THE STRUCTURE OF THE MAIN SAPONIN FROM *Clematis Songarica*

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UDC 547.918+547.597

In a preceding communication we described the isolation of triterpene glycosides from the leaves of *Clematis songarica* (Sungari clematis) [1]. Polar saponins predominate in the plant, and these were included in the group of main saponins. The less polar — minor — saponins are present in an amount of 10% of the total glycosides. Below we give a proof of the structure of one of these substances — songaroside* A (I).

The saponin was first subjected to acid hydrolysis. As the aglycone we identified hederagenin (II), and the carbohydrate moiety consisted of glucose, arabinose, and rhamnose (3:2:3).

To determine the number of carbohydrate chains in the saponin, the initial substance was subjected to alkaline saponification. This gave a glycoside (III) and an oligosaccharide (IV).

Acid hydrolysis of the saponified glycoside gave arabinose and rhamnose in a ratio of 1:2, and acid hydrolysis of the oligosaccharides (IV) gave 3 moles of glucose and 1 mole each of rhamnose and arabinose.

The saponin was then exhaustively methylated by Kuhn's [2] and Purdie's [3] methods. The product obtained was cleaved with perchloric acid in methanol, and the methyl glycosides formed were chromatographed on silica gel. Fully methylated methyl glucoside and methyl rhamnoside and also methyl 3,4-di-O-methyl-L-arabinoside, methyl 2,3-di-O-methyl-D-rhamnoside, methyl 2,3,6-tri-O-methyl-D-glucoside, and methyl 3,4-di-O-methyl-D-glucoside were identified in the presence of authentic markers by chromatography in a thin layer of silica gel and by gas—liquid chromatography. When the saponin was subjected to periodate oxidation, the monosaccharides were destroyed, which confirms the results of methylation.

To determine the localization of the monosaccharides with respect to the functional groups of the aglycone, the methylated saponin was treated with lithium tetrahydroaluminate. It was shown by this method that the methylated carbohydrate chain attached to the hydroxy group of the aglycone contained 3,4-di-O-methyl-L-arabinose, 2,3-di-O-methyl-L-rhamnose, and 2,3,4-tri-O-methyl-L-rhamnose. The acylosidic carbohydrate chain of the permethylate of the saponin included completely methylated glucose and rhamnose and the partially methylated 3,4-di-O-methyl-L-arabinose and 2,3,6-tri-O-methyl-D-glucose. Among the products of lithium tetrahydroaluminate cleavage, 3,4-di-O-methyl-D-sorbitol was identified. Consequently, the 3,4-di-O-methyl-D-glucose was attached directly to the carboxy group of the aglycone.

To determine the sequence of attachment of the monosaccharides to one another, the saponin was subjected to partial hydrolysis (see Experimental Method). In addition to hederagenin and the glycoside (III), four progenins (V-VIII) were obtained. Progenin (V) was less polar and the other more polar than the saponified glycoside (III).

The hydrolysis of compound (V) gave arabinose. Since in the saponin at the hydroxy groups of the aglycone there are two molecules of rhamnose in addition to arabinose, then, taking into account the results of methylation and of lithium tetrahydroaluminate reduction

*In the Consultants Bureau translation of the preceding paper [1] these compounds were called "dzhungarosides." [Translator].

Institute of Chemistry, Academy of Sciences of the Moldavian SSR. M. V. Frunze Simferopol' State University. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 470-474, July-August, 1975. Original article submitted March 27, 1974.

of the permethylate of saponin, the carbohydrate chain in the glycoside at the hydroxy group can be represented as:

$$L\text{-}Rha_p\text{-}(1^{\alpha} \rightarrow 4)\text{-}L\text{-}Rha_p\text{-}(1^{\alpha} \rightarrow 2)\text{-}LAra_p\text{-}(1^{\alpha} \rightarrow O)\text{-aglycone.}$$

Hydrolysis of the progenin (VI) led to arabinose and glucose; the alkaline saponification of (VI) gave hederagenin arabinoside (V).

