Sesquiterpene Lactone Glycosides from the Roots of *Ferula varia*

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Seven new sesquiterpene lactone glycosides (1–7) were isolated from the H_2O-soluble fraction from the MeOH extract of the roots of *Ferula varia*. Their structures were elucidated by extensive spectroscopic analyses. The absolute configurations of compounds 1 and 2 were determined by modified Mosher's method.

Key words *Ferula varia*; sesquiterpene lactone glycoside; Umbelliferae; Uzbekistan

We have been investigating medicinal plants of Uzbekistan in search of new drug leads.[1)] The roots of *Ferula varia* (Schrenk) Trautv. (Umbelliferae) have been used traditionally in Uzbekistan to treat fever and intestinal parasites and as a mouth rinse.[2)] We previously reported the isolation and structure elucidation of six new sesquiterpene lactones from the EtOAc-soluble fraction of the roots of this plant. Some of these lactones were found to enhance cytotoxicity against a multidrug-resistant cancer cell line (KB-C2) in the presence of 2.5 μM (a nontoxic concentration) colchicine.[3)] Our continuing chemical study of this plant has resulted in the isolation and characterization of seven new sesquiterpene lactone glycosides (**1**–**7**) from the H_2O-soluble fraction. In this paper, we describe the isolation and elucidate the structures of these compounds.

The H_2O-soluble fraction from the MeOH extract of the roots of *F. varia* was separated by repeated column chromatography to give seven new sesquiterpene lactone glycosides (**1**–**7**).

Compound **1** was obtained as a white amorphous powder. The molecular formula of **1** was assigned as $C_{21}H_{32}O_{10}$ by high-resolution electrospray ionization (HR-ESI) MS (*m/z* 467.1877 $[M+Na]^+$). The ^{1}H-NMR spectrum of **1** showed the presence of two *tert*-methyls (δ_H 0.95, 1.41), one vinyl methyl (δ_H 1.73), three methylenes [δ_H 1.28 (1H, ddd, J=5.4, 13.7, 13.7 Hz), 1.79 (1H, ddd, J=6.3, 13.7, 14.2 Hz), 2.05, 2.09, 2.21, and 2.49 (each 1H, m)], one methine [δ_H 2.23 (1H, d, J=11.7 Hz)], two oxygenated methines [δ_H 3.54 (1H, dd, J=6.8, 9.7 Hz), 4.38 (1H, d, J=11.7 Hz)], and one olefinic proton [δ_H 5.35 (1H, brs)], together with one anomeric proton [δ_H 4.25 (1H, d, J=7.8 Hz)]. The ^{13}C-NMR spectrum displayed 21 carbon resonances including three methyl carbons (δ_C 11.1, 22.1, 22.9), three sp^3 methylene carbons (δ_C 28.8, 32.9×2), one sp^3 methine carbon (δ_C 50.3), two oxygen-bearing sp^3 methine carbons (δ_C 85.4, 87.4), one sp^3 quaternary carbon (δ_C 39.8), two oxygen-bearing sp^3 quaternary carbons (δ_C 76.6×2), one sp^2 methine carbon (δ_C 123.9), one sp^2 quaternary carbon (δ_C 133.8), and one ester carbonyl carbon (δ_C 180.4). It also displayed six oxygenated sp^3 carbon resonances (δ_C 106.1, 75.6, 78.2, 71.7, 77.8, 62.8) assignable to a glucosyl moiety, which was confirmed by enzymatic hydrolysis to liberate glucose and an aglycone (**1a**). These data suggested that **1** was a sesquiterpene lactone glucoside. The ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum of **1** revealed the connectivity of C-1 to C-3, of C-5 to C-6, and of C-8 to C-9. The presence of an eudesmane skeleton with a γ-lactone between C-6 and C-7 was confirmed from the heteronuclear multiple bond correlations (HMBC) of H-6 [δ_H 4.38 (1H, d, J=11.7 Hz)] with C-7 (δ_C 76.6) and C-12 (δ_C 180.4); Me-13 (δ_H 1.41) with C-7, C-11 (δ_C 76.6), and C-12 (δ_C 180.4); Me-14 (δ_H 0.95) with C-1 (δ_C 85.4) and C-5 (δ_C 50.3), C-9 (δ_C 32.9), and C-10 (δ_C 39.8); and of Me-15 (δ_H 1.73) with C-3 (δ_C 123.9), C-4 (δ_C 133.8), and C-5. The location of the glucosyl moiety was assigned to C-1 from the HMBC correlation of H-1′ [δ_H 4.25 (1H, d, J=7.8 Hz)] with C-1, while its β-linkage was concluded from the J-value (7.8 Hz) of the anomeric proton signal. The nuclear Overhauser enhancement and exchange spectroscopy (NOESY) correlations of Me-14 with H-6 and H-8α [δ_H 1.79 (1H, ddd, J=6.3, 13.7, 14.2 Hz)] indicated that ring B adopted a chair conformation. The orientations of the hydroxyl groups at C-1, C-7, and C-11 were assigned as α in each case by the NOESY correlations of H-1 with H-9β [δ_H 1.28 (1H, ddd, J=5.4, 13.7, 13.7 Hz)], and of Me-13 with H-5 [δ_H 2.23 (1H, d, J=11.7 Hz)] and H-9β (Fig. 2B). The absolute configuration of **1** was determined by modified Mosher's method.[4)] Thus, the (*S*)-α-methoxy-α-trifluoromethyl phenylacetate (MTPA) ester of the aglycone (**1a**) was obtained by treatment of **1a** with (*R*)-MTPA chloride, while treatment of **1a** with (*S*)-MTPA chloride gave (*R*)-MTPA ester of **1a**. The 1*S*, 5*R*, 6*S*, 7*S*, 10*S*, 11*S* absolute configuration could be assigned based on the $\Delta\delta$ values ($\Delta\delta=\delta S-\delta R$)[4)] illustrated in Fig. 3. On the basis of these findings, the structure of **1** was elucidated as shown in Fig. 1.

