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TWO ISOQUINOLONES FROM THE ROOTS OF *PHELLODENDRON AMURENSE* VAR. *WILSONII*

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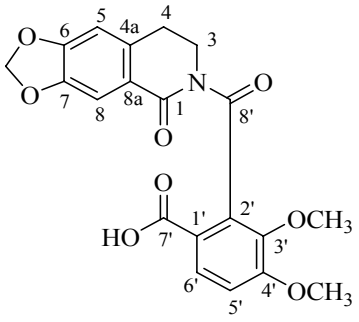
Abstract —Two isoquinolones, anhydroberberillic acid (**1**) and methyl anhydroberberillate (**2**) were isolated first time from the roots of *Phellodendron amurense* var. *wilsonii* as well as two related known compounds , berberine (**3**) and 8-oxyberberine (**4**). Their structures were elucidated by extensive 1D, 2D NMR and MS spectral analysis. Occurrence of the isoquinolones and protoberberines in the same plant indicated the definite possibility of these metabolites biogenetically originated from berberine alkaloids.

INTRODUCTION

There are about 10 species in the genus *Phellodendron*, distributed widely over the tropical and subtropical areas of Asia. The thick corky bark of *Phellodendron* has a long history of ethnobotanical use among native peoples in East Asia.[1] Diabetes mellitus, meningitis, pneumonia, anti stomachic, intestinal function control, anti-inflammatroy, anti bacterial, anti-psychic, heat relief, bacillary dysentery, diarrhea, tuberculosis and liver cirrhosis treatments are among the indications listed for Huangbai, the bark of *Phellodendron amurense* (amur cork tree).[2-6] Previous studies on the bark of *Phellodendron* species have shown the presence of alkaloids belonging to various classes such as isoquinolines,[3] quinolinones,[7] furoquinolines,[8] canthinones,[9] benzylisoquinolines,[10] protoberberines,[3,11] indolopyridoquinazolines[8,12] and chlorophylls.[13] Phenolic compounds,[10] flavonoids,[14-16] coumarins,[13] and lignans[10] have also been isolated from the bark and leaves together with phytosterols and limonoidal triterpenes.[17-19] *Phellodendron*

amurense var. *wilsonii*, is a variable species distributed widely in the northern and central parts of Taiwan.[20] It is a deciduous tree belonging to the family Rutaceae. In continuation of our investigation on the chemical components of *Phellodendron* species, we reported previously dihydroflavonoids, coumarins and chlorophylls from the leaves of titled plant.[13] The present work on the roots of this plant has resulted in the isolation and characterization of two new isoquinolones (**1**-**2**) along with two known compounds (**3**-**4**). This paper describes the structural elucidation of the new isoquinolones together with their possible biogenetic pathway from the berberine alkaloids.



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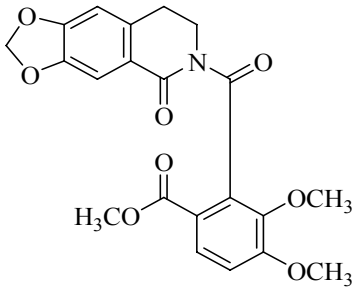

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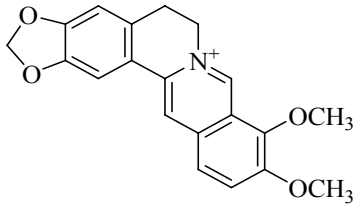

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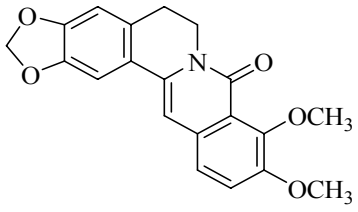

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Figure 1. Structures of Compounds (**1**-**4**)

RESULTS AND DISCUSSION

The air-dried and powdered leaves of *P. amurense* var. *wilsonii* were extracted with hot methanol and concentrated to give a dark brown syrup and a residual solid. This solid was crystallized in MeOH to afford the yellow crystals (**3**). A series of chromatographic separation of the remaining residue resulted in the isolation of **1**, **2** and **4**. The structures of new compounds (**1**) and (**2**) were elucidated on the basis of spectroscopic evidence as follow.

