Saikosaponin Homologues from *Verbascum* spp. The Structures of Mulleinsaponins I—VII

Toshio MIYASE,*[a] Chizuru HORIKOSHI,[a] Sachiyo YABE,[a] Setsuko MIYASAKA,[a] Farouk Rasmy MELEK,[b] and Genjiro KUSANO[c]

School of Pharmaceutical Sciences, University of Shizuoka,[a] 52–1 Yada, Shizuoka 422, Japan, National Research Centre,[b] Tahrir Street, Dokki, Cairo, Egypt, Osaka University of Phamaceutical Sciences,[c] 4–20–1 Nasahara, Takatsuki, Osaka 569–11, Japan.

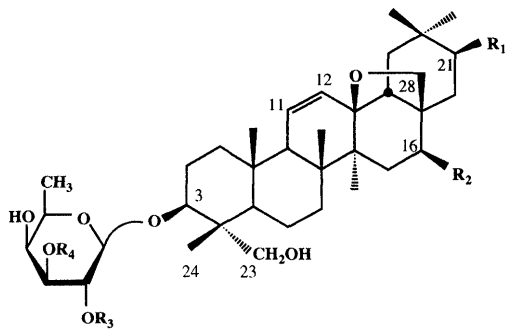
Received June 18, 1997: accepted August 22, 1997

From the aerial parts of *Verbascum* (*V.*) *sinaiticum*, *V. thapsiforme*, *V. fruticulosum* and *Celsia roripifolia*, seven new saikosaponin homologues, called mulleinsaponins I-VII, having 13,28-epoxy-olean-11-ene skelton were isolated together with eight known saikosaponin homologues, 3-*O*-β-D-fucopyranosyl saikogenin F, saikosaponin a, desrhamnosylverbascosaponin, songarosaponins C, D, mimengoside A and buddlejasaponins I, IV. The structures of mulleinsaponins I—VII were characterized as 3-*O*-β-D-glucopyranosyl-(1→3)-β-D-fucopyranosyl-6-deoxy-saikogenin F, 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-β-D-fucopyranosyl-16-deoxysaikogenin F, 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-β-D-fucopyranosyl-saikogenin F, 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-fucopyranosyl-21β-hydroxysaikogenin F, 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-fucopyranosyl-21β-acetoxysaikogenin F, 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-fucopyranosyl-16β-acetoxysaikogenin F and 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-fucopyranosylsaikogenin F 16-*O*-β-D-glucopyranoside, respectively, from chemical and spectroscopic evidence.

Key words *Verbascum sinaiticum*; *Verbascum thapsiforme*; *Verbascum fruticulosum*; *Celsia roripiforia*; mulleinsaponin; saikosaponin homologue

In the course of our chemotaxonomical and phytochemical studies on saikosaponin homologues of the family Buddlejaceae,[1] Labiatae,[2] and Scrophulariaceae[3] (order Solanales), we studied the saponin constituents of the genus *Verbascum* (*V.*) (Scrophulariaceae). With regard to the saponin of this genus, *V. songaricum* SCHRENK afforded songarosaponins C[4] and D,[5] *V. nigrum* L. afforded ilwensiasaponin A[3b] (mimengoside A[1b]) and *V. phlomoides* L. afforded verbascosaponin and desrhamnosylverbascosaponin[6] as a saikosaponin homologue. A genus *Verbascum* is believed to be a source of saikosaponin homologue. In this paper, we report the isolation and structure elucidation of saikosaponins isolated from the aerial parts of *V. sinaiticum* BENTH., *V. thapsiforme* SCHRAD., *V. fruticulosum* POST and *Celsia roripifolia* (*syn. Verbascum roripifolium* L. K. FERGUSON). Since the genus *Celsia* is very similar to the genus *Verbascum* except that the former has four stamens and the latter has five, the genus *Celsia* is regarded as a part of the genus *Verbascum*. From the polar and lipophilic fraction of these plants, saikosaponin homologues (**1**—**15**) were isolated by preparative HPLC using reversed phase [octadecyl silica (ODS), phenyl alkyl (PhA)] columns. Compounds **1**, **3**, **6**—**11** were identified by comparison of the ^{1}H- and ^{13}C-NMR data with reported data as 3-*O*-β-D-fucopyranosylsaikogenin F,[7] saikosaponin a,[1a] desrhamnosylverbascosaponin,[6] songarosaponins C,[4] D,[5] buddlejasaponin IV,[1a] ilwensiasaponin A[3b] (mimengoside A[1b]), buddlejasaponin I,[1a] respectively.

