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OLEANENE GLYCOSIDES FROM SEEDS OF *TRIFOLIUM ALEXANDRINUM*

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Key Word Index—*Trifolium alexandrinum*; Leguminosae; oleanene saponins; bersimosides I, II and dehydroazukisaponin V methyl esters.

Abstract—From the seeds of *Trifolium alexandrinum*, two new oleanene-type triterpene glycosides were isolated as their methyl esters, together with five known saponins. The structures of the isolated compounds were determined by NMR and mass spectral analyses.

INTRODUCTION

Trifolium alexandrinum (local name: bersim) is an annual plant cultivated in Egypt [1, 2]. Its aerial part is used as a cattle feed and the seeds are used as antidiabetic [3, 4]. Oleanane-type triterpene glycosides have been reported [5–7] from the genus *Trifolium*, but there is no report on the saponin constituents of *T. alexandrinum*. In this paper we describe the isolation and structural elucidation of two new triterpene glycosides (**4** and **6**) as their methyl esters, in addition to five known saponins (**1**–**3**, **5** and **7**), from the seeds of the titled plant.

RESULTS AND DISCUSSION

Seeds of *T. alexandrinum* were defatted with hexane followed by extraction with methanol, and the extract was then partitioned with ethyl acetate and water. The aqueous fraction was subjected to Diaion HP-20 column chromatography and eluted with water, 50% methanol and methanol, respectively. From the methanolic eluate, compound **7** was isolated and the rest of the fraction was filtered through Amberlite MB-3 followed by methylation with ethereal diazomethane, and the product was subjected to repeated silica gel, reversed phase RP-18 column chromatography and preparative HPLC using a polyamine column to isolate compounds **1**, **2**, **5** and **6**. The 50% methanolic eluate was treated also in the same way to afford compounds **3** and **4**.

The various techniques of NMR spectral analysis, viz. ^{1}H, ^{13}C, DEPT ^{13}C, H–H COSY and HSQC, in conjunction with FAB mass spectroscopy, revealed that compounds **1**–**7** are olean-12-ene-type triterpene glycosides with oxygenated carbons (C-3 and C-22) and a hydroxymethyl group (C-24). Acid hydrolysis of **1** and **2** using 10% aqueous HCl afforded a common aglycone identified as soyasapogenol B [8] by direct comparison with an authentic sample.

Compounds **1** and **2** were identified as 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 2)-β-D-glucuronopyranosyl soyasapogenol B methyl ester (soyasaponin I methyl ester) [6, 9–11] and 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 2)-β-D-glucuronopyranosyl soyasapogenol B methyl ester (azukisaponin V methyl ester) [12], respectively, by comparing the FAB mass spectra and NMR data (Tables 1 and 2) with those reported earlier.

Compound **3** has a molecular formula $C_{61}H_{100}O_{28}$, as calculated from the negative HR FAB mass spectrum, and the spectrum showed a molecular ion peak at *m/z* $[M - H]^-$ 1279, which was greater than those of **1** by two hexosyl units. The ^{13}C NMR data for **3** (Tables 1 and 2) supported the above suggestion in which signals assignable for five anomeric carbons have been displayed at δ_C 106.2, 105.5, 102.4, 101.7 and 100.4. The sapogenol obtained by acid hydrolysis of **3** was identified as soyasapogenol B, and the monosaccharide mixture revealed the presence of glucuronic acid methyl ester, galactose, rhamnose and glucose. The enzymic hydrolysis of **3** with glycyrrhizin hydrolase [13, 14] afforded

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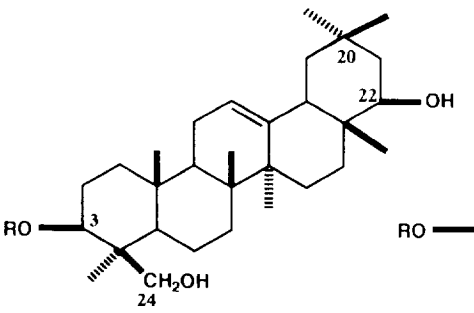
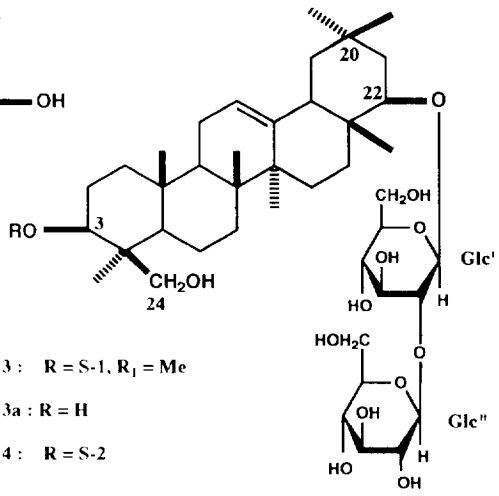


