UDC 547.597+547.918

TRITERPENE GLYCOSIDES OF LEONTICE EVERSMANNII

IV. Structure of Leontoside D

L. G. Mzhel'skaya and N. K. Abubakirov

Khimiya Prirodnykh Soedinenii, Vol. 4, No. 3, pp. 153-158, 1968

Five individual triterpene glycosides—leontosides A, B, C, D, and E—have been isolated from tubers of Leontice. The complete structure of the first three has been established [2, 3]. In the present paper we give the results of a study of the chemical structure of leontoside D (I).

Acid hydrolysis of the glycoside (I) showed that it contains hederagenin (V) as a glycone. The sugar moiety of leontoside D consists of D-glucose, L-arabinose, and L-rhamnose in a ratio of 3 : 1 : 1. Consequently, leontoside D is a hederagenin pentaoside with the empirical formula $C_{59}H_{96}O_{27}$, as was confirmed by the results of the elementary analysis of the full acetate (II)—$C_{91}H_{128}O_{43}$—and the product of exhaustive methylation (III)—$C_{75}H_{128}O_{27}$.

The hydrolysis of leontoside D previously treated with diazomethane gave hederagenin (V) and not its methyl ester. This shows the presence of a glycosidic carbohydrate chain attached to the genin by an O-acyl glycosidic bond. The results of the alkaline cleavage of the pentaoside (I) with Dowex-1 (OH^- form) confirmed our assumption [4]. On alkaline hydrolysis, the whole of the leontoside B was converted into a crystalline glycoside identical with leontoside B (IV) [2] in chromatographic behavior, melting point, specific rotation, and IR spectrum. The aqueous hydrolysate was found to contain an oligosaccharide which, as was shown by subsequent acid hydrolysis, consisted of two molecules of D-glucose and one molecule of L-rhamnose. Thus, the carbohydrate chain attached to the carboxy group of the genin is a trisaccharide.

We obtained further information on the structure of the glycoside through its exhaustive methylation by Kuhn's method [5]. The chromatographically homogeneous methylation product (III) was hydrolyzed with perchloric acid. The following methylated monosaccharides were identified in the hydrolysate: 2, 3, 4, 6-tetra-O-methyl-D-glucopyranose, 2, 3-di-O-Methyl-L-arabopyranose, 2, 3, 4-tri-O-methyl-D-glucopyranose, 2, 3, 6-tri-O-methyl-D-glucopyranose, and 2, 3, 4-tri-O-methyl-L-rhamnopyranose. Since in the hydrolysis products of completely methylated leontoside B (IV) we found 2, 3, 4, 6-tetramethylglucose and 2, 3-dimethylarabinose [2], it follows that the methylated trisaccharide attached to the carboxy group of hederagenin includes 2, 3, 4-trimethylglucose, 2, 3, 6-trimethylglucose, and 2, 3, 4-trimethylrhamnose. The information obtained also shows that the L-rhamnose occupies the end position in the sugar chain.

Substance	$[\alpha]_D^{20}$	$[M]_D$
	degrees	
Leontoside D	0±2	0±24
Leontoside B	+49±2	+381±15
Trisaccharide contribution to $[M]_D$		−381±39
Kalopanax saponin B [7]	−21	−255
Kalopanax saponin A [7]	+13.5	+102
Trisaccharide contribution to $[M]_D$		−357
Asiaticoside [6]	−14	−134
Asiatic acid	+51	+249
Trisaccharide contribution to $[M]_D$		−383

To elucidate the question of which of the two molecules of trimethylglucose is attached directly to the carboxy group of the sapogenin, we carried out the reductive cleavage of the O-acyl glycosidic bond of permethylated leontoside D with lithium aluminium hydride. A methylated bioside (VIII) and the product of the reduction of the methylated trisaccharide (VII) were obtained. After hydrolysis, the bioside gave 23-methoxyerythrodiol (IX), 2, 3, 4, 6-tetramethylglucose, and 2, 3-dimethylarabinose, while compound (VII) gave 2, 3, 4-tri-O-methylsorbitol, 2, 3, 6-trimethylglucose, and 2, 3, 4-trimethylrhamnose. The disappearance of 2, 3, 4-tri-O-methyl-D-glucose from the hydrolysis products and the appearance of the corresponding trimethylsorbitol showed that it is just this molecule of trimethylglucose that is attached to the hederagenin.

