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Saponins and Sapogenins. XI. Neotigogenin, a New Steroid Sapogenin

BY L. H. GOODSON AND C. R. NOLLER

During the course of the preparation of tigogenin acetate using acetic anhydride and pyridine,[1] a low melting fraction was accumulated which appeared to be a mixture of two or more components and could not be separated readily by repeated crystallization. When solutions of this fraction in ethyl alcohol were allowed to evaporate spontaneously, two types of crystals separated, a fine white powder and small regular octahedra. After mechanical separation it was found that the powder was chiefly tigogenin acetate. The octahedra were recrystallized from isopropyl alcohol and were found by melting point and mixed melting point to be a different compound. Analysis of this compound showed that it is isomeric with tigogenin acetate. Saponification of the new acetate gave a sapogenin which, although it has a melting point very close to that of tigogenin, produced a marked melting point depression when mixed with tigogenin. Oxidation of the sapogenin gave a ketone which was not identical with tigogenone[2] so the isomerism does not involve the carbon atom bearing the hydroxyl group. Moreover, the properties of the new compound and its derivatives are different from those of sarsasapogenin,[3] isosarsapogenin,[4] *epi*-sarsasapogenin[5] and smilagenin.[6] The compound does not appear to be related to tigogenin as isosarsasapogenin is related to sarsasapogenin[4] because tigogenin was practically unchanged by boiling with alcoholic hydrochloric acid. This conclusion is only tentative, however, because the tigogenin was altered partially to give a small amount of low melting material, but we have not yet succeeded in isolating the new compound from this altered tigogenin. Because the new sapogenin has been obtained in relatively small amounts and is difficult to isolate, we are unable to state at the present time whether it is one of the original hydrolysis products of the extract of *Chlorogalum pomeridianum* or is formed by isomerization during the acetylation. Because of the uncertainty concerning the seat of the isomerism we prefer to name the new compound "neotigogenin."

(1) Noller, Goodson and Synerholm, THIS JOURNAL, **61**, 1707 (1939) (paper X).
(2) Jacobs and Fleck, *J. Biol. Chem.*, **88**, 545 (1930).
(3) Jacobs and Simpson, *ibid*, **105**, 501 (1934).
(4) Marker and Rohrmann, THIS JOURNAL, **61**, 846 (1939).
(5) Marker and Rohrmann, *ibid.*, **61**, 943 (1939).
(6) Askew, Farmer and Kon, *J. Chem. Soc.*, 1399 (1936).

Experimental

Neotigogenin Acetate.—The acetylation of tigogenin, m. p. 198–203°, obtained from *Chlorogalum pomeridianum* was carried out at temperatures below 50° using acetic anhydride and pyridine. The yield of easily purified tigogenin acetate in this acetylation varied from 80–85%. The lower melting fractions which were more soluble in ethyl alcohol than tigogenin acetate were collected from several acetylations and combined. Fractional crystallization from ethyl alcohol yielded only a small amount of tigogenin acetate together with fractions melting over ranges of 10 to 30° and as low as 135°. Solutions of these low-melting fractions on spontaneous evaporation at room temperature over a period of weeks yielded two types of crystals, a white powder which appeared amorphous through a low-power microscope and small regular octahedra. The octahedra were separated mechanically and washed with isopropyl alcohol, when they melted at 172–174°. After one crystallization from isopropyl alcohol they melted at 174–176° and further crystallization from this solvent did not change the melting point. Approximately 40 mg. of neotigogenin acetate was obtained from the acetylation of 10 g. of tigogenin.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 76.10, 76.02; H, 10.21, 10.21.

A solution of 0.0414 g. in 3 cc. of chloroform gave $\alpha = -1.01°$ in a 1-dm. tube; $[\alpha]^{25}D$ −73.4°.

When this substance was mixed with tigogenin acetate, m. p. 202–208°, the mixture melted at 163.5–185°.

Neotigogenin.—A solution of 41 mg. of neotigogenin acetate, m. p. 174–176°, and 0.08 g. of potassium hydroxide in methyl alcohol was boiled under reflux for forty-five minutes and then a small quantity of water was added. On cooling needles separated which after recrystallization from 4 cc. of methyl alcohol weighed 35 mg. and melted at 200–203°. Further crystallization from methyl alcohol narrowed the melting point range to 202–203°. Neotigogenin when mixed with tigogenin, m. p. 206.5–209°, melted at 182–188° while a mixture with a sample of sarsasapogenin, m. p. 193.5–197.5°, melted below 140°. Neotigogenin separated with alcohol of crystallization and was dried for analysis at 135° and 2 mm.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.64. Found: C, 77.96; H, 10.45.

A solution of 0.0537 g. in 3 cc. of chloroform gave $\alpha = -1.16°$ in a 1-dm. tube; $[\alpha]^{26}D$ −64.9°.