1 : R = S-1, R_1 = Me

2 : R = S-2

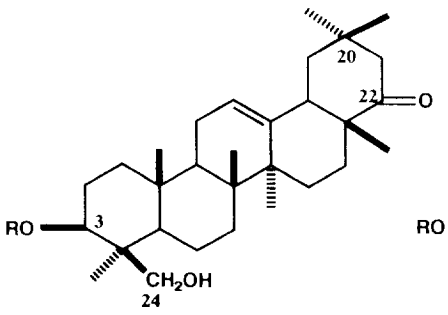
Soyasapogenol B : R = H



3 : R = S-1, R_1 = Me

3a : R = H

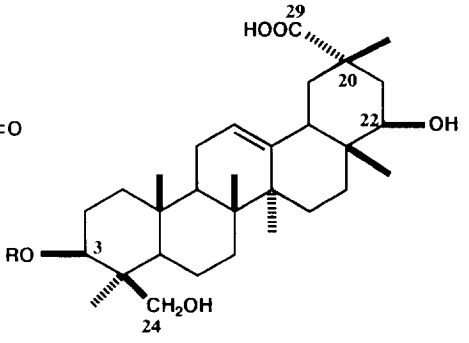
4 : R = S-2



5 : R = S-1, R_1 = Me

6 : R = S-2

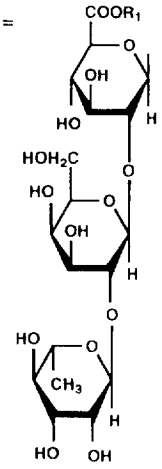
Soyasapogenol E : R = H



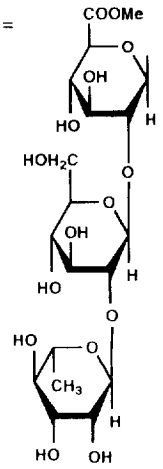
7 : R = S-1, R_1 = H

Oxytrogenin : R = H

S-1 =



S-2 =



a prosapogenin **3a** with the molecular formula $C_{42}H_{70}O_{13}$ and a molecular ion peak at *m/z* [M − H]⁻ 781 as determined from the negative FAB mass spectral data. The ^{13}C NMR signals of **3a** (Tables 1 and 2) at δ_C 100.5 and 106.3 indicated the presence of two β-D-glucopyranosyl units attached together by a (1 → 2) interglycosidic linkage as deduced from the downfield shift of C-2 of the inner glucose residue to δ_C 82.0. Their location at the aglycone C-22 was established from its downfield shift to δ_C 83.7 (glycosylation shift = 8.0 ppm) with upfield shift of C-21 to δ_C 36.7 in comparison with those of **1**. Similarly, the location of the other sugar units of **3** was established to be at C-3 based on the results of the enzymic cleavage, whereas C-3 of **3a** was shifted upfield to δ_C 80.2 (glycosylation shift = 11.1 ppm), while C-2 was shifted downfield to δ_C 28.5 in comparison with those of **3**. Therefore, the prosapogenin **3a** can be identified as 22-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl soyasapogenol B. From the ^{13}C NMR data for **3** (Tables 1 and 2) the signals corresponding to the sugar moiety linked at C-3 were superimposable on those of **1**. Based on the above evidence, the structure of the saponin **3** can be characterized as 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 2)-β-D-glucuro-

Table 1. ^{13}C NMR data for aglycone parts of saponins **1–9** (400 MHz, C_5D_5N, TMS)

