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

Sterols. CVI. Sapogenins. XXXV. The Supposed Trillarigenin

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Grove, Jenkins and Thompson[1] obtained a diglucoside of a sapogenin from an alcoholic extract of *Trillium erectum* from which they obtained a new sapogenin, $C_{25}H_{39}O_4$, which they called "trillarigenin." More recently the isolation and identification of diosgenin from *trillium erectum* was reported by us.[2] We failed to find any "trillarigenin." Because of the importance of diosgenin as a convenient starting material for the preparation of the steroidal hormones and its isolation from *trillium erectum*, we have investigated the "trillarigenin" isolated by Grove, Jenkins and Thompson.

The diglucoside, trillarin, was obtained by a mild treatment with hydrochloric acid of the residues from the alcoholic extract of the powdered roots. This was purified from a higher melting glycoside by fractional crystallization from methanol. We propose the name "trillin" for the latter glycoside.

Upon hydrolysis of trillarin with dilute acid, Grove, Jenkins and Thompson obtained a product ("trillarigenin") melting at 190–197° when crystallized from methyl alcohol. Using the same method of hydrolysis as described by these workers, we also obtained material melting at approximately the same temperature. This could not be separated by further crystallization from methanol. Carbon and hydrogen analysis checked closely with the values given by them for the composition $C_{25}H_{39}O_4$. However, it was found that upon treatment of this product with ether a considerable amount of material was insoluble. The ether insoluble material upon crystallization from methanol gave the higher melting glycoside, trillin, whereas the ether soluble material was identified as diosgenin. It thus appears that "trillarigenin" is a mixture of diosgenin and trillin. Carbon and hydrogen analyses indicate approximately 70% diosgenin and 30% trillin in the mixture.

In further experiments on the hydrolysis of the glucoside, trillarin, it was found that vigorous acid hydrolysis yielded almost entirely diosgenin, whereas very mild hydrolysis gave a good yield of trillin, a mono-glycoside. Grove, Jenkins and Thompson found that the sugar portion of trillarin was glucose. Vigorous acid hydrolysis of trillin gave diosgenin and glucose, the latter being identified as its osazone.

Trillin forms an acetate when treated with acetic anhydride. This is either a penta or tetraacetyl derivative but most probably the latter. When hydrogenated with platinum oxide catalyst in acetic acid it gave a new glycoside, which upon hydrolysis gave dihydrotigogenin, a product previously obtained by the catalytic reduction of tigogenin under the same conditions.[3] The formation of a glycoside of dihydrotigogenin by catalytic reduction of trillin indicated that the latter compound is a 3-glucoside of diosgenin.

Catalytic reduction of trillin in acidified methanol at room temperature gave the 3-glucoside of tigogenin, which upon acid hydrolysis gave tigogenin.

We thank Parke, Davis and Company for their assistance.

Experimental Part

The defatted alcoholic extract of 40 pounds (17 kg.) of *trillium erectum* (S. B. Penick and Company, New York) was concentrated to a tar. A solution of this in 1 liter of alcohol and 9 liters of water was treated with 500 cc. of concd. hydrochloric acid and kept at 80° for thirty minutes. The flask was cooled in ice. Dilution to 20 liters gave a brown precipitate. On standing overnight this settled to the bottom and the supernatant liquid was decanted and the precipitate filtered. This was crystallized from 3 liters of ethanol, and the filtered yellow product was washed well with alcohol and ether; yield, 220 g.

The filtrates were evaporated to 3 liters and hydrolyzed by refluxing for three hours with 400 cc. of concd. hydrochloric acid to give 74 g. of diosgenin by working it up in the usual way.[2]

The crude glycoside melted 180–195° and upon fractional crystallization from methanol yielded two colorless products, melting 197–200° and 275–280° with decomposition. The lower melting product appears to be identical with the diglucoside, trillarin, reported by Grove, Jenkins and Thompson.[1]

Hydrolysis of Trillarin.—To a solution of 3 g. of trillarin in 150 cc. of ethanol was added 3 cc. of concd. hydrochloric acid and the mixture was refluxed for one hour. It was poured into 500 cc. of water, filtered and washed with a small amount of alcohol and ether. Upon recrystallization from methanol it gave colorless crystals, m. p. 275–280°,

(1) Grove, Jenkins and Thompson, *J. Am. Pharm. Assoc.*, **27**, 457 (1938).

(2) Marker, Turner and Ulshafer, THIS JOURNAL, **62**, 2542 (1940).

(3) Marker and Rohrmann, *ibid.*, **61**, 1516 (1939).

which gave no depression when mixed with the higher melting product isolated above. We propose the name trillin for this product. Analysis showed it to contain one glucoside group; yield, 1.6 g.

Anal. Calcd. for $C_{33}H_{52}O_8 \cdot {}^1/_2H_2O$: C, 67.6; H, 9.1. Found: C, 67.6; H, 9.1.

A mixture of 3 g. of trillarin, 150 cc. of ethanol and 15 cc. of concd. hydrochloric acid was refluxed for three hours. Water was added and the product was extracted with ether. The ether was removed and the residue crystallized from acetone and methanol, m. p. 206–208°; yield practically quantitative. Mixed with tigogenin, 204–206° it melted at 185–195°. Mixed with diosgenin, m. p. 206–208°, it gave no depression in melting point.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.2; H, 10.2. Found: C, 78.5; H, 10.4.

