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Feroniellins A–C, novel cytotoxic furanocoumarins with highly oxygenated C_{10} moieties from *Feroniella lucida*

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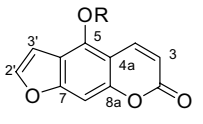
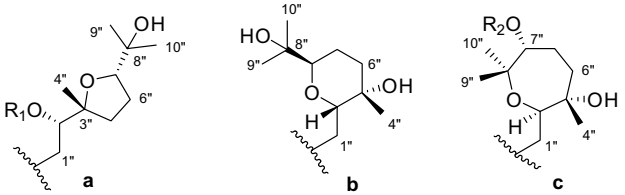
Abstract—Three new furanocoumarins, named feroniellins A (**1**), B (**2**), and C (**3**), were isolated from the roots of *Feroniella lucida*. Compounds **1**–**3** are novel structures having an oxolane, oxane, and oxepane moiety. Their overall structures and configurations were determined by spectral and chemical methods. The cytotoxicities of **1**–**3** were evaluated against human KB and HeLa carcinoma cells.

Feroniella lucida (Rutaceae) is a medium-sized tree distributed widely in the Northeast of Thailand. The genus *Feroniella* is categorized into the subtribe Balsamocitrinae, which includes the genera *Swinglea, Aegle*, *Afraegle*, *Aeglopsis*, *Balsamocitrus* and *Feronia*.[1] Anthranilic-derived alkaloids, such as quinolines and acridones[2] and coumarins encompassing modified C_5 and C_{10} moieties,[3] have been exemplified as principal secondary metabolites of this subtribe. Although *F. lucida* is now popularly cultivated as an ornamental plant, its ethnopharmacological use and phytochemical investigation have not been recorded. In our ongoing search for bioactive metabolites from Thai medicinal plants,[4] we have discovered distinct cytotoxicity in the CH_2Cl_2 extract of *F. lucida* roots. Bioassay-guided fractionation resulted in the isolation of three new isomeric furanocoumarins, named feroniellins A–C (**1**–**3**). The present paper describes the isolation and structure elucidation of **1**–**3**.

Feroniella lucida roots (3 kg), collected from Roi Et, in April 2005, were extracted with MeOH (4 L × 3). The combined extracts were partitioned between H_2O and CH_2Cl_2 to afford the cytotoxic CH_2Cl_2 extract. A portion (70 g) of the extract was chromatographed on silica gel with CH_2Cl_2–*n*-hexane (1:1–1:0) and MeOH–CH_2Cl_2 (1:99–10:90) to yield seven fractions. Fraction 4 (520 mg) was subsequently purified on Sephadex LH-20 [*n*-hexane–CH_2Cl_2–MeOH (2.5:2.0:0.5)] followed by ODS HPLC (80% MeOH–H_2O) to furnish three new isomeric furanocoumarins, named feroniellins A (94 mg), B (56 mg), and C (8 mg).



1 R = a, R_1 = H
1a R = a, R_1 = Ac
1b R = a, R_1 = *S*-(-)-MTPA
1c R = a, R_1 = *R*-(-)-MTPA
2 R = b
3 R = c, R_2 = H
3a R = c, R_2 = Ac



Feroniellin A (**1**)[5] was obtained as a pale yellow powder and had the molecular formula $C_{21}H_{24}O_7$ as established by HRESIMS. The UV (MeOH) absorbances at 252 and 309 nm suggested the presence of a coumarin moiety.[6] It also showed the characteristic ^{1}H NMR signals[7]

Keywords: *Feroniella lucida*; Rutaceae; Coumarin; Cytotoxic.
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of a 3,4-unsubstituted coumarin (δ 6.29, d, $J = 9.6$ Hz, H-3 and 8.22, d, $J = 10.0$ Hz, H-4), which had a furan moiety attached (δ 7.60, d, $J = 2.0$ Hz, H-2′ and 7.01, d, $J = 1.6$ Hz, H-3′). The ^{13}C NMR spectrum revealed 21 signals; eleven of, which were accounted for by the furanocoumarin nucleus (Table 1). The remaining proton and carbon signals were ascribable to a geranyl-derived portion on the basis of 2D NMR data analysis.