C	1	2	3	3a	4	5	6	7
1	38.8	38.7	38.6	38.8	38.5	38.6	38.5	38.8
2	26.7	26.7	26.7	28.5	26.6	26.7	26.6	26.7
3	91.4	91.8	91.3	80.2	91.7	91.3	91.7	91.2
4	44.0	43.9	43.9	43.2	43.7	43.9	43.7	43.9
5	56.3	56.4	56.2	56.5	56.3	56.1	56.2	56.2
6	18.6	18.7	18.5	19.1	18.5	18.5	18.5	18.6
7	33.4	33.4	33.5	33.8	33.5	33.0	33.0	33.3
8	40.1	40.1	39.6	39.7	39.6	39.8	39.8	39.9
9	47.9	47.9	47.8	48.2	47.8	47.7	47.6	47.8
10	36.6	36.6	36.5	37.1	36.4	36.5	36.4	36.5
11	24.1	24.1	24.0	24.0	24.0	24.0	24.0	24.1
12	122.4	122.5	122.8	122.9	122.8	123.9	123.9	123.1
13	144.9	144.9	144.0	144.0	144.0	141.9	141.9	144.3
14	42.5	42.5	42.5	42.5	42.5	42.0	42.0	42.5
15	26.5	26.5	25.8	25.9	25.8	25.4	25.4	26.4
16	28.9	28.8	29.1	29.2	29.2	27.4	27.3	28.9
17	38.0	38.0	37.7	37.7	37.7	47.8	47.8	38.0
18	45.5	45.5	46.1	46.3	46.2	47.9	48.0	44.7
19	46.9	46.9	46.4	46.5	46.4	46.7	46.7	41.5
20	30.9	30.9	30.4	30.4	30.4	34.1	34.1	42.6
21	42.4	42.4	36.8	36.7	36.8	50.9	50.9	37.8
22	75.7	75.7	83.6	83.7	83.6	215.6	215.6	75.4
23	23.0	22.8	23.0	23.6	22.8	23.0	22.8	23.0
24	63.6	63.4	63.6	64.6	63.4	63.6	63.4	63.7
25	15.9	15.7	15.9	16.4	15.7	15.8	15.6	15.9
26	17.1	17.1	17.0	17.2	17.0	16.7	16.7	17.1
27	25.7	25.7	24.7	24.7	24.6	25.5	25.5	25.5
28	28.7	28.7	29.1	29.2	29.2	20.9	20.9	20.9
29	33.2	33.2	31.4	31.4	31.4	31.9	31.9	181.4
30	21.0	21.1	20.6	20.6	20.6	25.3	25.3	24.9

nopyranosyl soyasapogenol B methyl ester 22-*O*-*β*-D-glucopyranosyl-(1 → 2)-*β*-D-glucopyranoside named as bersimoside I methyl ester. The structure of **3** was first reported by Jurzysta *et al.* [7]. However, the reported optical rotation (− 0.92°) differs from that of **3** (− 39.6°). Also, comparison of the ^{13}C NMR data between the two compounds showed that the reported chemical shift value of glc C-1′ was 105.4 ppm [7]. Our assignment was supported by the ^{13}C NMR signals of **3** from the upfield shift of the inner glucose moiety C-1′ to δ_C 100.4 with downfield shift of C-2′ to δ_C 81.9 in comparison with the reported values of the C-22 monoglucoside derivative at δ_C 102.6 and 75.3, respectively [10].

The negative HR FAB mass spectrum of **4** established its molecular formula as $C_{61}H_{100}O_{28}$ with fragment ion peaks similar to those of **3**. The acid hydrolysis of **4** afforded soyasapogenol B and the enzymic hydrolysis with glycyrrihizin hydrolase provided the prosapogenin **3a**. The ^{13}C NMR spectral data of **4** (Tables 1 and 2) clearly assigned the sugar moiety at C-3 the same as that of **2**. Consequently, the new saponin **4** can be formulated as 3-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-glucopyranosyl-(1 → 2)-*β*-D-glucuronopyranosyl soyasapogenol B methyl ester 22-*O*-*β*-D-glucopyranosyl-(1 → 2)-*β*-D-glucopyranoside and was named as bersimoside II methyl ester.

Compounds **5** and **6** have the same molecular formulae $C_{49}H_{78}O_{18}$. Comparison of the FAB mass spectrum and the ^1H and ^{13}C NMR spectral data (Tables 1 and 2) of **5** with those of reported compounds led to the identification of its structure as 3-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-galactopyranosyl-(1 → 2)-*β*-D-glucuronopyranosyl soyasapogenol E methyl ester (dehydrosoyasaponin I methyl ester) [11, 15]. The saponin **6** has the same aglycone as that of **5** as shown by the ^{13}C NMR data (Tables 1 and 2) and the remaining signals derived from the sugar residue located at C-3 were superimposable on those of **2**. Thus, the new triterpene glycoside **6** can be identified as 3-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-glucopyranosyl-(1 → 2)-*β*-D-glucuronopyranosyl soyasapogenol E methyl ester named as dehydroazukisaponin V methyl ester.

