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Two New *ent*-Kaurane Diterpenoids from the Roots of *Fritillaria thunbergii*

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Fritillaria thunbergii (Liliaceae) is a perennial herb that is widely distributed in mountainous regions of northeast Asia.[1] The roots of *F. thunbergii* have been used as a Chinese and Korean traditional medicine for the respiratory system.[2] Diterpenoids and steroidal alkaloids have been isolated from the MeOH extract of this source, and the isolated compounds showed *anti*-bacterial, *anti*-tumor, and *anti*-inflammatory effects.[3-5] In the course of our continuing search for diterpene derivatives from Korean tradition medicinal plants,[6-8] we studied the MeOH extract of the *F. thunbergii* roots and isolated two new *ent*-kaurane diterpenoids, fritillarinol A (**1**) and fritillarinol B (**2**), together with five known compounds (**3-7**).

The structures of known compounds were determined to be 16α,17-epoxy-*ent*-kaurane (**3**),[9] 16β,17-dihydroxyl-*ent*-kaurane (**4**),[10] 16β-methoxy-17-hydroxyl-*ent*-kaurane (**5**),[11] (−)-*ent*-kaur-16-ene (**6**),[10] and isopimara-7,15-dien (**7**)[12] by comparing their spectroscopic data with those in the literature.

Compound **1** was obtained as a white amorphous powder. The molecular formula was determined to be $C_{20}H_{32}O_2$ from the $[M + H]^+$ peak at *m/z* 305.2481 (calcd. for $C_{20}H_{33}O_2$: 305.2481) on the HR-FAB-MS spectrum. The IR spectrum at 3421 cm^{-1} indicated that **1** possessed a hydroxyl group. The ^1H NMR spectrum (Table 1) of **1** showed three tertiary methyl groups at δ_H 1.00 (s, H-20), 0.86 (s, H-19), and 0.81 (s, H-18) and one oxymethine proton at δ_H 2.95 (s, H-15), and a hydroxylated methylene groups at δ_H 4.03 (d, J = 12.5 Hz, H-17a) and 3.81 (d, J = 12.5 Hz, H-17b). The ^{13}C NMR and DEPT experiments displayed 20 carbon signals, including three methyls at δ_C 33.7 (C-19), 21.7 (C-18), and 17.6 (C-20); one oxygenated tertiary carbon at δ_C 65.4 (C-16), one oxygenated secondary carbon at δ_C 65.7 (C-15), one oxygenated methylene carbon at δ_C 59.2 (C-17), three tertiary carbons at δ_C 56.1 (C-5), 50.4 (C-9), and 35.9 (C-13); eight methylene carbons at δ_C 42.1 (C-3), 40.6 (C-1), 35.8 (C-14), 32.2 (C-7), 26.7 (C-12), 19.2 (C-6), 18.6 (C-2), and 18.1 (C-11); and three quaternary carbons at δ_C 43.3 (C-8), 39.3 (C-10), and 33.4 (C-4), suggesting that **1** was a *ent*-kaurane diterpenoid.[10] The ^{13}C NMR spectral data were similar to *ent*-15β,16β-epoxy-kauran-17-ol except for the chemical shifts at C-15, C-16, and C-17.[10] The α-configuration of the epoxy ring at C-15 and C-16 (δ_C 65.7 and 65.4) was assigned by the chemical shift of ^{13}C NMR spectrum (δ_C 65.7, 69.5, and 59.9 for an β-epoxy ring at C-15, C-16, and C-17; δ_C 65.3, 65.9, and 58.9 for an α-epoxy ring at C-15, C-16, and C-17).[10,13] The β-proton at C-15 was reconfirmed by the spectroscopy NOESY correlataion (H-15

Table 1. ^1H-, ^{13}C-NMR data of **1** and **2**

Position	**1**[a]		**2**[a]	
	δ_H	δ_C	δ_H	δ_C
1	1.81, m	40.6	1.82, m	40.7
	0.78, m		0.76, m	
2	1.41, m	18.6	1.40, m	18.9
	1.38, m		1.36, m	
3	1.40, m	42.1	1.41, m	42.3
	1.17, m		1.15, m	
4		33.4		33.4
5	0.79, m	56.1	0.82, m	56.4
6	1.57, m	19.2	1.55, m	20.9
	1.22, m		1.20, m	
7	1.63, m	32.2	1.40, m	41.7
	1.09, m		1.15, m	
8		43.3		41.7
9	1.16, m	50.4	1.08, m	56.4
10		39.3		39.5
11	1.60, m	18.1	1.53, m	18.8
	1.53, m		1.52, m	
12	1.62, m	26.7	1.45, m	31.7
	1.57, m		1.42, m	
13	2.29, br s	35.9	2.16, br s	38.2
14	1.74, m	35.8	1.83, br d (11.5)	38.1
	1.56, m		1.05, m	
15	2.95, s	65.7	1.79, q (5.5)	44.4
			1.38, m	
16		65.4	1.79, br d (8.5)	43.0
17	4.03, d (12.5)	59.2	4.05, d (8.5)	107.8
	3.81, d (12.5)			
18	0.81, s	21.7	0.83, s	21.8
19	0.86, s	33.7	0.88, s	33.8
20	1.00, s	17.6	1.12, s	17.7
-OCH_3			3.29, s	52.7
-OCH_3			3.29, s	52.8

