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Saponins and polar compounds from *Trifolium resupinatum*

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Abstract

In addition to the known soyasaponin I, 3-*O*-[α-L-rhamnopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 2-β-D-glucuronopyranosyl]-22-*O*-[β-D-glucuronopyranosyl(1 → 2)-β-D-glucopyranosyl]soyasapogenol B, soyasaponin II, 3-*O*-β-D-glucopyranosyl sitosterol, 3-*O*-β-D-glucopyranosyl stigmasterol and 3-*O*-α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranosyl]oct-1-ene-3-ol, the new saponin, 3-*O*-α-L-rhamnopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 2)-β-D-glucuronopyranosyl]-22-*O*-β-D-glucopyranosyl soyasapogenol B (**2**) was isolated and characterised from the seed saponins fraction of *Trifolium resupinatum*. The structural determination is based on spectroscopic methods (including DQF-COSY, HETCOR, TOCSY, ROESY and NOEs experiments). © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Persian clover; *Trifolium resupinatum*; Leguminosae; Clover seeds; Saponins; Glycosides

1. Introduction

Persian clover (*Trifolium resupinatum* L.) is an annual pasture crop originating from the Mediterranean region, where it has been cultivated for centuries. In northern altitudes, it has been cultivated from the beginning of the twentieth century. To overcome seed supply problems in some countries, including Poland, genetic selection has resulted in the creation of varieties which give the same yield of dry matter per hectare, as the original Mediterranean populations, but also are able to produce satisfactory yields of seeds (Kaszuba, 1984).

In spite of the progress in breeding not much is known on the secondary metabolites influencing the nutritional quality of this species (Bisby, Buckingham & Harborne, 1994). Thus, the goal of the present paper was to analyse the saponins and other polar compounds in the seeds of Persian clover.

2. Results and discussion

The seed saponins fraction of *Trifolium resupinatum* provided, after repeated column chromatography, seven glycosides. The new saponin, 3-*O*-[α-L-rhamnopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 2)-β-D-glucuronopyranosyl]-22-*O*-β-D-glucopyranosyl soyasapogenol B (**2**) and six known compounds which were identified by comparison of their NMR data with those reported previously. The known compounds were identified as soyasaponin I (**1**), 3-*O*-[α-L-rhamnopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 2)-β-D-glucuronopyranosyl]-22-*O*-[β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl] soyasapogenol B (**3**), soyasaponin II (**4**) (Jurzysta, Price, Ridout & Fenwick, 1989), 3-*O*-[α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranosyl]oct-1-ene-3-ol (**5**) (Wang, Ghisalberti & Ridsdill-Smith, 1998), 3-*O*-β-D-glucopyranosyl sitosterol (**6**) and 3-*O*-β-D-glucopyranosyl stigmasterol (**7**) (Simonet, 1997). The α, ^{1}H and ^{13}C-NMR values in pyridine-d_5 of compounds **5** are included, since the previous ones have been run in MeOH-d_4.

3-*O*-[α-L-rhamnopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 2)-β-D-glucuronopyranosyl]-22-*O*-β-D-gluco-

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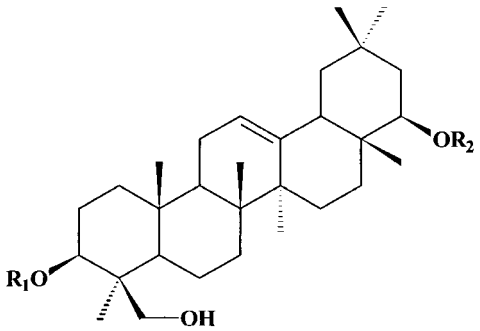

1 R_1=Rha-^{2}Gal-^{2}GlcA, R_2=H, Soyasaponin I

2 R_1=Rha-^{2}Gal-^{2}GlcA, R_2=Glc

3 R_1=Rha-^{2}Gal-^{2}GlcA, R_2=Glc-^{2}Glc

4 R_1=Rha-^{2}Xyl-^{2}GlcA, R_2=H, Soyasaponin II

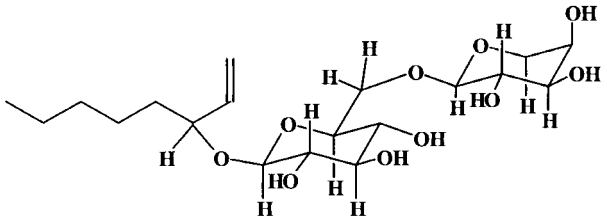

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Fig. 1. Isolated soyasaponins from *Trifolium resupinatum*.