A pseudomolecular ion peak at *m/z* 451.1951 $[M+Na]^+$ was observed in the positive ion HR-ESI-MS of **2**, indicating the molecular formula of $C_{21}H_{32}O_9$, which was 16 mass units smaller than that of **1**. The ^{1}H- and ^{13}C-NMR spectra were correlated with those of **1**, but differed in the appearance of a methine [δ_H 2.61 (1H, m); δ_C 45.7] instead of an oxygen-bearing sp^3 quaternary carbon seen in **1**; this suggested that **2** was also a eudesmane-type sesquiterpene lactone glucoside. This was further supported by the ^{1}H–^{1}H COSY correlations of H-5–H-6–H-7 (δ_H 2.61)–H_2–8-H_2-9, coupled with the HMBC cross peak of Me-13 with C-7 (δ_C 45.7). The enzymatic hydrolysis of **2** gave, along with glucose, an aglycone, which was identified as 1α,11α-dihydroxy-3,4-dehydro-5βH,6αH,7αH,10$\alpha$$CH_3$-eudesmane-6,12-olides by comparing the physical and spectral data with those described in the literature.[5)] The location of the glucosyl moiety was assigned to C-1 from the HMBC

The authors declare no conflict of interest.

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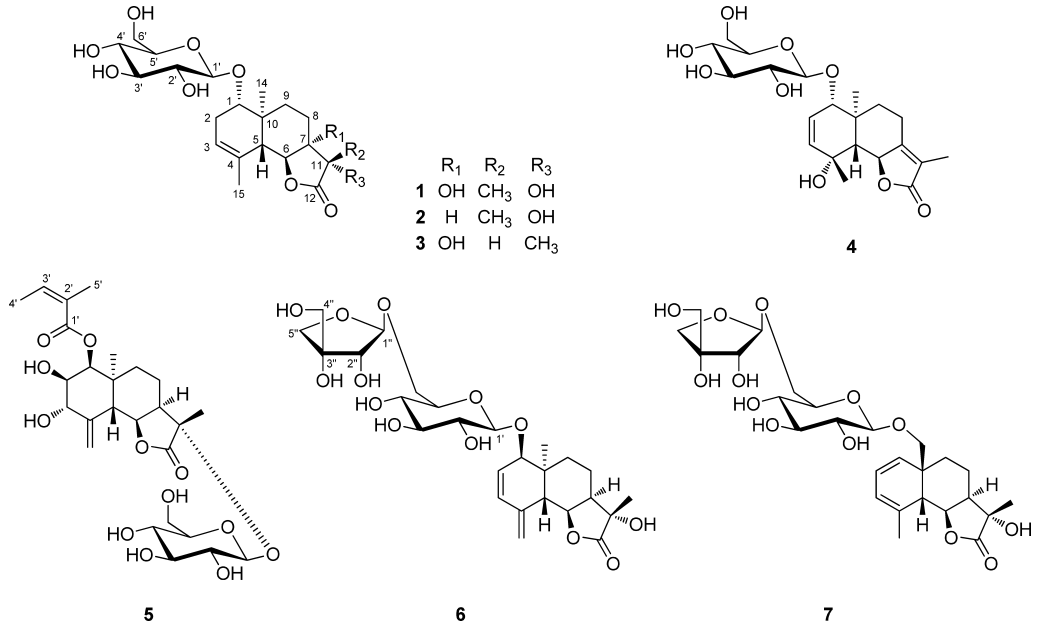

Fig. 1. New Sesquiterpene Lactone Glycosides from *F. varia*

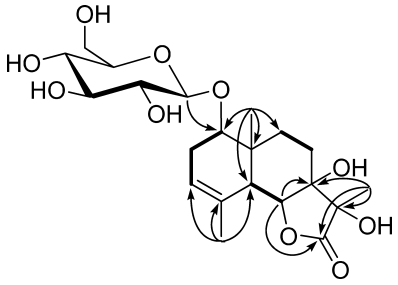

Fig. 2A. Key HMBC and ^{1}H–^{1}H COSY Correlations of Compound **1**

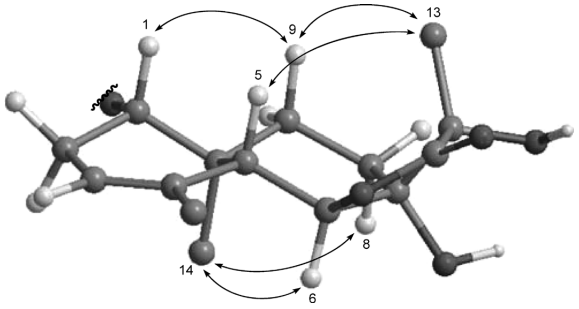

Fig. 2B. Key NOESY Correlations of **1**

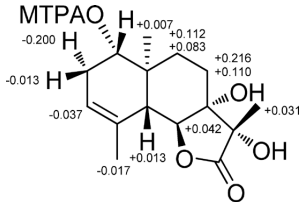

Fig. 3. $\Delta\delta$ Values of (*S*)- and (*R*)-MTPA Esters of **1a**

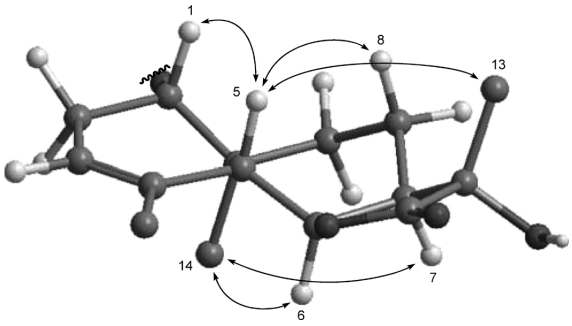

Fig. 4. Key NOESY Correlations of **2**

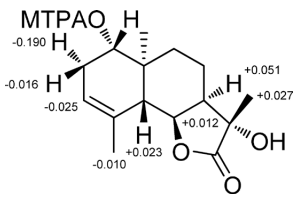

Fig. 5. $\Delta\delta$ Values of (*S*)- and (*R*)-MTPA Esters of **2a**

correlation of H-1' [δ_H 4.28 (1H, d, J=7.8 Hz)] with C-1 (δ_C 86.6), and its β-linkage was elucidated by the coupling constant of the anomeric proton signal (J=7.8 Hz). The boat conformation of ring B was suggested from the NOESY correlations of H-5 with H-1, H-8β, and Me-13 (Fig. 4). The absolute configuration of **2** was elucidated as 1*S*, 5*R*, 6*S*, 7*R*, 10*S*, 11*S* by the modified Mosher's method (Fig. 5). From these observations, the structure of **2** was determined as shown (Fig. 1).

