THE STRUCTURE OF SAPONIN B FROM *Clematis songarica*

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We have previously reported the isolation of saponin A from *Sungari clematis* (*Clematis songarica* Bge.) and the determination of its structure [1]. The present paper describes the determination of the structure of saponin B. This glycoside contains hederagenin as its aglycone, and its carbohydrate component consists of rhamnose, ribose, arabinose, and glucose in a ratio of 3:1:1:1:3.

To determine the type of link between the monosaccharides, saponin B was exhaustively methylated by the methods of Kuhn [2] and Purdie [3]. The permethylate was subjected to methanolysis followed by the separation of the methyl glycosides on a column of silica gel. The following glycosides were identified by gas–liquid chromatography (GLC) and thin-layer chromatography (TLC): methyl 2,3,4,6-tetra-O-methyl-D-glucoside, methyl 2,3,4-tri-O-methyl-D-riboside, methyl 2,4,6-tri-O-methyl-D-glucoside, methyl 2,4-di-O-methyl-L-rhamnoside, methyl 3,4-di-O-methyl-L-arabinoside, and methyl 2,3,6-tri-O-methyl-D-glucoside.

In a hydrolyzate of the saponin after periodate oxidation, glucose and rhamnose were identified by paper chromatography. These results are in harmony with the results of methylation.

Saponification of the saponin with alkali yielded a progenin and an oligosaccharide. The hydrolysis of the progenin gave rhamnose, ribose, and arabinose (2:1:1). Rhamnose and glucose (1:3) were identified in a hydrolyzate of the oligosaccharide. The saponin was then converted into the permethylated derivative which was subjected to $LiAlH_4$ cleavage. A reduced methylated glycoside was obtained the products of the methanolysis of which contained methyl 2,3,4-tri-O-methyl-D-riboside, methyl 2,4-di-O-methyl-L-rhamnoside, and methyl 3,4-di-O-methyl-L-arabinoside, and an oligosaccharide decomposing on methanolysis into methyl 2,4,6-tri-O-methyl-D-glucoside, methyl 2,4-di-O-methyl-L-rhamnoside, methyl 2,3,4,6-tetra-O-methyl-D-glucoside, and 2,3,6-tri-O-methyl-D-sorbitol.

The monosaccharides sequences in the carbohydrate chains were determined by means of partial hydrolysis of the saponin with oxalic acid. The reaction mixture yielded a monoside (I), two biosides (II, III), a trioside (IV), a tetraoside (V), and a heptaoside (VI) of hederagenin. The hydrolysis of (I) yielded hederagenin and arabinose. In the bioside (II) and the trioside, rhamnose was also identified, and in (V) ribose as well. The other bioside (III) split into glucose and arabinose. Alkaline saponification showed that in this bioside the glucose was attached to the carboxy group of the aglycone. The hederagenin heptaoside contained the same monosaccharides as the initial saponin but one molecule of glucose fewer. Its alkaline treatment yielded the progenin of saponin B.

The structure of saponin B was determined definitively by the methylation of hederagenin heptaoside. When the permethylate was cleaved with lithium tetrahydroaluminate followed by methanolysis, methyl 2,3,4-tri-O-methyl-D-riboside, methyl 2,4-di-O-methyl-L-rhamnoside, and methyl 3,4-di-O-methyl-L-arabinoside were found in the reduced glycoside, and 2,3,6-tri-O-methyl-D-sorbitol, methyl 2,4-di-O-methyl-L-rhamnoside, and methyl 2,3,4,6-tetra-O-methyl-D-glucoside were found in the oligosaccharide.

The configurations of the glycosidic centers are given in accordance with Klyne's rule

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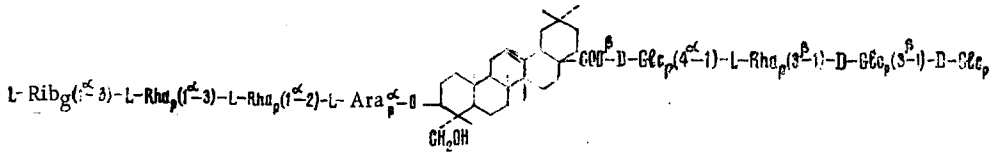
[4]. On the basis of these results, the structure of saponin B can be represented by the following formula:



EXPERIMENTAL

For chromatography we used FN-13 paper (GDR), type KS silica gel, and "Silufol" plates (Czechoslovakia) and the following solvent systems: 1) butan-1-ol—ethanol—water (10:2:5); 2) ethyl acetate—ethanol—water (10:2:5); 3) butan-1-ol—benzene—pyridine—water (5:1:3:3); 4) chloroform—methanol (9:1); 5) benzene—ethanol (9:1); 6) benzene—acetone (2:1).

The saponins were revealed on silica gel with concentrated H_2SO_4 and antimony trichloride and on the "Silufol" plates with a 25% ethanolic solution of phosphotungstic acid. The sugars were revealed on paper with aniline phthalate and on analysis by the GLC method in the form of the acetates of the aldononitriles on a Khrom-4 instrument, FID, glass columns (2 m × 0.35 cm), 5% of XE-60 on Chromaton N-AW-HDMS; programmed temperature from 180 to 225°C; $V_{progr.}$ = 3 deg/min; carrier gas helium at the rate of 50 ml/min.

