TRITERPENE GLYCOSIDES OF *Ladyginia bucharica*

III. STRUCTURE OF LADYGINOSIDE C

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In a preceding paper [1] we reported a determination of the structures of the simplest glycosides from Ladyginia bucharica Lipsky (family Umbelliferae) – ladyginosides A and B. In the present paper we give the results of a study of the chemical structure of ladyginoside C.

The acid hydrolysis of ladyginoside C showed that the aglycone of this glycoside is oleanolic acid, and the carbohydrate moiety consists of D-glucose, L-arabinose, and D-glucuronic acid [2]. It was established by the gas-liquid chromatography of the silylated methyl glycosides [3] that the monosaccharides are present in the glycoside in a ratio of 1 : 1 : 1. Thus, ladyginoside C is a trioside of oleanolic acid with the empirical formula $C_{47}H_{74}O_{18}$.

The partial cleavage of the glycoside with dilute sulfuric acid gave a glucuronoside of oleanolic acid. This shows that the glucuronic acid is attached directly to the aglycone.

Hydrolysis of ladyginoside C treated previously with diazomethane led to methyl oleanolate. The glycoside underwent no change on being treated with alkali. It follows from these facts that ladyginoside C is not an acyloside and the other two sugars (glucose and arabinose) are not attached to the carboxy groups either of the genin or of the glucuronic acid.

No free sugar residues were found after the periodate oxidation of ladyginoside C. Consequently there are no $1 \rightarrow 3$ bonds between the individual sugars.

In order to determine the position of attachment of the monosaccharides to one another, ladyginoside C was methylated exhaustively by Kuhn's method [4]. A hydrolyzate of ladyginoside C was shown in the presence of markers to contain 2,3,4,6-tetra-O-methyl-D-glucose, 2,3-di-O-methyl-L-arabinose, and 2,3-di-O-methyl-D-glucuronic acid. Hence, the D-glucose occupies the terminal position in the chain of sugars and is attached to the L-arabinose at the C_4 hydroxyl if the L-arabinose is present in the pyranose form, or at C_5 if the pentose has the furanose form. The choice between the two alternative forms for L-arabinose was made on the basis of the following facts: on being boiled with 10% oxalic acid, ladyginoside C was not hydrolyzed, and therefore the pyranose form is more probable for the L-arabinose residue. In both cases, the L-arabinose is attached to the D-glucuronic acid through the C_4 hydroxy group. A $1 \rightarrow 4$ bond was confirmed by the fact that after paper chromatography (PC) and thin-layer chromatography (TLC) on silica gel the methylated monosaccharides gave no reaction with Bonner's reagent for an α-glycol group.

The permethylate of ladyginoside C was reduced with lithium tetrahydroaluminate. Hydrolysis of the reduced glycoside gave 2,3-di-O-methyl-D-glucose, 2,3-di-O-methyl-L-arabinose, 2,3,4,6-tetra-O-methyl-D-glucose, and erythrodiol, which shows a linear sequence and the order given above for the sugars present.

On the basis of the results given, we propose the structural formula (I) for ladyginoside C.

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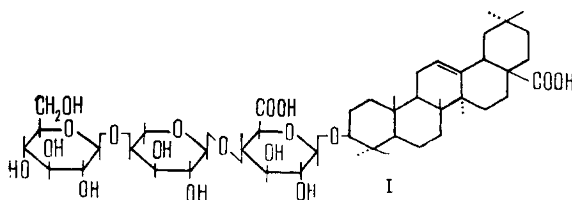

Ladyginoside C proved to be an isomer of araloside A – a glycoside from Aralia manschurica Rupr. et Max. (family Araliaceae) [5]. Both compounds are glycosides of oleanolic acid and they contain the same set of sugars. They differ from one another by the fact that in araloside A the D-glucose molecule is attached to the carboxy group of the genin, while in ladyginoside C it is attached to the L-arabinose.

In the D-glucuronoside of oleanolic acid, the sugar is attached to the aglycone by a β-glycosidic linkage [5]. The stepwise hydrolysis of ladyginoside C has not been performed, and therefore it was impossible to decide the configurations of the glycosidic linkages between the D-glucose and the L-arabinose and between the L-arabinose and the D-glucuronic acid by the method of molecular-rotation differences. However, ladyginoside C and araloside A have specific rotations of the same sign and of similar magnitudes: −17.1 and −26.7°, respectively. At the same time, the configuration of the glycosidic linkages, and not the position of attachment of the individual monosaccharides, exerts the main influence on the overall rotation of a glycoside. Taking this fact into account, it may be assumed with a high degree of probability that in ladyginoside C, as in araloside A, the D-glucose is attached by a β-glycosidic and the L-arabinose by an α-glycosidic linkage. This hypothesis is in complete harmony with Klyne's rule [6].

EXPFRIMENTAL

The conditions for chromatography have been given previously [1].

Isolation of Ladyginoside C. The fractions with ladyginosides BC and CD (for their preparation, see [1]), were combined and concentrated and were rechromatographed on a column of silica gel in system 1. The separation of the glycosides was monitored by chromatography in system 2. The eluates containing the ladyginoside C were combined and evaporated. Acicular crystals were obtained from aqueous butanol with mp 224-226°C, $[\alpha]_D^{20} -17.1 \pm 2°$ (c 1.8; methanol). The yield of glycoside on the raw material was ≈0.3%.

