Natural Product Sciences
26(2) : 171-175 (2020)
https://doi.org/10.20307/nps.2020.26.2.171

NMR Assignments of Rotameric Aporphine Alkaloids from *Liriodendron tulipifera*

InWha Park[1,2] and MinKyun Na[1,*]

[1]*College of Pharmacy, Chungnam National University, Daejeon 34134, Republic of Korea*
[2]*Natural Product Informatics Research Center, Korea Institute of Science and Technology (KIST) Gangneung Institute, Gangneung 25451, Republic of Korea*

Abstract – *Liriodendron tulipifera*, belonging to the family Magnoliaceae, is commonly called tulip tree. Four *N*-acetylated aporphine alkaloids, *N*-acetylnornuciferine (**1**), *N*-acetylanonaine (**2**), *N*-acetyl-3-methoxynornuciferine (**3**), and *N*-acetyl-3-methoxynornantenine (**4**) were isolated from the roots of *L. tulipifera.* Although the purity of each compound (**1 - 4**) was determined to be 97, 96, 99, and 98%, respectively, the ^{1}H and ^{13}C NMR spectroscopic data of the aporphine alkaloids **1 - 4** displayed all signals in duplicate, indicating the presence of two rotamers due to restricted rotation of *N*-$COCH_3$ functionality in solution status. The absolute configurations of **1 - 4** were established by measuring specific rotation and comparison with the reported data. This is the first report on the ^{1}H and ^{13}C NMR assignments of *N*-acetyl-3-methoxynornuciferine (**3**) and *N*-acetyl-3-methoxynornantenine (**4**). This study provides advanced NMR spectroscopic data for the structure determination of rotameric aporphine alkaloids.
Keywords – *Liriodendron tulipifera*, Magnoliaceae, Rotameric aporphine alkaloid, NMR assignment

Introduction

Aporphine alkaloids possess a tetracyclic skeleton. In the family Magnoliaceae, they are found in the genera of *Liriodendron, Elmerrillia, Magnolia, Michelia, Talauma,* and *Tsoongiodendron*.[1] Many studies showed that naturally occurring aporphine alkaloids exhibited diverse range of therapeutic potential, such as antibacterial, antiviral, antioxidant, and anticancer activities.[1,2]

Liriodendron is a genus in the subfamily Liriodendroidae (family Magnoliaceae) that encompasses only two species, *L. tulipifera* and *L. chinense. Liriodendron tulipifera*, a hardwood, is commonly called tuliptree or yellow-poplar. This plant, indigenous to the Northern United States, is cultivated for ornamental tree in Korea. The bark of *L. tulipifera* was utilized by the Native Americans as a febrifuge to treat the intermittent fevers associated with malaria.[3] The aporphine alkaloids, particularly liriodenine, anonaine, and oxoglaucine are considered as the major bioactive constituents of *L. tulipifera* based on their antiarrhythmic,[4] anticancer,[5] antiplasmodial[3] properties. *N*-Acetylated aporphine alkaloids such as tuliferoline exist in two isomeric forms due to rotation of *N*-$COCH_3$ functionality. In the ^{1}H and ^{13}C NMR spectroscopic data of these aporphine alkaloids, the spectra displayed all signals in duplicate, indicating the presence of two rotamers, which occur due to restricted rotation of the *N*-acetyl group. Four rotameric aporphine alkaloids (**1 - 4**) were afforded from the roots of *L. tulipifera*. To date, there are a few reports on the structure determination of *N*-acetyl aporphine alkaloids.[6-9] However, the NMR spectroscopic data assignments on each *E*- and *Z*-rotamer of *N*-acetyl aporphine alkaloids are limited.[6] The structures were elucidated by NMR and MS spectroscopic data interpretation. Absolute stereochemistry was identified by specific rotation measurement. This report details the isolation and structure elucidation of compounds **1 - 4**.