Mulleinsaponin I (**2**) revealed a pseudo molecular ion peak at *m/z* 765.4787 $[C_{42}H_{68}O_{12}+H]^+$ in the high resolution positive mode secondary ion (SI)-MS. The ^{1}H-NMR spectrum showed the presence of six singlet



	R1	R2	R3	R4
1	H	OH	H	H
2	H	H	H	Glc
3	H	OH	H	Glc
4	H	H	H	Glc (4←1) Rha
5	H	OH	H	Glc (4←1) Rha
6	H	H	Glc	Glc
7	H	H	Glc	Glc (4←1) Glc
8	H	OH	Glc	Glc (4←1) Glc
9	H	OH	Glc	Glc
10	H	H	Glc	Glc (4←1) Rha
11	H	OH	Glc	Glc (4←1) Rha
12	OH	OH	Glc	Glc (4←1) Rha
13	OAc	OH	Glc	Glc (4←1) Rha
14	H	OAc	Glc	Glc (4←1) Rha
15	H	O-Glc	Glc	Glc (4←1) Rha

Glc: β-D-glucopyranosyl
Rha: α-L-rhamnopyranosyl

Chart 1

* To whom correspondence should be addressed.

methyl (δ: 0.82, 0.92, 0.93, 0.99, 1.00, 1.34), one doublet methyl [δ: 1.44 (3H, d, J=6.5 Hz)], two sets of oxymethylene [δ: 3.33 (1H, d, J=7 Hz), 3.72 (1H, d, J=7 Hz); 3.72 (1H, d, J=9 Hz), 4.36 (1H, d, J=9 Hz)], two anomeric [δ: 4.96 (1H, d, J=8 Hz), 5.31 (1H, d, J=8 Hz)] and two olefinic proton signals [δ: 5.56 (1H, dd, J=10, 3 Hz), 5.97 (1H, d, J=10 Hz)]. On acid hydrolysis of **2**, D-fucose and D-glucose were detected as thiazolidine derivatives by GC. The ^{13}C-NMR spectrum showed 42 carbon signals and was similar to that of saikosaponin a (**3**), except for the D-ring carbons. In the aglycone moiety, the ^{13}C-NMR data were superimposable on those of desrhamnosylverbascosaponin (**6**), suggesting the aglycone of **2** to be 16-deoxysaikogenin F. In the nuclear Overhauser effect (NOE) experiments after assignment of all proton signals resulting from the sugar moiety by ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum, irradiation of the anomeric proton signal at δ 4.96 (H-1 of fucose) enhanced the proton signal at δ 4.27 (1H, dd, J=12, 3 Hz) which was due to the H-3 of the aglycone moiety, and irradiation of the other anomeric proton signal at δ 5.31 enhanced the proton signal at δ 4.03 (1H, dd, J=9, 3.5 Hz) from the H-3 of fucose. The heteronuclear multiple bond connectivity (HMBC) spectrum showed the correlations between the anomeric proton signal of glucose and the carbon signal of the C-3 of fucose, and between the anomeric proton signal of fucose and the carbon signal of the C-3 of the aglycone moiety (Chart 2). These NMR data led us to conclude that the structure of mulleinsaponin I is **2**.

Mulleinsaponins II (**4**) and III (**5**) revealed a pseudo molecular ion peak at m/z 933.5183 $[C_{48}H_{78}O_{16}+Na]^+$ and 949.5132 $[C_{48}H_{78}O_{17}+Na]^+$, respectively, in the high resolution SI-MS. On acid hydrolysis **4** and **5** afforded L-rhamnose, D-glucose and D-fucose. The ^{1}H- and ^{13}C-NMR spectra of these two compounds were very similar

