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

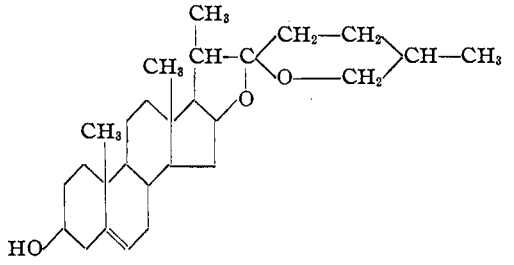
Sterols. CIV. Diosgenin from Certain American Plants

BY RUSSELL E. MARKER, D. L. TURNER AND PAUL R. ULSHAFER

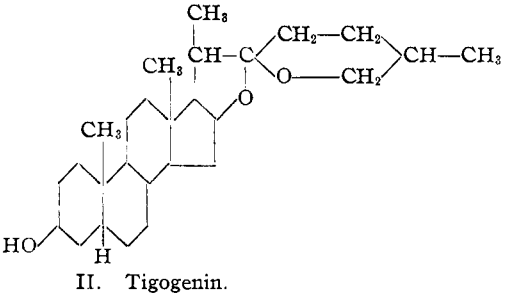
Diosgenin, isolated by Tsukamoto from the roots of *Dioscorea tokoro* (Makino), has been shown to be a steroidal sapogenin of structure I.[1,2,3,4] Recently sarsasapogenin was converted to progesterone.[5,6] An easier route to progesterone starts from diosgenin.[4] This suggested the desirability of finding an American source for diosgenin.

In the United States a single wild species of the *Dioscoreaceae* has been reported. This is *Dioscorea villosa* (L.). Other members of this family occur in Central and South America. Many plants of the genus *Dioscorea* are cultivated for ornamental use or for food.[7,8]

We have extracted the powdered rhizomes of *Dioscorea villosa* with a procedure similar to that used in the isolation of sarsasapogenin.[9] The genin was difficult to purify. Elementary analysis of the genin and of its acetate agreed with the formula of diosgenin. Mixed melting point determinations using a sample of diosgenin obtained from *Dioscorea tokoro* and kindly supplied by Professor Tsukamoto showed no depression. The acetates of the genin and diosgenin also showed no depression in mixed melting point.



I. Diosgenin.



II. Tigogenin.

By catalytic reduction tigogenin was obtained and identified by analysis and mixed melting point determinations with tigogenin from *Chlorogalum pomeridianum* bulbs,[10] and from diosgenin.[3] The acetates were also shown to be identical.

The occurrence of diosgenin is not confined, however, to the *Dioscoreaceae*. Reid[11] reported that the rhizomes of *Trillium erectum* (L.) (Liliaceae) contained a saponin. This was confirmed by other workers.[12]

We have now obtained diosgenin from the rhizomes of this plant. The yield was much higher than that obtained from *Dioscorea* roots. The crude material was also of greater purity. It was identified as diosgenin by the same method which was used for the sapogenin of *Dioscorea villosa*.

We have obtained none of the trillarigenin of Grove, Jenkins and Thompson[7] to which they gave the formula $C_{25}H_{39}O_4$.

We wish to thank Parke, Davis and Company for their generous assistance.

Experimental Part[13]

Isolation of Diosgenin from Trillium erectum.—The powdered root of *Trillium erectum* (L.) (25 pounds) purchased from S. B. Penick and Company was extracted with 9 gallons of ethanol. The extract on evaporation gave a brown gum which was dissolved in 4 liters of hot 85% ethanol and defatted with ligroin at 60–70°. After removal of the ligroin, 4 volumes of ether was added with stirring and the mixture allowed to stand overnight. The ether was decanted from the precipitated gum. The residual ether was distilled and the gum was dissolved in 4 liters of 20% ethanol and hydrolyzed by heating to 80°, adding 680 cc. of concentrated hydrochloric acid and keeping at this temperature for forty-five minutes. The mixture was stirred with a Hershberg stirrer. Foaming was prevented by the addition of a little capryl alcohol. The mixture was cooled rapidly by adding ice and after standing for a few hours the black precipitate was filtered, and washed with water. This material was dissolved in 1

(1) Tsukamoto, Ueno and Ohta, *J. Pharm. Soc., Japan*, **56**, 135 (1936).
(2) Tsukamoto, Ueno and Ohta, *ibid.*, **57**, 9 (1937).
(3) Tsukamoto, Ueno, Ohta and Tschesche, *ibid.*, **57**, 283 (1937).
(4) Marker, Tsukamoto and Turner, THIS JOURNAL, **62**, 2525 (1940).
(5) Marker and Rohrmann, *ibid.*, **62**, 518 (1940).
(6) Butenandt and Schmidt, *Ber.*, **67**, 1901 (1934).
(7) Bailey, "Manual of Cultivated Plants," 1924.
(8) R. A. Young, U. S. Dept. of Agriculture, Bulletin 1167 (1923).
(9) Jacobs and Simpson, *J. Biol. Chem.*, **105**, 501 (1934).
(10) Liang and Noller, THIS JOURNAL, **57**, 525 (1935).
(11) Reid, *Amer. J. Pharm.*, **64**, 69 (1892).
(12) Grove, Jenkins and Thompson, *J. Amer. Pharm. Assoc.*, **27**, 457 (1938).
(13) Microanalyses by Dr. George H. Fleming of this Laboratory.

liter of 95% ethanol, 100 cc. of concentrated hydrochloric acid was added and the mixture was refluxed for ninety minutes. It was cooled and the aglucone crystallized on standing a few hours. This was filtered and washed with 50% ethanol. It was light green in color. After crystallization from ethanol 90 g. of material melting at 192–200° was obtained. Material (30 g.) of the same melting point crystallized on concentrating the mother liquors to one-third volume.

