Structures of Three New Diterpenoids, Fritillebic Acid and Fritillebins A and B, from Bulbs of *Fritillaria ebeiensis* G. D. Yu *et* G. Q. Ji

Ji-Zhou Wu,[a] Chikako Morizane,[a] Akira Iida,[a] Shin-ichi Ueda,[a] Zuo-Ling Zhou,[b] Min Xu,[b] Min Zhang,[b] Rei-Min Li,[b] and Tetsuro Fujita*,[a]

Faculty of Pharmaceutical Sciences, Kyoto University,[a] Sakyo-ku, Kyoto 606–01, Japan and Faculty of Phamaceutical Sciences, Tongji Medical University,[b] Hangkong Rd., Wuhan 430030, China.
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A new diterpenoid, fritillebic acid (1) and four new diterpenoid dimers, fritillebins A (2) and B (3), and fritillebinides A and B, were isolated from a crude drug, bulbs of *Fritillaria ebeiensis* G. D. Yu *et* G. Q. Ji. The structures of compounds 1—3 were determined to be *ent*-3β-acetoxy-16β-kauran-17-oic acid (1), *ent*-16β-hydroxykauran-17-yl *ent*-3β-acetoxy-16β-kauran-17-oate (2), and *ent*-3β-acetoxy-16β-hydroxykauran-17-yl *ent*-3β-acetoxy-16β-kauran-17-oate (3), respectively, on the basis of spectroscopic and chemical evidence.

Key words *Fritillaria ebeiensis*; Liliaceae; fritillebic acid; fritillebin A; fritillebin B; *ent*-kaurane; diterpenoid; diterpenoid dimer; modified Mosher's method

Fritillaria ebeiensis G. D. Yu *et* G. Q. Ji is a liliaceous plant growing in the northwest district of Hubei Province, China. This plant, easily cultivable in the district, has a high alkaloid content and shows conspicuous antitussive and expectorant effects.[1] The bulbs, treated with lime and then bleached in the sun, are called Ebeibeimu, and are commercially available as a substitute crude drug for the principal Chinese traditional medicine Beimu.

With regard to the chemical constituents of the crude drug, we have reported the presence of alkaloids,[2] including peimine (verticine), peiminine (verticinone), hupehenidine, ebeinine, ebeinone and ebeiensine. In our continuing studies on the chemical constituents, five novel diterpenoids, designated as fritillebic acid (**1**), fritillebins A (**2**) and B (**3**), and fritillebinides A and B,[3] were isolated as non basic constituents from the crude drug, bulbs of *F. ebeiensis*. This paper describes the structure elucidation of compounds **1**—**3**.

Results and Discussion

The powdered crude drug (7.2 kg), produced in Suizhou City in Hubei Province, China, was extracted with MeOH. The extract was partitioned between 2% HCl and EtOAc. The EtOAc layer was fractionated by repeated column chromatography to yield **1**—**3** and fritillebinides A and B (Fig. 1).

Fritillebic acid (**1**), $C_{22}H_{34}O_4$ (HREI-MS *m/z*: 362.2460, M^+), mp 235—237 °C, $[\alpha]_D^{28}$ −60.6° (*c*=1.0, $CHCl_3$), showed IR absorptions due to an acetoxyl group at 1735 and 1250 cm^{-1} and a carboxyl group at 1700 cm^{-1}. The electron impact-mass spectra (EI-MS) showed the M^+ ion peak (weak) at *m/z* 362 and the base peak at *m/z* 302 ($M-CH_3COOH)^+$. Its ^{1}H-NMR spectrum indicated the presence of three tertiary methyl groups at δ 0.84, 0.85