In order to establish the structure of the carbohydrate chain attached to the carboxy group of the aglycone, we studied the glycoside (VII) in detail. Glucose, arabinose, and rhamnose were identified in a ratio close to 2:1:2. The alkaline saponification of substance (VII) formed the progenin (III). The products of the methanolysis of the permethylate of the glycoside (VII) were found by gas–liquid chromatography to contain methyl 2,3,4-tri-O-methyl-L-rhamnoside, methyl 2,3-di-O-methyl-L-rhamnoside, methyl 3,4-di-O-methyl-L-arabinoside, methyl 2,3,4-tri-O-methyl-D-glucoside, and methyl 2,3,4,6-tetra-O-methyl-D-glucoside. The production of methyl 2,3,4-tri-O-methyl-D-glucoside in place of the methyl 3,4-di-O-methyl-D-glucoside identified in the initial saponin shows that in the carbohydrate chain at the carboxy group the arabinose is attached to the C_2 atom of the glucose. On the basis of the results obtained, the structure of the carbohydrate chain of the carboxy group can be represented by two alternative formulas:

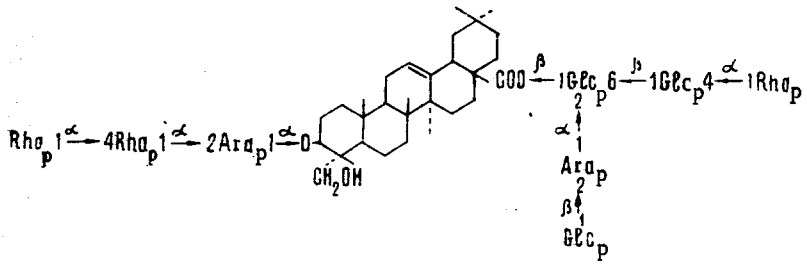
$$\text{aglycone-COO-}\overset{\beta}{}\text{Glc}\begin{matrix}6\overset{\beta}{-}\text{Glc}\overset{\beta}{-}\text{Glc}\\ 2\overset{\alpha}{-}\text{Ara}\overset{\alpha}{-}\text{Rha}\end{matrix} \quad \text{or aglycone -COO}\overset{\beta}{-}\text{Glc}\begin{matrix}6\overset{\beta}{-}\text{Glc}\overset{\alpha}{-}\text{Rha}\\ 2\overset{\alpha}{-}\text{Ara}\overset{\beta}{-}\text{Glc}\end{matrix}$$

The acid hydrolysis of the progenin (VIII) gave glucose, arabinose, and rhamnose (1:1:1).

The final structure of the saponin followed from the results of a study of the oligosaccharide (IX) obtained under the conditions of partial hydrolysis. Its acid hydrolysis gave two moles of glucose and one mole of rhamnose. This shows that the terminal rhamnose is attached to glucose and the terminal glucose is attached to arabinose. Thus, the oligosaccharide attached to the carboxy group of hederagenin has the form

$$\text{aglycone}\quad -COO-Glc_p\begin{matrix}6\overset{\beta}{-}1Glc_p4\overset{\alpha}{-}1Rha_p\\ 2\overset{\alpha}{-}1Ara_p2\overset{\beta}{-}Glc_p\end{matrix}$$

The configuration in the saponin is in harmony with Klyne's rule [4]. Saponin A is represented by the following structure:



EXPERIMENTAL METHOD

For column chromatography we used silica gels of types LS 100/250 and LS 40(100) (CzSSR), for paper chromatography type FN-13 paper (GDR), and the following solvent systems: 1) butanol–ethanol–water (10:2:5) 2) butanol–pyridine–benzene–water (5:3:1:3), 3) toluene–ethanol (9:1), 4) benzene–acetone (2:1), 5) chloroform–methanol (9:1), and 6) ethyl acetate–methanol–water (10:2:5).

The glycosides were revealed on the silica gel plates with concentrated H_2SO_4 and on Silufol with 25% phosphotungstic acid in methanol. Aniline phthalate was used to reveal the sugars. Gas–liquid chromatography was performed on a "Khrom-4" instrument (Czechoslovakia) with helium as the carrier gas at a programmed temperature of from 180 to 225°C in glass columns using as stationary phase 5% of XE-60 on Chromaton NAW HMDS (0.125-0.250 mm), $V_{program}$ = 3°C/min, V_{He} = 50 ml/min.

