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Alkaloids from the Stem Bark of *Phellodendron amurense* Rupr.

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Two isoquinolines and one quinolone were isolated from the stem bark of *Pellodendron amurense* Rupr. (Rutaceae). Two isoquinolines were elucidated as thalifoline (**2**) and pharmacological active berberine (**3**) has been blocking the release of Ca^{2+} from internal stores. One quinolone was identified as *N*-methylatanine (**1**). This is the first report on the isolation of *N*-methylatanine (**1**) and thalifoline (**2**) from this plant.

Key words – *Phellodendron amurense* Rupr., Rutaceae, alkaloid, *N*-methylatanine, thalifoline

Phellodendron amurense Rupr. belongs to the family Rutaceae, is native to an oriental country. The bark of this plant is used as a stomachic for intestinal function control, antipyretic, and anti-inflammatory. Recently, the following biological activities were reported on the extracts of this plant as anti-inflammatory[19,23], anti-gastric ulcer[17,20] and repression for experimental hypertension in rats[1]. The major chemical constituents of the crude extract reported so far are alkaloids and limonoids. Alkaloids have been the target of increased chemical and biological interest because some of their members have been found to possess potent biological activities[16]. In addition to, among those compounds, the isoquinoline and quinoline alkaloids showed a potential antimalarial activity, antiprotozoan parasites, and antiplasmodial activity[7,14,18]. Especially, berberine (**3**), an isoquinoline alkaloid has been shown to have pharmacological actions including anticerebral ischemic, inhibition of thromboxame A2 synthesis, antitumor, antibiotic, antiarrhythmic, and antidiarrheal activities[8,9,12,13,22]. Thalifoline (**2**) is an important component such as baluchistanamine and (-)-tejedine[5], which presumed to result from the biochemical oxidation of the more prevalent alkaloid components[3,4]. Also, owing to two alkaloids (**1** and **2**) was present in only minor amounts from natural sources, these compounds were attracted a considerable amount of interest by synthetic chemist in recent years[10,15,21]. These biological activities encouraged us to have examined the stem bark from *P. amurense*. In this paper, we report on the isolation and structural elucidation of three alkaloids, *N*-methylatanine (**1**), thalifoline (**2**) and berberine (**3**). Two alkaloids (**1** and **2**) have been isolated from this plant for the first time.

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Materials and Methods

Materials

The stem bark of *Phellodendron amurense* Rupr. was collected in July 2003, in Gyeongsangnam-do Southern Forest Research Center (SFRC), jinju province of Korea and identified by Gyeongsangnam-do Southern Forest Research Center.

Instruments

Melting points were measured on a Thomas Scientific Capillary Melting point Apparatus and are uncorrected. IR spectra were recorded on a Bruker IFS66 infrared Fourier transform spectrophotometer (KBr) and UV spectra were measured on a Beckman DU650 spectrophotometer. ^{1}H- and ^{13}C-NMR at 500 MHz, 125 MHz, respectively and 2D-NMR data were obtained on a Bruker AM 500 spectrometer in $CDCl_3$ and CD_3OD. EIMS were obtained on a JEOLJMS-700 mass spectrometer.

Extraction and Isolation

The air-dried stem bark of *P. amurense* (2.0 kg) were cut into pieces and were extracted at room temperature with MeOH (5L×3) for seven days, and then the methanolic extract was evaporated *in vacuo* to give a crude extract (300 g). The concentrated extract was suspended in water:MeOH (9:1) mixture and extracted successively with hexane (1L), $CHCl_3$ (1L), and BuOH (1.5 L). The $CHCl_3$ extract (40 g) was chromatographed over silica gel using hexane:EtOAc and $CHCl_3$:MeOH gradient to give 16 fractions (P1-P16). Fraction P7 (1.9 g) was submitted to a silica gel column chromatography eluted with hexane-EtOAc gradient (20:1→2:1) re-

sulting in 10 subfraction. Subfraction 4~5 was rechromatographed on silica gel with hexane-EtOAc gradient (25:1→6:1) to yield *N*-methylatanine **1** (14 mg). Fraction P11 (2.5 g) was chromatographed over silica gel as stationary phase using $CHCl_3$:acetone gradient (30:1→2:1) as mobile phase to afford 12 subfractions. Subfractions 10~12 was chromatographed on a silica gel column eluted with $CHCl_3$:acetone gradient (15:1→2:1) to give thalifoline **2** (16 mg). The BuOH extract (35 g) was chromatographed on silica gel eluting with $CHCl_3$:MeOH and 6 fraction were collected on the basis of TLC profiles. Fraction 5~6 was subjected to silica gel column chromatography $CHCl_3$:MeOH (40:1→1:1) to give 8 subfractions. The subfraction 4~7 was chromatographed on a silica gel column eluted with $CHCl_3$:MeOH (10:1→1:1) to give berberine **3** (580 mg).

