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8-BENZYLBERBINE AND *N*-OXIDE ALKALOIDS FROM *ARISTOLOCHIA GIGANTEA*

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Key Word Index—*Aristolochia gigantea*; Aristolochiaceae; alkaloids; 8-benzylberbine; 8-benzyltetrahydroprotoberberine; *N*-oxide alkaloids; cyclitol.

Abstract—From the ethanol extract of *Aristolochia gigantea* Mart an 8-benzylberbine-type alkaloid and an *N*-oxide were isolated together with pinitol and sequoyitol. Structural assignments were based on analysis of spectral data, mainly by 2D NMR spectroscopic techniques. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

As part of our ongoing studies on the chemical constituents in *Aristolochia gigantea* Mart (Aristolochiaceae), we reported the isolation and structural identification of ten 8-benzylberbine-type alkaloids [1], as well as allantoin, sitosterol and pinitol which are common in the Aristolochiaceae [2]. The present paper deals with the isolation and structural elucidation of two new 8-benzylberbine-type alkaloids (**1**, named 8-benzylberbine **A** and **2**, an unusual *N*-oxide derivative, named *N*-oxide 8-benzylberbine **B**) from this species.

RESULTS AND DISCUSSION

The EtOH extract of leaves of *A. gigantea* afforded two cyclitols: pinitol and sequoyitol, which were identified by comparison of their physical and spectroscopic data with authentic samples. In addition, the new alkaloids **1** and **2** were also isolated.

The IR spectrum of **1** exhibited a strong broad hydroxyl group (3434 cm^{-1}) absorption band. Its ^1H, ^{13}C and DEPT NMR spectra (Table 1) were very similar to those of other 8-benzylberbine-type alkaloids isolated from this species [1]. The ^1H-^{13}C COSY, ^1H-^{13}C COSY long-range coupling correlations [3] and reverse detected HMQC [4] spectra of **1** established the basic structure. The long range correlation between methoxyl protons and a carbon signal at $\delta \sim 147.6$ suggested that the methoxyl group could be linked to aromatic ring A or D. The structure of **1** was further corroborated by the use of ROESY [5], and TOCSY [6] techniques. The results of these experiments are summarised in Table 2. The correlation between H-4 and OCH_3, H-4 and H_2-5 led us to assign the C-3 position for the methoxyl group. The strong correlation between H-9 and the signals at $\delta \sim 4.37$ suggested that this proton was close to H-8 and H-1″ (anomeric proton). Besides, the NOEs observed between H-8 and H-α,2′,6′ led us to assign the resonances of H-8 and C-8, as well as to confirm the position of the *p*-hydroxybenzyl substituent at the berbine moiety. Thus, the ^1H and ^{13}C resonances were assigned as shown in Table 1 and the structure of this compound was deduced to be **1**.

The liquid secondary ion mass spectrum of **2** displayed a $[M+H]^+$ at *m/z* 598 (for $C_{31}H_{36}NO_{11}$ along with the base peak at *m/z* 328, the latter being due to the cleavage of C-8/C-α bond and the loss of a glucosyl moiety. These ions were 16 amu higher than those of **1**. Compound **2** also showed a strong hydroxyl absorption band (3401 cm^{-1}) in its IR spectrum. Its ^1H, ^{13}C, DEPT and HMQC [4] (Table 3) spectra showed the same number of carbons and protons as **1**, but with the N → O neighbouring protons and carbons shifted downfield. The $\Delta\delta$ observed between the carbons C-6, C-8 and C-14 of **3** [1] and **2** were 17.4, 17.8 and 14.0 ppm, respectively. This deshielding effect was analogous to that observed for tetrahydroisoquinoline, berbine and indole alkaloids in relation to their *N*-oxide derivatives [7–10]. A combination of ROESY [5] and TOCSY [6] experiments, summarised in Table 4, led us to deduce the structure **2** the oxide, as well as to assign the ^1H and ^{13}C NMR signals as indicated in Table 3. It is interesting that by ROESY experiments the aromatic substituents could also be assigned by taking into account the NOEs

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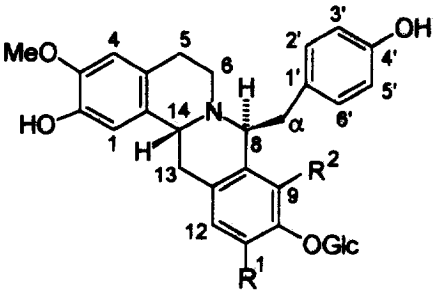
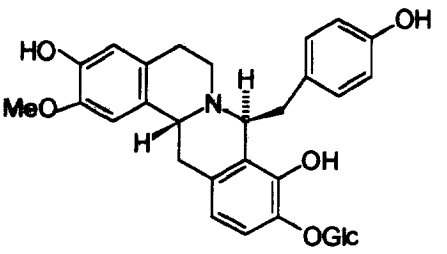