Hydrolysis of the Saponin. A solution of 20 mg of the saponin (I) [mp 228-230°C, $[\alpha]_D^{18}$ +2.70° (c 4,3; water)] in 30 ml of 5% H_2SO_4 was heated in a tube at 100°C for 5 h.

After hydrolysis, the contents of the tube were diluted with water and filtered. The precipitate was shown by thin-layer chromatography in system 5 to contain hederagenin: mp 330-332°C, $[\alpha]_D^{18}$ +79° (c 1,0; chloroform). Literature data for hederagenin: mp 332-333°C, $[\alpha]_D^{18}$ +81° (chloroform). The filtrate was neutralized with anion-exchange resin and evaporated, and the concentrated solution was chromatographed on paper in system 2 for 12 h. Glucose, arabinose, and rhamnose were identified. The residue was evaporated to dryness, an equivalent amount of hydroxylamine hydrochloride and 1 ml of freshly distilled pyridine were added, and the mixture was heated in the oil bath at 90°C for 1 h. Then 1 ml of freshly distilled acetic anhydride was added and it was heated for another 1 h. After the end of the reaction, the contents of the flask were dissolved in water, and the solution was cooled and was evaporated in vacuum at 45°C. This gave sugars in the form of the acetates of the aldononitriles, which were investigated by gas–liquid chromatography [5]. Glucose, arabinose, and rhamnose (3.1: 2: 3.2) were identified.

Alkaline Saponification. A mixture of 1.0 g of the saponin (I) and 10% NaOH was heated at 100°C for 5 h. After neutralization, solution was extracted with butanol, and the butanol was evaporated. This gave 500 mg of the saponified glycoside (III) [mp 216-218°C, $[\alpha]_D^{18}$ –60° (c 0.5; pyridine)] and 250 mg of the oligosaccharide (IV). After heating with 5% H_2SO_4 (100°C, 5 h) by paper chromatography in system 2 the hydrolyzate of (III) was shown to contain, in the form of the acetates of the aldononitriles, arabinose and rhamnose (1: 2.1), and the hydrolyzate of (IV) was found to contain glucose, arabinose, and rhamnose (3.2: 1: 1).

Methylation of the Saponin (I). The saponin (1 g) was methylated by Kuhn's method [1 g of saponin + 3 g of $Ba(OH)_2$ + 0.8 g of BaO + 50 ml of dimethylformamide + 20 ml of CH_3I] and was methylated to completion by Purdie's method (1 g of the partially methylated saponin + 30 ml of CH_3I + 1 g of Ag_2O). This gave 1.1 g of the permethylated saponin (I). Then the permethylate was heated in a solution of 72% $HClO_4$ in methanol (1:10) at 110°C for 7 h. The resulting mixture of methyl glycosides was separated on silica gel in system 4. Four partially methylated and two completely methylated methyl glycosides were detected. By thin-layer and gas–liquid chromatography in the presence of authentic markers the following were identified: methyl 2,3,4,6-tetra-O-methyl-D-glucoside, methyl 2,3,4-tri-O-methyl-L-rhamnoside, methyl 2,4-di-O-methyl-L-arabinoside, methyl 2,3-di-O-methyl-L-rhamnoside, methyl 3,4-di-O-methyl-D-glucoside, and methyl 2,3,6-tri-O-methyl-D-glucoside.

