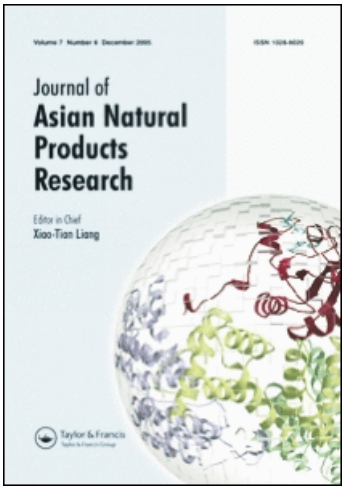

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Two new diterpenoid dimers, fritillebinide D and E, from bulbs of *Fritillaria ebeiensis*

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TWO NEW DITERPENOID DIMERS, FRITILLEBINIDE D AND E, FROM BULBS OF *FRITILLARIA EBEIENSIS*

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Two new *ent*-kaurane diterpenoid dimers, fritillebinide D (**1**) and fritillebinide E (**2**), were isolated from bulbs of *Fritillaria ebeiensis* G.D. Yu *et* G.Q. Ji. Their structures have been determined to be *ent*-3β-acetoxy-kauran-16β, 17-acetal *ent*-3β-acetoxy-16β-kauran-17(*R*)-aldehyde (**1**) and *ent*-3β-acetoxy-kauran-16β,17-acetal *ent*-3β-acetoxy-16β-kauran-17(*S*)-aldehyde (**2**) by means of spectral analysis.

Keywords: *Fritillaria ebeiensis*; *ent*-Kaurane; Diterpenoid dimer; Fritillebinide D; Fritillebinide E

INTRODUCTION

Fritillaria ebeiensis G.D. Yu *et* G.Q. Ji is a liliaceous plant growing in the northwest district of Hubei Province, China. As for the non-basic constituents, we have isolated twelve diterpenoids or diterpenoid dimers including fritillebic acid, fritilleninol, *ent*-kauran-16β, 17-diol, *ent*-kauran-16α, 17-diol, *ent*-kauran-15-en-17-ol, fritillebin A, fritillebin B, fritillebin C, fritillebin D, fritillebinide A, fritillebinide B and fritillebinide C [1–5]. In our continuous studies on the non-basic constituents, two new diterpenoid dimers, fritillebinide D and fritillebinide E were isolated Figs. 1 and 2. Both fritillebinide D and E are acetal dimers composed of two *ent*-kaurane skeletons. Their structures are very similar with that of fritillebinide A, B, C which are also acetal dimers of *ent*-kaurane isolated from the same plant before [3–5]. This paper describes the elucidation of their structures.

RESULTS AND DISCUSSION

The powdered bulbs (4.2 kg) were extracted with 95% EtOH. The extract was partitioned between EtOAc and H_2O. The EtOAc layer was fractionated by repeated column chromatography over silica gel to get fritillebinide D (**1**) and E (**2**).

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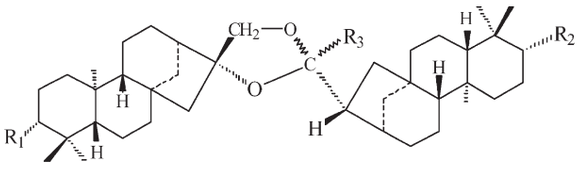

	R_1	R_2	R_3
1 Fritillebinide D	OAc	OAc	αH
2 Fritillebinide E	OAc	OAc	βH
3 Fritillebinide B	OAc	H	αH
4 Fritillebinide C	OAc	H	βH
5 Fritillebinide A	H	H	βH

FIGURE 1 Structures of fritillebinide D, E and related compounds.

Fritillebinide D (**1**), colorless needles (EtOAc), mp 247–249°C, $[\alpha]_D^{20}$ −86.3 (*c* 0.34, $CHCl_3$), molecular formula $C_{44}H_{68}O_6$ (FAB-MS *m/z* 692.5054, M^+; calcd. for $C_{44}H_{68}O_6$, 692.5016). IR (KBr, cm^{-1}): 1730, 1250 (–OAc). Its FAB-MS spectrum showed M^+ at *m/z* 692 and major fragments at *m/z* 574$[M - 2CH_3COO]^+$ and 346$[M - 346]^+$. The ^{1}H-NMR spectrum showed signals due to six tertiary methyl groups at δ 0.84 (6H, s), 0.85 (6H, s), 0.91 (3H, s) and 0.93 (3H, s), one oxymethylene group at δ 4.00, 4.07 (2H, AB, dd, $J = 8.1$), one dioxymethine group δ 4.86 (1H, d, $J = 5.8$ Hz), two acetyl groups at δ 2.06 (6H, s) and two protons on the carbons bearing the acetoxyl group at δ 4.63 (for each 1H, dd, $J = 11.3, 5.8$) (Table I).

