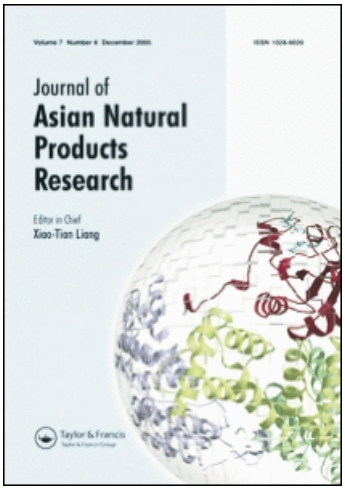

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STRUCTURES OF TWO DITERPENOID DIMERS FROM BULBS OF *FRITILLARIA EBEIENSIS*

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A new *ent*-kaurane diterpenoid dimer, fritillebinide C (**1**) together with one known diterpenoid dimer fritillebinide B (**2**) were isolated from the bulbs of *Fritillaria ebeiensis* G.D. Yu *et* G.Q. Ji. Compound **1** has been determined to be *ent*-3β-acetoxy-kauran-16β, 17-acetal *ent*-16β-kauran-17(*S*)-aldehyde (**1**) by means of spectral analysis and chemical evidence.

Keywords: Fritillaria ebeiensis; Fritillebinide C; Fritillebinide B; *ent*-kaurane; Diterpenoid dimer

INTRODUCTION

Fritillaria ebeiensis G.D. Yu *et* G.Q. Ji is a liliaceous plant growing in the northwest district of Hubei province, China. With regard to the chemical constituents of this bulbs, we reported six C-nor-D-homo steroidal alkaloids, including peimine (verticine), peiminine (verticinone), ebeinine, ebeinone, hupehenidine and ebeiensine [1–3]. As for the non-alkaloid

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constituents, we determined ten diterpenoids having *ent*-kaurane, i.e. *ent*-3β-acetoxy-kauran-17-oic acid (fritillebic acid), *ent*-3β-acetoxy-kauran-16β,17-diol (fritillebinol), *ent*-kauran-16β,17-diol, *ent*-kauran-16α,17-diol, *ent*-15-en-kau-17-ol and five dimers, including *ent*-16β-hydroxy-kauran-17-yl *ent*-3β-acetoxy-16β-kauran-17-oate (fritillebin A), *ent*-3β-acetoxy-16β-hydroxy-kauran-17-yl *ent*-3β-acetoxy-16β-kauran-17-oate (fritillebin B), *ent*-16β-hydroxy-kauran-17-yl *ent*-16β-kauran-17-oate (fritillebin C), *ent*-16β-hydroxy-kauran-17-yl *ent*-16α-kauran-17-oate (fritillebin D) and *ent*-kauran-16β, 17-acetal *ent*-16β-kauran-17(*S*)-aldehyde (fritillebinide A) [4–8]. In our continuing studies on the non-alkaloid constitents, a new acetal diterpenoid dimer fritillebinide C (**1**) was isolated from the bulbs of *Fritillaria ebeiensis* together with one known fritillebinide B (**2**). This paper describes the isolation and structural elucidation of **1** and **2**.

RESULTS AND DISCUSSION

The powdered crude drug (7.2 kg) was extracted with MeOH. The extract (467 g) was partitioned between 2% HCl and EtOAc. The EtOAc extract (58.5 g) was further fractionated by repeated column chromatography to yield fritillebinide C (**1**) and fritillebinide B (**2**).

Compound **1**, colorless needles (EtOAc), m.p. 206–208°C, $[\alpha]_D^{25}$ −69.3 (*c*, 0.21, $CHCl_3$), $C_{42}H_{66}O_4$ (anal. C, 79.06; H, 10.31; calcd. for $C_{42}H_{66}O_4$: C, 79.49; H, 10.41) showed the presence of acetoxyl group at 1730, 1250 cm^{-1} and geminal dimethyl at 1382, 1365 cm^{-1} in the IR spectrum. The FAB-MS contained $[M + Na]^+$ at *m/z* 657 and major fragments at *m/z* 633 $[M - H]^+$, 575 $[M - CH_3COO]^+$, 269 (100%). The ^{1}H-NMR spectrum of **1** showed signals due to six tertiary methyl groups at δ 0.80 (3H, s), 0.85 (9H, s), 0.99 (3H, s) and 1.05 (3H, s), one oxymethylene group at δ 3.77, 3.88 (2H, AB, dd, $J = 7.8$ Hz), one dioxymethine group at δ 4.69 (1H, d, $J = 6.0$ Hz), an acetyl group at δ 2.04 (3H, s) and the proton on a carbon bearing the acetoxyl group at δ 4.46 (1H, dd, $J = 10.4, 6.4$ Hz). As shown in Table 1, the chemical shifts of the protons in **1** were similar to those fritillebinide B (**2**), except for the *J* values of 17-H ($J = 7.8$ Hz) and 17′-H ($J = 6.0$ Hz) which were in accord with those of fritillebinide A (**3**). The ^{13}C-NMR spectrum of **1** showed 42 carbon signals, which were assigned to eight quaternary carbons including an ester carbonyl carbon at δ 170.9, and a carbon bearing an oxygenated methyl group and oxygen atom at δ 88.5, nine tertiary carbons including a carbon bearing the acetoxyl group at δ 80.9 and an acetal carbon at δ 105.8, eighteen secondary carbons including an oxymethylene carbon at