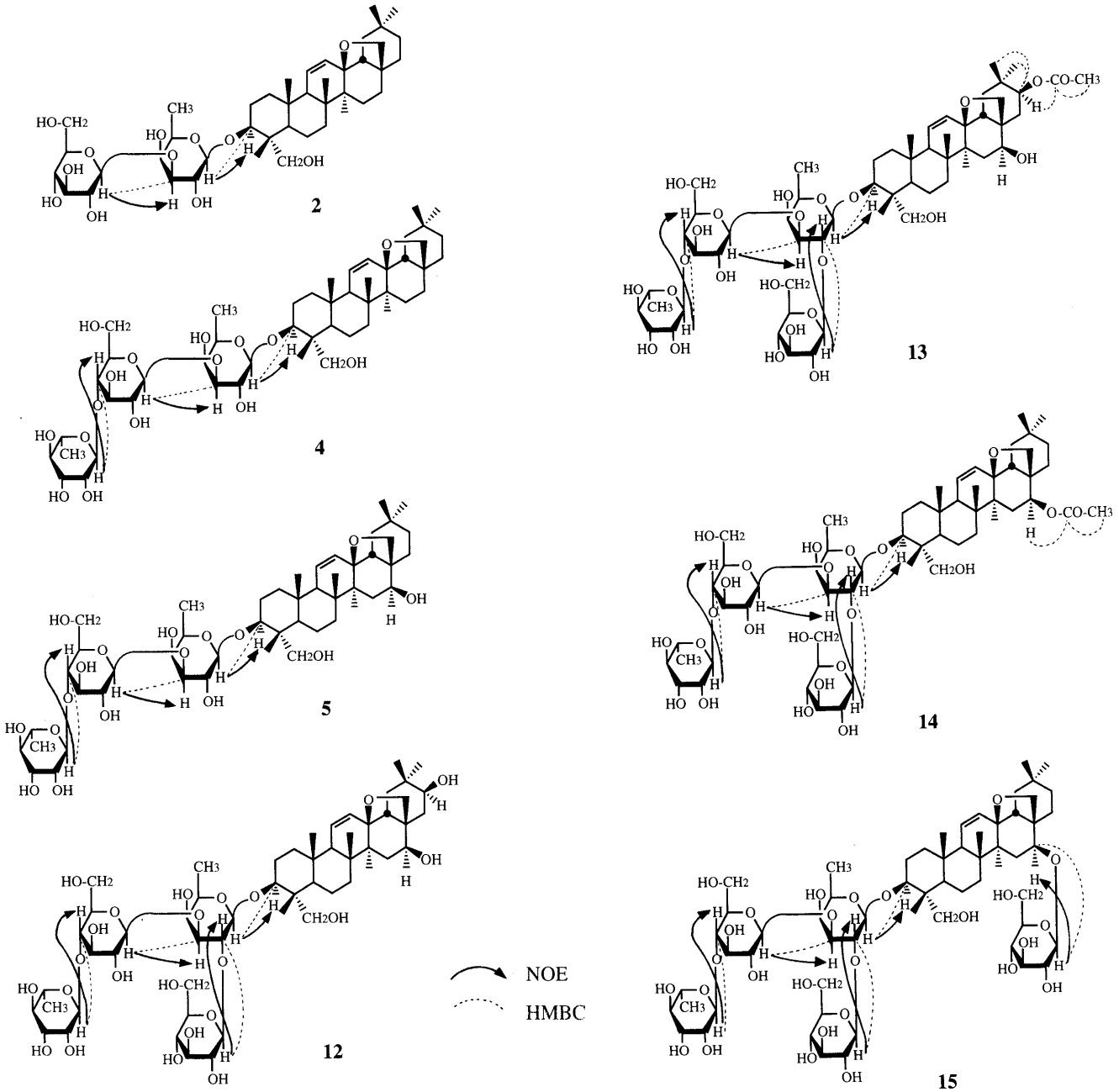


Chart 2

to each other especially in the sugar chain moiety, suggesting that **4** and **5** had the same sugar sequence. The NOE experiments after assignment of all proton signals by ^{1}H–^{1}H COSY and the HMBC spectrum (Chart 2) meant that the structure of the sugar chain of these two compounds is 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-β-D-fucopyranoside. The ^{13}C-NMR data of **4** and **5** showed the aglycone to be 16-deoxysaikogenin F[6] and saikogenin F,[1a] respectively. The structures of mulleinsaponins II and III were determined to be **4** and **5**, respectively.

Mulleinsaponin IV (**12**) revealed a pseudo molecular ion peak at *m/z* 1127.5609 $[C_{54}H_{88}O_{23}+Na]^+$ in high resolution SI-MS. On acid hydrolysis L-rhamnose, D-glucose and D-fucose were detected. The NMR spectra suggested that the sugar chain was composed of four monosaccharides [δ: 4.92 (1H, d, J=8 Hz), 104.0; 5.26 (1H, d, J=8 Hz), 104.9; 5.59 (1H, d, J=8 Hz), 104.0; 5.84 (1H, d, J=1 Hz), 102.8]. In the NOE experiments, irradiation of the anomeric proton signal of rhamnose (δ 5.84) enhanced the proton signal at δ 4.39 (1H, dd, J=9, 9 Hz) from the H-4 of glucose, irradiation of the anomeric proton signal of the same glucose (δ 5.26) enhanced the proton signal at δ 4.08 (1H, dd, J=9, 3 Hz) from the H-3 of fucose, irradiation of the anomeric proton signal of another glucose (δ 5.59) enhanced the proton signal at δ 4.66 (1H, dd, J=8, 8 Hz) from the H-2 of fucose and irradiation of the anomeric proton signal of fucose (δ 4.92) enhanced the proton signal at δ 4.15 (overlapped with other signals) from the H-3 of the aglycone moiety (Chart 2). These NMR data and the HMBC spectrum showed that the structure of the sugar chain was 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-fucopyranoside. In the aglycone moiety, on the other hand, the HMBC correlations were observed between the methyl proton signal at δ 1.16 (3H, s) and the carbinyl carbon signal at δ 73.2 and between the methyl proton signal at δ 1.27 (3H, s) and the carbinyl carbon signal at δ 73.2 (Chart 2). The ^{13}C-NMR data of the aglycone moiety were superimposable on those of the aglycone moiety of clinoposaponin XIII[2d] which was 21β-hydroxysaikogenin F. Therefore, the structure of mulleinsaponin IV was characterized as shown.