[a]500 MHz, $CDCl_3$; chemical shifts in ppm relative to TMS; coupling constants (J) in Hz.

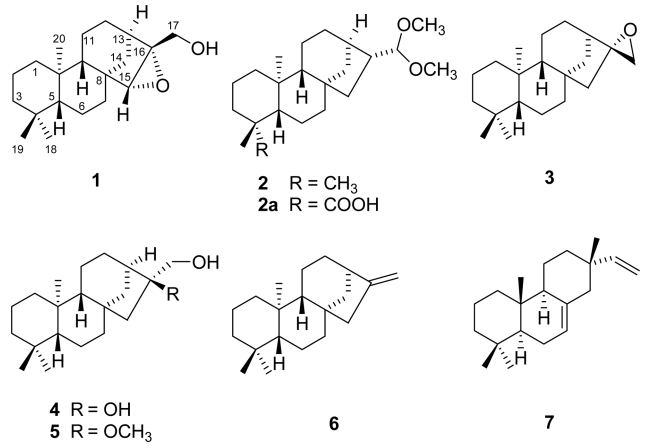

Figure 1. The structures of isolated compounds **1-7** *from F. thunbergii*.

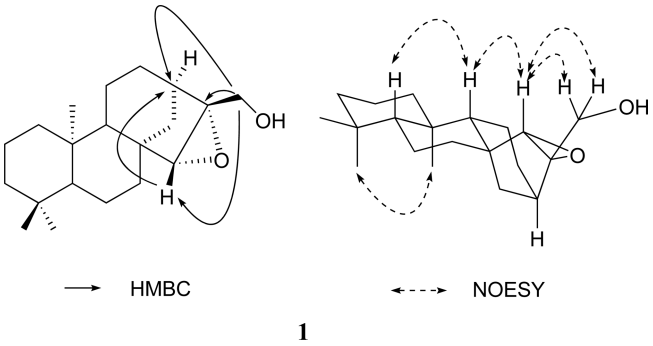

Figure 2. HMBC and NOESY correlations of **1**.

(δ_H 2.95)/H-9 (δ_H 1.16)) (Fig. 2). The positions of the epoxy ring and hydroxyl groups were confirmed in the HMBC spectrum by correlations H-17/C-13, H-17/C-15, and H-15/C-13 (Fig. 2). The other stereochemistries of ring junctions were identified by NOE correlations (Fig. 2). Thus, the structure of **1** was determined to be *ent*-15α,16α-epoxy-kauran-17-ol and named fritillarinol A.

Compound **2** was obtained as a white amorphous powder. The molecular formula was determined to be $C_{22}H_{38}O_2$ from the $[M + Na]^+$ peak at *m/z* 357.2771 (calcd. for $C_{22}H_{38}O_2Na$: 357.2770) on HR-FAB-MS spectrum. The ^1H NMR spectrum (Table 1) of **1** showed three tertiary methyl groups at δ_H 1.12 (s, H-20), 0.88 (s, H-19), and 0.83 (s, H-18) and two methoxy protons at δ_H 3.29 (6H, s, C-17-OCH_3), and one oxymethine protons at δ_H 4.05 (1H, d, J = 8.5 Hz, H-17). The ^{13}C NMR spectrum displayed 22 carbon signals including three methyls at δ_C 33.8 (C-19), 21.8 (C-18), and 17.7 (C-20), two methoxy carbons at δ_C 52.8 and 52.7, one oxygenated secondary carbon at δ_C 107.8 (C-17), four tertiary carbons at δ_C 56.4 (C-5 and 9), 43.0 (C-16), and 38.2 (C-13); nine secondary carbons at δ_C 44.4 (C-15), 42.3 (C-3), 41.7 (C-7), 40.7 (C-1), 38.1 (C-14), 31.7 (C-12), 20.9 (C-6), 18.9 (C-2) and 18.8 (C-11); and three quaternary carbons at δ_C 39.5 (C-10), 41.7 (C-8) and 33.4 (C-4), suggesting that **2** was a *ent*-kaurane diterpenoid.[10] The NMR data of **2** were very similar with those of (16*R*)-17-dimethoxy-*ent*-kauran-19-oic acid (**2a**) except for the presence of a carboxylic acid moiety.[14] The stereochemistry of **2** was assumed to be same as (16*R*)-17-dimethoxy-*ent*-kauran-19-oic acid (**2a**) by comparing the NMR data.[14] The stereostructure at C-16 was also determined to be same (16*R*) as that of (16*R*)-17-dimethoxy-*ent*-kauran-19-oic acid (**2a**), based on the chemical shifts and *J* values.[14] Thus, the structure of compound **2** was determined to be (16*R*)-17-dimethoxy-*ent*-kaurane and named fritillarinol B.

