Two New Saponins from Faba Bean (*Vicia faba* L.)

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Saponins, *Vicia faba*

Two new saponins were isolated from faba bean (*Vicia faba* L.) by column chromatography (Sephadex LH-20 and ODS) and semi-preparative HPLC. Their molecular weights determined by FAB-MS were 980 and 964, respectively. Results of TLC analyse showed that these compounds are similar to soya saponin group B. The presence of separated saponins was confirmed by TLC for seeds of four cultivars of faba bean.

Introduction

Saponins are a chemically complex group of compounds which occur naturally in plants. Due to their high biological activity, saponins have become the object of interest of many researchers from different scientific disciplines (Price and Fenwick, 1987). For example, a lowering of plasma cholesterol (Amarowicz *et al.* 1994), antioxidative properties (Ohminami *et al.*,1984), surface activity (Gothani *et al.*, 1990), and antiviral activity against HIV *in vitro* (Nakashima *et al.,* 1989) have been attributed to saponins.

Price *et al.* (1986) concluded on the basis of analysis of various edible beans that soybean contained the highest levels of saponins. After soya haricot, runner and kidney beans contained the most saponins. The presence of saponins was also reported for lentils, chickpea, broad bean, green pea, blackeye pea, and snow pea (Applebaum *et al.*, 1969; Knohar and Chauhan, 1986; Livingstone *et al.*, 1978, Sodipo and Arinze, 1985; Ruiz *et al.*, 1996). Saponins were detected in faba bean (*Vicia faba*) by Sharman and Seghgal (1992). Amarowicz *et al.*(1992; 1994) employed RP liquid chromatography on an ODS column and TLC to analyze the saponins of faba bean. TLC and FTIR analyses revealed that two saponins isolated from faba bean are similar to soya saponin group B. The results from partial characterization of two new saponins separated from faba bean, are presented in this paper.

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Materials and Methods

Seeds of faba bean (*Vicia faba* L.) of the Dino cultivar were purchased from the Institute of Plant Genetics and Breeding of the Agricultural University in Lublin (Poland). A portion of 300 g of seeds was milled and then extracted three times with 2 l of 70% ethanol at 80 °C for 3 h. The extract was evaporated to dryness at reduced pressure and dispersed in butanol-water (1:1, v/v) (Shiraiwa *et al.,* 1991). After standing overnight, the butanol layer was separated and evaporated to dryness. The residue was fractionated by gel filtration on Sephadex LH-20 (Shiraiwa *et al.* 1991). The fractions which were found to contain saponins by means TLC, were pooled, concentrated and freeze dried. A 100 mg portion of the the preparation obtained was dissolved in 5 ml of methanol – water (4:6, v/v) and loaded onto the chromatographic column (1.5 x 40 cm) packed with ODS gel (Yamamune Chemical Laboratories Co Ltd., Kyoto; 60/30 mesh). The elution was conducted with methanol-water, first at 4:6 and then at 6:4 (v/v) (Amarowicz *et al.*, 1991). Fractions eluated from the column with the second solvent were pooled and evaporated to dryness.

Saponins from the freeze dried extract were also separated using semipreparative HPLC (Shiraiwa *et al.*, 1991): chromatograph Hitachi 655–15, RI detector ERC 7520, column LiChromosorb RP-18-5 μm, 7.6 x 25 mm (Merck), mobile phase methanol-propanol-water-acetic acid (32.3:4.2:63.4:0.1, v/v/v/v).

The purity of separated saponins was examined by TLC using silica gel plates (Merck Germany) and a chloroform-methanol-water (65:35:10, v/v/v)

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mobile phase (Amarowicz *et al.*, 1992), and RP-18 gel plates (Merck) and the mobile phase used in the HPLC method. Spots on silica gel plates were visualized by spraying with 10% (w/v) sulfuric acid and heating at 120 °C for 10 min (Amarowicz *et al.*, 1992), and on reversed-phase plates were visualized by spraying with a solution of *p*-anisaldehyde-glacial acetic acid-concentrated sulfuric acid (1:100:2, v/v/v) (Muzquiz *et al.*, 1993).

Mass spectra (FAB-MS) of the saponins was determined using a JEOL DX-303 instrument (Nihon Denshi). UV spectrum was recorded on a Beckman DU 7500 diode array spectrophotometer. Hemolytical activity of the saponins was confirmed using sheep blood erythrocytes suspension (Kyoto Pharmaceutical Industries Co. Ltd., Kyoto) (Kabat and Mayer, 1964).

