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TRITERPENE GLYCOSIDES FROM SAPONARIA OFFICINALIS

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Only fragmentary information is available on the chemical composition of Saponaria officinalis L. (bouncingbet, fuller's herb). Thus, in 1906 Barger isolated from young shoots of bouncingbet a flavone glycoside saponarin $C_{21}H_{24}O_{12}$ decomposing on hydrolysis into glucose and the aglycone vitexin $C_{15}H_{14}O_7$ [1]. Kon and Soper, on heating a methanolic extract of the roots of this plant with dilute hydrochloric acid, obtained and identified gypsogenin $C_{30}H_{46}O_4$ [2]. There is information in the literature [3] that bouncingbet contains about 20% of a mixture of saponins, but we have found no reports of the isolation of individual triterpene glycosides.

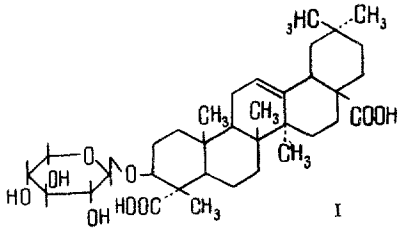
We have investigated the roots of this plant collected in the summer of 1967 in the Elabuzh region of the Tatar ASSR. The air-dry roots, previously defatted with chloroform, were extracted with boiling methanol to exhaustion. The yield of extractive substances was 25% of the weight of the roots. A preliminary investigation of the the composition of the extract by thin-layer chromatography on silica gel showed the presence of two substances giving a blue-violet coloration with antimony trichloride.

To isolate the less polar component A, the methanolic extract was dissolved in water, the solution was extracted with ethyl acetate, and the resulting extract was chromatographed on EDE-10P ion-exchange resin. The glycoside obtained, which we have called saponaroside is a colorless crystalline compound with mp 220–222° C. Yield 1% (of the weight of the extract). The retention of saponaroside on a basic ion-exchange resin, and also the presence in the IR spectrum of an absorption band in the 1700 cm^{-1} region showed that it has a free carboxyl group. When it was heated with mineral acids, the glycoside was split into xylose and a triterpene aglycone which, according to Kon and Soper [2], must be gypsogenin. However, the aglycone isolated had constants differing from those of gypsogenin; its IR spectrum lacked the absorption band at 1725 cm^{-1} characteristic for the aldehyde group, and its chromatographic behavior was completely different. The results of the elementary analysis of the compound and some of its derivatives agreed best with the empirical formula $C_{30}H_{46}O_5$, and on titration it was found that the substance contains two carboxyl groups. All this made it possible to assume that the aglycone is gypsogenic acid. A comparison with an authentic sample (kindly supplied by V. N. Belous and A. A. Ryabinin) showed their complete identity.

The fact that gypsogenic acid occurs in nature has recently been reported by V. N. Belous and A. A. Ryabinin [4] but we are apparently the first to have isolated a glycoside with this aglycone.

The titration of saponaroside (taking into account the presence of two free carboxyl groups) have a molecular weight of 620, which corresponds to monoside. The results of methylation confirmed this conclusion, since on hydrolysis of the completely methylated saponaroside only 2,3,4-trimethyl-D-xylose was found besides dimethyl gypsogenate.

The information above, and also a calculation of the configuration of the glycosidic centers by Klyne's method permits structural formula I to be proposed for saponaroside



I

For the isolation of glycoside B, the methanolic extract of the roots was first extracted with ethyl acetate and then with butan-1-ol and the butanol extract was chromatographed on EDE-10-P ion-exchange resin. The acid fraction obtained (yield 2% of the weight of the roots) was purified further by partition chromatography on silica gel. The glycoside, isolated in the form of an amorphous white powder, gave on hydrolytic cleavage an aglycone which was identical with an authentic sample of gypsogenin in respect of its constants, IR spectrum, and chromatographic behavior. D-Glucose, D-galactose, D-xylose, D-fucose, L-rhamnose, and L-arabinose were identified in the hydrolyzate by paper chromatography. On being heated with dilute alkali, the glycoside was converted into a less polar product cleaved by acids into gypsogenin, D-glucose, D-galactose, and L-arabinose. The fully methylated glycoside B, obtained by Kuhn's method, was cleaved by acids with the formation of 3-methyl-L-rhamnose, 2,4-dimethyl-D-xylose, 2,4-dimethyl-D-fucose,

2,3,4-trimethyl-L-arabinose, 2,3,6-trimethyl-D-glucose, 2,3,4,6-tetramethyl-D-glucose, 2,3,4-trimethyl-D-xylose, and methyl 2-methyl-D-glucuronate. On the basis of the reactions described above and its chromatographic behavior, the glycoside B is identical with the known gypsoside [5].