Compound **3** had the same molecular formula ($C_{21}H_{32}O_9$) as **2**. The ^{1}H- and ^{13}C-NMR spectroscopic data were closely correlated with those of **2** except for the observation of a secondary methyl signal [δ_H 1.07 (3H, d, J=7.1 Hz); δ_C 22.9] instead of one of the *tert*-methyl signals seen in **2**. The locations of the hydroxyl group and secondary methyl group were concluded to be at C-7 and C-11 (δ_C 41.5), respectively, from the HMBC cross peaks of the secondary methyl signal with C-7 (δ_C 78.4) and C-12, and of H-6 with C-7 and C-12, respectively. The NOESY correlations of Me-14 with H-6 and H-8α suggested that ring B adopted a chair conformation. The

β configurations of H-1, H-5, and H-11 were assigned by the NOESY correlations of H-1 with H-9β, and of H-11 with H-5 and H-9β (Fig. 6). The location of the glucosyl moiety was assigned to the C-1 hydroxyl group from the HMBC correlations of H-1′ [δ_H 4.26 (1H, d, J=7.8 Hz)] with C-1 (δ_C 85.1), and its β-linkage was concluded from the J-value (7.8 Hz) of H-1′. Based on these data, the structure of **3** was elucidated as shown in Fig. 1.

The molecular formula of compound **4** was assigned as $C_{21}H_{30}O_9$ by the HR-ESI-MS. The ^{1}H- and ^{13}C-NMR spectra revealed the presence of two *tert*-methyls, one vinyl methyl, two methylenes, one methine, two oxygenated methines, one quaternary carbon, one oxygen-bearing quaternary carbon, one disubstituted olefin, one trisubstituted olefin, one carbonyl group, and one glucosyl moiety, which implied that **4** also was a eudesmane-type sesquiterpene lactone glucoside. This was further confirmed by the 2D-NMR experiments. The presence of the double bonds at C-2 (C-3) was assigned from the ^{1}H–^{1}H COSY correlations of H-1–H-2 [δ_H 5.91 (1H, dd, J=5.5, 10.0 Hz)]–H-3 [δ_H 5.81 (1H, d, J=10.0 Hz)], while the ^{1}H–^{1}H COSY correlation of H_2-8–H_2-9, coupled with the HMBC correlations of Me-13 (δ_H 1.75) with C-7 (δ_C 166.8) and C-11 (δ_C 119.8), of Me-14 with C-1, and of Me-15 with C-3 (δ_C 140.1), indicated the location of the double bond at C-7 (C-11). The presence of the hydroxyl group at C-4 was revealed by the HMBC cross peaks of H-3 with C-4 (δ_C 69.1), and of Me-15 with C-4. The location and linkage of the glucosyl moiety were elucidated from the analysis of the HMBC spectrum and from the ^{1}H-NMR spectroscopic data (Fig. 7A). The orientations of H-6 and Me-14 were elucidated as α by the NOE correlations of Me-14 with H-6 and H-8α, while the β-orientations of H-1, H-5, and Me-15 were deduced from the NOESY cross peaks of H-1 with H-9β and of H-5 with Me-15 (Fig. 7B). Thus, the structure of **4** was elucidated as shown (Fig. 1).

Compound **5** gave a pseudomolecular ion peak at m/z 565.2257 ([M+Na]$^+$ Calcd for 565.2261) in the positive-ion HR-ESI-MS, suggesting the molecular formula $C_{26}H_{38}O_{12}$. The ^{1}H- and ^{13}C-NMR spectra showed the signals due to two *tert*-methyl groups, two vinyl methyl groups, two methylenes, two methines, four oxygen-bearing methines, one quaternary carbon, one oxygen-bearing quaternary carbon, one exo-methylene, one sp^2 methine, two sp^2 quaternary carbons, two carbonyl carbons, and a glucosyl moiety. The data for the aglycone moiety were similar to those of a eudesmane-type sesquiterpene lactone with an angeloyl group (1β-angeloyloxy-11β-hydroxy-5βH,6αH,7αH,10α-methyleudesm-2(3),5(15)-diene-6,12-olide, **I**) previously isolated from the EtOAc-soluble fraction of this plant,[3] except for the observation of the signals due to two additional oxygen-bearing methines and a sugar moiety, as well as the absence of a disubstituted olefinic signal. The existence of the eudesmane-type sesquiterpene lactone structure with three oxygen-bearing functions and an angeloyl group was also supported by 2D-NMR spectral analysis. The locations of the oxygen-bearing methines were determined to be C-1, C-2, and C-3 from the ^{1}H–^{1}H COSY correlations of H-1 [δ_H 5.04 (1H, d, J=3.1 Hz)]–H-2 [δ_H 3.64 (1H, dd, J=3.1, 9.9 Hz)]–H-3 [δ_H 4.04 (1H, d, J=9.9 Hz)], together with the HMBC correlations of Me-14 with C-1 (δ_C 80.0), and of H_2-15 with C-3 (δ_C 74.5). The angeloyl group and the glucosyl moiety were confirmed

