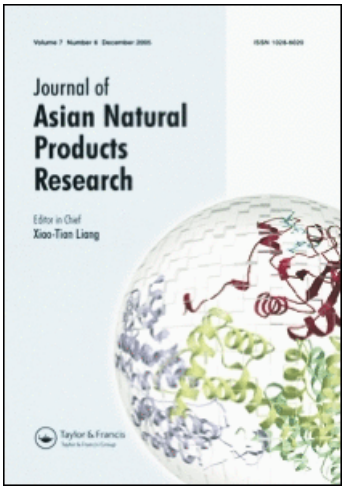

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STRUCTURAL ELUCIDATION OF FRITILLAHUPEHIN FROM BULBS OF *FRITILLARIA HUPEHENSIS* HSIAO *ET* K.C. HSIA

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A novel diterpenoid ester, fritillahupehin (**1**) and three known fatty acids, palmitic acid (**2**), lignoceric acid (**3**) and azelaic acid (**4**), have been isolated from the bulbs of *Fritillaria hupehensis* Hsiao *et* K.C. Hsia. The structure of fritillahupehin has been established to be *ent*-kauran-16β-yl lignocerate by means of spectroscopic and chemical evidence. Compounds **2**–**4** were isolated from *Fritillaria* sp. for the first time.

Keywords: *Fritillaria hupehensis*; Diterpenoid ester; Fritillahupehin

INTRODUCTION

Fritillaria hupehensis Hsiao *et* K.C. Hsia is a liliaceous plant growing in the southwest district of Hubei Province, China. Its bulbs have been recorded in the Pharmacopoeia of the People's Republic of China as a principal Chinese traditional medicine named "Hubeibeimu" [1].

Regarding the chemical constituents of the bulbs, we have reported ten C-nor-D-homo steroidal alkaloids, verticine (peimine), verticinone (peiminine), hupehunine, hupeheninoside, hupehenirine, hupehenizine, hupehenisine, hupehenidine, hupehemonoside and ebeiensine [2–8]. As for the non-basic constituents, we have reported the presence of β-sitosterol and four *ent*-kaurane diterpenoids: *ent*-kauran-16β,17-diol, *ent*-kauran-16α,17-diol, *ent*-16β-hydroxy-kauran-17-yl *ent*-16β-kauran-17-oate (fritillebin C), *ent*-16α-hydroxy-kauran-17-yl *ent*-16β-kauran-17-oate (fritillebin D) [9,10]. In our continuing studies on the non-basic constituents, a novel diterpenoid ester, fritillahupehin (**1**), and three known fatty acids, palmitic acid (**2**), lignoceric acid (**3**) and azelaic acid (**4**), have been isolated. This paper describes the structural elucidation of compound **1**.

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RESULTS AND DISCUSSION

The EtOH extract of *F. hupehensis* was partitioned between water and n-hexane. The n-hexane extract was fractionated by column chromatography to obtain compounds **1**–**4**.

Fritillahupehin (**1**): colorless needles (EtOAc), mp 183–185°C, $[\alpha]_D^{20}$ −78.2 (*c* 0.67, $CHCl_3$), its molecular formula was determined by elemental analysis to be $C_{44}H_{80}O_2$. The IR spectrum suggested the presence of ester carbonyl (1738 cm^{-1}), geminal dimethyl (1382, 1365 cm^{-1}) and $[(CH_2)_n, n > 4]$ (720 cm^{-1}) groups. Its FAB-MS spectrum showed M^+ at 731 (M − H + Gly) and its EI-MS spectrum exhibited major fragments of $M-(CH_2)_n$. The ^1H/NMR spectrum revealed the presence of five methyl groups at δ 0.84 (3H, s, H-18), 0.80 (3H, s, H-19), 1.00 (3H, s, H-20), 1.34 (3H, s, H-17) and 0.88 (3H, t, H-24′), the signals of δ 0.88 (3H, t, H-24′) and 1.26 (36H, m, H-4′–21′) suggested the presence of a long-chain hydrocarbon. The ^{13}C NMR spectrum of **1** showed signals characteristic of an ester composed of *ent*-kaurane diterpenoid and a long-chain fatty acid, which can be assigned to five quaternary carbons, including an ester carbonyl at δ 173.9 and a carbon-bearing the oxygen and methyl group at δ 79.6, three tertiary carbons, thirteen secondary carbons, including nine carbons of *ent*-kaurane diterpenoid, and four carbons of the hydrocarbon chain at δ 29.2–29.7, and five primary carbons, including a terminal methyl group of the hydrocarbon chain at δ 14.3 by a DEPT experiment.