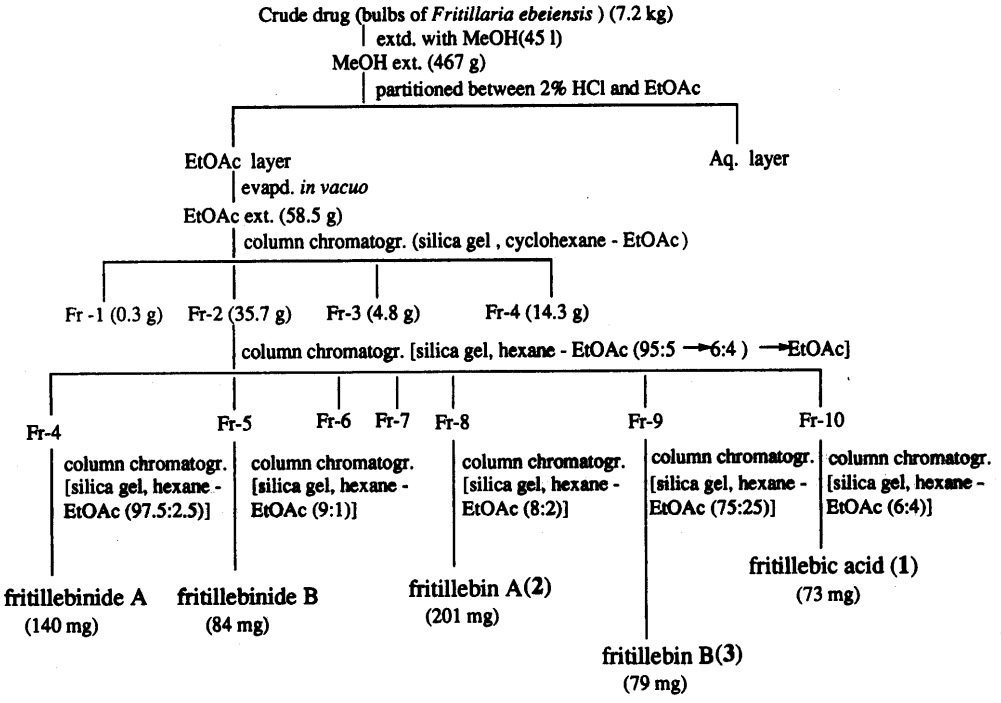

Fig. 1. Isolation Procedure of Diterpenoids from *Fritillaria ebeiensis*

* To whom correspondence should be addressed.

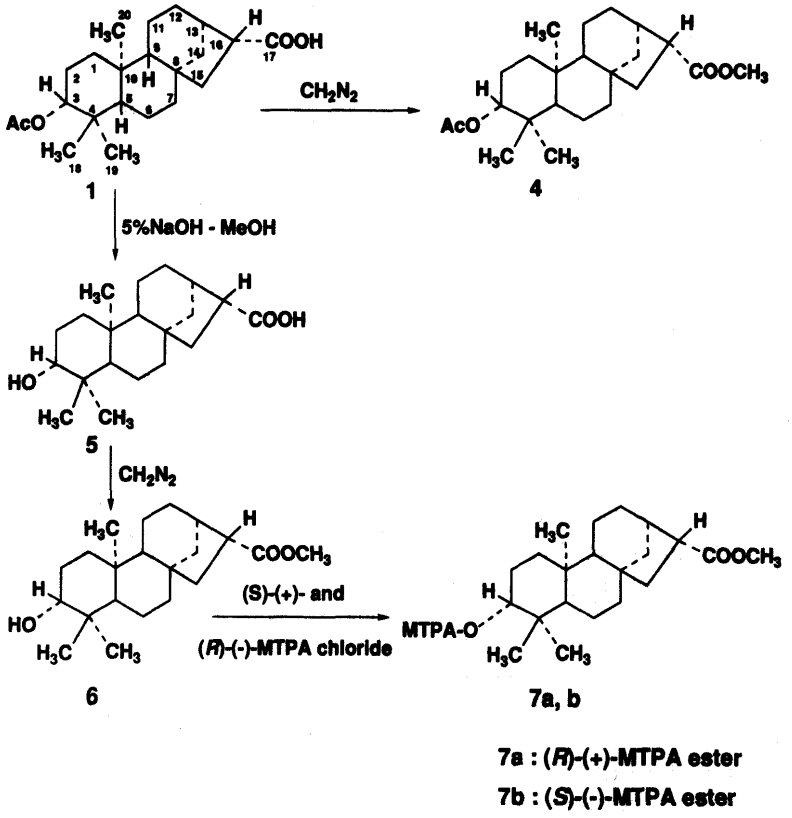

Fig. 2. Derivatives of Fritillebic Acid (1)

and 1.02, an acetyl group at δ2.05, and a proton on a carbon bearing the acetoxyl group at δ4.47 (dd, J=11.8, 5.5 Hz). The ^{13}C-NMR data of **1** revealed the presence of 22 carbon atoms, which were assigned as five quaternary carbons including an acetyl carbonyl at δ171.0 and a carboxyl carbon at δ183.3, five tertiary carbons including a carbon bearing the acetoxyl group at δ81.0, eight secondary carbons and four primary carbons by means of a distortionless enhancement by polarization transfer (DEPT) experiment. Methylation and alkaline hydrolysis of **1** yielded the monomethyl ester (**4**) and the alcohol (**5**), respectively, as shown in Fig. 2.

In view of the previous studies on the diterpenoids in *Fritillaria* sp.[4] together with the above results and the degree of unsaturation (=6) calculated from the molecular formula, it was predicted that **1** would be an *ent*-kaurane-type diterpenoic acid bearing an acetoxyl group. All ^{1}H- and ^{13}C-NMR signals of **1** were assigned by two-dimensional homo- and heteronuclear NMR experiments, as summarized in Table 1.