A mixture of 3 g. of trillarin, 150 cc. of ethanol and 3 cc. of concd. hydrochloric acid was refluxed for two hours according to the directions of Grove, Jenkins and Thompson[1] for the formation of "trillarigenin." Water was added and the product was filtered. The precipitate was crystallized from methanol. It melted at 195–196° with previous softening at 193°. The analysis is compared to that given by the previous workers for their "trillarigenin." Grove, Jenkins and Thompson: Found: C, 74.7, 74.6; H, 9.6, 9.7. Our values found: C, 74.4; H, 9.5. Further crystallization from methanol gave no separation of products. The "trillarigenin" was shaken with ether and filtered. The ether insoluble fraction was crystallized from methanol. It melted at 275–280° and gave no depression in melting point when mixed with trillin.

Anal. Calcd. for $C_{33}H_{52}O_8 \cdot {}^1/_2H_2O$: C, 67.6; H, 9.1. Found: C, 67.6; H, 9.1.

The ether soluble fraction was crystallized from methanol, m. p. 206–208°. It gave no depression in melting point with diosgenin, m. p. 206–208°. It gave an acetate, m. p. 197–199°, which gave no depression when mixed with diosgenin acetate.

Anal. Calcd. for $C_{29}H_{44}O_4$: C, 76.3; H, 9.7. Found: C, 76.2; H, 9.5.

Acetate of Trillin.—Trillin was refluxed with an excess of acetic anhydride. The solvent was removed and the residue was crystallized from methanol, m. p. 202–203°.

Anal. Calcd. for $C_{43}H_{62}O_{13}$: C, 65.6; H, 7.9. Calcd. for $C_{41}H_{60}O_{12}$: C, 66.1; H, 8.1. Found: C, 65.9; H, 8.2.

The pure acetate, 5.5 g., was refluxed for one hour with 300 cc. of 5% methanolic potassium hydroxide. It was crystallized twice from methanol, m. p. 275–283°; yield, 4.0 g. It gave no depression in melting point with trillin.

Anal. Calcd. for $C_{33}H_{52}O_8 \cdot {}^1/_2H_2O$: C, 67.6; H, 9.1. Found: C, 67.5; H, 9.0.

One gram of the glucoside was refluxed for two hours in 50 cc. of ethanol with 6 cc. of concd. hydrochloric acid. After standing overnight the mixture was poured into water and extracted with ether. The ethereal solution was washed well with water and evaporated to dryness. The residue weighed 670 mg. and melted at 202°. There was no depression in melting point with diosgenin. The sugar was isolated and identified as its osazone, m. p. 206°, dec.

Catalytic Reduction of the Acetate of Trillin.—One gram of the acetate of trillin, 0.5 g. of Adams catalyst and 100 cc. of acetic acid were shaken under hydrogen at 3 atm. and 70° for twelve hours. The catalyst was filtered off and the acetic acid was removed *in vacuo*. The residue was recrystallized from methanol; yield, 900 mg.; m. p. 151°.

Anal. Calcd. for $C_{43}H_{66}O_{13}$: C, 65.3; H, 8.4. Calcd. for $C_{41}H_{64}O_{12}$: C, 65.7; H, 8.6. Found: C, 65.6; H, 8.6.

A mixture of 500 mg. of the reduced glucoside, 50 cc. of ethanol and 5 cc. of concentrated hydrochloric acid was refluxed for ninety minutes. The solution was poured into water and was extracted with ether. The ether layer was washed with water and then evaporated. The residue crystallized from methanol as tiny white leaflets, m. p. 165°, which showed no depression with dihydrotigogenin, m. p. 166°, prepared by the catalytic reduction of tigogenin.

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.4; H, 11.1. Found: C, 77.4; H, 11.1.

Catalytic Reduction of Trillin.—One gram of trillin, m. p. 283°, was dissolved in 100 cc. of methanol containing two drops of acetic acid and shaken under hydrogen at one atm. pressure for ninety minutes with 100 mg. of platinum oxide catalyst. It was crystallized from methanol and melted at 270°; yield, 900 mg.

Anal. Calcd. for $C_{33}H_{54}O_8 \cdot {}^1/_2H_2O$: C, 67.5; H, 9.4. Found: C, 67.3; H, 9.2.

A solution of 500 mg. of dihydrotrillin in 50 cc. of ethanol containing 6 cc. of concd. hydrochloric acid was refluxed for two hours. The solution was diluted with water and extracted with ether. The solvent was removed and the residue was crystallized from methanol to yield a substance, m. p. 204°, which did not depress the melting point of tigogenin, m. p. 204–205°.

The identity of the tigogenin was confirmed by oxidation to tigogenone. This melted at 207° when crystallized from methanol, and gave no depression with an authentic sample of tigogenone.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.2; H, 10.2. Found: C, 78.1; H, 10.1.

Summary

"Trillarigenin" was identified as a mixture of diosgenin and trillin.

STATE COLLEGE, PENNA. RECEIVED JULY 5, 1940