TRITERPENE GLYCOSIDES OF Hedera pastuchovii

IV. THE STRUCTURE OF PASTUCHOSIDE B

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We have previously [1] reported triterpene glycosides from the leaves of Hedera pastuchovii – pastuchosides A, B, and C. For the simplest of them, pastuchoside C, a definitive structure has been proposed [2]. Pastuchoside A proved to be kalopanax saponin B.

The present paper gives information on the basis of which the full structure of pastuchoside B has been established.

Pastuchoside B contains hederagenin as the aglycone. The sugar moiety consists of D-glucose and L-rhamnose in a ratio of 2 : 1. Consequently, pastuchoside B is a hederagenin trioside, as is confirmed by the results of elementary analysis of both the glycoside itself, $C_{48}H_{78}O_{18}$, and its full acetate, $C_{68}H_{98}O_{28}$, and also by the molecular weight of the glycoside found from the yield of hederagenin on acid hydrolysis under analytical conditions.

The hydrolysis of the glycoside methylated with diazomethane formed the methyl ester of hederagenin [1, 3], showing the absence of an O-acyl glycosidic bond in the molecule of the glycoside. The glycoside underwent no change under the action of alkali.

In order to determine the site of attachment of the carbohydrate chain to hederagenin, pastuchoside B was exhaustively methylated by Kuhn's method [4] and then the methylation product was hydrolyzed. The crystalline substance isolated was identified as the methyl ester of 23-O-methylhederagenin [5, 6]. The presence of the latter shows that the carbohydrate moiety of pastuchoside B is attached to the hydroxy group of hederagenin in position 3.

In the hydrolysate we identified 2,3,4,6-tetra-O-methyl-D-glucopyranose, 2,3,6-tri-O-methyl-D-glucopyranose, and 2,4-di-O-methyl-L-rhamnopyranose. Thus, it was established that the carbohydrate moiety of pastuchoside B has a linear structure and one of the D-glucose residues occupies the terminal position.

The sequence of monosaccharides in the carbohydrate chain was established by partial hydrolysis. The reaction mixture yielded, in addition to the initial glycoside, the hederagenin bioside pastuchoside C, i.e., hederagenin 3-α-L-rhamnopyranoside [2] and a disaccharide. This shows that in the pastuchoside B molecule the L-rhamnose is directly attached to the hederagenin.

Since the disaccharide isolated was maltose, the glucose molecules are attached to one another by a $1 \rightarrow 4$ bond, which agrees with the results of exhaustive methylation and the negative reaction of the methylated glucoses with Bonner's reagent [7] for α-glycol groupings.

The results of methylation show that the D-glucose is attached to the L-rhamnose by a $1 \rightarrow 3$ bond, as is confirmed by the periodate oxidation [8] of pastuchoside B, in which of the sugars only rhamnose did not undergo destruction.

Pastuchoside B did not change under the action of dilute oxalic acid, which indirectly shows the pyranose form of the monosaccharides [6]. The configurations of the glycosidic centers were calculated by means of Klyne's rule [9].

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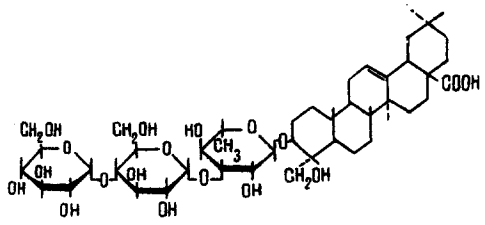
Substance		$[M]_D$, degrees
Pastuchoside C	(Mol. wt. 618; $[\alpha]_D^{20}$ + 43°)	+266
Bioside	(Mol. wt. 780; $[\alpha]_D^{20}$ + 74°)	+577.2
Pastuchoside B	(Mol. wt. 942; $[\alpha]_D^{20}$ + 92°)	+866.6
Methyl-α-D-glucopyranoside	[5, 10]	+307
Methyl-β-L-glucopyranoside	[5, 10]	–62

It follows from the calculations that all the sugars possess the α configuration of the glycosidic bond. On the basis of the facts presented, pastuchoiside B may be ascribed the following complete structure:



EXPERIMENTAL

Chromatography was carried out on type KSK silica gel (250 mesh), "M" ["slow"] paper of the Leningrad No. 2 paper mill, and the following solvent systems: 1) butan-1-ol–acetic acid–water (4:1:5); 2) butan-1-ol–ethanol–25% ammonia (10:2:5); 3) butan-1-ol–ethanol–water (1:1:1); 4) chloroform–methanol (10:1); 5) butan-1-ol–ethanol–water (5:1:4); and 6) chloroform–ethanol (1:1). On the chromatograms, the triterpene glycosides, the genins, and their derivatives were revealed by means of a 25% solution of phosphotungstic acid in ethanol and with a saturated solution of antimony trichloride, and the sugars and their derivatives by means of aniline phthalate. The elementary analyses were performed by workers of the microanalysis laboratory of VILR [All-Union Scientific-Research Institute of Medicinal Plants] and correspond to the calculated formulas.

Isolation of Pastuchoside B. The mixture of pastuchosides A and B obtained previously [1] (6.20 g) was passed through a column (4 × 80 cm) filled with silica gel, and elution was performed in system 1, 20-ml to 25-ml fractions being collected. The separation was monitored by TLC in systems 2 and 3. A total of 68 fractions was obtained. There were no glycosides in fractions 1-6; fractions 7-28 contained pastuchoside B; fractions 29-36 a mixture of pastuchosides A and B; and from the remainder, pastuchoside A was isolated. The fractions similar in composition were combined and evaporated to dryness. The pastuchoside B, $C_{48}H_{78}O_{18}$, had mp 218-221° C, $[\alpha]_D^{20}$ + 92° (c 1.8; methanol).

Deca-O-acetate of Pastuchoside B was obtained in the usual way. The completeness of the acetylation was checked from the IR spectrum by means of the disappearance of the absorption band in the 3200-3400 cm^{-1} region (OH) After recrystallization from ethanol, the melting point of the acetate $C_{68}H_{98}O_{28}$ 168-170° C, $[\alpha]_D^{20}$ + 76.5° (c 1.2; ethanol).

Acid Hydrolysis of Pastuchoside B. A solution of 0.25 g of pastuchoside B in 15 ml of 60% methanol was treated with 20 ml of 15% sulfuric acid solution. The mixture was boiled for 10 h. The precipitate of the aglycone that deposited was separated off, neutralized with sodium hydrogen carbonate, washed with water, and dried.

By its physicochemical properties, elementary composition, IR spectrum, and chromatographic behavior, the aglycone was identified as hederagenin [1].

After neutralization, the hydrolysate was found by paper chromatography in system 1 to contain D-glucose and L-rhamnose.

In order to determine the quantitative ratio of the monosaccharides, after being revealed with aniline phthalate the spots were cut out, eluted with glacial acetic acid, and subjected to colorimetry [11]. As a result, it was found that the D-glucose and L-rhamnose in the molecule of pastuchoside B are present in a ratio of 2:1.

Methylation of Pastuchoside B with Diazomethane. The glycoside (0.1 g) was dissolved in 10 ml of methanol and methylated with an ethereal solution of diazomethane. The product obtained was hydrolyzed with 8% sulfuric acid solution for 4 h. The crystalline substance isolated, with mp 235-237° C, $[\alpha]_D^{20}$ + 73.5° (c 0.3; chloroform) was identical with the methyl ester of hederagenin.

Methylation of Pastuchoside B by Kuhn's Method. A solution of 0.45 g of the glycoside in 10 ml of freshly distilled dimethylformamide was treated with 10 ml of methyl iodide and 3.2 g of barium oxide. The mixture was heated in the water bath for 12 h. Then 5 ml of dimethylformamide, 5 ml of methyl iodide, 1.5 g of barium oxide, and 1.2 g of barium hydroxide were added and the mixture was heated for another 10 h. The methylation process was repeated twice. The course of the methylation was monitored in a thin layer of silica gel in systems 3 and 4. The reaction mixture was poured into a saturated aqueous solution of sodium thiosulfate and extracted with chloroform (3 × 75 ml). The chloroform extracts were combined and distilled to dryness.