The presence of separated saponins in seeds of four cultivars of faba bean: Dino, RAH, Kamir and Nadwiślański was examined using TLC (Amarowicz *et al.*, 1991).

Results and Discussion

The separated saponin gave one peak from the semipreparative HPLC chromatogram. Retention time was similar to retention times of soyabean saponins group B. On silica gel plates one brown spot with a R_f value 0.19 was visualized (Fig. 1 A).

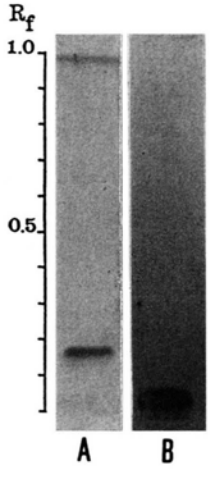

Fig. 1. TLC chromatograms of separated faba bean saponins; A – a silica gel plate, B – RP-18 plate.

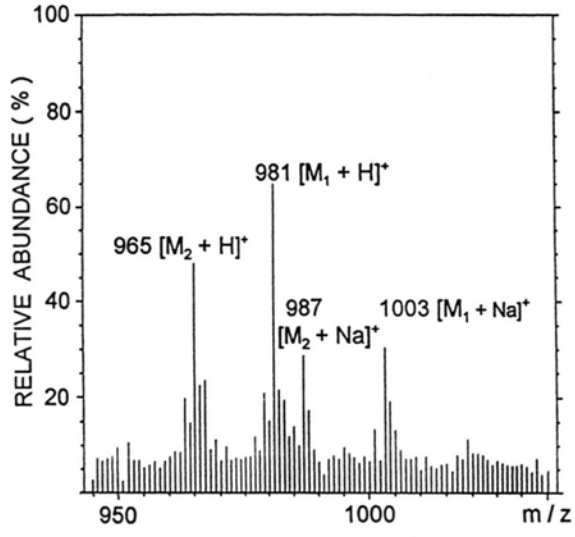

Fig. 2. FAB-MS spectra of separated faba bean saponins.

The location of the spot on the plate and its brown colour were similar to that of the soybean saponins from the B group. On the RP-18 TLC plate, only one green spot (R_f 0.05) was obtained from the separated saponin (Fig. 1 B). Positive reaction with *p*-anisaldehyde on TLC plate is typical vor saponins (Muzquiz *et al.*, 1993). UV spectrum of the separated saponin did not indicate absorption maximum what is also characterized for soybean saponins. Hemolytical activity of the saponin was noted against sheep blood erythrocytes.

FAB-MS (positive-ion mode) (Fig. 2) showed that the purified saponin was in reality a mixture

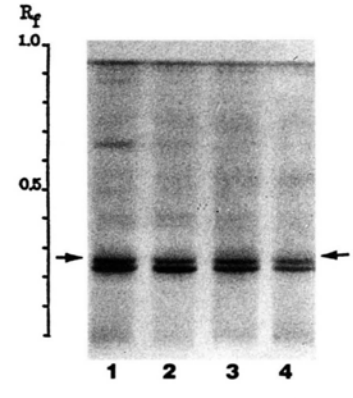

Fig. 3. Silica gel TLC chromatogram of separated saponins (marked with arrows) for seeds of four cultivars of faba bean: DINO (1), RAH (2), KAMIR (3) and NADWIŚLAŃSKI (4).

of two compounds not separated by the chromatographic techniques. Compound **1** (molecular weight 980) showed molecular ion peaks at *m/z* 981 and 1003 as $[M_1 + H]^+$ and $[M_1 + Na]^+$, respectively. Peaks at *m/z* 965 and 987 from compound **2** (molecular weight 962) may be interpreted as $[M_2 + H]^+$ and $[M_2 + Na]^+$, respectectively. The difference in the molecular weight between the two isolated saponins might be due to the presence of a $-CH_3$ group in the sugar ring of saponin 2 instead of a $-CH_2OH$ in saponin 1. In the early-ear work (Amarowicz *et al.*, 1997) the presence of two other saponins in faba bean with molecular weights 978 and 962 was noted. Results obtained are similar to the molecular weights of soybean saponin group B recorded by Shiraiwa *et al.* (1981): saponin Ba – 958, Bb – 942, Bc – 912, Bd 956 and Be 940.

The presence of separated saponins was confirmed by TLC for seeds of four cultivars of faba bean (*Vicia faba* L.): Dino, RAH, Kamir and Nadwiślański (Fig. 3).

In conclusion, FAB-MS and TLC data of the 2 saponins isolated from faba bean (*Vicia faba* L.) indicated that these saponins possess a chemical structure similar to that of soybean group B saponin.

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