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ALKALOIDS FROM LEAVES OF *ALANGIUM BUSSYANUM*

A. O. DIALLO, H. MEHRI, L. IOUZALEN and M. PLAT

Université Paris-Sud, Laboratoire de Chimie Thérapeutique II, Centre d'Etudes Pharmaceutiques, Rue Jean Baptiste Clément, 92296 Châtenay-Malabry Cedex, France

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Key Words Index—*Alangium bussyanum*; Alangiaceae; leaves; benzoquinolizidines; indolobenzoquinolizidines; alangiobussine; alangiobussinine.

Abstract—Eight alkaloids were isolated from the leaves of *Alangium bussyanum*, six of which were known, viz. tubulosine, 9-desmethyltubulosine, deoxydesmethyltubulosine, *O*-methyltubulosine, deoxytubulosine and 9- or 10-desmethylprotoemetinol. The two other alkaloids were new and their structures were established as alangiobussine and alangiobussinine by spectroscopic and chemical methods.

INTRODUCTION

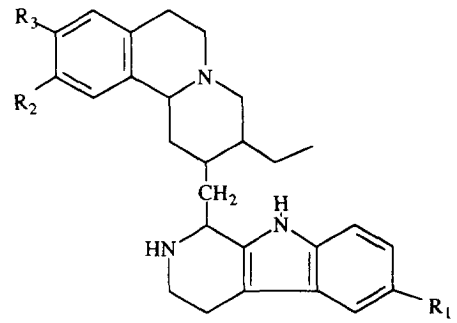
Alangium is the only genus in the Alangiaceae, which is composed of *ca* 20 species (shrubs or trees) occurring in the Tropics of the Eastern Hemisphere [1]. Among these species, *A. lamarckii* and *A. vitiense* have been widely studied, from which phenolic benzoquinolizidine and indolobenzoquinolizidine alkaloids are the most encountered compounds [2–4]. Pharmacological studies of extracts from these two species showed inhibiting effects on the biosynthesis of DNA and proteins on the one hand [5] and, on the other, stimulating effects on the parasympathetic system [6].

The present study concerns the isolation and structural determination of new alkaloids from the leaves of a New Caledonian species of *A. bussyanum* collected in October 1970 in the Riviere bleue forest, lot no. 265J. Among the eight alkaloids isolated from this batch, six are common to the two species mentioned above [2], and the other two are new alkaloids.

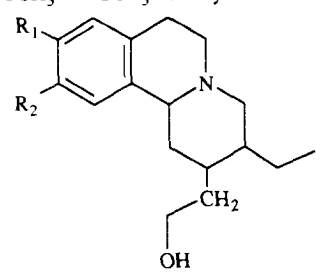
RESULTS AND DISCUSSION

Leaves were submitted to usual procedures for extraction and isolation of alkaloids. The six known alkaloids were identified as tubulosine (**1**), desmethyltubulosine (**2**), deoxydesmethyltubulosine (**3**), *O*-methyltubulosine (**4**), deoxytubulosine (**5**) and 9- or 10-desmethylprotoemetinol (**6**). Identifications were confirmed by comparison of their spectroscopic data with published data [6] and comparisons of R_f values with those of authentic samples. Besides these known compounds, two new alkaloids were isolated, alangiobussine (**7**) and alangiobussinine (**8**).

Compounds **7** and **8** were obtained as crystals from methanol and their high resolution mass spectra gave $[M]^+$ at *m/z* 356.1658 and 354.1494, respectively, compatible with the formulae $C_{22}H_{20}N_4O$ and $C_{22}H_{18}N_4O$, respectively. Their structures were established on the basis of spectroscopic analyses and confirmed by partial synthesis.



