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### Structural Elucidations of Two *Ent*-Kaurane Dimers from Bulbs of *Fritillaria ebeiensis* var. *purpurea*

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## STRUCTURAL ELUCIDATIONS OF TWO ENT-KAURANE DIMERS FROM BULBS OF *FRITILLARIA EBEIENSIS* VAR. *PURPUREA*

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A novel *ent*-kaurane diterpenoid dimer, fritillebinide B (**1**) together with one known diterpenoid dimer fritillebinide A (**2**) were isolated from the bulbs of *Fritillaria ebeiensis* var. *purpurea* G.D. Yu et P. Li. Compound **1** has been established to be *ent*-3 $\beta$ -acetoxy-kauran-16 $\beta$ ,17-acetal *ent*-16 $\beta$ -kauran-17(*R*)-aldehyde (**1**) by means of spectral analysis and chemical evidence.

**Keywords:** *Fritillaria ebeiensis* var. *purpurea*; Fritillebinide B; Fritillebinide A; *ent*-Kaurane; Diterpenoid dimer

### INTRODUCTION

*Fritillaria ebeiensis* var. *purpurea* G.D. Yu et P. Li is a variety of *Fritillaria ebeiensis* G.D. Yu et G.Q. Ji growing in the northwest district of Hubei province, China. With regard to the chemical constituents of this bulb, we have reported six C-nor-D-homo-steroidal alkaloids, including peimine (verticine), peiminine (verticinone), ebeinine, ebeinone, ebeiensinc and ziebeimine [1-3]. As for the non-alkaloid constituents, we isolated four *ent*-kaurane diterpenoids, including *ent*-3 $\beta$ -acetoxy-kauran-16 $\beta$ ,17-diol (fritillebinol),

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*ent*-kauran-16 $\beta$ -hydroxy-17-chloride (fritillaziebinol), *ent*-kauran-3,3,16 $\beta$ , 17-triol, *ent*-kauran-16 $\beta$ ,17-diol [4]. In our continuing studies on the non-alkaloid constituents, a noveol acetal diterpenoid dimer fritillebinide B (**1**) was isolated from the bulbs of *Fritillaria ebeiensis* var. *purpurea*, together with one known fritillebinide A (**2**). This paper describes the isolation and structural elucidation of **1** and **2**.

## RESULTS AND DISCUSSION

A 95% ethanolic extract from powdered bulbs of *Fritillaria ebeiensis* var. *purpurea* was partitioned between H<sub>2</sub>O and EtOAc. The EtOAc extract was further purified by repeated column chromatography over silica gel to yield a new acetal diterpenoid dimer fritillebinide B (**1**), together with one known acetal dimer fritillebinide A (**2**).

Compound **1**, colorless needles (EtOAc), m.p. 201–203°C,  $[\alpha]_D^{25}$  –48.3 (*c* 0.20, CHCl<sub>3</sub>), C<sub>42</sub>H<sub>66</sub>O<sub>4</sub> (Anal. C, 79.15; H, 10.32, *calcd.* for C<sub>42</sub>H<sub>66</sub>O<sub>4</sub>: C, 79.49; H, 10.41) showed the presence of acetoxy group at 1730, 1250 cm<sup>-1</sup> and geminal dimethyl at 1382, 1365 cm<sup>-1</sup> in its IR spectrum. The FAB-MS contained the ion [M + Na]<sup>+</sup> at *m/z* 657 and major fragments at *m/z* 633 [M – H]<sup>+</sup>, 575 [M – CH<sub>3</sub>COO]<sup>+</sup>, 269 (100%). The <sup>1</sup>H-NMR spectrum of **1** shown in Table I showed signals for six tertiary methyl groups at  $\delta$  0.80 (3H, s), 0.85 (9H, s), 0.99 (3H, s) and 1.05 (3H, s), one oxymethylene group at  $\delta$  3.78, 3.93 (2H, AB, dd, *J* = 8.1 Hz), one dioxymethine group at  $\delta$  4.64 (1H, d, *J* = 5.7 Hz), an acetyl methyl group at  $\delta$  2.04 (3H, s) and the proton on carbon bearing the acetoxy group at  $\delta$  4.45 (1H, dd, *J* = 10.4, 6.1 Hz). The <sup>13</sup>C-NMR spectrum of **1** showed 42 carbon signals, which were assigned to eight quaternary carbons including an ester carbonyl

TABLE I <sup>1</sup>H-NMR spectral data (600 MHz) of **1**, **2** and related compounds

