STEROID SAPOGENINS OF DIGITALIS CILIATA TRAUTV

E. P. Kemertelidze

Khimiya Prirodnykh Soedinenii, Vol. 1, No. 5, pp. 315-318, 1965

The first steroid saponin described in the literature, digitonin, was isolated by Schmiedeberg [1] from digitalin – a preparation of the seeds of Digitalis purpurea. Subsequently, Windaus and Schneckenburger [2] obtained a second saponin, which they called gitonin, from a commercial sample of digitonin of the firm of Merck and Co. Inc. Jacobs and Fleck [3] isolated a new sapogenin, tigogenin, from the leaves of D. purpurea. Tschesche [4] first obtained tigogenin and then the saponin corresponding to it, tigonin, from the leaves of D. lanata.

Tigogenin and gitogenin have been isolated from the leaves and seeds of D. purpurea, and also from the leaves of D. ferruginea and D. lutea [5-8]. Comparatively recently, new sapogenins, neodigitogenin [9] and digalogenin [10], have been found in a study of commercial digitogenin. In 1936, Szahlender [11] isolated crystalline digitonin from the seeds of D. lanata. This substance, which is the main saponin of the seeds of the foxglove, was not found in the leaves of this plant.

The present paper gives the results of an investigation of the steroid sapogenins of D. ciliata Trautv. (ciliated foxglove).

From the leaves and flowers of the ciliated foxglove we have isolated tigogenin and gitogenin, and from the seeds of this plant digitogenin and tigogenin.

Experimental

To obtain the sapogenins from the leaves and flowers of the ciliated foxglove, we used the method of direct hydrolysis of the saponins in the raw material proposed by Rothrock [12] and modified by L. Chetverikova and O. Madaeva [13]. From the seeds, which were first defatted, the saponins were extracted with methanol, and were then subjected to hydrolysis.

The separation of the mixture of unpurified sapogenins was carried out in both cases by adsorption chromatography on alumina.

In the investigation of the sapogenins by paper chromatography, we used a group I solvent system for the monohydroxysapogenins [isooctane – chloroform – acetic acid (100:4:1)] and a group II system for the polyhydroxysapogenins [the same solvents (100:40:4 or 100:10:2)]. Petroleum ether was sometimes used in place of isooctane. The sapogenins were detected on the paper chromatograms with Sannié's reagent [13, 14].

The compounds isolated were identified with authentic samples by mixed melting points, paper chromatography, and comparison of spectra.

The IR spectra of the sapogenins that we obtained from D. ciliata corresponded to the IR spectra of the acetates of tigogenin, gitogenin, and digitogenin given in [15].

Isolation of sapogenins from the leaves of D. ciliata. One hundred grams of the air-dry comminuted leaves was covered with 1 *l* of 2 N hydrochloric acid containing 8% butanol. The mixture was heated on a boiling water bath for 5 hours with constant stirring. After cooling, the liquid was filtered. The residue of plant material and sapogenins remaining on the filter was washed with water, neutralized with 5% sodium bicarbonate solution, and dried at 80°. This gave 29.5 g of hydrolyzate residue which was extracted with petroleum ether in a Soxhlet apparatus. The petroleum ether extract was concentrated to small bulk and left in the refrigerator. A greenish crystalline substance, the combined sapogenins, was precipitated (0.46 g). After the solvent had been distilled off from the mother liquor, 3.41 g of a dark-green oily mass was obtained.

The crystals and the mass obtained from the mother liquor had similar sapogenin compositions; they gave two spots on a chromatogram. From its R_f value (0.40), one spot in the dihydroxysapogenin region corresponded to gitogenin, and the other, in the region of the monohydroxysapogenins, appeared at the level of authentic tigogenin (R_f 0.62).

The mixture of unpurified sapogenins (0.43 g) was dissolved in 15 ml of benzene and was chromatographed on a column containing 15 g of alumina. The column was eluted with benzene, a mixture of benzene and ethyl ether, and finally methanol.

