GLYCOSIDES OF PATRINIA SIBIRICA. I

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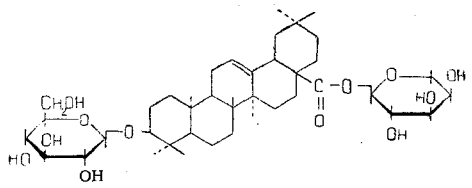
Essential oils, alkaloids, and ascorbic acid have been found in Patrinia sibirica (L.) Juss, a medicinal plant of the Valerianae family [1]. This paper presents the results of the isolation and structure determination of the glycosides of this plant. A study of a methanolic extract by means of thin-layer chromatography on silica gel revealed that it contained three glycosides, which we have named sibirosides A, B, and C, in order of increasing polarity. The most polar, sibiroside C, was separated from the enriched fraction obtained by gel-filtration through Sephadex. Glycoside A could not be separated by this method from the reserve sugars, probably because of the relatively small difference in their molecular weights. It was obtained in the pure state by partition chromatography on aluminum oxide. The glycosides isolated were amorphous powders, easily soluble in water and ethanol. Hydrolytic cleavage with mineral acids proved that glycosides A and C are derivatives of hederagenin.

According to their molecular weights, determined from the yield of hederagenin, sibiroside A is a bioside and sibiroside C a hexaoside of hederagenin.

The same monosaccharide units, D-glucose and L-arabinose, entered into the composition of the carbohydrate chains of both glycosides. In the hexaoside the ratio of glucose to arabinose, as determined photocolorimetrically [1], was 2 : 1.

An absorption band at 1740 cm^{-1} in the IR-spectra of the isolated glycosides indicated the presence of an ester bond. This was confirmed by the fact that both glycosides were easily cleaved by alkali to form the corresponding acid glucosides. Sibiroside A yielded a monoside (I) which decomposed on further acid hydrolysis into hederagenin and glucose. Consequently, in this glycoside the carboxyl group of hederagenin is linked to L-arabinose, and the hydroxyl group to D-glucose.

In order to ascertain the sizes of the oxide rings of the monosaccharide residues, the fully methylated ether of sibiroside A was prepared. When submitted to acid hydrolysis, this permethylate decomposed into 23-O-methyl-hederagenin [3], 2,3,4,6,tetra-O-methyl-D-glucopyranose and 2,3,4,tri-O-methyl-L-arabopyranose. The methyl ethers of the monosaccharides were identified by paper chromatography using known markers, as well as by preparative separation on paper. Calculation of the configuration of the glycosidic centers, carried out according to Klyne [4], showed that arabinose is linked by an α- and glucose by a β-glycosidic bond (see table). On the basis of all these facts, the complete formula of the bioside may be represented thus:



EXPERIMENTAL

ASK silica gel, Volodarskii Leningrad factory paper, as well as Schleicher und Schull No. 2043 paper were used for chromatography. The solvent systems employed were: a) butanil–acetic acid–water (4 : 1 : 5); 2) ethyl acetate–pyridine–water (2 : 1 : 2), and 3) butaniol–ethanol–water (5 : 1 : 4 and 4 : 1 : 5).

Roots and rhizomes of the plants were collected during the summer of 1965 in Gorno Altai. The air-dried and ground roots (1.3 kg), after preliminary defatting with chloroform, were exhaustively extracted with methanol. Upon prolonged standing of the condensed methanolic extract, 25 g of sucrose crystallized out.

The solution was evaporated to dryness under vacuum, and the residue (120 g) was dissolved in water and extracted with butan-1-ol. After the solvent had been distilled off, 60 g of dry residue was obtained.