Experimental

General experimental procedures – Specific rotations were measured on a JASCO DIP-370 polarimeter (Tokyo, Japan). UV spectra were recorded on a Shimadazu SPD-M20A PDA detector. NMR spectroscopic data were recorded on a Bruker DMX 600 (^{1}H-600 MHz, ^{13}C-150 MHz) spectrometers equipped with a 5 mm direct detection BBFO probe. All NMR experiments were performed at 294 K, using $CDCl_3$ as the solvent. Chemical shifts

*Author for correspondence
MinKyun Na, College of Pharmacy, Chungnam National University, Daejeon 34134, Republic of Korea.
Tel: +82-42-821-5925; E-mail: mkna@cnu.ac.kr

were given on the δ scale and referenced by $CDCl_3$ as an internal standard (δ_H = 7.26, δ_C = 77.2). Coupling constants (*J*) are in Hz. Data processing was carried out with MestReNova v6.0.2 program. Precoated TLC silica gel 60 F_{254} and RP-18 F_{254} plates from Merck was used for analytical purposes followed by detection under UV light at 254 and 365 nm wavelengths or after spraying with 10% sulfuric acid and dragendorff reagents. VLC was implemented on Merck silica gel (70 - 230 mesh), and MPLC (Biotage Isolera™) was carried out utilizing silica (KP-Sil, Biotage, Biotage, Uppsala, Sewden) and C18 (KP-C18-HS, Biotage, Uppsala, Sewden) cartridges. Preparative HPLC was accomplished on a Gilson system (PLC 2020) with a flow rate of 5.0 mL/min using a Phenomenex kinetex biphenyl column (250 × 21.2 mm, 5 μm), a Hector C_{18} column (250 × 21.2 mm, 5 μm), or Hector phenyl column (250 × 21.2 mm, 5 μm).

Plant materials – The roots of *Liriodendron tulipifera* were collected from Chungnam National University in August 2016. A voucher specimen (CNU201608) has been deposited at the Laboratory of Pharmacognosy of College of Pharmacy, Chungnam National University, Daejeon, Korea.

Extraction and isolation – The EtOH extract (239.3 g) of *L. tulipifera* roots was subjected to silica gel VLC and eluted with a stepwise gradient of *n*-hexane-EtOAc (8:2, 5:5, 3:7, 1:9; each step 6 L) and $CHCl_3$-MeOH (9:1, 7:3, 5:5, 2:8; each step 6 L; final washing with 10 L of 100% MeOH) to produce seven fractions (LT1 ~ LT7). Fraction LT4 (20.7 g) was separated by MPLC (Biotage SNAP cartridge, KP-C18-HS, 400 g) using a step gradient mixures of MeOH-H_2O (5:5, 7:3, 9:1, 10:0, MeCN 100%) to yield **1** (300.4 mg) and six subfractions (LT4-1 ~ LT4-6). LT4-5 (5.5 g) was separated by MPLC (Biotage SNAP cartridge, KP-C18-HS, 400 g) with a stepwise gradient elution using MeOH-H_2O (7:3, 8:2, 9:1, 10:0) to give seven fractions (LT4-5-1 ~ LT4-5-7). Fr. LT4-5-5 (461.0 mg) was repeatedly subjected to HPLC (Hector C_{18} column) with a gradient mixtures of MeOH-H_2O (80:20 → 90:10) and to yield three fractions (LT4-5-5-2 ~ LT4-5-5-4). LT4-5-5-3 (41.0 mg) and LT4-5-5-4 (207.1 mg) were subjected to HPLC [Hector phenyl column, MeOH-H_2O (80:20 → 90:10)] purification to obtain **4** (30.5 mg) and **3** (63.4 mg). Compound **2** (6.1 mg) was isolated from LT4-5-6 (907.4 mg) by HPLC (Phenomenex biphenyl column) eluting with isocratic solvent of MeOH-H_2O (90:10).