Experimental

Leningrad M paper and KSK silica gel were used for chromatography with the following solvent systems: 1) butan-1-ol–acetic acid–water (4:1:5), 2) butan-1-ol saturated with water, 3) benzene–ether (4:1), 4) benzene-butan-1-ol–pyridine–water (10:50:30:30), 5) butan-1-ol–ethanol–water (5:1:4), and 6) butan-1-ol–ethanol–water (40:11:19).

Extraction of the roots of the plant. The air-dry roots of Saponaria officinalis L. (1.8 kg), previously defatted with chloroform, were exhaustively extracted with boiling methanol. The residue obtained after the distillation of the solvent (450 g) was dissolved in 2 *l* of water and the solution was extracted successively with ethyl acetate and butan-1-ol (yields 15 and 35 g, respectively).

Saponaroside. The ethyl acetate fraction was filtered through a small layer (3–4 cm) of alumina and was deposited on EDE-10-P ion-exchange resin in a ratio of 1:35. The column was washed successively with 5% aqueous ethanol and a 10% aqueous methanolic solution of acetic acid. The fractions collected were monitored by thin-layer chromatography on silica gel in system 2. The fractions eluted by the second solvent gave, after recrystallization from 90% ethanol, saponaroside with mp 220–222° C, $[\alpha]_D^{20}$ − 9° (c 1.2; pyridine).

Found, %: C 66.03, 66.15; H 8.52, 8.66. Calculated for $C_{35}H_{54}O_9 \cdot H_2O$, %: C 66.03; H 8.80; mol. wt. 625, 637, (by titration).

Saponaroside acetate. A solution of 0.15 g of the glycoside in a mixture of 3 ml of pyridine and 1 ml of acetic anhydride was kept at room temperature for two days. Then it was poured into ice water and the precipitate was filtered off, deposited on a column of silica gel (1:20), and eluted with chloroform–ethyl acetate (4:1). After recrystallization from 60% aqueous ethanol, an acetate with mp 159–160° C, $[\alpha]_D^{20}$ −13° (c, 1.8; chloroform) was obtained.

Found, %: C 64.31, 64.55; H 7.95, 7.80; Calculated for $C_{41}H_{60}O_{12} \cdot H_2O$, %: C 64.68; H 8.13.

Hydrolysis of saponaroside. A mixture of 0.65 g of the substance and 35 ml of a 5% aqueous methanolic solution of hydrochloric acid was boiled in the water bath for 6 hr. After the distillation of the methanol, the precipitate that had deposited was filtered off, washed with water, dried, transferred to a column of silica gel (1:20), and eluted successively with 50-ml portions of benzene, ether, and butan-1-ol. The fractions were monitored by thin-layer chromatography on silica gel in system 3. The fractions eluted by ether (0.12 g) after recrystallization from 60% ethanol and drying in vacuum, had mp 337–338° C (melting point of a mixture with a sample of gypsogenic acid 345–350° C), $[\alpha]_D^{20}$ +70° ± 3 (c 1.7; ethanol).

IR spectrum: 1705 cm^{-1} (—COOH), 3440 cm^{-1} (—OH). After hydrolysis the filtrate was neutralized with AV-17 resin and evaporated to small volume. Paper chromatography in systems 1, 4, and 5 showed the identity of the sugar obtained with D-xylose.

Found, %: C 71.69, 71.89; H 9.54, 9.98. Calculated for $C_{30}H_{46}O_5 \cdot H_2O$, %: C 71.42; H 9.52.

Methylated saponaroside. A mixture of 0.15 g of the compound, 1.5 g of BaO, 8 ml of dimethylformamide, and 9 ml of methyl iodide was heated in a sealed tube in the water bath for 6 hr. Then the tube was opened, 0.5 g of BaO, 3 ml of dimethylformamide, and 3 ml of methyl iodide was added, and the mixture was heated for another 6 hr. The reaction mixture was poured into a saturated solution of sodium thiosulfate and extracted with chloroform (5 × 50 ml), and then the solvent was distilled off and the residue was transferred to a column of silica gel (1:10). The fully methylated compound (0.125 g), which was eluted with chloroform, consisted of an amorphous powder with decomp. p. 66–68° C, $[\alpha]_D^{20}$ −4° (c 2.5; chloroform). The IR spectrum lacked absorption bands in the OH group region.

Found, %: C 69.76, 69.36; H 9.45, 9.45. Calculated for $C_{40}C_{64}O_9$, %: C 69.76; H 9.33.

Methanolysis and hydrolysis of methylated saponaroside. A solution of 0.25 g of methylated saponaroside in 12 ml of a 3% methanolic solution of HCl was heated in the water bath for 9 hr. Then it was diluted twofold with water and the mixture was heated for another 1 hr. The precipitate (0.17 g) was filtered off, transferred to a column of silica gel (1:10), and eluted with ether. After recrystallization from 60% ethanol, dimethyl gypsogenate with mp 228–229° C, $[\alpha]_D^{20}$ +76° (c 1.1; chloroform), was obtained.

