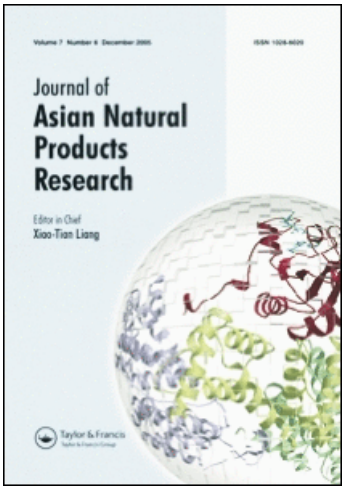

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Structures of Two New Diterpenoid Dimers from Bulbs of *Fritillaria ebeiensis*

Ji-Zhou Wu; Han-Li Ruan[a]; Chun-Lan Zeng[a]; Hua-An Cheng[a]; Fang Zhang[a]; Qin-Shi Zhao[b]; Han-Dong Sun[b]; Tetsuro Fujita[c]
[a] Faculty of Pharmaceutical Sciences, Tongji Medical University, Wuhan, China [b] Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China [c] Faculty of Pharmaceutical Sciences, Setsunan University, Osaka, Japan

To cite this Article Wu, Ji-Zhou , Ruan, Han-Li , Zeng, Chun-Lan , Cheng, Hua-An , Zhang, Fang , Zhao, Qin-Shi , Sun, Han-Dong and Fujita, Tetsuro(1999) 'Structures of Two New Diterpenoid Dimers from Bulbs of *Fritillaria ebeiensis*', Journal of Asian Natural Products Research, 1: 4, 251 — 257
To link to this Article: DOI: 10.1080/10286029908039873
URL: http://dx.doi.org/10.1080/10286029908039873

JANPR, Vol. 1, pp. 251–257
Reprints available directly from the publisher
Photocopying permitted by license only

STRUCTURES OF TWO NEW DITERPENOID DIMERS FROM BULBS OF *FRITILLARIA EBEIENSIS*

JI-ZHOU WU[a,*], HAN-LI RUAN[a], CHUN-LAN ZENG[a], HUA-AN CHENG[a], FANG ZHANG[a], QIN-SHI ZHAO[b], HAN-DONG SUN[b] and TETSURO FUJITA[c]

[a]*Faculty of Pharmaceutical Sciences, Tongji Medical University, Wuhan 430030, China;* [b]*Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China;* [c]*Faculty of Pharmaceutical Sciences, Setsunan University, Osaka 573-0101, Japan*

(Received 2 September 1998; Revised 25 September 1998; In final form 30 October 1998)

Two new *ent*-kauranoid diterpenoid dimers, fritillebin C (**1**) and fritillebin D (**2**), were isolated from the bulbs of *Fritillaria ebeiensis* G.D. Yu and G.Q. Ji. Their structures were determined to be *ent*-16β-hydroxy-kauran-17-yl *ent*-16β-kauran-17-oate (**1**); *ent*-16α-hydroxy-kauran-17-yl *ent*-16β-kauran-17-oate (**2**) by means of spectral analysis and chemical evidence.

Keywords: *Fritillaria ebeiensis*; *ent*-Kaurane; Diterpenoid dimer; Fritillebin C; Fritillebin D

INTRODUCTION

Fritillaria ebeiensis G.D. Yu and G.Q. Ji is a liliaceous plant growing in the northwest district of Hubei Province, China. With regard to the chemical constituents of the bulbs, we have reported the presence of six *C*-nor-*D*-homo steroidal alkaloids, i.e. peimine (verticine), peiminine (verticinone), ebeinine, ebeinone, hupehenidine, ebeiensine [1–3]. As for the non-basic constituents, we isolated seven *ent*-kaurane diterpenoids and determined the structures as *ent*-3β-acetoxy-16β-kauran-17-oic acid (fritillebic acid), *ent*-3β-acetoxy-kauran-16β,17-diol (fritillebinol), *ent*-kauran-16β,17-diol,

* Corresponding author. Tel.: +86-27-83692738. Fax: +86-27-83643050.