***N*-Acetylnornuciferine (1)** – yellow needles; $[\alpha]_D^{22}$ -311.6 (*c*=0.55, $CHCl_3$); ^1H and ^{13}C NMR spectroscopic data,

Table 1. ^1H NMR assignments for **1** - **4** in $CDCl_3$ (600 MHz)

proton	**1**		**2**		**3**		**4**	
	Z	*E*	*Z*	*E*	*Z*	*E*	*Z*	*E*
3	6.66, s	6.70, s	6.59, s	6.62, s	-	-	-	-
4	2.89, t (*J*=12.1), 2.68, overlap	2.83, m, 2.68, overlap	2.84, overlap, 2.69, m	2.78, overlap, 2.64, m	3.01, overlap, 2.56, overlap	3.01, overlap, 2.56, overlap	3.03, overlap, 2.56, m	2.94, d (*J*=16.1), 2.49, m
5	4.00, d (*J*=12.6), 3.31, t (*J*=12.6)	4.96, d (*J*=11.6), 2.76, overlap	3.99, d (*J*=11.7), 3.31, t (*J*=11.7)	4.96, d (*J*=8.9), 2.78, overlap	4.01, d (*J*=12.6), 3.24, t (*J*=12.6)	4.97, d (*J*=10.0), 2.70, m	3.99, d (*J*=11.9) 3.21, t (*J*=11.9)	4.95, d (*J*=11.0), 2.63, m
6a	5.09, dd (*J*=2.9, 13.4)	4.56, br d (*J*=13.5)	5.22, br d (*J*=11.3)	4.70, br d (*J*=11.9)	5.11, br d (*J*=12.7)	4.56, br d (*J*=13.3)	5.05, br d (*J*=12.4)	4.50, br d (*J*=13.6)
7	3.05, dd (*J*=2.9, 13.4), 2.76, overlap	3.10, t (*J*=13.5), 2.76, overlap	3.16, overlap, 2.84, overlap	3.16, overlap, 2.84, overlap	3.01, overlap, 2.78, overlap	3.13, t (*J*=13.3), 2.78, overlap	2.90, dd (*J*=3.2, 12.4), 2.69, t (*J*=12.4)	3.03, overlap, 2.63, m
8	7.38~7.25	7.38~7.25	7.40~7.21	7.40~7.21	7.37~7.19	7.37~7.19	6.75, s	6.75, s
9	7.24, m	7.38~7.25	7.40~7.21	7.40~7.21	7.37~7.19	7.37~7.19	-	-
10	7.38~7.25	7.38~7.25	7.40~7.21	7.40~7.21	7.37~7.19	7.37~7.19	-	-
11	8.42, d (*J*=7.7)	8.47, d (*J*=7.7)	8.10, br d (*J*=7.5)	8.12, overlap	8.32, d (*J*=7.2)	8.37, d (*J*=6.2)	7.89, s	7.92, s
1-OCH_3	3.67, s	3.67, s	-	-	3.74, s	3.74, s	3.74, s	3.74, s
2-OCH_3	3.90, s	3.90, s	-	-	3.91, s	3.91, s	3.89, s	3.89, s
3-OCH_3	-	-	-	-	3.96, s	3.96, s	3.95, s	3.95, s
N-$COCH_3$	2.22, s	2.17, s	2.24, s	2.20, s	2.24, s	2.17, s	2.22, s	2.16, s
-OCH_2O-	-	-	5.98, s	6.10, s	-	-	5.97, overlap	5.97, overlap

Table 2. ^{13}C NMR assignments for **1 - 4** in $CDCl_3$ (150 MHz)

