THE STRUCTURE OF A RIBOSE-CONTAINING SAPONIN – VITALBOSIDE F

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In a preceding paper the isolation from traveler's-joy of a group of saponins containing ribose has been reported [1]. Below we give a proof of the structure of the chief of these substances – vitalboside F.

A quantitative determination of the monosaccharide composition of the glycoside by a method which we have recently developed [2] showed that it contains glucose, rhamnose, arabinose, and ribose in a ratio close to 2:1:1:1.

To determine the types of bonds between the monosaccharides, the saponin was converted into the permethylated derivative [3], and this was then subjected to methanolysis. The structures of the completely methylated sugars were determined by gas-liquid chromatography and those of the partially methylated sugars by mass spectrometry [4, 5]. The following compounds were identified: methyl 2,3,4-tri-O-methyl-L-riboside, methyl 2,3,4-tri-O-methyl-L-rhamnoside, methyl 2,3,4- and 2,3,6-tri-O-methyl-D-glucosides, and methyl 3,4-di-O-methyl-L-arabinoside. The tetrahydroaluminate cleavage of completely methylated vitalboside F led to the formation of an oligosaccharide consisting of 2,3,4-tri-O-methylrhamnose, 2,3,4-tri-O-methylsorbitol, and 2,3,6-tri-O-methylglucose [6], and also a glycoside which hydrolyzed to 2,3,4-tri-O-methylribose and 3,4-di-O-methylarabinose.

Consequently, the acyloside component of the sugar had the form L-Rha_p-(1→4)-D-G_p-(1→6)-D-G_p, and the disaccharide attached to one of the hydroxy groups of the hederagenin has the structure L-Rib_p-(1→2)-L-Ara_p. It is interesting to note that while the trisaccharide is found comparatively frequently in triterpene glycosides [6-9], the second component is unusual.

The consumption of sodium periodate determined by the arsenite method [10] amounted to 8 moles per mole of the saponin, and the yield of formic acid, determined amperometrically [11], was 3 moles per mole. These results agree well with those of methylation.

To determine the position of localization of the carbohydrate chain attached to a hydroxyl of the aglycone, vitalboside F was oxidized with chromium trioxide in acetic acid [12], was then subjected to acid hydrolysis and was finally treated with diazomethane. After chromatography on alumina, the main aglycone was identified as dimethyl gypsogenate.* Thus, the disaccharide L-Rib_p-(1→2)-L-Ara_p is attached to the hydroxy group at C_3 of hederagenin.

The configuration of the glycosidic linkage between the ribose and the arabinose was determined by the partial hydrolysis of the hederagenin bioside obtained by the alkaline saponification of the saponin. The 3-O-α-arabopyranoside of this aglycone was isolated [13].

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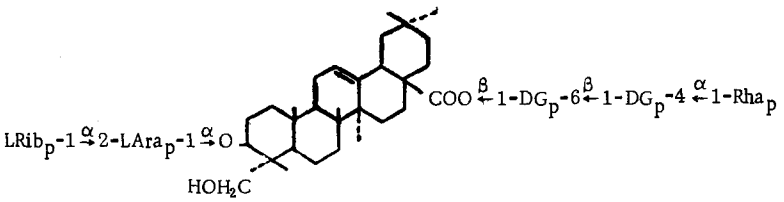

The definitive structure of vitalboside F is given above.

EXPERIMENTAL

Chromatography was performed on type C (fast) paper of the Volodarskii Leningrad Mill, on KSK silica gel, and on neutral alumina with the following systems of solvents: 1) butan-1-ol–benzene–pyridine–water (5:1:3:3), 2) benzene–acetone (2:1), 3) benzene–acetone (1:1), and 4) benzene–chloroform (4:1).

The mass spectra were taken on an MKh-1303 instrument, and gas-liquid chromatography was performed on a Hewlett-Packard model 5750G chromatograph with a flame-ionization detector and a Hewlett-Packard 3370A integrator. The stainless-steel column, 3 m long and 3 mm in diameter, was filled with 1% of XE-60 on Gas Chrom Z 80/100 mesh; the carrier gas was nitrogen at the rate of 36 ml/min. Temperature 280°C.