The structure of **7** (^{13}C NMR, Tables 1 and 2) was elucidated as 3-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-galactopyranosyl-(1 → 2)-*β*-D-glucuronopyranosyl oxytrogenin (sophoraflavoside II) by comparison with the published data [16] including FAB mass spectral and NMR spectral analyses.

Table 2. ^{13}C NMR data for sugar parts of saponins **1–7** (400 MHz, C_5D_5N, TMS)

C	1	2	3	3a	4	5	6	7
Glc A								
1	105.4	105.2	105.5		105.3	105.5	105.3	105.4
2	78.2	78.1*	78.2*		78.2*	78.2	78.1*	78.5
3	76.9*	76.7	76.9†		76.7	76.9*	76.7	77.6
4	74.4	74.4	74.3		74.3	74.4	74.3	74.4
5	77.6	77.9	77.9		77.9	77.7	77.7	77.8
6	170.2	170.2	170.4		170.4	170.4	170.4	172.4
$-CO_2Me$	52.1	52.1	52.1		52.1	52.1	52.1	
Gal								
1	101.9		101.7			101.8		101.9
2	76.5*		76.5†			76.5*		76.6*
3	76.4*		76.4†			76.5*		76.4*
4	71.3		71.1			71.2		71.3
5	76.9*		76.6†			76.6*		76.9
6	61.9		61.6			61.7		61.8
Glc								
1		101.9			101.9		102.0	
2		79.1			79.1		79.2	
3		78.6*			78.6*		78.4*	
4		69.9			69.7		69.8	
5		78.4*			78.4*		78.2*	
6		61.4			61.3		61.4	
Rha								
1	102.2	102.1	102.4		102.0	102.4	102.0	102.4
2	72.3†	72.3†	72.4†		72.3†	72.4†	72.3†	72.4†
3	72.7†	72.7†	72.7‡		72.7†	72.8†	72.7†	72.8†
4	73.5	73.3	73.6		73.5	73.6	73.4	73.8
5	69.4	69.5	69.4		69.4	69.4	69.4	69.4
6	18.9	18.9	19.0		18.9	18.9	19.0	18.9
Glc′								
1			100.4	100.5	100.4			
2			81.9	82.0	81.9			
3			78.0*	78.1*	78.1*			
4			71.7§	71.7†	71.7‡			
5			77.7	77.9	77.7			
6			62.2‡	62.7‡	62.7§			
Glc″								
1			106.2	106.3	106.2			
2			77.1	77.2	77.1			
3			78.6*	78.6*	78.6*			
4			71.8§	71.8†	71.8‡			
5			78.2*	78.2*	78.2*			
6			62.8‡	62.8‡	62.8§			

*†‡§ Assignment may be interchangeable in each column.

EXPERIMENTAL

^1H and ^{13}C NMR: TMS as int. standard. FAB MS: direct inlet method at an ionizing voltage of 70 eV. HPLC: Polyamine and D-ODS-5, each column (20 mm i.d. × 25 cm) with differential refractometer as detector; flow rate of mobile phase 6 ml min^{-1}, injection vol. 0.8–1.0 ml. CC: Kieselgel 60 (70–230 mesh, Merck) and Diaion HP 20 (Mitsubishi). TLC: Silica gel 60 precoated plates, F-244 (Merck), HPTLC using RP-18 precoated plates, F-254 s and NH_2F 254 s (Merck).

Plant material. Seeds of *T. alexandrinum* L. (local name: bersim) [1, 2] was collected from El-Fayoum, Egypt, in August 1992. A voucher specimen is deposited at the Herbarium, Department of Medicinal Chemistry and Natural Products, Institute of Pharmaceutical Sciences School of Medicine, Hiroshima University, Japan.