The ^{13}C-NMR spectrum of **1** showed 44 carbon signals on the basis of the DEPT experiment, they can be assigned to 9 quaternary carbons including two ester carbonyl carbons at δ 170.6 (overlapped) and a carbon bearing an oxygenated methyl group and an oxygen atom at δ 88.7, 10 tertiary carbons including two carbon bearing the acetoxyl group at δ 80.9 (overlapped) and an acetal carbon at δ 106.7, 17 secondary carbons including an oxymethylene carbon at δ 70.6 and 8 primary carbons including two acetyl group carbons at δ 21.1 (overlapped) (Table II).

As shown in Tables I and II, the ^{1}H- and ^{13}C-NMR signal patterns of **1** were very similar with those of fritillebinide B (**3**) [6] which has recently been reported and characterized by an AB-quartet with doublets centered at δ 3.78, 3.93 ($J = 8.1$ Hz) for H-17 and a doublet at δ 4.64 ($J = 5.7$ Hz) for H-17′, except for the presence of another acetyl group in **1**.This can be confirmed by the fact that the M.W of **1** is 58 amu more than that of **3** and **4**, which can be assigned to be the acetyl group. The above spectral characteristics and data of **1** suggested

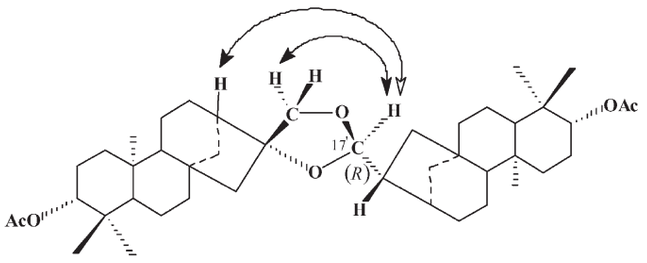

FIGURE 2 Diagnostic NOESY for fritillebinide D (**4**).

TABLE I ^{1}H-NMR spectral data of **1**, **2** and related compounds

H	**1***	**2***	**3**†	**4**†	**5**†	H	**1***	**2***	**3**†	**4**†	**5**†
H-3	4.63	4.63	4.45	4.46		H-3′	4.63	4.63			
(dd, *J*)	(11.3, 5.8)	(11.3, 5.8)	(10.4, 6.1)	(10.4, 6.4)		(dd, *J*)	(11.3, 5.8)	(11.3, 5.8)			
H-13 (br)	2.12	2.16	2.08	2.13	2.13	H-13′ (br)	2.20	2.27	2.18	2.22	2.22
						H-16′(d, br)	1.95	1.99	1.93	1.96	1.98
H-17	4.00	3.94	3.78	3.77	3.77	H-17′(d, *J*)	4.86	4.98	4.64	4.69	4.69
(dd, *J*)	4.07	4.06	3.93	3.88	3.88		(5.8)	(6.1)	(5.7)	(6.0)	(6.0)
	(8.1)	(7.8)	(8.1)	(7.8)	(7.8)						
H-18 (s)	0.85	0.85	0.85	0.85	0.84	H-18′ (s)	0.85	0.85	0.85	0.85	0.85
H-19 (s)	0.84	0.84	0.85	0.85	0.80	H-19′ (s)	0.84	0.84	0.80	0.80	0.80
H-20 (s)	0.91	0.90	1.05	1.05	1.01	H-20′ (s)	0.93	0.91	0.99	0.99	0.99
OAc (s)	2.06	2.06	2.04	2.04		OAc (s)	2.06	2.06			

* 400 MHz, in C_5D_5N.
† 600 MHz in $CDCl_3$.

