

BISBENZYLISOQUINOLINE ALKALOIDS FROM MAHONIA NEPALENSIS

Received 24 June 2008

NGUYEN THI MAI^{1,2}, TRAN ANH TUAN¹, HOANG THANH HUONG¹
CHAU VAN MINH¹, NINH KHAC BAN³, AND PHAN VAN KIEM¹

¹Institute of Natural Products Chemistry, VAST

²University of Transport and Communications

³Institute of Ecology and Biological Resources, VAST

ABSTRACT

From the wood of *Mahonia nepalensis* DC. 1821, two bisbenzylisoquinolines homoaromoline (**1**) and isotetrandrine (**2**) were isolated by using various chromatographies. Their structures were characterized on the basis of the spectroscopic data (1D-NMR, HSQC, HMBC, ESI-MS) in comparison with the literature. This is the first report of **1** - **2** from *Mahonia nepalensis*.

Keywords: *Mahonia nepalensis*, Isotetrandrine, Homoaromoline, Bisbenzylisoquinoline.

I - INTRODUCTION

Mahonia nepalensis DC. 1821 (hoang lien o ro), synonym *Mahonia annamica* Ganep. 1908 belonging to Berberidaceae family, is widely distributed in the high mountainous areas at altitudes 1700 — 1900 m of Vietnam as Lai Chau, Lao Cai, Ha Giang, Cao Bang, Bac Can and Lam Dong provinces. The stem bark and wood of *Mahonia nepalensis* were considered to have anti-inflammatory, anti-bacterial and anti-fungal activities and they are used particularly for the treatment of skin diseases. They are indicated for treatment of the eczema, psoriasis, and other skin conditions [1 - 3]. Alkaloids representing as the main compounds in *Mahonia* genus, belong to two major classes the protoberberines and the bisbenzylisoquinolines [2, 4-6]. However, no study on chemical components and biological activities of species of *M. nepalensis* has been previously reported. Herein, we report the isolation and structural elucidation of two bisbenzylisoquinolines homoaromoline (**1**) and isotetrandrine (**2**) from

the wood of *M. nepalensis*. Their chemical structures were elucidated based on the 1D-, 2D-NMR, ESI-MS experiments in comparison with the previous literature.

II - EXPERIMENTAL

1. General experimental procedures

Optical rotations were determined on a Jasco DIP-1000 KUY polarimeter. The Electrospray Ionization (ESI) mass spectrum was obtained using an AGILENT 1200 LC-MSD Trap spectrometer. The ¹H-NMR (500MHz) and ¹³C-NMR (125 MHz) spectra were recorded on Bruker AM500 FT-NMR spectrometer. Chemical shifts (δ) are reported in ppm using tetramethylsilane (TMS) as an internal standard. Column chromatography (CC) was performed on silica gel 230 - 400 mesh (0.040 - 0.063 mm, Merck) or YMC RP-18 resins (30 - 50 μm, Fujisilica Chemical Ltd.). Thin layer chromatography (TLC) was performed on DC-Alufolien 60 F₂₅₄ (Merck 1.05715) or RP₁₈ F_{254s} (Merck) plates. Spots

were visualized by spraying 10% H₂SO₄ aqueous and heating for 5 minutes and by Dragendoff reagent.

2. Plant material

The wood of *Mahonia nepalensis* species

was collected in Ngan Son district, Bac Can province in March 2008 and identified by Dr Ninh Khac Ban, Institute of Ecology and Biological Resources, VAST. A voucher specimen (EN5-CB2006) was deposited at this institute.

