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_{46}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→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→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].

**EXPERIMENTAL**

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, [α]D −17.1 ± 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, [α]D +79 ± 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% H2SO4 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, [α]D +31.2 ± 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% H2SO4 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.