The COSY spectrum of **1** displayed two spin systems, O–CH_2–CH–O and C–CH_2–CH_2–CH–O, which were flanked by an oxygenated quaternary C-3″, as suggested by the cross peaks of H-2″/C-3″ and H-5″/C-3″. Acetylation of **1** with Ac_2O in pyridine yielded feroniellin A acetate (**1a**),[8] indicating the presence of one secondary hydroxyl group, which was located at C-2″ based on HMBC correlation of H-2″/CO_2CH_3. Two singlet methyls (δ 1.16, CH_3-10″ and 1.26, CH_3-9″) were accommodated at C-8″ (δ 70.5), which was in turn connected to C-7″ (δ 87.6). The remaining singlet methyl (δ 1.24) was placed at C-3″ (δ 83.9) on the basis of HMBC cross peaks between these protons and C-2″, 3″, and 5″.

The slightly downfield shifts of oxygenated C-3″ (δ 83.9) and C-7″ (δ 87.6) coupled with the HMBC cross peak between H-7″ and C-3″ allowed us to construct a tetrahydrofuran or oxolane ring as a part of the C_{10} subunit. In fact, the NMR data of this portion were similar to those of dehydrovenustatriols, which were isolated from the red algae *Laurencia viridis*.[9] The C_{10} subunit was linked to the coumarin nucleus at C-5, as shown by an HMBC correlation of H_2-1″/C-5 and the presence of resonances typical of an unsubstituted C-8[7,10] (δ_C 94.7 and δ_H 7.17), thus completing the overall structure of **1**. The relative configuration of **1** was deduced by NOESY data

Table 1. ^{1}H 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)
2	161.2		
3	112.9	6.29 d (9.6)	C-2, 4a
4	139.3	8.22 d (10.0)	C-2, 5, 8a
4a	107.3		
5	148.6		
6	114.1		
7	158.1		
8	94.7	7.17 s	C-4a, 6, 7, 8a
8a	152.5		
2′	145.1	7.60 d (2.0)	C-6, 7, 3′
3′	104.8	7.01 d (1.6)	C-6, 7, 2′
1″	74.3	a 4.37 dd (2.8, 10.0)	C-5, 2″ C-5, 2″
		b 4.58 dd (2.8, 10.0)	
2″	75.9	4.04 dd (2.4, 8.0)	C-1″, 3″
3″	83.9		
4″	27.6	1.24 s	C-2″, 3″, 5″
5″	33.7	α 1.75 m	C-3″, 6″
		β 2.16 m	
6″	26.4	1.91 m	C-5″, 7″
7″	87.6	3.84 m	C-3″, 9″, 10″
8″	70.5		
9″	23.3	1.26 s	C-7″, 8″, 10″
10″	24.0	1.16 s	C-7″, 8″, 9″

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

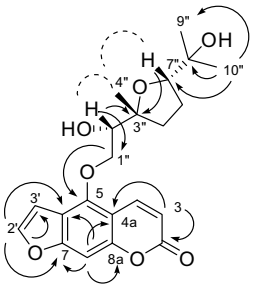

Figure 1. Key HMBC (solid arrows) and NOESY (dashed curves) correlations for **1**.

analysis (Fig. 1); the cross peaks between H-7″ and CH_3-4″, and between CH_3-4″ and H-2″ indicated that they were on the same face of the five-membered ring. To address the absolute configuration at C-2″, MTPA esters of **1** were prepared. Mosher analysis[11] of **1b** and **1c** resulted in the determination of the configuration as 2″*S*.[12]

Feroniellin B (**2**)[13] also had the molecular formula $C_{21}H_{24}O_7$, indicating that it was isomeric with **1**. The ^{1}H, ^{13}C NMR and COSY spectra resembled those of **1**, except for the subtle changes in the chemical shifts of the methyl and oxygenated protons and carbons in the C_{10} portion. Compound **2** did not yield any acetylated product on treatment with Ac_2O in pyridine, suggesting that the oxygenated resonances were that of ethers or tertiary hydroxyls. A downfield shift of C-2″ (δ_C 80.9 vs 75.9 in **1**), together with HMBC correlations between H-2″/C-7″ and H-7″/C-2″, indicated that C-2″ was fused to C-7″ through an ether bridge, thereby forming a tetrahydropyran or oxane moiety.[14]

The relative configuration of **2** (Fig. 2) was deduced from NOESY data and coupling constant analysis. CH_3-4″ (δ 1.24) was coupled to H-6″β (δ 1.61) and H-2″ (δ 4.12), which in turn was coupled to H-1″b (δ 4.72), thus indicating that CH_3-4″ and H-2″ resided in the same plane. The correlations of H-7″ to H-5″α and H-1″a to H-7″ along with a large coupling constant between H-7″ and H-6″β ($^3J_{7''/6''\beta} = 11.2$ Hz) indicated that H-7″ occupied a pseudo-axial position. Therefore, the relative configuration of **2** was described.