<i>H</i>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<i>H</i>	<b>1</b>	<b>2</b>	<b>5</b>
H-3 (dd, <i>J</i> )	4.45 (10.4, 6.1)	4.45 (10.4, 6.5)						
H-13 (br)	2.08	2.13	2.04	2.02	H-13' (br)	2.18	2.22	2.66
					H-16' (d, br)	1.93	1.98	2.55
H-17 (dd, <i>J</i> )	3.78 3.93 (8.1)	3.77 3.88 (7.8)	3.65 3.78 (11.2)	3.63 3.75 (11.0)	H-17' (d, <i>J</i> )	4.64 (5.7)	4.69 (6.0)	9.65 (1.9)
H-18 (s)	0.85	0.84	0.84	0.84	H-18' (s)	0.85	0.85	0.85
H-19 (s)	0.85	0.80	0.84	0.80	H-19' (s)	0.80	0.80	0.80
H-20 (s)	1.05	1.01	1.04	1.02	H-20' (s)	0.99	0.99	1.00
OAc (s)	2.04	2.05						

carbon at  $\delta$  170.9 and a carbon bearing oxygenated methyl group and an oxygen atom at  $\delta$  88.3, nine tertiary carbons including one carbon bearing an acetoxy group at  $\delta$  80.9 and one acetal carbon at  $\delta$  106.4, 18 secondary carbons including an oxymethylene carbon at  $\delta$  70.2 and seven primary carbons including an acetyl methyl group carbon at  $\delta$  21.3 on the basis of the DEPT experiment, shown in Table II.

The NMR spectral data and molecular formula suggested that compound **1** was a dimer with two *ent*-kaurane skeletons. In the HMBC spectrum of **1**, the proton signals of oxymethylene at  $\delta$  3.78, 3.93 correlates with the C-13, C-15, C-16 and C-17', H-13 ( $\delta$  2.08) with C-17, H-16' ( $\delta$  1.93) with C-17', H-17' ( $\delta$  4.64) with C-16', illustrated in Fig. 1. These correlations and  $J$  values ( $J=5.7$  and  $8.1$  Hz) of 17'-H and 17-H in **1** indicated that **1** is also an acetal dimer composed of two *ent*-kaurane skeletons. Hydrolysis of **1** with 40% TFA-H<sub>2</sub>O yielded **3** and **5**, shown in Fig. 2. Compound **3**, colorless needles (EtOAc), m.p. 163–164°C,  $[\alpha]_D^{20}$  -112.5 ( $c$  0.34, MeOH), C<sub>22</sub>H<sub>36</sub>O<sub>4</sub> (HREI-MS  $m/z$  364.2613, M<sup>+</sup>; *calcd.* for C<sub>22</sub>H<sub>36</sub>O<sub>4</sub>, 364.2612) was identified as *ent*-3 $\beta$ -acetoxy-kauran-16 $\beta$ ,17-diol by direct comparison with the authentic sample, which was also isolated from bulbs of *Fritillaria ebeiensis* [5]. Compound **5**, colorless gum, C<sub>20</sub>H<sub>32</sub>O (HREI-MS  $m/z$  288.2458, M<sup>+</sup>; *calcd.* for C<sub>20</sub>H<sub>32</sub>O 288.2453) was confirmed by

TABLE II <sup>13</sup>C-NMR spectral data (75 MHz) of **1**, **2** and related compounds

C	1	2	3	4	C	1	2
1	38.3	40.4	38.3	40.4	1'	40.5	40.5
2	23.6	18.6	23.6	18.3	2'	18.6	18.6
3	80.9	42.1	80.9	42.0	3'	42.1	42.1
4	37.7	33.3	37.7	33.2	4'	33.3	33.3
5	55.2	56.2	55.1	56.2	5'	56.2	56.2
6	20.0	20.4	20.0	20.5	6'	20.8	20.8
7	41.1	41.6	41.8	42.0	7'	41.3	41.4
8	44.0	45.0	44.4	44.7	8'	44.8	44.9
9	56.0	56.4	56.4	56.8	9'	56.3	56.4
10	38.9	39.4	38.9	39.4	10'	39.3	39.3
11	18.9	19.1	18.4	18.6	11'	18.7	18.7
12	26.8	27.3	26.2	26.3	12'	31.8	31.8
13	43.4	45.4	45.4	45.6	13'	38.2	38.1
14	38.4	38.5	37.2	37.3	14'	37.9	38.0
15	55.2	55.9	53.1	53.5	15'	43.5	43.5
16	88.3	88.6	81.7	81.7	16'	44.6	44.7
17	70.2	70.7	66.3	66.3	17'	106.4	105.7
18	28.3	33.6	28.2	33.5	18'	33.7	33.7
19	16.6	21.6	16.5	21.5	19'	21.6	21.6
20	17.8	17.8	17.8	17.7	20'	17.5	17.5
OAc	170.9	171.0					
	21.3	21.3					