Hydrolysis of Saponin B. The saponin, with mp 246-248°C, $[\alpha]_D^{18}$ 18—33° (c 0.83; methanol) (20 mg) was hydrolyzed with 5% H_2SO_4 at 100°C for 5 h. The hydrolyzate was found by paper chromatography in system 3 to contain glucose, arabinose, ribose, and rhamnose. Gas-liquid chromatography of the acetates of the aldononitriles showed that their ratio was 2.9 : 1 : 1 : 2.8. The aglycone, with mp 331-333°C (decomp.), $[\alpha]_D^{18}$ +81° was identified by comparison with an authentic sample of hederagenin by chromatography on SiO_2 in system 4, and by IR and mass spectrometry.

Methylation of Saponin B. The saponin (2 g) was methylated by Kuhn's method, and methylation was brought to completion by Purdie's method. The degree of methylation was checked by IR spectroscopy from the disappearance of the peak in the 3400-cm^{-1} region, and also by chromatography in a thin layer of silica gel in system 5. The product obtained was purified on a column of silica gel in the same system.

This gave 1.8 g of completely methylated product; 1 g of this compound was cleaved with 10 ml of 72% perchloric acid in absolute methanol (1:10) at 100°C for 5 h. After neutralization with an anion-exchange resin, the mixture was evaporated and chromatographed on a column of SiO_2 in system 6. The separation was monitored on plates of silica gel in the same system. This gave methyl 2,3,4-6-tetra-O-methyl-D-glucoside, methyl 2,3,4-tri-O-methyl-D-riboside, methyl 2,4-di-O-methyl-L-rhamnoside, methyl 3,4-di-O-methyl-L-arabinoside, methyl 2,4,6-tri-O-methyl-D-glucoside, and methyl 2,3,6-tri-O-methyl-D-glucoside.

Tetrahydroaluminate Reduction of Methylated Saponin B. The permethylated saponin was treated with an excess of $LiAlH_4$ in absolute tetrahydrofuran. After chromatographic purification on silica gel (system 5) of the cleavage products, 300 mg of reduced saponin and 100 mg of oligosaccharide were obtained. When the reduced preparation was subjected to methanolysis, GLC revealed the presence of methyl 2,4-di-O-methyl-L-rhamnoside, methyl 3,4-di-O-methyl-L-arabinoside, and methyl 2,3,4-tri-O-methyl-D-riboside. In the oligosaccharide after methanolysis we identified methyl 2,4,6-tri-O-methyl-D-glucoside, methyl 2,4-di-O-methyl-L-rhamnoside, and methyl 2,3,4,6-tetra-O-methyl-D-glucoside. In addition, 2,3,6-tri-O-methyl-D-sorbitol was identified by TLC in system 6.

Alkaline Saponification of Saponin B. The saponin (1 g) was heated with 10% NaOH at 100°C for 5 h. Then the reaction mixture was diluted with water and extracted with butanol (3 × 30 ml). The butanolic extracts were evaporated to dryness. This gave 700 mg of saponified glycoside with mp 234-236°C $[\alpha]_D^{18}$ −24° (c 2.34; methanol) and 250 mg of an oligosaccharide. An acid hydrolyzate of the saponified saponin was found to contain rhamnose, ribose, and arabinose (1.8 : 1.0 : 1.0; GLC). Rhamnose and glucose (1.0 : 2.9; GLC) were identified in a hydrolyzate of the oligosaccharide.

Partial Hydrolysis of Glycoside B. When 3 g of saponin B was heated with 5% H_2SO_4 at 78°C for 8 h, six progenins (I-VI) were obtained. They were separated by chromatography on a column of silica gel, elution being performed with systems 1 and 2.

Progenin (I) (yield 30 mg), with mp 226-228°C, $[\alpha]_D^{20}$ + 53° (c 1.1; dimethylformamide) was cleaved to form arabinose and hederagenin.

Literature information for hederagenin 3-O-α-L-arabinopyranoside: mp 228-230°C, $[\alpha]_D^{20}$ + 53.21° [5].

Progenin (II) (yield 50 mg) with mp 206-208°C, $[\alpha]_D^{20}$ −52° (c 1.0; methanol), on hydrolysis, gave rhamnose and arabinose (1:1); GLC.

The hydrolysis of progenin (III) (yield 100 mg) with mp 242-244°C, $[\alpha]_D^{20}$ + 20° (c 1.65; methanol) led to glucose, arabinose, and hederagenin. The saponification of the progenin (I) formed hederagenin arabinopyranoside.

Progenin (IV) (yield 140 mg) with mp 210-212°C, $[\alpha]_D^{20}$ −60° (c 1.0; methanol) decomposed on acid cleavage into rhamnose and arabinose (2.2 : 1.0; GLC) and hederagenin.

The acid hydrolysis of progenin (V) (yield 135 mg) with mp 234-236°C, $[\alpha]_D^{20}$ −24 (c 2.34; methanol) yielded rhamnose, ribose, and arabinose (1.9 : 1.0 : 1.0; GLC).

On hydrolysis, progenin (VI) (yield 500 mg) with mp 244-246°C, $[\alpha]_D^{20}$ −33° (c 0.8; methanol) gave rhamnose, ribose, arabinose, and glucose (2.8 : 1.0 : 1.9; GLC). This progenin (100 mg) was permethylated by Kuhn's method. The permethylated compound obtained was treated with $LiAlH_4$. On methanolysis of the reduced glycoside, methyl 2,3,4-tri-O-methyl-D-Riboside and methyl 2,4-di-O-methyl-L-arabinoside were identified by GLC and TLC in system 6, and methanolysis of the oligosaccharide gave methyl 2,3,4,6-tetra-O-methyl-D-glucoside, methyl 2,4-di-O-methyl-L-rhamnoside, and 2,3,6-tri-O-methyl-D-sorbitol.

SUMMARY

The structure of a triterpene glycoside from *Clematis songarica* Bge. has been established; it is a hederagenin octaoside.

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