ent-kauran-16α,17-diol, *ent*-kaur-15-en-17-ol, and two dimers, including *ent*-16β-hydroxy-kauran-17-yl *ent*-3β-acetoxy-16β-kauran-17-oate (fritillebin A) and *ent*-3β-acetoxy-16β-hydroxy-kauran-17-yl *ent*-3β-acetoxy-16β-kauran-17-oate (fritillebin B) [4,5]. In our continuing studies on the non-basic constituents, two new diterpenoid dimers, fritillebin C (**1**) and fritillebin D (**2**) were isolated from bulbs of *Fritillaria ebeiensis* G.D. Yu and G.Q. Ji. This paper describes the structure elucidation of fritillebin C (**1**) and fritillebin D (**2**).

RESULTS AND DISCUSSION

The powdered bulbs (4.2 kg) were extracted with 95% EtOH. The extract was partitioned between cyclohexane and H_2O. The cyclohexane layer was fractionated by repeated column chromatography to yield fritillebin C (**1**) and fritillebin D (**2**).

Fritillebin C (**1**), colorless needles (EtOAc), m.p. 210–212°C, $[\alpha]_D$-95.1 (*c* 0.25, $CHCl_3$), $C_{40}H_{64}O_3$ (HREI-MS *m/z*: 592.4860; calcd. for $C_{40}H_{64}O_3$: 592.4855) was isolated. Its IR spectrum showed the presence of hydroxyl group at 3450 cm^{-1} and ester carbonyl group at 1680 cm^{-1}. Its EI-MS spectrum showed M^+ at *m/z* 592 and major fragments at *m/z* 574 $[M-H_2O]^+$, 304 $[M-C_{20}H_{33}O]^+$, 287 $[M-C_{20}H_{33}O_2]^+$, 275, 231 and 123. The ^{1}H-NMR spectrum of **1** showed signals due to six tertiary methyl groups at δ 0.80 (6H, s), 0.85 (6H, s), 1.00 (3H, s) and 1.02 (3H, s), and one oxymethylene group at δ 4.17, 4.23 (2H, AB, dd, $J = 11.3$ Hz), which was shifted downfield because of the formation of the ester bond. ^{13}C-NMR spectrum of **1** showed 40 carbon signals, which were assigned to eight quaternary carbons including the ester carbonyl at 177.5 and carbon bearing the hydroxyl and oxygenated methyl group at δ 80.3, seven tertiary carbons, nineteen secondary carbons including an oxymethylene carbon at δ 68.4 and six primary carbons on the basis of the DEPT experiment. As shown in Tables I and II, the ^{1}H- and ^{13}C-NMR signal patterns of **1** were identical to those of fritillebin A (**3**) [4], except for the presence of the acetyl group at C-3 in fritillebin A (**3**). Therefore, **1** was suggested to be a dimer composed of two *ent*-kaurane skeletons. Furthermore, comparison of the spectral data of **1** with those of *ent*-16β-kauran-17-oic acid (**4**) [6] and *ent*-kauran-16β,17-diol (**6**) [4] suggested that **1** is a dimer derived from **4** and **6**.

Alkaline hydrolysis of **1** yielded **4** and **6**, as shown in Fig. 1. Compound **4**, m.p. 215–217°C, literature [6] 215–217°C. $C_{20}H_{32}O_2$ (HREI-MS found: 304.2386; calcd. for $C_{20}H_{32}O_2$: 304.2402). Its EI-MS spectrum showed M^+ at *m/z* 304 and major fragments at *m/z* 289 $[M-Me]^+$, 248, 231 and 123.

