

# THE STRUCTURE OF THE SAPONINS OF *Phaseolus vulgaris*

L. G. Kretsu, P. K. Kintya,  
and V. Ya. Chirva

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We have previously established the structures of phaseolosides D and E from *Phaseolus vulgaris* L. (kidney bean) [1]. Phaseoloside D is a hexaoside and E an octaoside of soyasapogenol C.

The sequence of bonds in the carbohydrate chains of these glycosides was deduced from the results of methylation. However, in some cases it is impossible unambiguously to determine the dimensions of the oxide rings of the monosaccharides by this method. The results of periodate oxidation did not satisfy us, either. To answer this question a set of progenins was obtained (Table 1). If a progenin contains a hexose in the furanose form present at the nonreducing end of the carbohydrate chain, its oxidation gives formaldehyde, the amount of which is easy to measure by a method recently proposed by V. E. Vas'kovskii and S. V. Isai [2]. The application of it to our progenins and to the initial glycosides gave negative results, which shows the absence of the furanose forms of the sugars in the saponins studied and confirms the results obtained by methylation and periodate oxidation.

To determine the position of attachment of the glucuronic acid to the aglycone [1] and, consequently that of the whole carbohydrate chain in saponins D and E, and also to show the configuration of the glycosidic center, we synthesized glucosiduronates of triterpene aglycones by a method which we had developed previously [3]. First, from the total saponins, the methyl glucosiduronate of soyasapogenol C was isolated by Smith's method. In order to accumulate the initial soyasapogenol C (I), which is the aglycone of phaseolosides D and E, instead of subjecting the glycosides to acid hydrolysis, which causes considerable destruction of the aglycone, we used periodate oxidation with subsequent alkali degradation by a published method [4] for obtaining native aglycones.

The methyl glucosiduronate of soyasapogenol C was synthesized by condensing the orthoester (III) with monotritylsoyasapogenol C (II). Triphenylmethyl chloride (TrCl) etherifies primary hydroxy groups but reacts slowly with secondary hydroxy groups. A model experiment with methyl oleanolate, which contains a secondary alcohol group at C<sub>3</sub>, which is analogous to the hydroxyl at C<sub>3</sub> in soyasapogenol C, showed that TrCl does in fact scarcely react with it. Conversely, the condensation of soyasapogenol C with TrCl under the same conditions led to a monotrityl derivative to which, on the basis of the considerations given, the structure of 23-O-Tr-soyasapogenol C (II) must be assigned.

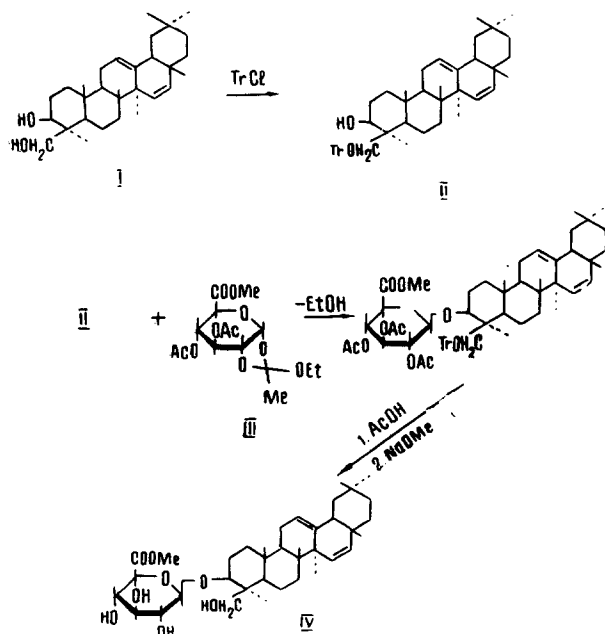
TABLE 1. Products of the Partial Acid Hydrolysis of Phaseolosides D and E