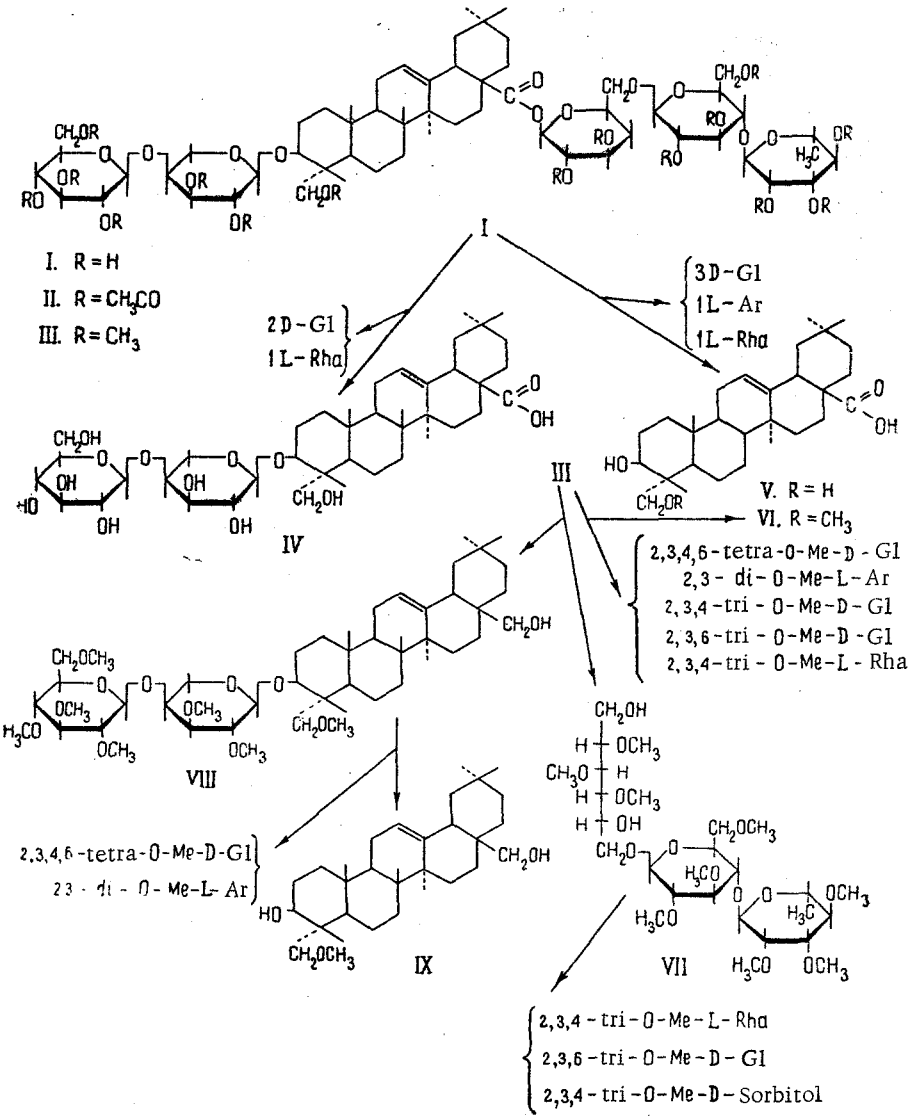
The contribution of the molecular rotation of the trisaccharide residue in leontoside D coincided (table) with the rotation of the trisaccharide L-Rha-(1 $\xrightarrow{\alpha}$ 4)-D-Gl-(1 $\xrightarrow{\beta}$ 6)-D-Gl-(1 $\xrightarrow{\beta}$) in asiaticoside from Centella asiatica [6] and Kalopanax saponin B [7] from Kalopanax septemlobum. Consequently, the O-acyl glycoside moiety of leontoside D not only has the same sugars in the same sequence as in the two glycosides mentioned but also the same configuration of the glycosidic centers, i.e., it is α-L-rhamnopyranosido-(1 → 4)-β-D-glucopyranosido-(1 → 6)-β-D-glucopyranose.