Anhydroberberillic acid (**1**) was obtained as white powder. The HREIMS spectrum showed a molecular ion peak at *m/z* 399.0954 corresponding to the molecular formula $C_{20}H_{17}NO_8$. It gave a positive reaction with Dragendorff's reagent indicating it to be an alkaloid. The UV absorption maxima at 224, 254, 299 and 335 nm were characteristic of isoquinolone type alkaloid with highly conjugated system.[21] The IR absorptions at 3528 and 1708 cm^{-1} indicated the presence of hydroxyl and carbonyl groups, respectively.

The ^{1}H-NMR spectrum contained two aromatic singlets at δ 7.26 (H-8) and 6.70 (H-5) together with two aliphatic triplets at δ 3.77 and 3.18 (each 2H, J = 6.3 Hz) for H-3 and H-4 and a two protons singlet δ 6.01, and its connectivity with a carbon at δ 101.9 in HMQC spectrum for a methylene-dioxy group represents 6,7-methylenedioxytetrahydroisoquinolone basic skeleton for **1**. This was further confirmed by the existence of NOEs spectrum from H-4 to H-3 and H-5 and ^3J-correlations of H-8 with a carbonyl carbon at δ 167.7 (C-1) and C-6 (δ 149.8); H-3 with C-4a (δ 136.4) in the HMBC spectrum. The ^{1}H-NMR spectrum of **1** also revealed the presence of the two *ortho*-coupled aromatic protons at δ 7.48 and 7.31 (each 1H, J = 8.1 Hz) as well as two methoxyl groups at δ 3.90 and 3.88 (each 3H, s). These resonances were confirmed to be those of a 2-carbonyl-3,4-dimethoxybenzoic acid moiety from long range correlations observed from H-5' to C-1' and C-3', H-6' to C-2', C-4' and a carboxylic carbon at δ 167.0 in the HMBC spectrum. The correlations between H-3 of 6,7-methylenedioxytetrahydroisoquinolone unit and carbonyl carbon (δ165.6) of 2-carbonyl-3,4-dimethoxybenzoic acid moiety confirmed that the two parts of the molecule were connected through the amide linkage. Thus the structure (**1**) was established for anhydroberberillic acid. The identification of **1** was also accomplished by the comparison of spectral data with those of reported synthetic sample.[22] Although **1** has been synthesized by Monito *et al.*, this represents its first isolation from a natural source.

Table 1 ^{1}H-NMR and ^{13}C-NMR spectral assignments for the compounds (**1**, **2**) (ppm, J in Hz)[a,b]

	1			**2**		
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
1		167.7			163.8	
3	3.77 t (6.3)	39.0	C-6, C-4a	4.26, 4.44 m	41.7	C-6, C-4a
4	3.18 t (6.3)	32.5	C-4, C-5 C-8a, C-4a	3.02 dd (6.4, 5.2)	27.6	C-4, C-5, C-8a, C-4a
4a		136.4			136.9	
5	6.70 s	111.1	C-4, C-6, C-7, C-8a	6.67 s	107.0	C-4, C-6, C-7
6		149.8			151.9	
7		145.9			147.2	
8	7.26 s	110.2	C-1, C-6, C-7, C-4a	7.38 s	108.8	C-1, C-6, C-7, C-4a
8a		124.0			122.6	
OCH_2O-6,7	6.01 s	101.9	C-6, C-7	5.99 s	101.7	C-6, C-7
1'		124.2			118.6	

2’		122.0			136.6	
3’		145.9			143.9	
4’		157.6			156.2	
5’	7.31 d (8.1)	116.9	C-1’, C-3’, C-4’	6.93 d (9.0)	110.9	C-1’, C-3’, C-4’
6’	7.48 d (8.1)	119.5	C-2’, C-4’, C-7’	7.82 d (9.0)	127.0	C-2’, C-4’, C-7’
7’		167.0			165.6	
8’		165.6			168.8	
3’-OCH_3	3.88 s	62.0	C-3’	3.77 s	61.1	C-3’
4’-OCH_3	3.90 s	56.8	C-4’	3.92 s	55.8	C-4’
7’-OCH_3				3.78 s	52.0	C-7’