This product was dissolved in an equal volume of acetic anhydride and refluxed thirty minutes. The colorless acetate was recrystallized from ethyl acetate and finally from acetone and melted at 199–200° with softening at 196°. The mixed m. p. with diosgenin acetate was 199–200°. The mixed m. p. with tigogenin acetate, m. p. 204°, was 183–195°.

Anal. Calcd. for $C_{29}H_{44}O_4$: C, 76.27; H, 9.71. Found: C, 76.44; H, 9.79.

By refluxing fifteen minutes with ethanolic potash the free genin was obtained, and crystallized from ethanol and then from acetone. It shrank slightly at 204° and melted at 206–208°. The mixture with diosgenin melting at 206–208° had m. p. 206–208° with shrinking at 204°.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.19. Found: C, 78.27; H, 10.29.

Reduction to Tigogenin.—A mixture of 1.5 g. of trillium genin acetate, 0.5 g. of Adams catalyst and 200 cc. of ether was shaken with hydrogen at 3 atm. at room temperature for ninety minutes. After filtering the catalyst, the product crystallized on concentrating the ether. It was washed with pentane; it crystallized from methyl alcohol as flat plates, m. p. 205–208°. The mixture with tigogenin acetate melted at 205–208°.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 75.91; H, 10.20.

Hydrolysis of the acetate by refluxing with ethanolic potash gave a product which crystallized from acetone as long needles, m. p. 207–208°. The mixed m. p. with tigogenin (m. p. 204–205°) was 205–208°. The mixed melting point with diosgenin was 192–200°.

Isolation of Diosgenin from Dioscorea villosa.—The procedure was identical with that described for *Trillium erectum;* yield of crude acetate, 73 g., m. p. 162–167° from 25 lb. of roots. By repeated recrystallization from ethyl acetate and then from acetone a product was obtained with m. p. 196–198°. It gave no depression in melting point when mixed with an authentic sample of diosgenin acetate.

Anal. Calcd. for $C_{29}H_{44}O_4$: C, 76.27; H, 9.71. Found: C, 76.09; H, 9.92.

The free genin was obtained by hydrolysis of the acetate. It crystallized from acetone as needles, m. p. 206–209°, and gave no depression in melting point when mixed with an authentic sample of diosgenin.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.19. Found: C, 77.95; H, 10.22.

The catalytic reduction to tigogenin was carried out as before using the genin acetate. The product was recrystallized from acetone, m. p. 196–199°. When mixed with tigogenin acetate there was no depression in m. p. Mixed with diosgenin acetate it melted at 173–195°.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 76.20; H, 9.89.

Hydrolysis gave a product which crystallized from acetone as needles, m. p. 206–208°. Mixed with tigogenin it showed no depression. When mixed with diosgenin the m. p. was 194–206°.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.64. Found: C, 78.10; H, 10.31.

Summary

Diosgenin has been isolated from *Dioscorea villosa* (L.) and from *Trillium erectum* (L.).

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Sterols. CV. The Preparation of Testosterone and Related Compounds from Sarsasapogenin and Diosgenin

BY RUSSELL E. MARKER

It has been shown that the steroidal sapogenins upon treatment with acetic anhydride at 200° are converted into pseudosapogenins,[1,2,3] which are readily oxidized to give Δ^{16}-20-keto-pregnane compounds. Because of the ready availability of the sapogenins and the high yields obtained, they make a very desirable starting material for the preparation of the steroidal hormones. The preparation of progesterone from sarsasapogenin and diosgenin already has been described.[1,2,3]

We now report one of the methods for the conversion of the steroidal sapogenins into testosterone and related compounds. Heretofore, these compounds have been generally prepared from cholesterol. It was shown that the action of persulfuric acid on *allo*-pregnanone-20[4] caused an oxidation between C-17 and C-20 to produce an acetoxy group at C-17, which apparently was a mixture of isomers under the conditions then used. We have now modified our original procedure, carrying out the reaction at room tempera-

(1) Marker and Rohrmann, THIS JOURNAL, **61**, 3592 (1939).
(2) Marker and Rohrmann, *ibid.*, **62**, 518 (1940).
(3) Marker, Tsukamoto and Turner, *ibid.*, **62**, 2525 (1940).
(4) Marker, Rohrmann, Wittle, Crooks and Jones, *ibid.*, **62**, 650 (1940).