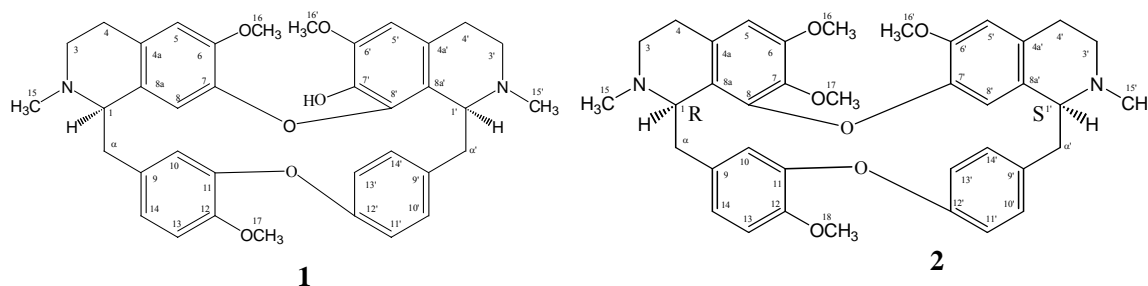


Fig. 1: The structures of 1 and 2

3. Extraction and Isolation

Dried wood pieces (3.0 kg) of *Mahonia nepalensis* were powdered and then extracted three times with MeOH in acetic acid (pH = 4). After removal the solvent, the extract (80 g) was suspended in water and partitioned with chloroform. After removal the chloroform layer, NH₄OH was added to the water layer (pH = 10) and then partitioned in turn with chloroform and *n*-butanol to obtained chloroform (15 g), *n*-butanol (30 g) fractions, and the water layer. The *n*-butanol (30 g) was crudely separated on normal silica gel column eluted with *n*-hexane/acetone (gradient from 50/1 to 50/50, v/v) to give three sub-fractions HLC2A (3g), HLC2B (5g) and HLC2C (5g). The HLC2A (3g) sub-fraction was further separated on normal silica gel column eluting with chloroform/methanol (15/1 v/v) to give 2 (35 mg) as yellow amorphous powder. The LIC2 (5g) sub-fraction was chromatographed on a silica gel column eluted with chloroform/methanol (20/1 v/v) to give 1 (25 mg) as yellow amorphous powder.

Homoaromoline (thalrugosamine, homothalicrine, N-methyl daphnandrine (1): Yellow amorphous powder; mp. 235 - 237°C; positive ESI-MS: m/z 609 [M+H]⁺, negative ESI-MS: m/z 607 [M-H]⁻, (C₃₇H₄₀N₂O₆, M=608). ¹³C-NMR (125MHz, CDCl₃) and ¹H-NMR (500

MHz, CDCl₃) see table 1.

Isotetrandrine (O-methylberbamine (2): Yellow amorphous powder; mp. 182-183°C; positive ESI-MS: m/z 623 [M+H]⁺, (C₃₈H₄₂N₂O₆, M= 622); ¹³C-NMR (125MHz, CDCl₃) and ¹H-NMR (500 MHz, CDCl₃) see Table 2.

III - RESULTS AND DISCUSSION

Compound 1 was obtained as a yellow amorphous powder. The molecular formula of 1 was suggested to be C₃₇H₄₀N₂O₆, from the exhibition of the quasi molecular ion peaks at m/z 609 [M+H]⁺ (positive ion mode) and 607 [M-H]⁻ (negative ion mode) with the strongest intensity in the ESI-MS. The ¹³C-NMR spectrum of 1 showed signals of 37 carbon atoms including 5 methyl, 6 methylene, 12 methine, and 14 quaternary carbons, determining from the DEPT 95° and DEPT 135° experiments. Two methyl groups bearing nitrogen atoms were at δ 44.13 and 42.42, four methylene groups at δ 51.52, 45.57, 28.92 and 25.13, in which signals at δ 51.52 and 45.57 suggested that these two methylene groups bearing nitrogen atoms. The above evidence together with two methine signals at δ 64.47 and 61.51 suggested the presence of a bisbenzylisoquinoline compound [6] having three methoxyl groups at δ 56.68, 56.35 and 55.73. In the ¹H-NMR spectrum, three aromatic signals at δ 6.38, 6.68, 6.34 as