R_1	R_2	R_3	
OH	OCH_3	OCH_3	tubulosine
OH	OH	OCH_3	desmethyltubulosine
H	OH	OCH_3	deoxydesmethyltubulosine
H	OCH_3	OCH_3	deoxytubulosine
OCH_3	OCH_3	OCH_3	methyltubulosine



$R_1 = R_2 = OCH_3$: protoemetinol
$R_1 = OH, R_2 = OCH_3$: desmethylprotoemetinol

The UV spectrum of **7** (see Experimental) is characteristic of a non-substituted indolic chromophore [7]; an important bathochromic shift (334.5 to 367.5 nm) was observed in an acidic medium. The UV spectrum of **8** however, remained unchanged in this medium. On the other hand, subtraction of the UV spectrum of tryptamine (**9**) from those of compounds **7** and **8** (run in neutral conditions) gave UV profiles with absorption bands at 204.5, 245 and 334 nm for **7** and 207.5, 302, 316 nm for **8**, revealing that the other moiety of **8** is a β-carboline [7]. Absorption bands at 1670 cm^{-1} (conjugated amide) [2, 3], and 3120, 3380 and 3475 cm^{-1} (NH groups), were observed in the IR spectrum [8]. The ^1H NMR data for **7** and **8** are given in Table 1 [7, 9].

Comparison between the mass spectra of **7** and **8** (Table 2) indicated that the two compounds possess an identical moiety, the second moiety of **8** having one extra degree of unsaturation [7, 8]; the ions *m/z* 143 and 130 are characteristic of **9**. These spectroscopic data suggest for alangiobussine and alangiobussinine the hypothetical structures **7** and **8**, respectively.

In order to confirm the structures assigned to compounds **7** and **8** isolated in minute amounts, synthesis was attempted using the Pictet Spengler reaction [10, 11]. Acid-catalysed reaction of glyoxal with **9** produced two minor compounds identical to compounds **7** and **8** (yield 10%). Unambiguous assignment of the carbon chemical shifts of the two compounds (Table 3), [7, 12, 13] was achieved by synthesis of compound **10** using the same method, in which the non-β-carboline moiety is phenylethylamine. From this evidence, the structures **7** and **8** for alangiobussine and alangiobussinine, respectively, were confirmed.

EXPERIMENTAL

UV were measured in 96% EtOH sol, IR in KBr discs. NMR are given in ppm and referenced to TMS as int. standard; *J* values are given in Hz. TLC was performed on silica gel 60 (Merck) with detection by UV light (254 and 366 nm).

Extraction and isolation. Dried leaves of *A. bussyanum* (Guill.) (3.68 kg) were finely crushed and extracted with 15 l of petrol, left to dry at room temp. and then extracted

Table 1. Comparative ^1H NMR data for compounds **7** and **8**

^1H	Alangiobussine (**7**)	Alangiobussinine (**8**)
H_3	3.85, *t*, *J* = 8.5 Hz, 2H	
H_4	2.75, *t*, *J* = 8.5 Hz, 2H	
$H_{3''}$	3.60, *t*, *J* = 7 Hz, 2H	3.85, *t*, *J* = 7 Hz, 2H
$H_{4''}$	2.95, *t*, *J* = 7 Hz, 2H	3.15, *t*, *J* = 7 Hz, 2H
NH	7.95, 8.10, *s*, 2H	8.10, 8.80, *s*, 2H
CO–NH	10.05, *s*, 1H	10.15, *s*, 1H
Ar. H	6.95–7.50, *m*, 9H	7.50–8.20, *m*, 11h

δ: ppm, 200 MHz, $CDCl_3$.

Table 2. Mass spectral fragmentation pattern (*m/z*) of compounds **7** and **8**

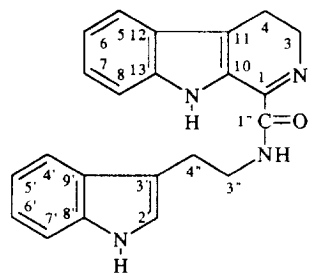
Alangiobussine (**7**)		Alangiobussinine (**8**)
$[M]^+$	356	354
	226	224
	197	195
	170	168

