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Complete NMR assignment and absolute configuration of feronielloside, a new acetylcholinesterase inhibitor from *Feroniella lucida*

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Feronielloside (**1**), a new furanocoumarin glycoside, was isolated from roots of *Feroniella lucida*. The structure of **1** was elucidated by combined spectroscopic and chemical analysis. The absolute configuration of the chiral center in geranyl-derived side chain was confirmed by modified Mosher's method. Feronielloside inhibited acetylcholinesterase with IC_{50} value of 24.7 mM.

Keywords: *Feroniella lucida*; feronielloside; furanocoumarin; acetylcholinesterase inhibitor

1. Introduction

Acetylcholinesterase (AChE) inhibitors are the major drugs approved for the symptomatic treatment of Alzheimer's disease. It has also been demonstrated that AChE could play an important role during the early stage in the development of the senile plaques by accelerating β-amyloid peptide deposition (Inestrosa et al., 1996). Inhibition of the peripheral binding site of AChE might prevent the deposition of β-amyloid peptide induced by AChE. Natural AChE inhibitors currently used for clinical Alzheimer's therapy are exemplified by physostigmine, an indole alkaloid from Calabar beans (*Physostigma venenosum*) (Robinson, 1988) and galanthamine, an azepine alkaloid from various *Amarylidaceae* plants (Hoshino, 1998). In our continuing investigation on bioactive compounds from *Feroniella lucida* (Phuwapraisirisan, Surapinit, Sombund, Siripong, & Tip-Pyang, 2006; Phuwapraisirisan, Surapinit, & Tip-Pyang, 2006; Phuwapraisirisan, Surapinit, Siripong, Tip-pyang, & Kokpol, 2007; Phuwapraisirisan, Surapinit, Jeenapongsa, Tip-Pyang, & Kokpol, 2007), we found inhibition activity against AChE in *n*-BuOH extract. Bioassay-guided isolation afforded a new furanocoumarin glycoside (**1**) named feronielloside, along with three known glycosides (**2–4**). In fact, the compound closely related to **1** was proposed, by Koul in 1979, based on chemical derivatization only (Koul, Dhar, & Thakur, 1979). The lack of NMR data of the originally proposed structure made an impossible direct comparison to our sample. To clarify this

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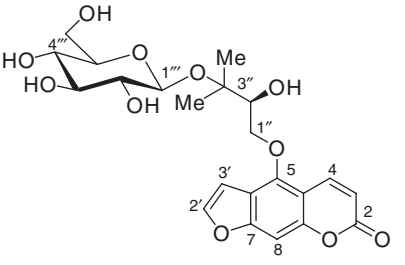
problem, we carried out structure elucidation of feronielloside through spectroscopic data and chemical transformation.



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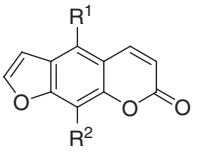
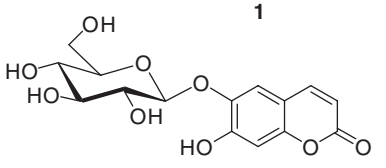


2 R^1=O–β–Glc, R^2=H

3 R^1=H, R^2=O–β–Glc



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2. Results and discussion

Feronielloside was isolated as white powder. The molecular formula $C_{22}H_{26}O_{11}$ was deduced by HRESIMS in conjuction with ^{13}C NMR data. The UV absorptions (log ε) at 250 (3.73) and 303 (3.61) were suggestive of coumarin moiety (Murray, Mendez, & Brown, 1982). The ^1H NMR spectrum of **1** in CD_3OD (Table 1) demonstrated signals typical to a 3,4-unsubstituted furanocoumarin: δ_H 6.28 (1H, d, $J = 10.0$ Hz,), 8.43 (1H, d, $J = 10.0$ Hz), 7.26 (1H, d, $J = 2.0$ Hz) and 7.69 (1H, d, $J = 2.0$ Hz). The ^{13}C NMR spectrum showed 22 signals: 11 of which and remaining protons in high-field region were ascribable to geranyl-derived and sugar moieties. Interpretation of 2D NMR data indicated that the spin system of O–CH_2–CH−O was flanked by a quaternary carbon (δ_C 78.6) which was in turn accommodated by two singlet methyls (δ_H 1.34 and 1.38). The sugar moiety was identified to be glucose by NMR and chemical methods. A typical large coupling constant ($J = 7.8$ Hz) of anomeric proton (δ_H 4.58) pointed out a β–OH orientation while methyl glucopyranosides (**1e**) obtained from hydrolysis of **1** (Scheme 1) were identical to those prepared from D-glucose.

