[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF HARVARD AND STANFORD UNIVERSITIES]

Saponins and Sapogenins. X. The Isolation of Gitogenin from Chlorogalum Pomeridianum

BY C. R. NOLLER, L. H. GOODSON AND MARTIN SYNERHOLM

In a recent paper Marker and Rohrmann[1] state that they "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." This interest of other workers in *Chlorogalum* as a source of steroid sapogenins prompts us to report the observations in regard to their isolation which we have made since our original publication.[2]

Hydrolysis of the alcoholic extracts of the ground bulbs or of the partially purified saponins followed by separation of the sapogenins by crystallization always has yielded an intermediate fraction melting between 230 and 255° which could be made to melt over the comparatively narrow range of 235–240°.[3] Oxidation of this material with Kiliani's mixture[7] gave an acid fraction which appeared to be identical with gitogenic acid. Three possibilities seemed likely: (1) that the material was a molecular compound of chlorogenin and tigogenin, the gitogenic acid being formed by oxidation of the latter; (2) that it was a new sapogenin which also gave gitogenic acid on oxidation; (3) that it contained gitogenin possibly as a molecular compound with chlorogenin or tigogenin. Melting point determinations of mixtures of pure tigogenin and chlorogenin eliminated the first possibility as shown in Fig. 1.[4] Moreover, an analysis checked almost exactly for the composition $C_{27}H_{44}O_4$. This could result from an equimolar mixture of tigogenin, $C_{27}H_{44}O_3$, and a trihydroxy compound, $C_{27}H_{44}O_5$, or from a mixture of the isomeric substances gitogenin and chlorogenin.

Further attempts to separate the mixture by crystallization of the sapogenins were without encouraging results. While the material crystallized well from benzene, carbon tetrachloride, ethylene chloride, and dioxane, there was no more evidence that a separation was taking place than when crystallized from methyl, ethyl or isopropyl alcohol. Separation was effected readily, however, after conversion to the benzoates or acetates. The mixed esters were extracted with ether in which one isomer is much more soluble than the other and this was followed by systematic crystallization of the two portions to constant melting point. The less soluble benzoate, its saponification product and the acetate had melting points almost identical with those of authentic samples of gitogenin dibenzoate, gitogenin and gitogenin diacetate.[5] Mixtures of the corresponding compounds gave slight but definitely observable depressions or elevations over that for the components even when present in the same bath, which, until a satisfactory explanation is found, is a disturbing fact. The specific rotations, however, of the two sapogenins and of their acetates were identical within experimental error and in spite of the melting behavior of the mixtures we feel certain that the two sapogenins are identical.

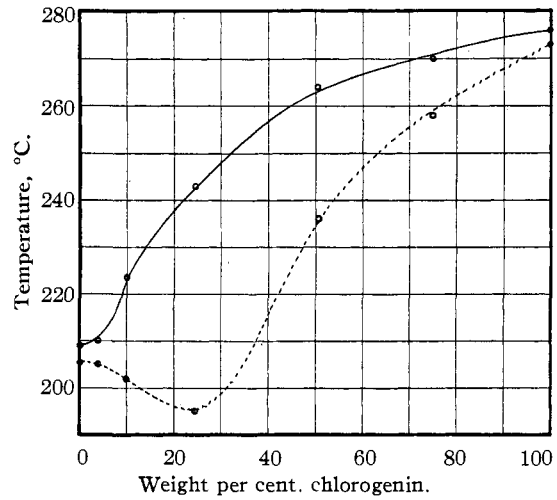

Fig. 1.—Melting point diagram for mixtures of tigogenin and chlorogenin.[4]

The more soluble benzoate, its saponification product and the acetate proved to have the same

(1) Marker and Rohrmann, THIS JOURNAL, **61**, 946 (1939).

(2) Liang and Noller, *ibid.*, **57**, 525 (1935).

(3) McMillan, Thesis, Stanford University, 1937, p. 40.

(4) In all of the melting point diagrams, the dotted curve represents the temperature at which the mixture first wets the walls of the capillary tube while the solid line gives the temperature at which the melt is entirely clear. In Figs. 1 and 3 the melting point diagram indicates that the components form a continuous series of solid solutions with the melting point passing through a minimum. The fact that the temperature at which the melt is clear does not pass through a minimum as does the softening point is probably caused by the system not being in equilibrium.