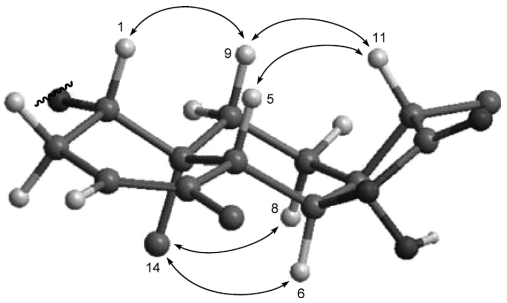

Fig. 6. Key NOESY Correlations of **3**

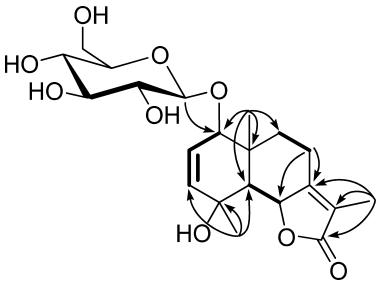

Fig. 7A. Key HMBC and ^{1}H–^{1}H COSY Correlations of Compound **4**

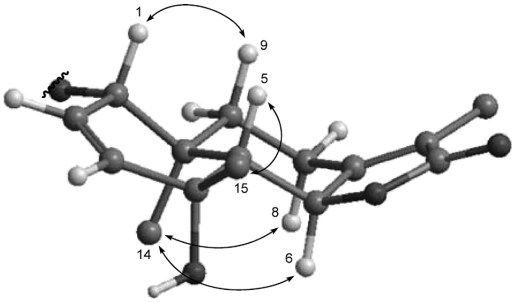

Fig. 7B. Key NOESY Correlations of **4**

to be attached at C-1 and C-11, respectively, by the HMBC correlations of H-1 with C-1′ (δ_C 168.7), and of H-1′ [δ_H 4.64 (1H, d, J=7.7 Hz)] with C-11 (δ_C 81.7). The linkage of the glucosyl moiety was assigned as β from the coupling constant value (J=7.7 Hz) of the anomeric proton signal (Fig. 8A). The NOESY correlations of Me-14 with H-2, H-6, H-7, and H-8α, and of H-1 and H-9α indicated that ring B adopted a half-chair conformation, and they were α-oriented. The β configurations of H-3, H-5, and Me-13 were elucidated from the NOESY cross peaks of H-5 with H-3, H-9β, and Me-13 (Fig. 8B). Based on this evidence, the structure of **5** was characterized as shown in Fig. 1.

Compound **6** was obtained as a pale yellow amorphous powder. The molecular formula of **6** was established as $C_{26}H_{38}O_{13}$ by HR-ESI-MS. The ^{1}H and ^{13}C NMR spectra were correlated with those of **I**,[3] except for the observation of signals due to two sugar moieties and the absence of signals arising from an angeloyl group. The two sugar moieties were confirmed as glucose and apiose by acid hydrolysis of **6**. The ^{1}H–^{1}H COSY and HMBC experiments revealed that the aglycone of **6** had the same planar structure as **I**.[3] The presence of β-apiosyl-(1→6)-β-glucosyl moiety at C-1 was confirmed from the HMBC correlations of H-1′ [δ_H 4.37 (1H, d, J=7.7 Hz)] with C-1 (δ_C 77.5), and of H_2-6′ [δ_H 3.57 (1H, dd, J=6.4, 11.3 Hz), 3.93

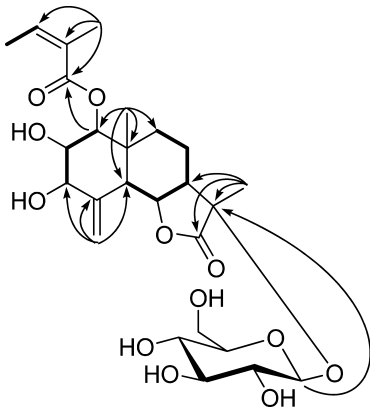

Fig. 8A. Key HMBC and ^{1}H–^{1}H COSY Correlations of Compound **5**

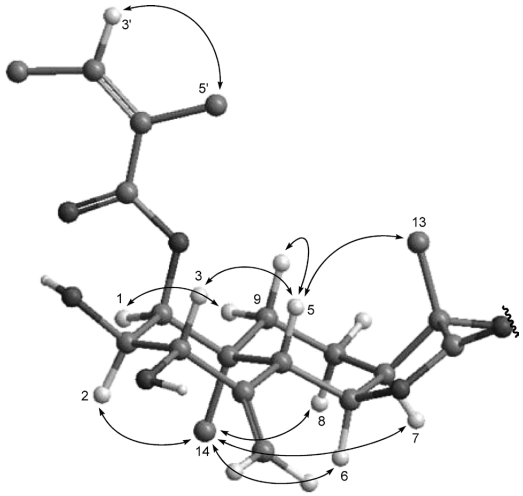

Fig. 8B. Key NOESY Correlations of **5**

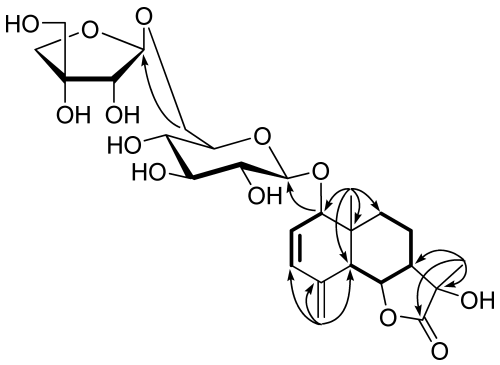

Fig. 9A. Key HMBC and ^{1}H–^{1}H COSY Correlations of Compound **6**

(1H, dd, J=1.6, 11.3 Hz)] with C-1" (δ_C 110.9) (Fig. 9A). The α configurations of H-1, H-6, H-7, and Me-14 were indicated by the NOE correlations of Me-14 with H-1, H-6, H-7, and H-8α, and of H-1 and H-9α. The β configurations of H-5 and Me-13 were elucidated by the NOE correlations of H-9β with H-5 and Me-13 (Fig. 9B). From these observations, the structure of **6** was assigned as shown (Fig. 1).

