TRITERPENE GLYCOSIDES OF LEONTICE EVERSMANNII. I.

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Leontice eversmannii Bge. is a perennial plant of the family Berberidaceae which is being studied in detail with respect to its alkaloids. Individual substances that have been found in it are leontidine, leontamine, leontine, pachycarpine, α-lupanine, thaspine, and isoleontine [1]. In an investigation of the plants for their saponin content, a fairly high hemolytic index (6000-10 000) [2] and good foam-forming properties were found for leontice tubers. From the related plant L. leontopetalum, in addition to alkaloids, a crystalline saponin with mp 235°-238°C, $[\alpha]_D^{20}$ +15.1°, which gave hederagenin, glucose and arabinose on hydrolysis [3] has been isolated.

We used tubers of L. eversmannii, collected in the Keless Massif of the South Kazakhstan Oblast in March 1965. After defatting with acetone, the comminuted air-dried tubers were extracted with methanol. A preliminary investigation of the methanolic extract, which amounted to about 38% of the weight of the tubers, by chromatography on paper and in a thin layer of fixed silica gel in various systems showed the presence of five individual substances of glycosidic nature in the extract. The glycosides were called leontosides A, B, C, D, and E (Fig. 1) in order of increasing polarity.

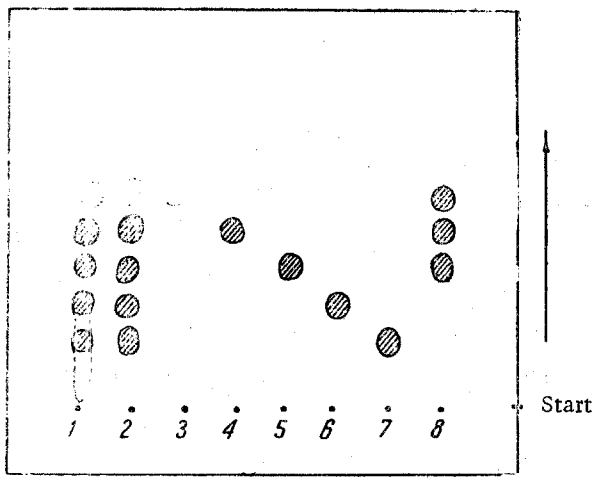

Fig. 1. Chromatogram of the triterpene glycosides in a thin-fixed layer of silica gel in system 2. 1) Initial methanolic extract; 2) total glycosides; 3-7) leontosides A, B, C, D, and E, respectively; 8) total glycosides after hydrolysis with a 10% alcoholic solution of KOH.

To determine the nature of the genin, the total saponins were subjected to acid hydrolysis with sulfuric acid. The aglycone which was obtained in the crystalline state, was identified as hederagenin [4] by its physicochemical constants and its derivatives (diacetate, methyl ether, and methyl ether of the diacetate).

The individual leontosides were isolated by partition chromatography on columns of cellulose and silica gel in systems 1 and 2 (cf. Experimental section). As a rule, on cellulose the initial combined leontosides could be separated into fractions each containing two or three glycosides. Subsequent chromatography on silica gel gave fractions of the individual substances. Leontoside A, the least polar of the glycosides, was present only in traces in the initial mixture and was isolated by preparative chromatography on plates with a fixed layer of silica gel (Table 1). The leontosides (separately) were subjected to acid hydrolysis. In all cases, hederagenin was obtained.

The qualitative and rough quantitative compositions of the carbohydrates were determined by paper chromatography with the subsequent colorimetry of the cut-out spots of the individual sugars [5](Fig. 2). The rough molecular weights of leontosides A, B, C, and E were found from the yield of genin on acid hydrolysis (Table 2).

In order to ascertain whether an O-acyl glycosidic bond was present in the glycosides investigated, the initial total leontoside was hydrolyzed with a 10% solution of caustic potash. Analysis of the hydrolyzate in a thin layer of silica gel in system 2 showed only the three less polar leontosides, A, B, and C. The spots of the glycosides D and E had disappeared. Consequently, the latter two glycosides contained an O-acyl glycosidic bond.