The HMBC correlation between H-1‴ and C-3″ confirmed the connectivity of glucose and geranyl-derived residue through ether linkage. However, further 2D NMR analysis addressing the position of geranyl derived moiety on the furanocoumarin was hampered because there was no HMBC cross peak observed between H-1″ and a carbon on the aromatic ring. This problem, which resulted from the signal overlapping with HOD residue, was circumvented by formation of peracetylated product. Treatment of **1** with AcCl in pyridine at ambient temperature afforded feronielloside pentacetate (**1a**). The ^1HNMR spectrum of **1a** in $CDCl_3$ displayed the sharp signal of H-1″ at δ_H 4.52 and 4.78, which revealed HMBC correlations with C-5 of coumarin moiety (Figure 1), thus completing the entire structure of feronielloside (**1**).

We attempted to address the absolute configuration of C-2″ since the previously proposed structure has remained unclear. Prior to applying Mosher's analysis (Ohtani, Kusumi, Kashman, & Kakisawa, 1991), removal of glucose moiety from **1** was required (Scheme 1), in order to eliminate unexpectedly combined anisotropic effects of four

Table 1. NMR data of feronielloside (**1**, CD_3OD) and feronielloside pentacetate (**1a**, $CDCl_3$).

	1		**1a**[a]	
Position	δ_C	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)
2	161.8		161.2	
3	111.4	6.28 d (10.0)	112.8	6.26 d (10.0)
4	140.3	8.43 d (10.0)	139.2	8.04 d (10.0)
4a	107.0		106.7	
5	149.4		148.5	
6	114.2		113.0	
7	158.3		158.2	
8	93.2	7.21 s	94.2	7.14 s
8a	152.2		152.6	
2′	145.2	7.69 d (2.0)	145.0	7.62 d (2.0)
3′	105.0	7.26 d (2.0)	105.1	7.03 d (2.0)
1″	74.3	4.41 dd (8.8, 8.2) 4.90[b]	71.1	4.52 dd (8.0, 10.2) 4.78 dd (1.2, 10.2)
2″	75.9	3.89 dd (1.6, 8.2)	76.7	5.40 dd (1.2, 8.0)
3″	78.6		78.8	
4″	20.9	1.34 s	22.1	1.27 s
5″	23.0	1.38 s	24.5	1.35 s
Glc				
1‴	97.0	4.58 d (7.8)	95.3	4.74 d (7.8)
2‴	73.7	3.16 dd (7.8, 8.8)	71.5	5.00 dd (7.8, 8.9)
3‴	76.5	3.30 m	5.02	5.02 m
4‴	76.3	3.38 m	72.6	5.24 m
5‴	70.1	3.28 m	71.8	3.72 m
6‴	61.3	3.62 d (10.6) 3.81 d (11.8)	62.2	4.08 dd (5.6, 11.8) 4.17 dd (5.6, 11.8)

[a]Signals of acetates resonated at δ_H 1.98, 1.99, 2.01, 2.02 and 2.09; δ_C 20.3, 20.6 ($4 \times CH_3$), 170.2 ($4 \times C{=}O$) and 169.4.
[b]Overlapped by HOD residue.