***N*-Methylatanine (1)**: colorless crystals; mp 128-131℃; UV (MeOH) λ_{max} 232, 273, 280, 338 nm; MS *m/z*: 257 (M^+), 242, 226, 214; IR (KBr) ν_{max} 2950, 1630 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.70 (3H, s, H-4′), 1.82 (3H, s, H-5′), 3.41 (2H, d, *J* = 6.0 Hz, H-1′), 3.70 (3H, s, N-CH_3), 3.91 (3H, s, -OCH_3), 5.26-5.29 (1H, m, H-2′), 7.22 (1H, m, H-6), 7.32 (1H, m, H-8), 7.51 (1H, m, H-7), 7.80 (1H, m, H-5); ^{13}C NMR data (Table 1).

Thalifoline (2): colorless needles; mp 208-210℃; UV (MeOH) λ_{max} 224, 263, 305 nm; MS *m/z*: 207 (M^+), 164, 136; IR (KBr) ν_{max} 3436, 1643 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.91 (2H, t, *J* = 6.7 Hz, H-4), 3.12 (3H, s, N-CH_3), 3.52 (2H, t, *J* = 6.7 Hz, H-3), 3.91 (3H, s, OCH_3), 6.10 (1H, s, H-7-OH), 6.60 (1H, s, H-5), 7.72 (1H, s, H-8); ^{13}C NMR data (Table 1).

Berberine (3): yellow needles; mp 210-213℃; UV (MeOH) λ_{max} 222, 265, 349, 429 nm; MS *m/z*: 337 (M^+), 321, 320, 278; IR (KBr) ν_{max} 3423, 2923, 2360, 1638 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 3.26 (1H, t, *J* = 6.5 Hz, H-5), 4.11 (3H, s, H-9′), 4.21 (3H, s, H-10′), 4.93 (2H, t, *J* = 6.3 Hz, H-6), 6.10 (2H, H-2′), 6.95 (1H, s, H-4), 7.65 (1H, s, H-1), 7.99 (1H, d, *J* = 9.1 Hz, H-12), 8.10 (1H, d, *J* = 9.1 Hz, H-11), 8.69 (1H, s, H-13), 9.76 (1H, s, H-8); ^{13}C NMR data (Table 1).

Results and Discussions

The MeOH extract obtained from stem bark of *P. amurense* was fractionated into hexane, $CHCl_3$, BuOH layer through solvent fractionation, two isoquinolines and one quinolone were isolated by the repeated chromatographic separation of $CHCl_3$ and BuOH fractions. Two isoquinolines were eluci-

Table 1. ^{13}C NMR of compound **1**, **2**, and **3** at 125 MHz (ppm, m)[a]

Position	Compounds		
	1	2	3
1		164.9 s	106.6 d
2	163.9 s		150.0 s
3	121.6 s	48.5 t	152.1 s
4	160.2 s	27.6 t	109.4 d
5	123.4 d	108.8 d	28.3 t
6	121.8 d	149.6 s	57.3 t
7	130.1 d	144.7 s	
8	114.1 d	114.5 d	146.4 d
9			145.9 s
10			152.2 s
11			128.2 d
12			124.5 d
13			121.5 d
1a			121.9 s
4a	117.8 s	130.8 s	132.0 s
8a	139.0 s	122.6 s	123.4 s
12a			135.3 s
13a			139.7 s
1′	24.3 t		
2′	122.5 d		103.7 t
3′	132.4 s		
4′	18.0 q		
5′	25.7 q		
9′(OCH_3)			57.7 q
10′(OCH_3)			62.6 q
OCH_3	61.7 q	56.0 q	
NCH_3	29.7 q	35.2 q	

[a]The chemical shifts of compound **1** and **2** were determined in $CDCl_3$. Compound **3** was measured in CD_3OD; multiplicity by DEPT.