singlets, signals at δ 5.65 (s), 6.78 (d, $J = 8.5$ Hz) and 6.80 (d, $J = 8.5$ Hz) suggesting a 1,2,4-trisubstituted aromatic ring, and four the other aromatic ring signals at δ 6.42 (dd, $J = 8.0, 2.0$ Hz), 6.92 (dd, $J = 8.0, 2.0$ Hz), 6.93 (dd, $J = 8.0, 2.0$ Hz) and 7.40 (dd, $J = 8.0, 2.0$ Hz) confirming the presence of the typical para-substituted aromatic ring [6]. Three methoxyl groups were recognized at δ 3.88, 3.76, 3.60 and

two N-Me groups were assigned at δ 2.59 and 2.55. All the carbons were assigned to relevant protons through an HSQC experiment and the NMR data of **1** were compared to those of homoaromoline [6] and found to match well (Table 1). Furthermore, the HMBC experiment was taken to determine all the chemical shifts of **1**. The key correlations observed in the HMBC spectrum were shown in table 1.

Table 1: The NMR data and HMBC results of **1**

C	Homoaromoline [6]		1		
	$\delta_C^\#$	$\delta_H^\#$	δ_C	δ_H	HMBC (H to C)
(1)	(2)	(3)	(4)	(5)	(6)
1	64.26	3.63 dd (4, 2.6)	64.77	3.61*	8
3	51.10	2.73 m / 3.02 m	51.52	2.45 m / 2.78 m	
4	28.45	2.40 m	28.92	2.40 m	
4a	130.56	-	131.73	-	
5	111.10	6.33 s	111.71	6.36 s	6, 7, 8a
6	148.50	-	148.85	-	
7	143.96	-	144.32	-	
8	116.93	6.66 s	117.78	6.68 s	4a, 6
8a	128.02	-	128.75	-	
α	38.32	2.94 dd (14, 1.4) / 2.68 m	38.64	3.05 m / 2.79 m	
9	130.95	-	131.73	-	
10	117.00	5.54 brs	117.78	5.6 s	
11	48.70	-	148.85	-	
12	146.64	-	147.28	-	
13	110.71	6.74 d (8.4)	111.45	6.80 d (8.5)	9, 11
14	123.65	6,70 dd (8.4, 2.3)	124.05	6,80 d (8.5)	α , 10, 12
15	43.72	2.53 s	44.13	2.59 s	1, 3
16	55.21	3.60 s	55.73	3.60 s	6
17	55.79	3.88 s	56.68	3.88 s	12
1'	60.46	4.13 d (6.5)	61.51	4.18 d (7.0)	α' , 8', 9', 4a'
3'	44.96	2.55 m / 2.86 m	45.57	2.89 m / 3.21 m	1', 4a', 4'
4'	24.96	3.08 m / 2.73 m	25.13	3.05 m / 2.67 m	8a'
4a'	122.99	-	123.54	-	
5'	104.50	6.20 s	105.26	6.34 s	4', 6', 7', 8a'
6'	147.61	-	147.28	-	
7'	133.39	-	134.10	-	
8'	142.37	-	141.82	-	

(1)	(2)	(3)	(4)	(5)	(6)
8a'	122.91	-	123.54	-	
α'	38.20	2.61 m / 3.22 d (13.8)	38.64	2.79 m / 3.3 d (14.0)	1', 8a', 9', 10', 14'
9'	138.17		137.82		
10'	131.48	6.95 dd (2.2, 8.4)	131.73	6.93 dd (2.5, 8.0)	α' , 9', 11', 12'
11'	121.12	6.40 dd (2.2, 8.4)	121.37	6.42 dd (2.0, 8.0)	9', 13'
12'	152.74		153.20		
13'	121.90	6.91 dd (2.2, 8.4)	122.32	6.92 dd (2.5, 8.0)	
14'	128.34	7.33 dd (2.2, 8.4)	128.75	7.40 dd (2.0, 8.0)	α' , 10', 12'
15'	41.50	2.43 s	42.42	2.55 s	1', 3'
16'	55.68	3.76 s	56.35	3.76 s	6'

[#] δ_C of homoaromoline [6], ^aMeasured in CDCl₃, ^b125 MHz, ^c500 MHz, δ : ppm.

