Four New Naphthylisoquinoline Alkaloids from *Ancistrocladus tectorius*

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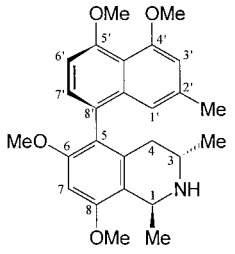
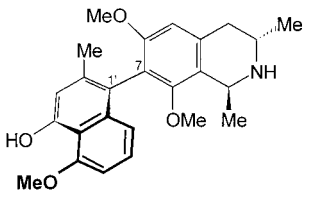
Four new naphthylisoquinoline alkaloids, ancistrotectoriline A (**1**), ancistrotectoriline B (**2**), 6-*O*-methyl-4′-*O*-demethylancistrocladine (**3**), and 6-*O*-methyl-4′-*O*-demethylhamatine (**4**), were isolated from the stems and leaves of *Ancistrocladus tectorius*, collected from Hainan Province, Southern China. Their structures were elucidated using MS and NMR methods.

Ancistrocladus tectorius (Lour.) Merr. belongs to the small monogeneric family Ancistrocladaceae,[1] which consists of ca. 30 species of tropical lianas and shrubs. Like the closely related Dioncophyllaceae, members of Ancistrocladaceae produce a unique class of naphthylisoquinoline alkaloids. Naphthylisoquinoline alkaloids can be divided into subtypes according to the linkage between their naphthyl and isoquinoline moieties. Some of these alkaloids have significant antifungal, antimalarial, or antiviral activity.[2−5] Previous work on *A. tectorius* resulted in the isolation of eight naphthylisoquinoline alkaloids including seven 5,1′-linked compounds[6−10] and one 7,3′-linked.[11] In the present paper, we report the isolation and structural elucidation of four new naphthylisoquinoline alkaloids (**1**−**4**) from *A. tectorius* grown in China.



1



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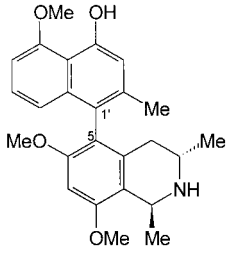
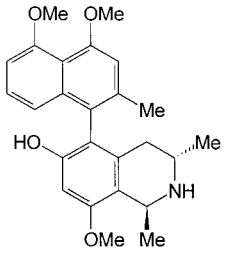


3 5,1'-axis: P

4 5,1'-axis: M



Ancistrocladine 5,1'-axis: P

Hamatine 5,1'-axis: M

Results and Discussion

The alkaloids were extracted by conventional methods. Four novel Dragendorff-positive compounds, ancistrotectoriline A (**1**), ancistrotectoriline B (**2**), 6-*O*-methyl-4′-*O*-demethylancistrocladine (**3**), and 6-*O*-methyl-4′-*O*-demethylhamatine (**4**), were obtained from the crude alkaloid residue by repeated column chromatography and preparative TLC.

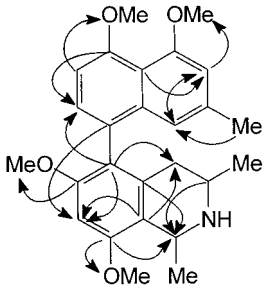


Figure 1. HMBC correlation of **1**.

Ancistrotectoriline A (**1**) gave a molecular formula of $C_{26}H_{31}NO_4$ by HRMS. The ^{1}H NMR spectrum indicated four methoxy groups (δ 3.50, 3.76, 3.78, and 3.84), one aromatic methyl group (δ 2.31), and two doublet methyl groups (δ 1.14 and 1.57). The upfield-shielding shift of the C-4 protons (δ 2.05 and 2.36) revealed that the naphthyl moiety was most probably attached to C-5. The normal chemical shift of the naphthyl-bound methyl group (δ 2.31) at C-2′ and the lack of three neighboring aromatic protons indicated that the isoquinoline moiety was attached to C-8′. The relationship between C-5 and C-8′ was further confirmed by HMBC experiments. Correlations between C-5 and H-7′ (δ 7.09), C-5 and H-7 (δ 6.46), and C-8′ and H-7 were clearly observed (Figure 1). Hence the basic structure of **1** was established as a 5,8′-coupled naphthylisoquinoline.