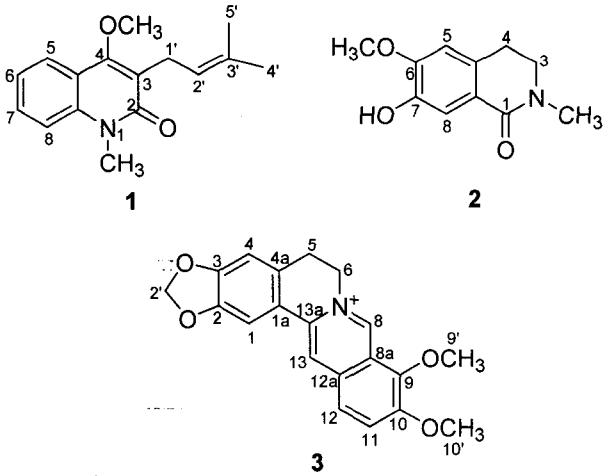
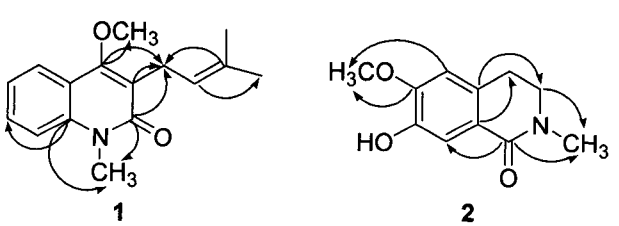


Fig. 1. Structures of compounds **1-3**.

dated as thalifoline (**2**) and berberine (**3**). One quinolone was identified as *N-methylatanine* (**1**). Among the isolated compounds, this is the first report of the isolation of *N*-methylatanine (**1**), thalifoline (**2**) from *P. amurense*.

Compound **1** was obtained as colorless crystals. IR spectrum showed absorption bands due to carbonyl (1630 cm^{-1}) group and the mass spectrum exhibited an ion at *m/z* 257 (M^+). The ^1H NMR spectrum indicated the presence of one methoxy proton at δ 3.90, one *N*-methyl proton at δ 3.70, two methyl protons at δ 1.70 and 1.82, one methylene proton at δ 3.41 (d, *J* = 6.0 Hz), one methine proton at δ 5.29~5.26, and four aromatic protons at δ 7.22 (m, H-6), 7.32 (m, H-8), 7.51 (m, H-7) and 7.80 (m, H-5). The ^{13}C-NMR and DEPT experimentals showed sixteen carbons which revealed one methoxy carbon, one *N*-methyl carbon, two methyl carbons, five methine carbons, one methylene carbon, as well as six quaternary carbons including carbonyl carbon. The 3,3-dimethylallyl group was determined on the basis of successive connectivities from C-1′ to C-5′ in ^1H-^1H COSY spectrum. The HMBC correlation of H-1′ with C-2 and C-4, and H-2′ with C-3 allowed 3,3-dimethylallyl group to site at C-3 (Fig. 2). On the basis of these spectral data and careful analysis of the HMQC and HMBC data, this compound **1** was identified as a *N*-methylatanine[6,15]. This compound has been isolated for the first time from this plant. Compound **2** was obtained as colorless needles. The IR spectrum showed presence of hydroxyl group at 3436 cm^{-1} and carbonyl group at 1643 cm^{-1}. The EIMS spectrum showed a molecular ion peak at *m/z* 207. The ^1H NMR spectrum showed one *N*-methyl singlet proton at δ 3.12 (N-CH_3) and one methoxy singlet proton 3.91 (OCH_3), two aromatic singlet protons (δ 6.60 and 7.73), two methylene protons (δ 2.91 and 3.52, both t, *J* = 6.7 Hz) and aromatic hydroxyl (δ 6.1). The ^{13}C-NMR and DEPT experiments showed the presence of eleven carbon signals for one methoxy carbon (δ 149.6), one *N*-methyl carbon (δ 35.2), two methylene carbons (δ 27.6 and 48.5), one carbonyl carbon (δ 164.9), two methine carbons (δ 108.8 and 114.5), and four quaternary carbons (δ 122.6, 130.8, 144.7, and 149.6). In the HMBC spectrum, the following correlations appeared: aromatic singlet proton at δ 7.73 (H-8) with C-4a, C-6, and C-1, methoxyl proton at δ 3.91 (OCH_3) with C-5 and C-6 and *N*-methyl proton at δ 3.12 (NCH_3) with C-1 and C-3 (Fig. 2). On the basis of the above mentioned data, structure of compound **2** was determined as thalifoline [5,10]. Also, this compound has been isolated for the first time from this plant. Compound **3** was obtained as yellow needles and in the EIMS, the molecular ion peak showed at at *m/z* 337. UV spectrum showed absorption bands at 222, 265, 349, 429 nm. The ^1H NMR spectrum revealed six aromatic methine protons at δ 6.95 (H-4), 7.65 (H-1), 7.99 (d, *J* = 9.1 Hz, H-12), 8.10 (d, *J* = 9.1 Hz, H-11), 8.69 (H-13), and 9.76 (H-8), two methylene protons at δ 3.26 [(t, *J* = 6.5 Hz, H-5), 4.93 (t, *J* = 6.3 Hz, H-6)], and two methoxy groups at δ 4.11 and 4.21. The ^{13}C-NMR and DEPT experiments showed twenty carbons, which revealed two methoxy carbons at δ 57.7 and 62.6, six aromatic methine carbons at δ 109.4, 106.6, 124.5, 128.0, 121.5, and 146.4, three methylene carbons at δ 28.3, 57.3, and 103.7 and nine quaternary carbons at δ 121.9, 123.4, 131.9, 135.3, 139.7, 145.9, 150.0, 152.1, and 152.2. Analysis of HMQC and HMBC spectra allowed the unequivocal assignment of all carbons. All data mentioned above indicate that the structure of compound **3** was berberine[2,11].



