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Saponins and Sapogenins of Chick Pea, Haricot Bean and Red Kidney Bean

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ABSTRACT

*The saponins of red kidney bean (*Phaseolus vulgaris*), chick pea (*Cicer arientinum*) and haricot bean (*Phaseolus vulgaris*) all contain soyasapogenol B as the only aglycone. The levels of soyasapogenol B estimated were: chick pea, 0·075%; haricot bean, 0·149%; and red kidney bean, 0·102%; on a defatted, dry weight basis. HPLC separation of the saponin preparations indicates the presence of at least five saponins in red kidney bean, five in haricot bean and two in chick pea. Retention time comparison of the saponin preparations indicates the possible presence of soyasaponin I in all three legumes and soyasaponin II in haricot and red kidney beans.*

INTRODUCTION

Saponins are steroid or triterpenoid glycosides which exhibit a wide range of biological properties and are known to occur in a wide variety of food plants (Oakenfull, 1981). The saponins from soya bean are, perhaps, the most widely documented and recently a number of methods have appeared for their quantitative determination (Kitagawa *et al.*, 1984*a,b*; Curl *et al.*, 1985; Ireland & Dziedzic, 1986*a*).

Four aglycones (Fig. 1), soyasapogenols A, B, C and E (Kitawaga *et al.*, 1982), have been isolated after the hydrolysis of soya saponins, five of which, soyasaponins I, II, III, A_1 and A_2, have been isolated and their structures elucidated (Kitagawa *et al.*, 1976, 1985*a,b*). However, three of the

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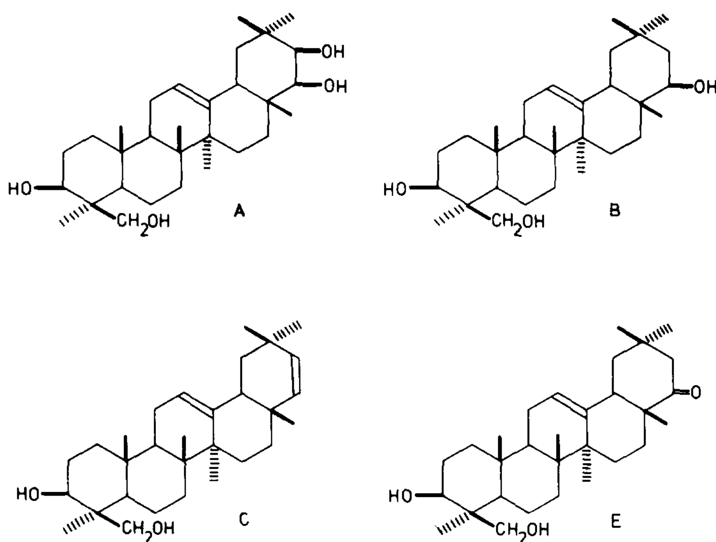

Fig. 1. Structures of soyasapogenols A, B, C and E.

soya saponins contain soyasapogenol B as the aglycone whilst the other two, soyasaponins A_1 and A_2, contain soyasapogenol A. Saponins containing soyasapogenols C or E have not been found in soya, and it appears that these sapogenins, together with a new sapogenol (tentatively named soyasapogenol B_1), may be artefacts formed during hydrolysis (Ireland & Dziedzic, 1986*b*). A number of other plants, some used as food, are reported to contain the soyasapogenols as aglycones and these need to be re-investigated in the light of the findings on artefact formation.

Five saponins have been reported in *Phaseolus vulgaris*, two of which have been isolated and their structures elucidated (Chirva *et al.*, 1970*a*,*b*). Both these saponins are stated to contain soyasapogenol C as the aglycone; however, the structures presented for the two saponins contain a 15,16 double bond (Chirva *et al.*, 1970*a*,*b*) rather than the 21,22 double bond generally accepted for soyasapogenol C (Fig. 1). Additionally, French beans (*P. vulgaris*) reportedly contain saponins which yield four aglycones on hydrolysis having the same R_f as the soyasapogenols when analysed by paper chromatography (Pantic *et al.*, 1974).