The question of the identity of leontoside B (IV) and hederacoside A [8] remains open. Tschesche et al. [9] were unable to detect hederacoside A in any of the samples of saponins from Hedera helix that they investigated. Instead of it they found α-hederin, identical with Kalopanax saponin A [7] which has the structure of hederagenin 3-α-L-ara-

bopyranosido-2-α-L-rhamnopyranoside. It is possible that it is just the absence of hederacoside A from the plant samples studied that also explains the failure of our attempt to compare by chromatography the glycosides of ivy (Hedera) and Leontice [2].

Experimental

The chromatography was carried out with type KSK silica gel, alumina (activity II-III), paper of type M from the Leningrad No. 2 paper mill, and the following solvent systems: 1) 1-butanol—ethanol—concentrated ammonia (10 : 2 : 5), 2) chloroform—ethanol (25 : 1), 3) toluene—ethanol (9 : 1), 4) methylethyl ketone saturated with a 1% solution of ammonia, and 5) 1-butanol—acetic acid—water (4 : 1 : 5). The glycosides were stained with a 25% solution of phosphomolybdic acid in ethanol, the methylated glycoside derivatives with concentrated sulfuric acid, and the sugars and their derivatives with aniline phthalate. The IR spectra were recorded on a UR-10 spectrophotometer.



Leontoside D (I) (for preparation, see [1]) formed a chromatographically homogeneous colorless amorphous substance with mp 202–204° C (from a mixture of ethanol and acetone), $[\alpha]_D^{20}$ 0 ± 2° (c 1.51; methanol).

Leontoside D hexadeca-O-acetate (II) formed a white amorphous powder with mp 155–157° C (precipitated with petroleum ether from benzene solution), $[\alpha]_D^{20}$ + 5 ± 2° (c 3.13; chloroform). The IR spectra of the substance had no band for a free hydroxyl (3400 cm^{-1}) while the absorption for ester groups (1260 cm^{-1}) was clearly expressed.

Found, %: C 56.90, 56.96; H 6.87, 6.82. Calculated for $C_{91}H_{128}O_{43}$, %: C 57.23; H 6.70.

Acid hydrolysis of leontoside D (I). Seventy-five milligrams of the glycoside was hydrolyzed with 7% sulfuric acid at the boiling point of the reaction mixture for 8 hr. The completeness of the hydrolysis was checked in a thin fixed layer of silica gel in system 1. The crystalline precipitate that deposited was filtered off and was twice recrystallized from aqueous ethanol. This gave crystals with mp 328°C, $[\alpha]_D^{20}$ + 78 ± 1° (c 2.11; pyridine), which were identified

as hederagenin (V) by chromatography on silica gel in systems 1-3 and by a mixed melting point. The hydrolysate was neutralized with EDE-10 P anion-exchanger (OH^- form), evaporated to a volume of 1 ml, and chromatographed quantitatively on paper in system 5. The spots of the sugars were revealed with aniline phthalate, and they were then cut out, and eluted with glacial acetic acid. By photocolorimetry it was found that the ratio of D-glucose to L-arabinose to L-rhamnose in the eluates was 3 : 1 : 1.

Action of diazomethane on leontoside D. Thirty milligrams of the glycoside in 3 ml of methanol was methylated with an ethereal solution of diazomethane under the usual conditions. After the hydrolysis of the product obtained with 6% sulfuric acid for 8 hr, a crystalline substance was isolated which was found by chromatography in systems 1 and 2 to be identical with hederagenin (V).

