TRITERPENE GLYCOSIDES OF Ladyginia bucharica

IV. STRUCTURE OF LADYGINOSIDES D AND F

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From the roots of Ladyginia bucharica Lipsky, in addition to ladyginosides A, B, and C, the structures of which we have established previously [1, 2], we have isolated more complex triterpene glycosides – ladyginosides D and F. The present paper gives information leading to a proof of their structures.

It was found by complete acid hydrolysis that both glycosides contain hederagenin as the aglycone. The results of a chromatographic study of the hydrolyzates showed that the qualitative sets of monosaccharides for D and F are also the same, consisting of D-glucose, D-galactose, and D-glucuronic acid (in a previous paper [1], D-galactose was erroneously omitted for ladyginoside D). It has been found by gas–liquid chromatography that in ladyginoside D these sugars are present in a ratio of 3:1:1 and in ladyginoside F 5:1:1. Thus, glycoside D is a pentaoside and F an heptaoside of hederagenin.

In both cases, the hydrolysis of ladyginosides D and F previously treated with diazomethane gave hederagenin, and not its methyl ester. Consequently, in both glycosides a carbohydrate chain is attached to the carboxy group of heragenin.

The mild alkaline cleavage of both ladyginosides D (I) and F (III) with AV-17 anion-exchange resin (OH^- form) led to the isolation of the same crystalline glycoside which was identified by its melting point, specific rotation, and migration in a thin layer of silica gel as ladyginoside B (II) [1]. It follows from this that the carbohydrate chains attached to the hydroxy group at C_3 of the aglycone have the same structure in the two glycosides, namely β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranuronic acid.

The similarity of the structures of ladyginosides D and F is also shown by the partial acid hydrolysis of the glycoside F: among the products of hydrolytic cleavage were found gentiobiose (IV) and a glycoside identical in chromatographic behavior with ladyginoside D (I).

On the basis of the results of the alkaline cleavage of the glycosides, the acyloside carbohydrate chain of ladyginoside D must be a trisaccharide and that of F a pentasaccharide differing from the trisaccharide of glycoside D by a molecule of gentiobiose.

On periodate oxidation, the carbohydrate chains of both glycosides decomposed completely, and therefore there is no 1→3 bond between the monosaccharides in ladyginosides D and F.

Further information on the structures of the two glycosides was obtained by their exhaustive methylation using Hakomori's method [3]. On hydrolysis with sulfuric acid, the chromatographically homogeneous completely methylated ladyginoside D gave 23-O-methylhederagenin and the following methylated monosaccharides: 2,3,4,6-tetra-O-methyl-D-glucose, 2,3-di-O-methyl-D-glucuronic acid (the accurate assignment of the latter was established as a consequence of reductive cleavage), 2,3,4-tri-O-methyl-D-glucose, 2,3,6-tri-O-methyl-D-glucose, and 2,3,4,6-tetra-O-methyl-D-galactose. The first two methylated compounds correspond to the structure of ladyginoside B [1] and were obtained from a disaccharide attached by an O-glycosidic link. The other three methylated monosaccharides were formed from the sugar chain attached to the carboxy group of the genin. It was established simultaneously that this chain has a linear structure and the terminal sugar in it is D-galactose.

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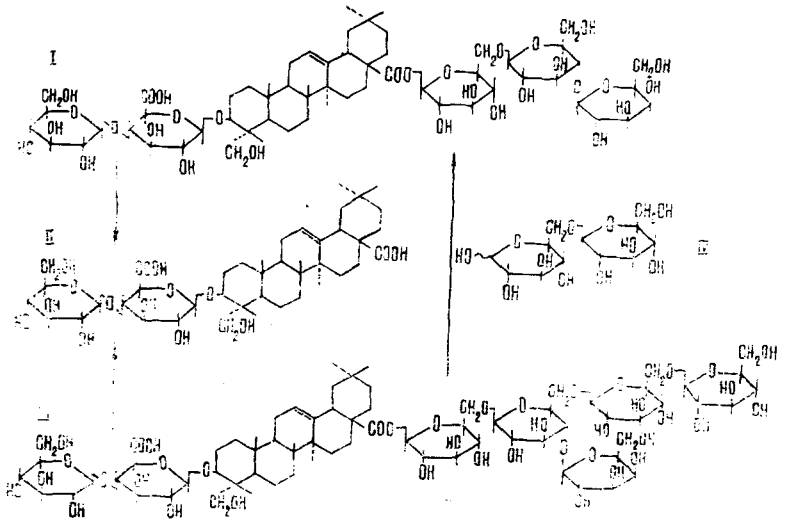
To determine the sizes of the oxide rings and the order of mutual attachment of the two molecules of D-glucose in the acyloside moiety of ladyginoside D, the methylated glycoside D was subjected to reductive cleavage with lithium tetrahydroaluminate. This reaction gave reduced methylated ladyginoside B and a reduced methylated trisaccharide. The sugar moiety of the bioside yielded 2,3,4,6-tetra-O-methyl-D-glucose and 2,3-di-O-methyl-D-glucose. The trisaccharide yielded 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,6-tri-O-methyl-D-glucose, and 2,3,4-tri-O-methyl-D-sorbitol. The appearance of the latter instead of 2,3,4-tri-O-methyl-D-glucose confirmed that D-glucose is directly attached to the carboxy group and to it a second molecule of D-glucose is attached by a 1 → 6 bond, and the D-galactose is attached to the middle molecule of D-glucose by a 1 → 4 bond.

