TRITERPENE GLYCOSIDES OF LEGUMINOSAE

III. STRUCTURE OF A MINOR GLYCOSIDE OF THE KIDNEY BEAN

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In addition to the main saponin, phaseoloside D (I) [1], kidney beans contain a more polar glycoside amounting to 10% of the weight of the glycoside fraction. This substance has the same aglycone as the preceding glycoside, namely soyasapogenol C. The densitometry of paper chromatograms of hydrolyzates [2] has shown that the carbohydrate moiety of the minor saponin, phaseoloside E (II), contains glucuronic acid, glucose, galactose, arabinose, and rhamnose (1 : 3 : 2 : 1 : 1).

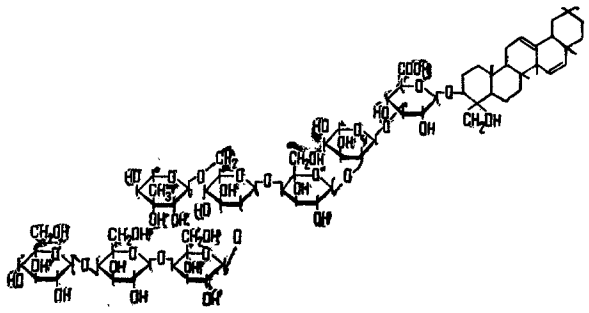
We have shown the structure of this compound in the same way as for I. When the saponin was methylated by Hakomori's method [3], a hydrolyzate of the methylated product was shown by chromatography to contain methyl 2,4-di-O-methyl-D-glucuronate, 3,4-di-O-methyl-L-arabinose, 3,4-di-O-methyl-D-glucose, 2,3,6-tri-O-methyl-D-glucose, 2,3,6-tri-O-methylgalactose, 2,3,4-tri-O-methyl-L-rhamnose, and 2,3,4,6-tetra-O-methyl-D-glucose. The authenticity of the results of the methylation of II was confirmed by periodate oxidation. The reaction practically ceased after 40 h, when 9 moles of oxidizing agent had been consumed and 2 moles of formic acid had been produced.

Examination of the methylation products of compounds I and II permits the assumption that the sequence of monosaccharides in the saponin under investigation is similar to that in I. In fact, the partial hydrolysis of II gave the same group of substances as those in phaseoloside D.

Furthermore, the treatment of compound II with a snail enzyme preparation gave phaseoloside D. The formation of 2,3,6-tri-O-methyl-D-galactose in place of 2,3,4,6-tetra-O-methyl-D-galactose on the methylation of II shows that the cellobiose residue in II is attached to the C_4 OH group of galactose.

The attachment position of the carbohydrate moiety to the aglycone was shown by the oxidation to a ketone of the 23-O-acetylsoyasapogenol C obtained from the glycoside. Therefore, the sugar chain in the saponin is attached to the secondary alcoholic group of soyasapogenol C.

Thus, the final structure of phaseoloside E appears as follows:



The configurations of the glycosidic centers were calculated by means of Klyne's rule [4].

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EXPERIMENTAL

Chromatography was carried out with KSK silica gel and "M" paper of the Leningrad Volodarskii Mill using the following systems: 1) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3); 2) chloroform–methanol–water (55 : 35 : 10); 3) toluene–ethanol (9 : 1); 4) ethyl acetate–methanol–water (10 : 2 : 5); 5) chloroform–ethyl acetate (4 : 1); and 6) benzene–acetone (2 : 1). The sugars were revealed on a paper chromatogram with aniline phthalate and on plates with conc. H_2SO_4.

Hydrolysis of Phaseoloside E (II). A 20-mg sample of compound II was subjected to cleavage with 1 ml of Kiliani's mixture at 110°C for 5 h. The hydrolyzate was shown by paper chromatography in system 1 to contain glucuronic acid, glucose, galactose, rhamnose, and arabinose. The aglycone was identified by comparative chromatography with an authentic sample on a SiO_2 plate in system 5, by its mp, and by IR spectroscopy.