TABLE II ^{13}C-NMR spectral data of **1**, **2** and related compounds

C	**1***	**2***	**3**†	**4**†	**5**†	C	**1***	**2***	**3**†	**4**†	**5**†
1	38.3	38.2	38.3	38.4	40.4	1′	38.3	38.3	40.5	40.5	40.5
2	24.0	24.0	23.6	23.6	18.6	2′	24.0	24.0	18.6	18.6	18.6
3	80.9	80.8	80.9	80.9	42.1	3′	80.9	80.8	42.1	42.1	42.1
4	38.0	37.9	37.7	37.7	33.3	4′	38.0	37.8	33.3	33.3	33.3
5	55.2	55.2	55.2	55.2	56.2	5′	55.2	55.2	56.2	56.2	56.2
6	20.2	20.2	20.0	20.0	20.4	6′	20.6	20.6	20.8	20.9	20.8
7	41.4	41.4	41.1	41.4	41.6	7′	41.4	41.6	41.3	41.3	41.4
8	44.3	44.9	44.0	44.7	45.0	8′	44.3	44.9	44.8	44.9	44.9
9	56.1	56.1	56.0	56.1	56.4	9′	56.0	56.1	56.3	56.3	56.4
10	38.1	37.9	38.9	38.9	39.4	10′	38.0	37.9	39.3	39.3	39.3
11	19.0	19.2	18.9	19.2	19.1	11′	18.9	18.9	18.7	18.7	18.7
12	26.9	27.4	26.8	27.2	27.3	12′	32.0	32.0	31.8	31.8	31.8
13	45.2	45.9	43.4	45.9	45.4	13′	38.9	38.5	38.2	38.1	38.1
14	38.6	38.8	38.4	38.4	38.5	14′	39.1	39.1	37.9	38.0	38.0
15	55.8	56.1	55.2	55.6	55.9	15′	43.6	43.7	43.5	43.4	43.5
16	88.7	88.9	88.3	88.5	88.6	16′	44.1	45.3	44.6	44.7	44.7
17	70.6	70.8	70.2	70.6	70.7	17′	106.7	106.1	106.4	105.8	105.7
18	28.4	28.3	28.3	28.3	33.6	18′	28.4	28.3	33.7	33.7	33.7
19	16.6	16.6	16.6	16.7	21.6	19′	16.6	16.6	21.6	21.7	21.6
20	17.9	17.9	17.8	17.9	17.8	20′	17.6	17.7	17.5	17.5	17.5
OAc	170.6	170.6	170.9	170.9		OAc	170.6	170.6			
	21.1	21.1	21.3	21.3			21.1	21.1			

* 100 MHz, C_5D_5N.
† 100 MHz, $CDCl_3$.

that compound **1** was an acetal dimer composed of two *ent*-kaurane skeletons, each of which has an acetyl group. In view of the previous studies on the acetal diterpenoid dimers from the bulbs of *Fritillaria ebeiensis* [3–5], together with the above results, it was predicted that one of two acetoxyl groups in **1** must be located at C-3, and the other acetoxyl group must be placed at C-3′.The proposed position is further supported by the following facts. The ^{1}H-NMR spectrum of compound **1** showed overlapped signals at δ 0.84 (6H, s, 19-CH_3, 19′-$_3$), δ 0.85 (6H, s, 18-CH_3, 18′-CH_3) and at δ 4.63 (2H, dd, H-3, H-3′), which were different from those of **3** [δ 0.85 (9H, s, H-18, H-19, H-18′, three signals overlapped), δ 0.80 (3H, s, H-19′), δ 4.45 (1H, dd, H-3)]; Similarly, in the ^{13}C-NMR spectrum of compound **1**, the signals were overlapped at δ 24.0 (C_2, C_2'), δ 80.9 (C_3, C_3') and δ 38.0 (C_4, C_4'), whereas these signals were not overlapped in compound **3** [δ 23.6 (C_2), δ 80.9 (C_3), δ 37.7 (C_4), δ 18.6 (C_2') δ 42.1 (C_3') δ 33.3 (C_4')]. Furthermore, the coupling constant (J = 11.3, 5.8 Hz) of H-3 with H-2 and H-3′ with H-2′ indicates that H-3 and H-3′ were axial, thus both acetoxyl groups were equatorial.

