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SOYA BEAN SAPONINS—VI*

COMPOSITION OF CARBOHYDRATE AND AGLYCONE MOIETIES OF SOYA BEAN SAPONIN EXTRACT AND OF ITS FRACTIONS†

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Abstract—A modified acid hydrolysis method, using sulphuric acid–water–dioxane for four hours results in no loss of the released monosaccharides, and has been used for the hydrolysis of total soya bean saponin extract and of four of its fractions. The sugar moieties of the extract and of its fractions has been shown by thin layer and paper chromatography to consist of glucose and xylose in addition to the previously reported galactose, arabinose, rhamnose and glucuronic acid. The quantitative determination of these sugars has been carried out by direct densitometric measurements of paper chromatograms stained with silver nitrate. The sapogenin composition of the aglycone moiety of the extract and of its fractions has been established by paper chromatography.

INTRODUCTION

SOYA BEAN saponin extract (SBSE) can be fractionated into four saponin fractions *a*, *b*, *c* and *e* which differ markedly in their haemolytic and foam-forming activities.[1] We felt that a better knowledge of the qualitative and quantitative composition of the aglycone and sugar constituents of these fractions would lead to better understanding of their different known and possible biological activities. Most of the earlier studies of soya bean saponins were devoted to the chemical composition and structure of the sapogenins,[2] and little attention has been paid to the accompanying sugar moieties. The present paper describes the elaboration of optimal conditions of acid hydrolysis necessary to release intact the aglycone and sugar residues of these saponins, the qualitative identification of the sapogenins and sugars, and a direct quantitative determination of the individual sugar residues.

EXPERIMENTAL AND RESULTS

Isolation and Fractionation of Soya Bean Saponins

Soya bean saponin extract (SBSE) was prepared from ether-extracted soya bean flour, Lincoln variety, according to Birk *et al.*[3] It was separated into four fractions, *a*, *b*, *c and e*, by means of column (Al_2O_3) and paper chromatography.[1]

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Acid Hydrolysis of SBSE and of its Fractions

Acid hydrolysis of SBSE and of its fractions was attempted first by using 1 N HCl or 1 N H_2SO_4 in 50% ethanol, methods which were mainly developed for analyses of the aglycones of saponins from soya bean and other legumes.[4,5] These methods were found to be useless for quantitative sugar determinations, since boiling glucose, xylose and other known carbohydrate constituents of soya bean saponins[6] in such media, for only three hours, gave 25–30 per cent losses of the reducing power as determined colorimetrically.[7] On the other hand, when ethanol was replaced by dioxane, as recommended for the breakdown of dioscin,[8] no loss of reducing power of the sugar was noted. The latter method[8] has therefore been adopted for the acid hydrolysis of SBSE and of its fractions, as follows: A sample of SBSE (10 mg/10 ml) or of each of its fractions was dispersed in 1 N H_2SO_4 (in dioxane–water 1:3) and boiled under reflux. The optimal time of hydrolysis was determined by withdrawing aliquots from the hydrolysates at different time intervals and measuring the amount of reducing sugars liberated. After 3 hr boiling the production of reducing sugar from the breakdown of SBSE reached a maximum that remained constant for a further 13 hr at least. Since traces of SBSE but no oligosaccharides were identified chromatographically in the 3 hr hydrolysates, a period of 4 hr was chosen as being the most convenient for the acid hydrolysis of SBSE and of its fractions.

The Composition of the Carbohydrate Moiety of SBSE and of its Fractions

The H_2SO_4 in the hydrolysates was neutralized with $BaCO_3$ and the $BaSO_4$ precipitates were removed by centrifugation and filtration. Aliquots of the filtrates were used for the identification, and quantitative determination of the sugar constituents of SBSE and of its fractions.

Identification of sugars. This was performed by paper and thin-layer chromatography (TLC).

The following solvent systems were used for paper chromatography: *n*-butanol–acetic acid–water (4:1:5), upper phase;[9] isopropanol–pyridine–acetic acid–water (8:8:1:4);[10] benzene–*n*-butanol–pyridine–water (1:5:5:3), upper phase.[11] TLC was performed on Kieselgur G plates prepared according to Stahl[12] with the following solvent systems: ethyl acetate–isopropanol–water (65:23:12);[12] ethylacetate–isopropanol–*n*-butanol–water (8:4:2:2).

Paper and thin-layer chromatograms were dried in air and stained with the silver nitrate reagent;[13] TLCs were also stained with anisaldehyde[14] which enables glucose (greyish) and galactose (green) to be better distinguished. In the acid hydrolysate of SBSE, galactose, arabinose, rhamnose and glucuronic acid—the known[6] sugar constituents of soya bean saponins—could be identified. In addition, two spots, with R_f values identical to those of glucose and xylose in all five solvent systems were present in the chromatograms.

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The presence of glucose in the carbohydrate moiety of SBSE was further established by subjecting SBSE and its various fractions to digestion with β-glucosidase. Samples of SBSE or of each of its fractions were suspended in 0·1 M acetate buffer, pH 5·0 (20 mg/5 ml) and incubated with commercial almond emulsin (1 mg) for 72 hr, at 32°. Considerable amounts of glucose could be found chromatographically in the reaction mixtures which contained SBSE, and soya bean saponin fractions *a*, *b* and *c* as substrates.

