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SAPONINS OF DIOSCOREA XV. CAUCASOSAPONIN AND CAUCASOPROSAPOGENIN FROM D. CAUCASICA

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As has been stated previously [1], the preparation diosponin used for the treatment of arteriosclerosis is a mixture of saponins. The latter were isolated from the yams Dioscorea polystachya and D. caucasica growing in the USSR. The composition and structure of the saponins of the first plant have been reported previously [1, 2]. The present paper gives the results of a study of the saponins of D. caucasica.

The saponins of D. caucasica were isolated by the method worked out for D. polystachya [1]. These saponins consist mainly of glycosides of diosgenin with small amounts of glycosides of gito- and hecogenin.

When water-methanol extracts of the saponins are allowed to stand or when they are concentrated, water-insoluble saponins with R_f 0.6 and 0.7 (system 1) deposit, in the main, and water-soluble saponins with R_f 0.53 and 0.57, together with some water-insoluble saponins that can be extracted with butanol, remain in the filtrate. A pure water-soluble saponin the aglycone of which is solely diosgenin has R_f 0.53. It could be obtained by repeated precipitation of the mixture of water-soluble saponins with acetone from their solution in methanol. From its elemental composition, this saponin corresponds to a tetraoside of diosgenin having three molecules of glucose and one molecule of rhamnose in the sugar moiety. Acetylation gave a dodecaacetate. The compound that we isolated has not been described in the literature; we have called it caucasosaponin.

The saponin obtained is easily hydrolyzed; heating its solution above 50°C leads to a less polar prosapogenin. Chromatography of the saponin acetate on neutral alumina splits out acetylated sugars.

We have studied the hydrolysis of caucasosaponin under various conditions. When it is heated with water, partial hydrolysis takes place with the splitting out first of rhamnose and then of glucose. Boiling with 1.78% sulfuric acid for 1 hr 30 min leads to the formation of a prosapogenin identical with the water-insoluble saponin with R_f 0.6 isolated directly from an extract of the rhizomes of D. caucasica. The prosapogenin is a triglucoside of diosgenin, as was confirmed by the production of a decaacetate from it. The new saponin has been called caucasoprosapogenin. On being boiled with 1 N sulfuric acid, caucasosaponin gives a diosgenin diglucoside. This diglucoside is also obtained by boiling caucasoprosapogenin with 2 N hydrochloric acid in 50% ethanol.

It was impossible to obtain the pure water-insoluble saponins from their mixture (R_f ~ 0.6 and 0.7) by repeated recrystallization from alcohol. The best results were achieved by chromatographing a mixture of the acetates of the saponins on a column of magnesium trisilicate. The eluates collected when the column was eluted with benzene–methylene chloride (1:1) had R_f 0.75 (system 4). After deacetylation, a saponin with R_f 0.7 containing traces of caucasoprosapogenin was obtained. Appropriate purification of this saponin led to a compound with R_f 0.67 (system 1) and R_f 0.58 (system 2). From its R_f value and elementary composition, this substance was identical with gracillin [3]. When the amount of glucose and rhamnose in the carbohydrate moiety of the saponin (after its complete hydrolysis) was determined by titration (Hagedorn [4]), a ratio of 1.89:1 was obtained. This confirms that the saponin had the structure of gracillin. Further elution of the column with more polar solvents, a mixture of chloroform and methanol (9:1), led to the isolation of acetates with R_f 0.65 (system 5) the deacetylation of which gave a precipitate of caucasoprosapogenin with traces of gracillin. Purification with 80% methanol gave pure caucasoprosapogenin, the composition of which was identical with that of the triglucoside of diosgenin obtained by the hydrolysis of caucasosaponin.

Experimental

The following chromatographic methods were used [1]: for the separation of the saponins, ascending chromatography on a plate of paper pulp [5] with 1-butanol saturated with 5% acetic acid (2:1) as solvent in system 1, and on a plate of silica gel [6] with chloroform–methanol–water (65:35:10) as solvent [7] in system 2.

For the sapogenins we used descending paper chromatography with pyridine–butanol–water (2:3:1.5) as solvent in system 6; sugars in system 3, and the same solvents in a ratio of 100:40:4 in system 4.

For the acetates of the saponins we used descending paper chromatography with iso-octane–chloroform–acetic acid (100:30:3) as solvent in system 5.

The saponins and their acetates and sapogenins were revealed with Sannié's reagent [8]. For the sugars we used ascending chromatography on paper with the solvents pyridine–butanol–water (2:3:1.5) in system 6; the sugars were revealed by being sprayed with solutions of silver nitrate and caustic soda [9].