Lithium Tetrahydroaluminate Cleavage of the Methylated Saponin. The permethylated saponin (500 mg) was treated with an excess of $LiAlH_4$ in absolute tetrahydrofuran with constant stirring and heating for 10 h. The excess of $LiAlH_4$ was decomposed with ethyl acetate, after which water was added. The organic layer was separated from the aqueous layer, and the latter was twice extracted with ether. The ethereal extracts were combined with the ethyl acetate. After chromatographic purification on a column of silica gel in system 3, the reduced glycoside, with mp 226-228°C, $[\alpha]_D^{18}$ –54° (c 1.1; methanol), and an oligosaccharide were obtained. In a hydrolyzate of the reduced glycoside the following methylated methyl glycosides were found by GLC and TLC: methyl 2,3,4-tri-O-methyl-L-rhamnoside, methyl 2,3-di-O-methyl-L-rhamnoside, and methyl 3,4-di-O-methyl-L-arabinoside, and in the oligosaccharide: methyl 3,4-di-O-methyl-D-sorbitol, methyl 3,4-di-O-methyl-L-arabinoside, methyl 2,3,4-tri-O-methyl-L-rhamnoside, methyl 2,3,6-tri-O-methyl-D-glucoside, and methyl 2,3,4,6-tetra-O-methyl-D-glucoside.

Partial Hydrolysis of the Saponin. A mixture of 3.0 g of the saponin and 300 ml of 5% oxalic acid was heated at 70°C for 3.5 h. The hydrolyzate was neutralized with Dowex anion-exchange resin (HCO_3^- form) and was exhaustively extracted with butanol. The organic layer was found by chromatography on silica gel in systems 1 and 6 to contain the initial substance (I), traces of (II), the glycoside (III), and progenins (V-VIII). The butanol layer, after evaporation, was chromatographed on silica gel in system 6. This gave 40 mg of (III), 50 mg of (V), 65 mg of (VI), 45 mg of (VII), and 70 mg of (VIII).

The glycoside (V) [mp 226-228°C, $[\alpha]_D^{18}$+ 53) c 1.1; dimethylformamide] was cleaved on hydrolysis into arabinose and hederagenin.

The progenin (VI) [(mp 210-212°C, $[\alpha]_D^{19}$–60° (c 1; methanol)] was cleaved on acid hydrolysis into arabinose and glucose. Its alkaline saponification gave the glycoside (V).

Substance (VII) (20 mg) was heated with 5% H_2SO_4 at 100°C for 5 h. In the hydrolyzate by gas–liquid chromatography we identified glucose, arabinose, and rhamnose (2.3: 1: 2.1).

Alkaline saponification of the glycoside formed the progenin (III).

Compound (VII) (20 mg) [mp 226-228°C, $[\alpha]_D^{19}$ −93° (c 0.85; pyridine)] was converted into the fully methylated product as described above.

After cleavage with a solution of 72% $HClO_4$ in methanol (1:10) compound (VII) yielded, as shown by gas–liquid chromatography in the presence of markers, methyl 2,3,4,-tri-O-methyl-L-rhamnoside, methyl 2,3-di-O-methyl-rhamnoside, methyl 3,4-di-O-methyl-L-arabinoside, methyl-2,3,4-tri-O-methyl-D glucoside, and methyl 2,3,4,6-tetra-O-methyl-D-glycoside.

In the aqueous layer from partial hydrolysis we detected the oligosaccharide (IX) on paper in system 2. On hydrolysis (2.5% H_2SO_4, 5 h, 100°C, compound (IX) formed glucose and rhamnose (2.2 : 1).

Periodate Oxidation of the Saponin. A solution of 30 mg of (I) in an aqueous solution of $NaIO_4$ was kept in the dark in the cold for 48 h. After this, the excess of periodate was decomposed with ethylene glycol, and after 1 h the mixture was deionized with resins and evaporated. No sugars were found by paper chromatography in a hydrolyzate of the product of periodate oxidation.

Oxidation of the Saponin. To 1.0 g of the saponin (I) in absolute pyridine was added 50 ml of a solution of chromium trioxide in acetic acid, and the mixture was stirred at room temperature for 24 h. Then, as described by Grimmer [6], the product was subjected to acid hydrolysis and was methylated with diazomethane. This gave the dimethyl ester of gypsogeninic acid with mp 244-245°C.

Literature data for the dimethyl derivative of gypsogeninic acid: 248-250°C [7].

SUMMARY

The structure of the main saponin from *Clematis songarica* Bge. has been established; it is an octaoside of hederagenin.

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