[a] Assignments were made with the aid of HMQC, HMBC, COSY and NOESY spectra

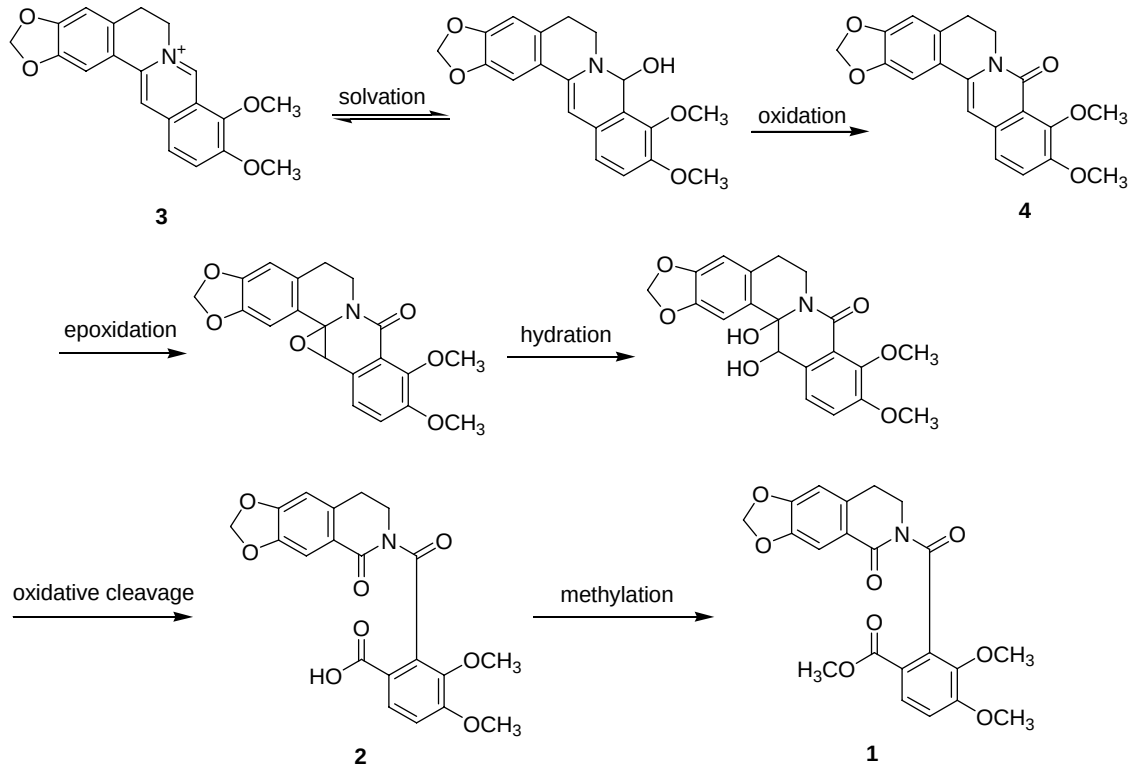
[b] Recorded in $CDCl_3$

Methyl anhydroberberillate (**2**) was obtained as white powder. The HREIMS spectrum showed a molecular ion peak at *m/z* 413.1111 consistent with the molecular formula $C_{21}H_{19}NO_8$. It responds positively with Dragendorff’s reagent suggesting that **2** is also an alkaloid. Compound (**2**) also gave UV spectrum indicative of a highly conjugated system and implied the presence of a chromophore very similar to that present in compound (**1**). The IR absorptions at 1708 and 1686 cm^{-1} indicated the presence of the conjugated amide and an ester carbonyl groups, respectively. The ^1H and ^{13}C NMR spectra of **2** included a subset of resonances corresponding to the 6,7-methylenedioxy tetrahydroisoquinolone moiety of **1**, and the remaining resonances for the other part of the molecule with an exception that the presence of an additional methoxyl group at δ_H 3.78 and δ_C 52.0. Finally, from the ^3J-HMBC connectivity of these methoxyl protons with an ester carbonyl at δ 165.6, it was apperent that the compound (**2**) was a methyl ester of **1**. Thus the structure (**2**) was established for methyl anhydroberberillate. The spectral data of **2** were in good agreement with those of synthetic sample.[22] However, it is the first time that it was isolated from a natural source. The full assignment of the ^1H and ^{13}C NMR resonances of **1** and **2** (Table **1**) was made using COSY, NOESY, HMQC and HMBC experiments.