Isolation of the water-insoluble saponins and caucasosaponin. Seven hundred grams of the air-dry comminuted and chloroform-defatted rhizomes of D. caucasica was exhaustively extracted with 80% methanol in an apparatus of the Soxhlet type. After the methanol had been distilled off, 9.27 g of water-soluble saponins ($R_f \sim 0.6$ and 0.71, contaminated with water soluble saponins) deposited. The aqueous filtrate was made alkaline with ammonia and extracted with butanol. Concentration of this solution in vacuum at a temperature not above 50° C gave a colored precipitate of a mixture of water-soluble saponins (R_f 0.53 and 0.57), which were dried in vacuum and were then freed from colored and waxlike impurities by extraction with chloroform. A chromatograph of the saponins after their complete hydrolysis by boiling with 2.5 N hydrochloric acid in the presence of benzene for 5 hr showed that the hydrolysis products contained mainly diosgenin (R_f 0.63, system 3) contaminated with heco- and gitogenins (R_f 0.33 and 0.447, system 4). With heating, 3.78 g of the mixture of water-soluble saponins was dissolved in 17 ml of methanol, and the saponins were gradually precipitated from this solution with acetone. The first precipitates corresponded to caucasosaponin with R_f 0.53; they were the purest and gave only diosgenin on hydrolysis. The subsequent precipitates contained saponins of other aglycones as impurities.

Caucasosaponin is readily soluble in water and has mp 218–220° C (decomp.), $[\alpha]_D^{20}$ −62.35° (c 1.0; pyridine).

Found, %: C 55.49; H 7.86. Calculated for $C_{51}H_{82}O_{22}$, %: C 55.64; H 8.05.

After the complete hydrolysis of caucasosaponin, only the one spot of diosgenin was found on the chromatogram, with R_f 0.63 (system 3), while in the sugar fraction there was a large glucose spot (R_f 0.21) and a smaller rhamnose spot (R_f 0.44), system 6.

The acetate of caucasosaponin was obtained under the usual conditions by allowing it to stand with acetic anhydride in the presence of pyridine at room temperature for 48 hr. For purification, the acetate was dissolved in ether and precipitated with acetone, mp 126–128° C, $[\alpha]_D^{20}$ −34.97° (c 0.1; chloroform).

Found, %: C 57.98, 57.90; H 6.94, 6.76. Calculated for $C_{75}H_{106}O_{24}$, %: C 58.05; H 6.89.

Caucasoprosapogenin. A mixture of 0.12 g of purified caucasosaponin and 3 ml of 1.78% sulfuric acid was boiled for 1 hr 20 min. The white precipitate that deposited was filtered off, dissolved in methanol, and purified by reprecipitation with acetone, mp 243–247° C (decomp.). After hydrolysis only one spot appeared on a chromatogram of the sugar fraction, with R_f 0.21, which corresponds to glucose, $[\alpha]_D^{20}$ −50.35° (c 1.0; pyridine).

Found, %: C 58.25, 58.25; H 8.03, 7.83. Calculated for $C_{45}H_{72}O_{18} \cdot 1\ 1/2\ H_2O$, %: C 58.26; H 8.14.

The acetate was obtained under the conditions described above, mp 163–164° C, $[\alpha]_D^{20}$ −25.54° (c 1.0; chloroform).

Found, %: C 59.04, 59.09; H 6.95, 7.11. Calculated for $C_{65}H_{98}O_{28}$, %: C 59.06; H 7.02.

Diosgenin diglucoside. A. Hydrolysis of caucasosaponin. A mixture of 1.63 g of the water-soluble saponin and 50 ml of 1 N sulfuric acid in 50% ethanol was boiled for 2 hr 30 min. The acidic water–ethanol solution was diluted with water, the sapogenins were extracted with benzene, and the saponins from the aqueous layer were extracted with butanol. After neutralization, the butanol extract was evaporated in vacuum, the residue (1.16 g) was acetylated, and the acetates were chromatographed on a column of 18 g of neutral alumina and eluted with 20-ml portions of benzene. The first eluates gave 0.85 g of a colored residue which was dissolved in carbon tetrachloride and was transferred to another column containing 13 g of alumina. The first eluates, with R_f 0.75 (system 4), were collected and saponified with ethanolic caustic potash, forming a water-soluble precipitate. On recrystallization from methanol, the diosgenin diglucoside precipitated in the form of clusters of needles, mp 262–264° C (decomp.), R_f 0.80 (system 1). The precipitate was dried at 105° C and then in vacuum at 10^{-2} mm for 5 hr.

Found, %: C 61.68; H 8.46. Calculated for $C_{39}H_{62}O_{13} \cdot H_2O$, %: C 61.86; H 8.52.

The acetate of the diosgenin diglucoside had mp 219–221° C.

Found, %: C 61.48; H 7.38. Calculated for $C_{51}H_{76}O_{20}$, %: C 61.59; H 7.41.

