STRUCTURE OF DIPSACOSIDE B – A TRITERPENE GLYCOSIDE FROM Dipsacus azureus

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As reported previously [1], dipsacoside B – the main glycoside of the roots of Dipsacus azureus Schrenk. (family Dipsacaceae) – is an acyloside [2]. It consists of hederagenin, L-arabinose, L-rhamnose, and D-glucose. The present paper gives information showing the structure of this glycoside.

Since dipsacoside was obtained only in the amorphous form, containing water and a small amount of ash residue, the results of elementary analysis of the glycoside were unreliable. The results of a

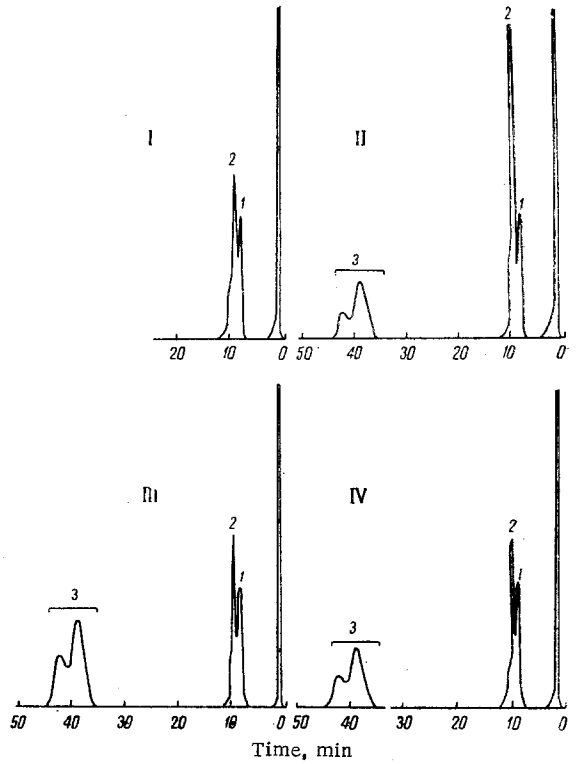

Fig. 1. Chromatogram of the trimethylsilyl ethers of methyl saccharides of kalopanax saponin A (I), kalopanax saponin B (II), leontoside D (III), and dipsacoside B (IV): 1) L-arabinose; 2) L-rhamnose; 3) D-glucose.

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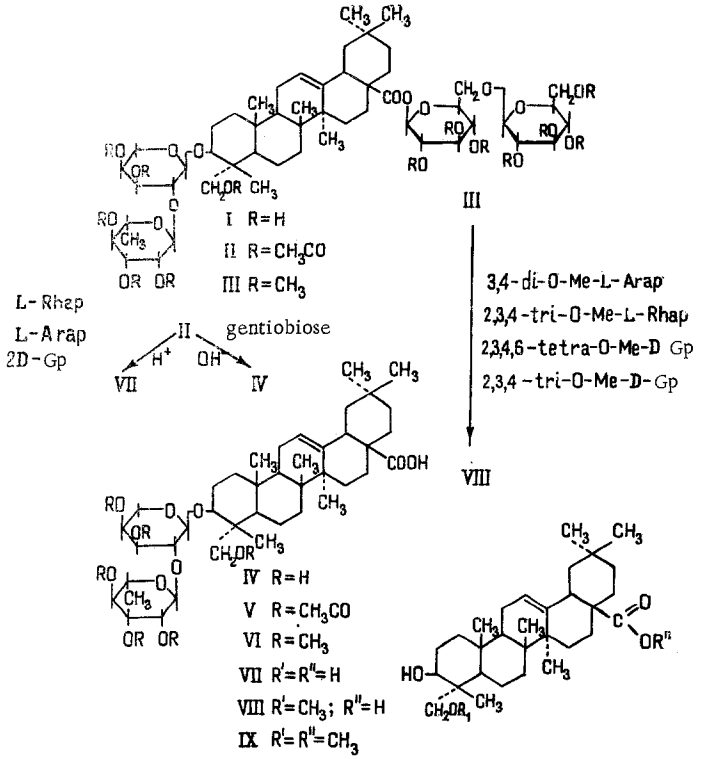
quantitative determination of the sugars by the aniline phthalate method also proved to be contradictory. The glycoside contains one molecule each of L-rhamnose and L-arabinose, but the amount of D-glucose could not be determined quantitatively: it could be two or three molecules.