In order to clarify the situation, the recently developed high-performance liquid chromatography (HPLC) methods for the analysis of soya saponins (Ireland & Dziedzic, 1986*c*) and sapogenins (Ireland & Dziedzic, 1986*a*) have been used to investigate the saponins and sapogenins of haricot (*P. vulgaris*) and red kidney (*P. vulgaris*) beans. The saponins and sapogenins of chick pea (*Cicer arientinum*) have also been investigated as part of our continuing studies on saponins in foods.

EXPERIMENTAL

Materials

Haricot beans, chick peas and red kidney beans were purchased locally. Each sample was milled (UDY cyclone sample mill, Tecator Ltd, Bristol, Great Britain) and defatted in a Soxhlet apparatus with light petroleum (boiling point 60–80°C). Soyasapogenol B was isolated from defatted soya flour as described below. C_{18} Sep-Pak™ cartridges were obtained from Millipore-Waters, Harrow, Great Britain. The HPLC column used for the quantitative determination of the aglycones packed with LiChrosorb Si-60 5μm was bought complete from Hichrom, Reading, Great Britain, and the column used for the separation of the saponin preparations was packed in our laboratory with Spherisorb 5μm silica (Phase Separations, Queensferry, Great Britain). Thin-layer chromatography (TLC) plates of silica gel were purchased from Merck (0·25 mm) or prepared in our laboratory (1 mm) from silica gel 60G (Merck, 7731).

METHODS

Isolation of soyasapogenol B

Defatted soya flour was extracted with methanol in a Soxhlet apparatus. After extraction, sufficient conc. sulphuric acid was carefully added to the methanol extract to give a 3% sulphuric acid in methanol solution (v/v) and hydrolysis was effected by refluxing for 3 h. The hydrolysis mixture was cooled, neutralised with conc. ammonia solution and the methanol removed under reduced pressure. The sample was dispersed in distilled water and the sapogenins extracted into diethyl ether. The ether extract was washed with 2% potassium hydroxide solution (m/v) and distilled water, and the sapogenin fraction isolated by removal of the diethyl ether. Soyasapogenol B was isolated by repeated preparative TLC on silica gel (1 mm) using light petroleum (boiling point 60–80°C)—ethyl acetate (4:3) as eluent. The soyasapogenol B was visualised by spraying with 0·01% Rhodamine 6G in ethanol and viewing under UV. The band corresponding to soyasapogenol B was removed, eluted with diethyl ether and rechromatographed until pure. The identity of the isolated soyasapogenol B was confirmed by mass spectrometry, NMR and chromatographic comparison with an authentic sample.

Saponin extraction procedure

A weighed quantity (about 20 g) of defatted sample was extracted for at least 36 h with methanol in a Soxhlet apparatus.

Saponin hydrolysis procedure

The methanolic extract was made up to 250 ml with methanol and conc. sulphuric acid (7·5 ml) carefully added to give approximately a 3% sulphuric acid in methanol solution (v/v). Hydrolysis was effected by refluxing for 3 h, this method of hydrolysis having been observed to give maximal yield of sapogenins with minimal artefact formation for soya (Ireland & Dziedzic, 1986*b*). The hydrolysis mixture was cooled, neutralised with conc. ammonia solution and the methanol removed under reduced pressure. The sample was then dispersed in distilled water (200 ml) and transferred to a separating funnel. The hydrolysis flask was washed with diethyl ether (200 ml) which was then used to extract the sapogenins from the aqueous solution. Two further portions of diethyl ether (2 × 100 ml) were used to ensure complete extraction. The combined ether extracts were washed with distilled water (2 × 100 ml), the ether removed and the sapogenin extracts made up to constant volume (5 ml for chick pea and red kidney bean; 10 ml for haricot bean) with chloroform–methanol (1:1, v/v).

Quantitative analysis of sapogenin fractions

This was carried out in triplicate for each sample using normal-phase HPLC with gradient elution as previously described (Ireland & Dziedzic, 1985).