Neotigogenone.—To a solution of 72 mg. of neotigogenin (alcohol-free) in 2 cc. of acetic acid at room temperature was added dropwise a solution of 0.012 g. of chromium trioxide in 2 cc. of 80% acetic acid. The mixture was allowed to stand until the solution was a light clear green in color. This was diluted with water and extracted with

ether. The ether solution was washed with 5% sodium carbonate solution and evaporated to dryness. The residue was boiled with 5 cc. of ligroin (b. p. 60–70°), the solution decanted and concentrated to approximately 1 cc. Cooling gave needles, m. p. 196–205°. After three recrystallizations from acetone platelets were obtained melting at 211–214°. Neotigogenone when mixed with tigogenone, m. p. 201–207°, melted at 191–205°.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.24; H, 9.90.

A solution of 0.0233 g. in 3 cc. of chloroform gave $\alpha = -0.47°$ in a 1-dm. tube; $[\alpha]^{25}D$ −60.6°.

Neotigogenone Oxime.—A mixture of 23 mg. of neotigogenone, m. p. 205–210°, with 0.04 g. of hydroxylamine hydrochloride, 0.46 g. of sodium acetate, 1 cc. of water and 4 cc. of ethyl alcohol was boiled for one hour. Water was added and the mixture was filtered, dried, and recrystallized by dissolving in 7 cc. of acetone, concentrating to 1.5 cc. and allowing to cool slowly. This gave 12 mg. of fine needles which melted with decomposition at 224.5°. Repeated recrystallization from acetone raised the melting point to 231–232° with decomposition.

Anal. Calcd. for $C_{27}H_{43}O_3N$: C, 75.48; H, 10.09; N, 3.25. Found: C, 75.20; H, 9.87; N, 3.33.

Attempted Isomerization of Tigogenin.—One gram of tigogenin, m. p. 200–202.5°, was dissolved in 100 cc. of 95% ethyl alcohol and this was added to a mixture of 66 cc. of 95% ethyl alcohol and 30 cc. of concentrated hydrochloric acid. After heating for thirty-six hours the mixture was concentrated, cooled and filtered. On fractional crystallization from ethyl alcohol almost all of the material was separated into fractions which melted close to the melting point of tigogenin and gave no depression when mixed with tigogenin. However, about 5 mg. of material melting from 189.5–194.5° was obtained from the mother liquors. This gave no Beilstein test for halogen and a mixture with tigogenin, m. p. 206.5–209°, melted at 193–206°.

Summary

A new steroid sapogenin has been obtained which is isomeric with tigogenin and has been named "neotigogenin." It has been isolated as the acetate and characterized by oxidation to a monoketone and conversion of the ketone into the oxime.

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Saponins and Sapogenins. XII. The Product of Direct Oxidation of Echinocystic Acid with Dichromic Acid

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When methyl echinocystate is oxidized with dichromic acid in glacial acetic acid solution, a diketo ester is formed which on saponification loses carbon dioxide to give a diketone, norechinocystenedione.[2] However, if free echinocystic acid is oxidized under the same conditions, one obtains instead of the expected norechinocystenedione an isomeric compound which was believed to be an isomeric diketone and was named "isonorechinocystenedione."

In the hope that a clue would be provided as to the difference in structure of these two compounds we have determined their ultraviolet absorption spectra (Figs. 2 and 3) as well as that of the monoketone, norechinocystenone (Fig. 1). All three compounds contain a carbon–carbon double bond and the nature of the absorption precludes the possibility that this is conjugated with a carbonyl group since such a structure would give an intense absorption band with a maximum near 2350 Å. (log E_m about 4.2) and a second band of lower intensity with a maximum near 3200 Å.[3] An α-diketone is excluded also since such a structure causes absorption in or near the visible region of the spectrum.

Norechinocystenone and norechinocystenedione gave typical carbonyl absorption bands in ethanol (Fig. 1 and Curve II, Fig. 2) with low-intensity maxima between 2900 and 3000 Å. This was not appreciably altered by the addition of alkali (Curve III, Fig. 2) or, in the case of the dione, by changing the solvent to ethyl ether (Curve I, Fig. 2). The second low-intensity maximum in the spectrum of the dione at 2450 to 2500 Å. cannot be attributed to simple carbonyl absorption and will be investigated further.

In the case of "isonorechinocystenedione" it was surprising to find no evidence of carbonyl absorption in ethyl ether (Curve I, Fig. 3). In absolute ethanol, however, it appeared as an inflection (Curve II) which developed in moist alcoholic sodium hydroxide solution into a clearly defined maximum at 2930 Å. (Curve III). This

(1) Commonwealth Fund Fellow.

(2) White and Noller, THIS JOURNAL, **61**, 983 (1939).

(3) Fieser, Fry and Jones, *ibid.*, **61**, 1849 (1939).