The relative *trans*-configuration at C-1 vs C-3 in **1** could be deduced from the chemical shift of H-3 (δ 3.30), which was in the typical range for a *trans*-substituted 1,3-dimethyltetrahydroisoquinoline, such as ancistrocladine and hamatine.[12] Considering the fact that all of the related alkaloids isolated to date from the Ancistrocladaceae (so-called Ancistrocladaceae-type alkaloids) have *S*-configuration at C-3,[12] we assumed the configuration to be 1*S*, 3*S*.

The CD spectrum of **1** exhibited a positive Cotton effect at 221 nm ($\Delta\epsilon$, +26.3) and a negative Cotton effect at 237 nm ($\Delta\epsilon$, −2.0). The CD curve coincided with that of the stereochemically well-known naphthylisoquinoline, korupensamine B, and was opposite those of atropisomerical korupensamine A and ancistroguinein A.[13,14] Thus the absolute configuration at the biaryl axis of **1** was assigned as identical with that of korupensamine B.

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Table 1. NMR Data of **1** and **2**[a]

	1			**2**		
position	^{13}C δ	^{1}H δ (J in Hz)	HMBC	^{13}C δ	^{1}H δ (J in Hz)	HMBC
1	47.5	4.12 (q, 6.2)	7-H, 1-Me	47.9	4.37 (q, 6.6)	3-H, 1-Me
3	44.5	3.30 (m)	1, 4-H, 3-Me	42.1	3.41 (m)	1, 4-H, 3-Me
4	31.8	2.05 (dd, 17.8, 12.0)	3-Me	37.9	2.57 (dd, 16.6, 11.1)	5-H, 3-Me
		2.36 (dd, 17.8, 4.2)			2.82 (dd, 16.6, 4.1)	
5	121.3		4, 7, 7′-H	106.7	6.47 (s)	4-H
6	158.1		4, 7-H, 6-OMe	156.8		5-H, 6-OMe
7	94.2	6.46 (s)		118.8		5-H
8	156.2		1, 7-H, 8-OMe	156.1		1-H, 8-OMe
9	114.6		1, 4, 7-H, 1-Me	125.7		1, 4, 5-H, 1-Me
10	132.8		1, 4-H, 3-Me	135.5		1, 4-H, 3-Me
1′	117.8	6.64 (s)	3′, 7′-H, 2′-Me	122.0		3′, 8′-H, 2′-Me
2′	137.8		2′-Me	137.4		2′-Me
3′	109.2		1′-H, 2′-Me, 4′-OMe	112.9	6.83 (s)	2′-Me
4′	157.1		3′-H, 4′-OMe	153.8		3′-H
5′	156.8		6′, 7′-H, 5′-OMe	156.3		6′, 7′-H, 5′-OMe
6′	105.0	6.82 (d, 7.5)	7′-H, 5′-OMe	103.2	6.69 (d, 7.6)	8′-H
7′	128.0	7.09 (d, 7.5)		125.5	7.14 (dd, 7.6, 8.5)	6′, 8′-H
8′	125.6		1′, 6′-H, 7-H	120.2	6.96 (d, 8.5)	6′-H
9′	136.2		1′, 7′-H	135.9		7′-H
10′	116.0		3′, 6′, 7′-H	113.8		3′, 6′, 8′-H
1-Me	18.5	1.57 (d, 6.6)	1-H	22.4	1.46 (d, 6.4)	1-H
3-Me	18.5	1.14 (d, 6.1)	4-H	22.8	1.27 (d, 8.5)	4-H
2′-Me	22.2	2.31 (s)	1′, 3′-H	20.6	2.13 (s)	3′-H
6-OMe	56.3	3.60 (s)		55.8	3.58 (s)	
8-OMe	55.4	3.90 (s)		59.8	3.09 (s)	
4′-OMe	56.6	3.94 (s)				
5′-OMe	56.4	3.97 (s)		56.2	4.02 (s)	
4′-OH					9.40 (s)	

[a] ^{1}H NMR spectra recorded at 400 MHz in $CDCl_3$. ^{13}C NMR spectra recorded at 100 MHz in $CDCl_3$.