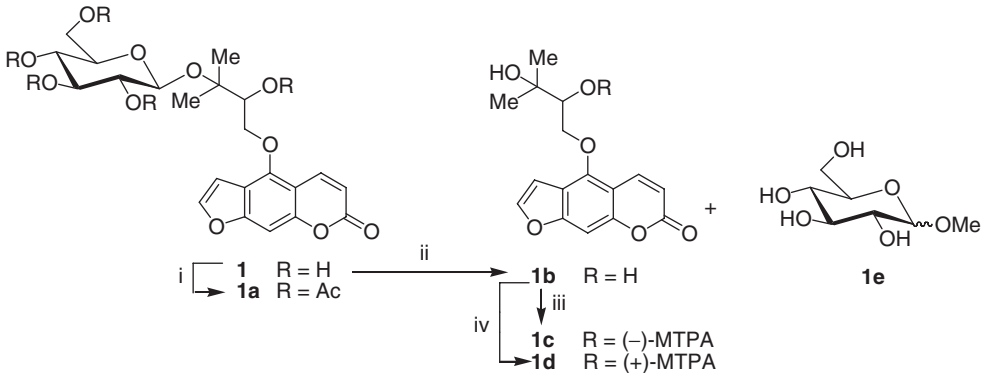
phenylacetic acid derivatives (Freire, Seco, Quiñoá, & Riguera, 2005). Acid hydrolysis of **1** under reflux condition yielded oxypeucedamin hydrate (**1b**) and glucose residue (**1e**). Treatment of **1b** with (−)- and (+)-MTPA chlorides gave (*S*)-(−)- and (*R*)-(+)-MTPA esters designated as **1c** and **1d**, respectively. The $\Delta\delta_{SR}$ distribution (Figure 2) indicated *S* configuration of C-2″.

Feronielloside (**1**) inhibited AChE with IC_{50} value of 24.7 mM. It is likely that furanocoumarin glycosides (**1–3**) are slightly less active than the hydroxylated coumarin glycoside (**4**) (Table 2). Recently, a number of 3,4-dimethyl coumarins have been reported as noncompetitive AChE inhibitors, suggesting that they are possibly beneficial in decreasing β-amyloid decomposition (Bruhlmann et al., 2001).

3. Experimental

3.1. *General procedure*

UV spectra were taken on a UV-160A spectrometer (SHIMADZU). ESIMS and HRESIMS were obtained by Micromass LCT mass spectrometer. NMR spectra were recorded on a Varian Mercury+400 spectrometer and chemical shifts were reported in



Scheme 1. Reagents and conditions: (i) AcCl, pyridine, rt; (ii) HCl/MeOH, reflux; (iii) (−)-MTPACl, pyridine; (iv) (+)-MTPACl, pyridine.