(5) The authors are greatly indebted to Dr. W. A. Jacobs of the Rockefeller Institute for Medical Research for a generous sample of gitogenin from *Digitalis* leaves.

melting points, respectively, as chlorogenin dibenzoate, chlorogenin and chlorogenin diacetate and the specific rotations of the sapogenins were identical within experimental error. Here again the mixture of the benzoates and of the acetates melted slightly lower than either component, while the mixture of the sapogenins melted higher. The fact that these phenomena occur with the chlorogenin fraction of the mixture supports our belief that they do not indicate non-identity and that the other component is indeed gitogenin.

The melting point curve for mixtures of gitogenin and chlorogenin given in Fig. 2 shows that the two components do not form a continuous series of solid solutions, but form a eutectic. This

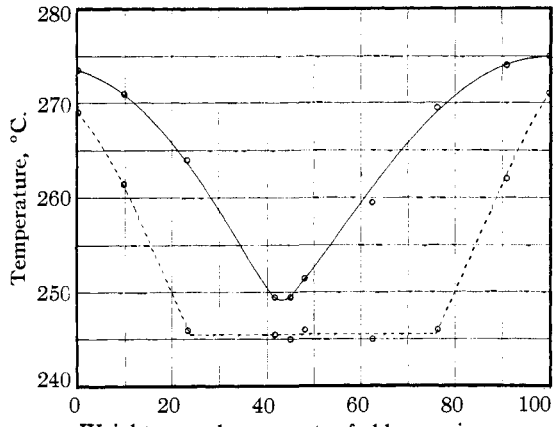

Fig. 2.—Melting point diagram for mixtures of gitogenin and chlorogenin.[4]

eutectic is the cause of the relatively sharp melting point of approximately equimolar quantities of gitogenin and chlorogenin. Apparently the solubility of the gitogenin and chlorogenin in the solvents used is practically identical and the solubility of the eutectic mixture is greater than that of either of the pure components with the result that with an excess of chlorogenin, crystallization leads to a separation of pure chlorogenin and the eutectic. One discrepancy in this explanation is the fact that the melting point of 235–240° commonly encountered in isolating the sapogenins is about 10° lower than that of the eutectic as determined by the mixed melting point curves. It is possible that the natural mixture contains some tigogenin which may lower the melting point of the eutectic.

Figure 3 gives the melting point diagram of mixtures of tigogenin and gitogenin which is very similar to Fig. 1 except that the lowering of the softening point of tigogenin by gitogenin is less than in the case of tigogenin and chlorogenin.

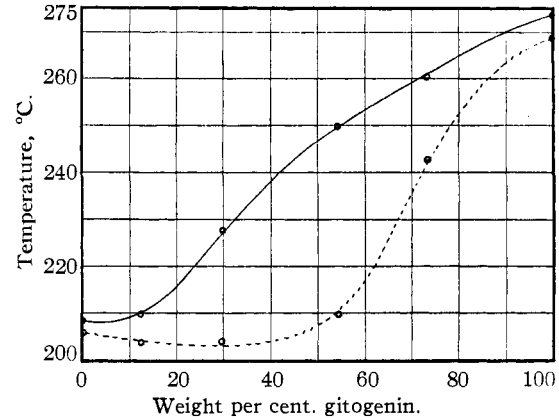

Fig. 3.—Melting point diagram for mixtures of tigogenin and gitogenin.[4]

The general procedure for the isolation of the sapogenins has been improved considerably over the method originally published[2] and our present procedure is recorded in the experimental part. The identity of tigogenin from *Chlorogalum* with that from *Digitalis* has been confirmed further by conversion into tigogenin acetate, tigogenone, tigogenone oxime, the C_{22} lactone acetate obtained by oxidation and the C_{22} hydroxy lactone, the melting points of which all checked with those recorded in the literature for derivatives of tigogenin isolated from *Digitalis purpurea* or *Digitalis lanata*.