Hydrolysis of the Saponin. A solution of 20 mg of vitalboside F (I) [mp 208-210°C, $[\alpha]_D^{20}$ −19.4° (c 1.27; methanol)] in 2% H_2SO_4 was heated in a sealed tube at 100°C for 5 h. After cooling, the mixture of sugars was neutralized with barium carbonate and was then converted into the trifluoroacetates of the polyols as described in the previous paper [2]. Glucose, arabinose, ribose, and rhamnose were identified, in a ratio of 2.10:1:1:0.95.

Methylation of Vitalboside F. The saponin (I) (1 g) was converted into the permethylated compound (II) [3], and this was heated with 7% $HClO_4$ in methanol at 110°C for 5 h. The mixture of sugars was separated by chromatography on silica gel in system 2. This gave substances (III-VII). Compounds (III) and (IV) had retention times corresponding to those of 2,3,4-tri-O-methyl-L-rhamnose and -ribose, and (V) and (VI) were shown by their mass-spectrometric decomposition [4] to be 2,3,4- and 2,3,6-O-methyl-D-glucoses. The structure of 3,4-di-O-methyl-L-arabinose (VII) was likewise shown by mass spectrometry [5] after the treatment of (VII) with trideuteromethyl iodide by Hakomori's method [3].

Tetrahydroaluminate Cleavage of (II). Permethylated vitalboside F (II) (1 g) was treated with an excess of $LiAlH_4$. After chromatographic purification of the decomposition products, 400 mg of the glycoside (VIII) and 250 mg of the oligosaccharide (IX) were obtained. Compounds (IV) and (VII) were found in a hydrolyzate of (VIII), and 2,3,4-tri-O-methyl-D-sorbitol (thin-layer chromatography in system 3), (III) and (VI) in a hydrolyzate of (IX).

Periodate Oxidation of the Saponin. A weighed amount of the saponin (I) (80.1 mg) was dissolved in 100 ml of 0.06 M sodium periodate. The consumption of the oxidizing agent was determined arsenometrically, and the yield of formic acid amperometrically. Aliquots were taken every 24 h. The oxidation was complete after 48 h. It was found that 1 mole of substance (I) absorbed 7.75 moles of $NaIO_4$ and liberated 2.83 moles of HCOOH.

Alkaline Saponification of the Saponin. A mixture of 1.5 g of (I) and a 10% solution of NaOH was heated at 100°C for 5 h. This gave 700 mg of saponified glycoside (X), mp 198-199°C, $[\alpha]_D^{20}$ −34° (c 1.5; methanol) and 400 mg of the oligosaccharide (XI). After acid cleavage (2% H_2SO_4, 100°C, 5 h), ribose and arabinose were found in a hydrolyzate of (X) by paper chromatography in system 1, and rhamnose and glucose were found in the hydrolyzate of (XI).

Partial Hydrolysis of (X). When compound (X) was heated with 5% $H_2C_2O_4$ (78°C, 5 h), hederagenin, (X), and substance (XII), mp 226-228°C, $[\alpha]_D^{20}$ +57.8° (c 1.5; methanol) were isolated. Literature data for hederagenin 3-O-α-arabopyranoside – mp 228°C, $[\alpha]_D^{20}$ +53.21° [13].

Oxidation of the Saponin. To 1.2 g of (I) in absolute pyridine, 50 ml of a solution of chromium trioxide in acetic acid was added, and the mixture was stirred at room temperature for 24 h. It was then diluted with water and extracted with chloroform and with chloroform–ethanol (1:1). The combined extracts were evaporated to dryness, and the residue was hydrolyzed as described above. The precipitate that deposited was filtered off and dried, and an ethereal solution of diazomethane was added to it. After

a day, the reaction product was purified on a column of alumina in system 4. Recrystallization from methanol gave 100 mg of compound (XIII) with mp 244-245.5°C, $[\alpha]_D^{20}$ 84° (c 1.3; chloroform). Literature data – mp 248-250°C, $[\alpha]_D^{20}$ +86.4° [14]. Substance (XIII) was shown in system 3 to be identical with an authentic sample of dimethyl gypsogenate.

SUMMARY

The structure of a ribose-containing triterpene glycoside consisting of a hederagenin pentaoside has been established.

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