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ISOQUINOLINE ALKALOIDS FROM *ANCISTROCLADUS TECTORIUS*

A. MONTAGNAC, A. HAMID A. HADI,* F. REMY and M. PAÏS†

Institut de Chimie des Substances Naturelles, C.N.R.S., 91198 Gif-sur-Yvette, France; *Department of Chemistry, University of Malaya, 59100 Kuala Lumpur, Malaysia

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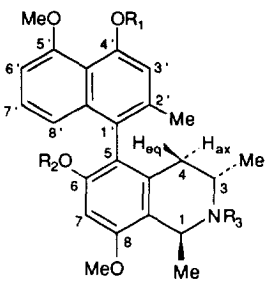
Key Word Index—*Ancistrocladus tectorius*; Ancistrocladaceae; bark; isoquinoline alkaloids.

Abstract—Two new alkaloids were extracted from the bark of *Ancistrocladus tectorius*, 6,8-dimethoxy-3-hydroxymethyl-1-methylisoquinoline and the naphthylisoquinoline, 4′-*O*-demethylancistrocladine, together with the known isoquinolines, 6,8-dimethoxy-1,3-dimethylisoquinoline and (*S*)-6,8-dimethoxy-1,3-dimethyl-3,4-dihydroisoquinoline, which, however, have never been isolated from a natural source. The structures of the new alkaloids, as well as the absolute stereochemistry of 4′-*O*-demethylancistrocladine were established by spectroscopic means and chemical correlations.

INTRODUCTION

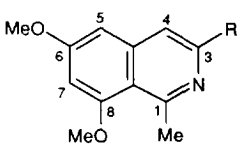
Various naphthylisoquinoline alkaloids, especially ancistrocladine (**1**) and hamatine (**2**), have previously been isolated from the roots, leaves and stems of *Ancistrocladus tectorius*, a southeast Asian liana [1]. We show in the present work that the bark of a sample collected in Malaysia contains a major alkaloid, which is a simple isoquinoline, namely 6,8-dimethoxy-3-hydroxymethyl-1-methylisoquinoline (**3**). Two other known isoquinolines, 6,8-dimethoxy-1,3-dimethylisoquinoline (**4**) [2] and (*S*)-6,8-dimethoxy-1,3-dimethyl-3,4-dihydrolisoquinoline (**5**) [3], which have never been described as natural products, were also isolated, together with a new naphthylisoquinoline alkaloid, 4′-*O*-demethylancistrocladine (**6**).



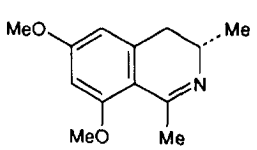
1 R_1 = Me, R_2 = R_3 = H, 1′-*S*
2 R_1 = Me, R_2 = R_3 = H, 1′-*R*
6 R_1 = R_2 = R_3 = H, 1′-*S*
7 R_1 = Me, R_2 = Me, R_3 = CHO, 1′-*S*

RESULTS AND DISCUSSION

The alkaloids were extracted using conventional methods. Recrystallization of the crude extract from methanol afforded almost pure **3** (yield 0.09%). The mother liquors were purified by CC on silica gel yielding **3** (0.04%), **4** (0.002%) and a complex mixture of alkaloids (0.03%). Chromatography of this mixture on alumina, followed by reverse-phase HPLC, afforded **5** (0.001%) and **6** (0.001%). ^{1}H NMR analysis of the other HPLC fractions showed the presence of several naphthylisoquinoline-type alkaloids, but attempts to purify them either on normal or reverse-phase were unsuccessful.



3 R = CH_2OH
4 R = Me



5

Alkaloid **3**, mp 164°, revealed UV maxima typical of an isoquinoline chromophore at 238, 321 and 306 nm (log ε 4.60, 3.74 and 3.75). The EI mass spectrum showed a $[M]^+$ ion at *m/z* 233 corresponding to the molecular formula $C_{13}H_{15}NO_3$. In the ^{1}H NMR spectrum, three methyl singlets at δ2.95 and 3.82, 3.85 were assigned, respectively, to a methyl group and two methoxy groups.