Thus, the trisaccharide is β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranose, and the structure of ladyginoside D corresponds to formula I.

Among the products of the hydrolytic cleavage of methylated ladyginoside F, in addition to the monosaccharides mentioned for glycoside D, 2,3-di-O-methyl-D-glucose and an additional amount of 2,3,4,6-tetra-O-methyl-D-glucose were found.

The results of a quantitative determination of the sugars in ladyginoside F showed that the pentasaccharide of the acyloside moiety consists of four molecules of D-glucose and one molecule of D-galactose. The permethylate of the glycoside F was subjected to reductive degradation under conditions similar to those for ladyginoside D. The reduced methylated pentasaccharide was hydrolyzed, and the following sugar derivatives were identified in the hydrolysis products: 2,3,4-tri-O-methylsorbitol, 2,3,4-tri-O-methyl-D-glucose, 2,3-di-O-methyl-D-glucose, 2,3,4,6-tetra-O-methyl-D-glucose, and 2,3,4,6-tetra-O-methyl-D-galactose. The appearance of 2,3,4-tri-O-methylsorbitol showed that the corresponding glucose molecule is attached to the carboxy group of the hederagenin. The presence of completely methylated D-glucose and D-galactose shows that they each have a terminal position and that branching exists in the pentasaccharide. The center of branching is D-glucose substituted at the C_4 and C_6 hydroxyls, the terminal D-galactose and gentiobiose being attached at these positions.

In view of the fact that among the products of the partial hydrolysis of ladyginoside F we found gentiobiose and ladyginoside D, it may be concluded that gentiobiose is attached to the center of branching at the C_6 hydroxyl and D at the C_4 hydroxyl. On the whole, the structure of ladyginoside F can be represented by formula (III).



The results of a comparison of molecular-rotation differences between ladyginosides D and B with the theoretically calculated values of $[M]_D$ for the anomeric forms of the trisaccharide show that these magnitudes are comparable only if the glycosidic bonds between the monosaccharides have the β configuration (absolute difference 92°). The increment of the molecular rotation of ladyginosides F and D agrees with literature data [4] for methyl β-gentiobioside (Table 1). Consequently, in ladyginoside F, as well, all the monosaccharides are connected by β linkages.

TABLE 1

Substance	$[\alpha]_D$, deg	M	$[M]_D = \frac{[\alpha]_D \cdot M}{100}$
Ladyginoside D	+ 12,0	1297	+155,6
Ladyginoside B	+ 22,5	811	+182,5
Proportion of $[M]_D$ of the trisaccharide in ladyginoside D	—	—	— 26,9
Methyl α-D-glucopyranoside [4]	+158,9	194	+308
Methyl β-D-glucopyranoside [4]	— 34,2	194	— 66
Methyl α-D-galactopyranoside [4]	+195	194	+378
Methyl β-D-galactopyranoside [4]	+2,6	194	+5
Calculated			
D-Glcp-D-Glcp-D-Galp			
β β β	—	—	—127
β β α	—	—	+246
α α β	—	—	+690
α α α	—	—	+1034
Ladyginoside F	+5	1621	+ 81
Proportion of $[M]_D$ of gentiobiose in ladyginoside F	—	—	— 74,6
Methyl β-gentiobioside [4]	—36	356	—128
Methyl α-gentiobioside [4]	+61,8	356	+219,7