In the long-range ^{13}C–^{1}H correlation spectroscopy (COSY) spectrum of **1**, the proton signal (δ4.47) on the carbon bearing the acetoxyl group correlates with the carbon signals of the geminal dimethyls at δ16.6 (C-19), 28.4 (C-18) and the acetyl carbonyl at δ171.0. The proton signals of the geminal dimethyl at δ0.84 (H-19) and 0.85 (H-18) also correlate with the signal at δ81.0 ppm, assignable to the carbon bearing the acetoxyl group. These correlations, illustrated in Fig. 3, show that the acetoxyl group is located at C-3. Furthermore, the coupling constant (J=11.8, 5.5 Hz) between H-3 and H-2 indicates that H-3 is axial, and thus the acetoxyl group is equatorial.

Some *ent*-kaurane-type diterpenoids possess a carboxyl group at C-16, C-18, or C-19.[5] Since **1** has the geminal dimethyls at C-18 and 19, the carboxyl group was suggested to be located at C-16. A signal at δ2.64, which was assignable to H-16 based on the chemical shift, correlated with one of H-15 in the double quantum filtered

Table 1. ^{1}H- (600 MHz) and ^{13}C-NMR (75 MHz) Spectral Data of Fritillebic Acid (**1**) in $CDCl_3$

H	δ, mult., J (Hz)	C	δ, mult.
1α	1.82 dt (13.4, 7.0)	1	38.4 t
1β	0.96 m	2	23.7 t
2α	1.62 m	3	81.0 d
2β	1.67 m	4	37.8 s
3	4.47 dd (11.8, 5.5)	5	55.2 d
5	0.83 d (2.1)	6	20.4 t
6α	1.37 dddd	7	40.8 t
	(12.1, 12.1, 4.2, 4.2)	8	44.9 s
6β	1.57 m	9	55.7 d
7α	1.55 m	10	38.9 s
7β	1.53 m	11	18.5 t
9	0.98 d (4.6)	12	31.2 t
11α	1.59 m	13	41.4 d
11β	1.59 m	14	38.0 t
12α	1.52 d (3.7)	15	44.5 t
12β	1.50 d (3.3)	16	45.4 d
13	2.54 br s	17	183.3 s
14α	1.88 d (11.7)	18	28.4 q
14β	1.21 dd (11.6, 4.9)	19	16.6 q
15α	1.72 dd (13.5, 5.9)	20	17.5 q
15β	1.64 m	OAc	171.0 s
16	2.64 dd (8.9, 6.1)		21.3 q
17	11.53 br s		
18	0.85 s		
19	0.84 s		
20	1.02 s		
OAc	2.05 s		

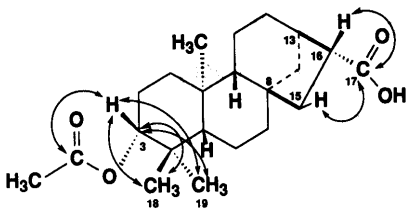

Fig. 3. Long-Range $^{13}C,^{1}H$-Connectivities of Fritillebic Acid (**1**) Observed in $CDCl_3$

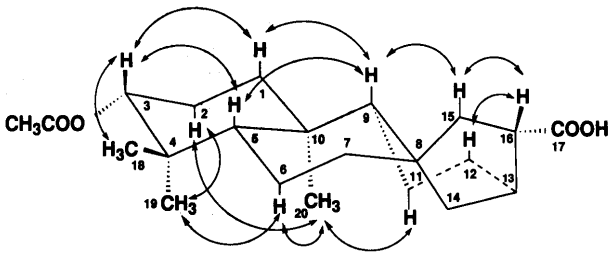

Fig. 4. Diagnostic NOEs of Fritillebic Acid (**1**) Observed in $CDCl_3$

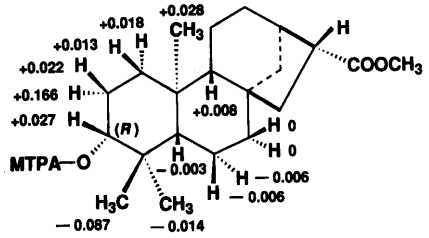

Fig. 5. $\Delta\delta$ ($\delta_S-\delta_R$) Values for the A and B Rings of the (*R*)- and (*S*)-MTPA Derivativs (**7a**, **7b**) ($CDCl_3$, 600 MHz)

(DQF)-COSY spectrum. However, neither of H-15 was correlated with any other protons except for H-16, indicating that the adjacent carbon (C-8) is quaternary. In addition, H-16 exhibited no correlation with H-13, presumably because the dihedral angle is near to 90°, as is generally observed in the kaurane-type diterpenoids. Furthermore, the carboxyl carbon signal at δ 183.3 showed a long-range coupling with H-15 and H-16 (Fig. 3). These results demonstrated that the carboxyl group is located at C-16.

