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STRUCTURE OF TRITERPENE SAPONINS FROM THE ROOTS OF GLYCYRRHIZA ECHINATA

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From the roots of Glycyrrhiza echinata L., family Leguminosae, we have isolated two individual saponins which we have called saponin A and saponin B. Their aglycones are triterpene acids: from saponin A, macedonic acid $C_{30}H_{46}O_4$, and from saponin B, echinatic acid $C_{30}H_{46}O_5$ [1]. The carbohydrate moiety of both saponins consists of L-rhamnose and D-glucuronic acid [2].

Molecular weights of the saponins were determined spectrophotometrically using the property of triterpene saponins of giving, in concentrated sulfuric acid, an intense red coloration changing to dark violet. In this reaction sugars, amino acids, and uronic acids do not affect the intensity of the color [3]. The optical density of the colored solutions was measured in an SF-4A spectrophotometer at a wavelength of 310 mμ. It was found that the molecular weights are within the following ranges: saponin A 785.5-828.5, and saponin B 807.5-837.5.

We have also performed the stepwise hydrolysis of the saponins with 2 N hydrochloric acid. The hydrolysis products were studied by paper chromatography, and it was found that in both saponins the D-glucuronic acid was split off first and then the L-rhamnose.

The amount of monosaccharides attached to the aglycones of saponins A and B was determined chromatographically by G. N. Zaitseva and T. P. Afanas'eva's method [4]. The molar ratios of L-rhamnose and D-glucuronic acid found in the saponins studied were 1 : 1. This enabled the molecular weights of the two saponins to be determined accurately (793 and 809, respectively), which is in agreement with the results of experiment for these saponins and shows that they are both biosides.

Experimental

Chromatography was carried out on papers of types B and M of the Volodarskii Leningrad mill. The following systems of solvents were used: 1) 1-butanol—benzene—pyridine—water (5 : 1 : 3 : 3), and 2) 1-butanol—acetic acid—water (4 : 1 : 5). The sugars were detected with p-anisidine and aniline phthalate.

Determination of the molecular weights of the saponins. Saponin A) A weighed sample (0.02 g) of saponin A was placed in a 50-ml measuring flask. A weighed sample (0.02 g) of the aglycone of saponin A was placed in a flask of the same capacity. To each flask was added 30 ml of concentrated sulfuric acid and they were shaken until the substances had dissolved completely. The solutions in both flasks were heated in the water bath at 60° C for 30 min. One-milliliter portions were transferred from each of the initial solutions to a 50-ml measuring flask and solutions of the substances in the new dilutions were obtained with concentrated sulfuric acid. The optical densities of the colored solutions were determined at a wavelength of 310 mμ (layer thickness in the cells 10 mm relative to pure concentrated sulfuric acid).

Table 1

Substance	Optical density of the solution		Calculated content of aglycone in the solution of saponin taken, %	Adjusted to molecular weight
	saponin	aglycone		
Saponin A	0.526	0.907	58.16	814.5
Saponin B	0.547	0.929	58.94	824.9

Table 2

Substance	Optical density of the solution		Molar ratio
	glucuronic acid	rhamnose	
Saponin A	0.405	0.439	1 : 0.93
Saponin B	0.392	0.397	1 : 0.90

Saponin B) The molecular weight was determined as described for saponin A. The results of the determinations of the molecular weights of both saponins are give in Table 1 (mean of five determinations).

Stepwise acid hydrolysis of the saponins. Saponin A) A mixture of 0.1 g of the substance and 20 ml of 1 N hydrochloric acid, and a little methanol (to dissolve the solid matter) was heated in the boiling water bath for 6 hr. Samples for analysis were taken every 10 min and were chromatographed in system 2. Glucuronic acid was found in the hydrolysate after 60 min and rhamnose after 4 hr.

Saponin B) Hydrolysis was carried out as described for saponin A. Glucuronic acid appeared in the hydrolysate after 60 min and rhamnose after 4.5 hr.

Quantitative determination of the monosaccharides in the saponins. Saponin A) A weighed sample of saponin A (0.01 g) was heated with 20 ml of 2 N hydrochloric acid and 10 ml of methanol in the boiling water bath for 8 hr and was then neutralized with barium carbonate. The precipitate of the aglycone of the saponin was filtered off, and the aqueous solution was evaporated in vacuum and chromatographed by the descending method in system 1. In addition to the saponin hydrolysate, 0.02-ml portions of 1% solutions of mixtures of rhamnose and glucuronic acid in molar ratios of 1 : 1, 1 : 2, and 2 : 1 were deposited on the chromatogram. The standard mixtures of monosaccharides were previously treated with 2 N hydrochloric acid under the same conditions as those under which the hydrolysis of the saponin was carried out.

The mixture of solvents was passed through the chromatogram three times (every 24 hr), and the chromatogram was then treated with active aniline phthalate and dried. The colored sections of the chromatogram with the spots of the sugars and the background not containing spots of monosaccharides were cut out in the form of squares of the same area (4 × 4 cm). Each was eluted in 5 ml of acetic acid for 3 days at room temperature. The optical densities of the eluates were measured at a wavelength of 413 mμ in comparison with the eluate of the background.

Saponin B) The analysis was carried out as described for saponin A. The molar ratios of the saccharides found in both saponins are given in Table 2 (mean of five determinations).

Conclusions

On the basis of a physicochemical study of the saponins of Glycyrrhiza echinata L., their molecular weights have been determined. It has been found that the two saponins are biosides of macedonic acid (saponin A) and echinatic acid (saponin B), the carbohydrate moieties of which contain L-rhamnose and D-glucuronic acid.

In both saponins, the L-rhamnose is bound directly to the aglycones.

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