Experimental Section

Plant Materials. The roots of *F. thunbergii* (Liliaceae) (2.6 kg) were purchased at Naemome Dah, Korea in January 2012. A voucher specimen of the plant (SKKU-NPL 1201) was deposited at the School of Pharmacy of Sungkyunkwan University.

Extraction and Isolation. The half dried roots of *F. thunbergii* (Liliaceae) (2.6 kg) were extracted with 80% MeOH three times under reflux for 4 h. The resulting MeOH extracts (200 g) were suspended in distilled water (800 mL) and then successively partitioned with *n*-hexane, $CHCl_3$, and hydrated *n*-BuOH, yielding 11 g, 7 g and 19 g, respectively. The hexane soluble fraction (11 g) was separated on a silica gel open column (230-400 mesh, 550 g), eluted in a gradient solvent system from Hex: EtOAc (20:1) to Hex: EtOAc (1:1) to give six fractions (fractions A–F). Fraction C (1.8 g) was separated over a RP-C_{18} silica gel column (230-400 mesh, 90 g) with a solvent system of 90% MeOH as the eluent to give seven fractions (fr. C1–C7). Fr. C5 (98 mg) was subjected to a Lobar®-A Si (240 × 10 mm) column, using a solvent system of Hex: EtOAc (7:1) to give five fractions (fr. C51–C55). Fr. C53 (21 mg) was purified further by preparative normal-HPLC, using a solvent system of Hex: EtOAc (4:1) to obtain **1** (14 mg). Fr. A (0.7 g) was separated on a silica gel open column (230-400 mesh, 35 g), and eluted with Hex: EtOAc (4:1) to give five fractions (fr. A1–A5). Fr. A5 (54 mg) was further purified by preparative reverse-phase HPLC, using a solvent system of 100% MeOH to obtain **2** (4 mg) and **3** (36 mg). Fr. A1 (20 mg) was purified further by preparative reverse-phase HPLC using a solvent system of 100% MeOH to obtain **6** (13 mg) and **7** (4 mg). Fr. F (2.6 g) was separated on a RP-C_{18} open column (230-400 mesh, 150 g), eluted with 85% MeOH to give five fractions (fr. F1–F5). Fr. F5 was separated on a Lobar®-A Si (240 × 10 mm) column, using a solvent system of Hex: EtOAc (4:1) to give three fractions (fr. F51–F53). Fr. F52 (21 mg) was purified further by preparative reverse-phase HPLC using a solvent system of 100% MeOH to obtain **4** (6 mg) and **5** (6mg).

15α,16α-epoxy-17-hydroxy-*ent*-karane (1): Amorphous white powder. mp 154-155 °C; $[\alpha]_D^{25}$ −19.50° (*c* = 0.7, $CHCl_3$); IR (KBr) ν_{max} : 3421, 2928, 1443, 1058 cm^{-1}; FAB-MS *m/z* (rel. int.) = 305 $[M + H]^+$ (100); HR-FAB-MS *m/z* = 305.2481 $[M + H]^+$ (calcd for: 305.2481); ^1H NMR ($CDCl_3$, 500 MHz): see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz): see Table 1.

16*R*-17-dimethoxy-*ent*-kaurane (2): Amorphous white powder. mp 155-156 ºC; $[\alpha]_D^{25}$ −7.85° (*c* = 0.2, $CHCl_3$); IR (KBr) ν_{max} 2926, 1462, 1089, 1040, 1026, 618 cm^{-1}; FAB-MS *m/z* (rel. int.) = 357.2771 $[M + Na]^+$ (100); HR-FAB-MS *m/z* = 357.2771 $[M + Na]^+$ (calcd for: 357.2770); ^1H NMR ($CDCl_3$, 500 MHz): see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz): see Table 1.

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Supporting Information. The spectral data of compounds **1-2** and the general experimental procedures are available upon request from the correspondence author.

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