Partial alkaline hydrolysis of leontoside D (I) to leontoside B (IV). A solution of 150 mg of the glycoside in 5 ml of water was transferred to a column containing 4 g of Dowex-1 × 2 (100/200 mesh) anion-exchanger in the OH^- form. After 16 hours' standing at room temperature, the sugar was eluted from the column with water and further elution was carried out with a 10% solution of acetic acid in methanol. The aqueous solution was evaporated to dryness in vacuum and the residue was hydrolyzed with 2% hydrochloric acid for 2 hr. The hydrolysate was neutralized with the same anion exchanger and concentrated. By paper chromatography in system 5, D-glucose and L-rhamnose were identified.

On evaporation, the methanolic acetic acid solution deposited a crystalline precipitate which, after recrystallization from anhydrous ethanol, had mp 243-244° C, $[\alpha]_D^{20}$ + 49.6 ± 1.6° (c 1.24; methanol). On hydrolysis with 7% sulfuric acid, this substance gave hederagenin, D-glucose, and L-arabinose. Chromatography on a thin layer of silica gel in systems 1 and 5 and a mixed melting point showed that the substance obtained was identical with leontoside B. The IR spectra of the bioside from leontoside D and an authentic sample of leontoside B coincided.

Methylation of leontoside D. A solution of 300 mg of the glycoside in 5 ml of dry dimethylformamide was treated with 5 ml of methyl iodide and, with stirring, 1.6 g of silver oxide was added to the mixture [5]. Methylation with the addition of fresh portions of the reagents was carried out until the bulk of the leontoside D had been methylated (35 hr). The course of the methylation process was checked in a thin fixed layer of alumina in system 3. After the end of the reaction, the precipitate of oxide and iodide was filtered off, and the solution containing the methylated product was evaporated to dryness.

The product obtained was purified on a column of alumina, being eluted first with pure benzene and then with a mixture of benzene and ethanol with a gradient of concentrations of ethanol increasing from 1 to 10%. The yield of hexadeca-O-methylleontoside D (III) was 210 mg. The substance formed a colorless amorphous powder with mp ~102° C, $[\alpha]_D^{20}$ − 8.7 ± 1.5° (c 1.36; chloroform). The IR spectrum of methylated leontoside D had no absorption band for a free hydroxyl.

Found, %: OCH_3 32, 43. Calculated for $C_{75}H_{128}O_{27}$, %: 15 OCH_3 31.84; 16 OCH_3 31.97.

Hydrolysis of hexadeca-O-methylleontoside D (III). Eighty milligrams of the product isolated in the preceding experiment was heated in 4 ml of a mixture of methanol and 42% perchloric acid (5 : 1) at the boiling point of the reaction mixture for 10 hr. The completeness of the hydrolysis was checked in a thin layer of silica gel and alumina in systems 2 and 3. After the end of the reaction, the mixture was diluted with water (threefold) and the precipitate of genin was filtered off. The hydrolysate was evaporated to a volume of 4 ml and was heated in the boiling water bath for 3 hr to hydrolyze the methyl glycosides of the methylated monosaccharides. After cooling, the solution was neutralized with the anion exchanger Dowex-1 (OH^- form). The column containing the exchanger was washed exhaustively with 50% aqueous methanol, and the combined aqueous methanolic solution was evaporated in vacuum to the state of a sirup. On paper chromatography in a system 4 in the presence of authentic samples, the following methylated sugars were identified: 2,3,4,6-tetra-O-methyl-D-glucose, 2,3-di-O-methyl-L-arabinose, 2,3,4-tri-O-methyl-D-glucose, 2,3,6-tri-O-methyl-D-glucose, and 2,3,4-tri-O-methyl-L-rhamnose.

By chromatography in a thin fixed layer of silica gel in system 3, the genin obtained in this experiment was identified as 23-O-methylhederagenin (VI)—the product of the alkaline hydrolysis of the methyl ester of 23-O-methylhederagenin [2].