In order to confirm the presence of an O-acyl glycosidic bond in the leontosides, each of them was methylated with diazomethane and was then hydrolyzed in 7% sulfuric acid. A chromatographic study of the hydrolysis products showed that the leontosides A, B, and C formed the methyl ether of hederagenin, while leontosides D and E formed hederagenin.

The fact that some of the glycosides of L. eversmannii contain an O-acyl glycosidic bond is also shown by the chromatographic behavior of the acetates of the leontosides in a thin fixed layer of alumina in system 3. The acetates were separated into two groups according to whether a free carboxyl was present or absent: the less polar (the acetates of leontosides D and E) and the more polar (the acetates of leontosides A, B, and C).

Experimental

Paper of types B and M of Leningrad Mill No. 2 and plates with silica gel of type KSK or alumina were used for

chromatography. The following systems of solvents were employed: 1) butan-1-ol–acetic acid–water (4:1:5); 2) butan-1-ol–ethanol–25% ammonia (10:2:5); 3) chloroform–ethanol (25:1). The glycosides were detected by means of a 25% alcoholic solution of phosphotungstic acid and concentrated sulfuric acid. The melting points are uncorrected.

Isolation of the glycoside fraction.

3.7 kg of the air-dry comminuted tubers of L. eversmannii was defatted with boiling acetone (three washings with 4–5 *l* of acetone each time), dried in the air, and extracted with methanol four times at room temperature and once with heating. The combined methanolic extract was evaporated to form a viscous sirup and dried in a vacuum desiccator. Yield 1.6 kg.

To bind the substances of basic nature, a solution of 200 g of the resulting extract in 1 *l* of water was acidified with 10% of sulfuric acid to pH 4–5, and it was then extracted with n-butyl alcohol (six to seven times with 200 ml of alcohol each time). The butanolic extracts were washed with water to neutrality and were concentrated in vacuum at 50°–60° C to near-dryness. The concentrated extract was dissolved in ethanol, and the saponins were precipitated with acetone and petroleum ether. The residue, dried at 50° C, had the form of a light yellow powder. Yield 89.8 g (19% of the weight of the air-dry tubers).

Table 1

Physicochemical Constants of the Triterpene Glycosides from L. eversmannii

Leontoside	State of aggregation	Mp, °C	$[\alpha]_D^{20}$, deg	Acetate	
				mp, °C	$[\alpha]_D^{20}$, deg
A	Crystals from ethanol	276–278	+ 47 ± 1 (c 1.18; pyridine)	166–168	+ 50 ± 1 (c 1.81; chloroform)
B	The same	242–244	+ 49 ± 2 (c 1.12; methanol)	261–262	+ 36 ± 2 (c 1.54; chloroform)
C	The same	222–224	+ 22 ± 2 (c 1.93; methanol)	178–180	+ 20 ± 1 (c 2.39; chloroform)
D	Amorphous powder precipitated from ethanol with acetone	202–204	0 (c 1.51; methanol)	155–157	+ 5 ± 1 (c 3.13; chloroform)
E	The same	186–195	0 (c 1.25; methanol)	154–156	0 (c 2.71; chloroform)

Table 2

Approximate Molecular Weights and Ratios of the Monosaccharides in the Triterpene Glycosides of L. eversmannii

Leontoside	Amount, mg		Molecular weights found	Ratio of the monosaccharides
	glycoside	aglycone		
A	20.0	15.0	630	L-arabinose (1)
B	107.0	62.0	816	L-arabinose
	50.0	30.0	788	D-glucose (1:1)
C	64.0	30.0	1020	L-arabinose D-glucose (1:2)
D	—	—	—	L-arabinose D-glucose L-rhamnose (1:3:1)
E	47.7	16.0	1409	L-arabinose D-glucose L-rhamnose (1:4:1 or 1:4:2)

Acid hydrolysis of the total leontosides.