In accordance with studies on the configuration of fritillebinide A (**5**), fritillebinide B (**3**) and fritillebinide C (**4**) at C-17′ previously reported [3–6], together with the above results, it was predicted that compound **1** would be the 17′-αH. Comparison of the J value of H-17 and H-17′ of **1** with those of **3** and **4** [6] showed that the J value of H-17 in **1** (δ 4.00, 4.07, J = 8.1 Hz) was identical with that of **3** (δ 3.78, 3.93, J = 8.1 Hz), but different from that of **4** (δ 3.77, 3.88, J = 7.8 Hz); Meanwhile, the J value of H-17′ in **1** (δ 4.86, J = 5.8 Hz) was also identical with that of **3** (δ 4.64, J = 5.7 Hz) and different from **4** (δ 4.69, J = 6.0 Hz). Furthermore, the NOEsy correlation between H-17′ and H-13, H-17α (δ 4.07) was observed, but there was no NOEsy correlation between H-17′ and H-17β (δ 4.00). All these evidences show that the configuration of $C_{17'}$ of **1** is identical with **3** and different from **4**, which can be assigned to be *R*.

From the evidences described above, the structure of **1** is established as *ent*-3β-acetoxy-kauran-16β,17-acetal *ent*-3β-acetoxy-16β-kauran-17(*R*)-aldehyde.

Fritillebinide E (**2**), colorless needles (EtOAc), mp 247–248°C, $[\alpha]_D^{20}$ −57.6 (*c* 0.29, $CHCl_3$), molecular formula $C_{44}H_{68}O_6$ (FAB-MS *m/z* 692.5051, M^+; calcd. For $C_{44}H_{68}O_6$, 692.5016). IR(KBr, cm^{-1}): 1730, 1250 (–OAc). Its FAB-MS spectrum showed M^+ at *m/z* 692 and major fragments at *m/z* 632 $[M - CH_3COOH]^+$, 572$[M - 2CH_3COOH]^+$. Its ^{1}H-NMR spectrum showed signals due to 6 tertiary methyl groups at δ 0.84 (6H, s), 0.85 (6H, s), 0.90 (3H, s) and 0.91 (3H, s), one oxymethylene group at δ 3.94, 4.06 (2H, AB, dd, $J = 7.8$ Hz), one dioxymethine group at δ 4.98 (1H, d, $J = 6.1$ Hz), two acetyl groups at δ 2.06 (6H, s) and two carbons bearing the acetoxyl group at δ 4.63 (for each 1H, dd, $J = 11.3$, 5.8 Hz) (Table I).

The ^{13}C-NMR spectrum of **2** showed 44 carbon signals. From the DEPT experiment, they can be assigned to 9 quaternary carbons including 2 ester carbonyl carbons at δ 170.6 and a carbon bearing the oxygenated methyl group and an oxygen atom at δ 88.9, 10 tertiary carbons including two carbons bearing the acetoxyl group at δ 80.8 (two signals are overlapped) and an acetal carbon at δ 106.1, 17 secondary carbons including an oxymethylene carbon at δ 70.8 and 8 primary carbons including two acetyl group carbons at δ 21.1.(Table II)

The formulas of **1** and **2** were the same, and their IR spectral characteristic were very similar, their R_f values on TLC were very close but different. This suggested that **2** was most probably an epimer of **1**.

The ^{1}H-NMR spectrum of **2** also showed the presence of one oxymethylene group, one dioxymethine group and two protons on the carbons bearing the acetyl groups. The ^{13}C-NMR spectrum of **2** was also very similar with that of **1**, both having 44 carbons, and the same number of primary, secondary, tertiary and quaternary carbons. Thus compound **2** is also an acetal dimer composed of two *ent*-kaurane skeletons.

The *J* value of H-17 in **2** (δ 3.94, 4.06, $J = 7.8$ Hz) was different from that of **1** (δ 4.00, 4.07, $J = 8.1$ Hz); Meanwhile, the *J* value of H-17′ in **2** (δ 4.98, $J = 6.1$ Hz) was also different from that of **1** (δ 4.86, $J = 5.8$ Hz). In the ^{13}C-NMR spectrum, the $C_{17'}$ chemical shift of **2** was δ 106.1, which was δ 106.7 in **1**. These differences of **1** and **2** were very similar with those of **3** and **4**. We have proved that the configuration of $C_{17'}$ of **1** was *R*, so the configuration of $C_{17'}$ of its epimer **2** was most probably ***S***.