carbon	1		2		3		4	
	Z	*E*	*Z*	*E*	*Z*	*E*	*Z*	*E*
1	146.0	145.8	143.3	143.2	150.9	150.9	150.2	150.2
2	152.2	152.5	147.0	147.3	145.5	145.5	145.4	145.4
3	111.4	111.8	107.4	108.0	150.1	150.1	149.5	149.5
3a	129.0	130.3	129.1	129.1	123.0	123.2	125.0	124.3
3b	126.7	125.7	126.2	125.2	128.7	128.4	129.4	128.5
4	30.9	30.0	30.9	30.1	24.6	23.8	24.5	23.8
5	42.1	36.5	42.3	36.9	41.8	36.2	41.8	36.4
6a	50.5	53.9	50.6	53.9	50.6	54.1	50.7	54.3
7	34.1	36.7	33.7	36.3	33.9	36.4	33.9	36.2
7a	136.9	136.3	135.9	136.9	136.4	135.8	130.8	130.1
8	128.0*	128.1*	128.5*	128.5*	128.0*	128.1*	109.0	108.4
9	127.9	127.6*	128.0*	128.1*	127.2*	127.6*	-	-
10	127.1*	127.5*	127.1*	127.2*	127.5*	127.1*	-	-
11	128.5	128.9	127.2	127.6	128.0	128.4	108.6	108.9
11a	131.6	131.8	130.7	131.9	131.6	130.1	129.4	128.5
11b	128.7	128.5	117.8	117.2	123.7	124.4	122.9	123.8
12	169.2	169.9	169.3	169.7	169.2	169.8	169.2	169.7
1-OCH_3	60.1	60.1	-	-	60.6	60.6	60.5	60.5
2-OCH_3	56.1	56.1	-	-	61.1	61.1	61.1	61.1
3-OCH_3	-	-	-	-	61.2	61.2	61.2	61.2
N-$COCH_3$	22.7	21.7	22.7	21.7	22.6	21.7	22.6	21.7
-OCH_2O-	-	-	101.1	101.2	-	-	101.0	101.2

*Because the chemical shifts are very similar, the signals may change.

see Tables 1 and 2; ESIMS, *m/z* 324 $[M+H]^+$, 346 $[M+Na]^+$

***N*-Acetylanonaine (2)** – yellow amorphous solid; $[\alpha]_D^{18}$ -322.0 (*c*=0.05, CH_2Cl_2); ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS, *m/z* 308 $[M+H]^+$, 330 $[M+Na]^+$

***N*-Acetyl-3-methoxynornuciferine (3)** – yellowish amorphous powder; $[\alpha]_D^{22}$ -333.5 (*c*=0.2, $CHCl_3$); ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS, *m/z* 354 $[M+H]^+$, 376 $[M+Na]^+$

***N*-Acetyl-3-methoxynornantenine (4)** – yellowish amorphous powder; $[\alpha]_D^{22}$ +429.0 (*c*=0.1, $CHCl_3$); ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS, *m/z* 398 $[M+H]^+$, 420 $[M+Na]^+$

Result and Discussion

Compounds **1 - 4** were determined as *N*-acetylated aporphine alkaloids (Fig. 1). HPLC analysis on compounds **1 - 4** revealed the purity should be 97, 96, 99, and 98%, respectively. However, the ^1H and ^{13}C NMR spectroscopic data of **1 - 4** displayed all signals in dupli-

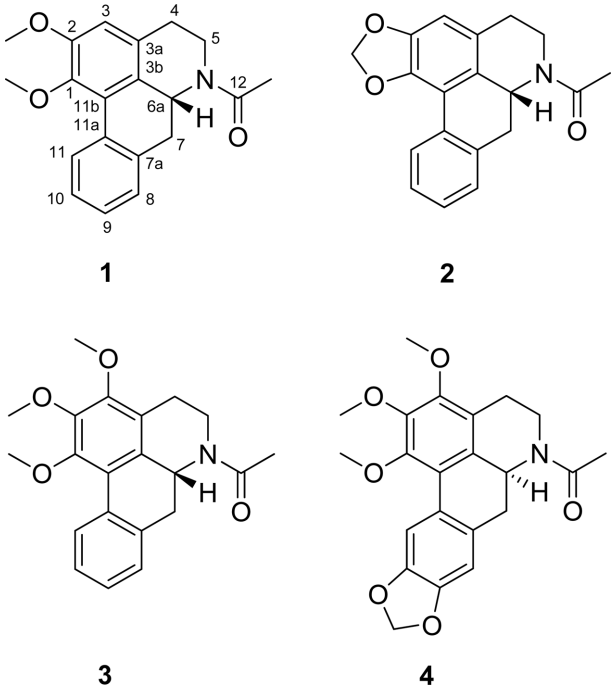

Fig. 1. Structures of compounds **1 - 4**.