TABLE I ^{1}H-NMR spectral data (600 MHz) of **1** and related compounds

H	**1**	**2**	**3**	**4**	*H*	**1**	**2**	**3**	**5**
H-3	4.46	4.45		4.45					
(dd, *J*)	(10.4, 6.4)	(10.4, 6.1)		(10.4, 6.5)					
H-13 (br)	2.13	2.08	2.13	2.04	H-13′ (br)	2.22	2.18	2.22	2.66
					H-16′ (d, br)	1.96	1.93	1.98	2.55
H-17	3.77	3.78	3.77	3.65	H-17′ (d, *J*)	4.69	4.64	4.69	9.65
(dd, *J*)	3.88	3.93	3.88	3.78		(6.0)	(5.7)	(6.0)	(1.9)
	(7.8)	(8.1)	(7.8)	(11.2)					
H-18 (s)	0.85	0.85	0.84	0.84	H-18′(s)	0.85	0.85	0.85	0.85
H-19 (s)	0.85	0.85	0.80	0.84	H-19′(s)	0.80	0.80	0.80	0.80
H-20 (s)	1.05	1.05	1.01	1.04	H-20′(s)	0.99	0.99	0.99	1.00
OAc (s)	2.04	2.04		2.05					

TABLE II ^{13}C-NMR spectral data (75 MHz) of **1** and related compounds

C	**1**	**2**	**3**	**4**	*C*	**1**	**2**	**3**
1	38.3	38.3	40.4	38.3	1′	40.5	40.5	40.5
2	23.6	23.6	18.6	23.6	2′	18.6	18.6	18.6
3	80.9	80.9	42.1	80.9	3′	42.1	42.1	42.1
4	37.7	37.7	33.3	37.7	4′	33.3	33.3	33.3
5	55.2	55.2	56.2	55.1	5′	56.2	56.2	56.2
6	20.0	20.0	20.4	20.0	6′	20.9	20.8	20.8
7	41.4	41.1	41.6	41.8	7′	41.3	41.3	41.4
8	44.7	44.0	45.0	44.4	8′	44.9	44.8	44.9
9	56.1	56.0	56.4	56.4	9′	56.3	56.3	56.4
10	38.9	38.9	39.4	38.9	10′	39.3	39.3	39.3
11	19.2	18.9	19.1	18.4	11′	18.7	18.7	18.7
12	27.2	26.8	27.3	26.2	12′	31.8	31.8	31.8
13	45.9	43.4	45.4	45.4	13′	38.1	38.2	38.1
14	38.4	38.4	38.5	37.2	14′	38.0	37.9	38.0
15	55.6	55.2	55.9	53.1	15′	43.4	43.5	43.5
16	88.5	88.3	88.6	81.7	16′	44.7	44.6	44.7
17	70.6	70.2	70.7	66.3	17′	105.8	106.4	105.7
18	28.3	28.3	33.6	28.2	18′	33.7	33.7	33.7
19	16.7	16.6	21.6	16.5	19′	21.7	21.6	21.6
20	17.9	17.8	17.8	17.8	20′	17.5	17.5	17.5
OAc	170.9	170.9		171.0				
	21.3	21.3		21.3				

δ 70.6 and seven primary carbons including an acetyl methyl group carbon at δ 21.3 on the basis of the DEPT experiment. As shown in Table II, the chemical shifts of carbons in **1** are identical with those of **2**, except for C-13 and C-17′, which are in accord with those of **3**.