Table 2. NMR Data of **3** and **4**[a]

	3		**4**		
position	^{13}C δ	^{1}H δ (J in Hz)	^{13}C δ	^{1}H δ (J in Hz)	HMBC
1	47.4	4.42 (q, 6.6)	47.4	4.59 (q, 6.5)	3, 7-H, 1-Me
3	42.2	3.11 (m)	42.3	3.30 (m)	1, 4-H, 3-Me
4	35.3	1.75 (dd, 17.0, 11.0)	35.1	1.87 (dd, 18.0, 14.0)	3-Me
		1.94 (dd, 17.0, 4.4)		2.10 (dd, 18.0, 2.0)	
5	119.4		119.4		4, 7-H
6	156.5		156.5		7-H, 6-OMe
7	93.4	6.45 (s)	93.5	6.46 (s)	
8	156.2		156.2		1-H, 8-OMe
9	121.3		121.0		1, 4, 7-H, 1-Me
10	135.8		135.6		1, 4-H, 3-Me
1′	124.3		124.4		3′, 8′-H, 2′-Me
2′	137.1		136.4		2′-Me
3′	113.1	6.84 (s)	112.9	6.84 (s)	2′-Me
4′	153.3		153.4		3′-H
5′	156.5		156.4		6′, 7′-H, 5′-OMe
6′	103.1	6.70 (d, 7.7)	103.1	6.68 (d, 7.7)	7′, 8′-H
7′	125.6	7.13 (dd, 7.7, 8.6)	125.6	7.11 (dd, 7.7, 8.5)	
8′	119.2	6.86 (d, 8.6)	119.6	6.86 (d, 8.5)	6′-H
9′	135.8		135.8		7′-H
10′	113.8		113.8		3′, 6′, 8′-H
1-Me	21.6	1.44 (d, 6.5)	21.5	1.52 (d, 6.7)	1-H
3-Me	22.6	0.97 (d, 6.2)	11.5	1.08 (d, 6.0)	4-H
2′-Me	20.2	2.03 (s)	20.4	2.03 (s)	3′-H
6-OMe	56.0	3.59 (s)	56.0	3.60 (s)	
8-OMe	55.3	3.89 (s)	55.3	3.89 (s)	
5′-OMe	56.1	4.04 (s)	56.1	4.04 (s)	
4′-OH		9.33 (s)		9.37 (s)	

[a] ^{1}H NMR spectra recorded at 400 MHz in $CDCl_3$. ^{13}C NMR spectra recorded at 100 MHz in $CDCl_3$.

Moreover, a diastereomer of compound **1** was already known as a synthetic analogue of korupensamines. The ^{1}H NMR data in the reference were similar to those of compound **1** except for slight differences in chemical shifts of Me-1, Me-3, H-1, H-3, and H-4.[15]

Ancistrotectoriline B (**2**) had molecular formula $C_{25}H_{29}NO_4$ (HRMS). The chemical formula and ^{1}H NMR spectrum suggested that it was also a naphthylisoquinoline alkaloid. The chemical shifts of the C-4 protons (δ 2.57 and 2.82), without shielding effect, indicated that the naphthyl moiety was attached to C-7. Three correlated aromatic protons on the naphthyl ring indicated that the isoquinoline ring was connected to C-1′ or C-3′. Considering that the protons resonating at δ 6.47 and 6.83 represented those neighboring the OH or OCH_3 group, the naphthyl and isoquinoline moieties of **2** were assumed to be connected at C-7 and C-1′.

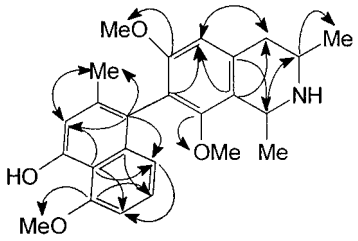

Figure 2. HMBC correlation of **2**.