Starting from the signals identified already, all the proton and carbon signals were assigned by through-bond and through-space coupling without any conflict, to give the relative configuration of **1** shown in Fig. 4. H-20 exhibited an nuclear Overhauser effect (NOE) cross-peak with H-11, but not with H-14. This indicates that ring C takes a boat-form conformation.

The absolute configuration of **1** was determined by the modified Mosher's method.[6] Methylation of **5** gave the methyl ester (**6**), which was transformed to (*R*)-(+)- and (*S*)-(−)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) esters (**7a** and **7b**). The ^{1}H-NMR signals of the two derivatives were assigned by $^{1}H,^{1}H$-COSY. The $\Delta\delta$ ($\delta_S-\delta_R$ ppm) values of the individual protons of rings A and B are shown in Fig. 5. The systematic arrangement of positive and negative $\Delta\delta$ values showed that the configuration at C-3 is *R*. Furthermore, the carboxyl group was found to occupy the C-16α position, because H-16 had NOE cross peaks with H-12β and H-15β. Therefore, the absolute configuration of fritillebic acid (**1**) was established to be *ent*-3β-acetoxy-16β-kauran-17-oic acid.

Fritillebin A (**2**), $C_{42}H_{66}O_5$, mp 237—239 °C, $[\alpha]_D^{28}$ −61.7° (c=1.3, $CHCl_3$), showed the presence of a hydroxyl group (3430 cm^{-1}), an acetoxyl group (1730, 1250 cm^{-1}) and an ester carbonyl group (1680 cm^{-1}) in its IR spectrum. The FAB-MS showed the $[M+Na]^+$ ion peak at m/z 673 and a strong fragment peak at m/z 633 $[(M+H)-H_2O]^+$. The ^{1}H-NMR spectrum of **2** showed signals due to six tertiary methyl groups at δ 0.80 (3H, s), 0.84 (6H, s), 0.85 (3H, s), 1.01 (3H, s) and 1.02 (3H, s), one oxymethylene group at δ 4.19 and 4.24 (2H, AB, dd, J=11.3 Hz), which was shifted downfield because of the formation of the ester bond, one acetyl group at δ 2.05 and one oxymethine group at δ 4.47 (dd, J=10.9, 6.0 Hz). As shown in Table 2, the chemical shifts of **2** at δ 0.80, 0.84 and 1.01 were similar to those of the tertiary methyl groups in the molecule of *ent*-kaurane-16β,17-diol (**8**)[4a] isolated from *F. thunbergii* MIQ. and the chemical shifts of the signals at δ 0.84, 0.85 and 1.02 were in accord with those of the tertiary methyl groups in the molecule of fritillebic acid (**1**). The ^{13}C-NMR spectrum of **2** showed

Table 2. ^{1}H-NMR Spectral Data (600 MHz) of Fritillebin A (**2**), Fritillebin B (**3**) and Their Derivatives

H	**2**[a]	**3**[a]	**4**[a]	**5**[b]	**6**[a]	**8**[a]	**9**[b]
tert-CH_3 (*S*)	0.80 0.84 0.84 0.85 1.01 1.02	0.84 0.84 0.85 0.85 1.02 1.04	0.84 0.85 1.05	0.73 0.97 1.00	0.77 0.97 1.00	0.80 0.84 1.02	0.77 0.97 1.05
H–C(–OAC)(OH) (dd, J[c])	4.47	4.47 4.47	4.47	3.08	3.19		3.51
–C–$OOCH_3$ (*S*)			3.65		3.65		
CH_3–COO– (*S*)	2.05	2.04 2.04	2.05				
–C(R)–CH_2–O–C=O	4.24 4.19	4.23 4.18					
(AB, dd, J=11.3) –CH_2OH						3.65 3.80	3.60 3.70
(AB, dd, J=11.0)							

a) In $CDCl_3$. *b*) In CD_3OD. *c*) **2**, J=10.9, 6.0; **3**, J=10.8, 5.6; **4**, J=11.5, 5.8; **5**, J=11.4, 5.8; **6**, J=11.3, 5.9; **9**, J=11.4, 6.0.

