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Feroniellides A and B, apotirucallane triterpenes with novel cyclic acetals from *Feroniella lucida*

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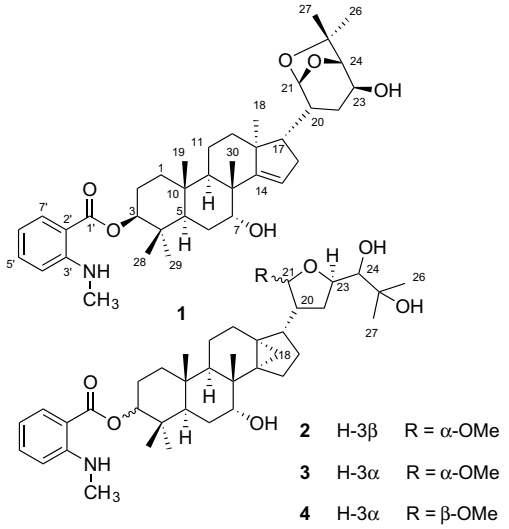
Abstract—The isolation and structure elucidation of two new triterpenes named feroniellides A (**1**) and B (**2**) from *Feroniella lucida* are described. Feroniellide A has a novel dioxabicyclic [3.2.1]octane moiety, and feroniellide B is the C-3 epimer of the known triterpenoid **3**. Their overall structures and relative configurations were established by combined spectral data analysis. The cytotoxicity of **1** and **2** was also evaluated against human KB and HeLa carcinoma cells.

Apotirucallane triterpenes have been found in several Meliaceae species and also in several of the Simaroubaceae and Rutaceae. These compounds can be categorized into two groups: 14,18-cycloapotirucallane and Δ^{14}-18-methylapotirucallane. Apotirucallane triterpenes have demonstrated several intriguing bioactivities, for example, dysobinin, a CNS depressant from *Dysoxylum alliaceum*[1] and meliavokin, a potent cytotoxin from *Melia volkensii*, which showed equivalent potency to adriamycin against the human breast tumor cell line.[2] In our search for biologically active metabolites from *Feroniella lucida* roots,[3] we recently reported the isolation of three new cytotoxic furanocoumarins, feroniellins A–C, bearing highly oxygenated side chains.

We also noticed the presence of other cytotoxic metabolites in the less polar fractions of the CH_2Cl_2 extract. An attempt to identify these active components led to the isolation of two new apotirucallane triterpenes named feroniellides A (**1**) and B (**2**).

F. lucida roots (3.8 kg), collected in Nakornpanom, in June 2005, were extracted as previously described.[3] The combined CH_2Cl_2 extracts were dissolved in MeOH and subsequently filtered to afford a residue. The residue (52 g) was chromatographed on silica gel eluting with CH_2Cl_2–*n*-hexane (1:1, 3:2, 4:1, and 1:0) and MeOH–CH_2Cl_2 (1:99→1:9) to yield eight fractions. The combined fractions 2 and 3 (530 mg) were further purified on Sephadex LH-20 [*n*-hexane–CH_2Cl_2–MeOH (6:3:1)] to yield feroniellide A (**1**, 20 mg). Fraction 4 (380 mg) was subsequently purified on Sephadex LH-20 [*n*-hexane–CH_2Cl_2–MeOH (5:4:1)] followed by ODS HPLC (85% MeOH–H_2O) to afford feroniellide B (**2**, 6 mg) and two known triterpenoids (**3** and **4**).[5]



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Feroniellide A (**1**)[4] was obtained as a colorless powder. The molecular formula $C_{38}H_{55}NO_6$ was determined by HRESIMS [*m*/*z* 622.4105 $[M+H]^+$, Δ −0.2 mmu]. The ^1H NMR spectrum displayed signals in three notable regions: 1,2-substituted benzene (δ 6.8–8.0), oxygenated and olefinic protons (δ 3.6–5.6), and methylene and methyl signals (δ 0.6–2.2). The UV (MeOH) absorbance at 255 and 354 together with the resonances in the downfield region [δ 7.84 (1H, dd, $J = 1.2$ and 8.0 Hz), 7.31 (1H, ddd, $J = 1.2$, 8.0, 8.2 Hz), 6.62 (1H, d, $J = 8.4$ Hz), and 6.53 (1H, t, $J = 7.6$ Hz)] and the singlet methyl [δ 2.80 (3H, s)] indicated the existence of an *N*-methyl anthranilate residue (Table 1).[5]

The ^{13}C NMR spectrum revealed 38 carbon signals, 30 of which were accounted for by a triterpenoid skeleton. In the upfield region, the ^1H NMR spectrum showed seven singlet methyls at δ 1.30, 1.23, 1.00, 0.98, 0.94, and 0.86 (6H), in addition to overlapped resonances of the methine and methylene protons (δ 1.4–2.2). These data suggested that the structure of **1** possessed an apotirucallane skeleton.