The full structure including methoxy groups at C-6, C-8, and C-5′ and a free phenolic hydroxyl group attached to C-4′ was established by HMBC experiments (Table 1, Figure 2).

The relative *trans*-configuration at C-1 vs C-3 in **2** was deduced from the chemical shift of H-3 (δ 3.41). Upon the basis of the same reasoning as in **1**, the two stereogenic centers in **2** were both established as *S*. The CD spectrum of **2** showed Cotton effects opposite those of the 7,1′-linked similar structure, ancistrobrevine C.[16] Compound **2** was thus assumed to have an axial configuration opposite that of ancistrobrevine C.

The alkaloids 6-*O*-methyl-4′-*O*-demethylancistrocladine (**3**) and 6-*O*-methyl-4′-*O*-demethylhamatine (**4**) were both light yellow powders. HRMS indicated the molecular formula of both as $C_{25}H_{29}NO_4$, which suggested that they were isomers of ancistrocladine and hamatine. Compounds **3** and **4** belong to the common 5,1′-coupled alkaloids isolated from the species. The characteristic upfield chemical shifts of the H-4 proton signals revealed that those protons were in the shielding zone of the naphthyl ring attached to C-5. Chemical shifts and the splitting patterns of five aromatic protons indicated that there were three neighboring protons in the naphthyl ring. Thus, the isoquinoline moiety was assumed to be connected to C-1′. Methoxy groups were placed at C-6, C-8, and C-5′ by HMBC (Table 2) (methoxy groups correlated to C-6, C-8, and C-5′ respectively); thus, the free hydroxyl group was placed at C-4′.

The relative configuration of **3** and **4** at the stereocenters could also be deduced by the chemical shift of the proton at C-3 (δ 3.11 and 3.30), which indicated that they both possessed a relative *trans*-configuration at C-1 vs C-3. As they both were Ancistrocladaceae-type alkaloids, we deduced the two stereocenters as 1*S*, 3*S*.

In the CD spectra, alkaloids **3** and **4** displayed opposite Cotton effects. Alkaloid **3** showed the same Cotton effect as ancistrocladine, while **4** had the same one as Hamatine.[17] So, **3** and **4** represent a pair of atropisomers. Their atropisomeric nature can also be observed from their NMR spectra. Their ^{13}C NMR spectra were almost superimposable, while most of the protons had very similar chemical shifts except for 1-, 3-, and 4-H (Table 2). Because proton signals are sensitive to shielding effects, the difference of the chemical shifts between the protons of **3** and **4** is caused by their opposite axial chirality.

Experimental Section

General Experimental Procedures. Melting points are uncorrected. ^1H NMR, ^{13}C NMR, HMBC, HMQC, and $^1H-^1H$ COSY spectra were recorded on Bruker AM-400, Bruker DRM-400, and Bruker AC-300 spectrometers in $CDCl_3$ or $(CD_3)_2CO$ with the solvent as internal standard. Chemical shifts are given in δ (ppm). EIMS and HRMS were performed on a Finnigan MAT-95. CD spectra were obtained with a JASCO DIP-181 digital polarimeter. CC and TLC were carried out using silica gel obtained from Qingdao Ocean Chemical Co.

Plant Material. The stems and leaves of *A. tectorius* (Lour.) Merr. were collected in 1996 from Hainan Province, China, and identified by Professor Yi Zhong. Voucher specimens have been deposited at the Herbarium in Shanghai Institute of Materia Medica, and at the Herbarium of the Department of Biology, Hainan Normal University.

Extraction and Isolation. The dried powdered stems and leaves of *A. tectorius* (ca. 2 kg) were macerated twice for 3-day periods with 95% EtOH, and the combined EtOH extracts were pooled and evaporated under reduced pressure. The residue was treated with 2% hydrochloric acid and then basified with concentrated ammonia. The solution was extracted three times with $CHCl_3$. The combined $CHCl_3$ extracts were evaporated under reduced pressure, yielding 54.0 g of crude alkaloid. The crude extract was purified by repeated CC and preparative TLC and yielded **1** (25 mg, 0.05%), **2** (70 mg, 0.13%), **3** (40 mg, 0.07%), and **4** (15 mg, 0.03%).

