

Two New *ent*-Kaurane Diterpenoids from the Roots of *Fritillaria thunbergii*Jong Eel Park, Seung Young Lee, Kyeong Wan Woo, Je Hyun Lee,[†] and Kang Ro Lee^{*}Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea
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Received December 13, 2012, Accepted February 27, 2013**Key Words** : *Fritillaria thunbergii*, Liliaceae, *ent*-kaurane diterpenoid

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 16 α ,17-epoxy-*ent*-kaurane (**3**),⁹ 16 β ,17-dihydroxyl-*ent*-kaurane (**4**),¹⁰ 16 β -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 C₂₀H₃₂O₂ from the [M + H]⁺ peak at *m/z* 305.2481 (calcd. for C₂₀H₃₃O₂: 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 ¹³C 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 ¹³C NMR spectral data were similar to *ent*-15 β ,16 β -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 ¹³C 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-, ¹³C-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
	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
	1.16, m	50.4	1.08, m	56.4
10		39.3		39.5
	1.60, m	18.1	1.53, m	18.8
12	1.53, m		1.52, m	
	1.62, m	26.7	1.45, m	31.7
13	1.57, m		1.42, m	
	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
	4.03, d (12.5)	59.2	4.05, d (8.5)	107.8
17	3.81, d (12.5)			
	0.81, s	21.7	0.83, s	21.8
19	0.86, s	33.7	0.88, s	33.8
	1.00, s	17.6	1.12, s	17.7
-OCH ₃			3.29, s	52.7
-OCH ₃			3.29, s	52.8

^a500 MHz, CDCl₃; chemical shifts in ppm relative to TMS; coupling constants (*J*) in Hz.

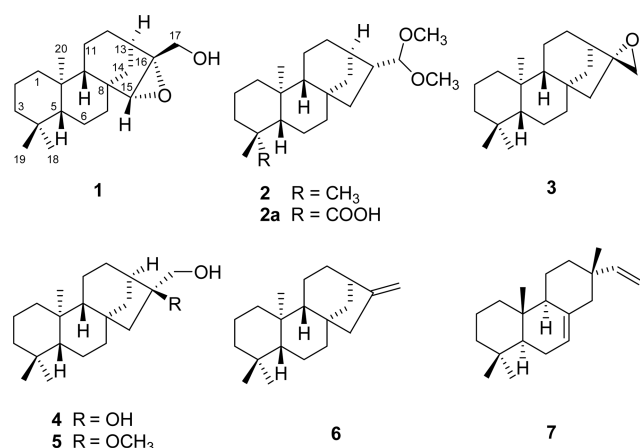


Figure 1. The structures of isolated compounds **1-7** from *F. thunbergii*.

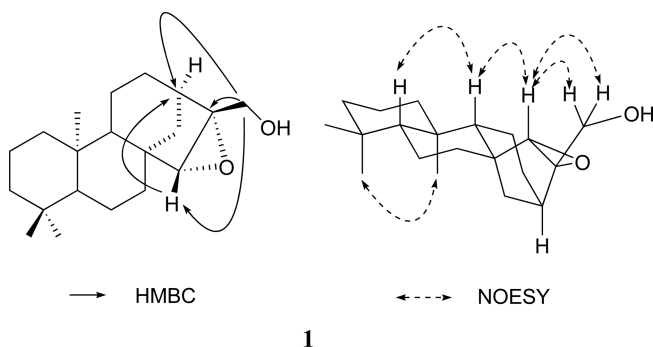


Figure 2. HMBC and NOESY correlations of **1**.

(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*-15 α ,16 α -epoxy-kauran-17-ol and named fritillarinol A.

Compound **2** was obtained as a white amorphous powder. The molecular formula was determined to be $\text{C}_{22}\text{H}_{38}\text{O}_2$ from the $[\text{M} + \text{Na}]^+$ peak at m/z 357.2771 (calcd. for $\text{C}_{22}\text{H}_{38}\text{O}_2\text{Na}$: 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 \times 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 \times 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).

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

16R-17-dimethoxy-ent-kaurane (2): Amorphous white powder. mp 155-156 °C; $[\alpha]_D^{25}$ -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 $[\text{M} + \text{Na}]^+$ (100); HR-FAB-MS $m/z = 357.2771$ $[\text{M} + \text{Na}]^+$ (calcd for: 357.2770); ^1H NMR (CDCl_3 , 500 MHz): see Table 1; ^{13}C NMR (CDCl_3 , 125 MHz): see Table 1.

Acknowledgments. This work was supported by grant no. 012172KDA989 from the Korea Food & Drug Administration in Korea. The authors would like to thank Mr. Do Kyun Kim, Dr. Eun Jung Bang, and Dr. Jung Ju Seo at the Korea Basic Science Institute for the NMR and MS spectral measurements.

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

References

1. Lee, T. B. *Coloured Flora of Korea*; Hyang-Mun Publishing Co.: Seoul, 2003; p 711.
2. Choi, Y. K. *The Characteristic and Use of Herbs*; Jeollabuk-do Agricultural Research and Extension Services: Jinan, 2004; p 308.
3. Atta, U. R.; Akhtar, M. N.; Choudhary, M. I.; Tsuda, Y.; Yasin, A.; Sener, B.; Parvez, M. *Nat. Prod. Res.* **2005**, *19*, 13.
4. Jianxing, Z.; Aina, L.; Rensheng, X. *Phytochemistry* **1993**, *33*, 946.
5. Li, Y.; Xu, C.; Zhang, Q.; Liu, J. Y.; Tan, R. X. *J. Ethnopharmacol.* **2005**, *98*, 329.
6. Kim, K. H.; Jin, M. R.; Choi, S. Z.; Son, M. W.; Lee, K. R. *Heterocycles* **2008**, *75*, 1447.
7. Kim, K. H.; Choi, J. W.; Choi, S. U.; Seo, E. K.; Lee, K. R. *Bull. Korean Chem. Soc.* **2010**, *31*, 1035.
8. Kim, C. S.; Choi, S. U.; Lee, K. R. *Planta Med.* **2012**, *78*, 485.
9. Aljancic, I.; Macura, N.; Juranic, N.; Andjelkovic, S.; Randjelovic, N.; Milosavljevic, S. *Phytochemistry* **1996**, *43*, 169.
10. Pacheco, A. G.; Oliveira, P. M.; Pilo-Veloso, D.; Alcantara, A. F. C. *Molecules* **2009**, *14*, 1245.
11. Kitajima, J.; Komori, T.; Kawasaki, T. *Chem. Pharm. Bull.* **1982**, *30*, 3912.
12. Piovano, M.; Gambaro, V.; Chamy, M. C.; Garbarino, J. A.; Nicoletti, M.; Guilhem, J.; Pascard, C. *Phytochemistry* **1988**, *27*, 1145.
13. Herz, W.; Kulanthaivel, P.; Watanabe, K. *Phytochemistry* **1983**, *22*, 2021.
14. Miyashita, H.; Nishida, M.; Okawa, M.; Nohhara, T.; Yoshimitsu, H. *Chem. Pharm. Bull.* **2010**, *58*, 765.