The *N*-methyl anthranilic acid unit was connected to C-3 as evident from the slightly downfield shifts[5] of 80.5 (C-3) and 4.64 (H-3) along with an HMBC cross peak between H-3 and C-1′ (δ_C 168.2). The location of the double bond at C-14 was determined from HMBC correlation of δ 5.38 (H-15) to δ 46.6 (C-13), 161.9 (C-14), 33.4 (C-16), and 55.2 (C-17).

The COSY spectrum (Fig. 1) demonstrated the contiguous spin system from H-24 to H-20, which was in turn coupled to H-21 and H-17. The occurrence of a cyclic

Table 1. ^1H and ^{13}C NMR data[a] for feroniellin A (**1**) in $CDCl_3$

No.	δ_C		δ_H (mult, *J* in Hz)	HMBC (H→C)
1	33.4	α	1.20 m	
		β	1.35 m	
2	23.6	α	1.71 m	
		β	1.85 m	
3	80.5		4.64 dd (4.8, 11.6)	C-1, 4, 5, 1′
4	37.6			
5	46.7		1.68 m	
6	27.6		1.58 m (2H)	
7	72.3		3.85 br s	C-5, 8
8	44.2			
9	41.8		1.98 m	
10	37.5			
11	16.9	α	1.52 m	
		β	1.70 m	
12	34.0	α	1.50 m	
		β	1.77 m	
13	46.6			
14	161.9			
15	119.7		5.38 br d (2.0)	C-13, 14, 16, 17
16	33.4	α	2.08 m	
		β	1.93 m	
17	55.2		1.58 m	
18	19.7		1.00 s	C-12, 13, 14, 17
19	15.5		0.86 s	C-1, 5, 9, 10
20	37.4		2.05 m	
21	102.9		5.47 br s	C-20, 22, 24, 25
22	31.1	α	1.66 m	C-21
		β	1.73 m	C-21
23	65.4		3.69 br s	C-20
24	83.3		3.77 br s	C-22, 23, 25, 26
25	79.2			
26	29.0		1.23 s	C-24, 25, 27
27	20.0		1.30 s	C-24, 25, 26
28	16.4		0.94 s	C-3, 4, 5, 29
29	27.6		0.86 s	C-3, 4, 5, 28
30	27.8		0.98 s	C-7, 8, 9, 14
1′	168.2			
2′	110.8			
3′	151.8			
4′	110.0		6.62 d (8.4)	C-2′, 6′
5′	134.4		7.31 ddd (1.2, 8.0, 8.2)	C-3′, 7′
6′	114.5		6.53 t (7.6)	C-2′, 4′
7′	131.4		7.84 dd (1.2, 8.0)	C-1′, 3′, 5′
NH*Me*	29.7		2.80 s	C-3′

[a] Measured at 400 MHz (^1H) and 100 MHz (^{13}C).

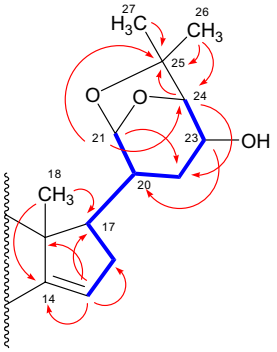

Figure 1. Connectivity of the dioxabicyclo[3.2.1]octane moiety and tetracyclic skeleton of **1**. Bold lines and arrows indicate selected COSY and HMBC cross peaks, respectively.

acetal was inferred from characteristic signals[6,7] at δ_H 5.47 and δ_C 102.9. In fact, the carbon chemical shifts of this portion were similar to those of meliavolin,[2] except for the striking downfield shifts of C-20, C-21, and C-24. The HMBC correlations (Fig. 1) of H-21 to C-24 and C-25 indicated that an additional ether bridge was formed between C-21 and C-25, thus completing the dioxabicyclic [3.2.1]octane moiety.

