UDC 547.597 + 547.918

TRITERPENE GLYCOSIDES OF LEONTICE EVERSMANNII

V. The Structure of Leontoside E

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Khimiya Prirodnykh Soedinenii, Vol. 4, No. 4, pp. 216–221, 1968

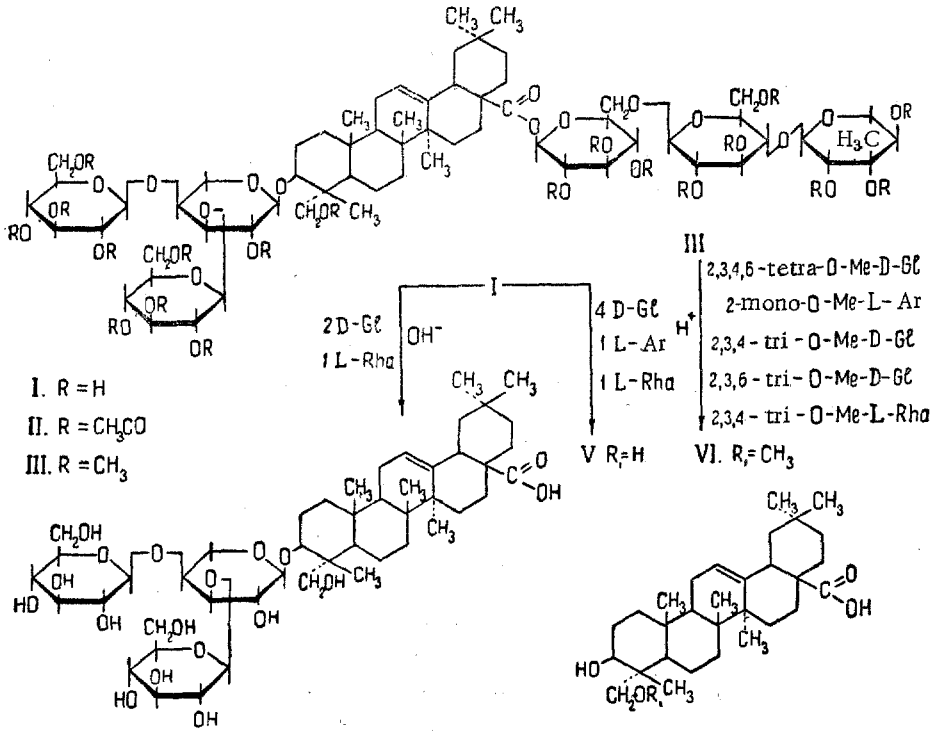
Leontoside E, $C_{65}H_{106}O_{32}$ **(I)**, is the most polar of the glycosides of Leontice [1]. The acid hydrolysis of this glycoside has shown that it consists of hederagenin, D-glucose, L-arabinose, and L-rhamnose in a ratio of 1 : 4 : 1 : 1, i.e., it is a hederagenin hexaoside.

The hydrolytic cleavage of leontoside E (I) methylated with diazomethane led to the formation of hederagenin (V) and not its methyl ether. Consequently, in the compound under investigation there is a carbohydrate chain attached to the carboxy group of the sapogenin.

In order to simplify the proof of the structure of the glycoside (I) and simultaneously to determine the composition of the acyl part of its molecule, we have performed the alkaline hydrolysis of the ester bond using the anion-exchanger Dowex-1 (OH^- form) [2]. An oligosaccharide consisting, according to the results of hydrolysis, of D-glucose and L-rhamnose was split off. Together with the saccharide we isolated a glycoside identical with leontoside C (IV) [3]. The convertion of leontoside E into leontoside C showed that the carbohydrate chain of leontoside E attached by a O-glycosidic bond is branched and has the structure of O-β-D-glucopyranosido (1→4)-O-β-D-glucopyranosido (1→3)-α-L-arabopyranoside.

To determine the order of attachment of the monosaccharides in the acyl part of the molecule, leontoside E was fully methylated and the compound obtained (III) was hydrolyzed with perchloric acid. In the reaction products we identified 23-O-methylhederagenin (VI) and the following methylated monosaccharides: 2, 3, 4, 6-tetra-O-methyl-D-glucopyranose, 2-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.

The first two methylated sugars have been found in the products of the hydrolysis of permethylated leontoside C and, consequently, were formed from the carbohydrates of the O-glycosidic moiety of the leontoside E molecule. The last three sugars must be ascribed exclusively to the carbohydrates of the O-acyl moiety of the molecule; they were found previously in the products of the hydrolysis of completely methylated leontoside D [3]. Hence, the carbohydrate chain attached to the carboxy group of the hederagenin in leontoside E has the same structure as in leontoside D. The structure of leontoside E is expressed by the structural formula (I).