In addition, two known alkaloids, berberine (**3**)[23] and 8-oxyberberine (**4**)[24] were also isolated and identified by the comparison of their spectroscopic data with those of authentic samples. Since we have obtained alkaloids (**1**-**4**) from the same source, *P. amurense var. wilsonii*, it was considered as a definite possibility that these metabolites were derived biogenetically from berberine (**3**) in general alkaloid catabolic process. The presence of the isoquinolone derivatives (**1**) and (**2**) in *P. amurense var. wilsonii* is

consistent with the previous works showing that this species is rich in alkaloids.[8-12] However, the biogenetic origins of isoquinolone alkaloids are still a matter of debate, although they were likely to be oxidative products of structurally more complex isoquinoline alkaloids.[25] Krane and Shamma suggested two possible routes for their formation, either as naturally occurring oxidation products of benzylisoquinolines or as the result of *in vivo* oxidation of protoberberines, phthalideisoquinolines or spirobenzylisoquinolines.[26] On the basis of this assumption, a possible biogenetic pathway of **1** and **2** from berberine (**3**) and relationships for these alkaloids (**1**-**4**) was proposed as shown in Scheme **1**. Anhydroberberillic acid (**1**) might be produced from berberine (**3**) and 8-oxyberberine (**4**) through intermediates (**5**-**7**). This pathway involves oxidation, epoxidation, hydration, oxidative cleavage and finally esterification.

Scheme 1. Possible biogenetic pathway of **1** and **2** from beberine



EXPERIMENTAL

General Experimental Procedures. Melting points were recorded on Yanaco MP-S3 melting point apparatus without correction. UV spectra obtained on a Hitachi UV-3210 spectrophotometer. IR spectra were determined in KBr discs on a Shimadzu FT-IRPrestige 21. ^{1}H, ^{13}C, HMQC, HMBC, and NOESY NMR spectra were measured on Bruker Avance NMR 300 spectrometer, using tetramethylsilane (TMS) as internal standard; all chemical shifts were reported in ppm (δ). All MS and HRMS spectra (EI) were obtained on a VG-70-250S mass spectrometer.

Plant Material. The roots of *P. amurense* var. *wilsonii* were collected in August, 2000 from Chiayi, Taiwan, and authenticated by Prof. C. S. Kuoh. A voucher specimen of the plant (TSWu 20000919) has been deposited at the herbarium of National Cheng Kung University, Tainan, Taiwan.

Extraction and Isolation. The air-dried and powdered roots (4.0 kg) of *P. amurense* var. *wilsonii* were extracted with methanol (5 L x 8) for 8 h at 70℃ and the extract was concentrated to give a dark brown syrup and residual solid (45 g). This residue was crystallized in MeOH to afford the yellow crystals (**3**) (6.92 g). The remaining residue (38 g) was chromatographed over silica gel using the gradients of chloroform and methanol to afford 10 fractions. Fraction 5 (80 mg) was rechromatographed over silica gel using mixture of *n*-hexane and ethyl acetate (9:1) as eluents, and purified by preparative TLC (silical gel, disopropyl ether:acetone, 9:1) to yield **2** (15.8 mg). Fraction 7 (20 mg) on column chromatography over silica gel with diisopropyl ether and acetone (9:1) yielded **4** (2.9 mg). Fraction 8 (150 mg) was chromatographed over silica gel using a gradient of chloroform and acetone (9:1) to afford **1** (29.4 mg).

Anhydroberberillic acid (1): $C_{20}H_{17}NO_8$, white powder, mp: 252-253°C (MeOH); UV λ_{max} (MeOH) (log ε) nm: 224, 254, 299, 335; IR (KBr) ν_{max} cm^{-1}: 3502 (OH), 1708 (C=O), 1606; EIMS *m/z* (*rel. int.* %): 399 (M+, 22), 220 (100), 206 (13), 192 (77); HR-EIMS *m/z* 399.0954 (M^+) (calcd for $C_{20}H_{17}NO_8$, 399.0954).

Methyl anhydroberberillate (**2**): $C_{21}H_{19}NO_8$, white powder, mp: 183-184°C (MeOH); UV λ_{max} (MeOH) (log ε) nm: 224, 260, 316; IR (KBr) ν_{max} cm^{-1}: 1708 (C=O), 1686 (C=O), 1597; EIMS *m/z* (*rel. int.* %): 413 (M+, 23), 382 (48), 354 (24), 223 (100); HR-EIMS *m/z* 413.1111 (M^+) (calcd for $C_{21}H_{19}NO_8$, 413.1111).

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