The relative configuration of **1** was determined from NOESY data and coupling constant analysis. A large coupling constant for H-3 (11.6 Hz) indicated its axial orientation, which was further confirmed by NOESY cross peaks between H-3 and CH_3-28. In the tetracyclic system, the NOESY correlations of CH_3-29/CH_3-19, CH_3-19/CH_3/30, and CH_3-30/H-7 indicated their β-positions while those of H-9/CH_3-18 and CH_3-18/H-20 confirmed their α-orientations. The correlations of H-23/CH_3-27 and H-24/CH_3-26 confirmed the configurations on the dioxabicyclic moiety (Fig. 2).

Feroniellide B (**2**)[8] was isolated as a colorless powder. High resolution mass spectral analysis suggested a molecular formula of $C_{39}H_{59}NO_7$, which was in accord with the spectral information provided by the ^{13}C NMR spectrum. The ^1H NMR spectrum exhibited diagnostic resonances similar to those of **1**, except for the signals in the upfield region. The presence of two doublet methylenes [δ 0.75 (1H, d, $J = 5.0$ Hz) and 0.50 (1H, d, $J = 5.0$ Hz)] and six singlet methyls (δ 1.29, 1.21, 1.03, 1.00, 0.93, and 0.92) suggested that a methyl group in **1** was replaced by a cyclopropane ring[5,6] in **2**. The HMBC cross peaks of H-18/C-13 and H-18/C-14 allowed connection of the cyclopropane moiety at C-13 and C-14. The existence of an acetal moiety was readily recognized from the signals of δ_H 4.80 and δ_C 108.5. Interpretation of HSQC, HMBC, and COSY data led to the assignment of a five-membered hemiacetal for these remaining resonances.

From 2D NMR data analysis, the overall structure of **2** was nearly identical to that of triterpene **3**, which was previously reported from *Raulinoa echinata* (Rutaceae).[5] Careful comparison of the ^1H NMR spectra of **2** and **3** indicated a noticeable difference in the resonance of H-3, in which that of **2** experienced a slightly downfield shift [δ 4.80 (br s) vs 4.60 (dd, $J = 11.0$, 1.7 Hz) in **3**]. These data indicated that **2** was the C-3 epimer of **3**. However, the orientation of H-3 in **2** could not be readily addressed from its coupling constant and NOESY data due to overlapping with H-21. This problem was circumvented by preparing acetylated product **2a**,[9] in which the overlapping signals of H-3 (δ 4.79) and H-21 (δ 4.89) were resolved. A small coupling constant for H-3 ($J = 2.5$ Hz) in **2a** confirmed its β-orientation.

The relative configuration of **2** was established by NOESY data. The cross peaks between H-3/CH_3-29, CH_3-29/CH_3-19, CH_3-19/CH_3-30, and CH_3-30/H-17 showed that H-3, CH_3-29, CH_3-19, CH_3-30, and H-17 were β-orientated. The correlations among H-20, OCH_3-21, and H-23 revealed that they occupied the same face of the tetrahydrofuran ring.

The occurrence of an *N*-methylanthranilic acid moiety in triterpenoids is rare. A recent example was reported from *R. echinata*.[5] To our knowledge, feroniellide A is the first example of a triterpenoid containing a dioxabicyclo[3.2.1]octane moiety isolated from plants. Compounds bearing similar heterobicyclic systems have been encountered particularly in insect pheromones such as brevicomin,[10] frontalin,[11] and sordidin.[12] The formation of dioxabicyclo[3.2.1]octane possibly arises from intramolecular nucleophilic attack of the hydroxyl (25-OH) at the cyclic hemiacetal carbon (C-21). Feroniellides A and B exhibited cytotoxicity toward KB cells with IC_{50} values of 60 and 49 μg/mL, and against HeLa cells with IC_{50} values of 46 and 40 μg/mL.

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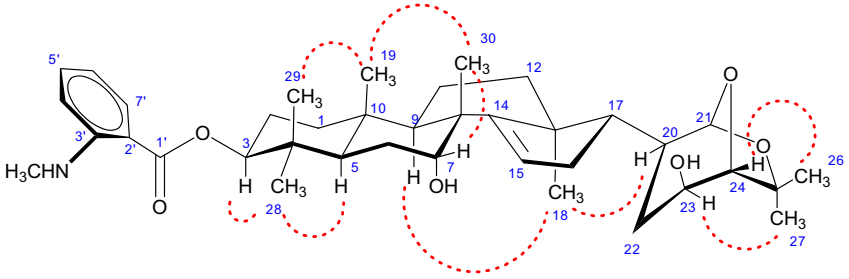

Figure 2. Key NOESY correlations for **1**.