Unlike the glycoside, the acetyl derivative, after purification by repeated reprecipitation from benzene with petroleum ether, proved to be fairly uniform. Consequently, the work on the determination of the structure of the glycoside was carried out mainly with the acetyl derivative.

In recent investigations on the structure of triterpene glycosides we have used the gas-chromatographic method of determining sugars. The sugars are determined in the form of the trimethylsilyl derivatives of their methyl glycosides [3-5].

Figure 1 gives comparative chromatograms of four related hederagenin glycosides. Leontoside D and dipsacoside B were analyzed in the form of the acetates. All the compounds contain one molecule each of L-arabinose. Kalopanax saponin A contains two sugars – L-arabinose and L-rhamnose – present in equimolecular proportions. Kalopanax saponin B contains five sugar molecules: two L-rhamnose, two D-glucose, and one L-arabinose. The chromatogram of the glycoside clearly reflects the quantitative ratio of the monosaccharides. Leontoside D is also a pentaoside, but here the ratio of L-rhamnose, D-glucose, and L-arabinose is different: 1 : 3 : 1. The changed proportion is reflected in the relative sizes of the individual peaks. The gas-liquid chromatography of the trimethylsilyl ethers methylated at the acetal hydroxyl of the carbohydrates present in dipsacoside B showed that the glycoside is a tetraoside that contains L-rhamnose, L-arabinose, and two molecules of D-glucose (1.05 : 0.90 : 2.00). The elementary analysis of the acetate and its molecular weight, determined spectrophotometrically [6], correspond to the empirical formula $C_{79}H_{112}O_{35}$. Consequently, the glycoside has the composition $C_{53}H_{86}O_{22}$.



The structure of dipsacoside B (I) was established on the basis of the following reactions. The hydrolysis of the glycoside previously treated with diazomethane gave hederagenin (VII) and not its methyl ester, which confirms the presence of a carbohydrate chain attached to the carboxy group of the genin. The acetate of the glycoside (II) was subjected to alkaline hydrolysis on Dowex-1 anion-exchange resin (OH form) [7]. At the end of the hydrolysis, a mixture of sugars consisting of gentiobiose and D-glucose was first eluted from the column and then, by changing the charge on the anion-exchange resin, a substance with a glycosidic nature (IV). This was identical with dipsacoside A, which is found as such in the plant [1]. The glycoside IV was exhaustively methylated. After the methanolysis of the methylation product

(VI), the methyl ester of 23-O-methylhederagenin (IX), 3, 4-di-O-methyl-L-arabinose, and 2, 3, 4-tri-O-methyl-L-rhamnose were isolated. The same methylated sugars were obtained in the hydrolysis of kalopanax saponin A – a glycoside from Kalopanax septemlobum Koidz., which has the structure of 3α-O--[O-L-rhamnopyranosyl-(1 → 2)-α-L-arabopyranosyl] hederagenin [8]. The results of a comparison of their physicochemical constants and chromatographic behavior confirmed the identity of dipsacoside A and kalopanax saponin A.

The structure of the acyl glycosidic moiety of dipsacoside B follows easily from the appearance of gentiobiose on alkaline hydrolysis and from the quantitative ratio of the sugars. In order to be sure, we methylated the glycoside by Kuhn's method. The products of the hydrolysis of the completely methylated dipsacoside B (III) contained – in addition to 23-L-methylhederagenin (VIII) – 3, 4-di-O-methyl-L-arabinose, 2, 3, 4-tri-O-methyl-L-rhamnose, 2, 3, 4-tri-O-methyl-D-glucose, and 2, 3, 4, 6-tetra-O-methyl-D-glucose. The first two methylated sugars were formed from the O-glycosidic moiety of the molecule, and the last two from the gentiobiose attached to the carboxy group of the hederagenin (VII). A calculation of molecular rotation differences (Table 1) showed that the gentiobiose is attached to the hederagenin by a β-glycosidic link.