EXPERIMENTAL

Chromatography was performed with type KSK silica gel, alumina of activity grade II, type "M" ["slow"] of the Leningrad No. 2 paper mill, and the following solvent systems for thin-layer chromatography (TLC): 1) butan-1-ol–ethanol–25% ammonia (10 : 2 : 5); 2) chloroform–methanol–water (65 : 35 : 8); 3) chloroform–methanol (25 : 1); 4) butan-1-ol–acetone–water (4 : 5 : 1); and 5) benzene–acetone (2 : 1); and for paper chromatography (PC): 6) butan-1-ol–pyridine–water (8 : 4 : 3); 7) butan-1-ol–ethanol–water (4 : 1 : 5); and 8) water-saturated methyl ethyl ketone. The glycosides and genins were revealed with an alcoholic solution of phosphotungstic acid and the sugars with o-toluidine salicylate. The quantitative determination of the monosaccharides in the form of silylated methyl glycosides was performed on a UKh-1 chromatograph with a copper column (1 m × 4 mm) filled with 5% of the silicone phase g-30M on Diaforit (0.2–0.315 mm) at a column temperature of 170°C with hydrogen as the carrier gas at a rate of flow of 55 ml/min [5].

Isolation of Ladyginosides D and F. The combined glycosides soluble in n-butanol (10 g) were separated into preliminary fractions as described previously [2]. The mixture containing ladyginosides CD and DEF (5 liters) was chromatographed on a column of silica gel in system 2. Fractions 6-14 contained the individual ladyginoside D, $C_{60}H_{96}O_{30}$, which, after recrystallization from aqueous butanol deposited in the form of acicular crystals with mp 208–212°C, $[\alpha]_D^{20}$ + 12 ± 2° (c 1.8; 50% aqueous methanol). The yield of ladyginoside D on the initial raw material was about 2.5%.

Eluates 27-35 yielded chromatographically homogeneous ladyginoside F, $C_{72}H_{11}O_{40}$,* in the form of a white amorphous powder with mp 202–204°C, $[\alpha]_D^{20}$ + 5 ± 2° (c 1.6; 50% aqueous methanol). The yield of ladyginoside F on the initial raw material was 2%.

Complete Acid Hydrolysis of the Glycosides. Ladyginoside D (150 mg) was hydrolyzed with 6% H_2SO_4 for 10 h at the boiling point of the reaction mixture. The completeness of hydrolysis was checked in systems 1 and 2. The precipitate that had deposited was filtered off and was twice recrystallized from aqueous ethanol. The resulting crystalline product with mp 328°C, $[\alpha]_D^{20}$ + 79 ± 1° (c 1.92; ethanol) was chromatographed on silica gel in system 3 and was identified by a mixed melting point as hederagenin. The hydrolyzate was neutralized with AV-17 anion-exchange resin (OH form) and concentrated, and D-glucose, D-galactose, and D-glucuronic acid were identified chromatographically in systems 4 and 6. Similar operations were performed with ladyginoside F. Its aglycone likewise proved to be hederagenin. The quantitative composition of the monosaccharides was the same as for ladyginoside D. It was established by gas–liquid chromatography that the quantitative ratio of D-glucose to D-galactose to D-glucuronic acid in ladyginoside D is 3.08 : 1.10 : 1.00 and in ladyginoside F 5.32 : 1.11 : 1.00 [5].

Methylation of Ladyginosides D and F. Each glycoside (50 mg) was dissolved in 10 ml of methanol and methylated with an ethereal solution of diazomethane. The product obtained was hydrolyzed with 6% H_2SO_4 at the boiling point of the reaction mixture for 6 h. The crystalline substance isolated, with mp 326°C, $[\alpha]_D^{20}$ + 78° (c 1.2; methanol) was identified as hederagenin.

*As in Russian original – Publisher.

Alkaline Hydrolysis of Ladyginosides D and F. Ladyginoside D (120 mg) and ladyginoside F (150 mg) were dissolved separately in 10-ml portions of water, and the solutions were deposited on columns containing 6 g of AV-17 anion-exchange resin (OH form) and were left at room temperature for 48 h. Then the oligosaccharides were eluted from the columns with water. The aqueous solutions were evaporated to dryness in vacuum, and the residues were heated with 2% H_2SO_4 for 3 h. The hydrolyzate was neutralized with the same anion-exchange resin and was concentrated. In each case, D-glucose and D-galactose were identified by chromatography in systems 4 and 6. The glycosidic fraction was eluted from the column with methanol. On evaporation, the methanolic solution deposited a crystalline precipitate which, after recrystallization from aqueous ethanol, had mp 224-226°C, $[\alpha]_D^{20}$ + 18 ± 2° (c 1.0; methanol). In its constants and its R_f values in systems 1 and 2 it coincided with an authentic sample of ladyginoside B. The alkaline hydrolysis of ladyginosides D and F with 10% aqueous ethanol (1 : 1) caustic soda gave the same products.