A solution of 3.0 g of the extract purified in the manner described above in 50 ml of aqueous methanol (1:1) was boiled with 50 ml of 6% sulfuric acid for 8 hr. A crystalline precipitate deposited which was separated off, washed with water, and dried. Yield 1.7 g. The filtrate after the removal of the precipitate of the aglycone was neutralized with barium carbonate (in another experiment, with the anion exchanger EDE-10P, OH^- form), evaporated, and used for the chromatographic analysis of the qualitative sugar composition (cf. Fig. 2).

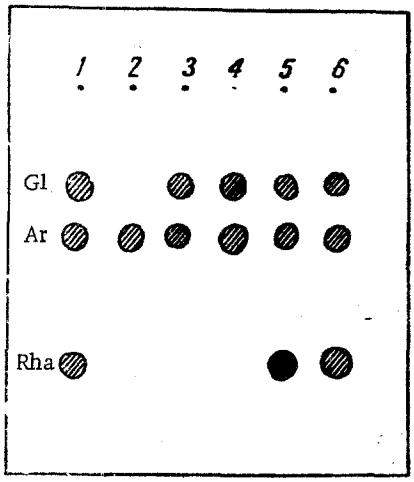

Fig. 2. Paper chromatogram in system 1 of the monosaccharides formed in the hydrolysis of the leontosides; time 80 hr, revealing agent aniline phthalate. 1) Mixture of D-glucose, L-arabinose, and L-rhamnose; 2–6) hydrolyzates of leontosides A, B, C, D, and E, respectively.

Hederagenin. The aglycone obtained in the preceding experiment was repeatedly recrystallized from ethanol using activated carbon. The acicular crystals had mp 326°–328°C, $[\alpha]_D^{20}$ +78 ± 1° (c 2.1; pyridine).

Found, %: C 76.20; 76.30; H 10.30; 10.20. Calculated for $C_{30}H_{48}O_4$, %: C 76.26; H 10.16.

The methyl ether of hederagenin was obtained by methylating the aglycone with an ethereal solution of diazomethane at room temperature for 24 hr; mp 232° C (from ethanol), $[\alpha]_D^{20}$ +74 ± 2° (c 0.60; chloroform). The aglycone was acetylated with acetic anhydride in pyridine at room temperature for 40 hr. The melting point of the diacetate of hederagenin was 169°–170° C (from aqueous ethanol), $[\alpha]_D^{20}$ +67 ± 2° (c 2.96; chloroform).

Found, %: C 72.89; 72.81; H 9.64; 9.54. Calculated for $C_{34}H_{52}O_6$, %: C 73.38; H 9.35.

The diacetate of the methyl ether of hederagenin was obtained by methylating hederagenin diacetate with diazomethane at room temperature. After the elimination of the excess of diazomethane and the evaporation of the ether, the methylation product was dissolved in methanol, boiled with carbon, and twice recrystallized from methanol. This gave crystals in the form of needles with mp 190° C, $[\alpha]_D^{20}$ +63.6 ± 1° (c 2.13; anhydrous ethanol with a few drops of chloroform).

Alkaline hydrolysis of the total leontosides.

10 ml of a 10% solution of caustic potash [water–ethanol (1:1)] was added to 0.1 g of the total leontosides in 20 ml of aqueous methanol, and the mixture was heated at 100° C for 3 hr. Then the cooled reaction mixture was neutralized with KU-1 cation exchanger (H^+ form) and used for chromatography in a thin layer of silica gel in system 2. Of the five glycosides, three were found which corresponded chromatographically to leontosides A, B, and C (cf. Fig. 1).

Separation of the mixture of leontosides.

3.0 g of the total leontosides was transferred to a column of 250 g of cellulose and chromatographed in system 1. Fractions with a volume of 10–15 ml were collected. The composition of the fractions was checked by paper chromatography in system 1 and in a thin layer of silica gel in system 2. Fractions of similar composition were combined. This gave seven fractions containing, respectively, the following mixtures of leontosides: AB, ABC, BC, BCD, BCDE, CDE, and DE. On evaporation in vacuum or on long standing, the fractions containing the mixtures AB, ABC, and BC deposited crystals of leontoside B. The eluates of the other fractions, each evaporated to dryness separately, were rechromatographed on columns of silica gel (ratio of dry residue to weight of silica gel not less than 1:100) in system 2. The BC fraction gave chromatographically homogeneous leontoside C and the DE fraction gave leontoside D and leontoside E. Leontoside A was obtained by the preparative chromatography in system 2 on fixed silica gel of the mother solutions from fractions AB and ABC.