Table 2: The NMR data of **2** in comparison with the literature

C	Tetrandrine [6]		2		
	$\delta_C^{\#}$	$\delta_H^{\#}$ mul (J in Hz)	$\delta_C^{\#\#}$ [8]	δ_C	δ_H mul (J in Hz)
(1)	(2)	(3)	(4)	(5)	(6)
1	61.19	3.72 d (9.5)	63.4	61.99	3.68 dd (10.0, 14.0)
3	43.91	2.87 m	45.5	45.72	2.79 - 2.95 *
4	21.28	2.39 m / 2.89 m	24.9	25.33	2.79 - 2.95 *
4a	127.72	-	130.5	129.40	-
5	105.55	6.26 s	107.1	106.00	6.27 s
6	151.18	-	153.5	151.86	-
7	137.63	-	138.4	137.07	-
8	148.19	-	149.3	148.20	-
8a	122.64	-	121.6	120.60	-
α	41.70	2.48 dd (1.8, 14) 2.67 dd (10, 14)	38.9	38.59	2.41* 2.62 dd (10.0, 14.0)
9	134.68	-	136.5	137.07	-
10	115.99	6.52 d (2.2)	117.4	116.00	6.54 s
11	149.12	-	151.0	149.51	-
12	146.83	-	148.9	147.00	-
13	111.33	6.82 d (8.2)	113.4	111.37	6.84 d (8.2)
14	122.56	6.86 dd (2.2, 8.2)	124.5	122.83	6.89 dd (2.2, 8.2)
15	42.05	2.3 s	43.1	42.62	2.26 s
16	55.55	3.70 s	56.3	55.71	3.75 s
17	59.99	3.15 s	61.0	60.40	3.13 s
18	55.87	3.88 s	56.8	56.05	3.91 s
1'	63.64	3.84 dd (5.9, 11.0)	64.4	63.61	3.88*
3'	45.00	2.83 m / 3.39 m	46.2	45.72	2.79 - 2.95*
4'	25.92	2.91 m / 3.47 m	26.2	25.33	2.79 - 2.95*
4a'	127.72	-	129.9	128.52	-
5'	112.50	6.48 s	112.6	111.23	6.41 s
6'	148.39	-	151.3	149.96	-

(1)	(2)	(3)	(4)	(5)	(6)
7'	143.57	-	145.0	143.66	-
8'	119.96	5.96 s	121.2	119.80	5.98 s
8a'	127.84	-	127.22	127.22	-
α'	37.93	2.75 dd (11, 12.3) 3.22 dd (5.9, 12)	37.3	37.61	2.77 3.42 m
9'	134.91	-	136.4	135.00	-
10'	132.42	6.27 dd (2.3, 8.2)	133.5	132.04	6.41 dd (2.3, 8.2)
11'	121.63	6.76 dd (2.3, 8.2)	122.5	120.00	6.79 dd (2.3, 8.2)
12'	153.58	-	155.9	154.14	-
13'	121.63	7.10 dd (2.3, 8.2)	122.3	121.60	7.14 dd (2.3, 8.2)
14'	129.90	7.30 dd (2.3, 8.2)	131.5	130.52	7.33 dd (2.3, 8.2)
15'	42.32	2.58 s	42.7	42.41	2.57 s
16'	55.56	3.33 s	55.9	55.53	3.61 s

[#] δ of tetrandrine [6], ^{##} δ_c of isotetrandrine [8] ^aMeasured in CDCl₃, ^b125 MHz, ^c500 MHz, δ : ppm.