Partial Hydrolysis of Ladyginosides D and F. Ladyginoside D (100 mg) was hydrolyzed with 0.5% H_2SO_4 in the boiling water bath. The progress of the hydrolysis was monitored in systems 1 and 4. After the end of the reaction (6 h), the mixture was diluted with water and the precipitate was filtered off. By TLC in systems 1 and 2 in comparison with reference samples, the precipitate was shown to contain ladyginoside B, hederagenin glucosiduronic acid, and hederagenin, and TLC of the hydrolyzate in systems 4 and 6 showed the presence of D-glucose, D-galactose, and glucuronolactone.

Ladyginoside F (120 mg) was hydrolyzed under the same conditions. After 40 min, by a chromatographic comparison in systems 1 and 2 with reference samples, the reaction mixture from ladyginoside F was found to contain ladyginoside D, ladyginoside B, and hederagenin glucosiduronic acid, while in system 4 gentiobiose was identified. It was hydrolyzed for another 7 h, after which, by the same method, only hederagenin glucosiduronic acid and hederagenin and, in the hydrolyzate, the same set of monosaccharides as after the degradation of ladyginoside D, were identified.

Periodate Oxidation of Ladyginosides D and F. The glycosides (50 mg each, separately) were oxidized with a 1% solution of sodium metaperiodate at 6°C for 48 h. After the addition of ethylene glycol to decompose the excess of periodate, the reaction mixture was evaporated with butanol, the salts that deposited were separated off, and the reaction product was hydrolyzed with 6% H_2SO_4. Chromatography of the neutralized hydrolyzate in systems 4 and 6 showed that free monosaccharides were absent.

Methylation of Ladyginosides D and F. Ladyginosides D and F (1.5 g in each case) were methylated by Hakomori's method [4]. In the case of ladyginoside D, 800 mg of the chromatographically homogeneous permethylate with mp 114-118°C, $[\alpha]_D^{20}$ + 21 ± 2° (c 1.8; methanol) and in the case of ladyginoside F 950 mg of permethylate with mp 98-100°C, $[\alpha]_D^{20}$ + 12 ± 2°C (c 1.96; methanol) were obtained. To 300 mg of methylated ladyginoside D was added 5% H_2SO_4 in methanol, and the mixture was heated at the boiling point for 6 h. Then it was diluted with water (twofold) and heated for another 2 h. The hydrolyzate was neutralized with AV-17 anion-exchange resin (OH^- form), concentrated, and chromatographed on a column (2.5 × 50 cm) of silica gel. Elution was performed with mixtures of benzene and acetone (0-10%). Fractions of 10-15 ml were collected. Eluates 3-7 contained 2,3,4,6-tetra-O-methyl-D-glucopyranose (22 mg) with $[\alpha]_D^{20}$ + 76 ± 2° (c 2.0; water), and fractions 10-16 contained 2,3,4,6-tetra-O-methyl-D-galactopyranose (20 mg) with $[\alpha]_D^{20}$ + 112 ± 3° (c 1.8; water). Eluates 17-30, consisting of a mixture of trimethylglucoses, were separated preparatively on plates of silica gel in system 5. The zones corresponding to the individual methylated monosaccharides were removed from the plate and eluted with methanol. In this way we isolated 2,3,4-tri-O-methyl-D-glucopyranose (18 mg) with $[\alpha]_D^{20}$ + 60 ± 2° (c 1.6; water) and 2,3,6-tri-O-methyl-D-glucopyranose (16 mg) with $[\alpha]_D^{20}$ + 67 ± 2° (water).