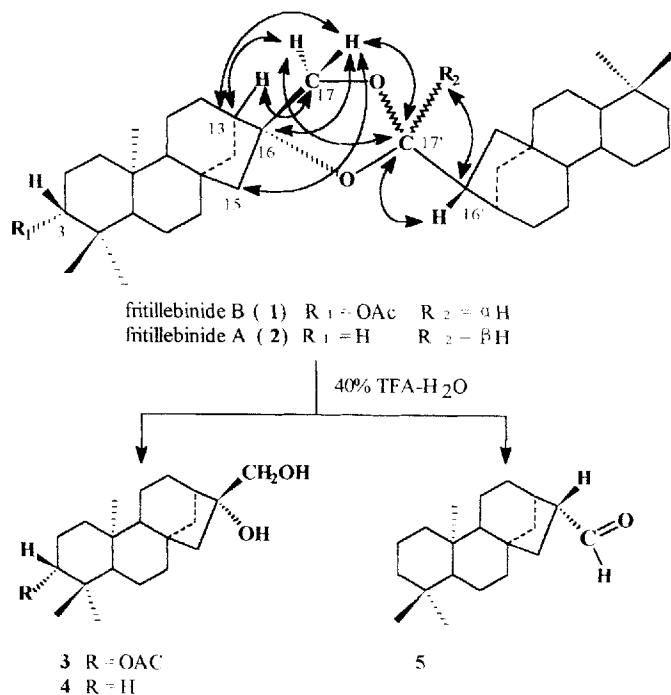


FIGURE 1 HMBC analysis and derivatives of **1** and **2**.

comparison of spectral properties with those of *ent*-16 $\beta$ -kauran-17-al, derived from fritillebinide A (**1**) [6]. As shown in Fig. 2, in compound **1**, the NOESY between H-17' and H-13, H-17 $\alpha$  ( $\delta$  3.93) were observed, but no NOESY between H-17' and H-17 $\beta$  ( $\delta$  3.78), which are different from NOESY results of fritillebinide A [6]. Therefore, the absolute configuration of **1** at C-17' was unequivocally determined to be *R*.

From the evidence described above, the structure of compound **1**, named fritillebinide B was established as *ent*-3 $\beta$ -acetoxy-kauran-16 $\beta$ ,17-acetal *ent*-16 $\beta$ -kauran-17(*R*)-aldehyde. Compound **2**, colorless needles (EtOAc), m.p. 199–201°C,  $[\alpha]_D^{22} -76.8$  (*c* 0.99, CHCl<sub>3</sub>), C<sub>40</sub>H<sub>64</sub>O<sub>2</sub> (Anal. C, 83.21; H, 11.25. *calcd.* for C<sub>40</sub>H<sub>64</sub>O<sub>2</sub>: C, 83.33; H, 11.11) gave *ent*-kauran-16 $\beta$ , 17-diol (**4**) and *ent*-16 $\beta$ -kauran-17-al (**5**) on hydrolysis with 40% TFA-H<sub>2</sub>O. The <sup>1</sup>H- and <sup>13</sup>C-NMR spectral characteristic data were identical with those of fritillebinide A (**2**) which was established as *ent*-kauran-16 $\beta$ ,17-acetal *ent*-16 $\beta$ -kauran-17(*S*)-aldehyde, to report as new *ent*-kaurane diterpenoid which was also isolated from bulbs of *Fritillaria chei*ensis [6].

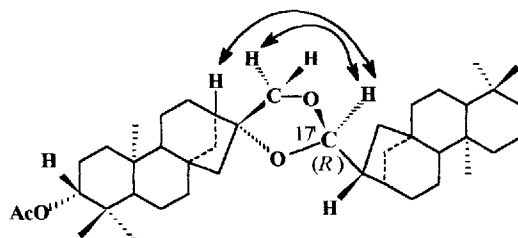


FIGURE 2 Diagnostic NOESY for Fritillebinide B (1).

## EXPERIMENTAL

### General Experimental Procedures

Melting points were determined on X<sub>4</sub> apparatus and are uncorrected. Optical rotations were taken on a Jasco DIP-181 Digital polarimeter. IR spectra were taken on Shimadzu 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 on silica gel (100–200 mesh, Qingdao, China).

### Plant Material

The bulbs of *Fritillaria ebeiensis* var. *purpurea* G.D. Yu *et* P. Li were collected in June, 1991 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 (5 kg) of *Fritillaria ebeiensis* var. *purpurea* were extracted with 95% EtOH (55 L) under reflux. The extract (1240 g) was partitioned between EtOAc and H<sub>2</sub>O. The EtOAc extract (97 g) was fractionated by column chromatography over silica gel, and eluted with petroleum ether–EtOA containing increasing contents of EtOAc. Combined fractions eluted with petroleum ether–EtOA (90 : 10, fr-2) were concentrated and further isolated over silica gel and eluted with petroleum ether–EtOA (95 : 5), to yield fritillebinide B (1) (450 mg) and fritillebinide A (2) (150 mg).