42 carbon signals (Table 3), which were assigned to nine quaternary carbons including two ester carbonyl carbons at δ 170.9 and 177.4, and a carbon bearing the hydroxyl and oxygenated methyl groups at δ 80.2, eight tertiary carbons including a carbon bearing the acetoxyl group at δ 80.9, eighteen secondary carbons including an oxymethylene carbon at δ 68.4 and seven primary carbons including an acetyl methyl carbon at δ 21.3 on the basis of a DEPT experiment. Comparison of the ^{1}H- and ^{13}C-NMR chemical shifts of **2** with those of **1** and **8** suggested that **2** is a dimer derived from **1** and **8**. Alkaline hydrolysis of **2** yielded two compounds, **6** derived from **1** and **8** (Fig. 6).

The structure of **8**, $C_{20}H_{34}O_2$ (HREI-MS m/z: 306.2554, M^+) was identified as *ent*-kaurane-16β,17-diol[4a] by di-

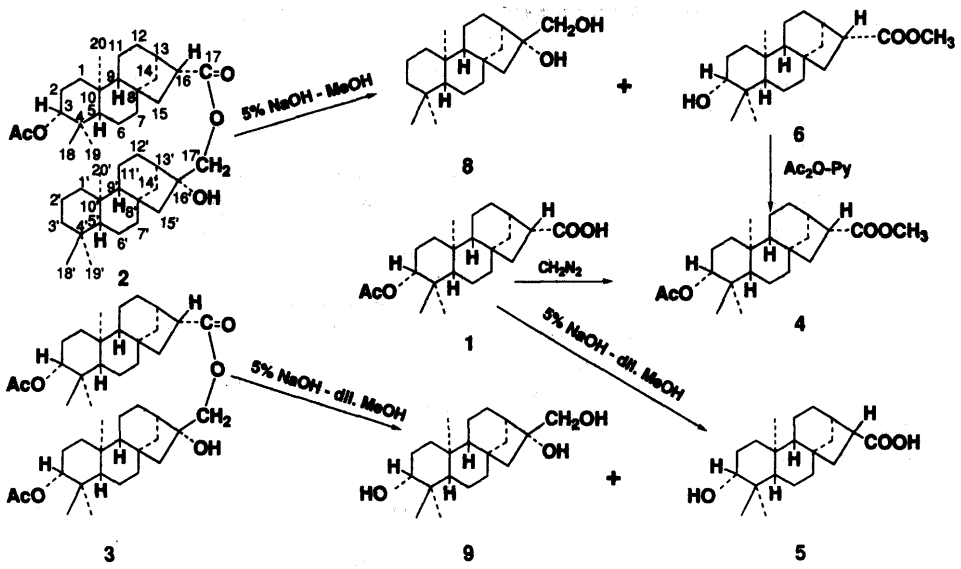

Fig. 6. Derivatives of Fritillebin A (2) and Fritillebin B (3)

Table 3. ^{13}C-NMR Spectral Data (75 MHz) of Fritillebin A (2), Fritillebin B (3) and Their Derivatives

C	2[a]	3[a]	5[b]	6[a]	C	2[a]	3[a]	8[a]	8[c]	9[b]
1	38.3	38.4	40.0	38.7	1′	40.3	38.3	40.3	42.0	40.0
2	23.6	23.6	28.0	27.4	2′	18.2	23.7	18.3	18.2	28.0
3	80.9	80.9	79.7	79.0	3′	41.8	80.9	42.1	42.0	79.7
4	37.7	37.8	39.9	38.8	4′	33.2	37.8	33.3	33.4	40.2
5	55.2	55.1	56.6	55.1	5′	56.1	55.2	56.2	56.1	56.5
6	20.4	20.4	21.6	20.5	6′	20.4	20.1	20.5	20.5	21.3
7	40.8	40.9	42.3	40.9	7′	42.0	41.7	42.1	37.2	43.3
8	44.9	44.9	46.1	44.9	8′	44.6	44.6	44.8	44.6	45.6
9	55.7	55.8	57.4	55.9	9′	56.6	56.3	56.7	56.7	58.2
10	38.9	38.9	42.3	39.0	10′	39.4	39.0	39.4	39.4	39.9
11	18.5	18.5	19.5	18.5	11′	18.6	18.3	18.6	18.3	19.4
12	31.2	31.3	32.4	31.3	12′	26.4	26.3	26.3	26.3	27.3
13	41.3	41.4	42.8	41.2	13′	46.2	46.2	45.5	45.5	46.4
14	38.1	38.1	39.0	38.0	14′	37.1	37.0	37.3	40.4	38.2
15	44.8	44.7	45.8	44.7	15′	53.1	52.9	53.4	53.4	53.8
16	45.6	45.6	46.9	45.4	16′	80.2	80.2	81.9	81.6	82.8
17	177.4	177.4	181.1	177.9	17′	68.4	68.4	66.2	66.2	66.9
18	28.3	28.3	29.0	28.4	18′	33.6	28.4	33.6	33.4	28.9
19	16.6	16.6	16.2	15.5	19′	21.5	16.5	21.6	21.5	16.2
20	17.5	17.5	18.1	17.5	20′	17.7	17.8	17.8	17.7	18.4
OAc	170.9	170.9			OAc′		170.9			
	21.3	21.3					21.3			
OCH_3				51.6						

a) In $CDCl_3$. *b*) In CD_3OD. *c*) Literature values in $CDCl_3$.