Ancistrotectoriline A (1): white amorphous material; mp 103−105 °C; $[\alpha]^{25}_D$ +1.34° (*c* 0.75, $CHCl_3$); CD (MeOH) 277 ($\Delta\epsilon$, +0.8), 237 ($\Delta\epsilon$, −21.8), 221 ($\Delta\epsilon$, +26.3), 203 nm ($\Delta\epsilon$, −2.0); IR (KBr) ν_{max} 3417, 1585, 1456, 1328, 1278, 1078, 813 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; EIMS *m*/*z* 421 $[M]^+$, 406, 392, 362, 346, 361, 280, 215, 149, 105 (100), 81; HRMS *m*/*z* 421.2240 $[M]^+$ (calcd for $C_{26}H_{31}NO_4$ 421.2253).

Ancistrotectoriline B (2): light yellow powder; $[\alpha]^{25}_D$ +79.14° (*c* 0.46, $CHCl_3$); CD (MeOH) 284 ($\Delta\epsilon$, −6.3), 256 ($\Delta\epsilon$, +1.6), 247 ($\Delta\epsilon$, −0.6), 232 ($\Delta\epsilon$, +20.8), 205 ($\Delta\epsilon$, −21.1), 199 nm ($\Delta\epsilon$, −16.4); IR (KBr) ν_{max} 3396, 1630, 1614, 1576, 1390, 1259, 1180, 1105, 752, 613 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; EIMS *m*/*z* 407 $[M^+]$, 392 (100), 346, 361, 302, 288, 218, 203, 196, 175, 101, 95, 56; HRMS *m*/*z* 407.2069 $[M]^+$ (calcd for $C_{25}H_{29}NO_4$ 407.2096).

6-*O*-Methyl-4′-*O*-demethylancistrocladine (3): light yellow powder: $[\alpha]^{25}_D$ −3.14° (*c* 0.38, MeOH); CD (MeOH) 315 ($\Delta\epsilon$, +2.0), 241 ($\Delta\epsilon$, +11.4), 238 ($\Delta\epsilon$, −19.9), 202 ($\Delta\epsilon$, +23.5), 192 nm ($\Delta\epsilon$, +6.5); IR (KBr) ν_{max} 3404, 1630, 1612, 1589, 1390, 1319, 1203, 1082, 810, 754 cm^{-1}; ^1H and ^{13}C NMR data, Table 2; EIMS *m*/*z* 407 $[M^+]$, 406, 392 (100), 377, 362, 330, 287, 261, 231, 217, 199, 181, 149, 115, 101, 91, 71; HRMS *m*/*z* 407.2082 $[M]^+$ (calcd for $C_{25}H_{29}NO_4$ 407.2097).

6-O-Methyl-4′-*O*-demethylhamatine (4): light yellow powder; $[\alpha]^{25}_D$ +12.4° (*c* 0.36, MeOH); CD (MeOH) 284 ($\Delta\epsilon$, +3.0), 240 ($\Delta\epsilon$, −21.4), 227 ($\Delta\epsilon$, +38.2), 198 ($\Delta\epsilon$, −15.7),190 nm ($\Delta\epsilon$, +6.1); IR (KBr) ν_{max} 3404, 1728, 1612, 1591, 1390, 1321, 1205, 1080, 966, 810, 754, 604 cm^{-1}; ^1H and ^{13}C NMR data, Table 2; EIMS *m*/*z* 407 $[M^+]$, 406, 392 (100), 362, 347, 316, 280, 245, 199, 196, 167, 149, 115, 101, 91, 71, 59; HRMS *m*/*z*: 407.2082 $[M]^+$ (calcd for $C_{25}H_{29}NO_4$ 407.2097).

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