Acid Hydrolysis of Ladyginoside C. A. Complete hydrolysis of the glycoside. Ladyginoside C (50 mg) was heated in 10 ml of 18% hydrochloric acid at 100°C for 6 h. The reaction mixture was diluted with water, and the precipitate that deposited was filtered off and recrystallized from absolute ethanol. This gave crystals with mp 306-308°C, $[\alpha]_D^{20} +79 \pm 2°$ (c 1.6; methanol), identified by a mixed melting point with an authentic sample of oleanolic acid and by chromatography (TLC) in system 3. The hydrolyzate was neutralized with AV-17 anion-exchange resin (OH^- form). The neutral solution was evaporated and chromatographed on silica gel (TLC) impregnated with a 0.2 M solution of sodium dihydrogen phosphate [7] in systems 4 and 7. D-Glucose, L-arabinose, and D-glucuronic acid and its lactone were detected.

B. Partial hydrolysis of the glycoside. Ladyginoside C (100 mg) was hydrolyzed with 0.5% H_2SO_4 at 70-75°C for 6 h. The reaction mixture was diluted with water and was exhaustively extracted with n-butanol. The extract was washed free from acid and, after concentration, was chromatographed in system 2. This gave oleanolic acid, the initial glycoside, a bioside, and a monoside. The latter (20 mg), after purification and recrystallization from ethanol, had mp 212-214°C, $[\alpha]_D^{20} +31.2 \pm 2°$ (c 1.2; ethanol). From its chromatographic behavior and its constants, the substance corresponded to oleanolic acid glucuronoside [1, 8]. On acid hydrolysis of the glucuronoside with 18% HCl, the hydrolyzate was found in system 7 to contain D-glucuronic acid and its lactone.

Methylation of Ladyginoside C with Diazomethane. The glycoside (50 mg) was dissolved in 6 ml of absolute methanol and was methylated with an ethereal solution of diazomethane at room temperature for 48 h. The products obtained were hydrolyzed in 10 ml of 6% H_2SO_4 at the boil for 8 h. The cooled reaction mixture was extracted with chloroform and the chloroform extract was chromatographed in system 3. Among other hydrolysis products, methyl oleanolate was present, as a chromatogram showed.

Alkaline Hydrolysis of Ladyginoside C. The glycoside (20 mg) was dissolved in 10 ml of methanol, 10 ml of a 10% aqueous ethanolic (1 : 1) solution of caustic soda was added, and the mixture was heated at 100°C for 6 h. Then it was cooled and was neutralized with acetic acid. The results of chromatography of the neutral solution in system 2 confirmed that the ladyginoside C had undergone no change under the action of the alkali.

Periodate Oxidation of Ladyginoside C. The glycoside (50 mg) was oxidized with a 1% solution of sodium periodate at 6°C for 48 h. After the addition of ethylene glycol, the reaction mixture was extracted with n-butanol. The butanolic solution was evaporated in vacuum and the salts that deposited were separated off. The reaction product was hydrolyzed with 6% H_2SO_4. No free monosaccharides were found in the neutralized hydrolyzate in systems 4 and 7.

Kuhn Methylation of Ladyginoside C. The substance (110 mg) was dissolved in 3 ml of dimethylformamide that had been freshly distilled over phosphorus pentoxide and previously heated to 40°C, and then 3 ml of methyl iodide was added. After this, with stirring, 1.0 g of silver oxide was added in portions over 30 min.

After 16 h, another 2 ml of methyl iodide and 0.5 g of silver oxide were added to the reaction mixture. The completeness of the methylation was monitored in system 6. After the end of the reaction the unchanged silver oxide and the silver iodide were filtered off. The residue was washed on the filter with chloroform. The chloroform solution was treated with a saturated solution of sodium thiosulfate and with water, and the solvent was distilled off. The product obtained (64 mg) was purified on a column of silica gel, with successive elution by pure benzene and then by mixtures of benzene and ethanol with the concentration of ethanol increasing from 1 to 10%. The separation was monitored in system 6. Fractions 8-10 contained the completely methylated glycoside. The permethylate (30 mg) was hydrolyzed in 3 ml of 5% methanolic hydrogen chloride at the boil for 6 h, and then the solution was diluted with water and the aglycone was filtered off. The filtrate was boiled for another 4 h and was then neutralized with AV-17 anion-exchange resin (OH^- form). The hydrolyzate was found by the use of systems 5 and 8 to contain 2,3,4,6-tetra-O-methyl-D-glucose, 2,3-di-O-methyl-L-arabinose, and 2,3-di-O-methyl-D-glucuronic acid. The aglycone consisted of methyl oleanolate (TLC, system 3).

Reduction of the Permethylate of Ladyginoside C. The product of the methylation of ladyginoside C (30 mg) was reduced in ethereal solution with lithium tetrahydroaluminate. The mixture was heated at the boil for 6 h, and then the excess of $LiAlH_4$ was destroyed with ethyl acetate and 2% sulfuric acid. The ethereal layer was washed with water and dried, and the residue was hydrolyzed with 6% H_2SO_4, the precipitate that deposited being separated off. In the hydrolyzate in system 8, 2,3,4,6-tetra-O-methyl-D-glucose, 2,3-di-O-methyl-L-arabinose, and 2,3-di-O-methyl-D-glucose were identified. The precipitate was recrystallized from absolute ethanol and was identified chromatographically in system 3 in the presence of an authentic sample as erythrodiol.

SUMMARY

The structure of ladyginoside C – a trioside of oleanolic acid – has been established. The carbohydrate chain is attached to the hydroxyl at C_3 of the aglycone and consists of β-D-glucopyranosyl-(1→4)-α-L-arabopyranosyl-(1→4)-β-D-glucopyranuronic acid.

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