Reduction of hexadeca-O-methylleontoside D (III). Seven milliliters of a freshly-prepared 2.5% ethereal solution of lithium aluminum hydride was added to 70 mg of compound III in 7 ml of absolute tetrahydrofuran. The mixture was heated to the boil for 8 hr. The excess of lithium aluminum hydride was decomposed with ethyl acetate. Then 1 N sulfuric acid was added to the reaction mixture until the precipitate had dissolved completely, the tetrahydrofuran layer was separated off, and the aqueous layer was extracted with ether (4 × 10 ml). The combined ethereal solution was washed with sodium bicarbonate solution and with water and was dried over anhydrous sodium sulfate and evaporated to dryness. This gave 40 mg of the bioside (VIII). The aqueous layer was extracted with a mixture of chloroform and ethanol (1 : 1), the solution was washed with water, and the solvent was distilled off in vacuum to leave ~25 mg of the reduced methylated trisaccharide (VII).

Hydrolysis of the methylated bioside (VIII). Compound VIII was hydrolyzed under the same conditions as the methylated glycoside (III). By paper chromatography in system 4, 2,3,4,6-tetramethylglucose and 2,3-dimethylarabinose were identified. The precipitate of sapogenin, which was 28-hydroxy-23-methoxy-β-amyrin (IX) was recrystallized from ethanol. The crystals obtained had mp 190° C, $[\alpha]_D^{20}$ + 56 ± 3° (c 1.07; chloroform). The IR spectrum of compound (IX) showed the absence of carbonyl absorption in the 1700–1760 cm^{-1} region and the presence of the band of hydroxy groups at 3400–3500 cm^{-1}.

Found, %: C 78.60; H 10.95. Calculated for $C_{31}H_{52}O_3$, %: C 78.81; H 11.01.

Hydrolysis of the methylated trisaccharide (VII). The reduced trisaccharide was hydrolyzed under conditions analogous to those for the hydrolysis of compound (III). 2,3,6-Trimethylglucose, 2,3,4-trimethylglucose, and 2,3,4-trimethylsorbitol were identified by paper chromatography in system 4 in the presence of the appropriate reference samples. The sorbitol derivative was revealed by periodate in system 4.

Conclusions

The structure of leontoside D—a hederagenin pentaoside—has been established. The O-glycosidic moiety of leontoside D is O-β-D-glucopyranosido-(1 → 4)-O-α-L-arabopyranose, and the O-acyl moiety is O-α-L-rhamnopyranosido-(1 → 4)-O-β-D-glucopyranosido-(1 → 6)-O-β-D-glucopyranose.

REFERENCES

1. L. G. Mzhel'skaya, V. K. Yatsyn, and N. K. Abubakirov, KhPS [Chemistry of Natural Compounds], 2, 421, 1966.
2. L. G. Mzhel'skaya and N. K. Abubakirov, KhPS [Chemistry of Natural Compounds], 3, 101, 1967.
3. L. G. Mzhel'skaya and N. K. Abubakirov, KhPS [Chemistry of Natural Compounds], 3, 218, 1967.
4. V. G. Bukharov, V. V. Karlin, and V. A. Talan, KhPS [Chemistry of Natural Compounds], 3, 17, 1967.
5. R. Kuhn, H. Trischmann, and I. Low, Angew. Chem., 67, 32, 1955.
6. J. Polonsky, E. Sach, and E. Lederer, Bull. Soc. Chim. France, 880, 1959.
7. A. Ya. Khorlin, A. G. Ven'yaminova, and N. K. Kochetkov, DAN SSSR, 155, 619, 1964; Izv. AN SSSR, ser. khim., 1447, 1964; 1588, 1966.
8. J. J. Scheidegger and E. Cherbuliez, Helv. Chim. Acta, 38, 547, 1955.
9. R. Tschesche, W. Schmidt, and G. Wulff, Z. Naturforsch., 208, 708, 1965.

27 February 1967

Institute of the Chemistry of Plant Substances AS UzSSR