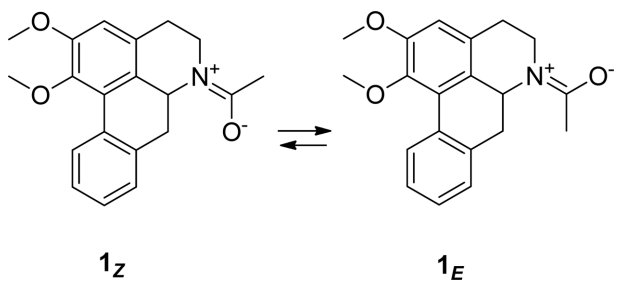

Fig. 2. *Z*- and *E*-rotamers of compound **1**.

cate, indicating the presence of two rotamers, *Z*- and *E*-isomers, due to rotation about *N*-$COCH_3$ functionality in solution status (Fig. 2). The ^1H and ^{13}C NMR signals at C-4, C-5, C-6a, C-7, and *N*-acetyl group were distinguished because of the shielding effect of *N*-acetyl group (Fig. 2), which enabled us to determine the structures of *Z*- and *E*-forms, respectively. The absolute configurations of rotameric aporphine alkaloids **1** - **4** were established by measuring specific rotation.

The ^1H NMR spectroscopic data of **1** was complex, because of the resonances of the two rotational isomers arising from restricted rotation about the *N*-acetyl group. Two separate signals in the ratio of 1.3/1 (**1*Z*/1*E***) were estimated based on the integration of aromatic signals. The ^1H NMR spectroscopic data of **1*Z*** displayed signals of two benzene protons at δ 8.42 (d, J = 7.7 Hz, H-11) and 6.66 (s, H-3), while these two benzene protons revealed in **1*E*** at δ 8.47 (d, J = 7.7 Hz, H-11) and 6.70 (s, H-3) in the same spectrum. Other aromatic protons at δ 7.38 - 7.24 for H-8, H-9, and H-10 were observed. In addition, the ^1H NMR spectrum revealed two methoxy groups and one *N*-acetyl moiety at δ 3.90, 3.67, and 2.22 for **1*Z*** as well as at δ 3.90, 3.67, and 2.17 for **1*E*** (Table 1). The presences of the two methoxy and an *N*-acetyl groups were further confirmed by the ^{13}C NMR spectroscopic data, which showed characteristic signals at δ 60.1, 56.1, 169.2, and 22.7 for **1*Z*** as well as at 60.1, 56.1, 169.9, and 21.7 for **1*E***. The resonances of one methine proton at δ 5.09/4.56 (dd, J = 13.4 and 2.9 Hz, H-6a of **1*Z***/br, J = 13.5 Hz, H-6a of **1*E***) and three methylenes at δ 4.96 (d, J = 11.6 Hz, H_2-5α of **1*E***), 4.00 (d, J = 12.6 Hz, H_2-5α of **1*Z***), 3.31 (t, J = 12.6 Hz, H_2-5β of **1*Z***), 3.10 (t, J = 13.5 Hz, H_2-7α of **1*E***), 2.89 (t, J = 12.1 Hz, H_2-4α of **1*Z***), 2.83 (m, H_2-4α of **1*E***), 2.76 (m, overlap, H_2-7β of **1*E*** and **1*Z***, H_2-5β of **1*E***), and 2.68 (m, H_2-4β of **1*E*** and **1*Z***) were observed (Table 2). Each structure of **1*E*** and **1*Z*** was confirmed by ^{13}C NMR, HSQC, and HMBC spectra. The absolute configuration at C-6a was assigned as *R* based on the specific rotation value $[\alpha]_D^{22}$ -311.6 (c=0.55, $CHCl_3$) in com- parison with the reported data.[6] The structure of **1** was determined as *N*-acetylnornuciferine, whose NMR spec- troscopic data was displayed in Tables 1 and 2.