Substance	M	$[\alpha]_D$, degrees	$[M]_D$, degrees
Sibiroside A	766.9	−47∓3	−360∓23
Monoside (I)	634.7	−41∓3	−260∓19
Hederagenin	472.7	+80∓3	+378∓14
$[M]_D$ contribution of L-arabinose	—	—	−100
$[M]_D$ contribution of D-glucose	—	—	−638
Methyl α-L-arabopyranoside	164.1	+ 17.3	+27.8
Methyl β-L-arabopyranoside	164.1	+245.5	−394
Methyl α-D-glucopyranoside	194.2	+158.9	+309
Methyl β-D-glucopyranoside	194.2	− 34.2	−66.4

Preliminary separation of the butanol extract. Into a column (5.5 × 130 cm) filled with silica gel (grain size 10 mμ), 60 g of the extract was introduced and eluted with the chloroform–methanol system with a gradient increase in the fraction of the latter. One-liter fractions were collected. They were monitored by chromatography in a thin fixed layer of silica gel.

Fractions 1–3 (6.2 g), eluated by the system with a ratio of 25 : 2, did not contain glycosides; 4–7 (20.4 g), eluated by the system with a ratio of 10 : 2, were rich in sibiroside A; 8–13 (19 g) consisted of a mixture of sibirosides A and B; and 14–21 (17 g) contained mainly sibiroside C. The subsequent fractions (7.5 g), eluated with butan-1-ol–acetic acid (5 : 2), represented reserve carbohydrates.

Purification of sibiroside A. A 20.4 g of amount of the fractions rich in sibiroside A (obtained in the previous experiment) was introduced into a column (5 × 35 cm) filled with alumina and was eluated with the acetate–methanol–water (10 : 2 : 3) system. 100-ml fractions were collected. They were monitored in a thin fixed layer of silica gel. Then, 8.8 g of an individual substance was obtained, which after crystallization from butan-1-ol melted at 245–250° C, $[\alpha]_D^{20}$ − 47° (c 2.4; ethanol).

Found, %: C 63.78; H 8.84. Calculated for $C_{41}H_{66}O_{13}$, %: C 64.20; H 8.68.

Purification of sibiroside C. A 10.6 g amount of the fractions enriches in sibiroside C (obtained previously) was introduced into a column with 140 g of Sephadex G-25 and eluted with water, 20-ml portions being collected. Fractions 2–10 contained sibiroside C free from reserve sugars. Total weight 5.4 g. After recrystallization from aqueous methanol it melted at 199–203° C (with decomp.). $[\alpha]_D^{20}$ − 20° (c 3.8; pyridine).

Found, %: C 55.26; H 7.73. Calculated for $C_{64}H_{104}O_{32}$, %: C 55.49; H 7.55.

Acid hydrolysis of the total glycosidic fraction. 1.8 g of the butanol extract was dissolved in 15 ml of methanol–water (2 : 1) containing 5% of H_2SO_4 and the solution was heated for 3 hr at 65–75° C. 0.2 g of hederagenin was obtained with mp 325–327° C $[\alpha]_D^{20}$ +80° (c 4.1; pyridine). A mixture mixed with authentic hederagenin showed no melting point depression.

Hederagenin acetate: mp 169–170° C. According to the literature, mp 173–175° C [8].

The methyl ester of hederagenin melted at 234–235° C. According to the literature, 240° C [9]. When submitted to chromatography, it behaved exactly like a known sample of the methyl ester of hederagenin.*

Acid hydrolysis of sibiroside A. A 0.33 g amount of the substance was dissolved in 20 ml of 3% HCl was heated for 5 hr at 100° C. The precipitate was filtered off, and the hydrolysate was extracted with chloroform. The total w weight of the aglycone was 0.1964 g. The molecular weight, determined from the yield of aglycone, was 804. Calculated for $C_{41}H_{60}O_{13}$, 760. Paper chromatography in the solvent systems 1 and 2, using known markers, revealed the presence in the hydrolysate of D-glucose and L-arabinose.

*The samples of hederagenin, its acetate and of the acetate of its methyl ester were kindly supplied by N. K. Abubakirovy and L. G. Mzhel'skaya.