Experimental

Isolation of the Mixed Sapogenins.—Several hundred pounds of bulbs of *Chlorogalum pomeridianum* from which the outer husks had been removed were put through a power food grinder at the San José plant of the California Prune and Apricot Growers Association. The resultant mash was mixed thoroughly with about an equal volume of methyl alcohol to act as a preservative and the saponin extracted as opportunity and facilities permitted. Addition of the methyl alcohol caused a thickening of the mash presumably due to a precipitation of plant gums by the alcohol. For isolating the saponins the mixture of ground bulb and methyl alcohol was pressed in a cider press. The expressed liquid yielded very little sapogenin on hydrolysis so that it was hardly profitable to work it up other than to recover the methyl alcohol. The press cake was extracted in 12-liter flasks with boiling methyl alcohol, the hot solution filtered and allowed to cool overnight. The saponins which separated were filtered and stored moist in tightly stoppered wide-mouthed bottles. The extraction with hot methyl alcohol was repeated until no saponin separated on standing overnight. Each of the mother liquors was concentrated and additional crops of saponin were obtained.

For hydrolysis a saturated solution of the saponins in hot methyl alcohol was prepared and an equal volume of 10% aqueous hydrochloric acid added. A flocculent precipitate formed which after twelve hours of refluxing became crystalline and was filtered. The advantage of using the saponins which have been isolated as above rather than the crude extracts is that about half the time is required for hydrolysis, and the hydrolysis product is light colored and almost pure sapogenins, very little residue being left after exhaustive extraction with hot carbon tetrachloride.

It was found that a partial separation of the sapogenins could be obtained in the next step, the extraction of the crude hydrolysis product with carbon tetrachloride. Extraction was carried out in Soxhlet extractors until a copious crop of crystals had formed in the boiling flask. These were filtered and the extraction continued and crops removed until no more sapogenin separated. The carbon tetrachloride was then concentrated and successive crops of crystals removed. The melting point of each crop was taken and the various crops combined into three groups: those melting below 230°, those melting between 230 and 245° and those melting above 245°.

Isolation of Tigogenin.—The combined fractions melting below 230° were extracted in a Soxhlet extractor with a petroleum hexane fraction (Viking Distributing Company, b. p. 60–70°), separating crops of crystals until the rate of extraction is relatively slow. The crops melting up to 220° could be purified readily by several crystallizations from isopropyl alcohol when they melted at 203–208°. Concentration of the hexane mother liquors gave material melting as low as 180–186°. This low melting point was found to be caused by the presence of a non-crystallizable oil. The melting point of these fractions readily could be brought to that of tigogenin by one crystallization from hexane, in which the oil is very soluble, followed by recrystallization from isopropyl alcohol.

A special effort was made to obtain a sample of tigogenin of maximum purity. A sample that had been purified by crystallization from isopropyl alcohol was converted to the acetate by means of acetic anhydride and pyridine. After two crystallizations from ethyl alcohol the acetate melted at 202–208° while Jacobs and Fleck[6] report 200–202°. Saponification of the acetate and two crystallizations from isopropyl alcohol brought the melting point of tigogenin to 206.5–209° which could not be raised nor the range narrowed by subsequent recrystallizations. This value compares favorably with the 206.5–210° previously reported.[2]

Oxidation of a less pure sample of tigogenin, m. p. 199–204°, gave tigogenone which after two crystallizations from acetone melted at 203–205°. This was converted to the oxime, which after three crystallizations from acetone melted at 256–258°.

Isolation of Chlorogenin.—The crude fractions melting above 245° readily gave pure chlorogenin by repeated crystallization from methyl alcohol. The purest sample of chlorogenin that we have obtained was prepared by crystallizing a product, m. p. 270–275°, once from methyl alcohol, twice from dioxane and twice from methyl alcohol, after which it melted at 273–276°. No change in the melting point was observed during the last three crystallizations.

(6) Jacobs and Fleck, *J. Biol. Chem.*, **88**, 546 (1930).

Separation of Gitogenin and Chlorogenin.—All attempts to separate the crude fraction melting between 230 and 245° into its components by crystallization or partial extraction with hot solvents failed. The solvents tried were benzene, dioxane, ethylene dichloride, carbon tetrachloride, methyl alcohol and 95% ethyl alcohol. While there were minor changes in the melting point, such as spreading or narrowing of the range, successive crops of the dissolved and undissolved portions would melt at so nearly the same temperatures that no practical separation could be made.