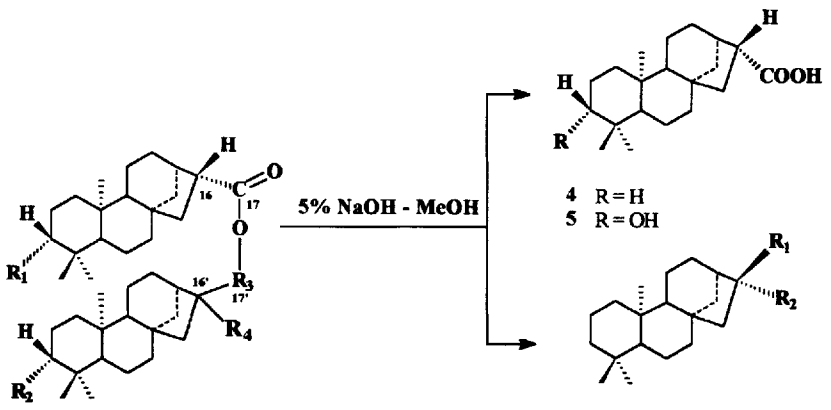

FIGURE 1 Derivatives of fritillebin C (**1**) and fritillebin D (**2**).

Its ^{1}H-NMR showed three methyl signals at δ 0.80, 0.85, 1.00 and 2.65 (1H, m, H-16). All these facts were consistent with the structure of **4** being *ent*-16β-kauran-17-oic acid [6]. But, the present ^{13}C-, ^{1}H-COSY and NOE experimental results of **4** indicate that the literature [6] assigments of the chemical shifts of **4**, δ 42.2 (C-1), 39.4 (C-7), 41.7 (C-14), and 42.7 (C-5), 46.7 (C-9), 57.3 (C-13), 57.4 (C-16) should be revised to 41.7 (C-1), 42.7 (C-7), 39.4 (C-14), and 57.3 (C-5), 57.4 (C-9), 42.7 (C-13), 46.7 (C-16) (Table II). Compound **6**, $C_{20}H_{34}O_2$ (HREI-MS *m/z*: 306.2547, calcd. for $C_{20}H_{34}O_2$: 306.2535) was identified as *ent*-kauran-16β,17-diol (**6**) by direct comparison with the authentic sample.

From the evidences described above, the structure of fritillebin C (**1**) was determined to be *ent*-16β-hydroxy-kauran-17-yl *ent*-16β-kauran-17-oate.

Fritillebin D (**2**), colorless needles (EtOAc), m.p. 231–233°C, $[\alpha]_D$-86.4 (*c* 0.16, $CHCl_3$), $C_{40}H_{64}O_3$ (HREI-MS *m/z*: 592.4898 M^+; calcd. for $C_{40}H_{64}O_3$: 592.4855) was isolated. Its IR spectrum showed the presence of hydroxyl group at 3400 cm^{-1} and ester carbonyl group at 1714 cm^{-1}. Its EI-MS spectrum showed the M^+ at *m/z* 592 and major fragments at *m/z* 574 $[M\text{-}H_2O]^+$, 304 $[M\text{-}C_{20}H_{33}O]^+$, 287 $[M\text{-}C_{20}H_{33}O_2]^+$, 275, 231 and 123. The ^{1}H-NMR spectrum of **2** showed signals due to six tertiary methyl groups at δ 0.80 (6H, s), 0.84 (6H, s), 0.99 (3H, s) and 1.02 (3H, s), and one oxymethylene group at δ 3.89, 4.02 (2H, AB, dd, J = 11.3 Hz), which was shifted downfield because of the formation of the ester bond as shown in

TABLE I ^{1}H-NMR spectral data (400 MHz) of **1**, **2**, **3** and their derivatives

H	1	2	3	4	4*	6	6*	7	7*
tert CH_3 (*s*)	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
	0.80	0.80	0.84	0.85	0.85	0.84	0.84	0.84	0.84
	0.85	0.85	0.84	1.00	1.00	1.02	1.02	1.03	1.02
	0.85	0.85	0.85						
	1.00	0.99	1.01						
	1.02	1.02	1.02						
H–C–OAc (dd, J = 10.9, 6.0 Hz)			4.47						
CH_3–COO (*s*)			2.05						
R CH_2–O–R′	4.23	4.02	4.24						
(AB, dd, J = 11.3 Hz)	4.17	3.89	4.19						
R CH_2OH (AB, dd, J = 11.0 Hz)						3.65	3.65	3.39	3.39
						3.80	3.80	3.47	3.47

* literature values.