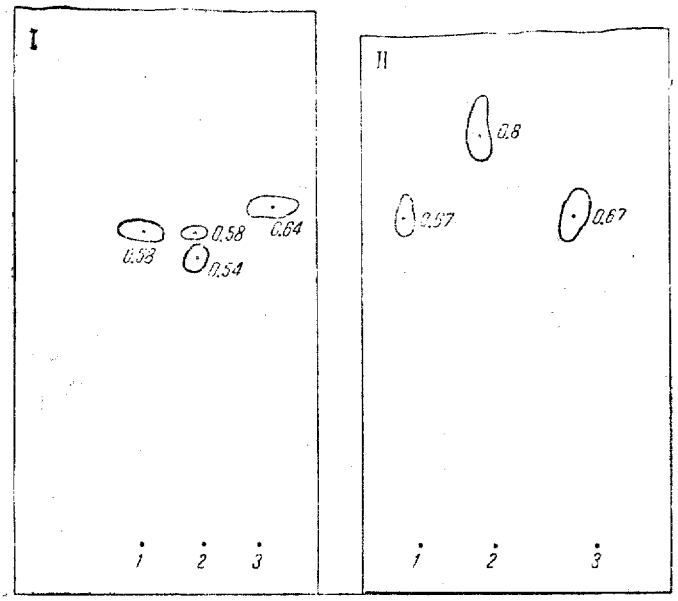
B. Hydrolysis of caucasoprosapogenin. 0.58 g of caucasoprosapogenin containing a small amount of saponin (R_f 0.7) was boiled with 2 N hydrochloric acid in 50% ethanol for 5 hr in the presence of benzene. A precipitate of 0.22 g of diosgenin diglucoside was obtained. It was recrystallized from aqueous butanol and dried at 110° C in a vacuum of 10^{-2} mm for 10 hr, mp 262–264° C (decomp.).

Found, %: C 63.42; H 8.67. Calculated for $C_{39}H_{62}O_{13}$, %: C 63.41; H 8.46.

The acetate, with R_f 0.75 (system 4) and mp 223–224° C, was obtained under the usual conditions.

Gracillin. Two grams of the mixture of water-soluble saponins ($R_f \sim 0.6$ and 0.7) was acetylated under the usual conditions. 1.56 g of the dried precipitates of acetates was dissolved in a mixture of benzene and methylene chloride

(1:1) and chromatographed on a column containing 2.27 g of magnesium trisilicate. The elution of this mixture gave 0.68 g of substance. Deacetylation of the eluates with R_f 0.75 (system 4) by boiling them with 5% methanolic caustic potash for 45 min gave a compound with R_f 0.7 containing slight traces of an impurity (R_f 0.6). When the product was



Chromatogram of the water-insoluble saponin from D. caucasica. I) Silica gel plate, system 2: 1) Gracillin; 2) water-insoluble saponins from D. caucasica; 3) diosgenin diglucoside. II) Paper pulp plate, system 1: 1) Gracillin; 2) diosgenin diglucoside; 3) water-insoluble saponin from D. caucasica.

boiled with 80% methanol, the product obtained did not all pass into solution. The methanolic solution was filtered and evaporated to dryness, and the residue was recrystallized from 50% acetic acid. The crystals which deposited in the form of clusters of thin needles with R_f 0.67 (in system 1) and R_f 0.58 (in system 2) were identical with gracillin (figure). After the complete hydrolysis of the sample of gracillin obtained with 2 N hydrochloric acid in 50% ethanol for 5 hr, the sugar fraction gave, by Hagedorn titration [4], 20 γ of glucose and 8.79 γ of rhamnose. The molecular ratio of glucose and rhamnose was 1.89:1. The melting point after drying in vacuum at 106° C and 10^{-2} mm for 5 hr was 281–282° C (decomp.).

Found, %: C 59.84; H 8.02. Calculated for $C_{45}H_{72}O_{17} \cdot H_2O$, %: C 59.86; H 8.25.

After additional drying for 5 hr:

Found, %: C 60.68; H 8.15. Calculated for $C_{45}H_{72}O_{17} \cdot 0.5\ H_2O$, %: C 60.47; H 8.22.

Isolation of caucasoprosapogenin from the mixture of water-insoluble saponins. Subsequent elution of the column with a mixture of chloroform and methanol (9:1) gave eluates of acetates with $R_f \sim 0.65$ (system 4). After deacetylation, the precipitate was recrystallized from 80% methanol and dried in vacuum at 100° C and 10^{-2} mm for 5 hr. The caucasoprosapogenin obtained had mp 267–267.5° C.

Found, %: C 58.70; H 7.95. Calculated for $C_{45}H_{72}O_{18} \cdot H_2O$, %: C 58.82; H 8.11.

Summary

Two new saponins have been isolated from the rhizomes of D. caucasica: the water-soluble caucasosaponin—a rhamnosotriglucoside of 25 D-22α-spirost-22α-en-3β-ol—and the water insoluble caucasoprosapogenin—a glucosotrioside of 25 D-22α-spirost-5-en-3β-ol—, and also the previously known rhamnosidodiglucoside of 25 D-22α-spirost-5-en-3β-ol—gracillin.

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