From above results, **1** was predicted to be an ester composed of an *ent*-kaurane diterpenoid and a long-chain fatty acid. Both ^1H and ^{13}C NMR signals of **1** were assigned by two-dimensional homo- and heteronuclear NMR experiments, as shown in Tables I and II.

In the HMBC spectrum of **1**, the fact that the carbon signal of ester carbonyl group at δ 173.9 was only correlated with H-2′ at δ 2.34 indicated that this ester carbonyl group

TABLE I ^1H NMR spectral data of fritillahupehin (**1**), **1a** and fatty acids **2**–**4**

H	*1*	*1a*	*H*	*1*	*1b*	*2*	*3*	*4*
1α	1.76	d (12.8)	2′	2.34 t	2.51	2.52	2.51	2.46
	1.74 d							
1β	0.70	td (13.0, 3.8)	3′	1.64 m	1.78	1.78	1.78	1.71
	0.70 d							
2α	1.48 m		4′		1.37	1.38	1.37	
2β	1.56 m							
3α	1.36 m		$(CH_2)n$	1.26 m	1.26	1.24	1.26	1.29
3β	1.10	td(13.6, 4.4)						
5	0.75	dd (6.3, 1.7)	16′					0.85
	0.73dd							
6α	1.28	dd (13.8, 4.8)	24′		0.88 t	0.85	0.85	
6β	1.52 m							
7α	1.46	dd (13.0, 6.4)						
7β	1.57 m							
9	0.96	d (7.0)						
	0.94d							
11α	1.40 m							
11β	1.60 m							
12α	1.55 m							
12β	1.55 m							
13	1.81	br, s						
	1.80 br, s							
14α	1.94	d (11.8)						
	1.91 d							
14β	1.54 m							
15α	1.56 m							

TABLE II ^{13}C NMR spectral data of fritillahupehin (**1**), **1a** and fatty acids **2**–**4**

C	*1*	*1a*	*C*	*1*	*1b*	*2*	*3*	*4*
1	40.6	40.5	1′	173.9	176.0	176.0	176.0	176.0
2	18.2	18.0	2′	34.1	34.9	34.9	34.9	34.9
3	42.3	42.1	3′	24.9	25.7	25.7	25.7	25.7
4	33.4	33.2	$(CH_2)_n = 18$		18	10	18	3
5	56.5	56.3		29.2–29.7	30.0	30.0	30.0	29.5
6	20.7	20.5	7′					25.7
7	42.3	42.1	8′					34.9
8	45.6	45.4	9′					176.0
9	57.1	57.0	14′			32.2		
10	39.6	39.4	15′			23.0		
11	18.9	18.7	16′			14.3		
12	27.2	27.0	22′	32.0	32.1		32.1	
13	49.3	49.2	23′	22.7	23.0		23.0	
14	37.9	37.8	24′	14.3	14.3		14.3	

must derive from the carboxylic group of fatty acid, another oxygen atom must come from *ent*-kaurane skeleton. Since the proton signal at δ 1.36 (a methyl group) correlated with C-13, C-15 and C-16 at δ 49.3, 58.3 and 79.6, the methyl group at δ 1.36 can most probably be assigned to 17-CH_3.