In the HMBC spectrum of **1**, the proton signal of oxymethylene at δ 3.77, 3.88 correlates with the C-13, C-15, C-16 and C-17′, H-13 (δ 2.13) with C-17, H-16′ (δ 1.96) with C-17′, H-17′ (δ 4.69) with C-16′. These correlations

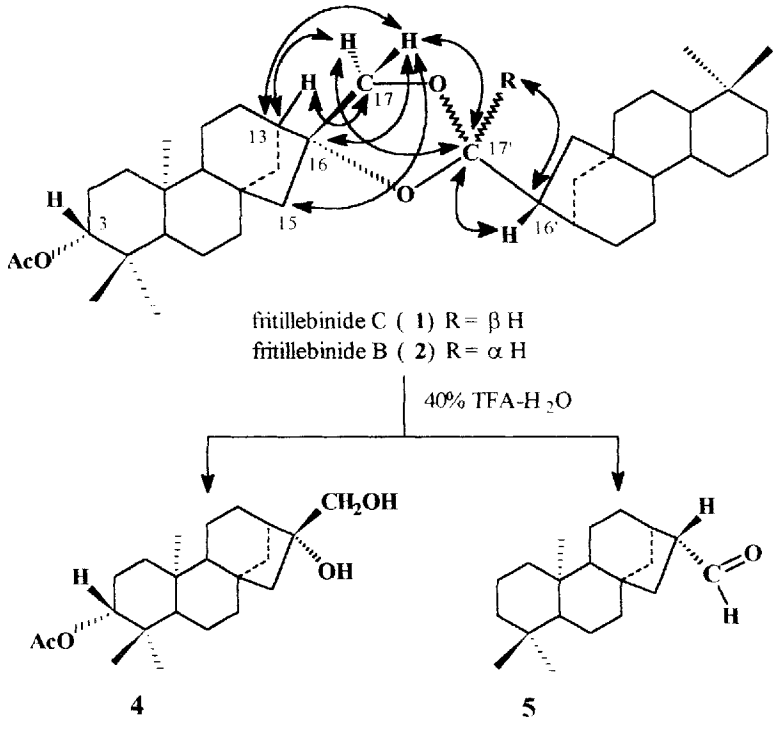

FIGURE 1 HMBC analysis and derivatives of **1** and **2**.

illustrated in Fig. 1 and NMR spectral data suggested that **1** is a acetal dimer composed of two *ent*-kaurane skeletons. Hydrolysis of **1** with 40% TFA–H_2O yielded two compounds derived from *ent*-3β-acetoxy-kauran-16β,17-diol (**4**) and *ent*-16β-kauran-17-al (**5**) (Fig. 1), which are identical to those of **2**. In view of the previous studies on the **2** and **3** [8,9], together with the above results, it was predicted that **1** would be the C-17′ epimer of **2**. Furthermore, it was further confirmed by comparison the NOESY of **1** with **2** and **3** (Fig. 2), because H-17′ exhibited the NOE cross peak with H-17β (δ 3.77), H-17α (δ 3.88) with H-13, but no NOESY between H-17′ and H-17α (δ 3.88), which are in accord with those of **3**. However, the NOESY correlation between H-17′ and H-13, H-17α (δ 3.93) in **2** were exhibited, but no NOESY between H-17′ and H-17β (δ 3.78). Therefore, the absolute configuration of **1** at C-17′ was unequivocally assigned to be *S*.

From the evidence described above, the structure of compound **1**, named fritillebinide C was established as *ent*-3β-acetoxy-kauran-16β,17-acetal *ent*-16β-kauran-17(*S*)-aldehyde.

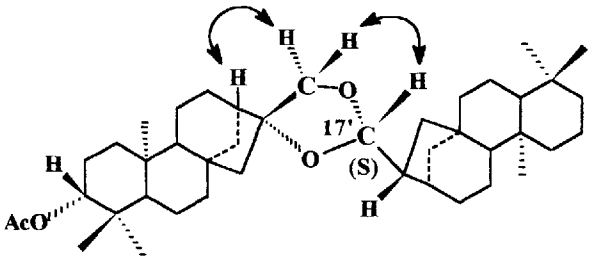

FIGURE 2 Diagnostic NOESY for Fritillebinide C (**1**).