Alkaline hydrolysis of sibiroside A. A 0.28 g amount of the substance was dissolved in 10 ml of 10% aqueous potassium hydroxide and the solution was heated for 3 hr. The reaction mass was acidified to pH 4–5, the product was extracted with chloroform, and the extract was washed with water. After the solvent had been distilled off, the residue was introduced into a column (0.8 × 15 cm) filled with silica gel (grain size 20 mμ) and eluated with the ethyl acetate–methanol (10 : 1) system. 0.1 g of the monoside (I) melting at 215–218° C (ex aqueous methanol) and with $[\alpha]_D^{20}$ – 41° (c 1.2: pyridine) was obtained. 0.015 g of the product was dissolved in 1 ml of methanol–water (3 : 1) containing 5% of HCl, and the solution was heated for 4 hr. The reaction mixture was diluted with 20 ml of butanol and evaporated to dryness under vacuum. D-glucose was identified by paper chromatography in solvent systems 1 and 2.

Total methyl ether of sibiroside A. A mixture of 0.8 g of the substance, 9 ml of methyl iodide, 8 g barium oxide and 27 ml of absolute dimethylformamide was boiled on the water-bath; 10 ml water was then added and the heating 150 ml of water containing 0.5 g of sodium thiosulfate: the product was extracted with chloroform, the extract was washed with water, and the solvent was evaporated. The residue was introduced into a column (0.8 × 20 cm) filled with silica gel and eluated first with the chloroform–ethyl acetate system (1 : 1), followed by pure ethyl acetate. The first fraction (0.3 g) was the total methyl ether, with an IR spectrum almost completely free of the OH-group absorption band. The second fraction (0.32 g) was submitted to a second methylation by the previously described procedure. The total yield of permethylate was 0.46 g.

Acid hydrolysis of the total methyl ether of sibiroside A. Then, 0.33 g of permethylate dissolved in 30 ml of a methanol–conc HCl (10 : 1) mixture was heated for 3 hr on a water-bath: 10 ml water was then added and the heating was continued for 4 hr more, after which 50 ml of butanol was added to the reaction mass and the solvent was distilled off under vacuum. The dry residue was introduced into a column (0.8 × 15 cm) filled with silica gel, and was eluted first with chloroform (30 ml) and then with ethyl acetate (50 ml). The fractions extracted with ethyl acetate contained 23-O-methylhederagenin (mp 227–229° C). The chloroform eluate was evaporated and then submitted to preparative chromatography on Schleicher und Schüll paper using solvent system 3. 2,3,4,6-tetra-O-methyl-D-glucose (0.37 g), $[\alpha]_D^{20}$ – 82° (c 0.8; acetone) was isolated. According to the literature, $[\alpha]_D^{20}$ – 83.9° [9]. The substance was chromatographically identical with an authentic sample.

The tetramethylglucose (0.015 g) was heated for 5 min in 0.5 ml of 47% HBr; then the reaction mass was diluted with 15 ml butan-1-ol and the solvent was distilled off under vacuum. Glucose was identified among the decomposition products, 2,3,4-Tri-O-methyl-L-arabinose (0.035 g) was also obtained, R_g 0.83 [10], $[\alpha]_D^{20}$ +133.4° [6]. L-Arabinose was identified by means of paper chromatography among the decomposition products obtained on demethylation by heating with 47% HBr.

Acid hydrolysis of sibiroside C. 0.304 g of the substance dissolved in 50 ml of 5% aqueous H_2SO_4 was heated for 5 hr on a boiling water-bath. The reaction mass was extracted with chloroform (2 × 30 ml). The yield of hederagenin was 0.107 g. In another experiment 0.115 g of the aglycone was obtained from 0.331 g of glycoside. The molecular weights determined from the yields of aglycone were 1340 and 1360, respectively. Calculated for $C_{64}H_{104}O_{32}$; 1385.5.

D-Glucose and L-arabinose in the ratio 2 : 1, determined photocolorimetrically [2], were identified in the hydrolysate by paper chromatography in systems 1 and 2 using known standards.

CONCLUSIONS

Three glycosides, named sibirosides A, B, and C, have been detected in the roots of Patrinia sibirica (L.) Juss. All three are derivatives of hederagenin. Sibirosides A and C were isolated in the pure state and the complete structure of the former was established. It was shown that sibiroside C is a hexaoside of hederagenin, with carbohydrate chains consisting of two L-arabinose and four D-glucose residues.

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