The molecular formula of mulleinsaponin V (**13**) was determined to be $C_{56}H_{90}O_{24}$ by high resolution SI-MS. On acid hydrolysis, **13** afforded L-rhamnose, D-glucose and D-fucose. The sugar sequence was determined to be 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyramosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-fucopyranoside by comparison of the ^{13}C-NMR data with those of **12**. The NOE experiments and the HMBC spectrum also supported this sugar chain structure. The ^{1}H- and ^{13}C-NMR spectra showed the presence of an acetyl group [δ: 2.05 (3H, s), 21.0, 170.4]. An oxymethine proton signal was observed at δ 5.35 (1H, dd, J=12, 4 Hz). This was correlated with the carbon signal at δ 76.1 in the heteronuclear single quantum coherence (HSQC) spectrum and with the carbonyl carbon signal at δ 170.4 in the HMBC spectrum. Two methyl proton signals at δ 0.99 and 1.03 were correlated with the carbon signal at δ 76.1 in the HMBC spectrum. From these data, the aglycone of **13** was characterized as 21β-acetoxysaikogenin F.

Mulleinsaponin VI (**14**) revealed a pseudo molecular ion peak at *m/z* 1153.5765 $[C_{56}H_{90}O_{23}+Na]^+$ in high resolution SI-MS and afforded L-rhamnose, D-glucose and D-fucose as a sugar moiety on acid hydrolysis. The ^{1}H-NMR spectrum showed the presence of an acetyl group at δ 2.11 (3H, s) and a downfield shifted oxymethine proton signal at δ 5.71 (1H, dd, J=10, 6 Hz). The ^{13}C-NMR data of the sugar chain moiety were almost the same as those of **13** and those of the aglycone moiety were similar to those of buddlejasaponin I (**11**) except for C-15, C-16 and C-17. C-16 of **14** was shifted downfield by 4.8 ppm and the C-15 and C-17 were shifted upfield by 3.8 ppm and 1.4 ppm, respectively, by comparing the ^{13}C-NMR chemical shifts with those of **11**. The oxymethine proton signal (δ 5.71) was correlated with the carbon signal at δ 68.9 in the HSQC spectrum and correlated with the carbonyl carbon signal at δ 170.6 in the HMBC spectrum. Therefore, the structure of mulleinsaponin VI was decided as **14**.

The high resolution mass spectral data of mulleinsaponin VII (**15**) suggested the molecular formula $C_{60}H_{98}O_{27}$. The ^{1}H-NMR spectrum revealed five anomeric proton signals at δ 4.91 (1H, d, J=8 Hz), 5.04 (1H, d, J=7 Hz), 5.25 (1H, d, J=8 Hz), 5.58 (1H, d, J=7.5 Hz), 5.83 (1H, br s). Comparison of the ^{13}C-NMR data of the aglycone moiety with those of buddlejasaponi I (**11**) suggested that the aglycone of **15** was saikogenin F. In the NOE experiments after assignment of most proton signals by ^{1}H–^{1}H COSY, NOEs were observed as follows: H-1 of fucose (δ 4.91)/H-3 of aglycone (δ 4.12); H-1 of glucose (δ 5.58)/H-2 of fucose (δ 4.64); H-1 of glucose (δ 5.25)/H-3 of fucose (δ 4.07); H-1 of rhamnose (δ 5.83)/H-4 of glucose attached to the C-3 of fucose (δ 4.38); H-1 of glucose (δ 5.04)/H-16 of aglycone (δ 4.59) (Chart 2). In the HMBC spectrum, the following correlations were observed: H-1 of fucose (δ 4.91)/C-3 of aglycone (δ 82.5); H-1 of glucose (δ 5.58)/C-2 of fucose (δ 77.3); H-1 of glucose (δ 5.25)/C-3 of fucose (δ 84.8); H-1 of rhamnose (δ 5.83)/C-4 of glucose (δ 78.5) attached to C-3 of fucose; H-1 of glucose (δ 5.04)/C-16 of aglycone (δ 75.8) (Chart 2). The above mentioned spectral and chemical data showed the structure of mulleinsaponin VII to be **15**. *Verbascum* and *Celsia* spp. (Scrophulariaceae) are also a valuable source of saikosaponin homologue.

Experimental

General The instruments used in this work: JASCO DIP-1000 digital polarimeter for optical rotation; JEOL α-400 FT-NMR spectrometer for NMR spectra (^{1}H, 400 MHz; ^{13}C, 100 MHz, in pyridine-d_5 at 35 °C); JEOL JMS-SX102 spectrometer for positive mode FAB-MS; Hitachi M-80 spectrometer for positive mode high resolution SI-MS; Hitachi G-3000 gas chromatograph for GC; JASCO System 800 for HPLC.

