ALBIFLOMANTHINE - A CRINANE ALKALOID FROM *HAEMANTHUS ALBIFLOS* (JACQ.)

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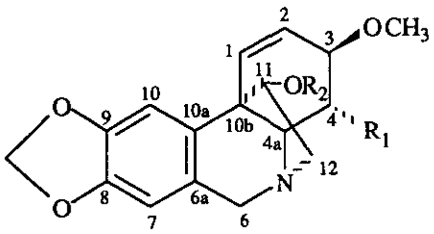
Abstract - A new crinane alkaloid, albiflomanthine (1) has been isolated from *Haemanthus albiflos* Jacq. Its structure has been elucidated on the basis of spectral analyses. It is the first crinane alkaloid to present an oxygen substituent at C-4 .

The recent description of the strong antiviral activity[1-3] of bulb extracts of *Haemanthus albiflos* Jacq. led us to reinvestigate the alkaloid contents of this *Amaryllidaceae* species.[4, 5] The two alkaloids tazettine [6, 7] and lycorenine [6] were previously described from this plant, whereas the very closely related species *Haemanthus albomaculatus* Bak. led to the obtention of albomaculine, coccinine, and lycorenine.[8] We report here the structural elucidation of a new alkaloid isolated from *Haemanthus albiflos* bulbs [9, 10] and named albiflomanthine.

Albiflomanthine (1) was obtained as a colourless amorphous solid, $[\alpha]^{20}_{D} = +31°$ (EtOH, *c* = 0.05) (contents : 0.04 % from the dried plant material). The empirical formula was established by high resolution mass spectrometry as $C_{17}H_{19}NO_5$ (Found : 317.1261; Calcd : 317.1263). The uv spectrum displayed characteristic absorptions at λ^{MeOH}_{max} nm (log ε): 219 (3.73), 240 (sh.) (3.49), and 294 (3.65) associated with a methylenedioxyaryl chromophore.[11, 12] The ir spectrum afforded typical bands at ν^{KBr}_{max} cm $^{-1}$: 3300 (OH), 2830 (aliph. OCH_3), 1485 (arom. C=C), 930 and 730 (methylenedioxy). The general feature of the ms (ie), m/z (%) : 317 (M^+) (100), 316 (6), 302 (2), 288 (10), 286 (15), 285 (20), 284 (12), 273 (10), 256 (7), 241 (5), 240 (12), 225 (22), 197 (21), 115 (58) suggested the structure of a 5,10b-ethanophenanthridine derived alkaloid having a hydroxyl group on the ethylene bridge at C-11,[13,14] and therefore related to haemanthamine (**2**). Chemical evidence for a haemanthamine derivative bearing an additional hydroxyl group was obtained by acetylation (Ac_2O/C_5H_5N/48 h/20°C), which led to a di-*O*-acetyl derivative (**3**), M^+= 401, in almost quantitative yield. Location of two oxygenated substituents at C-3 and C-4, both in pseudo-axial position, could be deduced from the ^1H nmr data (Table I) of albiflomanthine (**1**) compared with those of haemanthamine (**2**).[15] Of particular interest were the lack of vinylic coupling between H-1 and H-3 indicating a pseudo-axial oxygen substituent at C-3, and the small couplings excluding trans-diaxial relationships between H-3 and H-4 (J=1.5 Hz) and H-4 and H-4a (J=2.5 Hz). Assignments of the ^{13}C nmr signals of **1** , **2** and **3** (Table II) were unambiguously deduced from 2D ^{13}C-^1H correlation experiments, HETCORR [16,17] and COLOC.[17,18] Cross peaks observed on the COLOC spectra of **1** and **3** between the signals of C-3 and OCH_3 gave evidence for the location of the methoxy substituent at C-3. In addition, these correlation experiments led to a reassignment of the ^{13}C nmr data previously published for haemanthamine.[19] Finally, the absolute configurations of both the ethylene bridge and the hydroxyl group at C-11 were established by cd.[20,21] Albiflomanthine displayed a negative dichroism at 244 nm similar in sign and magnitude to that observed for haemanthamine (**2**) (Scheme 1). This similarity demonstrated identical absolute configurations of the asymmetric centres at C-10b and C-11 in these two alkaloids. These data permitted depicting the structure of albiflomanthine as **1** .