Substance	R <sub>f</sub>	mp, °C	[α] <sub>D</sub> , deg	Monosaccharide comp.
Phaseoloside D				
Monooside	0,86	136-8	+19	GA
Bioside	0,60	132-5	-15	GA, Ara
Trioside	0,34	135-8	+34,2	GA, Ara, Gal
Pentaoside	0,26	206-8	+7	GA, Ara, Gal, Rha
Phaseoloside E				
Monooside	0,85	132-4	+21	GA
Bioside	0,65	136-8	-12	GA, Ara
Trioside	0,30	140-1	+30	GA, Ara, Gal
Pentaoside	0,23	203-4	+10	GA, Ara, Gal, G
Phaseoloside	0,20	218-20	+38	GA, Rha, Ara, Gal, G, Rha

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Then compound (II) was condensed with the methyl glucuronate orthoester (III). The reaction was performed as in the synthesis of the glucuronoside of methyl oleanolate [3, 5].



The resulting product, without isolation in the pure state, was subjected to detritylation, methanolysis, and saponification followed by purification by chromatography on a column of silica gel. The structure of the progenin (IV) synthesized was confirmed by its hydrolysis, after which glucuronic acid was detected chromatographically and electrophoretically. The glycoside synthesized did not differ from a natural sample of the methyl ester of the monooside of soyasapogenol C by its melting point, IR spectrum, and specific rotation.

## EXPERIMENTAL

Nitromethane was redistilled at 100–200 mm over urea and then twice over phosphorus pentoxide; chloroform and carbon tetrachloride were redistilled twice over calcium carbonate; alumina was neutralized by being boiled with a 50-fold volume of distilled water (to activity grade III according to Brockmann).

Thin-layer chromatography was performed on alumina in system 1 [chloroform–methyl ethyl ketone (98.5 : 1.5)] and on silica gel in system 2 [chloroform–ethanol (6 : 1)], and the substances were revealed by means of a solution of  $\text{SbCl}_3$  + 5% of  $\text{SbCl}_5$  in chloroform or conc.  $\text{H}_2\text{SO}_4$ . Sugars were revealed by chromatography on paper in system 3 [butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3)] and treatment of the chromatogram with aniline phthalate. The solvent systems are shown at the end of each experiment. The melting points were determined on a Kofler block. The elementary analysis agreed with the calculated figures for all the substances.

**Size of the Oxide Rings in Phaseoloside D and E.** In a 25-ml measuring flask, 20 mg of arabitol was dissolved in 2 ml of a 0.03 M solution of  $\text{HIO}_4$  and 2 ml of 1 M sodium bicarbonate solution, and the mixture was kept at room temperature for 20 min. Then 0.6 ml of 5% phenylhydrazine hydrochloride solution was added and, after 3 min, 1 ml of 40% NaOH solution. After 5 min, 7.5 ml of isopropanol was added, and the volume of the mixture was made up to the mark with distilled water. The solution had a bright red color.

Under similar conditions, samples of phaseolosides D and E, and also of their progenins (see Table 1), remained pale yellow. A similar coloration was observed in a blank experiment performed with the reagents mentioned in the absence of glycosides and sugars.

**Preparation of the Methyl Glucosiduronate of Soyasapogenol C.** To a solution of 150 mg of a mixture of phaseolosides D and E in 14 ml of 30% (by volume) methanol in water was added 360 mg of sodium metaperiodate in 70 ml of 30% methanol. The mixture was left in the dark at 22°C for 90 h. After the end of oxidation, the solution was extracted with chloroform, washed with water, dried, and evaporated to dryness. The residue was dissolved in a mixture of 5 ml of water and 10 ml of dioxane, and 20 mg of  $\text{NaBH}_4$  was added, and then it was again left at 22°C for 24 h. After this, it was diluted with water and extracted with