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4. Feroniellide A (**1**): $[\alpha]_D^{25}$ +12.9 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ε) 223 (4.02), 255 (3.59), 354 (3.60); ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) (see Table 1); HRESIMS *m*/*z* $[M+H]^+$ 622.4105 (calcd for $C_{38}H_{56}NO_6$, 622.4107).
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8. Feroniellide B (**2**): $[\alpha]_D^{25}$ −34.0 (*c* 0.19, MeOH); UV (MeOH) λ_{max} (log ε) 225 (4.00), 255 (3.59), 354 (3.60); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.92 (1H, dd, $J = 1.6$, 8.2 Hz, H-7′), 7.40 (1H, m, H-5′), 6.87 (1H, dd, $J = 1.7$, 8.0 Hz, H-4′), 6.78 (1H, m, H-6′), 4.80 (2H, br s, H-3 and H-21), 4.02 (1H, m, H-23), 3.70 (1H, br s, H-7), 3.55 (1H, d, $J = 5.0$ Hz, H-24), 3.31 (3H, s, OCH_3-21), 2.90 (3H, s, NH*Me*), 2.05 (1H, m, H-20), 2.02 (1H, m, H-5), 2.00 (1H, m, H-17), 1.92 (1H, m, H-15), 1.91 (1H, m, H-22), 1.88 (1H, m, H-2), 1.84 (1H, m, H-12), 1.82–1.80 (2H, m, H-12 and H-22), 1.66 (2H, m, H-16), 1.64 (1H, m, H-6), 1.59 (1H, m, H-2), 1.56 (1H, m, H-6), 1.54 (1H, m, H-15), 1.36 (1H, m, H-1), 1.31 (2H, m, H-11), 1.30 (1H, m, H-9), 1.29 (3H, s, CH_3-26), 1.21 (3H, s, CH_3-27), 1.17 (1H, m, H-1), 1.03 (3H, s, CH_3-30), 1.00 (3H, s, CH_3-28), 0.93 (3H, s, CH_3-19), 0.92 (3H, s, CH_3-29), 0.75 (1H, d, $J = 5.0$ Hz, H-18a), 0.50 (1H, d, $J = 5.0$ Hz, H-18b); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.0 (C-1′), 152.0 (C-3′), 134.4 (C-5′), 131.3 (C-7′), 114.0 (C-6′), 110.6 (C-4′), 110.4 (C-2′), 108.5 (C-21), 80.5 (C-3), 80.0 (C-24), 77.8 (C-23), 74.4 (C-7), 72.1 (C-25), 55.4 (OCH_3-21), 49.5 (C-20), 48.6 (C-17), 46.0 (C-5), 43.6 (C-9), 38.5 (C-14), 37.8 (C-8), 37.1 (C-10), 37.0 (C-4), 34.1 (C-1), 32.4 (C-16), 29.6 (NH*Me*), 28.5 (C-13), 27.6 (C-28), 26.6 (C-26), 26.0 (C-22), 25.7 (C-15), 25.0 (C-27), 24.0 (C-12), 23.6 (C-6), 23.0 (C-2), 19.2 (C-30), 16.2 (C-11), 15.5 (C-19 and C-29), 13.7 (C-18); HRESIMS *m*/*z* $[M+H]^+$ 654.4350 (calcd for $C_{39}H_{60}NO_7$, 654.4370).
9. Feroniellide B diacetate (**2a**) was prepared as follows. To a solution of **2** (3 mg) in dry pyridine (200 μL) was added acetyl chloride (four drops), and the mixture was stirred at room temperature for 1 h. The reaction mixture was evaporated and partitioned between CH_2Cl_2 and H_2O. The organic layer was concentrated and purified on a short silica gel column using CH_2Cl_2–*n*-hexane (4:1) to afford the major product (**2a**, 2 mg), in which C-7 and C-24 were acetylated. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.89 (1H, d, $J = 3.0$ Hz, H-21), 4.79 (1H, br d, $J = 2.5$ Hz, H-3), 4.10 (1H, br s, H-7), 4.07 (1H, m, H-23), 3.95 (1H, d, $J = 5.0$ Hz, H-24), 2.92 (3H, br s, NH*Me*), 1.30 (3H, s, CH_3-26), 1.25 (3H, s, CH_3-27), 1.05 (6H, s, CH_3-28 and CH_3-30), 0.95 (3H, s, CH_3-29), 0.94 (3H, s, CH_3-19), 0.80 (1H, d, $J = 5.0$ Hz, H-18a), 0.58 (1H, d, $J = 5.0$ Hz, H-18b); ESIMS *m*/*z* $[M+H]^+$ 738.
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