Although the presence of xylose in the acid hydrolysates of SBSE has been chromatographically demonstrated, it was doubted whether it indeed exists as a part of the carbohydrate moiety, since it may also be formed by the decarboxylation of glucuronic acid in an acidic medium.[15] When pure glucuronic acid was subjected to the conditions of hydrolysis of SBSE, no xylose could be identified, and moreover, when SBSE was subjected to milder conditions of acid hydrolysis, by using 0·1 N instead of 1 N H_2SO_4, 5 hr of hydrolysis were sufficient for the liberation of xylose, whereas glucuronic acid could only be identified chromatographically after 8–9 hr of hydrolysis. Had xylose arisen from the decarboxylation of glucuronic acid, the order of their appearance in the partial acid hydrolysate of SBSE would have been reversed.

Quantitative determination of sugars. The quantitative determination of the individual sugars present in SBSE and in its fractions, was carried out on paper chromatograms according to the method of McFarren *et al.*,[16] and the chromatograms were stained with the silver nitrate reagent as described by Trevelyan *et al.*[13] The absorbancies of the developed spots were measured with a Photovolt Densitometer, using filter No. 445. All quantitative determinations of the sugars present in SBSE were performed on chromatograms developed in the benzene solvent system, since it has been found to possess the best resolving power of the five solvent systems examined. It was found that in the range of 2–10 μg of all the sugars investigated the logarithm of the concentrations is linearly proportional to the absorbancy.

TABLE 1. QUANTITATIVE ANALYSIS OF SUGARS IN ACID HYDROLYSATES OF SBSE AND OF ITS FRACTIONS

	Saponins						
	SBSE		Fractions *a*+*b**		Fraction *c*		Fraction *e*
Sugar	(μg/mg)	molar ratio	(μg/mg)	molar ratio	(μg/mg)	molar ratio	(μg/mg)
Xylose	22	1·0	13	1·0	25	1·0	—
Arabinose	64	2·9	110	8·1	52	2·1	—
Galactose	105	4·0	—	—	148	4·9	—
Glucose	106	4·0	210	12·9	58	1·9	173
Rhamnose	120	5·0	46	3·1	150	5·0	—
Glucuronic acid	60	2·1	54	3·1	70	2·1	—
Total	477		433		503		173

* Fractions *a* and *b* were analysed together, due to difficulties in their separation and because of their relatively small amounts in SBSE.[1]

The amount of sugars determined in 4 hr acid hydrolysates of SBSE and of its fractions are given in Table 1. As can be seen the four saponin fractions differ markedly in the composition of their carbohydrate moieties.

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The Composition of the Aglycone Moiety of SBSE and of its Fractions

The acid hydrolysates of SBSE and of its fractions (10 ml each) were diluted with equal volumes of water and the sapogenins were extracted with 25 ml ether. The ether extracts were washed with water until neutral, dried with Na_2SO_4 and the ether was then removed.

The residues were dissolved in a small amount of chloroform and the soya sapogenins were identified by circular paper chromatography.[17] All the five soya sapogenins,* i.e. soya sapogenols A, B, C, D, and E, were found to be among the constituents of the aglycone moieties of saponin fractions *a*, *b* and *c*. In fraction *e*, however, only soyasapogenol A could be identified.

DISCUSSION

Most of the earlier investigations of soya bean saponins were directed towards the elucidation of the structure of the sapogenins. Since the latter compounds are rather stable under conditions prevailing during acid hydrolysis no precautions were taken to prevent losses of the relatively unstable liberated monosaccharides[18] which might interfere with their quantitative determination. Losses of 25–30 per cent were encountered during acid hydrolysis in ethanol. This difficulty was overcome by using dioxane based mixtures.[8] The use of the H_2SO_4–water–dioxane system also reduced the time of hydrolysis, and a period of 3 hr boiling was found to be sufficient to liberate the maximal amounts of sugars whereas in previous reports,[4, 5] 60–72 hr of hydrolysis were recommended for soya bean saponins.

In addition to galactose, arabinose, rhamnose and glucuronic acid—the four known[6] sugar constituents of soya bean saponins—xylose and glucose have been also identified. The finding of considerable amounts of glucose in soya bean saponins (Table 1) is somewhat surprising, since except for one early report,[19] glucose has not been identified in the sugar moiety of soya bean saponins. Quantitative and qualitative analyses of the acid hydrolysates of the saponin fractions (Table 1) indicated the absence of galactose from the carbohydrate moieties of fractions *a* and *b*, which are composed mainly of glucose and arabinose, whereas the carbohydrate moiety of fraction *c* contains mainly galactose and rhamnose. In fraction *e* only glucose could be identified.

The molecular weight of soya bean saponins has been found to be approximately 950.[2] Since the average molecular weight of the soya sapogenin is 460 ($C_{30}H_{48-50}O_{2-3-4}$), it may be concluded that an average of three carbohydrate residues are attached to each sapogenin. The presence of 5–6 different sugars and five different sapogenins (soyasapogenols A, B, C, D and E) in each of the saponin fractions *a*, *b* and *c* clearly indicates that these fractions are mixtures, differing in their sugar components and possibly also in their mode of attachment. It seems, however, that these differences between the various soya bean saponins are not sufficient to allow their separation by the available chromatographic and electrophoretic methods.[1]

* In this and in the following paper the term "soya sapogenins" will be used for the aglycone moiety of soya saponins as a whole, whereas the individual aglycones will be defined as soya sapogenols A, B, C, D and E.

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