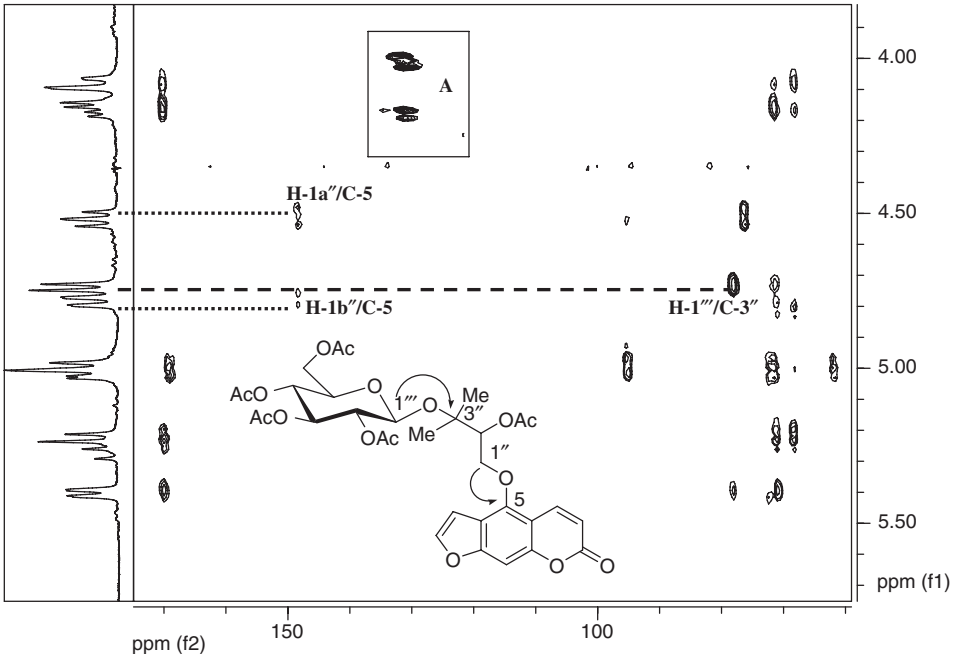


Figure 1. Diagnostic HMBC correlations observed in **1a**. Expansion (A) shows cross peaks of H-1a″/C-5 and H-1b″/C-5.

ppm referenced to solvent residues (δ_H 7.25 and δ_C 77.0 ppm for $CDCl_3$ and δ_H 3.34 and δ_C 49.2 ppm for CD_3OD).

3.2. *Plant material*

The roots of *F. lucida* were collected in April 2005 from Roi-Et province. The specimens (voucher number BCUOT 968) were identified by Professor Thaweesakdi Boonkerd, Department of Botany, Faculty of Science, Chulalongkorn University.

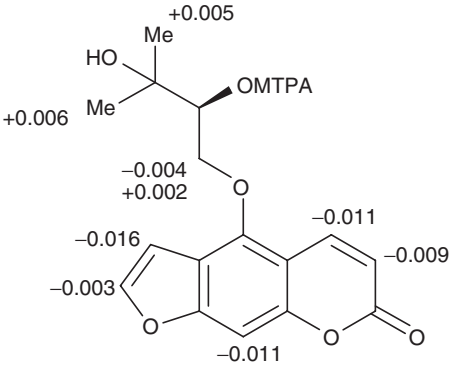


Figure 2. $\Delta\delta_{SR}$ values (in ppm) for the MTPA esters (**1c** and **1d**) of **1b**.

Table 2. Acetylcholinesterase (AchE) inhibitory effect of **1**–**4**.

Compound	AchE inhibitory effect (IC_{50}, Mm)
1	24.7
2	41.6
3	24.5
4	16.3

3.3. *Extraction and isolation*

The air dried chopped roots of *F. lucida* (3.8 kg) were extracted with MeOH in Soxhlet extractor. The solvent was removed under vacuum to yield the crude extract, which was suspended in MeOH : H_2O (1 : 1, 1 L) and extracted with CH_2Cl_2 (3 × 1 L). The aqueous layer was concentrated and extracted with saturated *n*-BuOH (3 × 700 mL). A portion (30 g) of BuOH extract was subjected to silica gel VCC using MeOH–CH_2Cl_2 (1 : 9, 1 : 4, 1 : 1 and 1 : 0) to yield four fractions. Fraction 2 was further purified on Sephadex LH-20 (3 : 1.5 : 0.5 and 2 : 2 : 0.5 *n*-hexane–CH_2Cl_2–MeOH) followed by silica gel CC (1 : 9 MeOH–CH_2Cl_2). Final purification was performed on an ODS column using 1 : 1 MeOH–H_2O (UV 254 nm; flow rate 6 mL min^{-1}), affording compounds **1** (t_R 31.5 min, 50 mg), **2** (t_R 22.0 min, 13 mg), **3** (t_R 24.3 min, 60 mg) and **4** (t_R 10.5 min, 17 mg).