The benzene and the benzene/ether eluate gave 0.32 g of a yellowish crystalline residue. After recrystallization, 0.19 g of a white crystalline powder with mp 200-203° was obtained. On paper chromatography, the R_f of this substance coincided with the R_f of an authentic sample of tigogenin (0.62). A mixture with authentic tigogenin gave no

depression of the melting point, $[\alpha]_D^{20}$ −64.3° (c 0.77; chloroform).

Acetylation of 110 mg of the substance that we had isolated gave 89 mg of a monoacetate with mp 203-205°.

The properties of tigogenin are given in the literature as: mp 196-198°[8], 201-202° [14], 203-206° [17], and 205-207° [16]; $[\alpha]_D$ −59.4° [14], −67.2° [4], and −69° (chloroform) [17]. Mp of tigogenin acetate; 194-196° [8], 195-197° [14], 202-204° [17], 205-206° [16].

Found, %: C 75.64; H 9.22. Calculated for $C_{29}H_{46}O_4$, %: C 75.94; H 10.11.

The ether eluates gave very small amounts of residue. The residue from the distillation of the methanol eluates (0.025 g) was treated with carbon and recrystallized from methanol. This gave 0.011 g of needle-like crystals. On paper chromatography, this substance gave a single spot with R_f 0.41 at the level of a standard sample of gitogenin.

The substance isolated had mp 268-271° and gave an acetate with mp 241-242°.

The melting points of gitogenin and gitogenin acetate according to [18], are 272 and 243-244° respectively.

Thus, tigogenin (0.19%) and gitogenin (0.011%) have been isolated from the leaves of the ciliated foxglove. No digitogenin was found in the leaves.

As we have reported previously [19], steroid sapogenins, in particular, tigogenin, can also be obtained from an alcoholic aqueous extract of the leaves of the ciliated foxglove after the isolation of the cardiac glycosides from it.

In a similar way as from both the leaves and the flowers of this plant (after preliminary defatting), we obtained 0.055% of tigogenin and 0.009% of gitogenin.

Isolation of sapogenins from the seeds of D. ciliata Trautv. Three hundred grams of the air-dry comminuted seeds were extracted in a Soxhlet apparatus first with petroleum ether and then with chloroform. The defatted material was extracted with 80% methanol. The solvent was distilled off from the methanolic extracts. The resulting syrupy mass (49 g) was dissolved in 200 ml of methanol, and the solution was diluted with 140 ml of water and treated with 60 ml of concentrated hydrochloric acid (d 1.19). With continuous stirring, the mixture was heated on a boiling water bath for 5 hours. After cooling, the liquid was filtered. The residue was washed with water and was neutralized with 5% sodium bicarbonate solution. The aqueous alcoholic filtrate was concentrated until the methanol had been eliminated. The additional amount of precipitate separated was treated as described above and added to the first precipitate. After drying (at 80°), 5.57 g of a greyish black powder – the hydrolyzate residue – was obtained. To isolate the sapogenins, this mass was extracted in a Soxhlet apparatus first with petroleum ether and then with benzene. The petroleum-ether extracts gave 0.295 g of a yellowish crystalline residue, and the benzene extract gave 2.36 g of a yellowish-brown crystalline mass. The two residues had similar compositions. The chromatograms showed three spots: one in the region of the monohydroxysapogenins at the level of tigogenin, and two in the region of the polyhydroxysapogenins. Of these, one large spot had an R_f value corresponding to the R_f of digotogenin, and the other, weak, spot corresponded to the R_f value of gitogenin.

The mixture of unpurified sapogenins (2.65 g) obtained from the petroleum-ether and benzene extracts was dissolved in 50 ml of chloroform and passed through a column containing 100 g of alumina. The column was washed with pure chloroform and then with chloroform containing, successively, 1, 2, 5, and 10% of methanol.

Fractions 35-60, eluted with chloroform containing 1% of methanol, gave 0.56 g of a yellowish crystalline powder. This substance was dissolved in methanol and treated with carbon, and after recrystallization from methanol and then from acetone 0.19 g of white lustrous crystals was obtained. Mp 202°, $[\alpha]_D^{20}$ −70° (c 0.53; chloroform). On paper chromatography, this compound showed one spot at the level of a standard sample of tigogenin.