pyranosyl soyasapogenol B (**2**) had a molecular peak *m/z* 1104 (FAB-MS) indicating the molecular formula $C_{54}H_{88}O_{23}$. Acidic hydrolysis (1 h) of **3** gave glucose, rhamnose, galactose and glucuronic acid as sugars and soyasapogenol B as the aglycone. The ^{1}H and ^{13}C NMR data (Table 1) were in agreement with this result, the anomeric configurations of the carbohydrate units, α-L-rhamnose, β-D-galactose, β-D-glucuronic acid and β-D-glucose were determined from J_{H1-H2} values and ^{13}C NMR data of C-1, C-2, C-3 and C-5 (Agrawal, 1992; Shujiro, Yutaka, Kazuo & Yohko, 1978). The ROESY NMR experiment showed the sugar sequences after a total assignment of sugar proton and carbon signals found with a combination of DQF-COSY, TOCSY and HETCOR NMR experiments. ROESY spectra of **2** showed ROE correlations between the following pairs of signals: δ 4.95 (*d*, $J=7$ Hz, H-1 of GlcA) and δ 3.45 (*dd*, $J=11$ Hz, $J=5$ Hz, H-3 of soyasapogenol B), δ 5.64 (*d*, $J=7$ Hz, H-1 of Gal) and δ 4.45 (*m* H-2 of GlcA), δ 6.14 (*s*, H-1 of Rha) and δ 4.43 (*m*, H-2 of Gal), δ 4.79 (*d*, $J=8$ Hz) and δ 3.78 (*dd*, $J=7$ Hz, $J=3$ Hz). These correlations allow us to establish the structure of **2** as 3-*O*-[α-L-rhamnopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 2)-β-D-glucuronopyranosyl]-22-*O*-β-D-glucopyranosyl soyasapogenol B.

3. Experimental

Mass spectra were obtained by using a VG 1250 or a Kratos MS-80-RFA using 70 eV. Optical rotations were determined using a Perkin-Elmer polarimeter (Model 241) set on the sodium D line. ^{1}H and ^{13}C

Table 1
^{1}H and ^{13}C-NMR data of saccaride units of compound **2** (399.95 and 100.25 MHz, respectively, 60°C, pyridine-d_5, signal of residual pyridine centred at δ 7.55 and 135.5 *t* ppm, respectively)

	^{13}C	^{1}H	^{13}C	^{1}H	
GlcA			Rha		
1	105.3	4.95 *d*, $J_{1,2}=7$ Hz	1	102.2	6.14 *bs*
2	77.2[a]	4.45 *o*[b]	2	72.2	4.68 *dd*, $J_{2,3}=3$ Hz, $J_{2,1}=1$ Hz
3	78.4	4.51 *o*	3	72.7	4.55 *dd*, $J_{3,4}=9$ Hz, $J_{3,2}=3$ Hz
4	73.6	4.37 *dd*, $J_{4,3}=9$ Hz, $J_{4,5}=9$ Hz	4	74.4	4.20 *dd*, $J_{4,3}=9$ Hz, $J_{4,5}=9$ Hz
5	77.4	4.53 *d*, $J_{5,4}=9$ Hz	5	69.4	4.86 *dq*, $J_{5,4}=9$ Hz, $J_{5,6}=6$ Hz
6	172.1	—	6	18.8	1.71 *d*, $J_{6,5}=6$ Hz
Gal			Glc		
1	101.9	5.64 *d*, $J_{1,2}=7$ Hz	1	102.5	4.79 *d*, $J_{1,2}=8$ Hz
2	77.7[a]	4.46 *o*	2	75.2	3.93 *dd*, $J_{2,1}=8$ Hz, $J_{2,3}=8$ Hz
3	76.5	4.01 *dd*, $J_{3,2}=10$ Hz, $J_{3,4}=3$ Hz	3	78.8	4.16 *dd*, $J_{3,2}=8$ Hz, $J_{3,4}=8$ Hz
4	71.3	4.29 *o*	4	72.3	4.12 *dd*, $J_{4,3}=8$ Hz, $J_{4,5}=8$ Hz
5	76.3	3.85 *o*	5	78.0	3.85 *o*
6	62.0	4.44 *o*, 4.30 *o*	6	66.3	4.45 *o*, 4.26 *o*

[a] Values may be interchanged.
[b] Signals unresolved due to overlapping are indicated by *o*.

NMR spectra were made at 399.952 MHz and 100.577 MHz, respectively, using pyridine-d_5 as solvent, and one or two drops of D_2O to dissolve some saponins, on a VARIAN UNITY-400 spectrometer. The resonance signals of residual pyridine centred at δ 7.55 and 135.5 *t* ppm, respectively, were used as internal reference for ^1H and ^{13}C spectra, respectively.