The molecular formula of **7** was assigned as $C_{26}H_{38}O_{13}$ by the HR-ESI-MS experiment. The presence of two sugar moieties was indicated by two anomeric resonances. In addition, the ^{1}H- and ^{13}C-NMR spectroscopic data for the sugar

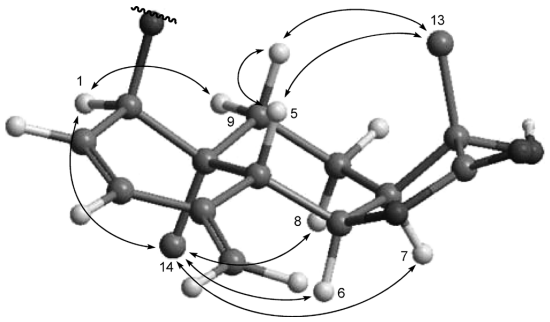

Fig. 9B. Key NOESY Correlations of **6**

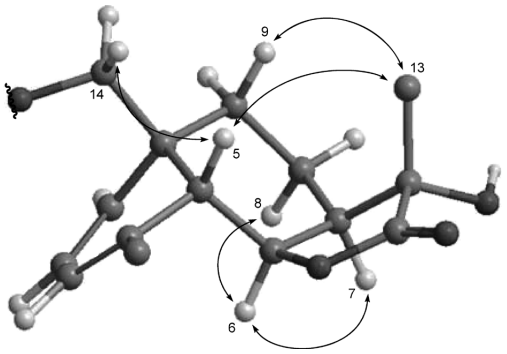

Fig. 10. Key NOESY Correlations of **7**

moieties in **7**, similar to those found in **6**, were in good agreement with the presence of the β-apiosyl-(1→6)-β-glucosyl moiety. In contrast, the signals arising from the aglycone moiety were similar to those found in **2** except for the observation of signals due to one oxygenated methylene [δ_H 3.30 (1H, d, J=9.5 Hz), 3.40 (1H, d, J=9.5 Hz); δ_H 73.5] and one disubstituted olefin [δ_H 5.23 (1H, d, J=9.4 Hz), 5.94 (1H, dd, J=4.0, 9.4 Hz); δ_C 128.1, 130.0], along with the absence of one *tert*-methyl signal. The HMBC correlations of Me-15 with C-3, C-4, and C-5, together with the ^{1}H–^{1}H COSY correlations of H-1 [δ_H 5.23 (1H, d, J=9.4 Hz)]–H-2 [δ_H 5.94 (1H, dd, J=4.0, 9.4 Hz)]–H-3 [δ_H 5.72 (1H, d, J=4.0 Hz)] indicated the presence of the double bonds at C-1(2) and C-3(4). In addition, the oxygenated methylene could be assigned to C-14 from the HMBC correlations of the oxygenated methylene signal with C-1, C-5, C-9, and C-10. The ^{1}H–^{1}H COSY and HMBC correlations revealed that **7** had the same partial structure on ring B and the γ-lactone unit as **2**. The location of the β-apiosyl-(1→6)-β-glucosyl moiety at C-14 was elucidated by the HMBC correlations of H-1′ [δ_H 4.06 (1H, d, J=7.8 Hz)] with C-1. The NOESY correlations of H-5 with Me-13 and H_2-14, and those of Me-13 with H-9β, revealed that H-5, Me-13, and H_2-14 were β-oriented. The α configurations of H-6 and H-7 were determined from the NOESY cross peaks of H-6 with H-7 and H-8α (Fig. 10). On the basis of these observations, the structure of **7** was assigned as shown in Fig. 1.

Experimental

General Experimental Procedures Optical rotations were measured with a JASCO DIP-370 digital polarimeter. MS were obtained on a Waters LCT PREMIER 2695. NMR spectra were measured on Bruker AVANCE-400 Fourier transform spectrometers (^{1}H-NMR: 400 MHz, ^{13}C-NMR: 100 MHz)

Table 1. ^{1}H- and ^{13}C-NMR Data for Compounds **1**–**4** in CD_3OD

Position	**1**		**2**		**3**		**4**	
	$^{1}H^{a)}$	$^{13}C^{b)}$	$^{1}H^{a)}$	$^{13}C^{b)}$	$^{1}H^{a)}$	$^{13}C^{b)}$	$^{1}H^{a)}$	$^{13}C^{b)}$
1	3.54 (1H, dd, 6.8, 9.7)	85.4	3.51 (1H, dd, 6.5, 10.2)	86.6	3.51 (1H, dd, 6.8, 9.1)	85.1	3.84 (1H, d, 5.5)	75.9
2	2.49 (1H, m)	32.9	2.47 (1H, m)	32.8	2.49 (1H, m)	33.0	5.91 (1H, dd, 5.5, 10.0)	123.7
	2.21 (1H, m)		2.08 (1H, m)		2.08 (1H, m)			
3	5.35 (1H, brs)	123.9	5.36 (1H, brs)	123.4	5.34 (1H, brs)	123.9	5.81 (1H, d, 10.0)	140.1
4	—	133.8	—	134.1	—	133.8	—	69.1
5	2.23 (1H, d, 11.7)	50.3	2.15 (1H, d, 10.2)	48.8	2.05 (1H, m)	50.4	1.95 (1H, d, 11.1)	52.0
6	4.38 (1H, d, 11.7)	87.4	4.70 (1H, dd, 7.2, 10.2)	80.2	4.29 (1H, d, 11.0)	87.8	5.24 (1H, d, 11.1)	82.0
7	—	76.6	2.61 (1H, m)	45.7	—	78.4	—	166.8
8	2.05 (1H, m)	28.8	1.50 (1H, m)	19.5	1.92 (1H, m)	29.7	2.76 (1H, dd, 4.7, 14.2)	23.3
	1.79 (ddd, 6.3, 13.7, 14.2)				1.81 (1H, dd, 4.8, 14.1)		2.46 (1H, ddd, 4.3, 12.7, 14.2)	23.3
9	2.09 (1H, m)	32.9	1.87 (1H, m)	33.1	2.19 (1H, m)	31.9	2.03 (1H, ddd, 4.7, 12.7, 13.7)	36.3
	1.28 (ddd, 5.4, 13.7, 13.7)		1.55 (1H, m)		1.21 (1H, ddd, 4.1, 13.8, 14.1)		1.49 (1H, dd, 4.3, 13.7)	
10	—	39.8	—	38.4	—	40.0	—	38.9
11	—	76.6	—	75.7	2.81 (1H, q, 7.1)	41.5	—	119.8
12	—	180.4	—	180.2	—	180.7	—	177.4
13	1.41 (3H, s)	22.1	1.32 (3H, s)	20.8	1.07 (3H, d, 7.1)	6.7	1.75 (3H, s)	8.1
14	0.95 (3H, s)	11.1	0.91 (3H, s)	14.6	0.90 (3H, s)	10.7	1.08 (3H, s)	19.4
15	1.73 (3H, s)	22.9	1.75 (3H, s)	22.4	1.73 (3H, s)	22.9	1.39 (3H, s)	31.4
1′	4.25 (1H, d, 7.8)	106.1	4.28 (1H, d, 7.8)	106.2	4.46 (1H, d, 7.8)	106.0	4.32 (1H, d, 7.7)	100.7
2′	3.12 (1H, t, 7.8)	75.6	3.14 (1H, t, 7.8)	76.0	3.13 (1H, t, 7.8)	75.5	3.05 (1H, t, 7.7)	75.2
3′		78.2		78.2		78.2		78.1
4′		71.7		71.7		71.7		71.8
5′		77.8		77.7		77.7		77.9
6′	3.78 (1H, d, 11.5)	62.8	3.80 (1H, dd, 2.1, 11.8)	62.8	3.79 (1H, dd, 2.0, 11.9)	62.8	3.76 (1H, m)	62.9
	3.59 (1H, dd, 4.9, 11.5)		3.61 (1H, dd, 5.1, 11.8)		3.60 (1H, dd, 5.3, 11.9)		3.60 (1H, dd, 5.6, 11.8)	