Extraction and Isolation The aerial parts of (500 g) *V. sinaiticum* (collected in the northern part of Sinai, Egypt in 1995 and plant identification confirmed by Dr. M. El-Gibally, Plant Taxonomy Department, National Research Centre, Cairo) were extracted with ether twice (each 10 h) and the residue was extracted with methanol at room temperature twice (each 10 h). The methanol solution was passed through an Amberlyst 27 column and the eluate was concentrated *in vacuo* at 50 °C. The residue was dissolved in water and the solution was passed through a porous polymer gel column (Mitsubishi Diaion HP-20). After washing with water, 60% methanol eluate (9 g) and methanol

Table 1. ^{1}H-NMR Spectral Data of Compounds **2**, **4**, **5**, **12**—**15** in Pyridine-d_5

	2	**4**	**5**	**12**	**13**	**14**	**15**
Aglycone							
3	4.27 (dd, 12, 3)	4.27[a)]	4.27 (dd, 12, 2.5)	4.15[a)]	4.15[a)]	4.16[a)]	4.12[a)]
11	5.97 (d, 10)	5.98 (d, 10.5)	6.01 (d, 10)	6.01 (d, 10.5)	6.00 (d, 10)	5.97 (d, 10)	5.95 (d, 10)
12	5.56 (dd, 10, 3)	5.56 (dd, 10.5, 3)	5.65 (dd, 10, 2.5)	5.71 (dd, 10.5, 3)	5.66 (dd, 10, 2)	5.57 (dd, 10, 4)	5.59 (dd, 10, 2)
16			4.51[a)]	4.58 (m)	4.51[a)]	5.71 (dd, 10, 6)	4.59 (dd, 9.5, 3)
21				4.10[a)]	5.35 (dd, 12, 4)		
23	3.72 (d, 9)	3.72 (d, 10)	3.71 (d, 10)	3.73 (d, 11)	3.70 (d, 10)	3.70[a)]	3.68 (d, 10.5)
	4.36 (d, 9)	4.36 (d, 10)	4.38 (d, 10)	4.38 (d, 11)	4.37 (d, 10)	4.37[a)]	4.35 (d, 10.5)
24	0.93 (s)	0.93 (s)	1.01 (s)	1.07 (s)	1.07 (s)	1.05 (s)	1.03 (s)
25	1.00 (s)	1.00 (s)	0.94 (s)	0.99 (s)	0.98 (s)	0.96 (s)	0.93 (s)
26	1.34 (s)	1.34 (s)	1.40 (s)	1.40 (s)	1.37 (s)	1.32 (s)	1.10 (s)
27	0.99 (s)	0.99 (s)	1.11 (s)	1.13 (s)	1.06 (s)	1.08 (s)	1.25 (s)
28	3.33 (d, 7)	3.33 (d, 6.5)	3.34 (d, 7)	3.43 (d, 7)	3.39 (d, 7)	3.32 (d, 7)	3.27 (d, 7)
	3.72 (d, 7)	3.72 (d, 6.5)	4.38 (d, 7)	4.39[a)]	4.35[a)]		4.28[a)]
29	0.92 (s)	0.92 (s)	0.93 (s)	1.27 (s)	0.99 (s)	0.89 (s)	0.93 (s)
30	0.82 (s)	0.82 (s)	0.90 (s)	1.16 (s)	1.03 (s)	0.85 (s)	0.87 (s)
					2.05 (s)	2.11 (s)	
C-3 sugar							
Fuc							
1	4.96 (d, 8)	4.95 (d, 8)	4.96 (d, 8)	4.92 (d, 8)	4.93 (d, 8)	4.90 (d, 7.5)	4.91 (d, 8)
2	4.51 (dd, 9, 8)	4.49 (dd, 9, 8)	4.32 (dd, 9, 8)	4.66 (dd, 8, 8)	4.67 (dd, 8, 8)	4.64 (dd, 8, 7.5)	4.64 (dd, 8, 8)
3	4.03 (dd, 9, 3.5)	4.00 (dd, 9, 3.5)	3.99 (dd, 9, 3.5)	4.08 (dd, 9, 3)	4.07 (dd, 8, 3)	4.05 (dd, 8, 3.5)	4.07[a)]
4	4.14 (d, 3)	4.10 (d, 3)	4.09 (d, 3)	4.16[a)]		4.15[a)]	
5	3.68 (q, 6.5)	3.96 (q, 6.5)	3.69 (m)	3.62 (q, 6)	3.62 (q, 6)	3.61 (q, 7)	3.61 (q, 6.5)
6	1.44 (d, 6.5)	1.44 (d, 6.5)	1.45 (d, 6.5)	1.40 (d, 6)	1.41 (d, 6)	1.41 (d, 7)	1.39 (d, 6.5)
Glc (C-2 of Fuc)							
1				5.59 (d, 8)	5.59 (d, 7)	5.57 (d, 7)	5.58 (d, 7.5)
2				4.10[a)]			4.09[a)]
3				4.21[a)]	4.20[a)]	4.19[a)]	4.19[a)]
4							
5				4.17[a)]			
6				4.29 (dd, 11, 5)			4.26[a)]
				4.35 (dd, 11, 3.5)			4.36[a)]
Glc (C-3 of Fuc)							
1	5.31 (d, 8)	5.25 (d, 8)	5.25 (d, 8)	5.26 (d, 8)	5.25 (d, 8)	5.24 (d, 8)	5.25 (d, 8)
2	4.02 (dd, 8, 8)	3.97 (dd, 9, 8)	3.96 (dd, 9, 8)	3.95 (dd, 8, 8)	3.95 (dd, 8, 8)	3.92 (dd, 8, 8)	3.93 (dd, 8, 8)
3	4.00 (m)	4.20 (dd, 9, 9)	4.21 (dd, 9, 9)	3.66[a)]	3.67[a)]	3.64[a)]	3.65[a)]
4	4.20 (dd, 8.5, 8.5)	4.40 (dd, 9, 9)	4.41 (dd, 8.5, 8.5)	4.39 (dd, 9, 9)	4.38 (dd, 9, 9)	4.37 (dd, 9, 9)	4.38 (dd, 9, 9)
5	4.26 (m)	3.80 (m)	3.81 (m)	3.75 (m)	3.74 (m)	3.73 (m)	3.73 (m)
6	4.37 (dd, 11.5, 5.5)	4.14 (dd, 11, 3)	4.14 (dd, 11, 3)	4.11 (dd, 10, 3)	4.10 (dd, 11, 4)	4.08[a)]	4.08[a)]
	4.54 (dd, 11.5, 2.5)	4.27[a)]	4.40[a)]	4.21[a)]	4.19[a)]	4.18 (dd, 10, 2.5)	4.19[a)]
Rha							
1		5.86 (br s)	5.86 (br s)	5.84 (d, 1)	5.83 (br s)	5.82 (br s)	5.83 (br s)
2		4.67[a)]	4.68 (dd, 3.5, 1.5)	4.67[a)]	4.06[a)]	4.66 (dd, 3, 1.5)	4.66[a)]
3		4.54 (dd, 9.5, 3)	4.54 (dd, 9.5, 3.5)	4.54 (dd, 9, 3)	4.54 (dd, 9, 3)	4.52 (dd, 9, 3)	
4		4.32 (dd, 9.5, 9.5)	4.32 (dd, 9.5, 9.5)	4.33 (dd, 9, 9)	4.34 (dd, 9, 9)	4.32 (dd, 9, 9)	4.34 (dd, 9, 9)
5		4.93 (m)	4.92 (m)	4.93 (m)	4.92[a)]	4.92 (m)	4.94 (m)
6		1.69 (d, 6)	1.70 (d, 6)	1.73 (d, 6)	1.73 (d, 6)	1.72 (d, 6.5)	1.73 (d, 6)
C-16 sugar							
Glc							
1							5.04 (d, 7)
2							4.06[a)]
3							4.05[a)]
4							4.32[a)]
5							3.96 (m)
6							4.43 (dd, 12, 4)
							4.52 (dd, 12, 2.5)

