Phytochemistry, Vol 28, No 4, pp 1241–1244, 1989
Printed in Great Britain

0031-9422/89 $3.00+0.00

ALKALOIDS FROM *ALSTONIA CONGENSIS*

CATHERINE CARON, ALAIN GRAFTIEAUX, GEORGES MASSIOT, LOUISETTE LE MEN-OLIVIER and CLEMENT DELAUDE*

Faculte de Pharmacie (U A au CNRS No. 492), 51 rue Cognacq-Jay, 51096 Reims Cédex, France; *Universite de Liège, Sart-Tilman, 4000 Liège, Belgium

(*Received in revised form* 28 *September* 1988)

Key Word Index—*Alstonia congensis*; Apocynaceae, indole alkaloids, ^{1}H and ^{13}C NMR.

Abstract—Fifteen alkaloids were found in the root bark, stem bark and leaves of *Alstonia congensis*. They are echitamidine, echitamine, *nor*-echitamine, 17-acetoxy-*nor*-echitamine, akuammicine, 12-methoxyakuammicine, 12-methoxy-*N*(4)-methylakuammicine, tubotaiwine, 12-methoxytubotaiwine, angustilobines A and B, 6,7-seco angustilobines A and B, angustilobine B-*N*(4)-oxide and akuammidine

INTRODUCTION

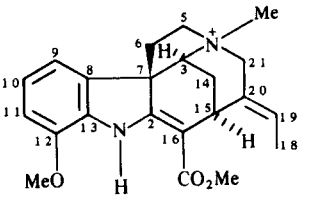
Monachino's classification of the genus *Alstonia* retains two African species, *A. boonei* and *A. congensis* [1]. These plants are closely related and resemble the widely distributed *A. scholaris*. *Alstonia congensis* Engl. has been the object of previous chemical investigations limited to the isolation of echitamine [2, 3], of echitamidine [3, 4] and of triterpenes [5]. A recent report on the isolation of rhazine from the stem bark of *A. congensis* [6] prompts us to disclose our results on the alkaloid content of the root bark, stem bark and leaves of the plant.

RESULTS AND DISCUSSION

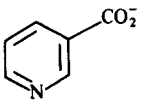
Material was collected from a large ornamental tree growing in the suburbs of Kinshasa, Zaire. Tertiary alkaloids were isolated in the usual fashion [7], with the following yields: 1.55 g/kg (root bark), 0.48 g/kg (stem-bark) and 2.5 g/kg (leaves) Owing to the small quantities of available material, alkaloids were purified by CC followed by prep. TLC. After the preliminary extraction of the tertiary alkaloids, quaternary ammonium salts were extracted from the remaining solid with *n*-butanol Fifteen pure compounds were obtained from the three parts of the plant, their occurrence and the means of identification are listed in Table 1.

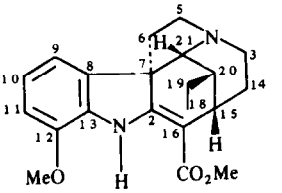
Echitamine (**2**) is the major alkaloid of the root bark; it is accompanied by two minor bases with the same characteristic Ph-N-C-N- chromophore: *nor*-echitamine (**3**) and its 17-*O*-acetate (**4**) Compounds **3** and **4** were identified by comparison of their spectra with those of the same compounds available from other *Alstonia* species [8, 9] Structural elucidation of the antimalarial echitamine has been an area of intense research in the fifties and the problem was solved by X-ray analysis, [10]

To the best of our knowledge, only partial NMR data on **2** are available [11]. To fill this gap we have investigated the ^{1}H and ^{13}C NMR spectra of **2** using 2D techniques; Table 2 gives ^{13}C assignments and ^{1}H NMR is detailed in the Experimental. Quaternarization of N(4) induces *ca* 10 ppm ^{13}C and *ca* 1 ppm ^{1}H deshieldings; N(4)-Me thus appears as a singlet at δ 3.6 instead of 2.24 as reported [11]. Protonated carbons are assigned through a direct C–H correlation and non-protonated carbons through a 'long range' correlation optimized for $J = 10$ Hz This latter experiment allows distinction between C-7 and C-16, which shows ^3J coupling with H-14