Thus, dipsacoside B is represented by structure I.

EXPERIMENTAL

Chromatography was carried out with type KSK silica gel, alumina (activity grade II-III), type "M" ["slow"] paper of the Leningrad No. 2 paper mill, and the following solvent systems: 1) butan-1-ol–ethanol–conc. amononia (10 : 2 : 5); 2) chloroform–ethanol (25 : 1); 3) toluene–ethanol (9 : 1); 4) methyl ethyl ketone saturated with 1% ammonia solution; and 5) butan-1-ol–acetic acid–water (4 : 1 : 5). The glycosides were detected with a 25% solution of phosphotungstic acid in ethanol or with a saturated solution of antimony trichloride in chloroform, the acetates with conc. H_2SO_4, and the sugars and their derivatives with aniline phthalate.

The molecular weights of the glycosides were determined on a Hitachi spectrophotometer at a wavelength of 195-210 nm.

The quantitative ratio of the sugars was found by the internal-standard method on a UKh-1 chromatograph with a copper column (1 m × 4 mm) filled with Diaphorit (0.2-0.315 mm) containing 5% of G-30 M silicone phase. The column temperature was 170° C and the carrier gas was hydrogen (55 ml/min).

The results of elementary analysis corresponded to the calculated figures.

Trideca-O-acetate of Dipsacoside B (II). Dipsacoside B (I) (1.0 g) was acetylated with acetic anhydride in pyridine at room temperature for 24 h. The amorphous precipitate that deposited when the reaction mixture was decomposed with ice water was dried (1.35 g) and dissolved in 3 ml of benzene. Petroleum ether was added to the benzene solution. A colored precipitate deposited at first, and when the volume of petroleum ether was increased a colorless substance with mp 140-144° C was obtained. On TLC (Al_2O_3) in system 2, the acetate of the dipsacoside migrated at the same level as the acetates of leontosides D and E [10]. The IR spectrum of compound II showed no absorption in the hydroxy group region.

Found: mol. wt. 1584.9 (spectrophotometrically [6] in 95% ethanol in comparison with hederagenin diacetate). $C_{79}H_{112}O_{35}$. Calculated: mol. wt. 1621.7

TABLE 1

Substance	M	$[\alpha]_D$, deg (in water)	$[M]_D$
Dipsacoside B	1075,2	+11,0	+118
Kalopanax saponin A [8]	769,0	+13,5	+104
Fraction of $[M]_D$ due to gentiobiose			— 14
Methyl α-gentiobioside [9]	356,3	+65,5	+233
Methyl β-gentiobioside [9]	356,3	—36,0	—128

Action of Diazomethane on Dipsacoside. A suspension of 30 mg of the glycoside in ether was methylated with an ethereal solution of diazomethane under the usual conditions. The reaction product was hydrolyzed with 6% sulfuric acid. TLC on silica gel in systems 1 and 2 showed the identity of the precipitate with hederagenin (VII).

Hydrolysis of the Acetate of Dipsacoside B (II). A solution of 620 mg of the acetate (II) in 20

ml of ethanol was mixed with 25 g of Dowex 1 × 2 anion-exchange resin (100/200 mesh) in the OH^- form. After careful shaking, the suspension was left for 48 h and was then transferred to a column. The column was first washed with 1 liter of water (until the reaction for sugars was negative), and then it was eluted with a 10% solution of acetic acid in methanol (300 ml).

The aqueous solution was evaporated to dryness in vacuum, and the residue was chromatographed on a column of silica gel (20 g). The column was washed with the butan-1-ol–water (90 : 10) system. A very small amount of D-glucose was eluated and also 87 mg of a disaccharide identified by PC in system 5 (with an exposure time of 96 h) as gentiobiose mp 190-194° C (from methanol), $[\alpha]_D^{24}$ + 9.4° (c 2.15; water). The phenylosazone had mp 166-172° C.