Qualitative analysis of sapogenin fractions

The aglycone fractions were separated by silica gel TLC (0·25 mm) using light petroleum (boiling point 60–80°C)—ethyl acetate (1:1, v/v) and light petroleum (boiling point 60–80°C)—chloroform–acetic acid (7:2:1, v/v/v) as eluents. The following spray reagents were all freshly prepared and development was by heating at 110°C for 5 min: (i) Ekkert reagent—anisaldehyde (0·5 ml) dissolved in a mixture of acetic acid (49 ml) and conc. sulphuric acid (1 ml); (ii) vanillin–phosphoric acid reagent—vanillin (1 g) dissolved in a mixture of ethanol (40 ml) and orthophosphoric acid (10 ml); (iii) ceric sulphate in sulphuric acid—saturated solution of ceric(IV) sulphate in 65% sulphuric acid; and (iv) Liebermann–Burchard reagent—acetic anhydride (5 ml) and conc. sulphuric acid (5 ml) in ethanol (50 ml).

Periodate oxidation of saponin extract (based on Dugan & de Mayo, 1965)

Methanolic saponin extracts of chick pea, red kidney and haricot beans were obtained as described above and the methanol removed under reduced pressure. The extract was suspended in water (100 ml) and cooled

in an ice-bath. Sodium periodate (4 g) was slowly added to the extract at 0°C over 30 min with constant stirring. The reaction mixture was then kept in the dark for 24 h at room temperature (*ca.* 18°C). Excess periodate was destroyed by the addition of 1 ml of ethan-1,2-diol and stirring for 1 h. Potassium hydroxide (5 g) was added and the mixture heated at 90°C for 30 min. After cooling to room temperature and careful acidification (pH 4 with HCl) the liberated aglycones were extracted with diethyl ether (2 × 100 ml). The ether extracts were washed with 2% potassium hydroxide solution (2 × 50 ml), 2% sodium thiosulphate solution (2 × 50 ml) and water (2 × 50 ml). The aglycone fraction was obtained by removal of the ether and dissolving in chloroform–methanol (1:1, v/v).

Isolation of saponin preparations

Duplicate methanolic saponin extracts of chick pea, red kidney and haricot beans (*ca.* 5 g) were obtained as described above and the methanol removed under reduced pressure. The extracts were dispersed in distilled water (10 ml) and passed through a Sep-Pak C-18 cartridge which had been preconditioned by washing with methanol (2 ml) followed by water (5 ml). The cartridge was washed with a further portion of water (10 ml) and the saponin preparation eluted from the cartridge with methanol (5 ml). The solvent was removed under reduced pressure and the saponin preparations dissolved in dry methanol (1 ml).

Throughout the extraction and isolation procedure, to ensure no loss of compound, the location of the saponins was monitored by TLC on silica gel (0·25 mm) using chloroform–methanol–water (6:4:1, v/v/v) as eluent and Ekkert reagent for visualisation.

Zémplen deacetylation of saponin preparations

A solution of sodium methoxide in dry methanol (1M) was prepared by carefully dissolving sodium (2·3 g) in dry methanol (100 ml). Deacetylation was accomplished by the addition of ten drops of the sodium methoxide solution to one of the duplicates of each of the saponin preparations isolated and stirring at room temperature for 20 min. The sample was neutralised by the addition of a small quantity of Dowex 50WX8 resin which was subsequently filtered off to yield the deacetylated saponin preparations.

Qualitative analysis of saponin preparations

This was carried out using normal-phase HPLC with gradient elution and a mass detector as previously described for soya saponins (Ireland & Dziedzic, 1986*c*).

RESULTS AND DISCUSSION

The chromatograms of the aglycone preparations of chick pea, haricot bean and red kidney bean obtained using the HPLC method previously developed (Ireland & Dziedzic, 1985) are shown in Figs 2, 3 and 4, together with the chromatogram of the aglycone fraction of soya bean (Fig. 5). From the chromatograms it can be seen that red kidney bean, chick pea and haricot bean all contain soyasapogenol B as the sole sapogenin. This was confirmed by spiking the aglycone preparations with authentic soyasapogenol B. Similarly, the presence of sterols, possibly originating from sterol glycosides, was confirmed in all the samples. The identity of the third main component in the chick pea aglycone preparation is, as yet, unknown.

The identity of the sapogenin in each of the aglycone preparations was further proven by separating the components on silica gel TLC and visualising with a number of spray reagents. A component with the same R_f and same colour production on reaction with the various visualisation reagents as authentic soyasapogenol B was found in each sample (chick pea, red kidney and haricot beans), thus confirming the compound as soyasapogenol B.