TABLE II ^{13}C-NMR spectral data (100 MHz) of **1**, **2**, **3** and their derivatives

C	1	2	3	4	4′	5	C	1	2	3	6	6′	7	7′
1	40.4	40.4	38.3	40.5	42.2	40.0	1′	40.5	40.5	40.3	40.4	40.3	40.5	40.5
2	18.3	18.4	23.6	18.4	19.6	28.8	2′	18.4	18.6	18.2	18.3	18.3	18.7	18.8
3	41.0	41.0	80.9	41.0	43.3	79.7	3′	41.4	41.9	41.8	42.0	42.1	41.9	42.0
4	33.3	33.3	37.7	33.3	34.5	39.9	4′	33.3	33.3	33.2	33.2	33.3	33.3	33.3
5	56.2	56.1	55.2	56.1	42.7	56.6	5′	56.2	56.2	56.1	56.2	56.2	56.2	56.3
6	20.8	20.8	20.4	20.8	22.0	21.6	6′	20.5	20.1	20.4	20.5	20.5	20.1	20.1
7	42.1	42.1	40.8	42.1	39.4	42.3	7′	41.9	42.1	42.0	42.0	42.1	42.1	41.9
8	45.2	45.2	44.9	45.2	46.0	46.1	8′	45.0	43.8	44.6	44.7	44.8	43.6	43.6
9	56.7	56.2	55.7	56.3	46.7	57.4	9′	56.2	57.1	56.6	56.8	56.7	57.1	57.1
10	39.4	39.3	38.9	39.4	40.5	42.3	10′	39.4	39.4	39.4	39.4	39.4	39.4	39.5
11	18.6	18.4	18.5	18.7	19.9	19.5	11′	18.7	18.7	18.6	18.6	18.6	18.8	18.7
12	31.4	31.3	31.2	31.3	32.6	32.4	12′	26.3	26.8	26.4	26.3	26.3	26.8	26.8
13	41.5	41.4	41.3	41.4	57.3	42.8	13′	46.4	41.6	46.2	45.6	45.5	41.0	41.0
14	38.3	38.4	38.1	38.2	41.7	39.0	14′	37.2	38.3	37.1	37.3	37.3	38.3	38.4
15	44.9	45.1	44.8	44.9	46.4	45.8	15′	53.2	52.7	53.1	53.5	53.4	52.9	52.9
16	45.8	45.6	45.6	45.4	57.4	46.9	16′	80.3	78.8	80.2	81.7	81.9	79.8	79.8
17	177.5	177.8	177.4	182.5	184.2	181.1	17′	68.4	71.1	68.4	66.3	66.2	70.0	70.0
18	33.6	33.6	28.3	33.6	34.9	29.0	18′	33.6	33.7	33.6	33.5	33.6	33.6	33.6
19	21.6	21.6	16.6	21.7	22.9	16.2	19′	21.6	21.6	21.5	21.5	21.6	21.6	21.6
20	17.5	17.6	17.5	17.5	18.7	18.1	20′	17.8	17.5	17.7	17.7	17.8	17.6	17.6
OAc		170.9												
		21.3												

3′: literature [6] values (75.5 MHz). **4**, **5**′: literature [4,5] values (75, 100 MHz).

Table I. ^{13}C-NMR spectrum of **2** showed 40 carbon signals (Table II), which were assigned to eight quaternary carbons, including an ester carbonyl carbon at δ 177.8 and a carbon bearing the hydroxyl and oxygenated methyl group at δ 78.8, seven tertiary carbons, nineteen secondary carbons

including an oxymethylene carbon at δ 71.1 and six primary carbons on the basis of the DEPT experiment. The ^{1}H- and ^{13}C-NMR signal patterns of **2** are identical to those of **1**, except for signals due to C-16′ and C-17′ in **2**. Therefore, **2** was also suggested to be a dimer composed of two *ent*-kaurane skeletons. Furthermore, comparison of the ^{1}H- and ^{13}C-NMR chemical shifts of **2** with those of *ent*-16β-kauran-17-oic acid (**4**) and *ent*-kauran-16α,17-diol (**7**) [5] suggested that **2** is a dimer derived from **4** and **7**.

