TRITERPENE GLYCOSIDES OF LEONTICE EVERSMANNII

II. The Structure of Leontoside A and Leontoside B

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We have previously [1] reported the isolation from the tubers of L. eversmannii Bge. of five triterpene glycosides called leontosides A, B, C, D, and E. Leontosides A and B have the simplest compositions; they are both glycosides of hederagenin. The sugar moiety of leontoside A consists of L-arabinose, and that of leontoside B of L-arabinose and D-glucose. When the glycosides are methylated with diazomethane and the methylated glycosides are hydrolyzed, the products are found to contain an ester of hederagenin methylated at the carboxy group. This shows that the glycosides described are not O-acyl glycosides.

In the present paper we give additional information showing the structures of leontosides A and B. A quantitative determination of the arabinose obtained by the acid hydrolysis of leontoside A showed that one molecule of the sapogenin contains one molecule of the sugar. To determine the size of the oxide ring of the carbohydrate moiety, leontoside A was methylated first with dimethyl sulfate and then with methyl iodide in the presence of silver oxide. The completeness of the methylation was checked by the IR spectrum. The methylation product was hydrolyzed with sulfuric acid and 2,3,4-tri-O-methyl-L-arabinose was found in the hydrolysate, which shows the pyranose form of the L-arabinose.

Substance	M	$[\alpha]_D$ deg	Solvent	$(M)_D = \frac{M \cdot [\alpha]_D}{100}$, deg
Leontoside B	766.9	+49±2	Methanol	+381±15
Leontoside A	604.8	+47±1	Pyridine	+287±6
Hederagenin	472.7	+78±1	Pyridine	+368±4
Contribution (M) of D-glucose				+94
Contribution (M) of L-arabinose				−80
Methyl α-D-glucopyranoside [5]	194.2	+158	Water	+307
Methyl β-D-glucopyranoside [5]	194.2	−32	Water	−62
Methyl α-L-arabopyranoside [6]	164.1	+17.6	Water	+28
Methyl β-L-arabopyranoside [6]	164.1	+246	Water	+404

Consequently, leontoside A has the structure hederagenin L-arabopyranoside.

To establish the position of the attachment of the carbohydrates in leontoside B, the latter was hydrolyzed with an enzyme preparation from Aspergillus oryzae [2]. Leontoside A and free D-glucose were found in the hydrolysis products, in addition to the initial leontoside B. This shows that in the sugar chain the D-glucose occupies the terminal position and the L-arabinose is directly attached to the sapogenin, three variants of the linkage between the D-glucose and L-arabinose being possible: 1→2, 1→3, and 1→4.

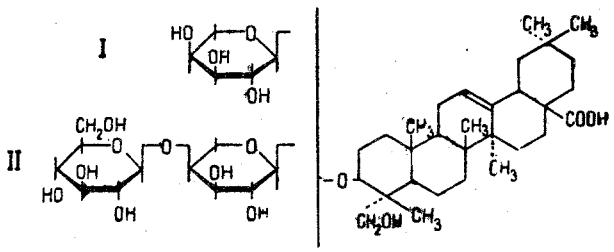
When leontoside B was oxidized with periodic acid and the oxidation product was subsequently hydrolyzed with sulfuric acid, the sugar moiety of leontoside B decomposed completely and no free monosaccharides were found in the hydrolyzate. Consequently, the 1→3 bond is excluded. Further information on the structure of the carbohydrate moiety of the leontoside B was obtained by exhaustive methylation. Methanolysis of the methylated product gave a crystalline substance identified by its physicochemical constants as the methyl ester of 23-O-methylhederagenin [3]. This shows that in leontosides A and B the carbohydrate moiety is attached to the secondary hydroxyl at C-3.

Subsequent hydrolysis of the methyl glycosides formed with sulfuric acid led to free methylated monosaccharides among which the presence of 2,3,4,6-tetra-O-methyl-D-glucopyranose and 2,3-dimethyl-L-arabinose was shown by paper chromatography. The results of the experiments again confirm that in the chain of sugars the D-glucose occupies the terminal position and that it is linked to the arabinose by a 1→4 linkage. The 1→4 linkage is also shown by the fact that when chromatographed on paper the methylated sugars are not revealed by Bonner's reagent [4] for the α-glycol group.

We have established the nature of the glycosidic bonds by the usual method from the differences in molecular

rotation between leontoside B and leontoside A, and between leontoside A and hederagenin (table). Although the specific rotations necessarily have to be determined in different solvents (so that no clear picture was obtained, it is impossible not to see that the absolute value of the difference between the molecular rotations of leontoside B and leontoside A is closer to the molecular rotation of methyl β-D-glucopyranoside (the difference between +94° and −62° is 156°) than to that of methyl α-D-glucopyranoside (the difference between +94° and +307° is 213°). This show, with a high degree of probability, the presence of a β-glycosidic linkage in the substances. In the second case, the difference between the molecular rotations of leontoside A and hederagenin shows the presence of an α-glycosidic linkage fairly clearly (see table).