A fraction having the narrow melting range of 235–239° occasionally was obtained, usually when using carbon tetrachloride as a solvent. Five grams of this fraction on oxidation at room temperature by the procedure of Kiliani and Merk[7] gave 4 g. of crude oxidation product. This was extracted with cold benzene, giving 1.5 g. of insoluble material. The benzene extract after washing with 10% sodium carbonate solution and evaporation of the benzene gave 0.72 g. of neutral product. The sodium carbonate extracts on acidification gave 1.3 g. of acidic material. The benzene insoluble material was completely soluble in sodium carbonate solution. This behavior on oxidation is in marked contrast to that of chlorogenin[8] which yields very little acidic material. The benzene insoluble acid was crystallized eight times from glacial acetic acid, the melting point then remaining constant at 243–245°. Windaus and Schneckenburger[9] report the melting point of gitogenic acid as 242–243° and Jacobs and Simpson[10] as 244° with preliminary sintering.

The remainder of the sapogenin sample, m. p. 235–239°, was recrystallized for analysis.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.96; H, 10.25. Found: C, 74.77; H, 10.24.

The separation of the mixture was first effected through the benzoates. A mixture of 9 g. of material, m. p. 230–240°, 17 g. of benzoyl chloride, 9.5 g. of pyridine and 500 cc. of benzene was refluxed for fifteen minutes, allowed to stand at room temperature for three hours and the benzene distilled from a steam-bath until a sirup remained. The residue was rubbed up with 6 *N* hydrochloric acid and then three times with 25-cc. portions of 80% ethyl alcohol, decanting from the residue. The product is still pasty at this point but, on the addition of 25 cc. of ether, a portion goes into solution leaving a solid which can be filtered. The undissolved portion weighed 4 g. and melted at 211-227°. It was only slightly soluble in hot methyl alcohol, 95% ethyl alcohol or hexane, very soluble in dioxane and could be crystallized from isopropyl alcohol, ethyl acetate or acetone. After two crystallizations from each of the last three solvents, the product melted at 223–228°.

Anal. Calcd. for $C_{41}H_{52}O_6$: C, 76.84; H, 8.18. Found: C, 76.89; H, 8.22.

A mixture with an equal amount of gitogenin benzoate, m. p. 223–227°, melted at 222–227°. All three samples were placed at the same time in a Hershberg[11] apparatus so that the lowering though slight was definitely observable. The specific rotation for the dibenzoate isolated from the

(7) Kiliani and Merk, *Ber.*, **34**, 3564 (1901).
(8) Noller, THIS JOURNAL, **59**, 1092 (1937).
(9) Windaus and Schneckenburger, *Ber.*, **46**, 2628 (1913).
(10) Jacobs and Simpson, *J. Biol. Chem.*, **110**, 429 (1935).
(11) Hershberg, *Ind. Eng. Chem., Anal. Ed.*, **8**, 312 (1936)

mixture was $[\alpha]^{25}D$ −75.9° in pyridine. The sample of gitogenin benzoate available was not sufficiently large for a determination of its rotation.

The dibenzoate was saponified by refluxing 1.8 g. with 0.8 g. of potassium hydroxide in 60 cc. of ethyl alcohol for three hours. The sapogenin was precipitated by the addition of water and crystallized twice from 95% ethyl alcohol and three times from 90% ethyl alcohol, when it melted at 269–274°. A recrystallized sample of gitogenin melted at 270–274.5° and a mixture of equal amounts of the two preparations melted at 271–275°. Here again all three tubes were in the same melting point bath. The specific rotation for the sample from *Chlorogalum* was $[\alpha]^{25}D$ −61.3° in pyridine, while that for gitogenin was $[\alpha]^{25}D$ −60.9°.

The sapogenin was converted to the diacetate by heating 0.2 g. with 1 cc. of acetic anhydride and 1 cc. of pyridine on the steam-bath for two hours and allowing to stand overnight. After precipitation with water the product was crystallized from methyl alcohol when it melted at 239–244°. This sample was not used for determination of the specific rotation or a mixed melting point with gitogenin diacetate, the sample obtained below from the acetylation of the mixed sapogenins being used for this purpose.

The filtrate from the above ether extraction of the crude benzoate gave on evaporation an oil which partially solidified on cooling. On rubbing up with 25 cc. of 80% ethyl alcohol, the mass solidified sufficiently to be pulverized and filtered. The product, which weighed 8 g. and melted at 130–165°, probably contained considerable benzoic acid. Two crystallizations from isopropyl alcohol and one from ethyl acetate raised the melting point to 180–197° and two more crystallizations from acetone brought it to 196–203.5°.