Compound **2**, colorless needles (EtOAc), m.p. 201–203°C, $[\alpha]_D^{25}$ −48.3 (*c* 0.20, $CHCl_3$), $C_{42}H_{66}O_4$ (Anal.: C, 79.15; H, 10.32; calcd. for $C_{42}H_{66}O_4$: C, 79.49; H, 10.41) gave *ent*-3β-acetoxy-kauran-16β,17-diol (**4**) and *ent*-16β-kauran-17-al (**5**) on hydrolysis with 40% TFA–H_2O. The ^{1}H-, ^{13}C-NMR spectral characteristic and spectral data were identical with those of fritillebinide B (**2**), which was established as *ent*-kauran-16β,17-acetal *ent*-16β-kauran-17(*R*)-aldehyde, to report as new diterpenoid isolated from bulbs of *Fritillaria ebeiensis var. purpurea* [9].

EXPERIMENTAL

General Experimental Procedures

Melting Ponits were determined on X_4 apparatus and are uncorrected. Optical rotations were taken on a Jasco DIP-181 Digital polarimeter. IR spectra were recorded on Shimatzu IR-460 spectrometer. MS spectra were measured on a JEOL JMS-HX 110/11A mass spectrometer. NMR spectra were run on a Bruker AM-600 and Bruker AC-300 spectrometer. TLC was performed on silica gel (Qingdao, China) using anisaldehyde reagent for detection. Column chromatography was carried out over silica gel (100–200 mesh, Qingdao, China).

Plant Material

The bulbs of *Fritillaria ebeiensis* G.D. Yu *et* G.Q. Ji were collected in June 1990 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 (7.2 kg) of *Fritillaria ebeiensis* G.D. Yu *et* G.Q. Ji were extracted with MeOH under reflux. The extract (467 g) was partitioned between 2% HCl and EtOAc. The EtOAc extract (58.5 g) was fractionated by column chromatography over silica gel, and eluted with cyclohexane–EtOAc containing increasing contents of EtOAc. Combined fractions eluted with cyclohexane–EtOAc (80 : 20, fr-2) were concentrated and further isolated by dry column chromatography over silica gel and eluted with hexane–EtOAc (90 : 10) to yield fritillebinide C (**1**) (110.1 mg) and fritillebinide B (**2**) (84.7 mg).

Compound **1** Colorless needles (EtOAc), m.p. 206–208°C, $[\alpha]_D^{25}$ −69.3 (*c* 0.21, $CHCl_3$), $C_{42}H_{66}O_4$ (Anal.: C, 79.06; H, 10.31; calcd. for $C_{42}H_{66}O_4$: C, 79.49; H, 10.41.); IR ν_{max}^{KBr} (cm^{-1}): 1730, 1250 (OAc), 1382, 1365 (geminal dimathyl); FAB-MS *m/z* 657 $[M + Na]^+$, 633 $[M - H]^+$, 575 $[M - CH_3COO]^+$, 269 (100%); ^{1}H-NMR ($CDCl_3$) δ: see Table I; ^{13}C-NMR ($CDCl_3$) δ: see Table II.

Compound **2** Colorless needles (EtOAc), m.p. 201–203°C, $[\alpha]_D^{25}$ −48.3 (*c* 0.20, $CHCl_3$), $C_{42}H_{66}O_4$ (Anal.: C, 79.15; H, 10.32; calcd. for $C_{42}H_{66}O_4$: C, 79.49; H, 10.41); IR ν_{max}^{KBr} (cm^{-1}): 1730, 1250 (OAc), 1382, 1365 (geminal dimathyl); FAB-MS *m/z* 657 $[M + Na]^+$, 633 $[M - H]^+$, 575 $[M - CH_3COO]^+$, 269 (100%); ^{1}H-NMR ($CDCl_3$) δ: see Table I; ^{13}C-NMR ($CDCl_3$) δ: see Table II.

Hydrolysis of **1** *and* **2** A solution of each sample (30 mg) in 40% TFA–H_2O [0.5 ml, i.e. $CHCl_3$ 0.2 ml, TFA 0.2 ml, H_2O 0.1 ml] was stirred at room temperature for 15 min. Saturated water solution of $NaHCO_3$ was added to reaction mixture at 0°C to pH = 7 and extracted with $CHCl_3$, the solvent was removed. The residue was purified by column chromatography over silica gel, eluted with hexane–EtOAc (8 : 2), yielded *ent*-3β-acetoxy-kauran-16β,17-diol (**4**) and *ent*-16β-kau-ran-17-al (**5**) for both **1** and **2**. The structures of **4** and **5** were identified by comparison of thier physical properties and spectral data with those reported in the literature [5,9], and were also demonstrated by comparison with authentic samples.

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