This work has been carried out in the framework of a collaborative program between CNRS (France) and the University of Malaya (Kuala Lumpur, Malaysia).

†Author to whom correspondence should be addressed.

The *meta*-coupled aromatic protons appeared as two doublets (δ6.50 and 6.70, J = 2 Hz) and the hydroxymethylene protons as a singlet at δ3.70. In the ^{13}C NMR spectrum, the two methoxy groups resonated at δ54.8, the methyl group at δ26.5 and the methylene at δ63.8. Detailed analysis of the HMBC spectrum (Table 1) defined the assignments and the connectivity of all carbons leading to the structure depicted in **3**. Further evidence for the latter structure was provided by the conversion of **3** into the known alkaloid **4** using successive mesylation (MsCl, NEt_3, CH_2Cl_2) and reduction with $LiAlH_4$.

Alkaloid **6**, $[\alpha]_D$ + 2.5°, showed the characteristic UV maxima of a naphthylisoquinoline at 230, 290, 306, 320 and 335 nm (log ε 4.65, 3.89, 3.93, 3.82, 3.76). The HREI mass spectrum exhibited a $[M]^+$ ion at m/z 393.1935 (calcd 393.1940) corresponding to the molecular formula $C_{24}H_{27}NO_4$. The ^{1}H NMR spectrum displayed only two methoxy groups at δ4.08 and δ3.82, instead of three for ancistrocladine, and one aromatic methyl group at δ2.08. Two doublets (J = 6.5 Hz) typical of the methyl groups of the heterocyclic ring (3-Me at δ0.96 and 1-Me at δ1.42), were also observed. The downfield shift of the 1-methyl group revealed that it was inside the shielding zone of the nearby naphthyl ring, which therefore was most probably attached to C-5, as in ancistrocladine (**1**) [4]. The chemical shift and splitting pattern of the protons attached to C-1, C-3 and C-4 were very similar to those found in **1** showing the same relative *trans*-stereochemistry between the two methyl groups (see Table 1 and [5]). The aromatic region (Table 1) was also similar to that of **1**, indicating the same substitution pattern by OH or OMe groups at C-6, C-8, C-4′ and C-5′, and by a Me at C-2′. Thus, **6** was an *O*-demethyl derivative of ancistrocladine (**1**) or its atropisomer hamatine (**2**). The extra OH showed hydrogen bonding with an oxygen, as deduced from the downfield shift of the proton at δ9.45 and, thus, could be located only at C-4′ or C-6′. The NOESY spectrum displayed a cross-peak from one of the methoxy groups (δ4.08) to H-6′, which therefore indicated a 4′-OH. Furthermore, a NOESY experiment (Table 1) showed the correlations of 1-Me/H-3 and H-3/H-4eq, which confirm-

Table 1. ^{13}C (62.5 MHz) and ^{1}H NMR (400 MHz) data for 6,8-dimethoxy-3-hydroxymethyl-1-methylisoquinoline (**3**)*† and 4′-*O*-demethylancistrocladine (**6**)†‡

	3			**6**			
Position	δ_C	δ_H (J Hz)	HMBC	δ_C	δ_H (J Hz)	HMBC	NOESY
1	157.2		1-Me	47.4	4.30 *q* (6.5)	1-Me	1-Me
3	152.3		CH_2O	42.1	3.10 *m*	3-Me	1-Me, 4eq, 3-Me
4	114.3	7.42 *s*	5, 10, CH_2O	35.6	*ax.* 1.75 *dd* (17.5, 11)	3-Me	4eq, 3-Me
					eq. 1.95 *dd* (17.5, 4.5)		4ax, 8′
5	97.4	6.70 *d* (2)	6,7	120.3		4eq, 7,1-Me	
6	161.3		6-OMe	152.4		7	
7	98.7	6.50 *d* (2)	5, 6, 8	96.0	6.45 *s*		8-OMe
8	159.2		8-OMe	156.8		7,8-OMe	
9	115.1		1-Me	115.8		7,4eq	
10	140.8			135.2		4eq, 4ax	
1′				120.3		3′,2′-Me	
2′				139.7		2′-Me	
3′				113.3	6.88 *s*	2′-Me	2′-Me
4′				154.7			
5′				156.7		6′,7′,5′-OMe	
6′				103.9	6.80 *d* (8)	8′	5′-OMe
7′				126.7	7.20 *dd* (8, 8)		
8′				118.9	6.95 *d* (8)	6′	
9′				136.4		7′	
10′				114.2		3′,6′,8′	
1-Me	26.5	2.95 *s*		21.6	1.42 *d* (6.5)		
3-Me				22.5	0.96 *d* (6.5)		
3-CH_2O	63.8	4.70 *br s*					
2′-Me				20.8	2.08 *s*		
6-OMe	54.8	3.90 *s*					
8-OMe	54.8	3.90 *s*		55.3	3.82 *s*		
5′-OMe				56.3	4.08 *s*		
4′-OH					9.45 *br s*		