The isolation of albiflomanthine is interesting from a biogenetic point of view. Its



1 $R_1 = OH \quad R_2 = H$

2 $R_1 = R_2 = H$

3 $R_1 = OCOCH_3 \quad R_2 = COCH_3$

Table I : ^{1}H Nmr spectra of albiflomanthine (**1**) and di-*O*- acetylalbiflomanthine (**3**) (300 MHz, $CDCl_3$ / TMS, δppm, J in Hz)

H	**1**			**3**		
1	6.58	d	J = 10	6.43	d	J = 10
2	6.23	ddd	J = 10, 5, 1	6.07	ddd	J = 10, 5, 1
3	3.71	dd	J = 5, 1.5	3.72	dd	J = 5, 2
4	4.24	ddd	J = 4, 1.5, 1	5.36	ddd	J = 4, 2, 1
4a	3.39	d	J = 4	3.55	d	J = 4
6	3.67	d	J = 17	3.78	d	J = 17
	4.34	d	J = 17	4.48	d	J = 17
7	6.46	s		6.49	s	
10	6.88	s		6.48	s	
11	4.05	dd	J = 6.5, 4	5.08	dd	J = 7, 4
12	3.29	m		3.37	dd	J = 14, 7
				3.46	dd	J = 14, 4
O-CH_2-O	5.90	d	J = 1.5	5.92	d	J = 1.5
	5.92	d	J = 1.5	5.93	d	J = 1.5
OCH_3	3.40	s		3.48	s	
$COCH_3$				2.03	s	
				2.10	s	

Scheme 1: Cd curves of albiflomanthine (**1**) and haemanthamine (**2**)

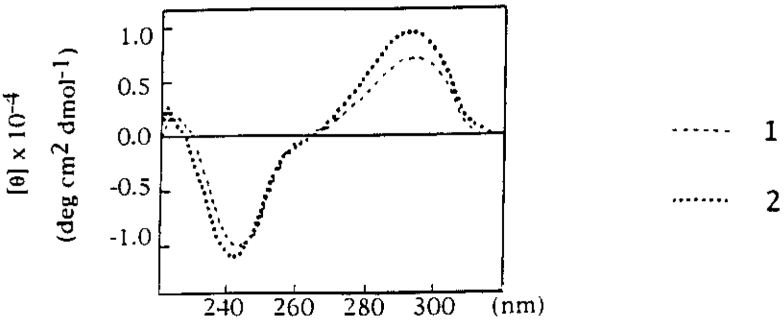


Table II : ^{13} C Nmr spectra of haemanthamine (**2**), albiflomanthine (**1**) and di-*O*-acetylalbiflomanthine (**3**) (75 MHz, $CDCl_3$ / TMS, δ ppm)

	haemanthamine (**2**)	albiflomanthine (**1**)	**3**
1	127.3	130.1	127.7
2	131.6	125.6	127.2
3	72.7	77.6	74.2
4	28.1	67.7	68.9
4a	62.6	66.8	65.1
6	61.2	62.8	62.9
6a	126.5	124.7	125.0
7	106.7	106.8	106.8
8	146.3*	146.8*	146.9*
9	146.0*	146.4*	146.7*
10	103.2	103.8	103.6
10a	135.2	136.7	136.5
10b	49.9	49.4	47.5
11	80.0	80.2	79.9
12	63.5	65.7	62.4
O-CH_2-O	100.7	100.9	101.0
OCH_3	56.5	56.8	57.7
CO-*C*H_3			21.4
			21.1
*C*O-CH_3			170.3
			169.8

* Assignments may be reversed on the same column.

oxidation level at C-ring may lead to consider two *ortho*-dioxygenated C_6-C_1 and C_6-C_2 units as its biogenetic precursors.[22, 23] This oxidation level is commonly encountered in several classes of *Amaryllidaceae* alkaloids.[24] In contrast, it is described here for the first time in a crinane derivative bearing a 1,2-double bond. All the representatives of this class previously isolated bear only one oxygen substituent at C-ring which is therefore recognized to arise from a mono-oxygenated C_6-C_2 tyrosine-derived unit .

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9. The plant material was obtained from the botanical garden of the Faculté de

Pharmacie, Université René Descartes, Paris, where *Haemanthus albiflos* is cultivated under greenhouse conditions. It was collected in december 1990, just before flowering. An herbarium sample is kept in the Musée de Matière Médicale of the Université René Descartes, Paris.

10. The dried bulbs of *Haemanthus albiflos* afforded 1.85 % of crude alkaloids after extraction by standard means. In addition to albiflomanthine, fractionation of the alkaloid extract yielded the known compounds albomaculine, haemanthamine, haemanthidine (as a mixture of 6α-6β epimers), galanthamine, and lycoramine.
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