
Digitonin	.0213							

[a] For the pure samples of β-cholestanol, m. p. 143–145°, *epi*-dihydrocholesterol, m. p. 185–186°, and *epi*-coprosterol, m. p. 111°, all of which had been purified by means of digitonin, we are indebted to Dr. R. Schoenheimer, for sarsasapogenin, m. p. 192–194°, to Dr. L. F. Fieser, and for gitogenin, m. p. 270–274°, to Dr. W. A. Jacobs. The tigogenin, m. p. 202–207°, and chlorogenin, m. p. 273–276°, were isolated from *Chlorogalum pomeridianum*. [b] For the first three compounds the temperature of the solubility determinations was 23 ± 0.5° and the volume of the mixed solutions 10 cc. For the last five compounds the solubility determinations were made at 25 ± 0.5° and the volume of the mixed solutions was 5 cc.

of 1228, the weight of compound and of digitonin precipitated could be calculated and these values subtracted from the weights originally present gave the amounts of compound and of digitonin remaining in solution. From these data approximate solubility products for the digitonides could be calculated. The results are given in the table.

The solubility products are approximate only since they have been calculated from a single pair of concentrations in each case and no attempt was made to make the determinations more than roughly quantitative. The precipitates were not washed because of their known high solubility; hence they include the material dissolved in the adherent mother liquors. This may be partly compensated by the difficulty of quantitative transfer of the precipitates to the funnels. Where a second filtration was necessary, as in the case of chlorogenin, the weight of precipitate may be too high because of evaporation of the solvent during the first suction filtration, although such evaporation was held to a minimum. It is believed from several duplicate determinations that the error may be of the order of 5 to 10%. That the values are fairly accurate is indicated by the last column of the table which gives the products of the concentrations on mixing equal volumes of saturated solutions of the compounds and of digitonin. In the case of tigogenin and gitogenin these products exceed the solubility product and it is known that these compounds give precipitates under these conditions, whereas the concentration products are less than the solubility products in the case of *epi*-dihydrocholesterol, sarsasapogenin and chlorogenin.

The table shows clearly that tigogenin forms a stable digitonide as previously reported[4] although its precipitation can hardly be called "almost quantitative." Gitogenin, which has been reported as being not precipitated by digitonin,[4] forms a less soluble digitonide than sarsasapogenin whose epimer gives a more soluble digitonide.[3] Hence until the epimers of gitogenin have been prepared and their properties studied, gitogenin should be considered as being precipitable by digitonin. Chlorogenin digitonide on the other hand is considerably more soluble than sarsasapogenin digitonide, and hence tentatively should be considered as belonging to the group not precipitated by digitonin. The necessity of comparing pairs of epimers is shown clearly by the case of *epi*-dihydrocholesterol, which gives a digitonide having a solubility product the same as that of sarsasapogenin, yet they must be considered as belonging to two different stereochemical groups.

Precipitability by digitonin long has been considered to be specific for steroids having an hydroxyl group at C-3 with the same stereochemical configuration as that present in cholesterol.[5] In recent years it has been shown that not only are some compounds which lack this hydroxyl group precipitated by digitonin,[6] but that others con-

(4) Tschesche and Hagedorn, *Ber.*, **68**, 2247 (1935).

(5) Fieser, "Chemistry of Natural Products Related to Phenanthrene," Reinhold Pub. Corp., New York, N. Y., 1937, p. 118.

(6) Butenandt and Mamoli, *Ber.*, **68**, 1847 (1935); Wintersteiner, THIS JOURNAL, **59**, 765 (1937).

taining a C-3 hydroxyl group of the proper configuration are not precipitated by digitonin.[4,7] It thus may even be questionable whether precipitation by digitonin has the same significance for the steroid sapogenins as for the sterols.

Summary

1. The solubility of the digitonides of steroid sapogenins varies considerably and is much greater than that of the digitonides of cholesterol and β-cholestanol. Thus the solubility product of the digitonide of sarsasapogenin, which is known to be the less soluble of two epimeric forms, is practically the same as that of the digitonide of *epi*-dihydrocholesterol, which ordinarily is considered as not precipitable by digitonin.

2. Gitogenin digitonide has a smaller solubility product than sarsasapogenin digitonide and hence gitogenin provisionally must be considered as being the epimer that is precipitated by digitonin.

3. The digitonide of chlorogenin has a much larger solubility product than that of sarsasapogenin and until its epimer is prepared and shown to give a more soluble digitonide, chlorogenin must be considered as the isomer not precipitable by digitonin.

4. It has been shown that the solubility products of steroid digitonides vary so widely that qualitative tests for precipitation are without meaning unless the behavior of both isomers can be compared.

(7) Dimroth, *Ber.*, **69**, 1123 (1936); Reichstein and Grätzi, *Helv. Chim. Acta*, **21**, 1185 (1938).

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Sterols. LXXI. Urane Derivatives

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In papers XXIX[1] and XXXVII[2] of this series the isolation from mares' pregnancy urine of uranetriol and uranediol was described. These substances were shown to possess hydroxyl groups at C-11 and both compounds were converted to the same parent hydrocarbon, urane (I), which is believed to differ from pregnane in the configuration at C-9.

In spite of the close relationship[1,2,3] existing between uranetriol and uranediol, they nevertheless show some unusual and striking differences. We have pointed out previously the fact that on the basis of precipitability with digitonin uranetriol is a 3-α while uranediol is a 3-β compound. There is also a pronounced difference in the reaction of bromouranetrione and bromouranedione with pyridine, the former yielding urenetrione directly while the latter gives largely pyridinium salt which yields urenedione upon destructive distillation. The fact that on the debromination of bromouranedione only one product was obtained suggests that the bromine occupied the C-4 position. Although it appears that the double bond in urenetrione and urenedione may be in the 4,5-position, no definite proof of this point has as yet been obtained.

In the catalytic hydrogenation of uranedione-3,11 (V) the carbonyl group at C-3 is much more easily reduced than that at C-11 and consequently the preparation of uraneol-3(β)-one-11 (VIII) is possible. There was no noticeable *epi* fraction obtained in this reduction. The same product was formed by the reduction of uranedione with aluminum isopropylate. Oxidation of the product with an excess of chromic anhydride gave uranedione.

Uranediol on partial oxidation with chromic anhydride yielded some uraneol-3(β)-one-11 (VIII) in addition to uranedione. No uraneol-11-one-3 was isolated from the oxidation. In the oxidation of uranediol with aluminum isopropylate, however, the oxidation proceeds readily at C-3 and the only ketonic product obtained was uraneol-11-one-3 (IX). This product formed a monoacetate and a monosemicarbazone.

By the Clemmensen reduction of uranedione-3,11 with unamalgamated zinc in ethanol solution, an almost quantitative yield of uraneone-11 (IV) was obtained. This product formed no semicarbazone, but on catalytic hydrogenation yielded uraneol-11 (VII) which formed a monoace-

(1) Marker, Kamm, Oakwood, Wittle and Lawson, THIS JOURNAL, **60**, 1061 (1938).
(2) Marker, Rohrmann and Wittle, *ibid.*, **60**, 1561 (1938).
(3) Marker, Wittle and Oakwood, *ibid.*, **60**, 1567 (1938).