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SOYASAPONIN IV, AN ADDITIONAL MONODESMOSIDIC SAPONIN ISOLATED FROM SOYABEAN

JEREMY C. BURROWS, KEITH R. PRICE and G. ROGER FENWICK

AFRC Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich NR4 7UA, U.K.

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Key Word Index—*Glycine max*; Leguminosae; soya; triterpenoid saponin; 3-*O*-[α-L-arabinopyranosyl(1 → 2)β-D-glucuronopyranosyl-(1 →)]-3β,22β,24-trihydroxyolean-12-ene.

Abstract—A new saponin has been isolated from the methanolic extract of soybean meal and its structure elucidated as 3-*O*-[α-L-arabinopyranosyl(1 → 2)β-D-glucuronopyranosyl(1 →)]-3β,22β,24-trihydroxyolean-12-ene.

INTRODUCTION

Five soyasaponins, namely I, II, III, A_1 and A_2 have been isolated from soyabean and recently fully characterized by Kitagawa and co-workers [1–3].

The presence of these saponins in foodstuffs has attracted considerable interest over the last few years [4, 5] due to their physiological properties, including hypocholesterolaemic activity [6], gut wall permeabilization [7] and adverse sensory characteristics [8]. Further biological studies in this area require relatively large quantities of isolated pure saponins and during the course of large scale extraction and isolation of the monodesmosidic soyasaponins I, II and III from soyabean meal a fourth saponin, described here, was isolated and characterized.

RESULTS AND DISCUSSION

The methanol extract of defatted soya was partially purified by flash chromatography on octadecyl silanized silica using water and methanol. The methanol eluate was then subjected to chromatography on silica gel using a chloroform, methanol, water mixture [5] where the soyasaponins were found to elute in the order soyasaponin IV, III, II and I. The isolated soyasaponin IV gave a single spot in two different thin layer chromatographic systems.

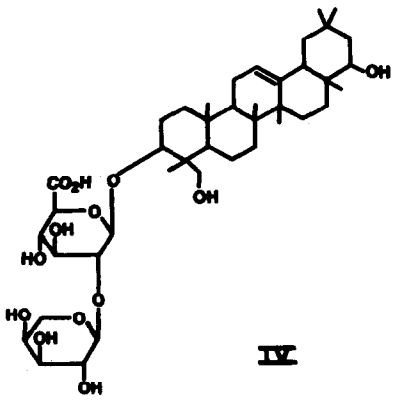
Mass spectrometry using fast atom bombardment (FAB) in the negative ion mode produced a spectrum containing ions at m/z 765, 633, 421 corresponding to the [M − H]-ion and sequential losses of a pentose (132 mu), a uronic acid (176 mu) and two molecules of water (36 mu) from the aglycone. The positive ion FAB mass spectrum similarly exhibited ions at m/z 767, 635, 441, 423 corresponding to the MH^+ ion and sequential losses of pentose, uronic acid and two molecules of water. The last two ions are consistent with the presence of soyasapogenol B (M_r 458) as the aglycone. Further comparison of the aglycone with an authentic sample using TLC, GC and GC/MS confirmed this identity.

The 300 MHz ^{1}H-NMR spectrum of the permethylated saponin exhibited three signals of interest, two doublets at δ4.40, J = 7.2 Hz and 4.62, J = 6.0 Hz (anomeric proton signals indicating a β-linked uronic acid and an α-linked L-arabinose, respectively [1]). The third signal δ5.21 (*br s*) corresponded to the C-12 olefinic proton of soyasapogenol B [9].

Standard GC sugar analysis on the alditol acetates of the acid hydrolysed disaccharide showed only the pentose, L-arabinose (uronic acid not being detected by this method). The permethylated saponin was reduced using $LiAlD_4$ (whereby the uronic acid moiety was converted to a deuterated hexose), and the sugars hydrolysed, reduced and acetylated to their respective partially methylated alditol acetates. The two sugars shown to be present by FAB-MS, namely a uronic acid and a pentose, were identified using a combination of retention time and coupled GC-MS analysis. Modes of linkage were also determined using these data. Two peaks were observed at 34 and 62 min with ions at m/z 162, 161, 118, 117 and m/z 191, 190, respectively. These are characteristic of a terminal L-arabinose and a 1,2-linked deuterated D-glucose [10].

The known structures of soyasaponins I, II and III, together with that proposed here for soyasaponin IV, show a relationship whereby III is an analogue of I (by a loss of the terminal sugar, L-rhamnose); similarly IV can be seen as an analogue of II (by the loss of the terminal sugar, L-rhamnose).

From the data presented here, IV was concluded to be 3-*O*-[α-L-arabinopyranosyl(1 → 2)β-D-glucuronopyranosyl-(1 →)]-3β,22β,24-trihydroxyolean-12-ene (**1**).



IV

EXPERIMENTAL

Isolation of saponin. Soyabean meal (100 g) was defatted by Soxhlet extraction with $CHCl_3$ (800 ml, 16 hr). The air-dried defatted meal was then Soxhlet extracted with redistilled MeOH (900 ml, 30 hr). The MeOH extract was concentrated to dryness, dissolved in H_2O (50 ml) and eluted through a column of octadecylsilane (C_{18}) bonded silica gel (100 g) with H_2O (500 ml) and MeOH (500 ml). The MeOH eluate was evaporated to dryness (1 g) and treated with 5% methanolic KOH by reflux (30 min) to remove any acetyl groups. The reaction mixture was neutralised with ion exchange resin (Dowex 50W), filtered, evaporated to dryness and applied to a silica gel column (100 g) in $CHCl_3$–MeOH–H_2O (7:3:1) and eluted with the same solvent. Fractions were monitored by TLC on silica gel $(COOH)_2$ ($CHCl_3$–MeOH–H_2O, 65:35:10, lower layer) and on silica gel (C_{18}) (MeOH–H_2O, 3:2). A chromatographically homogeneous sample of IV (16 mg) was obtained, as a non-crystalline glass.

Structural analysis. A 5 mg sample was taken for standard sugar analysis, hydrolysed (HCO_2H, H_2SO_4), reduced ($NaBH_4$), acetylated (Ac_2O) and the resultant alditol acetates analysed by GC.

A further 10 mg sample was permethylated with dimsyl sodium (in DMSO) and MeI and submitted for ^1H NMR spectroscopy ($CDCl_3$). After reduction of the uronic acid with $LiAlD_4$ the permethylated products were hydrolysed to the individual sugars (HCO_2H, H_2SO_4), reduced to alditols ($NaBD_4$), acetylated (Ac_2O) and the partially permethylated alditol acetates thus formed analysed by GC and combined GC/MS. GLC was carried out on a Perkin–Elmer Sigma 3B instrument using a 1 m × 3 mm ID glass column packed with 3% OVI on Diatomite C-AW 60-80#. Column temperature was 280°, injector 285° and detector 300°. Argon carrier gas flow rate was 35 ml/min. NMR spectroscopy was carried out using a 300 MHz Bruker spectrometer using $CDCl_3$ as solvent.

FAB MS were obtained using a Kratos MS9/50TC spectrometer by bombardment of the sample with a fast atom beam of xenon produced by an Ion-Tech 11NF atom gun operating at 9 kV (nominal). The spectra were recorded using a UV galvanometer recorder.

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