Found, %: C 78.00, 77.80; H 10.51, 10.59. Calculated for $C_{27}H_{44}O_3$, %: C 77.84; H 10.64.

The acetate of the genin obtained melted at 195-198°.

Fractions 70-105, eluted with chloroform containing 2% of methanol, gave a single spot with a R_f corresponding to the R_f of digitogenin. These fractions yielded 1.49 g of a yellowish crystalline residue. Treatment with carbon and recrystallization from methanol gave 0.85 g of snow-white needle-shaped crystals with mp 283°, $[\alpha]_D^{20}$ −80° (c 1; chloroform).

According to [4], the melting point of digitogenin is 280-283°, $[\alpha]_D$ −81° (in chloroform).

Found, %: C 72.40, 72.86; H 10.15, 9.82. Calculated for $C_{27}H_{44}O_5$, %: C 72.28; H 9.89.

The authentic samples of the sapogenins were given to us by O. S. Madaeva (VNIKhFI [All-Union Chemical and Pharmaceutical Scientific Research Institute]).
The IR spectra of the acetates of the sapogenins that we obtained were measured by colleagues of the physicochemical laboratory of VNKhFI.

Summary

Tigogenin and gitogenin have been isolated from the leaves and flowers, and digitogenin (0.283%) and tigogenin (0.062%) from the seeds of D. ciliata Trautv. The presence of gitogenin in the seeds has also been established by paper chromatography.

REFERENCES

1. O. Schmiedeberg, Archiv. exper. Pathol. Pharmacol., 3, 16, 1875.
2. A. Windaus and A. Schneckenburger, Ber., 46, 2628, 1913.
3. N. A. Jacobs and E. E. Fleck, J. Biol. Chem., 88, 545, 1930.
4. R. Tschesche and A. Hogedorn, Ber., 68, 1412, 1090, 1935; 69, 166, 1936.
5. F. Weiss and O. Manns, Pharmaz. Zentralhalle f. Deutschland, 98, 437, 1959.
6. M. Appel and O. Gisvold, J. Am. Pharm., Ass., 43, 215, 1954.
7. S. M. Schwartz and O. Gisvold, J. Am. Pharm. Ass., 46, 324, 1957.
8. L. Kier and O. Cisvold, J. Am. Pharm. Ass., 45, 58, 1956.
9. L. Klass, M. Fieser, and L. Fieser, J. Am. Chem. Soc., 77, 3829, 1955.
10. R. Tschesche and G. Wulff, Ber., 94, 2019, 1961.
11. K. Szahlender, Archiv. d. Pharmazie, 274, 446, 1936.
12. J. W. Rothrock, P. A. Hammes, and W. J. McAleer, Ind. Eng. Chem., 49, 186, 1957.
13. L. S. Chetverikova and O. S. Madaeva, Med. prom. SSSR, 8, 28, 1958.
14. Ch. Sannié, S. Heitz, and H. Lapin, C.R. Acad. Sci., 233, 1670, 1951; Bull. Soc. Chim., 1080, 1952.
15. C. R. Eddy, M. E. Wall, and M. K. Scott, Anal. Chem., 25, 266, 1953.
16. V. Černy and L. Labler, Coll., 24, 3468, 1959.
17. A. A. Dawidar and M. B. E. Fayez, J. Chem. UAR, 4, 101, 1961.
18. M. E. Wall, M. M. Krider, S. Rothman, and C. R. Eddy, J. Biol. Chem., 583, 198, 1952.
19. E. P. Kemertelidze, Sbornik trudov Tbilisskogo n.-i. khimiko-farmatsevticheskogo in-ta [Collection of papers from the Tiflis Scientific Research Chemical and Pharmaceutical Institute], 9, 47, 1960.

7 July 1965

Kutateladze Institute of Pharmaceutical Chemistry,
AS Georgian SSR