On evaporation, a methanolic-acetic acid solution deposited a crystalline precipitate (352 mg), which was shown by chromatography on silica gel in system 1 and on paper in system 5 to be identical with dipsacoside A and kalopanax saponin A (IV). mp 224-228° C (from ethanol), $[\alpha]_D^{92}$ + 12.5 ± 3° (c 2.40; ethanol).

Found: mol. wt. 748.8 (spectrophotometrically [6]). $C_{41}H_{66}O_{12} \cdot H_2O$. Calculated: mol. wt. 769.0.

The hexa-O-acetate of dipsacoside A (V) was obtained in the usual way. mp 254-258° C (from ethanol).

Substance (IV) (10 mg) was hydrolyzed with 3% H_2SO_4 in methanol. A crystalline substance deposited which was identified by TLC on silica gel in systems 1 and 2 as hederagenin. The acid hydrolyzate was treated with freshly-precipitated $BaCO_3$. The filtrate was found by PC in system 5 (48 h) to contain L-rhamnose and L-arabinose. Gas-chromatographic analysis of the glycoside confirmed that the molar ratio of the sugars was 1 : 1 (1.00 : 1.11).

The glycoside (IV) (250 mg) was methylated, and the completely-methylated product (VI) was hydrolyzed under the conditions used for kalopanax saponin A [8]. This gave 32 mg of a crystalline substance with mp 224-227° C identified by TLC on silica gel in systems 2 and 3 as the methyl ester of 23-O-methylhederagenin (IX).

After neutralization and the appropriate working up, the hydrolyzate was chromatographed on paper in systems 4 and 5 in the presence of markers, and was shown to contain 3,4-di-O-methyl-L-arabinose and 2, 3, 4-tri-O-methyl-L-rhamnose.

Methylation of Dipsacoside B. A solution of 0.340 g of the acetate II in 5 ml of dimethylformamide which had been redistilled over P_2O_5 was treated with 2 ml of methyl iodide and 2 g of silver oxide. Methylation was performed at 26-28° C for 18 h with the addition of fresh portions of reagents. The course of the reaction was checked by TLC on silica gel in system 3. After the spot of the initial glycoside had disappeared, the precipitate of silver oxide and iodide was separated off and washed with chloroform. The chloroform extract was combined with the main filtrate, shaken with saturated aqueous sodium thiosulfate solution, washed with water, dried over Na_2SO_4, and distilled to dryness. The residue was dissolved in benzene and the solution was passed through a column containing 20 g of alumina and was then evaporated. This gave 240 mg of a colorless chromatographically homogeneous permethylate of dipsacoside B (III) the IR spectrum of which lacked the absorption band of hydroxy groups.

The product III isolated in the preceding experiment (110 mg) was dissolved in 10 ml of methanol, and the solution was treated with 2 ml of 42% perchloric acid and heated in the boiling water bath for 4 h. The completeness of the hydrolysis was confirmed by TLC on silica gel in systems 2 and 3. After the end of the reaction, the mixture was diluted with water (1 : 3), the precipitate of genin that had deposited was filtered off, and it was identified by TLC on silica gel in systems 2 and 3 as 23-O-methylhederagenin, which has also been obtained by the hydrolysis of fully-methylated leontosides D and E [21.

The hydrolyzate was evaporated to a volume of 3 ml (to destroy the 1-methyl ethers of the methylated sugars) and dissolved in 30 ml of water, and the solution was neutralized on a column of Dowex-1 anion-exchange resin (OH^- form) resin; the column was washed with 50% aqueous methanol (200 ml) and the solution was concentrated in vacuum to a sirupy consistency. The precipitate was chromatographed on paper in system 4. 3,4-Di-O-methyl-L-arabinose, 2,3,4-tri-O-methyl-L-rhamnose, 2,3,4-tri-O-methyl-D-glucose, and 2,3,4,6-tetra-O-methyl-D-glucose were identified in the presence of authentic samples.

The samples of kalopanax saponins A and B were kindly given to us by A. G. Ven'yaminova.

SUMMARY

The structure of dipsacoside B – a heteragenin tetraoside from Dipsacus azureus Schrenk. – has been established. The O-glycosidic moiety consists of O-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabopyranose, and the O-acyl moiety consists of gentiobiose.

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