	R1	R2	
1	OH	H	N
2	H	OH	N → O



3

Table 1. ^{1}H and ^{13}C NMR spectroscopic data of **1** with ^{1}H multiplicities established by *J*-resolved and ^{13}C multiplicities by DEPT pulse sequences MeCN-d_3–D_2O (7 : 3)

Position	^{1}H (δ	*J* (Hz)	^{13}C (δ)	HMQC correlations (^{1}H)†
1	6.71 *s*		113.5	1
2			145.6	(4)
3			147.7*	(4, OMe)
4	6.75 *s*		113.0	4
4a			126.0	(1)
5*ax*	3.02 *ddd*	18.0, 11.7, 5.0	29.2	5*ax*
5*eq*	3.5–3.5 *m*			
6*ax*	3.27 *m*	17.6, 11.7, 5.9	48.5	6*ax*
6*eq*	3.4–3.5 *m*			
8	4.37 *dd*	10.1, 3.6	67.2	8
8a			131.8	
9	6.25 *s*		119.2	9
10			144.3	(12)
11			147.6*	(9)
12	6.69 *s*		116.7	12
12a			128.6	(9)
13*ax*	2.80 *dd*	17.8, 11.4	32.5	13*ax*
13*eq*	3.30 *dd*	17.8, 5.5	32.5	13*eq*
14	4.68 *dd*	11.4, 5.5	52.0	14
14a			125.5	(4)
αa	2.92 *dd*	13.7, 10.1	39.7	αa
αb	3.32 *dd*	13.7, 3.6	39.7	αb
1′			129.8	(3′, 5′)
2′,6′	6.95 *d*	8.3	131.9	2′, 6′
3′,5′	6.74 *d*	8.3	116.1	3′, 5′
4′			156.4	(2′,6′)
1″	4.38 *d*	7.4	105.0	1″
2″	3.3–3.5 *m*		74.6	2″
3″,5″	3.3–3.5 *m*		77.3, 77.7	3″,5″
4″	3.3–3.5 *m*		70.8	4″
6″a	3.71 *dd*	12.5, 5.5	62.0	6″a
6″b	3.75 *dd*	12.5, 2.5	62.0	6″b
OMe	3.81 *s*		56.3	OMe

*Values bearing the same sign may be reversed.

†Long range coupling between ^{1}H and ^{13}C are shown in parentheses.

observed between H-11 and H-1″; H-4 and $OC\underline{H}_3$ protons.

There are six isomeric pairs possible [1, 11] for the compounds: two *trans* B/C ring (**4a**, **b**) and four *cis* B/C ring (**4c–f**), due to asymmetric centres: C-8, C-14 and N-7. The stereochemistry of **1** and **2** were then deduced by TOCSY and ROESY experiments. The spectra of **1** showed a coupling and NOE correlation

Table 2. Summary of the 2D ^{1}H NMR spectroscopic correlations of **1**

Proton	TOCSY (proton)	ROESY (proton NOEs)	ROESY (coupling)
1	14, 13*ax*, 13*eq*	14, 13*eq*	5*eq*
4	5*eq*	5*ax*, 5*eq*	5*ax*, 5*eq*, OMe
5*ax*	5*eq*, 6*ax*	4, 5*eq* and/or 6*eq*	5*eq*, 6*ax*, 4
8, 1″	αa, αb, 2″	αa, 6*ax*, 6*eq* and/or 3″, 9, 6′, 2′	9, 6*ax*, αa, αb, 6*eq* and/or 2″
9	12	8 and/or 1″	8 and/or 1″
13*ax*	13*eq*, 1	12, 13*eq* and/or 6*ax*	13*eq*, 14
14	13*ax*, 6*eq*, 1	αa, 1	13*ax*, 13*eq*
αa	αb, 8	8, 14, 2′, 6′	8, 2′, 6′
2′, 6′	3′, 5′	3′, 5′, 8, αa, αb	3′, 5′, 8, αa, αb
1″	2″		2″

Table 3. ^{1}H and ^{13}C NMR spectroscopic data of **2** with ^{1}H multiplicities established by *J*-resolved and ^{13}C multiplicities by DEPT pulse sequence; MeCN-d_3–D_2O (7 : 3)