*In $CDCl_3$–CD_4O.
†Assignments based on 2D experiments.
‡In $CDCl_3$.

ed the relative stereochemistry at C-1/C-3 as described previously [5, 6]. Finally, a cross-peak was observed between H-4eq, *cis* to H-3, and H-8′ which revealed that **6** possessed the same *trans* spatial relationship between H-8′ and the 3-methyl as in ancistrocladine (**1**).

The relative stereochemistry of alkaloid **6** was confirmed and its 1′-*S* and 3-*S* configuration was established, by correlation with ancistrocladine. Treatment of **6** with CH_2N_2 gave only partial methylation of the chelated OH-4′. Therefore, the NH was protected by a formyl group and subsequent methylation with MeI (DMF, NaH) yielded the known (−)-*N*-formyl-*O*-methylancistrocladine (**7**). The *S* configuration at C-1′ was further supported by the CD spectrum, which displayed a negative Cotton effect at 225 nm [7]. Thus, both alkaloids **5** and **6** have the same 3-*S* configuration, as found previously for the 5-1′ coupled naphthylisoquinoline alkaloids of *A. tectorius* and other Asian species of *Ancistrocladus* [8].

EXPERIMENTAL

General. Mp: uncorr. Optical rotation and IR were measured in $CHCl_3$, UV and CD spectra in MeOH. EIMS 70 eV. ^{1}H NMR were recorded at 400 MHz, ^{13}C NMR at 62.5 MHz; chemical shifts are given in ppm with TMS as int. standard. 2D NMR expts were carried out with standard pulse sequences. Semi-prep. HPLC: C-18 (25 × 100 mm).

Plant material. Bark of *A. tectorius* (Lour.) Merr was collected in Mersing Johor on 30 April, 1991. Identification was made by F. R. Voucher specimens (KL 4019) are deposited at the Muséum National d'Histoire Naturelle in Paris and at the Herbarium of Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia.

Extraction and isolation of alkaloids. Dried ground bark (2 kg) was extracted exhaustively with MeOH at room temp. The concd extract was diluted with CH_2Cl_2 and re-extracted with 5% HCl. The aq. layer was basified to *ca* pH 11 with NH_4OH and re-extracted with CH_2Cl_2 until a negative Mayer's test was obtained. The CH_2Cl_2 extracts were pooled, washed with H_2O, dried (Na_2SO_4) and evapd, yielding a crude alkaloid fr. (4.6 g). This crude product (3.7 g) was recrystallized from MeOH yielding alkaloid **3** (1.39 g). The mother liquors were chromatographed on silica gel with mixts of heptane–Me_2CO and then CH_2Cl_2–MeOH as eluant, yielding alkaloid **4**, (30 mg) (heptane–Me_2CO, 7:3) which recrystallized from Et_2O, mp 71–73° [lit. [2], mp 65–67°], alkaloid **3** (0.65 g) (heptane–Me_2CO, 3:2) and a mixt. (0.48 g) (CH_2Cl_2–MeOH, 9:1). This mixt. was chromatographed on alumina. Alkaloid **5** (21 mg) was eluted first with Et_2O and converted into its HBr salt, mp 197° (Me_2CO). $[\alpha]_D$ − 138° (MeOH; *c* 0.7) [lit. [3] mp 202°; $[\alpha]_D$ − 141°]. The following frs (Et_2O, Et_2O–MeOH, 49:1, and CH_2Cl_2–MeOH, 99:1) contained complex mixts of naphthylisoquinoline alkaloids (^{1}H NMR). The fr. eluted with CH_2Cl_2–MeOH (99:1) on purification by semi-prep. reverse-phase HPLC using MeOH–1% NH_4Cl adjusted to pH 5.6 with HOAc (9:13), yielded **6** (14 mg). Identification of **4** and **5** was further carried out by comparison of their spectral data with lit. values [2].