Peaks in common: 143, 130, 103, 77, 43

Table 3. Comparative ^{13}C NMR data for compounds **7**–**10**

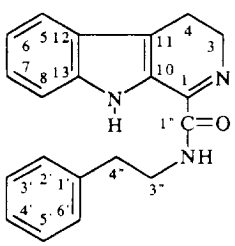
C	**7**	**8**	**9**	**10**
1	151.5 *s*	165 *s*	—	151.6 *s*
3	47 *t*	137.2 *d*	—	47.3 *t*
4	19.2 *t*	123.23 *d*	—	19.38 *t*
5	120.43 *d*	120.57 *d*	—	120.58 *d*
6	120.74 *d*	129.6 *d*	—	120.9 *d*
7	124.59 *d*	118.94 *d*	—	124.5 *d*
8	112.75 *d*	113.8 *d*	—	112.8 *d*
10	126.46 *s*	134.8 *s*	—	126.5 *s*
11	120.01 *s*	120.34 *s*	—	120.5 *s*
12	127.33 *s*	131.3 *s*	—	126.8 *s*
13	138.3 *s*	142.04 *s*	—	138.3 *s*
1′	—	—	—	138.7 *s*
2′	122.15 *d*	122.34 *d*	122.16 *d*	128.8 *d*
3′	112.75 *s*	112.3 *s*	113.65 *s*	128.8 *d*
4′	119.45 *d*	118.9 *d*	119.42 *d*	128.8 *d*
5′	118.77 *d*	118.42 *d*	119.10 *d*	128.8 *d*
6′	122.15 *d*	121.66 *d*	122.55 *d*	128.8 *d*
7′	111.35 *d*	112.03 *d*	111.55 *d*	—
8′	136.4 *s*	136.7 *s*	136.78 *s*	—
9′	127.33 *s*	127.71 *s*	127 75 *s*	—
1″	163 *s*	172 *s*	—	163 *s*
3″	39.9 *t*	39.9 *t*	42.55 *t*	41.1 *t*
4″	25.2 *t*	25.7 *t*	29.67 *t*	35.7 *t*

δ: ppm, 50 MHz, $CDCl_3$.



7

8 = Δ_{3-4} **7**



10

with 15 l of MeOH. The MeOH extract, concd under red. pres., was acidified with 3% HOAc, washed with Et_2O, then made alkaline with Na_2CO_3, and the free alkaloids extracted with EtOAc then CH_2Cl_2 to give 18.32 g total alkaloids (yield 0.497%). Alkaloid residue (8.65 g) was chromatographed on an alumina column (basic, 0.063–0.2 mm) and eluted with a gradient of MeOH–CH_2Cl_2 (1–30%); 25–30 ml frs were collected. Fraction D (CH_2Cl_2–MeOH, 49:1) (0.086 g) contained two minor alkaloids which were sepd by prep. TLC on silica gel. After three successive elutions with CH_2Cl_2–hexane–NH_4OH (17:3:0.1), 20 mg of **7** and 14 mg of **8** were isolated.

Frns E (CH_2Cl_2–MeOH, 19:1) (2.24 g) and F–I (CH_2Cl_2–MeOH, 9:1) (2.048 g) were combined after TLC analysis on basic silica gel to give 4.288 g, which was then submitted to flash CC (basic silica gel 60, 0.063–0.2 mm) and eluted with a gradient of MeOH–CH_2Cl_2 (0.5–3%). The collected subfrs led to the isolation of **2** (0.957 g), which was recrystallized from $CHCl_3$–MeOH, **6** (0.117 g), **4** (0.023 g) and **5** (0.036 g). The mother liquor (0.985 g) from **2** was treated in the same way and led to the isolation of **1** (0.015 g), **2** (0.110 g) and **3** (0.078 g).