Alkaline hydrolysis of **1** with 5% NaOH–MeOH yielded two compounds, **1a** and **1b**, as shown in Fig. 1. Compound **1a** colorless needles, mp 216–217°C, $C_{20}H_{34}O$ (HR-EIMS: 290.2598; calcd. for $C_{20}H_{34}O$: 290.2610). The EI-MS spectrum of **1a** showed M^+ at *m/z* 290 and major fragments at *m/z* 272 ($M - H_2O$, 100%), 257 ($M - H_2O - CH_3$), 232 ($M - C_3H_6O$), 217 ($M - C_3H_6O - CH_3$). Its ^1H NMR spectrum revealed signals of four methyl groups at δ 0.83 (3H, s, H-18), 0.78 (3H, s, H-19), 1.00 (3H, s, H-20) and 1.35 (3H, s, H-17). The ^{13}C NMR spectrum of **1a** showed signals of twenty carbons, which were assigned to four quaternary carbons, including a carbon bearing the hydroxyl and methyl group at δ 79.3, three tertiary carbons, nine secondary carbons and four primary carbons by a DEPT experiment. The spectral characteristics of **1a** were identical to those of *ent*-kauran-16β-ol isolated from *Xylopia aethiopica* [11]. Compound **1b**, a colorless powder, mp 69–71°C, $C_{24}H_{48}O_2$ (FAB-MS: *m/z* 367), was identified as lignoceric acid by direct comparison with an authentic sample.

From the evidence described above, the structure of fritillahupehin (**1**) was established as *ent*-kauran-16β-yl lignocerate.

Compound **2**: white hemispheric crystals (EtOAc), mp 51–53°C, $C_{16}H_{32}O_2$ [FAB-MS *m/z* 257 $(M + H)^+$]. Its IR spectrum suggested the existence of carboxyl (3400–2500 cm^{-1}), carbonyl (1700 cm^{-1}) and $-(CH_2)_n-$ ($n > 4$) (720 cm^{-1}), while the FAB-MS spectrum showed M^+ at 257 $(M + H)^+$ and 239 ($M + H - H_2O$), 213 (239 − CO), and 157 [213 − 4(CH_2)]. The ^1H NMR spectrum showed a methyl group at δ 0.85 (3H, t), 11 methylene groups at δ 1.24 (11 × CH_2, 22H, m), connected with the methyl group, one γ-methylene of carboxyl at δ 1.38 (2H, m), one β-methylene of carboxyl at δ 1.78 (2H, m) and one α-methylene of carboxyl at δ 2.52 (2H, t). The DEPT spectrum shows the presence of one quaternary carboxylic carbon at δ 176 and one methyl carbon at δ 14.3; the others are all methylene carbons (Table II). From these spectral characteristics we deduce that **2** is palmitic acid.

Compound **3**: white powdered crystals (EtOAc), mp 69.5–71°C, $C_{24}H_{48}O_2$ [FAB-MS *m/z* 367 $(M - H)^-$]. Its IR spectrum showed bands due to carboxyl (3300–2500 cm^{-1}), carbonyl (1705 cm^{-1}) and $-(CH_2)_n-$ ($n > 4$) (720 cm^{-1}). The FAB-MS spectrum