Compound **1**, colorless needles (EtOAc), m.p. 201–203°C,  $[\alpha]_D^{25} -48.3$  ( $c$  0.20, CHCl<sub>3</sub>), C<sub>42</sub>H<sub>66</sub>O<sub>4</sub> (Anal. C, 79.15; H, 10.32, *calcd.* for C<sub>42</sub>H<sub>66</sub>O<sub>4</sub>: C, 79.49; H, 10.41). IR  $\nu_{\max}^{\text{KBr}}$  (cm<sup>-1</sup>): 1730, 1250 (OAc), 1382, 1365 (geminal dimethyl); FAB-MS  $m/z$  657 [M + Na]<sup>+</sup>, 633 [M – H]<sup>-</sup>, 575 [M – CH<sub>3</sub>COO]<sup>+</sup>, 269 (100%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>) $\delta$ : see Table I; <sup>13</sup>C-NMR (CDCl<sub>3</sub>) $\delta$ : see Table II.

Compound **2**, colorless needles (EtOAc), m.p. 199–201°C,  $[\alpha]_D^{22} -76.8$  ( $c$  0.99, CHCl<sub>3</sub>), C<sub>40</sub>H<sub>64</sub>O<sub>2</sub> (Anal. C, 83.21; H, 11.25, *calcd.* for C<sub>40</sub>H<sub>64</sub>O<sub>2</sub>: C, 83.33; H, 11.11); IR  $\nu_{\max}^{\text{KBr}}$  (cm<sup>-1</sup>): 1382, 1365 (geminal dimethyl), 1092, 1020; FAB-MS  $m/z$  577 [M + H]<sup>+</sup>, 289 [M + H – 288]<sup>-</sup>, 271 [289 – H<sub>2</sub>O]<sup>+</sup> (100%); <sup>1</sup>H-NMR (CDCl<sub>3</sub>) $\delta$ : see Table I; <sup>13</sup>C-NMR (CDCl<sub>3</sub>) $\delta$ : see Table II.

Hydrolysis of **1** and **2**. A solution of each sample (25 mg) in 40% TFA-H<sub>2</sub>O [2.5 ml, i.e. CHCl<sub>3</sub> 1.0 ml, TFA 1.0 ml, H<sub>2</sub>O 0.5 ml] was stirred at room temperature for 15 min. Saturated water solution of NaHCO<sub>3</sub> was added to reaction mixture at 0°C to pH = 7 and extracted with CHCl<sub>3</sub>, the solvent was removed. The residue was purified by column chromatography over silica gel, eluted with hexane–EtOAc (8:2), yielded *ent*-16 $\beta$ -kauran-17-al (**5**) for both **1** and **2**. However, the alcoholic fraction on hydrolysis of **1** was different from **2**, which was identified as *ent*-3 $\beta$ -acetoxy-kauran-16 $\beta$ ,17-diol (**3**) for **1** and *ent*-kauran-16 $\beta$ ,17-diol (**4**) for **2**. The structures of compounds **3–5** were identified by comparison of their physical properties and spectral data with those reported in the literature [5,6], and were also demonstrated by comparison with authentic samples.

## References

- [1] J.Z. Wu, X.P. Pan, M.A. Lou, X.S. Wang and D.K. Ling. *Acta Pharm. Sinica*, 1989, **24**, 600–605.
- [2] J.Z. Wu, B. Kou and Y.E. Zhang. *Acta Universitatis Medicinæ Tongji*, 1991, **20**, 89–92.
- [3] J.Z. Wu, L.R. Sheng and B.S. Luo. *Chemical Journal of Chinese Universities*, 1992, **13**, 658–661.
- [4] J.Z. Wu, Y.P. Wen, H.L. Ruan, N.H. Yao, Q.S. Zhao, H.D. Sun, C. Morizane, A. Iida and T. Fujita. *Zhongcaoyao* 1999, **30**, 803–806.
- [5] H.L. Ruan, J.Z. Wu, S.K. Deng, J. Ma, G.L. Liu, H.A. Cheng, H.D. Sun and H.J.H.D. Zhang. *Zhongcaoyao* 1999, **30**, 404–407.
- [6] J.Z. Wu, H.L. Ruan, N.H. Yao, H.D. Sun, Q.S. Zhao, C. Morizane, A. Iida and T. Fujita. *Acta Pharm. Sinica*, 1999, **34**, 581–588.