After a similar treatment of the permethylate of ladyginoside F (420 mg), 2,3,4,6-tetra-O-methyl-D-glucopyranose (48 mg), 2,3,4,6-tetra-O-methyl-D-galactopyranose (20 mg), 2,3,4-tri-O-methyl-D-glucopyranose (18 mg), and 2,3-di-O-methyl-D-glucopyranose (16 mg) with $[\alpha]_D^{20}$ + 60 ± 2° (c 1.2; water) were isolated. Literature data: 2,3,4,6-tetra-O-methyl-D-glucopyranose, $[\alpha]_D^{20}$ + 83.3° (water), 2,3,4,6-tetra-O-methyl-D-galactopyranose, $[\alpha]_D^{20}$ + 110° (water), 2,3,4-tri-O-methyl-D-glucopyranose $[\alpha]_D^{20}$ + 66.8° (water), 2,3,6-tri-O-methyl-D-glucopyranose $[\alpha]_D^{20}$ + 70° (water), and 2,3-di-O-methyl-D-glucopyranose $[\alpha]_D^{20}$ + 48.3° (acetone) [6].

Reductive Cleavage of the Permethylates of Ladyginosides D and F. The permethylate of glycoside D (500 mg) was dissolved in 10 ml of absolute ether, 200 mg of $LiAlH_4$ was added, and the reaction mixture was heated at the boiling point of ether for 10 h. The excess of $LiAlH_4$ was decomposed with ethyl acetate, 2% H_2SO_4, and 20 ml of water. By extraction with ether, the reduction products were separated into ether-soluble and ether-insoluble fractions. The ethereal extract was washed with water and evaporated. The

residue (200 mg) was deposited on a column (1.5 × 30 cm) of silica gel and was eluted with benzene–acetone (2:1). This gave 180 mg of the reduced permethylate of glycoside B with mp 78°C (decomp.), $[\alpha]_D^{20}$ + 26 ± 2° (c 1.4; methanol). This product was hydrolyzed in 10 ml of 5% H_2SO_4 in methanol. 23-Methoxyerythrodiol with mp 186°C, $[\alpha]_D^{20}$ + 54 ± 2° (c 1.02; chloroform) was detected. The hydrolyzate was neutralized with AV-17 anion-exchange resin (OH^- form) and concentrated. The residue (50 mg) was chromatographed on a plate of silica gel in system 5, and 2,3,4,6-tetra-O-methyl-D-glucopyranose and 2,3-di-O-methyl-D-glucopyranose were identified.

The reduced permethylate of the trisaccharide remaining in the aqueous phase was purified on a column of silica gel in system 5. The chromatographically homogeneous product was hydrolyzed with 5% H_2SO_4 (4 h). In the neutralized hydrolyzate, 2,3,4,6-tetra-O-methyl-D-galactopyranose, 2,3,6-tri-O-methyl-D-glucopyranose, and 2,3,4-tri-O-methylsorbitol were identified in systems 5, 7, and 8. The last-mentioned compound was revealed by means of Bonner's periodate-containing reagent [7].

The reductive cleavage of 500 mg of the permethylate of ladyginoside F was performed in the same way. The ether-soluble fraction of the reduced permethylate of ladyginoside F (150 mg) was hydrolyzed with 5% H_2SO_4 in methanol. After a working-up similar to that for methylated ladyginoside D, 23-methoxyerythrodiol was found in system 3, and 2,3,4,6-tetra-O-methyl-D-glucopyranose and 2,3-di-O-methyl-D-glucopyranose were identified in systems 5, 7, and 8. The aqueous solution was concentrated in vacuum and the residue was deposited on a column of silica gel and eluted with system 5. The fractions were checked by TLC in the same system. The eluates containing the chromatographically individual reduced methylated pentasaccharide were combined, evaporated, and hydrolyzed with 5% H_2SO_4 (4 h). The hydrolyzate was neutralized and the solvent was distilled off. In the residue, by comparison with authentic samples in systems 5, 7, and 8, 2,3,4,6-tetra-O-methyl-D-glucopyranose, 2,3,4,6-tetra-O-methyl-D-galactopyranose, 2,3,4-tri-O-methyl-D-glucopyranose, 2,3-di-O-methyl-D-glucopyranose, and 2,3,4-tri-O-methylsorbitol were identified.

SUMMARY

The structures of ladyginoside D – a pentaoside of hederagenin – and of ladyginoside F – a heptaoside of hederagenin – isolated from the roots of Ladyginia bucharica have been established. The O-glycosidic carbohydrate chains of the two glycosides are identical and agree with that of ladyginoside B, corresponding to the disaccharide β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranose. The acyl moiety of ladyginoside D is β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranose. In ladyginoside F, the carbohydrate chain attached to the carboxy group of the genin is branched and has the structure of [β-D-galactopyranosyl-(1 → 4)]-[β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranose.

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