rect comparison with an authentic sample. The present ^{13}C,^{1}H–COSY and NOE experimental results indicate that the literature assignments of the chemical shifts of **8**, δ 42.0 (C-1), 37.2 (C-7) and 40.4 (C-14), should be revised to δ 40.3 (C-1), 42.1 (C-7) and 37.3 (C-14) (Table 3).

From the evidence described above, the structure of fritillebin A (**2**) was established as *ent*-16β-hydroxykauran-17-yl *ent*-3β-acetoxy-16β-kauran-17-oate.

Fritillebin B (**3**), $C_{44}H_{68}O_7$, mp 243—245 °C, $[\alpha]_D^{28}$ −56.9° (c=0.4, $CDCl_3$), showed the presence of a hydroxyl group (3430 cm^{-1}), an acetoxyl group (1730, 1250 cm^{-1}) and an ester carbonyl group (1680 cm^{-1}) in its IR spectrum. The FAB-MS showed the $(M+Na)^+$ ion peak (weak) at m/z 731 and a strong fragment peak at m/z 691 $[(M+H)-H_2O]^+$. The ^{1}H-NMR spectrum of **3** showed signals due to six tertiary methyl groups at δ 0.84 (6H, s), 0.85 (6H, s), 1.02 (3H, s) and 1.04 (3H, s), one esterified oxymethylene group at δ 4.18, 4.23 (2H, AB, dd, J=11.0 Hz), two acetyl groups at δ 2.04 (6H, s) and two oxymethine groups at δ 4.47 (2H, dd, J=10.8, 5.6 Hz) (Table 2). The ^{13}C-NMR spectrum of **3** showed 44 carbon signals (Table 3), which were assigned to ten quaternary carbons including three ester carbonyl carbons at δ 170.9 (two signals are overlapping) and 177.4 ppm, and a carbon bearing the hydroxyl and oxygenated methyl groups at δ 80.2, nine tertiary carbons including two carbons bearing the acetoxyl group at δ 80.9 (two signals are overlapping), seventeen secondary carbons including an oxymethylene carbon at δ 68.4 and eight primary carbons including two acetyl methyl groups at δ 21.3 (two signals are overlapping)

by means of a DEPT experiment. The ^{1}H- and ^{13}C-NMR signal patterns of **3** are identical to those of **2**, except for the presence of another acetyl group in **3**. Therefore, **3** is suggested to be a dimer composed of two *ent*-kaurane skeletons, each of which has an acetyl group.

Alkaline hydrolysis of **3** with 5% NaOH–MeOH yielded two compounds, **5** (derived from **1**) and **9**, as shown in Fig. 6. The structure of **9**, $C_{20}H_{34}O_3$ (HREI-MS *m/z*: 322.2505, M^+), was identified as *ent*-kaurane-3β,16β,17-triol,[7] which had been isolated from *Croton lacciferus*. The physical and spectral data of **9** were in good agreement with those of *ent*-kauran-3β,16β,17-triol, although its ^{13}C-NMR data have not been reported. Therefore, the structure of fritillebin B (**3**) was elucidated as *ent*-3β-acetoxy-16β-hydroxykauran-17-yl *ent*-3β-acetoxy-16β-kauran-17-oate.

Experimental

Melting points are uncorrected. Optical rotations were taken on a Jasco DIP-181 digital polarimeter. IR spectra were obtained on an Shimadzu IR-435 spectrometer. ^{13}C-NMR data were obtained on a Bruker AC-300 spectrometer. ^{1}H-NMR spectra were recorded on a Bruker AM-600 spectrometer. HREI-MS was measured on a JEOL JMS-HX110/110A mass spectrometer. TLC was performed on silica gel (Kieselgel 60 F_{254}, Merck) using the anisaldehyde reagent for detection. Column chromatography was carried out on Silica gel 60 (70—230 mesh, Merck).