The ^1H NMR spectrum of **2** closely resembled those of **1**. However, the two methoxy groups at δ 3.90 and 3.67 of **1** were replaced by one dioxymethylene at δ 6.10 (s, -OCH_2O- of **2*E***) and 5.98 (s, -OCH_2O- of **2*Z***). Two separate peaks for **2*Z*** and **2*E*** were observed with relative intensities of 1.5/1. The specific rotation of **2** was levo-rotatory ($[\alpha]_D^{18}$ -322.0). By comparison with the literature data,[7] **2** was identified as *N*-acetylanonaine.

The ^1H NMR spectroscopic data for **3** was similar to those of **1** except for the absence of one benzene proton and the presence of one additional methoxy group at δ 3.96 (s, 3-OCH_3 of **3*Z/E***). The ^1H and ^{13}C NMR spectra showed **3*Z*** and **3*E*** with integrated intensities of 1.5/1. The structure of compound **3** was determined as *N*-acetyl-3-methoxynornuciferine by comparison of specific rotation value with that in the literature.[8]

Compound **4** was also identified as an *N*-acetylated aporphine alkaloid based on the NMR spectroscopic data. The differences in **4,** compared with **3,** was the absence of two benzene protons and the presence of one oxymethylene protons at δ 5.97 (m, -OCH_2O- of **4*Z/E***). The ratio of two rotamers, **4*Z*** and **4*E***, was 2/1 based on ^1H NMR spectrum. The *S* configuration at C-6a was deduced by the positive specific rotation value ($[\alpha]_D^{22}$ +429.0).[9] Based on the above evidence, the structure of compound **4** was assigned as *N*-acetyl-3-methoxynornantenine.

Although *N*-acetyl aporphine alkaloids have often been found in natural products, the NMR assignments for *E*- and *Z*-rotamers are limited. The ^1H and ^{13}C NMR spec-troscopic data on *N*-acetyl-3-methoxynornuciferine (**3**) and *N*-acetyl-3-methoxynornantenine (**4**) were assigned for the first time in this study. Our results provide advanced NMR spectroscopic data for the structure determination of rotameric aporphine alkaloids.

Acknowledgment

This work was supported by research fund of Chungnam National University

References

(1) Chen, J.; Gao, K.; Liu, T.; Zhao, H.; Wang, J.; Wu, H.; Liu, B.; Wang, W. *Asian J. Chem.* **2013**, *25*, 10015-10027.

(2) Kang, Y. F.; Liu, C. M.; Kao, C. L.; Chen, C. Y. *Molecules* **2014**, *19*, 4234-4245.

(3) Graziose, R.; Rathinasabapathy, T.; Lategan, C.; Poulev, A.; Smith,

P. J.; Grace, M.; Lila, M. A.; Raskin, I. *J. Ethnopharmacol*. **2011**, *133*, 26-30.
(4) Chang, G. J.; Wu, M. H.; Wu, Y. C.; Su, M. J. *Br. J. Pharmacol*. **1996**, *118*, 1571-1583.
(5) Chen, B. H.; Chang, H. W.; Huang, H. M.; Chong, I. W.; Chen, J. S.; Chen, C. Y.; Wang, H. M. *J. Agric. Food Chem*. **2011**, *59*, 2284-2290.
(6) Pachaly, P.; Adnan, A. Z.; Will, G. *Planta Med*. **1992**, *58*, 184-187.
(7) Chu, C. W.; Liu, C. M.; Chung, M. I.; Chen, C. Y. *Molecules* **2015**, *20*, 12166-12174.
(8) Hufford, C. D. *Phytochemistry* **1976**, *15*, 1169-1171.
(9) Hufford, C. D.; Funderburk, M. J. *J. Pharm. Sci*. **1974**, *63*, 1338-1339.

Received May 12, 2020
Revised June 25, 2020
Accepted June 25, 2020