Alkaline hydrolysis of **2** yielded **4** and **7**, as shown in Fig. 1. The structure of **3** was identical to that of *ent*-16β-kauran-17-oic acid (derived from **1**). Compound **5**, $C_{20}H_{34}O_2$ (HREI-MS *m/z*: 306.2564; calcd. for $C_{20}H_{34}O_2$; 306.2535) was identified as *ent*-kauran-16α,17-diol by direct comparison with the authentic sample.

From the evidences described above, the structure of fritillebin D (**2**) was determined to be *ent* 16α-hydroxy-kauran-17-yl *ent*-16β-kauran-17-oate.

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were determined on a X_4 apparatus and are uncorrected. Optical rotations were taken on a WZZ-1 digital polarimeter. IR spectra were taken on Shimadzu IR-460 spectrometer. EI-mass spectra were measured on an Auto-Spe mass spectrometer, 70 eV. ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker AM-400 spectrometer. TLC was performed on silica gel (Qingdao, China) using anisaldehyde reagent for detection. Column chromatography was carried out on silica gel (100–200 mesh, Qingdao, China).

Plant Material

The bulbs of *Fritillaria ebeiensis* G.D. Yu and G.Q. Ji were collected in June from plants cultivated in Suizhou city of Hubei Province, China, and was taxonomically identified by Associate Prof. G.Q. Ji in Hubei Institute of Chinese Materia Medica, China.

Extraction and Isolation

The powdered bulbs (4.2 kg) were extracted with 95% EtOH. The extract (410 g) was partitioned between cyclohexane and H_2O. The cyclohexane extract (51.0 g) was fractionated by column chromatography on silica gel with petroleum ether/EtOAc containing increasing contents of EtOAc.

Combined fractions eluted with petroleum ether/EtOAc (90 : 10, fr-2, 7.7 g) were concentrated and further subjected to column chromatography on silica gel with petroleum ether/EtOAc containing increasing contents of EtOAc, to yield fritillebin C (**1**) (89 mg) and fritillebin D (**2**) (51.6 mg), respectively.

Fritillebin C (**1**) Colorless needles (EtOAc), m.p. 210–212°C, $[\alpha]_D$-95.1 (*c* 0.25, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3450 (OH), 1680 (ester carbonyl); HREI-MS *m/z*: 592.4860 (M^+, calcd. for $C_{40}H_{64}O_3$: 592.4855), 574 [M-H_2O]$^+$, 304 [M-$C_{20}H_{33}O$]$^+$, 287 [M-$C_{20}H_{33}O_2$]$^+$, 275, 231, 123; ^{1}H-NMR ($CDCl_3$) δ: see Table I; ^{13}C-NMR ($CDCl_3$) δ: see Table II.

Alkaline Hydrolysis of **1** Compound **1** (40 mg) was refluxed with 5% NaOH–MeOH (10 ml) for 4 h at 70°C. After usual work-up, the residue was purified by dry silica gel column chromatography (silica gel 15 g, solvent: petroleum ether/EtOAc = 6 : 4) to give *ent*-16β-kauran-17-oic acid (**4**) (15.3 mg) and *ent*-kauran-16β,17-diol (**6**) (18.5 mg).

Ent-16β-kauran-17-oic acid (**4**) Colorless needles (EtOAc), m.p. 215–217°C, $[\alpha]_D$-54.8 (*c* 0.36, $CHCl_3$) (literature m.p. 215–217°C, $[\alpha]_D$-65.7 (*c* 0.70, $CHCl_3$)) [6]; IR (KBr) ν_{max} cm^{-1}: 3400–2500, 1700 (COOH); HREI-MS *m/z*: 304.2386 (M^+, calcd. for $C_{20}H_{32}O_2$: 304.2402), 289 [M-CH_3]$^+$, 248, 231, 123; ^{1}H-NMR ($CDCl_3$) δ: see Table I; ^{13}C-NMR ($CDCl_3$): see Table II.