Extraction and Isolation of Diterpenoids The powdered crude drug, bulbs (7.2 kg) of *F. ebeiensis* G. D. Yu. *et* G. Q. Ji, was extracted with MeOH. The extract was partitioned between 2% HCl and EtOAc. The EtOAc layer was fractionated by column chromatography on silica gel with cyclohexane–EtOAc containing increasing contents of EtOAc. Combined fractions eluted with cyclohexane–EtOAc (80 : 20, fr-2) were concentrated and further subjected to column chromatography on silica gel (hexane–EtOAc with increasing content of EtOAc). Each combined fraction, frs. 10, 9, 8, 5 and 4, was concentrated and chromatographed on silica gel with hexane–EtOAc, the mixture ratio of which is shown in Fig. 1, to yield pure fritillebic acid (**1**) (73 mg), fritillebin B (**3**) (79 mg), fritillebin A (**2**) (201 mg), fritillebinide B (84 mg, $C_{42}H_{66}O_4$, mp 193.5—194.5 °C), and fritillebinide A (140 mg, $C_{40}H_{64}O_2$, mp 199—201 °C), respectively.

Fritillebic Acid (1) Colorless flakes (EtOAc), mp 235—237 °C, $[\alpha]_D^{28}$ −60.6° (*c* = 1.0, $CHCl_3$). IR (KBr): 1735, 1250 (OAc), 3400—2500, 1700 (carboxyl C=O) cm^{-1}. HREI-MS *m/z*: 362.2458 (M^+, $C_{22}H_{34}O_4$). EI-MS *m/z*: 362 $(M)^+$, 347 $(M-CH_3)^+$, 302 $[(M-CH_3COOH)^+$, base peak], 287 $(347-CH_3COOH)^+$, 247, 136, 121. *Anal.* Calcd for $C_{22}H_{34}O_4$: C, 72.89; H, 9.45. Found: C, 72.95; H, 9.39. ^{1}H- and ^{13}C-NMR δ: see Table 1.

Fritillebin A (2) Colorless sand (EtOAc), mp 237—239 °C, $[\alpha]_D^{28}$ −61.7° (*c* = 1.3, $CHCl_3$). IR (KBr): 3430 (OH), 1730, 1250 (OAc), 1680 (ester, C=O) cm^{-1}. FAB-MS *m/z*: 673 $(M+Na)^+$, 633 $[(M+H)-H_2O]^+$, 573 $[(M+H)-AcOH-H_2O]^+$, 271 (base peak). *Anal.* Calcd for $C_{42}H_{66}O_5$: C, 77.49; H, 10.22. Found: C, 77.54; H, 10.15. ^{1}H-NMR ($CDCl_3$) δ: see Table 2. ^{13}C-NMR ($CDCl_3$) δ: see Table 3.

Fritillebin B (3) Colorless sand (EtOAc), mp 243—245 °C, $[\alpha]_D^{28}$ −56.9° (*c* = 0.40, $CHCl_3$). IR (KBr): 3430 (OH), 1730, 1250 (OAc), 1680 (ester, C=O) cm^{-1}. FAB-MS *m/z*: 731 $(M+Na)^+$, 691 $[(M+H)-H_2O]^+$, 631 $[(M+H)-AcOH-H_2O]^+$, 269 (base peak). *Anal.* Calcd for $C_{44}H_{68}O_7$: C, 74.54; H, 9.67. Found: C, 74.57; H, 9.60. ^{1}H-NMR ($CDCl_3$) δ: see Table 2. ^{13}C-NMR ($CDCl_3$) δ: see Table 3.

Methyl Ester of 1 Treatment of **1** (2 mg) with CH_2N_2 at room temperature gave *ent*-3β-acetoxy-16β-kauran-17-oic acid methyl ester (**4**), $[\alpha]_D^{28}$ −61.8° (*c* = 0.25, $CHCl_3$). HREI-MS *m/z*: 376.2654 (M^+, $C_{23}H_{36}O_4$). EI-MS *m/z*: 376 $(M)^+$, 316 $[(M-CH_3COOH)^+$, base peak], 301, 261, 136, 121. ^{1}H-NMR δ: see Table 2.

Alkaline Hydrolysis of 1 Compound **1** (20 mg) was refluxed with 5% NaOH–MeOH (5 ml) for 4 h. After usual work-up, the residue was purified by dry silica gel column chromatography (20 g, solvent, *n*-hexane : EtOAc = 6 : 4) to afford *ent*-3β-hydroxy-16β-kauran-17-oic acid (**5**) (12 mg). Colorless needles (MeOH), mp 210—212 °C, $[\alpha]_D^{28}$ −57.6° (*c* = 0.42, MeOH). IR (KBr): 3400 (OH), 1700 (C=O) cm^{-1}. HREI-MS *m/z*: 320.2350 (M^+, $C_{20}H_{32}O_3$). EI-MS *m/z*: 320 $(M)^+$, 302 $[(M-H_2O)^+$, base peak], 287 $(302-CH_3)^+$, 276 $(M-COO)^+$. ^{1}H- and ^{13}C-NMR δ: see Tables 2 and 3.