a) Overlapped.

eluate (5.68 g) were obtained as a pale brown powder. Part (2.8 g) of the methanol eluate was chromatographed on Develosil Lop-ODS column (5 × 50 cm × 2) using a methanol-water system (linear gradient) and Develosil PhA (2 × 25 cm) column using a acetonitrile-water system to give compounds **2** (5 mg), **3** (4 mg), **4** (4 mg), **5** (3 mg), **10** (241 mg), **11** (457 mg), **14** (63 mg) and **15** (2.5 mg). The aerial parts (1.2 kg) of *V. thapsiforme* [The seeds were purchased from CHILTERN SEEDS, Bortree Stile, Ulverston, Cumbria, England (catalogue No. 1275J) and the plants were cultivated in our botanical garden in 1996] gave 60% methanol eluate (13.5 g) and methanol eluate (9.63 g) and part (3.2 g) of the methanol eluate afforded compounds **6** (40 mg), **7** (5 mg), **8** (5 mg), **9** (1 mg), **10** (35 mg) and **11** (12 mg) in the same manner. The aerial parts (500 g) of *V. fruticulosum* (collected in the northern part of Sinai, Egypt in 1995 and identified by Dr. M. El-Gibally) gave 60% methanol eluate (6.2 g) and methanol eluate (7 g) and part (2.3 g) of the methanol eluate afforded compounds **11** (44 mg), **12** (4 mg) and **13** (10 mg). The aerial

Table 2. ^{13}C-NMR Spectral Data of Compounds **2**, **4**, **5**, **12**—**15** in Pyridine-d_5