Ent-kauran-16β,17-diol (**6**) Colorless needles (EtOAc), m.p. 187–188°C, $[\alpha]_D$-38.4 (*c* 0.76, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3390 (OH); HREI-MS *m/z*: 306.2547 [M^+, calcd. for $C_{20}H_{32}O_2$: 306.2535], 288 [M-H_2O]$^+$, 275 [M-CH_2OH]$^+$ (100%), 257 [M-CH_2OH-H_2O]$^+$, 231, 123; ^{1}H-NMR ($CDCl_3$) δ: see Table I; ^{13}C-NMR ($CDCl_3$) δ: see Table II.

Fritillebin D (**2**) Colorless needles (EtOAc), m.p. 231–233°C, $[\alpha]_D$-86.4 (*c* 0.16, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3400 (OH), 1710 (ester carbonyl); HREI-MS *m/z*: 592.4898 (M^+, calcd. for $C_{40}H_{64}O_3$: 592.4855), 574 [M-H_2O]$^+$, 304 [M-$C_{20}H_{33}O$]$^+$, 287 [M-$C_{20}H_{33}O_2$]$^+$, 275, 231, 123. ^{1}H-NMR ($CDCl_3$): see Table I. ^{13}C-NMR ($CDCl_3$) δ: see Table II.

Alkaline Hydrolysis of **2** Compound **2** (20 mg) was refluxed with 5% NaOH–MeOH (5 ml) for 4 h at 70°C. After usual work-up, the residue was purified by dry silica gel column chromatography (silica gel 10 g, solvent: petroleum ether/EtOAc = 6 : 4) to give *ent*-16β-kauran-17-oic acid (**4**) (8.5 mg) and *ent*-kauran-16α,17-diol (**7**) (7 mg). The structure of **3** was identical to that *ent*-16β-kauran-17-oic acid derived from **1**.

Ent-kauran-16α,17-diol (**7**) Colorless needles (EtOAc), m.p. 177–178°C, $[\alpha]_D$-43.2 (*c* 0.12, $CHCl_3$); IR (KBr) cm^{-1}: 3380 (OH); HREI-MS *m/z*: 306.2564 (M^+, calcd. for $C_{20}H_{32}O_2$: 306.2535), 288 [M-H_2O]$^+$, 275

$[M\text{-}CH_2OH]^+$ (100%), 257 $[M\text{-}CH_2OH\text{-}H_2O]^+$, 231, 123; ^1H-NMR ($CDCl_3$) δ: see Table I; ^{13}C-NMR ($CDCl_3$) δ: see Table II.

References

[1] Wu, J.Z., Li, H.B. and Zhu, J.X. *Zhongcaoyao*, 1989, **20**, 533–535; *Chem Abstr.*, 1990, **113**, 20848n.
[2] Wu, J.Z., Kou, B. and Zhang, Y.E. *Acta Univ. Med. Tongji*, 1991, **20**, 89–91; *Chem Abstr.*, 1992, **116**, 170082j.
[3] Wu, J.Z., Sheng, L.R. and Luo, B.S. *Chem. J. Chinese Uni.*, 1992, **13**, 658–601; *Chem Abstr.*, 1993, **118**, 19193s.
[4] Wu, J.Z., Morizane, C., Iida, A., Ueda, S., Zhou, Z.L., Xu, M., Zhang, M., Li, R.M. and Fujita, T. *Chem. Pharm. Bull.*, 1995, **43**, 1448–1453.
[5] Ruan, H.L., Wu, J.Z., Deng, S.K., Ma, J., Cheng, H.A., Zhang, H.J. and Sun, H.D. *Zhongcaoyao*, 1999, **30** (in press).
[6] Bandana, B.M.R., Wimalasiri, W.R. and Macleod, J.K. *Phytochemistry*, 1988, **27**, 869–871.