6,8-*Dimethoxy*-3-*hydroxymethyl*-1-*methylisoquinoline* (**3**). Crystals, mp 164° (MeOH–CH_2Cl_2). UV λ_{max} nm (log ε) 238 (4.60), 321 (3.74), 306 nm (3.75). EIMS *m/z* (rel. int.): 233 $[M]^+$ (60), 232 $[M-1]^+$ (100). ^{1}H and ^{13}C NMR: Table 1. Analyt: found C 66.66, H 6.59, N 6.01, O 20.34; $C_{13}H_{15}NO_3$ requires C 66.93, H 6.48, N 6.01, O 20.58.

6,8-*Dimethoxy*-1,3-*dimethylisoquinoline* (**4**) *from* **3**. To a stirred soln of **3** (0.1 g, 0.43 mM) in anhydrous CH_2Cl_2 (2 ml) was added at − 20° NEt_3 (0.90 ml, 0.65 mM) and MsCl (0.045 ml, 0.50 mM). The mixt. was stirred for 20 min at − 20° and then diluted with CH_2Cl_2, washed with H_2O and evapd yielding the crude mesylate [0.11 g. ^{1}H NMR: δ3.10, *s* (MeS)]. To a stirred soln of this product in THF (5 ml) was added $LiAlH_4$ (0.5 g) at 0°. The temp. was raised to 20° and the mixt. stirred for 30 min. The mixt. was then diluted with Et_2O and filtered after addition of a satd NH_4SO_4 soln. The filtrate was washed with H_2O and then with brine. The solvent was evapd and the residue purified by CC on silica gel (heptane–Me_2CO, 4:1) yielding **4** (50 mg) which recrystallized from Et_2O, mp 85–90°.

4′-O-*Demethylancistrocladine* (**6**). Gum. $[\alpha]_D$ + 2.5° (*c* 1). UV λ_{max} nm (log ε) 230 (4.65), 290 (3.89), 306 (3.93), 320 (3.82), 335 (3.76). EIMS *m/z* (rel. int.) 393 $[M]^+$ (7), 392 $[M-1]^+$ (7), 378 $[M-15]^+$ (100), 188 (10). CD λ_{ext} (Δε) 225 nm (− 25), 240 nm (+ 7). ^{1}H and ^{13}C NMR: Table 1.

(−)-N-*Formyl*-O-*methylancistrocladine* (**7**) *from* **6**. To a stirred soln of **6** (10 mg, 0.025 mM) in DMF (1 ml) chilled to 0° was added *N*-diethyldiisopropylamine (15 μl, 0.085 mM) and 4-nitrophenylformate (5 mg, 0.030 mM). After 30 min at 0°, the mixt. was diluted with CH_2Cl_2, washed with a satd $NaHCO_3$ soln (× 4) and then with a citric acid soln, and evapd, yielding *N*-formyl-4′-*O*-demethylancistrocladine, which was not purified. To a stirred soln of this crude formamide in DMF (0.8 ml) was added NaH (50 mg) and MeI (0.5 ml). After 1 h, MeI (0.3 ml) was added again and the mixt. stirred for one more hr. Ice and CH_2Cl_2 were then added, the organic layer washed with H_2O and evapd, giving crude **7**, which was submitted to CC on a small silica gel column. Elution with CH_2Cl_2–MeOH (99:1) yielded **7** (5 mg). $[\alpha]_D$ − 78° (*c*; 0.4) [lit. [9] $[\alpha]_D$ − 91.8° ($CHCl_3$; *c* 3.24)]. Spectral data identical in all aspects to lit. [9] and to those of **7** prepd from a sample of ancistrocladine (**1**) in the same manner as from **6**.

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