a) δ ppm (mult., J in Hz), 400 MHz. *b*) δ ppm, 100 MHz.

using tetramethylsilane (TMS) as an internal standard. Column chromatography: silica gel 60N (63–210 μm, Kanto Kagaku, Japan), Diaion HP-20 (Mitsubishi Chemical, Japan), Sephadex LH-20 (25–100 μm; GE Health Care, U.K.), MCI-gel CHP 20P (75–150 μm; Mitsubishi Chemical, Japan), YMC-pack ODS-A (S-50 μm; YMC Co., Ltd., Japan). Preparative HPLC: CAPCELL PACK C18 SG120 (250×20 mm; 5 μm; Shiseido, Japan), GPC (Gel-Permeation Chromatography) [Asahi pack GS-310 2G (MeOH, SHOWA DENKO)]. TLC: silica gel 60 F_{254} (Merck, Germany).

Plant Material The roots of *Ferula varia* were collected in Kyzylkum, Uzbekistan, in April 2001. Herbarium specimens (ESM-01ky-10) are deposited in the herbarium of the Graduate School of Pharmaceutical Sciences, Kyoto University.

Extraction and Isolation The dried roots of *F. varia* (1.4 kg) were crushed and extracted with MeOH for 4 h at 60°C three times. The MeOH extracts were concentrated *in vacuo* to give a gum (227 g), which was partitioned between EtOAc and H_2O. The H_2O-soluble fraction (115 g) was subjected to chromatography over Diaion HP-20 [MeOH–H_2O (0 : 1→1 : 0)] to give 6 fractions. Fr. 2 was fractionated by Sephadex LH-20 (H_2O) to afford fractions 2.1–2.8. Fr. 2.3 was applied to a YMC ODS-A column [MeOH–H_2O (0 : 1→1 : 0)] to yield fraction 2.3.1–2.3.13. Compoud **4** (2 mg) was isolated from fr. 2.3.5 by MCI gel CHP 20P CC [MeOH–H_2O (1 : 4→1 : 0)], and then GPC on HPLC (MeOH). Fr. 2.3.9 was subjected to a MCI gel CHP 20 column [MeOH–H_2O (1 : 1→1 : 0)] to afford 5 fractions (2.3.9.1–2.3.9.5). Fr. 2.3.9.2 was further purified by GPC on HPLC (MeOH), followed by ODS HPLC (CAPCELL