	2	**4**	**5**	**12**	**13**	**14**	**15**
Aglycone							
1	38.7	38.7	38.7	38.6	38.6	38.6	38.7
2	25.6	25.6	26.1	26.0	26.0	26.0	26.0
3	81.7	81.7	81.7	82.6	82.6	82.5	82.5
4	43.7	43.7	43.8	43.8	43.8	43.8	43.8
5	47.4	47.4	47.4	47.9	47.8	47.8	47.8
6	17.6	17.6	17.6	17.6	17.6	17.6	17.6
7	31.7	31.5	31.6	31.6	31.6	31.6	31.5
8	41.7	41.7	42.3	42.1	42.2	42.3	42.2
9	53.7	53.1	53.1	53.1	53.0	53.0	53.1
10	36.3	36.3	36.3	36.3	36.2	36.3	36.3
11	131.9	131.9	132.2	132.2	132.5	132.7	132.3
12	131.7	131.7	131.2	131.1	130.7	130.4	131.0
13	84.9	84.9	84.0	83.7	83.7	84.0	83.9
14	44.1	44.1	45.7	45.7	45.6	45.7	45.6
15	25.8	25.8	36.2	36.3	36.4	32.4	35.5
16	26.0	26.0	64.1	65.5	65.0	68.9	75.8
17	42.0	42.0	47.0	49.3	49.0	45.6	47.2
18	51.4	51.4	52.2	51.7	51.2	52.1	52.3
19	37.3	37.3	37.8	37.4	37.2	37.6	37.8
20	31.5	31.7	31.6	37.1	35.9	31.5	31.7
21	35.0	35.0	34.7	73.2	76.1	34.7	35.2
22	31.0	31.0	25.8	34.9	31.0	25.7	25.5
23	64.1	64.1	64.1	64.7	64.6	64.7	64.6
24	13.0	13.0	13.0	12.7	12.7	12.7	12.7
25	18.7	18.7	18.8	18.6	18.5	18.5	18.6
26	19.5	19.5	20.0	20.0	19.9	20.0	20.7
27	19.8	19.8	20.8	20.7	20.5	20.6	19.9
28	77.0	77.0	73.0	72.6	72.3	73.3	73.6
29	33.6	33.6	33.6	30.5	29.5	33.3	33.5
30	23.5	23.5	23.8	18.0	18.7	23.7	23.9
OAc					170.4	170.6	
					21.0	21.1	
Sugar							
Fuc							
1	105.9	105.9	106.5	104.0	104.1	104.1	104.0
2	71.6	71.8	71.8	77.2	77.2	77.3	77.3
3	85.3	85.3	85.4	84.7	84.8	84.8	84.8
4	71.8	72.1	72.2	72.2	72.3	72.3	72.0
5	71.0	71.0	71.0	70.5	70.5	70.5	70.5
6	17.2	17.2	17.2	17.2	17.2	17.2	17.2
Glc (C-2 of Fuc)							
1				104.0	104.0	104.0	104.0
2				76.2	76.2	76.3	76.3
3				78.8	78.8	78.8	78.9
4				72.0	72.0	72.0	72.3
5				76.4	76.4	76.5	76.5
6				63.1	63.2	63.2	63.2
Glc (C-3 of Fuc)							
1	106.6	106.4	106.0	104.9	104.9	105.0	105.0
2	75.8	76.0	76.0	75.5	75.5	75.6	75.6
3	78.7	76.5	76.6	77.5	77.5	77.5	77.5
4	72.1	78.4	78.4	78.4	78.4	78.5	78.5
5	78.4	77.4	77.4	77.2	77.1	77.2	77.3
6	62.7	61.4	61.5	61.3	61.4	61.4	61.4
Rha							
1		102.7	102.8	102.8	102.8	102.8	102.8
2		72.8	72.8	72.8	72.8	72.8	72.8
3		72.5	72.6	72.6	72.6	72.6	72.6
4		74.0	74.0	73.9	73.9	74.0	74.0
5		70.4	70.4	70.4	70.4	70.4	70.4
6		18.5	18.5	18.5	18.6	18.6	18.5
Glc (C-16)							
1							106.6
2							75.8
3							78.8
4							71.8
5							78.1
6							63.0

parts (550 g) of *Celsia roripifolia* [The seeds were purchased from CHILTERN SEEDS, England (catalogue No. 300E) and the plants were cultivated in our botanical garden in 1995] gave 60% methanol eluate (117 g) and methanol eluate (20 g). From the methanol eluate, compound **10** (3.3 g) was obtained as crystal and part (2.5 g) of the filtrate afforded compounds **1** (4 mg), **3** (7 mg), **5** (7 mg), **10** (16 mg) and **11** (37 mg).