To free the residue from the partially methylated glycoside and from other impurities, it was transferred to a column of silica gel (4 × 35 cm) and eluted with chloroform, 10-ml fractions of the eluate being collected. The fractions were monitored by TLC in system 4. From the first fractions, the fully methylated pastuchoside B was isolated in the form of an amorphous white powder with mp 108-110° C; $[\alpha]_D^{20}$ + 98.5° (c 1.5; chloroform). The IR spectrum lacked bands in the 3200-3400 cm^{-1} region (OH).

Hydrolysis of the Fully-Methylated Glycoside. A solution of 0.2 g of the methylated glycoside in 20 ml of a mixture of methanol and 72% perchloric acid (10:1) was heated in the boiling water bath for 10 h. The completeness of methanolysis was checked by TLC in system 4. Then an equal volume of water was added to the mixture and the precipitate of the genin that had deposited was filtered off, washed with water, dried, and recrystallized repeatedly from ethanol. This gave about 0.1 g of a crystalline substance, $C_{32}H_{52}O_4$ with mp 220-223° C, $[\alpha]_D^{20}$ + 70.3° (c 0.85; chloroform).

Literature data for the methyl ester of 20-O-methylhederagenin: mp 218-220° C, $[\alpha]_D^{20}$ + 69° (c 1.5; chloroform) [6]; mp 190-192° C, $[\alpha]_D^{20}$ + 70° ± 1° (c 1.9; chloroform) [12]; mp 228-230° C; $[\alpha]_D^{20}$ + 68.6 ± 2° (c 0.4; chloroform) [5].

The aqueous hydrolysate was evaporated, and the residue was treated with 5 ml of 2% sulfuric acid and hydrolyzed. After cooling, the mixture was neutralized and evaporated. The following methylated monosaccharides were identified by paper chromatography in systems 1 and 5: 2,3,4,6-tetra-O-methyl-D-glucopyranose, 2,3,6-tri-O-methyl-D-glucopyranose, and 2,4-di-O-methyl-L-rhamnopyranose.

Partial Hydrolysis of Pastuchoside B. A solution of 1.8 g of the substance in 40 ml of a mixture of methanol and water (3:1) was treated with 4 ml of conc. sulfuric acid and the mixture was heated in the boiling water bath for 40 min. Then 100 ml of water was added, and the precipitate that deposited was filtered off, washed, and dried. This gave 1.22 g of a mixture of glycosides, which was separated on a column (2 × 50 cm) of silica gel, being eluted with system 6. Fractions of 20-25 ml were collected, being monitored by the TLC method in systems 4 and 6.

Fractions 3-8, eluted by system 6, consisted of pastuchoside C, $C_{36}H_{58}O_8$, mp 254-257° C; $[\alpha]_D^{20}$ + 43° (c 0.8; methanol).

Fractions 11-38 (same system) contained a bioside with the composition $C_{42}H_{68}O_{13}$, mp 236-238° C, $[\alpha]_D^{20}$ + 74° (c 1.2; ethanol)

On the hydrolysis of the bioside, glucose and rhamnose were identified. The subsequent fractions yielded the initial glycoside.

The hydrolysate obtained after the separation of the mixture of glycosides was neutralized with barium hydroxide and evaporated. Chromatography on paper in the solvent system 1 showed the presence of maltose and glucose.

In order to separate the sugars, the mixture was passed through a column of cellulose powder (2 × 50 cm), 40-ml fractions being collected and monitored by paper chromatography.

Fractions 3-12 yielded a disaccharide with mp 162-163° C, $[\alpha]_D^{20}$ + 139.5° (c 0.5; water), identified as maltose.

Periodate Oxidation of the Glycoside. A solution of 0.05 g of the substance in 15 ml of acetate buffer with pH 4.5 was treated with 0.15 g of potassium periodate and was kept in the dark at room temperature for 5 days. The excess of potassium periodate was destroyed with ethylene glycol, the solvent was distilled off, and the oxidation product was extracted with methanol. The methanol was evaporated, and the residue was hydrolyzed with 5% sulfuric acid for 4 h. The hydrolysate was diluted with water, the precipitate of the aglycone was separated off, and the aqueous phase, after neutralization, was chromatographed on paper; only L-rhamnose was identified.

SUMMARY

Pastuchoside B has been isolated and shown to be O-α-D-glucopyranosyl-(1 → 4)-O-α-D-glucopyranosyl-(1 → 3)-hederagenin.

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