Table 2. ^{1}H- and ^{13}C-NMR Data for Compounds **5**–**7** in CD_3OD

Position	**5**		**6**		**7**	
	$^{1}H^{a)}$	$^{13}C^{b)}$	$^{1}H^{a)}$	$^{13}C^{b)}$	$^{1}H^{a)}$	$^{13}C^{b)}$
1	5.04 (1H, d, 3.1)	80.0	3.74 (1H, d, 4.6)	77.5	5.23 (1H, d, 9.4)	128.1
2	3.64 (1H, dd, 3.1, 9.9)	73.6	5.91 (1H, d, 4.6, 9.7)	125.3	5.94 (1H, 4.0, 9.4)	130.0
3	4.04 (1H, d, 9.9)	74.5	6.29 (1H, d, 9.7)	135.1	5.72 (1H, d, 4.0)	141.3
4	—	146.4	—	144.7	—	119.7
5	2.32 (1H, d, 10.3)	45.2	2.69 (1H, d, 9.8)	42.3	2.33 (1H, d, 9.7)	43.4
6	5.07 (1H, dd, 1.9, 10.3)	77.5	4.88 (1H, dd, 8.6, 9.8)	79.6	4.46 (1H, t, 9.7)	84.1
7	3.00 (1H, dd, 6.2, 13.9)	42.9	2.62 (1H, dd, 5.7, 13.9)	45.5	2.47 (1H, m)	45.1
8	1.78 (1H, m)	19.5	1.77 (2H, m)	19.2	1.86 (1H, m)	19.7
	1.69 (1H, m)				1.78 (1H, m)	
9	1.59 (1H, m)	31.6	2.36 (1H, m)	30.8	2.03 (1H, ddd, 4.7, 4.7, 13.8)	29.0
	1.21 (1H, m)		1.14 (1H, dt, 5.4, 14.3)		1.38 (1H, m)	
10	—	39.5	—	38.1	—	41.0
11	—	81.7	—	75.7	—	75.2
12	—	178.5	—	181.1	—	181.6
13	1.44 (3H, s)	19.6	1.41 (3H, s)	22.1	1.43 (3H, s)	22.5
14	0.90 (3H, s)	19.6	0.78 (3H, s)	19.7	3.40 (1H, d, 9.5)	73.5
					3.30 (1H, d, 9.5)	
15	5.49 (1H, s)	108.9	5.32 (1H, s)	116.5	1.95 (3H, s)	24.1
	5.11 (1H, s)		5.15 (1H, s)			
1′	—	168.7	4.37 (1H, d, 7.7)	101.3	4.06 (1H, d, 7.8)	104.8
2′	—	129.1	3.07 (1H, t, 7.7)	75.3	3.09 (1H, t, 7.8)	75.3
3′	6.11 (1H, q, 7.1)	139.7	3.29 (1H, m)	78.2		78.4
4′	1.95 (3H, d, 7.1)	16.1	3.20 (1H, t, 9.2)	71.9		71.7
5′	1.88 (3H, s)	21.0	3.34 (1H, m)	77.0		76.9
6′	—	—	3.93 (1H, dd, 1.6, 11.3)	68.6	3.90 (1H, m)	68.6
			3.57 (1H, dd, 6.4, 11.3)		3.54 (1H, m)	
1″	4.64 (1H, d, 7.7)	99.1	5.00 (1H, d, 2.2)	110.9	4.94 (1H, d, 2.3)	111.0
2″	3.11 (1H, d, t, 7.7)	75.0	3.85 (1H, d, 2.2)	78.0		78.0
3″		77.9	—	80.5		78.4
4″		71.5	3.91 (1H, d, 9.3)	75.0		75.0
			3.72 (1H, d, 9.3)			
5″		77.8	3.53 (1H, m)	65.7		65.7
6″	3.59 (1H, dd, 4.7, 10.8)	62.7	—	—	—	—
	3.78 (1H, d, 10.8)					

a) δ ppm (mult., J in Hz), 400 MHz. *b*) δ ppm, 100 MHz.

PAK C18 SG120) [MeOH : H_2O=1 : 1] to give **5** (2 mg) and **6** (18 mg). Fr. 2.3.9.3 was separated by GPC on HPLC (MeOH), and then silica gel CC [$CHCl_3$–MeOH–H_2O (8 : 2 : 0.2→6 : 4 : 1)] to yield **7** (6 mg). Fr. 2.4 was applied to a YMC ODS-A column [MeOH–H_2O (0 : 1→1 : 0)] to give 9 fractions (2.4.1–2.4.9). Fr. 2.4.7 was fractionated by Sephadex LH-20 CC [$CHCl_3$–MeOH (1 : 1)] and MCI gel CHP 20P CC [MeOH–H_2O (1 : 1→1 : 0)] to yield fractions 2.4.7.1–2.4.7.6. Fr. 2.4.7.3 was separated by YMC ODS-A CC [MeOH–H_2O (1 : 1→1 : 0)] and silica gel CC [$CHCl_3$–MeOH–H_2O (6 : 1 : 0.1→7 : 3 : 0.5)] to give 3 fractions (2.4.7.3.1–2.4.7.3.3). Purification of fr. 2.4.7.3.2 by GPC (MeOH) and ODS HPLC (CAPCELL PAK C18 SG120) [MeOH–H_2O=1 : 1] gave compound **1** (5 mg). Fr. 2.4.7.4 was repeatedly fractionated by YMC ODS-A CC [MeOH–H_2O (1 : 1→1 : 0)], silica gel CC [$CHCl_3$–MeOH–H_2O (6 : 1 : 0.1→7 : 3 : 0.5)] and GPC (MeOH) to obtain **3** (14 mg). Fr. 2.4.8 was repeatedly chromatographed over sphadex LH-20 (EtOH), silica gel ($CHCl_3$–MeOH–H_2O=6 : 1 : 0.1) and YMC ODS-A (MeOH–H_2O=4 : 1) to afford **2** (26 mg).

Compound **1**: White amorphous powder; $[\alpha]_D^{19}$ −17.5 (c=0.4, MeOH); ^{1}H- and ^{13}C-NMR data (CD_3OD) see Table 1; HR-ESI-MS m/z 467.1877 $[M+Na]^+$ (Calcd for $C_{21}H_{32}O_{10}Na$, 467.1893).

Enzymatic Hydrolysis of 1 A solution of compound **1** (10 mg) in water (2 mL) was treated with Cellulase from *Trichoderma viride* (3–10 units/mg solid, Sigma) (20 mg) at 37°C for 3 weeks. The reaction mixture was diluted with MeOH, and the resulting precipitates were filtrated off. The filtrate was purified by silica gel column chromatography [benzene–isopropanol (15 : 1→8 : 1)] to give an aglycone (**1a**) (1 mg) and a

sugar (0.7 mg). The sugar moiety was identified as glucose by the TLC analysis [*Rf*: 0.33, *n*-BuOH–pyridine–H_2O (6 : 4 : 3) on Avicel SF cellulose].

Preparation of (*S*)-MTPA or (*R*)-MTPA Esters of 1a Compound **1a** (0.5 mg) was dissolved with pyridine-d_5 (0.4 mL) in a NMR tube. The solution of (*S*)-MTPA or (*R*)-MTPA chloride (2 drops) was added to the NMR tube to kept for 12 h.

(*R*)-MTPA ester of **1a** (**1ar**): ^{1}H-NMR (pyridine-d_5) δ: 5.289 (1H, m, H-3), 5.269 (1H, m, H-1), 4.849 (1H, d, J=11.0 Hz, H-6), 2.642 (1H, brd, J=17.6 Hz, H-2a), 2.538 (1H, d, J=11.0 Hz, H-5), 2.214 (1H, m, H-2b), 2.167 (1H, dt, J=5.4, 15.3 Hz, H-9a), 2.004 (1H, m, H-8a), 1.864 (3H, s, H_3-15), 1.799 (1H, m, H-8b), 1.707 (3H, s, H_3-13), 1.540 (1H, m, H-9b), 1.168 (3H, s, H_3-14).