Consequently, leontoside A is hederagenin 3-α-L-arabopyranoside (I) and leontoside B is hederagenin 3-α-L-arabopyranosido-4-β-D-glucopyranoside (II) and their structures are expressed by



Hederacoside A, a triterpene glycoside isolated from Hedera helix [3], has the same aglycone and sugar compositions. The Swiss authors have assigned to it the structure of hederagenin glucosido-5-arabinose, the furanoside form being assigned to the L-arabinose, although no experimental data whatever on the presence of the L-arabinose in the furanose form are given. We have been unable to obtain an authentic sample of hederacoside A. However, in a rough comparison, in a thin layer of silica gel, of extracts of the tubers of L. eversmannii and of the leaves and stems of H. helix, a spot of a substance close to that of leontoside B but slightly less polar was found. However, even if the spot of leontoside B and hederacoside A were found at the same level this would not be a proof of their identity. A difference in the size of the oxide bridges of the L-arabinose might not affect the chromatographic behavior of the triterpene glycosides compared.

Experimental

Silica gel of type KSK, alumina (activity grade II, III), cellulose powder obtained by the hydrolysis of cotton cellulose with hydrochloric acid, and type M paper from the Leningrad No. 2 mill, were used for chromatography. The following systems of solvents were used (by volume): 1) butan-1-ol – ethanol – 25% ammonia (7:2:5) or (10:2:5) for a thin layer of silica gel; 2) toluene – ethanol (9:1) for a thin layer of alumina; and 3) butan-1-ol – acetic acid – water (4:1:5) for paper.

The triterpene glycosides were revealed on the chromatograms with a 25% solution of phosphotungstic acid, and the sugars and their derivatives with aniline phthalate. The melting points are uncorrected.

Leontoside A. This was obtained as described previously [1], mp 276°–278° C (from ethanol), $[\alpha]_D^{20}$ +47 ± 1° (c 1.18; pyridine).

Found, %: C 69.18, 69.41; H 9.24, 9.35. Calculated for $C_{35}H_{56}O_8$, %: C 69.50; H 9.33.

Leontoside A tetra-O-acetate, mp 166°–168° C, $[\alpha]_D^{20}$ +50 ± 1° (c 1.81; chloroform).

Found, %: C 66.52, 66.46; H 8.26, 8.41. Calculated for $C_{43}H_{64}O_{12}$, %: C 66.84; H 8.36.

Methylation of leontoside A. To a solution of 35 mg of the substance in 4 ml of dimethyl sulfoxide were added 2 ml of methyl iodide, 0.2 g of barium oxide, and 15 mg of barium hydroxide. The reaction was carried out with constant stirring at room temperature for two days. After every 8 hr, fresh portions of methyl iodide, barium oxide, and barium hydroxide were added in the same proportions. Since the IR spectrum still showed the band of a free hydroxyl (3460 cm^{-1}), the product obtained was further methylated with a mixture of methyl iodide and silver oxide. The methylation with methyl iodide was repeated three times until the methylation product showed only one slightly diffuse spot in a thin layer of alumina in system 2. A benzene extract of the reaction product was filtered through a small amount of alumina. After the benzene had been distilled off, chromatographically homogeneous methylated leontoside A was obtained in the form of a viscous sirup.

Hydrolysis of the methylated leontoside A. The substance obtained was treated with 2 ml of 6% sulfuric acid in

methanol and the mixture was heated at 70° C for 6 hr. The acid solution was filtered from the precipitate and neutralized with EDE-10P anion exchanger (OH^- form) and evaporated to 0.3 ml. The sirupy residue was shown by paper chromatography in system 3, compared with an authentic sample, to contain 2,3,4-tri-O-methyl-L-arabopyranose.

Leontoside B. Obtained in the manner described previously [1], mp 242°–244° C, $[\alpha]_D^{20}$ +49.8 ± 2° (c 1.12; methanol).

Found, %: C 63.61, 63.40; H 8.78, 8.84. Calculated for $C_{41}H_{66}O_{18} \cdot 1/2\ H_2O$, %: C 63.42; H 8.57.

Leontoside B penta-O-acetate, mp 261°–262° C, $[\alpha]_D^{20}$ +36.2 ±2° (c 1.54; chloroform). The IR spectrum contained no band corresponding to an OH group. A determination of the number of acetyl groups from the integral intensity of the ester band showed the presence of 7 or 8 acetyl groups in the acetate [7].

Found, %: C 61.41, 62.33; H 7.60, 7.74. Calculated for $C_{55}H_{80}O_{20} \cdot H_2O$, %: C 61.28; H 7.60.

Enzymatic hydrolysis of leontoside B. A solution of 100 mg of the glycoside in 50 ml of water was mixed with 125 mg of an enzyme preparation from Aspergillus oryzae. The mixture was kept in a thermostated vessel at 37° C for 4 days. The course of the fermentation was followed by chromatography in a thin layer of silica gel in system 1. After the time mentioned, a spot corresponding to leontoside A had appeared, in addition to the spot of the initial leontoside. The glycosides were extracted from the reaction mixture with butanol. After evaporation, the aqueous solution was chromatographed on paper in system 3, and D-glucose was identified.