In a separate experiment, a sample of the methanolic extract of each of chick pea, red kidney and haricot beans was subjected to periodate oxidation to liberate the aglycones. This method was used to ensure that there was no artefact formation on hydrolysis (Ireland & Dziedzic, 1986*b*). The aglycones produced were analysed by HPLC and TLC and all three

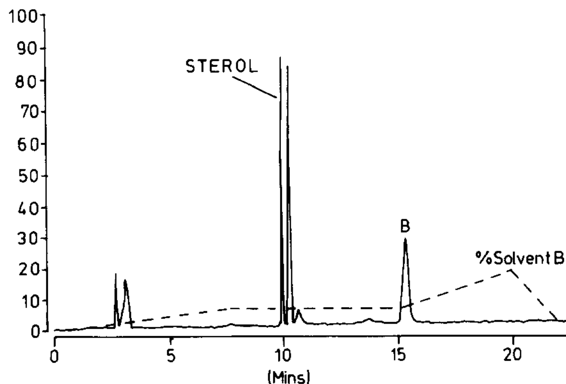

Fig. 2. HPLC separation of sapogenin extract of chick pea. Conditions: 25 × 0·46 cm normal-phase silica column; flow rate, 1·5 ml/min; Solvent A—light petroleum 60–80°C boiling point, solvent B—ethanol; mass detector. For full details see Ireland & Dziedzic (1985). For identity of component see Fig. 1.

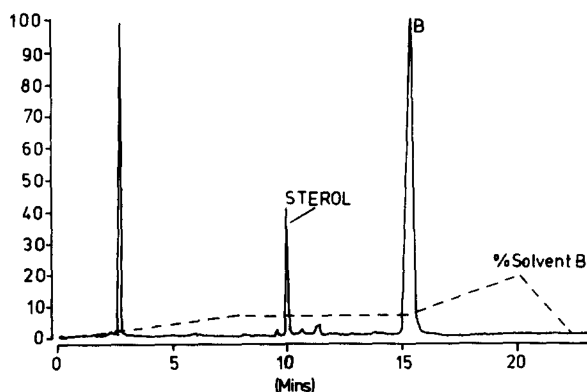

Fig. 3. HPLC separation of sapogenin extract of haricot bean. Conditions as Fig. 2. For identity of component see Fig. 1.

preparations contained soyasapogenol B as the only aglycone. However, the yield from chick pea was considerably lower than haricot or red kidney beans, possibly indicating that the sugar moiety directly attached to the sapogenin may be resistant to periodate oxidation, as has been found in soyasaponins A_1 and A_2 (Ireland & Dziedzic, 1986*b*).

Table 1 shows the estimates of lipid, moisture and soyasapogenol B in each sample. It should be stressed that the estimate of soyasapogenol B in Table 1 is an estimate of the sapogenin, not saponin, content. A sapogenin/carbohydrate ratio of 1:1 (m/m) for soya saponins has been reported (Gestetner *et al.*, 1966) and can be used to estimate total saponin content from sapogenin content. However, the sapogenin/carbohydrate ratios of the saponins of haricot bean, chick pea and red kidney bean are not known. Indeed, two saponins reportedly isolated from *P. vulgaris*

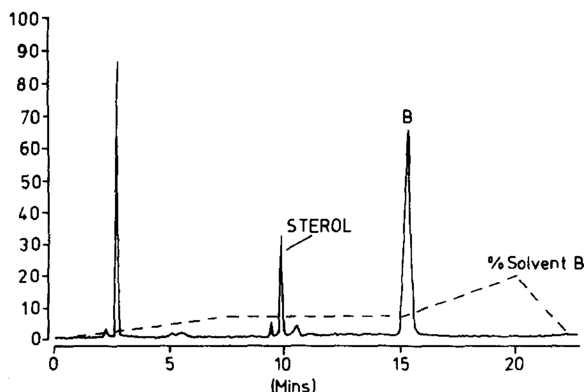

Fig. 4. HPLC separation of sapogenin extract of red kidney bean. Conditions as Fig. 2. For identity of component see Fig. 1.