Position	^{1}H (δ)	*J* (Hz)	^{13}C (δ)	HMQC correlations (^{1}H)
1	6.75 *s*		113.5	1
2			143.1*	
3			146.7†	
4	6.80 *s*		111.7	4
4a			127.8	
5*ax*	2.77 *dd*	18.0, 5.0	25.0	5*ax*
5*eq*	3.38 *m*		25.0	5*eq*
6*ax*	3.70 *m*		63.4	6*ax*
6*eq*	3.43–3.65 *m*		63.4	6*eq*
8	5.20 *dd*	8.7, 4.0	78.9	8
8a			123.5‡	
9			142.7*	
10			144.5†	
11	7.20 *d*	7.8	118.6	11
12	6.83 *d*	7.8	120.6	12
12a			122.2‡	
13*ax*	3.07 *dd*	16.5, 12.0	31.6	13*ax*
13*eq*	3.43–3.65 *m*		31.6	13*eq*
14	4.94 *dd*	12.0, 4.0	63.3	14
14a			126.6	
αa	3.36 *dd*	16.0, 4.0	38.8	αa
αb	3.32 *dd*	16.0, 8.7		
1′			130.2	
2′,6′	7.08 *d*	7.8	129.9	2′,6′
3′,5′	6.77 *d*	7.8	115.1	3′,5′
4′			155.2	
1″	4.85 *d*	7.5	102.2	1″
2″	3.43–3.65 *m*		75.2	2″
3″, 5″	3.43–3.65 *m*		78.3, 77.5	3″, 5″
4″	3.43–3.65 *m*		72.6,	4″
6″a	3.75 *dd*	12.0, 5.2	61.8	6″a
6″b	3.88 *dd*	12.5, 2.5	61.8	6″b
OMe	3.85 *s*		55.3	OMe

*†‡Values bearing the same sign may be reversed.

between H-14 and H-1 establishing that H-14 had an equatorial position in relation to ring B. Therefore, the *trans* conformations (**4a**, **b**) and the *cis* conformations **4e** and **4f** could be excluded. In addition, the ROESY spectra of both compounds showed NOE contours between H-14 and H-αa establishing that H-14 and C-α methylene group were oriented to the same side of ring C. Thus, both structures adopted the *cis*

Table 4. Summary of the 2D ^{1}H NMR spectroscopic correlations of **2**

Proton	TOCSY (proton)	ROESY (proton)
1	14	14, 13*eq*
4	5*eq*, 5*ax*	5*ax*, 5*eq*, OMe
5*ax*	4, 5*eq*, 6*ax*, 6*eq*	6*eq*, 4
6*ax*	6*eq*, 5*ax*	6*eq*
8	αa, αb	αa, 6*eq*, 2′, 6′
11	12	1″
13*ax*	13*eq*, 14	13*eq*
14	13*ax*, 13*eq*, 1	αa, 13*eq*, 1
2′, 6′	3′, 5′	3′, 5′, 8, αa
1″	2″	3″, 5″, 11
6″a, 6″b	5″	5″

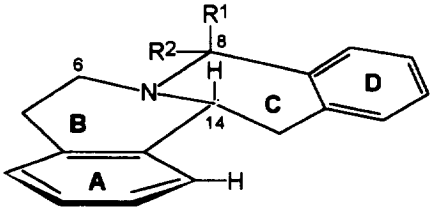
form at the B/C ring juncture (**4c**). The correlation observed between H-8 and H-6*eq* of **2** led us to confirm the relative configuration of N-7, in which H-6*ax* shifted to a lower field than H-6*eq* due to an *anti* interaction with its N → O group [8, 9].

EXPERIMENTAL

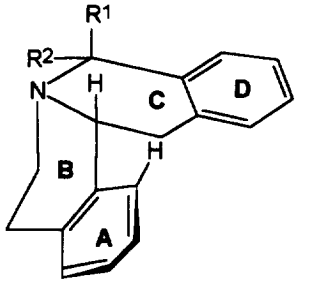
General. Melting points were determined on a Kofler hot-stage microscope and are uncorr. NMR spectra were measured on Bruker spectrometers, ^{1}H NMR, *J*-resolved, COSY, ROESY, and TOCSY spectra were obtained at 500 MHz; ^{13}C NMR and DEPT were obtained at 50 MHz; HETCOR spectra ^{1}H-^{13}C COSY (optimised for $J = 7$ Hz) and HMQC were taken at 200/50 MHz and at 500/126 MHz, respectively [locked to the major deuterium signal of the solvent (MeCN-d_3–D_2O, 7 : 3); with presaturation at D_2O absorption frequency; room temp]. Chemical shifts are given in ppm relative to TMS, with coupling constants in Hz. The mass spectra were obtained on a ITD 800 Finnigan MAT (ion trap detector) spectrometer. The LSIMS were recorded on a VG Quattro spectrometer by bombardment of samples (dissolved in a glycerol matrix) with Cesium, 20 kV. IR; KBr discs. Optical rotation: $[\alpha]_D$ values were recorded in units of 10^{-1} deg cm^2 g^{-1} and λ in nm, and were measured on a Polamat-A. UV absorption was measured using a Hewlett Packard 8452A, Diode array spectrophotometer. TLC: silica gel 60 PF_{254}.