Feroniellin C (**3**)[15] was another isomeric coumarin of **1** and **2**. Interpretation of its COSY and HMBC data suggested that it differed from **1** and **2** in the nature of the

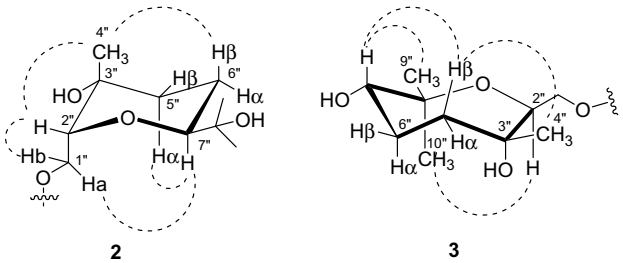

Figure 2. Key NOESY correlations in compounds **2** and **3**.

C_{10} subunit. The presence of a secondary hydroxyl group at C-7″ was clarified by the formation of feroniellin C acetate (**3a**)[16] and HMBC correlations of H-7″ to CH_3-9″, CH_3-10″ and CO_2CH_3. The oxygenated methine at δ 4.16 (H-2″) demonstrated correlations to C-8″ (δ 78.4), in addition to C-1″ (δ 73.7) and C-3″ (δ 74.1). These data allowed the connection of C-2″ to C-8″ through an ether linkage in **3**. The relative configuration in **3** was determined from NOESY cross peaks (Fig. 2). The key correlations were from CH_3-4″ to H-5″β, H-5″β to H-7″, H-7″ to CH_3-9″, and CH_3-10″ to H-2″. An attempt to deduce the absolute configuration of **3** was unsuccessful due to decomposition of the MTPA esters soon after isolation.

Furanocoumarins having modified C_{10} side chains have been consistently encountered in plants of the family Rutaceae; however, highly oxygenated C_{10} subunits are rare. To our knowledge, this is the first report of coumarins possessing an oxolane, an oxane, and an oxepane from terrestrial plants, although metabolites bearing these moieties are common among marine algae and dinoflagellates.[17] From a biogenetic point of view,[17–19] furanocoumarin bearing geranyl diepoxide may be a putative precursor of feroniellins A–C, which arise from different cyclization routes.

Feroniellins A and B exhibited in vitro cytotoxicity[20] against human KB carcinoma cells, with IC_{50} values of 0.13 and 0.23 mM, respectively. They were also cytotoxic toward human HeLa carcinoma cells with IC_{50} values of 0.14 and 0.19 mM, respectively. Feroniellin C did not exhibit cytotoxic activity.