Mulleinsaponin I (2) An amorphous powder, $[\alpha]_D^{25}$ +57.2° (c=0.56, MeOH). High resolution positive mode SI-MS: Calcd for $C_{42}H_{69}O_{12}$: 765.4785 $(M+H)^+$. Found: 765.4787. ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Mulleinsaponin II (4) An amorphous powder, $[\alpha]_D^{25}$ +25.1° (c=0.37, MeOH). High resolution positive mode SI-MS: Calcd for $C_{48}H_{78}NaO_{16}$: 933.5183 $(M+Na)^+$. Found: 933.5179. ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Mulleinsaponin III (5) An amorphous powder, $[\alpha]_D^{23}$ +18.7° (c=2.08, MeOH). High resolution positive mode SI-MS: Calcd for $C_{48}H_{78}NaO_{17}$: 949.5132 $(M+Na)^+$. Found: 949.5115. ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Mulleinsaponin IV (12) An amorphous powder, $[\alpha]_D^{25}$ +31.5° (c=1.15, MeOH). High resolution positive mode SI-MS: Calcd for $C_{54}H_{88}NaO_{23}$: 1127.5609 $(M+Na)^+$. Found: 1127.5628. ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Mulleinsaponin V (13) An amorphous powder, $[\alpha]_D^{25}$ +33.7° (c=1.49, MeOH). High resolution positive mode SI-MS: Calcd for $C_{56}H_{90}NaO_{24}$: 1169.5714 $(M+Na)^+$. Found: 1169.5729. ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Mulleinsaponin VI (14) An amorphous powder, $[\alpha]_D^{25}$ +36.2° (c=1.04, MeOH). High resolution positive mode SI-MS: Calcd for $C_{56}H_9NaO_{23}$: 1153.5765 $(M+Na)^+$. Found: 1153.5761. ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Mulleinsaponin VII (15) An amorphous powder, $[\alpha]_D^{25}$ +14.3° (c=0.14, MeOH). High resolution positive mode SI-MS: Calcd for $C_{60}H_{98}NaO_{27}$: 1273.6187 $(M+Na)^+$. Found: 1273.6185. ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Sugar Analysis of Mulleinsaponins I—VII Each saponin (1 mg) was dissolved in 5% H_2SO_4 (0.05 ml) and dioxane (0.05 ml) and heated at 100 °C for 30 min. The reaction mixture was diluted with water and passed through an Amberlite IRA-60E column and the eluate was concentrated. To the residue D-cysteine (0.5 mg) in 0.02 ml of sodium acetate water solution (1 g/ml) was added and the reaction mixture was stirred for 1 h at 60 °C then overnight at room temperature. After the reaction mixture was concentrated to dryness, the residual sugar thiazolidine derivatives were trimethylsilylated with pyridine (0.015 ml), hexamethyldisilazane (0.015 ml) and trimethylsilylchloride (0.015 ml) at 50 °C for 30 min. The supernatant of the mixture was analyzed by GC. GC conditions: column, Supelco SPB™-1, 0.25 mm × 27 m, column temperature, 230 °C. D-Fucose and D-glucose were detected from **2**, L-rhamnose, D-fucose and D-glucose were detected from **4**, **5** and **12**—**15**. Retention times: D-fucose (13.2 min), L-fucose (12.4 min), D-glucose (17.9 min), L-glucose (17.2 min), D-rhamnose (11.9 min), L-rhamnose (12.3 min).

References

1) *a*) Yamamoto A., Miyase T., Ueno A., Maeda T., *Chem. Pharm. Bull.*, **39**, 2764—2766 (1991); *b*) Ding N., Yahara S., Nohara T., *Chem. Pharm. Bull.*, **40**, 780—782 (1992).
2) *a*) Yamamoto A., Miyase T., Ueno A., Maeda T., *Chem. Pharm. Bull.*, **41**, 1270—1274 (1993); *b*) Yamamoto A., Suzuki H., Miyase T., Ueno A., Maeda T., *Phytochemistry*, **34**, 485—488 (1993); *c*) Mori F., Miyase T., Ueno A., *ibid.*, **36**, 1485—1488 (1994); *d*) Miyase T., Matsushima Y., *Chem. Pharm. Bull.*, **45**, 1493—1497 (1997).
3) a) Yamamoto A., Miyase T., Ueno A., Maeda T., *Chem. Pharm. Bull.*, **41**, 1780—1783 (1993); *b*) Calis I., Zor M., Basaran A. A., Wright A. D., Sticher O., *Helv. Chim. Acta*, **76**, 1352—1360 (1993).
4) Seifert K., Preiss A., Johne S., Schimdt J., Lien N. T., Lavaud C., Massiot G., *Phytochemistry*, **30**, 3395—3400 (1991).
5) Hartleb I., Seifert K., *Phytochemistry*, **35**, 1009—1011 (1994).
6) Klimek B., *Phytochemistry*, **43**, 1281—1284 (1996).
7) Ding J.-K., Fujino H., Kasai H., Fujimoto N., Tanaka O., Zhou J., Matsuura H., Fuwa T., *Chem. Pharm. Bull.*, **34**, 1158—1167 (1986).