The filtrate after hydrolysis was neutralized with AV-17 ion-exchange resin and evaporated to dryness, giving a syrup of 2,3,4-trimethyl-D-xylose, $[\alpha]_D^{20}$ +50° (c 1.0; chloroform). Literature data [6]; $[\alpha]_D$ +55.8° (chloroform). It was identical with an authentic sample in its chromatographic behavior on paper.

Found, %: C 74.64; H 9.91. Calculated for $C_{32}H_{50}O_5$, %: C 74.67; H 9.97.

Glycoside B. A methanolic extract of the roots previously extracted with ethyl acetate and butan-1-ol, amounting to 180 g, was transferred to a column of EDE-10P anion-exchanger in the OH form (600 g) and eluted first with water and then with a 10% aqueous methanolic solution of acetic acid. The fraction eluted by the latter solvent (30 g) was transferred to a column of silica gel (1 : 10) and then the column was first washed with system 2, after which the substance was eluted with system 1 and was recrystallized from the same solvent. This gave 25 g of the glycoside B with mp 242–246° C, $[\alpha]_D$ −20° (c 2.6; water). Mol. wt. 1840 (by tritration).

Acetate of glycoside B. This was obtained by the method described above, mp. 175–176° C, $[\alpha]_D^{20}$ −23 ± 3° (c 2; chloroform).

Found, %: C 55.33, 55.31; H 6.57, 6.58. Calculated for $C_{122}H_{168}O_{65}$, %: C 54.83; H 6.26.

Hydrolysis of the glycoside B. A) A mixture of 0.5 g of the glycoside and 50 ml of 5% HCl was heated in the water bath for 6 h. The precipitate that deposited (0.15 g) was filtered off, dried over P_2O_5, transferred to a column of silica gel (2 g), and eluted with system 3. After recrystallization from 80% ethanol, gypsogenin was obtained with 265° C, $[\alpha]_D^{20}$ +85 ±3° (c 1.1; ethanol).

Found, %: C 74.69, 73.41; H 9.48, 9.43. Calculated for $C_{30}H_{46}O_4 \cdot H_2O$, %: C 73.72, H 9.83.

The filtrate after hydrolysis was neutralized with AV-17 ion-exchange resin and evaporated to small volume. By paper chromatography in systems 1, 4, and 5, the sugars contained in it were shown to be identical with authentic samples of D-glucose, D-galactose, D-xylose, D-fucose, L-rhamnose, and L-arabinose.

B) A mixture of 0.3 g of the glycoside and 30 ml of 5% caustic potash solution was heated in a sealed tube at 90° C for 6 hr. The reaction mixture was neutralized with KU-2 ion-exchange resin and evaporated to dryness, and the residue (0.13 g) was transferred to a column of silica gel and eluted with system 2. The process was monitored by thin-layer chromatography on silica gel. The fractions free from sugars were hydrolyzed with 5% HCl as described above. This gave gypsogenin, and in the hydrolysate D-glucose, D-galactose, and L-arabinose were identified with reference samples.

Methylated glycoside B. A mixture of 2 g of the glycoside, 35 ml of dimethylformamide, and 11 g of barium oxide was heated to 100° C with vigorous stirring for 4 hr. Then 12 ml of methyl iodide and 4 g of barium oxide were added and stirring and heating were continued for another 2 hr. The reaction mixture was poured into water and exhaustively extracted with chloroform, and the extract was washed with sodium thiosulfate solution and with water and was evaporated to dryness. The residue (1.4 g) was transferred to a column of silica gel (30 g) and eluted with chloroform containing 5% of ethanol. This gave the fully methylated glycoside B in the form of an amorphous powder with decomp. p. 137–140° C. The IR spectrum lacked absorption bands in the region of free hydroxyl groups. A solution of 0.2 g of the product obtained in 20 ml of 3% HCl in methanol was heated in the water bath for 5 hr. Then the reaction mixture was diluted twofold with water and was heated for another 2 hr. After neutralization with EDE-10P ion-exchange resin, the hydrolyzate was shown by paper chromatography in systems 5 and 6 to contain 3-methyl-L-rhamnose, 2,4-dimethyl-D-xylose, 2,4-dimethyl-D-fucose, 2,3,4-trimethyl-L-arabinose, 2,3,4,6-tetramethyl-D-galactose, 2,3,6-trimethyl-D-glucose, 2,3,4-trimethyl-D-xylose, and methyl 2-methyl-D-glucuronate.

Conclusions

The roots of Saponaria officinalis L. have yielded the known triterpene glycoside gypsoside and the first glycoside of gypsogenic acid, which has been named saponaroside. Saponaroside is the 3-β-D-xylopyranoside of gypsogenic acid.

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