TRITERPENE GLYCOSIDES OF LEGUMINOSAE

II. STRUCTURE OF THE MAIN GLYCOSIDE OF THE KIDNEY BEAN

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In preceding communications [1, 2], we have given information on the isolation of glycosides from Phaseolus vulgaris (kidney bean). In this paper we report the determination of the structure of phaseoloside D, the main component of the glycoside fraction. On acid hydrolysis with Kiliani's mixture, the phaseoloside was cleaved into a sapogenin and the following monosaccharides: glucuronic acid, galactose, glucose, arabinose, and rhamnose. The aglycone was identified by its melting point, specific rotation, and chromatographic behavior, and the corresponding properties of its derivatives, as soyasapogenol C. The structure of this substance has been confirmed by mass spectrometry.*

To determine the type of bonds between the monosaccharides, the phaseoloside was exhaustively methylated by Hakomori's method [3]. The resulting permethylated derivative was cleaved with perchloric acid, and the hydrolysis products were separated on a column of silica gel. Six individual substances were detected. 3,4-Di-O-methylglucose was identified by paper chromatography and chromatography in a thin layer of silica gel in the presence of a reference sample. Permethylated galactose and rhamnose, and also 2,3,6-tri-O-methylgalactose and 3,4-di-O-methylarabinose, were identified by gas-liquid chromatography in the presence of reference samples. From its chromatographic behavior, an unidentified substance must be regarded as a dimethyl derivative. Its demethylation gave glucuronic acid. In addition, the methylated sugar cannot be detected on paper by Bonner's reagent [4], which would show the presence of a methoxy group of C_2 of the glucuronic acid. The reaction with triphenyltetrazolium chloride is also negative [5]. The glucuronic acid in the initial saponin is unaffected by periodate oxidation. Consequently, the compound may be ascribed the structure of methyl 2,4-di-O-methylglucuronate.

The sequence of monosaccharides in the carbohydrate chain was determined by means of partial hydrolysis with oxalic acid. This gave a glucuronoside (II), a bioside (III), a trioside (IV), and a pentaoside (V) of soyasapogenol C.

In addition to glucuronic acid, the bioside contained arabinose and the trioside contained arabinose and galactose. The cleavage of the pentaoside gave glucuronic acid, galactose, glucose, arabinose, and rhamnose.

To obtain more complete information on the structure of the saponin, enzymatic hydrolysis with diastase was used. This gave a disaccharide consisting of glucose and galactose having the glucose at the reducing end. The reaction with triphenyltetrazolium chloride was negative for glucose, which shows the presence of a $1 \rightarrow 2$ bond between the monosaccharides.

The structure of the saponin was finally established by methylating the pentaoside of soyasapogenol C. On subsequent hydrolysis, 2,4-di-O-methylglucuronic acid, 3,4-di-O-methylarabinose, 2,3,6-tri-O-methylgalactose, 2,3,4-tri-O-methylglucose, and 2,3,4-tri-O-methylrhamnose were identified. The configurations of the glycoside centers in phaseoloside D were determined from the differences in the molecular rotation of the glycoside and its progenins (Table 1) using Klyne's rule [10]. The position of attachment of the carbo-

*The mass spectra of soyasapogenol C and its acetate were recorded and interpreted by O. S. Chizhov (Institute of Organic Chemistry, Moscow).

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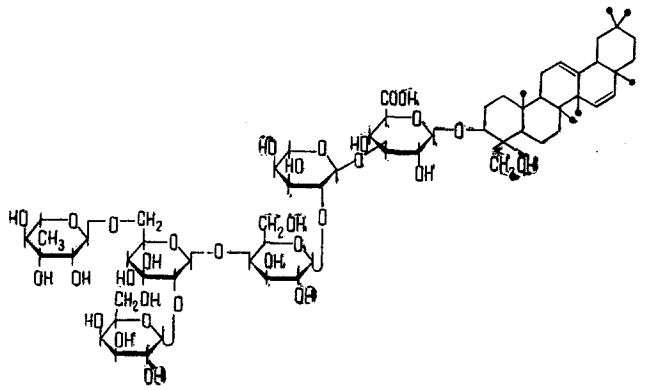
TABLE 1.