1 **2**

Fig. 2. Important HMBC correlations of compounds **1** and **2**.

P. amurense led to the isolation of two isoquinoline alkaloids thalifoline (**2**) and berberine (**3**) and one quinolone alkaloid *N-methylatanine* (**1**). The Rutaceae alkaloids have possessed various biological activities. Expecially, pharmacological actions of berberine (**3**) have been blocked the release of Ca^{2+} from internal stores. Therefore, the isolated alkaloids from *P. amurense* can be useful as a potentially applicable for various biological activities.

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References

1. Arakawa, K., Y. Otsuka and J. Cyong. 1985. Inhibition of the metyrapone and heat-stress induced hypertension by the phellodendri cortex on rats. *Shouyakugaku Zasshi* **39**, 162-164.

2. Blasko, G., G. A. Cordell, S. Bhamarapravati and C. W. W. Beecher. 1988. Carbon-13 NMR assignments of berberine and sanguinarine. *Heterocycles* **27**, 911-916.
3. Cava, M. P., K. Bessho, B. Douglas, S. Markey and J. A. Weisbach. 1966. Hernandia alkaloids. II. Hernandaline, a new, elaborated aporphine structural type. *Tetrahedron Lett.* **36**, 4279-4282.
4. Cava, M. P. and K. T. Buck. 1969. Some synthetic studies in the isoquinoline series. *Tetrahedron* **25**, 2795-2805.
5. Doskotch, R. W., JR. P. L. Schiff and J. L. Beal. 1969. Two new alkaloids from *T. Minus* Var. *Adiantifolium*: noroxyhydrastinine and thalifoline. *Tetrahedron* **25**, 469-475.
6. Fauvel, M. T., J. Gleye, C. Moulis, F. Blasco and E. Stanislas. 1981. Alkaloids and flavonoids of *Melicope indica*. *Phytochemistry* **20**, 2059-2060.
7. Fischer, D. C. H., N. C. A. Gualda, D. Bachiega, C. S. Carvalho, F. N. Lupo, S. V. Bonotto, M. O. Alves, A. Yogi, S. M. D. Santi, P. E. Avila, K. Kirchgatter and P. R. H. Moreno. 2004. In vitro screening for antiplasmodial activity of isoquinoline alkaloids from Brazilian plant species. *Acta Tropica* **92**, 261-266.
8. Huang, C. G., Z. L. Chu, S. J. Wei, H. Jiang and B. H. Jiao. 2002. Effects of berberine on arachidonic acid metabolism in rabbit platelets and endothelial cells. *Thrombosis Research* **106(4-5)**, 223-227.
9. Huang, W. M., Z. D. Wu and Y. Q. Gan. 1989. Effects of berberine on ischemic ventricular arrhythmia. *Zhonghua Xin Xue Guan Bing Za Zhi* **17(5)**, 300-301.
10. Irie, H., A. Shiina, T. Fushimi, J. Katakawa, N. Fujii and H. Yajima. 1980. New synthesis of isoquinoline alkaloids, thalifoline, corypalline, and cherylline. *Chem. Lett.* 875-878.
11. Kim, J. P., M. Y. Jung, J.-P. Kim and S. Y. Kim. 2000. Antiphotooxidative activity of protoberberines derived from *Coptis japonica* Makino in the chlorophyll-sensitized photooxidation of oil. *J. Agric. Food Chem.* **48**, 1058-1063.