Plant material. The fresh leaves (2.2 kg) of *A. gigantea* cultivated in Araraquara, SP [1], were collected, dried, ground and extracted exhaustively at room temp. and then by Soxhlet with EtOH.

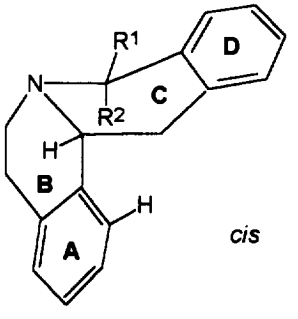
Isolation. The EtOH solution obtained at room temp were conc. The extract (13.8g) was chro-



		R1	R2
trans	**4a**	CH_2Ar	H
	4b	H	CH_2Ar



		R1	R2
cis	**4c**	CH_2Ar	H
	4d	H	CH_2Ar



		R1	R2
cis	**4e**	CH_2Ar	H
	4f	H	CH_2Ar

matographed over a silica gel flash column using CH_2Cl_2–EtOAc with increasing polarity, leading to 8 frs. Fr. 2 (8.1 g) by CC (silica gel, 35g, $CHCl_3$–MeOH–NH_4OH, 94.5 : 5.0 : 0.5) followed by HPTLC ($CHCl_3$–MeOH–NH_4OH, 69 : 30 : 1) afforded **2** (30 mg). After prep. TLC ($CHC)_3$–MeOH–NH_4OH, 69 : 30 : 1), fr. 5 (378 mg) afforded **1** (70 mg). Fr. 6 yielded pinitol (1.52 g) and fr. 7 afforded the sequoyitol (1.02 g) which were purified further by recrystallisation from MeOH.

(−)-10-O-[*β-Glucopyranosyl*]-8-[4′-*hydroxybenzyl*]-3-*methoxyberbin*-2,11-*diol* (*8-benzylberbine A*, **1**). Amorphous yellow solid, 201–204° (MeOH). $[\alpha]_D^{25}$ (MeOH; *c* 0.04) (*λ*): −50.8° (578), −75.2° (549), +250.0° (436). (Found: C, 64.9; H, 5.9; N, 2.6. $C_{31}H_{35}NO_{10}$ requires: C, 64.0; H, 6.0; N, 2.4%). UV λ_{max}^{MeOH} nm (log *ε*): 210 (3.65), 230 (3.71), 250 (3.69), 270 (3.71), 290 (3.71), 325 (3.63), 385 (3.22). IR ν_{max}^{KBr} cm^{-1}: 3434, 1633, 1516, 1451, 1383, 1271, 1175, 1105, 1073, 1040. EIMS 70 eV *m/z* (rel. int): 481 (< 1), 474 (1.3), 431 (1.0), 381 (< 1), 342 (< 1), 331 (< 1), 323 (< 1), 319 (< 1), 281 (1.1), 243 (1.1), 231 (1.7), 219 (2.5), 181 (7.8), 149 (18.5), 131 (18.5), 122 (45.0), 121 (100), 108 (21.2), 107 (41.6).

(−)-N-*Oxide*-8-*benzylberbine B* (**2**). Amorphous yellow solid, 220–224° decomp. (MeOH). $[\alpha]_D^{25}$ (MeOH; *c* 0.12) (*λ*): −24.6° (578), −41.0 (546), −90.2° (436), −114.8° (406). UV λ_{max}^{MeOH} nm (log *ε*): 215 (3.22), 230 (3.31), 250 (3.30), 265 (3.29), 275 (3.31), 295 (3.31), 325 (3.08) sh, 385 (2.88). IR ν_{max}^{KBr} cm^{-1}: 3401, 2956, 2849, 1616, 1517, 1500, 1464, 1384, 1275, 1246, 1074. EIMS 70 eV *m/z* (rel. int): 380 (< 1), 337 (< 1), 323 (< 1), 309 (< 1), 295 (< 1), 281 (1.0), 279 (1.1), 267 (1.0), 253 (1.1), 211 (1.0), 197 (1.1), 183 (2.0), 169 (1.9), 167 (4.4), 161 (75.6), 149 (28.6), 115 (23.4), 97 (23.5), 87 (37.5), 85 (35.9), 77 (36.9), 71 (59.3), 69 (46.6), 57 (100.0).

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