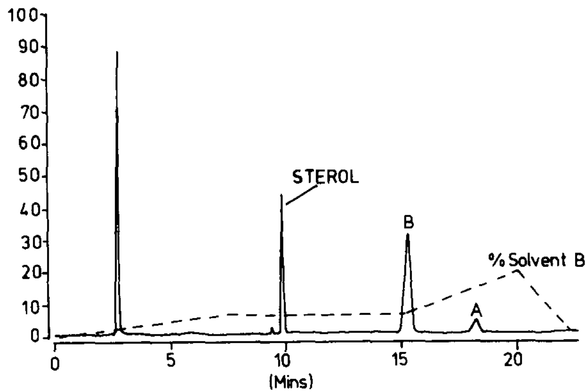

Fig. 5. HPLC separation of sapogenin extract of soya bean. Conditions as Fig. 2. For identity of component see Fig. 1.

contain six and eight monosaccharide residues (Chirva *et al.*, 1970*a*,*b*), giving approximate sapogenin/carbohydrate ratios of 1:2·1 (m/m) and 1:2·9 (m/m), respectively.

Fenwick & Oakenfull (1983) estimated the saponin content of chick peas, haricot beans and red kidney beans as 5·6%, 1·9% and 1·6%, respectively, on a dry weight basis using TLC/densitometry of the intact saponins. However, it has recently been suggested that when this method is used non-saponin material may co-elute and result in elevated estimates of saponin content or that the assignment of certain spots on the chromatogram as saponins may be incorrect (Curl *et al.*, 1985). When soya was analysed by a modified method using TLC/densitometry (Curl *et al.*, 1985) the value obtained was in close agreement with other recent estimates of soya saponin content (*inter alia* Ireland & Dziedzic, 1986*a*) and considerably

TABLE 1
Sapogenin, Moisture and Lipid Contents of Chick Pea, Haricot Bean, Red Kidney Bean and Soya

Sample	*Moisture* (% *m/m*)	*Lipid* (% *m/m*)	*Soyasapogenol B*[a] (% *m/m*)
Haricot bean	10·0	1·9	0·149
Chick pea	10·9	5·2	0·075
Red kidney bean	12·8	1·5	0·102
Soya bean[b]	11·4	19·7	0·245 (0·083[c])

[a] Defatted, dry weight basis.
[b] Ireland & Dziedzic (1986*a*).
[c] Soyasapogenol A.

lower than the level reported by Fenwick & Oakenfull (1981). The possibility that the results of Fenwick and Oakenfull are artificially high explains the discrepancy between their results and the estimates of sapogenin content presented here, even if a 3:1 carbohydrate/sapogenin ratio were used to estimate saponin content from sapogenin content.

Although the quantitative determination of sapogenin content yields information on the aglycones present and their content (and on saponin content by inference), no information is obtained on the saponins themselves. Different saponins, even from the same plant source, can differ markedly in their biological properties; thus data on the content of individual saponins is highly desirable. Consequently, the methanolic extracts obtained from each of the samples were subjected to HPLC analysis as previously described for soya saponins (Ireland & Dziedzic, 1986*c*).

The chromatograms of the saponin preparations of haricot bean, red kidney bean and chick pea are shown in Figs 6, 7 and 8, respectively. The saponins in soya bean have been observed to be present in both free and acetylated forms (Ireland & Dziedzic, 1986*c*); consequently, the saponin preparations of each sample were also subjected to HPLC after deacetylation to determine the extent, if any, of acetylation.

It can be seen (Fig. 6) that haricot bean contains three main saponins with at least two minor saponins which are poorly resolved from the main saponins. No changes in the chromatogram were apparent after deacetylation of the saponin preparation, indicating the absence of saponins in an acetylated form. Spiking of the saponin preparation with