Methylation of II, Hydrolysis and Separation of the Cleavage Products. One gram of II was methylated by Hakomori's method as described previously [1]. This gave 0.8 g of permethylated product.

The methylated product (0.5 g) was hydrolyzed with 5 ml of 72% perchloric acid in methanol (110°C, 4.5 h). A 0.2-g sample of the mixture of methyl glycosides obtained on methanolysis was chromatographed on a column of SiO_2 (d = 30 mm, h = 190 mm), being eluted with benzene containing from 1 to 10% of acetone. The separation was monitored chromatographically in a thin layer of silica gel in systems 3 and 6. This gave 40 mg of a mixture of 2,3,4,6-tetra-O-methylglucose and 2,3,4-tri-O-methylrhamnose, 20 mg of 2,3,6-tri-O-methylglucose, 40 mg of 2,3,6-tri-O-methylgalactose, 20 mg of 3,4-di-O-methylglucose, 18 mg of 3,4-di-O-methylarabinose, and 15 mg of methyl 2,4-di-O-methylglucuronate.

Periodate Oxiation of II. A solution of 20 mg of II in 1 ml of water was treated with 30 mg of sodium metaperiodate in 9 ml of water. The consumption of oxidizing agent was determined by Barneby's method as applied by Likhosherstov to the analysis of sugars [6].

Partial Hydrolysis of II. A mixture of 0.5 g of II and 100 ml of 10% oxalic acid was boiled at 100°C for 5 h. After treatment under the usual conditions, the mixture of saponins was separated on silica gel (d = 25 mm, h = 300 mm) in systems 2 and 4. This gave 50 mg of the glucuronoside (III), 60 mg of the bioside (IV), 35 mg of the trioside (V), and 42 mg of the pentaoside (VI) of soyasapogenol C, and 50 mg of I.

Action of the Enzyme from the Gastric Juice of the Snail Helix pomatia on Phaseoloside E. A few drops of an enzyme preparation from the snail was added to a solution of 20 mg of II in 2 ml of water, and the mixture was kept at 30°C for 30 min. Glucose was found in the substrate by chromatography on paper in system 1, and phaseoloside D in a thin layer of silica gel.

Action of Diastase on II. A solution of 20 mg of II in 2 ml of water was treated with 5 mg of diastase. In addition to glucose, the substrate was found to contain a disaccharide whose hydrolysis with 2 ml of 4% H_2SO_4 at 110°C for 3 h yielded glucose and galactose.

Preparation of 23-O-Acetylsoyasapogenol C. A mixture of I and II (250 mg) was acetylated with 14 ml of acetic anhydride in 20 ml of pyridine at 23°C for 24 h. This gave 300 mg of the acetates of I and II. A solution of 300 mg of the acetates in 5 ml of 4% H_2SO_4 was heated at 110°C for 5 h. The hydrolyzate was diluted with water and extracted with ether. The ethereal extracts were combined and evaporated to give 100 mg of saponified product, which was purified on a column of SiO_2 (d = 15 mm, h = 170 mm) in a chloroform–methanol gradient. This gave 22 mg of the monoacetate of soyasapogenol C (VII) in the form of a powder identified in systems 3 and 5.

3-Oxosoyasapogenol C. A solution of 20 mg of VII in 0.36 ml of pyridine was added to the complex obtained from 0.1 g of chromic anhydride and 1 ml of anhydrous pyridine, and the mixture was left for a day at 23°C. After the addition of 3 drops of HCl, the mixture was extracted with chloroform and the extract was evaporated. This gave 12 mg of oxosoyasapogenol C monoacetate (VIII), chromatographically homogeneous in system 5. Compound VIII (12 mg) was saponified with 0.1 ml of sodium methoxide in 1 ml of absolute methanol. After the purification on IR-120 resin (H^+ form), 5 mg of a substance giving an absorption band in the 1710-cm^{-1} region was obtained.

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