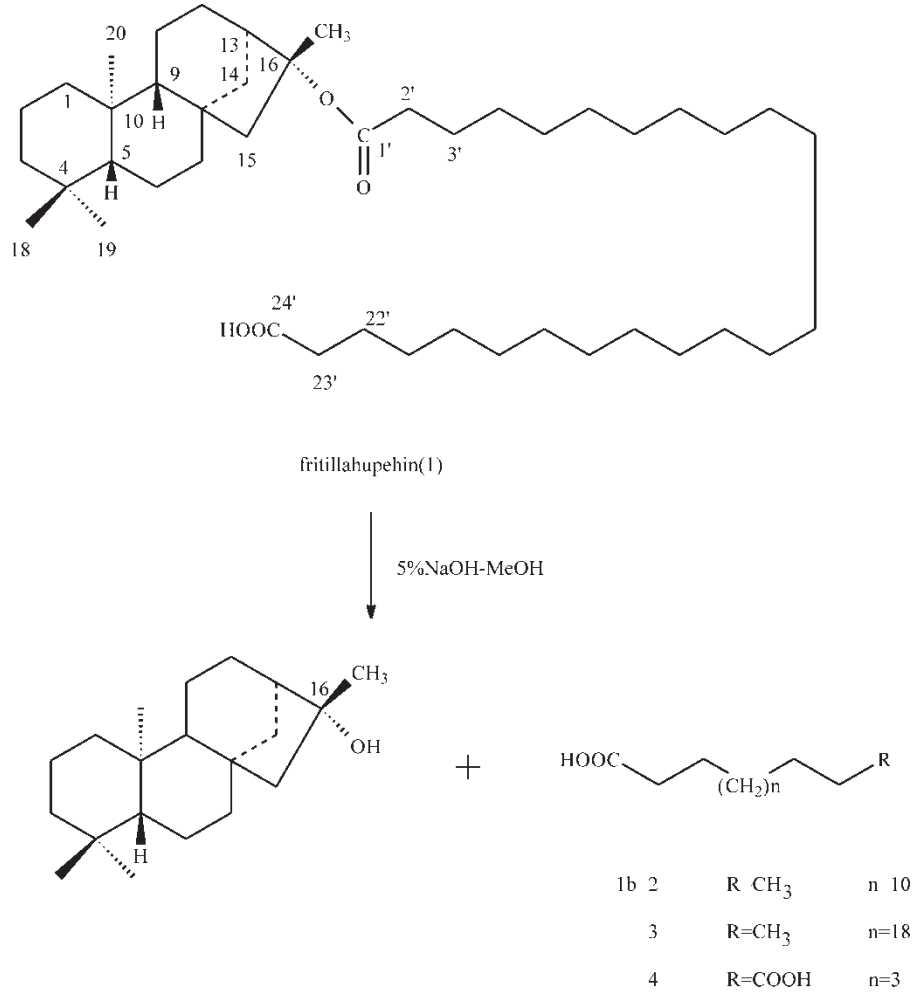

FIGURE 1 Derivatives of fritillahupehin.

showed M^+ at *m/z* 367 $(M - H)^-$ and *m/z* 353 $(M - H - CH_2)$, 339 $(353 - CH_2)$, 311 $(339 - 2 \times CH_2)$. The ^1H NMR showed a methyl group at δ 0.85 (3H, t), 19 methylene groups at δ 1.26 ($19 \times CH_2$, 38H, m), connected with the methyl group, one γ-methylene of carboxyl at δ 1.37 (2H, m), one β-methylene of carboxyl at δ 1.78 (2H, m) and one α-methylene of carboxyl at δ 2.51 (2H, t). The DEPT spectrum showed a quaternary carboxylic carbon at δ 176 and one methyl carbon at δ 14.3; the others are all methylene carbons (Table II). From above evidences, **3** was determined to be lignoceric acid.

Compound **4**: white hemispheric crystals (EtOAc), mp 105–107°C, $C_9H_{16}O_4$ [FAB-MS *m/z* 189 $(M + H)^+$]. Its IR spectrum showed bands due to carboxyl (3400–2400 cm^{-1}), carbonyl (1700 cm^{-1}) and $-(CH_2)_n-$ ($n > 4$) (720 cm^{-1}). The FAB-MS spectrum showed M^+ at *m/z* 189 $(M + H)^+$ and a characteristic ion peak at 171 $(M + H - H_2O)$. The ^1H NMR spectral data are δ 1.29 (6H, m), δ 1.71 (4H, m), δ 2.46 (4H, t). The DEPT spectrum of **4** showed two quaternary carbons at δ 176 (carboxylic carbon, overlapped); the others are all methylene carbons (Table II). Consequently, **4** is azelaic acid.

EXPERIMENTAL

General Experimental Procedures

Melting points were determined on a X_4 apparatus and are uncorrected. Optical rotations were taken on a WZZ-1digital polarimeter. IR spectra were recorded on a Shimadzu IR-460 spectrometer. EI-MS spectra were measured on an Auto-Spec mass spectrometer (70 eV). ^{1}H and ^{13}C NMR data were recorded on a Bruker-400 spectrometer using $CDCl_3$ as solvent. 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 hupehensis* Hsiao *et* K. C. Hsia. were from the HuBei Institute of Chinese Materia Medica and were identified by Peng De Tai, Lichuan Institute of Chinese Materia Medica, China.