3.1. Extraction and isolation

The authenticated seeds of *Trifolium resupinatum* var. Ira were supplied by the breeder, Dr Kaszuba, from IHAR Bartazek, Poland. Dried seeds (1 kg) was extracted 2× with MeOH–H_2O (7:3) under reflux after defatting using Soxhlet extraction (CH_2Cl_2 and EtOAc). The extract was concd under red. pres., the concd extract was diluted with H_2O (1 l) and the soln was passed through RP-18 (6 × 7 cm). The column was washed with H_2O and the adsorbed materials were eluted with MeOH–H_2O mixs increasing the proportions of MeOH to give five frs (A–E). Fr. C (5 gr) was sepd using an RP-18 column (30 × 3.5 cm) with MeOH–H_2O mixs (4:6, 5:5) and after purification by silica gel CC ($CHCl_3$–MeOH–H_2O) provided **1** (20 mg), **2** (221 mg), **3** (105 mg) and **5** (30 mg). After purification by silica gel CC ($CHCl_3$–MeOH–H_2O; 70:27:5), fr. D provided **1** (119 mg) and **4** (27 mg). Chromatography of fr. E (1 gr) by silica gel CC ($CHCl_3$–MeOH; 95:5) gave **6** (1 mg) and **7** (4 mg).

*3.2. 3-O-[α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl]-22-O-β-D-glucopyranosyl soyasapogenol B (**2**)*

White needles, $[\alpha]_D^{25}$ −13 (MeOH *c* 0.4). FAB-MS 1104 $[M]^+$, 957 $[M\text{-}147]^+$, 795 $[M\text{-}147\text{-}162]^+$. ^1H and ^{13}C NMR: see Table 1.

*3.3. 3-O-[α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl]oct-1-ene-3-ol (**5**)*

Colourless oil, $[\alpha]_D^{25}$ −27 (MeOH *c* 0.3). FAB-MS (matrix: thioglycerol + NaI): 445 $[M+Na]^+$, 467 $[M-H+2Na]^+$. ^1H NMR (399.95 MHz, pyridine-d_5): δ 0.77 (3H, *t*, $J_{8,7}=7$ Hz, H-8), δ 1.16 (2H, *m*, H-6), δ 1.16 (2H, *m*, H-7), δ 1.43 (2H, *m*, H-5), δ 1.62 (1H, *m*, H-4), δ 1.75 (1H, *m*, H-4), δ 3.73 (1H, *dd*, $J_{5b'',5a''}=12$ Hz, $J_{5b'',4''}=2$ Hz, H-5″), δ 3.97 (1H, *dd*, $J_{2',1'}=8$ Hz, $J_{2',3'}=8$ Hz, H-2′), δ 4.02 (1H, *ddd*, $J_{5',4'}=8$ Hz, $J_{5',6b'}=6$ Hz, $J_{5'6'a}=2$ Hz, H-5′), δ 4.12 (1H, *dd*, $J_{4',3'}=9$ Hz, $J_{4',5'}=9$ Hz, H-4′), δ 4.17 (1H, *dd*, $J_{3'',2''}=8$ Hz, $J_{3'',4''}=3$ Hz, H-3″), δ 4.18 (1H, *dd*, $J_{3',2'}=9$ Hz, $J_{3',4'}=9$ Hz, H-3′), δ 4.27 (1H, *dd*, $J_{6b',6'a}=11$ Hz, $J_{6b',5'}=6$ Hz, H-6′), δ 4.28 (1H, *dd*, $J_{5a'',5b''}=12$ Hz, $J_{5a'',4''}=3$ Hz, H-5″), δ 4.31 (1H, *ddd*, $J_{4'',3''}=3$ Hz, $J_{4'',5''a}=3$ Hz, $J_{4'',5b''}=2$ Hz, H-4″), δ 4.44 (1H, *dd*, $J_{2'',1''}=7$ Hz, $J_{2'',3''}=8$ Hz, H-2″), δ 4.46 (1H, *m*, H-3), δ 4.74 (1H, *dd*, $J_{6a',6b'}=11$ Hz, $J_{6a',5'}=2$ Hz, H-6′), δ 4.88 (1H, *d*, $J_{1',2'}=8$ Hz, H-1′), δ 4.96 (1H, *d*, $J_{1'',2''}=7$ Hz, H-1″), δ 5.18 (1H, *d*, $J_{1a,2}=11$ Hz, H-1), δ 5.42 (1H, *d*, $J_{1b,2}=17$ Hz, H-1), δ 6.15 (1H, *ddd*, $J_{2,1b}=17$ Hz, $J_{2,1a}=11$ Hz, $J_{2,3}=6$ Hz, H-2); ^{13}C NMR (100,577 MHz, pyridine-d_5): δ 14.1 (C-8), δ 22.8 (C-7), δ 24.8 (C-5), δ 32.1 (C-6), δ 35.1 (C-4), δ 66.3 (C-5″), δ 69.1 (C-4″), δ 69.4 (C-6′), δ 71.8 (C-4′), δ 72.3 (C-2″), δ 74.3 (C-3″), δ 75.2 (C-2′), δ 77.0 (C-5′), δ 78.5 (C-3′), δ 80.7 (C-3), δ 103.6 (C-1′), δ 105.2 (C-1″), δ 115.2 (C-1), δ 140.7 (C-2).

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