Methyl glycosides of the monosaccharides	$[M]_D^{20}$, deg		Glycosides	$[M]_D^{20}$, deg	Δc	Form of bond
	α	β				
Methyl D-galactopyranoside [6]	+380	0	Phaseoloside D	+524 + 84	+440	α
Methyl-L-rhamnopyranoside [7]	—111	+170	Pentaoside V	+ 84	—220	α
Methyl D-glucopyranoside [8]	+309	— 66	Trioside IV	+304		β
Calculated for Methyl + Methyl D-gluco L-rhamno						
α α					+198	
α β					+479	
β α					+177	
β β					+104	
Methyl D-galactopyranoside	+380	0	Trioside IV	+304	+416	α
			Bioside III	—112		
Methyl L-arabopyranoside [9]	+28	+402	Bioside III	—112	—420	α
			Soyasapogenol C	+308		β
Methyl D-glucopyranosiduronic acid	+307	—62				β
Calculated for Methyl L-arabo Methyl D-glucur.						
α α					+335	
α β					— 34	
β α					+709	
β β					+340	

hydrate chain to the aglycone was established by a number of chemical reactions which we shall describe in a subsequent paper.

Combining all these results, the structure of phaseoloside D may be represented in the form of the formula



EXPERIMENTAL

Chromatography was carried out with KSK silical gel, 'M' [slow] paper of the Leningrad Volodarskii Mill, and the following solvent 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 the paper chromatograms with aniline phthalate and on the plates with conc. H_2SO_4. Gas-liquid chromatography (GLC) was performed on a Pye argon chromatograph [column 120 × 0.5 cm, 5% poly(neopentyl glycol succinate) on Chromosorb W]. The methyl glycosides of the methylated sugars were chromatographed at 142, 153, and 161°C at a flow rate of argon of 80 ml/min.

Isolation of Phaseoloside D. One kilogram of the dry seeds of the kidney bean (1968 crop, Moldavian SSR) was extracted with methanol until the reaction for saponins was negative. The methanolic extracts,

after concentration, were suspended in water and extracted with butanol. After the butanol had been driven off, the saponin fraction was reprecipitated with acetone from methanol. This gave 2 g of total glycosides.

The total glycosides (1 g) were chromatographed on a column of silica gel. The column was eluted with system 2. The fractions containing the phaseoloside D were evaporated, and the residue was crystallized from methanol. Yield 0.1 g, mp 218-220°C (methanol), $[\alpha]_D$ +38° (c 1.05, methanol).

Hydrolysis of Phaseoloside D. A solution of 100 mg of I in 5 ml of Kiliani's mixture was heated at 110°C for 5 h. The precipitate of the aglycone that deposited was filtered off and recrystallized from methanol. Yield 30 mg, mp 238-240°C, $[\alpha]_D$ +70° (chloroform); acetate, mp 192-193°C. Literature data for soyasapogenol C, mp 239-240°C, $[\alpha]_D$ +71° (chloroform); acetate, mp 197-198°C [11]. With respect to chromatography in a thin layer of silica gel in system 5, the soyasapogenol C and its acetate were identical with authentic samples.

The filtrate was shown by paper chromatography in system 1 to contain glucuronic acid, galactose, glucose, arabinose, and rhamnose.

Methylation of I. A solution of 500 mg of I in the minimum volume of dimethyl sulfoxide was treated with 3 ml of methanesulfinyl carbanion. The mixture was stirred in a current of argon at room temperature for 10 min, after which an excess of methyl iodide was added and stirring was continued for another 20 min. Then the reaction mixture was diluted with water and extracted several times with chloroform. The completeness of the methylation was checked by chromatography on alumina in system 3 and by IR spectroscopy in the 3440-cm^{-1} region. The product was freed from the initial saponin by chromatography in a column of alumina in benzene containing 5% ethanol. The yield of permethylated product was 350 mg.