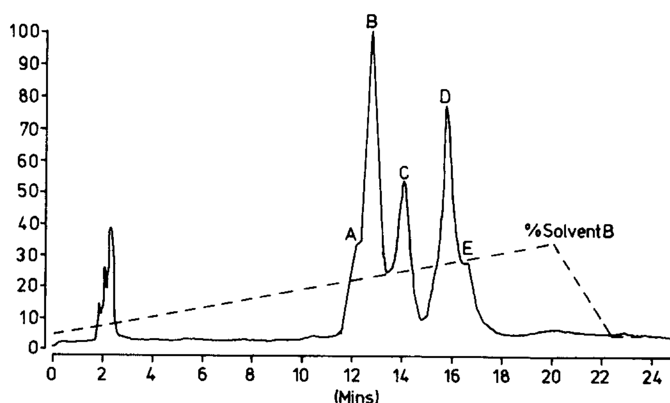

Fig. 6. HPLC separation of saponin preparation of haricot bean. Conditions: 25 × 0·46 cm normal-phase silica column; flow rate, 1·5 ml/min; solvent A—chloroform–acetic acid (99:1), solvent B—methanol–water–acetic acid (95:4:1); mass detector. For full details see Ireland & Dziedzic (1986*c*). For identity of component see text.

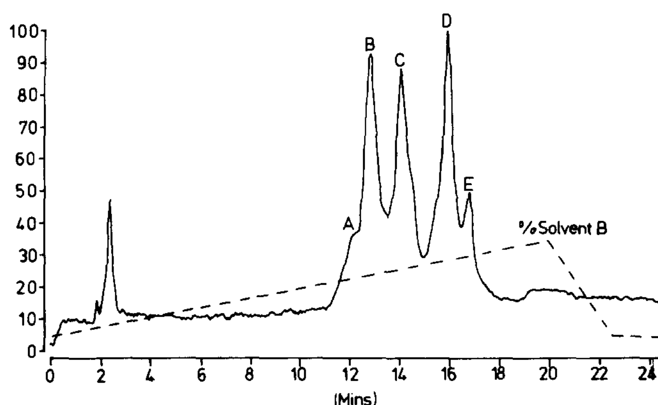

Fig. 7. HPLC separation of saponin preparation of red kidney bean. Conditions as Fig. 6. For identity of component see text.

authentic samples of soyasaponins I, II and III indicated the presence of two components in the haricot bean saponin preparation, B and C, in Fig. 6, having the same retention times as soyasaponins II and I, respectively, and the absence of soyasaponin III. Thus two of the components of the haricot bean saponin preparation may be soyasaponins I and II but the other three components are unidentified, and probably previously unknown, saponins.

Red kidney bean, the other variety of *P. vulgaris* investigated, showed a very similar saponin profile (Fig. 7) to haricot bean (Fig. 6). In a similar manner to haricot bean there was no apparent difference between the chromatograms of the deacetylated and native saponin preparations and two components, B and C (Fig. 7), had the same retention times as soyasaponins II and I, respectively.

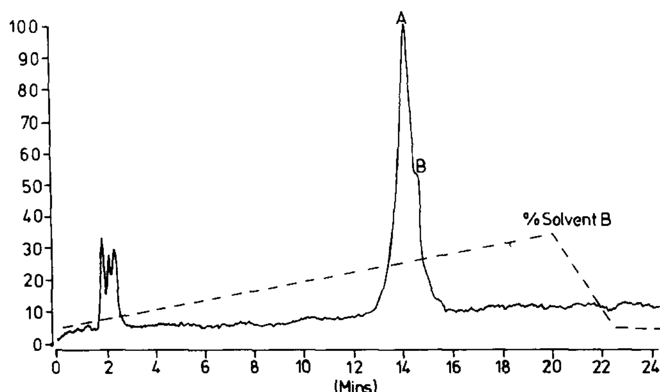

Fig. 8. HPLC separation of saponin preparation of chick pea. Conditions as Fig. 6. For identity of component see text.

The chromatogram of the saponin preparation of chick pea (Fig. 8) indicates the presence of two saponins, although poorly resolved. Again, no change is apparent in the chromatogram on deacetylation of the saponin preparation and spiking with authentic standards of the soyasaponins indicates that one of the two component saponins, A in Fig. 8, may be soyasaponin I.

The results indicate that the saponins in the legumes studied are dissimilar to those in soya bean in that no acetylation is present. The presence of soyasaponin I is indicated in all three legumes and soyasaponin II in red kidney and haricot beans. Red kidney and haricot beans, both varieties of *Phaseolus vulgaris*, have very similar saponin profiles, which also contain three unidentified saponins with soyasapogenol B as the aglycone. Chick pea, red kidney bean and haricot bean all contain soyasapogenol B as the sole aglycone and the presence of this aglycone in legumes may be more extensive than previously recognised.

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