Anal. Calcd. for $C_{41}H_{62}O_6$: C, 76.84; H, 8.18. Found: C, 76.72; H. 8.27.

When mixed with an equal amount of chlorogenin dibenzoate, m. p. 203.5–207.5°, the melting point was 195.5–205.5°, a slight but noticeable depression.

Saponification gave the sapogenin which after two crystallizations from methyl alcohol melted at 268–274°. A mixture with an equal part of chlorogenin, m. p. 268–275°, melted at 269–276°, a slight but definite elevation. The specific rotation of the sapogenin was $[\alpha]^{25}D$ −47.7° in dioxane while that for pure chlorogenin was $[\alpha]^{25}D$ −46.3°.

Conversion to the acetate by acetic anhydride and pyridine and crystallization from methyl alcohol gave a product, m. p. 154–156°. A mixture with an equal part of chlorogenin diacetate, m. p. 153.5–156.5°, melted at 152.5–155°.

The extreme solubility of chlorogenin diacetate and the slight solubility of gitogenin diacetate in hot methyl alcohol suggested that the mixture could be separated more conveniently through the acetates. A mixture of 10 g. of the mixed sapogenins, m. p. 230–245°, with 50 cc. of acetic anhydride and 80 cc. of pyridine was heated on a hot-plate until completely homogeneous and then allowed to stand overnight. Decomposition with an excess of water and filtering gave 11.5 g. of air-dried product. This was rubbed up with 50 cc. of ether and filtered. The air-dried insoluble portion weighed 5 g. and melted at 225–235°. It was recrystallized five times from methanol when it melted at 239–243°. No change in melting point took place during the last two crystallizations. A mixture with a sample of gitogenin, m. p. 239–243°, melted at the same temperature as far as measurement with the thermometer indicated, although the mixture appeared to shrink just before the individual samples and was definitely clear before them. The specific rotation of the acetate separated from the mixture was $[\alpha]^{25}D$ −80.2° in pyridine compared with −80.7° for an authentic sample of gitogenin diacetate.

The ether extract contained the chlorogenin diacetate contaminated by a small amount of gitogenin diacetate which was difficult to remove by crystallization because of its insolubility. This is true also when separating through the benzoates and the best procedure is to saponify the partially purified esters and continue the purification by crystallization of the chlorogenin.

Determination of Melting Point Diagrams for Mixtures.—The fact that some decomposition takes place at the melting point and that only limited amounts of material were available made it necessary to mix the components mechanically and to take the melting points in capillary tubes. Only slightly more material was weighed out than was necessary to fill the capillary tube in order to ensure adequate mixing. The data used in Fig. 1 were obtained on samples carefully mixed mechanically with a small spatula. It was later found more convenient to dissolve the sample in a few drops of dioxane, allow the solvent to evaporate and to grind the residue with a spatula, this method being used for the data of Fig. 2 and Fig. 3. Melting points in this and the previously recorded work were taken in a Hershberg melting point apparatus[11] with completely immersed calibrated Anschütz thermometers having 0.2° scale divisions. The points of the lower curves are the temperatures at which the sample first wet the sides of the capillary tubes while those of the upper curves represent the temperatures at which the melts were completely clear. The rate of rise of temperature was less than 1° per minute in the neighborhood of the melting point. In spite of the precautions taken it is obvious that this procedure could not be expected to give more than roughly quantitative results.

Summary

1. An improved procedure is given for the isolation of steroid sapogenins from *Chlorogalum pomeridianum*.

2. Besides the previously reported tigogenin and chlorogenin a third sapogenin has been isolated which has been shown beyond reasonable doubt to be identical with gitogenin from *Digitalis* in spite of an unexplainable behavior of mixed melting point determinations.

3. Melting point diagrams for mixtures of tigogenin and chlorogenin and for tigogenin and gitogenin indicate that these pairs form a continuous series of solid solutions, while mixtures of gitogenin and chlorogenin form a eutectic at approximately 42% gitogenin.

CAMBRIDGE, MASS. RECEIVED APRIL 21, 1939