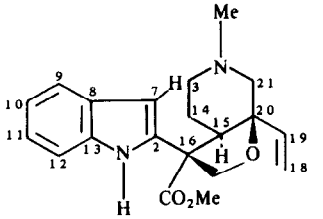


7





9



11

Table 1 Occurrence and identification of alkaloids in *Alstonia congensis*

Alkaloid	Yield (%) Root bark	Stem bark	Leaves	ccm	IR	UV	MS	H NMR	^{13}C NMR
Echitamidine **1**	3	2		+		+	+		
Echitamine **2**	10	5		+	+	+	+	+	+
nor-Echitamine **3**	1			+			+	+	
17-*O*-acetyl-*nor*-echitamine **4**	1		0 25	+		+	+		
Akuammicine **5**	1			+		+	+		
12-Methoxyakuammicine **6**	0 5	0 5		+		+	+		
12-Methoxy-*N*(4)-methylakuammicine **7**	10				+	+	+	+	+
Tubotaiwine **8**	1			+		+	+		
12-Methoxytubotaiwine **9**			0 25		+	+	+	+	+
Angustilobine A **10**		0 5	3	+		+	+	+	
6,7-Seco-angustilobine A **11**			3		+	+	+	+	+
Angustilobine B **12**		0 5		+		+	+	+	
Angustilobine-B-N-oxide **13**		0 5			+	+	+	+	
6,7-Seco-angustilobine B **14**	10		6			+	+	+	+
Akuammidine **15**		0 3		+		+	+		

Besides echitamidine **1** [12], five alkaloids colour blue upon Ce-IV spraying; among them are the ubiquitous (−)akuammicine (**5**) and (+)tubotaiwine (**8**) and their 12-methoxylated derivatives **6** and **9** These latter structures are proposed on the basis of high field ^{1}H NMR spectra whose lower frequencies parts are superimposable on those of the parent compounds and whose aromatic parts consist of three-spin systems identical with the one observed in the spectrum of 12-methoxy compactinervine [7] Analysis of the ^{13}C NMR spectrum of **9** and comparison with literature data [13] confirm the hypothesis. Compound **6** has previously been isolated from *Vinca ervincea* [14] and prepared by partial synthesis [7] To the best of our knowledge 12-methoxytubotaiwine is described here for the first time and is only the second derivative of tubotaiwine isolated until now [15]

Table 2 ^{13}C NMR data for the major alkaloids of *Alstonia congensis* (75 MHz, $CDCl_3$ except for compounds **2** and **7** whose spectra were measured in $CDCl_3$–CD_3OD)

C	**2**	**7**	**9**	**11**	**14**
2	100 0	165 4	—	132 9	- -
3	68 6	72 1	45 2	53 6	56 5
5	62 0	63 1	53 6		--
6	40 8	40 5	43 2	—	--
7	60 7	55 9	—	101 7	100 7
8	128 9	133 1	—	127 7	128
9	126 3	114 1	112 1	120 0	120 1
10	119 9	123 4	121 9	120 5	120 5
11	128 6	111 5	109 9	122 3	122 3
12	110 4	144 5	—	110 7	110 8
13	146 6	131 8	—	136 2	137 7
14	30 9	28 0	28 1	24 6	28 9
15	34 5	28 1	30 6	46 1	45 1
16	55 4	101 3	-	58 6	—
17	64 6	—	--	69 7	70 9
18	14 1	13 2	11 5	115 3	69 2
19	129 9	128 7	23 7	141 1	123 5
20	131 5	128 7	40 8	84 2	136 8
21	65 4	64 3	65 5	61 3	66 8
N-Me	49 1	49 9	--	46 8	46 4
C=O	172 6	166 6	—		
OMe	51 2	51 5	51 2	52 9	52 8
ArOMe	55 9	55 6			