3.4. *Feronielloside (1)*

White powder; $[\alpha]_D^{27} = +6.0^\circ$ ($c = 0.05$, MeOH); UV (MeOH) λ_{max} (log ε) 221 (3.88), 250 (3.73), 309 (3.61); ^{1}H- and ^{13}C-NMR (Table 1); HRESIMS *m*/*z* $[M + Na]^+$ 489.1370 (Calcd for $C_{22}H_{26}O_{11}Na$, 489.1373).

3.5. *Preparation of feronielloside pentaacetate (1a)*

To a solution of compound **1** (8 mg) in dry pyridine (500 µL) acetyl chloride (20 µL) was added, and the mixture was stirred at room temperature overnight. The reaction mixture

was dissolved in CH_2Cl_2 (500 μL) and extracted with 0.5 M HCl (2 × 1 mL) and H_2O (2 × 1 mL). The CH_2Cl_2 layer was evaporated to give feronielloside pentaacetate (**1a**, 4.3 mg).

Feronielloside pentaacetate (1a)

White morphous powder; $[\alpha]_D^{27} = -45.6^\circ$ ($c = 0.05$, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.19), 249 (4.02), 310 (3.89); ^{1}H- and ^{13}C-NMR (Table 1); HRESIMS m/z $[M + Na]^+$ 699.1902 (Calcd for $C_{32}H_{32}O_{16}Na$, 699.1901).

3.6. *Hydrolysis of feronielloside*

A solution of compound **1** (1 mg) in 2 M HCl in MeOH (500 μL) was refluxed for 4 h. The resulting solution was neutralized with Na_2CO_3 and evaporated to dryness. After removal of MeOH, the reaction mixture was dissolved in CH_2Cl_2 and extracted with H_2O. The aqueous layer was concentrated and analysed by TLC, in which the hydrolysate of **1** gave R_f value [0.56, SiO_2, CH_2Cl_2–MeOH–H_2O (6 : 4 : 0.5)] identical to methyl glucopyranosides (**1e**) prepared from authentic D-glucose.

3.7. *Mosher's esters of 1b*

To a solution of compound **1b** (3 mg) in pyridine (100 μL) was added (−)-MTPA chloride (5 μL), and the reaction mixture was stirred at room temperature. After 3 h, the reaction mixture was diluted with CH_2Cl_2 (2 mL) and washed with H_2O (2 × 2 mL). The CH_2Cl_2 layer was dried over anhydrous Na_2SO_4 and evaporated to give *S*-(−)-MTPA derivative (**1c**). The *R*-(+)-MTPA derivative (**1d**) was also prepared using the same protocol. The $\Delta\delta_{SR}$ values were shown in Figure 2.

4. Acetylcholinesterase inhibitory assay

Acetylcholinesterase inhibition was assessed by modifications of the Ellman method (Ellman, Courtney, Andres, & Featherstone, 1961), which is based on the reaction of released thiocholine to give a coloured product with 5,5′-dithio-bis(2-nitrobenzoic acid) or DTNB. In a 96-well plate, 15 mM acetylthiocholine iodine (ATCI, 25 μL), 3 mM DTNB (125 μL) in buffer C (50 mM Tris-HCl, pH 8, 0.1 M NaCl, 0.02 M $MgCl_2 \cdot 6H_2O$), 50 μL of buffer B (50 mM Tris-HCl, pH 8, 0.1% bovine serum albumin) and 25 μL of sample in buffer A (50 mM Tris-HCl, pH 8) was added, and the absorbance of resulting solution was measured five times at 415 nm for every 30 s. After the addition of enzyme solution (25 μL, 0.22 U mL^{-1}), the absorbance was measured again eight times for every 30 s. The rate of reaction was calculated by Microsoft Excel®. An increase in absorbance due to the spontaneous hydrolysis of substrate (ATCI) was corrected by subtracting the rate of reaction before adding the enzyme from the rate after assign the enzyme. Percentage of inhibition was calculated by comparing the rates for the sample to the blank (10% MeOH in buffer A). The IC_{50} values were determined from a plot of percent of inhibition against – log (concentration).

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