Compound **2** was obtained as a yellow amorphous powder. The molecular formula of **2** was suggested to be C₃₈H₄₂N₂O₆, from the exhibition of the quasi molecular ion peak at *m/z* 623 [M+H]⁺ (positive ion mode) with the strongest intensity in the ESI-MS. The ¹³C-NMR spectrum of **2** showed signals of 38 carbon atoms including 6 methyl, 6 methylene, 12 methine, and 14 quaternary carbons, determining from the DEPT 95° and DEPT 135° experiments. Two methyl groups bearing nitrogen atoms were at δ 42.41 and 42.62, four methylene groups at δ 45.72 \times 2, and 25.33 \times 2, in which signals at δ 45.72 suggested that these two methylene groups bearing nitrogen atoms. The above evidence together with two methine signals at δ 61.98 and 63.61 suggested the presence of a bisbenzylisoquinoline compound [6]. In the ¹H-NMR spectrum, singlet signals at δ 2.26 and 2.57 (3H $\dot{\iota}$ 2) correspond to two NMe groups, four singlet signals at δ 3.91, 3.75, 3.61 and 3.13 (3H \times 4) were assigned for 4 methoxyl groups. Aromatic singlet signals at δ 6.41, 6.27, and 5.98, a *para*-substituted aromatic ring was at δ 7.28, 7.11, 6.64, and 6.41 (4 \times dd, *J* = 2.3, 8.2 Hz), and signals at δ 6.80 (2H) and 6.54 (1H) displayed for the 1,2,4-trisubstituted aromatic ring of the bisbenzylisoquinoline compound [6]. This evidence led to suggest that compound **2** was isotetrandrine or tetrandrine. The NMR data of **2** were further compared to those of isotetrandrine and tetrandrine (table 2).

The chemical shift and the *J* coupling constant of H-1 and H- α of **2** [δ 2 3.68 dd (*J* = 10.0, 14.0 Hz)] differed from that of tetrandrine [δ 3.72 d (*J* = 9.5 Hz)] confirming that the configuration at C-1 was *R*. Furthermore, the melting point of **2** was 235 - 237°C, similar to that of isotetrandrine (1*R*,1'*S*) [8], and differed from that of tetrandrine (1*S*,1'*S*) 215 - 217°C [6]. From all the above data, compound **2** was identified as isotetrandrine, which was isolated from many *Mahonia* species such as *M. japonica*, *M. lomariifolia*, *M. morrisonensis*, *M. philippinensis*, [7], however, which was first isolated from *M. nepalensis*. Besides, this compound was also isolated from *Atherosperma moschatum*, *Cyclea barbata*, *Doryphora aromatica*, *Isopyrum thalictroides*, *Laurelia sempervirens* and many *Berberis* species, and it has been reported showing *in vitro* antineoplastic activity against HeLa cells and Ehrlich ascites, anti-inflammatory, antihypertensive agent, and active against gram-positive bacteria [7].

REFERENCES

1. Vietnam Red Data Book, Science and Technology Publishing House p. 133 (2007).
2. Bich, D. H., Chung, D. Q., Chuong, B. X., Dong, N. T., Dam, D. T., Hien, P. V., Lo, V.

- N., Mai, P. D., Man, P. K., Nhu, D. T., Tap, N., Toan, T., Medicinal Plants and Animals in Vietnam. Hanoi Science and Technology Publishing House, Vol. 2, 130 - 133 (2004).
3. Chi, V. V., Vietnamese Medical Plant Dictionary. Medicinal Publishing House, 566 - 567 (1997).
 4. R. H. A. M. Janssen, R. J. J. C. Lousberg, P. Wijkens, C. Kruk, and H. G. Theuns. *Phytochemistry*, Vol. 28(10), 2833 - 2839 (1989).
 5. R. Marek, P. Seckarova, D. Hulova, J. Marek, J. Dostal, and V. Sklenar. *J. Nat. Prod.* Vol. 66, 481 - 486 (2003).
 6. P. L. Schiff. *J. Nat. Prod.*, 60, 934 - 953 (1997).
 7. Dictionary of Natural Products on CD-ROM, Version 15:1, Copyright @ 1982-2007 Chapman & Hall/CRC
 8. T. Tanahashi, Y. Su, N. Nagakura, and H. Nayeshiro. *Chem. Pharm. Bull.*, Vol. 48(3), 370 - 373 (2000).

Corresponding author: **Phan Van Kiem**

Vietnam Academy of Science and Technology.