Acknowledgements

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5. Feroniellin A (**1**): $[\alpha]_D^{25}$ +18.8 (*c* 0.85, MeOH); UV (MeOH) λ_{max} (log ε) 228 (4.45), 252 (4.40), 309 (3.52); ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) (see Table 1); HRESIMS *m/z* $[M+Na]^+$ 411.1422 (calcd for $C_{21}H_{24}O_7Na$, 411.1420).
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8. Feroniellin A acetate (**1a**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.07 (1H, d, $J = 10.0$ Hz, H-4), 7.57 (1H, d, $J = 2.4$ Hz, H-2′), 7.12 (1H, s, H-8), 6.99 (1H, d, $J = 2.4$ Hz, H-3′), 6.26 (1H, d, $J = 10.0$ Hz, H-3), 5.38 (1H, d, $J = 2.0$, 6.4 Hz, H-2″), 4.77 (1H, dd, $J = 2.0$, 10.4 Hz, H-1″), 4.53 (1H, dd, $J = 8.4$, 10.0 Hz, H-1″), 4.13 (1H, m, H-7″), 2.09 (3H, s, OAc), 1.92 (2H, m, H-5″), 1.75 (2H, m, H-6″), 1.47 (3H, s, CH_3-10″), 1.43 (3H, s, CH_3-9″), 1.29 (3H, s, CH_3-4″); ESIMS *m/z* $[M+H]^+$ 431.
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13. Feroniellin B (**2**): brown liquid; $[\alpha]_D^{25}$ +21.7 (*c* 0.92, MeOH); UV (MeOH) λ_{max} (log ε) 224 (4.20), 249 (4.05), 310 (3.37); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.18 (1H, d, $J = 9.6$ Hz, H-4), 7.62 (1H, d, $J = 2.0$ Hz, H-2′), 7.17 (1H, s, H-8), 6.95 (1H, d, $J = 2.0$ Hz, H-3′), 6.29 (1H, d, $J = 10.0$ Hz, H-3), 4.72 (1H, dd, $J = 8.4$, 10.4 Hz, H-1″b), 4.49 (1H, dd, $J = 4.4$, 10.4 Hz, H-1″a), 4.12 (1H, dd, $J = 4.4$, 8.0 Hz, H-2″), 3.44 (1H, dd, $J = 2.4$, 11.2 Hz, H-7″), 1.83 (1H, m, H-6″α), 1.79 (1H, m, H-5″α), 1.68 (1H, m, H-5″β), 1.61 (1H, m, H-6″β), 1.24 (3H, s, CH_3-4″), 1.21 (3H, s, CH_3-10″), 1.19 (3H, s, CH_3-9″); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.0 (C-2), 158.1 (C-7), 152.6 (C-8a), 148.4 (C-5), 145.3 (C-2′), 139.0 (C-4), 113.5 (C-6), 113.1 (C-3), 107.1 (C-4a), 104.6 (C-3′), 94.7 (C-8), 80.9 (C-2″), 76.1 (C-7″), 71.9 (C-8″), 69.7 (C-1″), 68.0 (C-3″), 32.8 (C-5″), 26.3 (C-10″), 25.0 (C-4″), 24.2 (C-9″), 20.8 (C-6″); HRESIMS *m/z* $[M+Na]^+$ 411.1418 (calcd for $C_{21}H_{24}O_7Na$, 411.1420).
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15. Feroniellin C (**3**): white amorphous solid; mp 105–106 °C; $[\alpha]_D^{25}$ +10.6 (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ε) 228 (4.42), 249 (4.40), 310 (3.58); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.16 (1H, d, $J = 9.6$ Hz, H-4), 7.48 (1H, d, $J = 2.0$ Hz, H-2′), 7.02 (2H, br s, H-8 and H-3′), 6.18 (1H, d, $J = 9.6$ Hz, H-3), 4.68 (1H, br d, $J = 8.4$ Hz, H-1″b), 4.21 (1H, m, H-1″a), 4.16 (1H, m, H-2″), 3.72 (1H, br d, $J = 7.6$ Hz, H-7″), 2.04 (1H, br t, $J = 12.4$ Hz, H-5″α), 1.86 (1H, m, H-6″α), 1.76 (1H, m, H-6″β), 1.59 (1H, br dd, $J = 5.6$, 14.0 Hz, H-5″β), 1.28 (3H, s, CH_3-9″), 1.22 (3H, s, CH_3-4″), 1.44 (3H, s, CH_3-10″); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.5 (C-2), 158.3 (C-7), 152.6 (C-8a), 149.3 (C-5), 144.6 (C-2′), 139.7 (C-4), 113.4 (C-6), 112.2 (C-3), 106.7 (C-4a), 105.5 (C-3′), 93.8 (C-8), 78.4 (C-8″), 76.8 (C-7″), 76.1 (C-2″), 74.1 (C-3″), 73.7 (C-1″), 37.9 (C-5″), 28.9 (C-10″), 25.9 (C-6″), 22.1 (C-4″), 21.5 (C-9″); HRESIMS *m/z* $[M+Na]^+$ 411.1423 (calcd for $C_{21}H_{24}O_7Na$, 411.1420).

16. Feroniellin C acetate (**3a**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.14 (1H, d, $J = 10.0$ Hz, H-4), 7.42 (1H, d, $J = 2.4$ Hz, H-2′), 7.04 (d, 1H, $J = 2.6$ Hz, H-3′), 7.00 (1H, s, H-8), 6.15 (1H, d, $J = 10.0$ Hz, H-3), 4.82 (1H, m, H-7″), 4.70 (1H, d, $J = 8.5$ Hz, H-1″b), 4.28 (1H, m, H-1″a), 4.20 (1H, m, H-2″), 2.18 (1H, m, H-5″α), 2.02 (3H, OAc), 1.95 (2H, m, H-6″), 1.72 (1H, m, H-5″β), 1.41 (3H, s, CH_3-9″), 1.39 (3H, s, CH_3-10″), 1.27 (3H, s, CH_3-4″); ESIMS *m*/*z* $[M+H]^+$ 431.
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