Synthesis of alkaloids **7** *and* **8**. An aq. soln (100 ml) of 3.93 g of **9** chlorohydrate (20 meq) and 1.5 ml 40% glyoxal (10 meq) was stirred for 6 hr at room temp. The mixt. was then made basic with NH_4OH and extracted ×3 with 200 ml of CH_2Cl_2. The CH_2Cl_2 soln, when dried (Na_2SO_4) and evapd under red. pres. gave 2.297 g of a brownish crude mixt. Further purification by silica gel CC (CH_2Cl_2–MeOH, 49:1) and prep. TLC using system CH_2Cl_2–MeOH–NH_4OH (98:1.5:0.5) gave 80 mg **7** and 47 mg **8** (yield 10%).

Alangiobussine (**7**). $C_{22}H_{20}N_4O$, $[M]^+$ *m/z* 356 (found $[M]^+$ 356.1658). (%calc.: C 74.22; H 5.66, N 15.73; % found.: C 74.19; H 5.66; N 15.73). Recrystallized from MeOH (54 mg), mp 152–154°. UV (EtOH) λ_{max} nm (log ε), (neutral), 220.5 (4.93), 250 (4.49)*sh*, 290.5 (4.18), 334.5 (4.31); (0.1 N HCl), 217.5 (4.91), 250 (4.41), 367.5 (4.37), (0.1 N NaOH), 221 (4.49), 250 (4.41), 332 (4.19). IR, *v*: cm^{-1} (KBr), 3380, 3120, 2940, 1670, 1640, 775. EIMS 70 eV, *m/z* (rel. int.): 356 $[M]^+$ (41.06), 357 (18.06), 358 (0.528), 226 (100), 224 (7.71), 214 (16.14), 197 (12.03), 169 (51.77), 143 (72.11), 130 (61.57), 115 (22.07), 77 (14.26). NMR in Tables 1 and 3.

Alangiobussinine (**8**). $C_{22}H_{18}N_4O$, $[M]^+$ *m/z* 354 (found $[M]^+$ 354.1494). (% calc.: C 74.64; H 5.12; N 15.82; % found: C 74.61; H 5.12; N 15.81). Recrystallized from MeOH (28 mg), mp 188–190°. UV (EtOH) λ_{max}: nm (log ε), (0.1 N HCl), 216.5 (4.72), 273 (4.28), 291 (4.09), 305 (*sh*), 365 (4.75); (0.1 N. NaOH), 217 (4.72), 272.5 (4.28), 291.2 (4.09), 305 (*sh*), 363.5 (4.75). IR (KBr) ν_{max} cm^{-1}: 3460, 3380, 2940, 2935, 1668, 1620, 1580, 1525, 775. EIMS, 70 eV, *m/z* (rel. int.): 354 $[M]^+$ (10), 355 (3), 356 (1), 224 (30), 195 (18), 168 (29), 167 (70), 144 (13), 143 (100), 140 (22), 130 (37). NMR in Tables 1 and 3.

Alkaloid (**10**). Following the same conditions, 1 meq of **9** chlorohydrate, 1 meq of phenylethylamine and 1 meq of glyoxal gave alkaloid **10** (yield = 10%). $C_{20}H_{19}N_3O$, $[M]^+$ *m/z* 317. Amorphous. UV (EtOH), λ_{max}: nm (log ε), (neutral), 210 (3.82), 250 (3.89), 275 (3.94)*sh*, 332 (4.02), (0.1 N.HCl), 209.5 (3.82), 246.5 (3.89), 275 (3.94)*sh*, 369 (4.02); (0.1 N NaOH), 214.5 (3.83), 250 (3.89), 275 (3.94)*sh*, 334 (4.02). IR, (KBr), ν_{max} cm^{-1}: 3440, 3390, 3100, 2870, 1680, 1620, 1580, 750, 700. EIMS 70 eV, *m/z* (rel. int.): 317 $[M]^+$ (11), 318 (4), 260 (6), 226 (21), 197 (8), 170 (27), 169 (23), 143 (8), 105 (14), 91 (39), 18 (100), 19 (48), 30 (65). CIMS (NH_3) *m/z* (rel. int.): 318 (100), 317 (10), 226 (4), 170 (4), 148 (5). ^{13}C NMR in Table 3.

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