12. Mirska, I., H. Kedzia, Z. Kowalewski and W. Kedzia. 1972. The effect of berberine sulfate on healthy mice infected with *Candida albicans*. *Arch. Immunol. Ther. Exp. (Warsz)* **20(6)**, 921-929.
13. Nishino, H., K. Kitagawa, H. Fujiki and A. Iwashima. 1986. Berberine sulfate inhibits tumor-promoting activity of teleocidin in two-stage carcinogenesis on mouse skin. *Oncology* **43(2)**, 131-134.
14. Park, B. S., D. Y. Kim, P. J. Rosenthal, S. C. Huh, B. J. Lee, E. J. Park, S. M. Kim, J. E. Kim, M. H. Kim, T. L. Huh, Y. J. Choi, K. H. Suh, W. S. Choi and S. E. Lee. 2002. Synthesis and evaluation of new antimalarial analogues of quinoline alkaloids derived from *Cinchona ledgeriana* Moens ex Trimen. *Bioorg. Med. Lett.* **12**, 1351-1355.
15. Ramesh, M. and P. Shanmugam. 1984. A convenient synthesis of the naturally occurring alkaloid N-methylatanine. *Indian J Chem.* **23B**, 110-113.
16. Suffness, M. and G. A. Cordell. 1985. pp. 178-188, Brossi (eds.), *The Alkaloids*, Vol. XXL, Academic Press Inc., NewYork.
17. Takase, H., K. Imanishi, O. Miura, E. Yumioka and H. Watanabe. 1989. Features of the anti-ulcer effects of Orengedoku-to (a traditional Chinese medicine) and its component herb drugs. *Jpn J. Pharmacol.* **49**, 301-308.
18. Tempone, A. G., S. E. Treiger Borborema, H. F. de Andrade Jr, N.C. de Amorim Gualda, A. Yogi, C. Salerno Carvalho, D. Bachiega, F. N. Lupo, S. V. Bonotto and D. C. H. Fischer. 2005. Antiprotozoal activity of Brazilian plant extracts from isoquinoline alkaloid-producing families. *Phytomedicine* **12**, 382-390.
19. Uchiyama, T., H. Kamikawa and Z. Hagita. 1989. Anti-inflammatory effect of extract from phellodendri cortex. *Wakan Iyaku Gakkaishi* **6**, 158-164.
20. Uchiyama, T., H. Kamikawa and Z. Ogita. 1989. Anti-ulcer effect of extract from phellodendri cortex. *Yakugaku Zasshi* **109**, 672-676.
21. Wang, Y.-C. and P. E. Georghiou. 2002. An efficient synthesis of thalifoline. *Syntheis* 2187-2190.
22. Yamamoto, K., H. Takase, K. Abe, Y. Saito and A. Suzuki. 1993. Pharmacological studies on antidiarrheal effects of a preparation containing berberine and geranii herba. *Nippon Yakurigaku Zasshi* **101(3)**, 169-175.
23. Zasshi, Y. 1970. Screening test of anti-inflammatory action on the constituents of crude-drugs and plants. *Yakugaku Zasshi* **90**, 782-783.

초록 : 황백나무로부터 생리활성물질인 alkaloids 화합물의 분리 및 탐색

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운향과(Rutaceae)인 황백나무(*Phellodendron amurense* Rupr.)의 줄기 껍질에서 2종의 isoquinoline 화합물과 1종의 quinolone 화합물을 분리하였다. Isoquinoline 화합물은 thalifoline (**2**)과 칼슘 방출을 저해하여 혈압조절에 관여하는 berberine (**3**)으로 구조동정 되었다. 또한 quinolone 화합물은 *N*-methylatanine (**1**)로 구조동정 되었다. 이들의 화합물 중에서 *N*-methylatanine (**1**)과 thalifoline (**2**)는 황백나무에서 처음으로 분리하였다.