chloroform, and the extract was evaporated. The solid residue was transferred to a tube, 10 ml of 0.1 N HCl in absolute methanol was added, and the mixture was heated at 80°C for 17 h. The hydrolysis product, after evaporation in vacuum with methanol (2-3 times), was chromatographed on a column of alumina (d 2 cm; h 12 cm; gradient elution from benzene to chloroform). This gave 35 mg of a chromatographically homogeneous compound (system 2). After recrystallization from a mixture of diethyl ether and petroleum ether, the substance, with the composition  $C_{37}H_{58}O_8$ , had mp 248-250°C,  $[\alpha]_D^{22} + 28^\circ$  (c 1.5; chloroform). The IR spectrum showed absorption bands at 1710-1740  $cm^{-1}$ , corresponding to an ester group. A solution of 10 mg of the product in a mixture of 4 ml of 3% sulfuric acid and 2 ml of methanol was boiled under reflux at 100°C for 4 h and was then neutralized with barium carbonate. Glucuronic acid was detected by paper chromatography in system 3 and by electrophoresis.

**Action of TrCl on Methyl Oleanolate.** To 1 ml of a solution of 50 mg of methyl oleanolate in dry pyridine was added 25 mg of TrCl, and the mixture was allowed to stand at room temperature for 24 h. Then it was poured into ice water and was exhaustively extracted with chloroform. After evaporation, the starting material was recovered. Yield 42 mg.

**Preparation of 23-O-Tr-Soyasapogenol C (II).** To a solution of 150 mg of soyasapogenol C in 3 ml of dry pyridine was added 125 mg of TrCl, and the mixture was kept at 22°C for 24 h. After working up as in experiment 3, the product was chromatographed on a column of silica gel (d 2.2 cm, h 11 cm; elution gradient from benzene to chloroform). This gave 60 mg (25%) of compound (II) with the composition  $C_{49}H_{82}O_2$ , mp 197-197.5°C (from ethanol,  $[\alpha]_D^{23} - 17^\circ$  (c 2; chloroform). The IR spectrum of (II) had absorption bands with wavelengths of 610  $cm^{-1}$ , 1600-1700  $cm^{-1}$ , and 3400-3600  $cm^{-1}$ , corresponding to phenyl and hydroxy groups.

**Methyl 3-O- $\beta$ -D-Glucosiduronate of Soyasapogenol C (IV).** To 0.340 g (0.5 mmole) of compound (II) was added 0.290 g (0.75 mmole) of the orthoester (III) [3], and the mixture was boiled in nitromethane under the conditions of the orthoester method of glycosylation [6, 7]. After purification on silica gel by gradient elution from benzene to chloroform, 0.150 g (30.6%) of the glucuronic acid derivative IV, chromatographically homogeneous in system 1, was obtained. A mixture of 0.135 g of this substance, 0.3 ml of water, and 3.7 ml of acetic acid was heated in the boiling water bath for 2.5 h, and then the mixture was evaporated with toluene until the acetic acid had been eliminated. After this, the dry residue was treated with 10 ml of 0.1 N HCl in methanol, this mixture was heated at 80°C for 10 h and evaporated, the resulting residue was dissolved in 10 ml of 0.1 N sodium methoxide in absolute methanol, and the solution was left at 22°C for 24 h. Then it was neutralized with IR-120 cation-exchange resin ( $H^+$  form) and evaporated, and by chromatography on a column of silica gel (d 2 cm, h 10 cm with gradient elution from benzene to chloroform) it yielded 0.35 g (43%) of synthetic (IV). On crystallization from a mixture of diethyl ether and petroleum ether, white needles deposited with the composition  $C_{37}H_{58}O_8$ , mp 248-250°C,  $[\alpha]_D^{22} + 28^\circ$  (c 1; chloroform). A mixture of synthetic and natural samples of the glucosiduronates of soyasapogenol C showed no depression of the melting point. The IR spectra of the two samples were identical. Substance (IV) (0.01 g) was hydrolyzed under the conditions of experiment 2. After similar working up, glucuronic acid was identified by paper chromatography in system 3.

## SUMMARY

1. The dimensions of the monosaccharide rings have been confirmed by the phenylhydrazine method.
2. An independent synthesis of the methyl glucosiduronate of soyasapogenol C, the progenin of phase-oliosides D and E, has been performed.

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