The carbohydrate chain L-Rha(1 → 4)D-Gl (1 → 6)D-Gl has been found as the acyl residue in the glycosides of four plant genera: in asiaticoside from Centella (Hydrocotyle) asiatica (L.) Urban. (family Umbelliferae) [4], in kalopanax-saponin B from Kalopanax septemlobum (Thunb.) Koidz. (family Araliaceae) [5], in hederasaponins B and C from Hedera helix L. (family Araliaceae) [6], and now in leontosides D and E from Leontice eversmannii Bge. (family Berberidaceae). While the first three plants are related to one another to some extent phylogentically, this does not apply to L. eversmannii. Apparently, moreover, the aglycone moiety has no decisive significance and is represented by sapogenins both of the α-amyrin series (asiatic acid) and of the β-amyrin series (hederagenin, oleanolic acid).

Leontoside A: L-Ara1(α)→R—H

Leontoside B: D-Gl(1(β)→4) L-Ara1(α)→R—H

Leontoside C: D-Gl(1(β)→4) L-Ara1(α)→R—H, 3(β)↑1D-Gl

Leontoside D: D-Gl(1(β)→4) L-Ara1(α)→R(β)←1D-Gl(6(β)←1)D-Gl(4(α)←1) L-Rha

Leontoside E: D-Gl(1(β)→4)L-Ara 1(α)→R(β)←1D-Gl(6(β)←1)D-Gl(4(α)←1) L-Rha, 3(β)↑1D-Gl

R—hederagenin residue

As a result of the establishment of the structure of five successively more complicated molecules of triterpene glycosides with the same aglycone it has become possible to consider some questions of a biogenetic nature. The increase in the size of the carbohydrate chain in the sequence leontoside A → leontoside B → leontoside C takes place by the addition of one sugar molecule in the O-glycosidic chain of carbohydrates each time. In counterbalance to this complication, in the pairs leontoside B–leontoside D and leontoside C–leontoside E, i.e., in the transition from glycosides of a comparatively simple structure to oligosides [7] a whole trisaccharide residue—L-Rha(1 → 4)D-Gl(1 → 6)D-Gl-1—is introduced by the esterification of the carboxy group. The hypothetical intermediate compounds esterified by monoses and bioses have not been detected. These observations permit the hypothesis that the O-acyl glycosides are formed in the plants most frequently by the direct addition to the carboxy group of a large oligosaccharide molecule. It is possible that carbohydrate fragments of this type will be encountered frequently in the triterpene saponins.

With an increase in the number of glycosides containing carbohydrate chains attached to the carboxy and the alcohol groups of the genin, the necessity arises for seeking appropriate terms enabling them to be distinguished from the ordinary glycosides formed by the combination of the semiacetal hydroxyl with the alcoholic hydroxyl. For brevity, we recommend that such compounds should be called acylosides.

Experimental

Chromatography was carried out with type KSK silica gel prepared by the usual method, alumina (activity grade II–III), type M paper of 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) 1-butanol–acetic acid–water (4 : 1 : 5); 4) toluene–ethanol (9 : 1); and 5) methyl ethyl ketone saturated with 1% ammonia solution. The glycosides were revealed with phosphotungstic acid and the sugars and their derivatives with aniline phthalate.

Leontoside E (I). (For preparation, see [1]). The substance was chromatographically homogeneous and had mp 186–195°C (reprecipitated from ethanol with acetone), $[\alpha]_D^{20}$ 0 ± 3°(c 1.25; methanol).

Leontoside E nonadeca-O-acetate (II). The substance was obtained by the usual method. The completeness of the acetylation was followed from the disappearance of the spot of the initial compound on a fixed layer of alumina in system 2 and by the IR spectrum. The melting point of the substance obtained was 154–156° C (reprecipitated with petroleum ether from benzene), $[\alpha]_D^{20}$ 0 ± 3° (c 2.7; chloroform).

Found, %: C 56.17, 56.30; H 7.05, 6.85. Calculated for $C_{103}H_{144}O_{51}$, %: C 56.27; H 6.60.

Acid hydrolysis of leontoside E (I). A solution of 47 mg of the substance in 2 ml of methanol was treated with 3 ml of 10% sulfuric acid and boiled for 10 hr. The hydrolysis was complete when the spot corresponding to the initial glycoside in a thin layer of silica gel in systems 1 and 2 disappeared. The yield of the genin, worked up as far as possible without losses, was 16 mg, mp 326–328° C.

Found: mol. wt. 1409. Calculated for $C_{65}H_{106}O_{32}$: mol. wt. 1399.6.

The hydrolysate was neutralized with EDE-10P anion-exchanger (OH^- form), and after evaporation the solution was brought to a volume of 1 ml and was chromatographed quantitatively on paper in system 3. The spots of the sugars were revealed with aniline phthalate, cut out, eluted with glacial acetic acid, and subjected to colorimetry. It was found that D-glucose, L-arabinose, and L-rhamnose were present in a ratio of 4 : 1 : 1,

Methylation of leontoside E with diazomethane. A suspension of 40 mg of the glycoside in ether was methylated with an ethereal solution of diazomethane under the usual conditions and was then hydrolyzed with 7% sulfuric acid. The precipitate obtained was identified with chromatography in a thin layer of silica gel in systems 1 and 2 as hederagenin (V).