Extraction and Isolation

The powdered bulbs (12 kg) of *Fritillaria hupehensis* Hsiao *et* K. C. Hsia. were extracted with EtOH. The extract (484.75 g) was then partitioned between n-hexane and water. The n-hexane extract was fractionated by column chromatography over silica gel, and eluted with n-hexane–EtOAc containing increasing amounts of EtOAc to give Fr-II (155 g). Part of Fr-II (86 g) was eluted with light petroleum–EtOAc to give fritillahupehin (**1**) (53 mg), palmitic acid (**2**) (158.7 mg), lignoceric acid (69.0 mg) (**3**) and azelaic acid (**4**) (75.3 mg).

Fritillahupehin (**1**): colorless needles (EtOAc), mp 183–185°C, $[\alpha]_D^{20}$ −78.2 (*c* 0.67, $CHCl_3$). Elemental analysis: C, 82.92; H, 12.24 (calcd. for $C_{44}H_{80}O_2$: C, 82.50; H, 12.50). IR (KBr) ν_{max} (cm^{-1}): 1738 (carbonyl group), 1382, 1365 (geminal dimethyl group), [(—CH_2—)$_4$]. FAB-MS *m/z*: 731 (M − H + Gly). ^{1}H NMR ($CDCl_3$): see Table I; ^{13}C NMR: see Table II.

Alkaline hydrolysis of **1**. Compound **1** was refluxed with 5% NaOH–MeOH for 4 h at 70°C. After the usual work-up, the residue was purified by dry silica gel column chromatography (silica gel 20 g, solvent: light petroleum–EtOAc = 6:4) to give *ent*-kauran-16β-ol (**1a**) and lignoceric acid (**1b**).

ent-Kauran-16β-ol (**1a**): colorless needles, mp 216–217°C. $C_{20}H_{34}O$ (HREI-MS: *m/z* 290.2598; calcd. for $C_{20}H_{34}O$: 290.2610). EI-MS *m/z*: 290 (M^+), 272 (M − H_2O, 100%), 257 (M − H_2O − CH_3), 232 (M − C_3H_6O), 217 (M − C_3H_6O − CH_3). ^{1}H NMR: see Table I; ^{13}C NMR: see Table II.

Lignoceric acid (**1b**): colorless powders, mp 69–71°C. IR (KBr) ν (cm^{-1}): 3300–2500, 1705, 720. FAB-MS *m/z*: 367 (M^+), 353 (M − H − CH_2), 339 (353 − CH_2), 311 (339 − 2 × CH_2). ^{1}H NMR: see Table I; ^{13}C NMR: see Table II.

Palmitic acid (**2**): white hemispheric crystals (EtOAc), mp 51–53°C, IR (KBr) (cm^{-1}): 3400–2500, 1700, 720. FAB-MS *m/z*: 257 $(M + H)^+$, 239 (M + H − H_2O), 213 (239 − CO), 157 [213 − 4(CH_2)]. ^{1}H NMR: see Table I; ^{13}C NMR: see Table II.

Lignoceric acid (**3**): white powdered crystals (EtOAc), mp 69.5–71°C, IR (KBr) ν (cm^{-1}): 3300–2500, 1705, 720. FAB-MS *m/z*: 367 $(M - H)^-$, 353 (M − H − CH_2), 339 (353 − CH_2), 311 (339 − 2 × CH_2). ^{1}H NMR: see Table I; ^{13}C NMR: see Table II.

Azelaic acid (**4**): white hemispheric crystals (EtOAc), mp 105–107°C, IR (KBr) ν (cm^{-1}): 3400–2500, 1700, 720. FAB-MS *m/z*: 189 $(M + H)^+$, 171 (M + H − H_2O). ^{1}H NMR: see Table I; ^{13}C NMR: see Table II.

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