Extraction and isolation of saponins. Air-dried and powdered seeds (3 kg) were defatted (× 3) by reflux with hexane followed by extraction (× 3) with hot MeOH. The combined methanolic extracts were filtered, evapd under

red. pres. and the residue suspended in H_2O and partitioned with EtOAc. The aq. fr. was applied to a column of Diaion HP 20 and eluted with H_2O, 50% MeOH and MeOH, respectively. The MeOH eluate (13.6 g) was chromatographed by silica gel CC using EtOAc–EtOH–H_2O (4:2:1) to give 3 frs. Fr. 3 (8.6 g) was sepd by a column of reversed phase RP-18 using 70–80% MeOH gradient followed by prep. ODS HPLC using 65% MeOH–0.05% TFA to afford **7** in the yield (0.0009%). The rest of fr. 3 was methylated with CH_2N_2 after filtration through Amberlite MB-3 and sepd by normal and reversed phase CC and prep. polyamine HPLC using 87%–90% MeCN to yield **1** (0.043%), **2** (0.0098%), **5** (0.0014%) and **6** (0.0007%). The 50% MeOH eluate (16 g) was treated with Amberlite MB-3 and methylated by the same procedure mentioned above, followed by sepn with silica gel CC using CH_2Cl_2–MeOH–H_2O (25:10:1 and 20:10:1) successively, RP-18 CC using 75% MeOH and prep. polyamine HPLC using 80% MeCN to afford **3** (0.034%) and **4** (0.028%).

Soyasoponin I methyl ester (**1**). Amorphous powder $[\alpha]_D^{19}$ −4.5° (MeOH; *c* 3.3). FAB MS (negative) *m/z*: 955 $[M-H]^-$ $C_{49}H_{79}O_{18}$, 810 $[M-H-rha]^-$, 457 $[M-H-rha-gal-glcA\ Me]^-$. ^1H NMR (pyridine-d_5): 0.74, 0.96, 0.98, 1.19, 1.26, 1.27, 1.43 (each 3H, *s*, −MeX7), 1.70 (3H, *d* *J* = 6.3 Hz, rha Me-6), 3.26 (1H, *d*, *J* = 11.4 Hz, H-24a), 3.41 (1H, *dd*, *J* = 4.8, 11.4 Hz, H-3), 3.74 (3H, *s*, glcA Me-6), 3.77 (1H, *t*, *J* = 5.6 Hz, H-22), 4.24 (1H, *d*, *J* = 11.4 Hz, H-24b), 4.90 (1H, *d*, *J* = 7.3 Hz, glcA H-1), 5.30 (1H, *t*, *J* = 3.5 Hz, H-12), 5.66 (1H, *d*, *J* = 7.6 Hz, gal H-1), 6.17 (1H, *br s*, rha H-1). ^{13}C NMR (Tables 1 and 2).

Azukisaponin V methyl ester (**2**). Amorphous powder $[\alpha]_D^{19}$ +2.1° (MeOH; *c* 3.3). FAB MS (negative) *m/z*: 955 $[M-H]^-$ $C_{49}H_{79}O_{18}$, 810 $[M-H-rha]^-$, 457 $[M-H-rha-glc-glcA\ Me]^-$. ^1H NMR (pyridine-d_5): 0.74, 0.96, 0.98, 1.19, 1.25, 1.26, 1.44 (each 3H, *s*, −MeX7), 1.72 (3H, *d*, *J* = 6.3 Hz, rha Me-6), 3.33 (1H, *d*, *J* = 11.2 Hz, H-24a), 3.41 (1H, *dd*, *J* = 4.6, 11.5 Hz, H-3), 3.70 (1H, *t*, *J* = 3.4 Hz, H-22), 3.75 (3H, *s*, glcA Me-6), 4.22 (1H, *d*, *J* = 11.2 Hz, H-24b), 4.92 (1H, d, *J* = 7.8 Hz, glcA H-1), 5.30 (1H, *t*, *J* = 3.4 Hz, H-12), 5.75 (1H, *d*, *J* = 7.6 Hz, glc H-1), 6.28 (1H, *d*, *J* = 1.5 Hz, rha H-1). ^{13}C NMR (Tables 1 and 2).