(*S*)-MTPA ester of **1a** (**1as**): ^{1}H-NMR (pyridine-d_5) δ: 5.252 (1H, m, H-3), 5.247 (1H, m, H-1), 4.891 (1H, d, J=11.0 Hz, H-6), 2.629 (1H, brd, J=17.5 Hz, H-2a), 2.551 (1H, d, J=11.0 Hz, H-5), 2.250 (1H, dt, J=5.1, 14.2 Hz, H-9a), 2.114 (1H, m, H-8a), 2.015 (1H, m, H-8b), 2.014 (1H, m, H-2b), 1.847 (3H, s, H_3-15), 1.738 (3H, s, H_3-13), 1.658 (1H, m, H-9b), 1.175 (3H, s, H_3-14).

Compound **2**: White amorphous powder; $[\alpha]_D^{18}$ −66.2 (c=2.5, MeOH); ^{1}H- and ^{13}C-NMR (CD_3OD) see Table 1; HR-ESI-MS m/z 451.1951 $[M+Na]^+$ (Calcd for $C_{21}H_{32}O_9Na$, 451.1944).

Enzymatic Hydrolysis of 2 A solution of compound **2** (10 mg) in water (2 mL) was treated with β-glucosidase from almonds (4.8 units/mg solid, Sigma) (10 mg) at 37°C for 9 d. The reaction mixture was worked up as for **1**, and was purified by silica gel column chromatography [$CHCl_3$–MeOH –H_2O (20 : 1 : 0→7 : 3 : 0.5)] to give an aglycone (**2a**) (6 mg) and a glucose (3 mg). **2a** was identified as 1β,11α-dihydroxy-3,4-dehydro-5βH,6αH,7αH,10$\alpha$$CH_3$-eudesmane-6,12-olides by comparison of the physical and spectral data with described in the literature.[5] Glucose was identified by the TLC analysis [*Rf*: 0.33, *n*-BuOH–pyridine–H_2O (6 : 4 : 3) on Avicel SF cellulose].

Preparation of (*S*)- or (*R*)-MTPA Ester of 2a **2a** was treated with (*S*)-MTPA or (*R*)-MTPA chloride in a NMR tube as for **1a**.

(*R*)-MTPA ester of **2a** (**2ar**): ^{1}H-NMR (pyridine-d_5) δ: 5.224 (1H, brs, H-3), 5.145 (1H, dd, J=6.5, 10.2 Hz, H-1), 4.955 (1H, dd, J=6.0, 9.7 Hz, H-6), 2.836 (1H, m, H-7), 2.508 (1H, brd, J=17.2 Hz, H-2a), 2.304 (1H, d, J=9.7 Hz, H-5), 2.127 (1H, m, H-2b), 1.811 (3H, s, H_3-15), 1.725–1.328 (4H, m, H_2-8, H_2-9), 1.577 (3H, s, H_3-13), 0.891 (3H, s, H_3-14).

(*S*)-MTPA ester of **2a** (**2as**): ^{1}H-NMR (pyridine-d_5) δ: 5.199 (1H, brs, H-3), 5.131 (1H, dd, J=6.4, 10.0 Hz, H-1), 4.967 (1H, dd, J=6.6, 9.7 Hz, H-6), 2.887 (1H, m, H-7), 2.492 (1H, brd, J=17.3 Hz, H-2a), 2.327 (1H, d, J=9.7 Hz, H-5), 1.937 (1H, m, H-2b), 1.801 (3H, s, H_3-15), 1.604 (3H, s, H_3-13), 1.583–1.357 (4H, m, H_2-8, H_2-9), 0.891 (3H, s, H_3-14).

Compound **3**: Pale yellow amorphous powder; $[\alpha]_D^{18}$ −9.6 (c=1.6, MeOH); ^{1}H- and ^{13}C-NMR (CD_3OD) see Table 1; HR-ESI-MS m/z 451.1954 $[M+Na]^+$ (Calcd for $C_{21}H_{32}O_9Na$, 451.1944).

Compound **4**: White amorphous powder; $[\alpha]_D^{20}$ −88.6 (c=1.2, MeOH); ^{1}H- and ^{13}C-NMR (CD_3OD) see Table 1; HR-ESI-MS m/z 449.1794 $[M+Na]^+$ (Calcd for $C_{21}H_{30}O_9Na$, 449.1788).

Compound **5**: White amorphous powder; $[\alpha]_D^{20}$ −15.0 (c=0.3, MeOH); ^{1}H- and ^{13}C-NMR (CD_3OD) see Table 2; HR-ESI-MS m/z 565.2257 $[M+Na]^+$ (Calcd for $C_{26}H_{38}O_{12}Na$, 565.2261).

Compound **6**: Pale yellow amorphous powder; $[\alpha]_D^{20}$ −177.6 (c=1.4, MeOH); ^{1}H- and ^{13}C-NMR (CD_3OD) see Table 2; HR-ESI-MS m/z 557.2233 $[M-H]^-$ (Calcd for $C_{26}H_{38}O_{13}Na$, 557.2234).

Acid Hydrolysis of 6 Compound **6** (1 mg) was hydrolyzed with 1 M HCl for 12 h at 80°C. The reaction mixture was diluted with H_2O, and extracted with EtOAc. The H_2O layer was neutralized with Amberlite IRA-400 resin and evaporated. The residue was directly analyzed by TLC [*Rf*: 0.33 (glucose), 0.55 (apiose), respectively, *n*-BuOH–pyridine–H_2O (6 : 4 : 3) on Avicel SF cellulose] to detect glucose and apiose.

Compound **7**: White amorphous powder; $[\alpha]_D^{20}$ −142.0 (c=0.6, MeOH); ^{1}H- and ^{13}C-NMR (CD_3OD) see Table 2; HR-ESI-MS m/z 581.2213 $[M+Na]^+$ (Calcd for $C_{26}H_{38}O_{13}Na$, 581.2210).

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