Alkaline hydrolysis of leontoside E (I) to leontoside C (IV). A solution of 300 mg of the glycoside in 3 ml of water was transferred to a column containing 8 g of Dowex-1 × 2 anion-exchanger (100–200 mesh) in the OH^- form and was left at 20–22° C for 18 hr. The carbohydrate fraction was eluted from the column with water (until the reaction for sugars was negative) after which elution was continued with a 10% solution of acetic acid in methanol.

The aqueous solution was evaporated to dryness in vacuum. The residue was hydrolyzed with 2% hydrochloric acid, and the hydrolysate was neutralized and evaporated. By paper chromatography in system 3, the residue was shown to contain D-glucose and L-rhamnose.

When the methanolic acetic acid solution was evaporated, crystals deposited with mp 222–224° C (from ethanol) $[\alpha]_D^{20}$ + 24 ± 2° (c 2.15; methanol). A chromatographic comparison of the glycoside obtained and leontoside C isolated previously from the plant in a thin layer of silica gel in systems 1 and 3 showed that they were identical. A mixture showed no depression of the melting point. The IR spectra coincided. The glycoside formed was hydrolyzed with 7% sulfuric acid, and hederagenin, D-glucose, and L-arabinose were found.

The glycoside (100 mg)—the product of the alkaline cleavage of leontoside E—was methylated under the conditions for the methylation of leontoside D [3]. This gave 80 mg of permethylate, which was hydrolyzed. 2, 3, 4, 6-Tetramethylglucose and 2-methylarabinose were detected in the hydrolysis products by paper chromatography in system 5. The methyl glycoside of the latter gave a violet coloration with dimethylaniline trichloroacetate [8] and reacted with Bonner's periodate reagent for an α-glycol grouping [9].

Methylation of leontoside E (I). With gentle heating (30–40° C), 110 mg of substance was dissolved in 2 ml of dimethylformamide that had been dried over BaO and distilled over P_2O_5, and to this solution were added 2 ml of methyl iodide and, with stirring, over 20 min, 0.75 g of silver oxide. Methylation with the addition of fresh portions of reagents took 35 hr. The course of the reaction was followed on a thin layer of alumina in system 4 from the disappearance of the spot of the initial glycoside and of the products of partial methylation. Then the precipitate of silver oxide and iodide was separated off and washed with chloroform. The chloroform extracts were combined with the main filtrate and, after being washed with saturated aqueous sodium thiosulfate solution and water, were dried with sodium sulfate and evaporated to dryness. The residue was dissolved in benzene, and the solution was filtered through a layer of alumina and evaporated. This gave 80 mg of colorless chromatographically homogeneous leontoside E permethylate with a diffuse melting point (125–135° C). The IR spectrum of this substance contained no absorption band for hydroxy groups.

Hydrolysis of nonadeca-O-methylleontoside E (III). Seventy milligrams of the product of the preceding experiment was heated for 10 hr in 3 ml of a mixture of methanol and 42% perchloric acid (5 : 1) at the boiling point of the reaction mixture. The completeness of the hydrolysis was followed by thin-layer chromatography on alumina in system 4. After the end of the hydrolysis, the reaction mixture was diluted threefold with water, and the genin that precipitated was filtered off and was identified by chromatography on silica gel in system 4 as 23-O-methylhederagenin (IV).

The hydrolysate was evaporated to a volume of 3 ml and heated at 100° C for 2 hr in order to hydrolyze the methyl glycosides of the methylated monosaccharides. The solution was diluted with water, neutralized in a column containing Dowex-1 anion-exchanger (OH^- form), the column was washed with 50% aqueous methanol (total volume) 200 ml), and the solution was concentrated in vacuum to a syrupy consistency. The residue was chromatographed on paper in system 5, and 2, 3, 4, 6-tetra-O-methyl-D-glucose, 2-O-methyl-L-arabinose, 2, 3, 4-tri-O-methyl-D-glucose, and 2, 3, 6-tri-O-methyl-L-rhamnose were identified.

Conclusions

The structure of leontoside E has been established as a hexaoside of hederagenin. Its O-glycosidic moiety is branched and consists of O-β-D-glucopyranosido(1 → 4)-O-β-D-glucopyranosido(1 → 3)-O-α-L-arabopyranose and its O-acyl glycosidic moiety is O-α-L-rhamnopyranosido(1 → 4)-O-β-D-glucopyranosido(1 → 6)-O-β-D-glucopyranose.

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27 March 1967

Institute of the Chemistry of Plant Substances AS UzSSR