Bersimoside I methyl ester (**3**). Amorphous powder $[\alpha]_D^{19}$ −39.6° (MeOH; *c* 3.3). HR FAB MS (negative) *m/z*: 1279.6340 $[M-H]^-$ $C_{61}H_{99}O_{28}$ req. 1279.6321, 1133 $[M-H-rha]^-$, 971 $[M-H-rha-gal]^-$, 781 $[M-H-rha-gal-glcA\ Me]^-$. ^1H NMR (pyridine-d_5): 0.70, 0.88, 0.89, 1.10, 1.15, 1.19, 1.43 (each 3H, *s*, −MeX7), 1.76 (3H, *d*, *J* = 5.9 Hz, rha Me-6), 3.23 (1H, *d*, *J* = 10.9 Hz, H-24a), 3.39 (1H, *br d*, *J* = 10.7 Hz, H-3), 3.75 (3H, *s*, glcA Me-6), 4.25 (1H, *d*, *J* = 10.9 Hz, H-24b), 4.94 (1H, *d*, *J* = 6.9 Hz, glcA H-1), 4.96 (1H, *d*, *J* = 7.7 Hz, glc′ H-1), 5.27 (1H, *br s*, H-12), 5.32 (1H, *d*, *J* = 7.6 Hz, glc″ H-1), 5.77 (1H, *d*, *J* = 7.4 Hz, gal H-1), 6.27 (1H, *br s*, rha H-1). ^{13}C NMR (Tables 1 and 2).

Enzymic hydrolysis of **3**. To a soln of **3** (200 mg) in acetate buffer (pH 5.0, 5 ml) was added glycyrrihizin hydrolase (2 ml), and the mixt. was incubated with stirring at 45° for 2 weeks. The reaction mixt. was then subjected to CC on Diaion HP 20 and eluted with H_2O and MeOH, respectively. The MeOH fr. was subjected to RP-18 CC using 80% MeOH to isolate the prosapogenin **3a** (7 mg).

Prosapogenin (**3a**). Amorphous powder $[\alpha]_D^{19}$ +60.8° (MeOH; *c* 0.46). FAB MS (negative) *m/z*: 781 $[M-H]^-$ $C_{42}H_{69}O_{13}$, 457 $[M-H-glc-glc]^-$. ^1H NMR (pyridine-d_5): 0.90, 1.22, 1.57 (each 3H, *s*, −MeX3), 0.95 (6H, *s*, −MeX2), 1.13 (6H, *s*, −MeX2), 3.72 (1H, *d*, *J* = 11.0 Hz, H-24a), 4.53 (1H, *d*, *J* = 11.0 Hz, H-24b), 4.95 (1H, *d*, *J* = 7.5 Hz, glc′ H-1), 5.32 (1H, *br s*, H-12), 5.34 (1H, *d*, *J* = 7.9 Hz, glc″ H-1). ^{13}C NMR (Tables 1 and 2).

Bersimoside II methyl ester (**4**). Amorphous powder $[\alpha]_D^{19}$ +28.8° (MeOH; *c* 3.3). HR FAB MS (negative) *m/z*: 1279.6360 $[M-H]^-$ $C_{61}H_{99}O_{28}$ req. 1279.6326, 1133 $[M-H-rha]^-$, 971 $[M-H-rha-glc]^-$, 781 $[M-H-rha-glc-glcA\ Me]^-$. ^1H NMR (pyridine-d_5): 0.69, 0.87, 0.89, 1.10, 1.14, 1.19, 1.46 (each 3H, *s*, −MeX7), 1.78 (3H, *d*, *J* = 6.4 Hz, rha Me-6), 3.32 (1H, *d*, *J* = 11.2 Hz, H-24a), 3.39 (1H, *dd*, *J* = 4.4, 11.4 Hz, H-3), 3.76 (3H, *s*, glcA Me-6), 4.24 (1H, *d*, *J* = 11.2 Hz, H-24b), 4.94 (1H, *d*, *J* = 7.6 Hz, glcA H-1), 4.97 (1H, *d*, *J* = 8.0 Hz, glc′ H-1), 5.27 (1H, *br s*, H-12), 5.32 (1H, *d*, *J* = 7.8 Hz, glc″ H-1), 5.87 (1H, *d*, *J* = 7.8 Hz, glc H-1), 6.40 (1H, *br s*, rha H-1). ^{13}C NMR (Tables 1 and 2).

Enzymic hydrolysis of **4**. A sample of compound **4** was hydrolysed by glycyrrihizin hydrolase in the same way as **3** to give **3a**.

Acid hydrolysis of compounds **1**–**4**. A sample of each compound in 10% HCl in dioxan–H_2O (1:1) (10 ml) was refluxed at 80° for 4 hr. The reaction mixt. in each case was diluted with H_2O and then extracted with CH_2Cl_2. From the CH_2Cl_2 layer the aglycone was isolated and identified as soyasapogenol B by direct comparison with authentic sample. In the aq. phase, the sugar part of each saponin was identified by comparison with authentic substances.