The configuration of $C_{17'}$ of **2** can be further confirmed by the NOESY spectrum (Fig. 3), the NOESY cross peak of H-17′ with H-17β (δ 3.94), H-17α (δ 4.06) with H-13 was observed in **2**, but there was no NOESY between H-17′ and H-17α (δ 4.06). These were in accord with those of **4** and **5** [3,5] but different from that of **1**. Therefore, the absolute configuration of **2** at C-17′ was unequivocally assigned to be *S*.

From the evidences described above, the structure of **2** is established as *ent*-3β-acetoxy-kauran-16β,17-acetal *ent*-3β-acetoxy-16β-kauran-17(*S*)-aldehyde, which is an epimer of **1**.

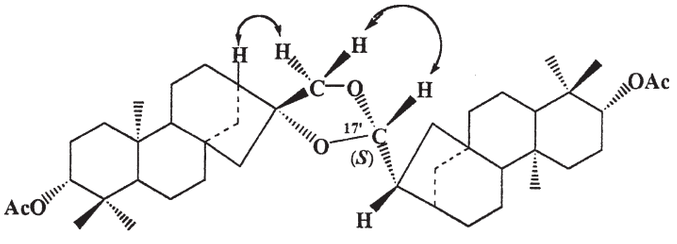

FIGURE 3 Diagnostic NOESY for fritillebinide E (**2**).

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points are uncorrected. Optical rotations were taken on WZZ-1 polarimeter. IR spectra were obtained on IR-460 spectrometer. ^{1}H-NMR and ^{13}C-NMR data were recorded on Bruker-400 spectrometer. HREI-MS was measured on Autospec mass spectrometer. Thin layer chromatography was performed on silica gel (Qingdao, China) using anisaldehyde reagent as detector. Column chromatography was carried out on silica gel (100–200 mesh, Qingdao, China).

Plant Material

The bulbs of *Fritillaria ebeiensis* G. D. Yu *et* G. Q. Ji were purchased in Suizhou city of Hubei province and were identified by Associate Prof. G.Q. Ji in Hubei Institute of Chinese Materia Medica, China.

EXTRACTION AND ISOLATION

The powdered bulbs (4.2 kg) of *Fritillaria ebeiensis* were extracted with 95% EtOH under reflux. The extract (410 g) was partitioned between EtOAc and H_2O. The EtOAc extract (51.0 g) was fractionated by repeated column chromatography over silica gel, and eluted with petroleum ether/EtOAc containing increasing contents of EtOAc, to get fritillebinide D (**1**) (66.9 mg) and fritillebinide E (**2**) (86.4 mg).

Fritillebinide D (1). Colorless needles (EtOAc), mp247–249°C, $[\alpha]_D^{20}$ −86.3 (*c* 0.34, $CHCl_3$).MF: $C_{44}H_{68}O_6$ (FAB-MS *m/z* 692.5054, M^+; calcd. for $C_{44}H_{68}O_6$, 692.5016). FAB-MS *m/z* 692.5054, M^+, 574[M − $2CH_3COO]^+$, 346[M − 346]$^+$. IR(KBr, cm^{-1}): 1730,1250 (–OAc), 1382, 1365 (geminal dimethyl). ^{1}H-NMR (C_5D_5N, 400 MHz) δ: Table I. ^{13}C-NMR (C_5D_5N, 100 MHz) δ: see Table II.

Fritillebinide E (2). Colorless needles (EtOAc), mp247–248°C, $[\alpha]_D^{20}$ − 57.6 (*c* 0.29, $CHCl_3$). MF: $C_{44}H_{68}O_6$ (FAB-MS *m/z* 692.5051, M^+; calcd. for $C_{44}H_{68}O_6$, 692.5016). FAB-MS *m/z* 692.5051, $[M]^+$, 632 [M − $CH_3COOH]^+$, 572 [M − $2CH_3COOH]^+$. IR (KBr, cm^{-1}): 1730, 1250 (–OAc), 1382, 1365 (geminal dimethyl). ^{1}H-NMR (C_5D_5N, 400 MHz) δ: see Table I. ^{13}C-NMR (C_5D_5N, 100 MHz) δ: see Table II.

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