A 90-mg sample of the substance obtained was treated with 10 ml of absolute methanol and 1 ml of 72% perchloric acid. After heating in a water bath (100°C, 5 h), the solution was diluted with water, the aglycone was filtered off, and the filtrate was analyzed for sugars.

Separation and Identification of the Methylated Monosaccharides. A 200-mg sample of the mixture of methylated sugars was deposited on a column of SiO_2 (d 2.5 cm, h 20 cm) charged with benzene and was eluted with benzene containing 1-50% of acetone (gradient). Six fractions, numbered VI-XI in order of the increasing polarity of the sugars, were obtained.

On demethylation with boron chloride in methylene chloride [12], rhamnose, galactose, glucuronic acid, galactose, arabinose, and glucose were identified in system 1. 2,3,4-Tri-O-methyl-L-rhamnopyranoside, 2,3,4,6-tetra-O-methylgalactopyranoside, 2,3,6-tri-O-methylgalactopyranoside, and 3,4-di-O-methylarabopyranoside were identified by GLC in the presence of markers. On demethylation, fraction XI gave glucose. 3,4-Di-O-methylglucopyranoside was identified by paper and thin-layer chromatography in the presence of markers. Compound XI was revealed by Bonner's reagent and also by triphenyltetrazolium chloride.

Partial Hydrolysis of I. A mixture of 1 g of I and 30 ml of 10% oxalic acid was heated at 78°C for 10 h. After cooling, the reaction mixture was diluted with water and exhaustively extracted with isoamyl alcohol. The residue after the evaporation of the alcohol was chromatographed on a column of silica gel (d 2.5 cm, h 25 cm) in system 4, giving four compounds (II-V).

A mixture of 10 mg of II and 1 ml of 2% H_2SO_4 was heated at 110°C for 5 h, and glucuronic acid was found in the hydrolyzate: Under similar conditions the cleavage of III (mp 132-135°C, $[\alpha]_D$ −15°, c 1.35, methanol) gave glucuronic acid and arabinose; IV (mp 135-138°C, $[\alpha]_D$ +34.2°, c 1.17, methanol) gave glucuronic acid, arabinose, and galactose; and V (mp 206-208°C, $[\alpha]_D$ +7°, c 1.44, methanol) gave glucuronic acid, arabinose, galactose, glucose, and rhamnose.

Methylation of V. Compound V (50 mg) was methylated by Hakomori's method as described above. This gave 70 mg of permethylated glycoside. In the presence of markers, by chromatography on paper in system 1 and by GLC, methyl 2,4-di-O-methylglucuronate, 3,4-di-O-methylarabinose, 2,3,6-tri-O-methylgalactose, 2,3,4-tri-O-methylglucose, and 2,3,4-tri-O-methylrhamnose were identified.

Enzymatic Hydrolysis of I. A solution of 500 mg of phaseoloside D in 50 ml of phosphate buffer was treated with 2-4 mg of diastase and heated at 30°C for 24 h. The substrate was found to contain the oligosaccharide XII, which was purified by preparative paper chromatography in system 1. This gave 30 mg of pure XII, 10 mg of which was hydrolyzed with 1% H_2SO_4 at 110°C for 4 h. The hydrolyzate was found to con-

tain glucose and galactose. Another 10 mg of XII was reduced with sodium borohydride and hydrolyzed as described above. This gave galactose and sorbitol. The reaction of XII with triphenyltetrazolium chloride was negative.

CONCLUSIONS

The main component of the glycoside fraction of the kidney bean has been isolated; it consists of crystalline phaseoloside D, whose complete chemical structure has been shown.

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