Dehydrosoyasaponin I methyl ester (**5**). Amorphous powder $[\alpha]_D^{19}$ −32.3° (MeOH; *c* 2.6). HR FAB MS (negative) *m/z*: 953.5110 $[M-H]^-$ (calc.) $C_{49}H_{77}O_{18}$, 807 $[M-H-rha]^-$, 455 $[M-H-rha-gal-glcA\ Me]^-$. ^1H NMR (pyridine-d_5): 0.71, 0.85, 0.87, 0.96, 1.17, 1.29, 1.44 (each 3H, *s*, -MeX7), 1.76 (3H, *d*, *J* = 6.1 Hz, rha Me-6), 2.17 (1H, *dd*, *J* = 5.6, 8.0 Hz, H-21a), 2.58 (1H, *brd*, *J* = 13.9 Hz, H-21b), 3.25 (1H, *d*, *J* = 11.4 Hz, H-24a), 3.39 (1H, *dd*, *J* = 4.4, 11.7 Hz, H-3), 3.76 (3H, *s*, glcA Me 6), 4.26 (1H, *d*, *J* = 11.4 Hz, H-24b), 4.94 (1H, *d*, *J* = 7.8 Hz, glcA H-1), 5.25 (1H, *t*, *J* = 3.4 Hz, H-12), 5.77 (1H, *d*, *J* = 7.6 Hz, gal H-1), 6.27 (1H, *d*, *J* = 1.2 Hz, rha H-1). ^{13}C NMR (Tables 1 and 2).

Dehydroazukisaponin V methyl ester (**6**). Amorphous powder $[\alpha]_D^{19}$ −14.2° (MeOH; *c* 1.3). HR FAB MS (negative) *m/z*: 953.5102 $[M-H]^-$ $C_{49}H_{77}O_{18}$, req. 953.5094, 807 $[M-H-rha]^-$, 455 $[M-H-rha-glc-glcA\ Me]^-$. ^1H NMR (pyridine-d_5): 0.70, 0.85, 0.86, 0.96, 1.17, 1.27, 1.47 (each 3H, *s*, -MeX7), 1.78 (3H, *d*, *J* = 6.3 Hz, rha Me-6), 2.15 (1H, *m*, H-21a), 2.58 (1H, *dd*, *J* = 13.9 Hz, H-21b), 3.33 (1H, *d*, *J* = 11.4 Hz,

H-24a), 3.40 (1H, *br d*, J = 4.7, 11.8 Hz, H-3), 3.77 (3H, *s*, glcA Me 6), 4.23 (1H, *d*, J = 11.4 Hz, H-24b), 4.97 (1H, *d*, J = 7.8 Hz, glcA H-1), 5.24 (1H, *t*, J = 3.6 Hz, H-12), 5.86 (1H, *d*, J = 7.8 Hz, glc H-1), 6.40 (1H, *d*, J = 1.2 Hz, rha H-1). ^{13}C NMR (Tables 1 and 2).

Sophoraflavoside II methyl ester (**7**). Amorphous powder $[\alpha]_D^{19}$ −21.6° (MeOH; *c* 1.8). HR FAB MS (negative) *m/z*: 971.4876 [M − H]$^-$ $C_{48}H_{75}O_{20}$ req. 971.4852, 825 [M − H − rha]$^-$, 663 [M − H − rha − gal]$^-$, 487 [M − H − rha − gal − glcA]$^-$. ^{1}H NMR (pyridine-d_5): 0.76, 1.00, 1.28, 1.32, 1.46, 1.83 (each 3H, *s*, -MeX6), 1.78 (3H, *d*, J = 6.4 Hz, rha Me-6), 3.29 (1H, *d*, J = 12.5 Hz, H-24a), 3.44 (1H, *dd*, J = 4.6, 11.4 Hz, H-3), 3.92 (1H, *t*, J = 5.8 Hz, H-22), 4.31 (1H, *d*, J = 12.5 Hz, H-24b), 5.00 (1H, *d*, J = 7.1 Hz, glcA H-1), 5.40 (1H, *t*, J = 3.6 Hz, H-12), 5.77 (1H, *d*, J = 7.4 Hz, gal H-1), 6.38 (1H, *br s*, rha H-1). ^{13}C NMR (Tables 1 and 2).

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