Methyl Ester of 5 Treatment of **5** (3 mg) with CH_2N_2 at room temperature gave *ent*-3β-hydroxy-16β-kauran-17-oic acid methyl ester (**6**). Colorless needles (EtOAc), mp 125—127 °C, $[\alpha]_D^{28}$ −60.7° (*c* = 0.98, $CHCl_3$). IR (KBr): 3400 (OH), 1720 (C=O) cm^{-1}. HREI-MS *m/z*: 334.2521 (M^+, $C_{21}H_{34}O_3$). EI-MS *m/z*: 334 $(M)^+$, 316 $[(M-H_2O)^+$, base peak], 290, 273, 247, 213, 192, 122. ^{1}H- and ^{13}C-NMR δ: see Tables 2 and 3.

(*S*)- and (*R*)-MTPA Ester of 6 Triethylamine (0.6 μl, 4.35 μmol) and (*S*)-(+)-MTPA chloride (1.1 μl, 60 μmol) were added to a solution of **6** (1 mg, 3 μmol) and dimethylaminopyridine (1.5 mg, 12 μmol) in 0.3 ml of dichloromethane (distilled over P_2O_5) at 0 °C. The mixture was stirred for 5 min at 0 °C and allowed to stand at room temperature for 3 h. 3-(Dimethylamino)propylamine (0.77 μl, 6 μmol) was added to the mixture and after 10 min, the solvent was removed. The residue was purified by preparative TLC [hexane–EtOAc (8 : 2)] to give the (*R*)-MTPA ester (**7a**). The (*S*)-MTPA ester (**7b**) was obtained in the same manner.

Alkaline Hydrolysis of 2 A solution of **2** (40 mg) in 5% NaOH–MeOH (8 ml) was refluxed for 4 h at 70° C. The reaction mixture was neutralized with 1% HCl–MeOH and concentrated *in vacuo*. The residue was purified by dry silica gel column chromatography (silica gel, 15 g, solvent, *n*-hexane : EtOAc = 6 : 4) to give *ent*-3β-hydroxy-16β-kauran-17-oic acid methyl ester (**6**) (17 mg) and *ent*-kaurane-16β,17-diol (**8**) (15 mg). *ent*-Kaurane-16β,17-diol (**8**), colorless needles (MeOH), mp 189—191 °C (lit. 188—189 °C[6]), $[\alpha]_D^{28}$ −38.4° (*c* = 0.76, $CHCl_3$) (lit. −47.0° (*c* = 2.1, $CHCl_3$)[4a]). IR (KBr): 3390 (OH) cm^{-1}. HREI-MS *m/z*: 306.2583 (M^+, $C_{20}H_{34}O_2$). EI-MS *m/z*: 306 (M^+), 288 $(M-H_2O)^+$, 275 $[(M-CH_2OH)^+$, base peak], 257 $(M-CH_2OH-H_2O)^+$, 123. ^{1}H-NMR ($CDCl_3$) δ: see Table 2. ^{13}C-NMR ($CDCl_3$) δ: see Table 3.

Acetylation of *ent*-3β-Hydroxykauran-17-oic Acid Methyl Ester (6) Acetylation of **6** (1 mg) with Ac_2O–pyridine (1 : 1, each 0.2 ml) at room temperature overnight gave the monoacetate **4**.

Alkaline Hydrolysis of 3 A solution of **3** (40 mg) in 5% NaOH–MeOH (10 ml) was refluxed at 70 °C for 4 h. The reaction mixture was poured into water and neutralized with 1% HCl and the solvent was evaporated *in vacuo*. The residue was purified by dry silica gel column chromatography (silica gel, 15 g, solvent, *n*-hexane : EtOAc = 6 : 4) to give *ent*-3β-hydroxykauran-16β-17-oic acid (**5**) (15.5 mg) and *ent*-kaurane-3β,16β,17-triol (**9**) (16.9 mg). *ent*-Kaurane-3β,16β,17-triol (**9**), colorless needles (MeOH), mp 214—216 °C (lit. 217 °C[7]), $[\alpha]_D^{21}$ −39.1° (*c* = 0.47, MeOH) (lit. −47.0° (*c* = 2.1, $CHCl_3$)[7]). IR (KBr): 3400 (OH), 1030 cm^{-1}. HREI-MS *m/z*: 322.2514 (M^+, $C_{20}H_{34}O_3$). EI-MS *m/z*: 322 (M^+), 304 $[(M-H_2O)]$, base peak], 291 $(M-CH_2OH)^+$, 273 $(M-CH_2OH-H_2O)^+$, 255. ^{1}H-NMR (CD_3OD) δ: see Table 2. ^{13}C-NMR (CD_3OD) δ: see Table 3.

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