Acid hydrolysis of the individual leontosides.

An accurately weighed sample of leontoside was boiled in a mixture of 7% sulfuric acid and methanol (1:1) for 10 hr. The disappearance of the spot corresponding to the initial leontoside in a thin layer of silica gel in system 2 showed that hydrolysis was complete. The precipitate that deposited was filtered off on to a weighed filter, washed with water, and dried over phosphorus pentoxide. The precipitate from all the leontosides was identified chromatographically as hederagenin. After neutralization with EDE-10P anion exchanger (OH^- form) and evaporation, the hydrolyzate was chromatographed on paper. The spots of the sugars were revealed with aniline phthalate, cut out, and subjected to colorimetry. An idea of the molecular weights and quantitative ratios of the monosaccharides found can be gained from the figures of Table 2.

Acid hydrolysis of the methylated leontosides.

40–50 mg of one of the chromatographically homogeneous leontosides was dissolved in 5 ml of anhydrous ethanol and methylated with an ethereal solution of diazomethane under the usual conditions. The resulting products were hydrolyzed with 8–10 ml of 7% sulfuric acid at the boil for 8 hr. The reaction mixture was extracted with chloroform and the chloroform extracts were chromatographed on a thin layer of silica gel in system 3 (Figs. 3 and 4).

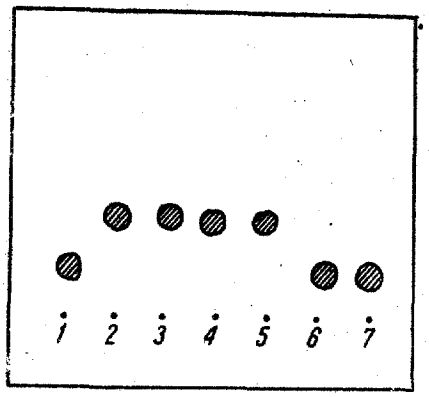

Fig. 3. Chromatogram of the products of the hydrolysis of the leontosides methylated with diazomethane in a thin bound layer of silica gel in system 3. 1) Hederagenin 2) methyl ether of hederagenin; 3–7) the genins of leontosides A, B, C, D, and E, respectively.

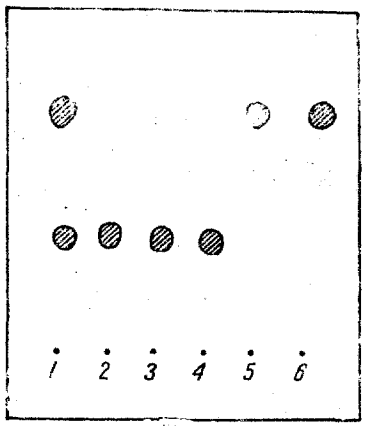

Fig. 4. Chromatogram of the acetates of the leontosides in a thin fixed layer of alumina in system 3; revealing agent concentrated H_2SO_4. 1) Acetates of the total leontosides; 2–6) acetates of leontosides A, B, C, D, and E, respectively.

Preparation of the acetates of the leontosides.

Each leontoside separately was dissolved in dry pyridine, acetic anhydride was added, and the reaction mixture was left at room temperature for 24 hr. After the usual working up, the acetates of leontosides A and B were recrystallized from aqueous ethanol, and the acetates of leontosides C, D, and E were reprecipitated from benzene with petroleum ether (cf. Table 1).

Summary

Triterpene glycosides, which we have called leontosides A, B, C, D, and E, have been isolated from the tubers of Leontice eversmannii Bge. The monosaccharide composition and the approximate quantitative ratio of the sugars in the carbohydrate moiety of each leontoside have been established. In leontosides A, B, and C, the carbohydrate moiety is bound only to the alcoholic group of hederagenin, while in leontosides D and E there is also an O-acyl glycosidic bond to the carboxy group.

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