Oxidation of leontoside B with periodic acid. A solution of 40 mg of the glycoside in 2 ml of methanol was mixed with 175 mg of periodic acid previously dissolved and neutralized with sodium hydrogen carbonate to pH 3. The reaction mixture was left at room temperature for ten days and was then extracted with butanol. The butanol solution was purified by filtration through a 2-cm layer of silica gel and was evaporated to dryness in vacuum. The residue was dissolved in 3 ml of methanol, 3 ml of 6% sulfuric acid added, and the mixture heated at 65°–70° C for 6 hr. The precipitate of hederagenin that deposited was filtered off, and the acid hydrolysate was neutralized with EDE-10P anion exchanger (OH^- form) and evaporated to the sirupy state. On paper chromatography in the presence of samples of D-glucose and L-arabinose, no monosaccharides were found.

Methylation of leontoside B. A solution of 1.380 g of the glycoside in 25 ml of methanol was treated with 13 ml of methyl iodide and 15 g of silver oxide. The reaction mixture was kept at a gentle boil with constant stirring for 48 hr. Then the silver oxide was filtered off, fresh portions of silver oxide and methyl iodide in the same ratio were added to the reaction mixture, and it was left at room temperature for five days. The operation with the addition of fresh silver oxide and methyl iodide was repeated. The subsequent methylation to completion was carried out in dimethylformamide (10 ml) with 10 ml of methyl iodide in the presence of 6 g of barium oxide and 0.2 g of barium hydroxide [8]. The reaction took place at room temperature, with the methylation process being repeated three times. As a result, a mixture of fully and partially methylated leontoside was obtained as a chromatogram in a fixed layer of alumina in system 2. Then the reaction mixture was poured into 100 ml of a saturated aqueous solution of thiosulfate, and 100 ml of chloroform was added. The precipitate that deposited was separated off and washed with chloroform (3 × 50 ml). All the chloroform extracts were combined and the chloroform was distilled off. For purification from partially methylated products, the mixture was dissolved in benzene and transferred to a column of alumina (34 × 3 cm) which was eluted with benzene, 10-ml fractions being collected. A check on the separation of the substances was carried out by chromatography in a thin layer of alumina in system 3. Fractions 9 and 10 contained the exhaustively methylated leontoside and fraction 16 the partially methylated glycoside, while fractions 10–15 gave two spots. The completely methylated, chromatographically homogeneous, leontoside B in the form of a colorless amorphous powder had, after drying in vacuum, mp 72°–74° C, $[\alpha]_D^{20}$ +44.1 ± 1° (c 2.22; chloroform).

Found, %: C 66.60, 66.51; H 9.57, 9.65; OCH_3 26.85. Calculated for $C_{49}H_{82}O_{13}$, %: C 66.94; H 9.40; 8 OCH_3 28.23.

Hydrolysis of methylated leontoside B. The methylated product (0.5 g) was subjected to methanolysis in a mixture of methanol and 42% perchloric acid (6:1) heated on a boiling water bath for 8 hr. A check on the methanolysis was carried out chromatographically in system 2. After completion of the reaction the mixture was treated with 20 ml of water. The precipitate that deposited was filtered off, washed with water, dried, and recrystallized twice from aqueous ethanol. This gave 0.30 g of a crystalline substance with mp 228°–230° C, $[\alpha]_D^{20}$ +68.6 ± 2° (c 2; 0.4; chloroform). Literature data for the methyl ether of 23-O-methylhederagenin gave mp 223°–229° C [3].

Found, %: C 77.01, 77.19; H 10.48, 10.60. Calculated for $C_{32}H_{52}O_4$, %: C 76.76; H 10.46.

The hydrolysate was evaporated in vacuum, 3 ml of 3% sulfuric acid added to the reaction vessel, and concentration was continued. At the end of hydrolysis the solution was neutralized with EDE-10P anion exchanger (OH^- form) and evaporated to the sirupy state. Chromatography on paper in system 3 in the presence of authentic samples showed that the mixture contained 2,3,4,6-tetra-O-methyl-D-glucopyranose and 2,3-di-O-methyl-L-arabopyranose. The

mixture of methylated sugars was dissolved in 3 ml of system 3, transferred to a column of cellulose powder (11 × 2 cm), and eluted by the same system, fractions of 8–10 ml being collected. Fractions 1 and 2 contained 2, 3, 4, 6-tetra-O-methyl-D-glucopyranose, fractions 3 and 4 a mixture, and fractions 4–6 consisted of 2, 3-dimethyl-L-arabopyranose.

Summary

The structures of the triterpene glycosides leontiside A and leontoside B from Leontice eversmannii have been established. It has been shown that the leontoside A is hederagenin 3-α-L-arabopyranoside and leontoside B is hederagenin 3-α-L-arabopyranosido-4-β-D-glucopyranoside.

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