Compound **7** is a quaternary alkaloid obtained from butanol extracts Its UV spectrum displays three maxima at 225, 289 and 333 nm and is reminiscent of the UV spectra of **6** and of **9** The ^{1}H NMR spectrum of **7** is fully assignable by means of a COSY experiment, it shows signals for an ethylidene chain with allylic and homoallylic couplings to a methylene, signals for a tryptamine CH_2–CH_2 unit and signals for a CH(3)–CH_2(14) –CH(15) unit Three methyls are also characterized by singlets at δ 3 87, 3 8 and 3 76 (^{1}H NMR) and by signals at 55 6, 51 5 and 49 9 ppm (^{13}C NMR), they are thus assigned to a methyl ether, a methyl ester and a quaternary *N*-methyl group, respectively The overall aspect of the ^{1}H NMR spectrum of **7** suggests a parenthood with 12-methoxyakuammicine Differences regard protons adjacent to nitrogen and may be explained by quaternarization of N(4) by a methyl group This is also demonstrated by the ^{13}C NMR spectrum (Table 2). Beside the three aromatic indole protons, four coupling resonances appear at low field in the ^{1}H NMR spectrum, they gradually disappear during repeated TLC purification using Verpoorte's ammonium nitrate solvent mixtures [16] These signals belong to the negatively charged counterion and are tentatively assigned to a nicotinate ion according to the ^{13}C NMR spectrum The ^{1}H NMR spectra of native **7**, of its chloride and nitrate forms show pronounced chemical shift differences, thus implying that in $CDCl_3$ and in $CDCl_3$–CD_3OD mixtures, the molecules form a tight ion pair.

A series of vallesamine derivatives is also present in all parts of the plant, they are angustilobines A and B (**10** and **12**) and 6,7-secoangustilobine B **14** isolated from two asian Alstonia species, *A pneumatophora* and *A angusti-*

loba [9, 17]. Angustilobine B has been independently isolated from *A. scholaris* and named alstonamine [18]. Alkaloid **11** is a novel vallesamine derivative, isomeric with **14** according to its mass spectrum and for which we propose a structure of 6,7-secoangustilobine A. The ^{1}H NMR spectrum of **11** shows four aromatic protons plus a singlet at δ 6.39, long-range coupling to the indole NH and featuring H-7 This spectrum also shows an isolated three-spin system for a vinyl group, two AX pairs of doublets (CH_2-17 and CH_2-21) and a five-spin system assigned to the CH_2-3 CH_2-14 CH-15 unit. Observation of signals for a methyl ester (δ3 6) and for a *N*-methyl group (δ2.24) leads to structure **11** with stereocenters configurations identical to those proposed for angustilobine A. In compounds **11** and **14**, opening of the eight-membered ring brings strain release and deshieldings of the ^{13}C resonances of C-3 and C-21 (suppression of γ effects). These vallesamines are accompanied by small quantities of the *N*(4)-oxide of angustilobine B whose structure is secured by a synthesis from the parent amine. The last compound identified in this study is akuammidine (**15**) [19] which sometimes is named rhazine [6].

Comparison of the alkaloid contents of *A. congensis* and of *A. boonei* does not allow answering the question of their being identical or not. Both species yield echitamine and echitamidine as major alkaloids as well as akuammidine [20]. *A boonei* also contains voacangine, the sole type III indole alkaloid isolated from an *Alstonia* species [20] and derivatives of N(1)-formyl echitamidine [21, 22] which were not found in the present study.

EXPERIMENTAL

Extraction and separation Dried powdered root bark (245 g) was wetted with 200 ml of NH_4OH half dild in H_2O and lixiviated overnight with 2.5 l of EtOAc. The organic soln was extracted with 2% H_2SO_4 and the aq. phase made alkaline with NH_4OH and extracted with $CHCl_3$ The $CHCl_3$ layers were dried (Na_2SO_4) and evapd *in vacuo* to give 0.381 g of crude alkaloid mixt. (AM). The alkaline aq phase was acidified to pH 6 with HOAc and extracted with *n*-BuOH, drying and evapn of solvent yielded 0 22 g of extract

Crude AM was purified by CC on 12 g of silica gel packed in $CHCl_3$; 10 ml fractions were collected Elution was performed with $CHCl_3$ 400 ml, $CHCl_3$–MeOH (99 . 1) 200 ml, (49 1) 280 ml and (19 · 1) 300 ml

Alkaloids **1**, **5** and **6** were in frs 63–70, alkaloids **8** in frs 71–90, **3** and **4** in frs 91–115 and **14** in frs 125–140 Alkaloids **2** and **7** were sepd by prep TLC (MeOH–0 2 M NH_4NO_3) Alkaloids from the leaves and stem bark were isolated and sepd in a similar fashion Polarity order of the alkaloids is angustilobine A, 6,7-*seco*angustilobine A, 17-acetoxy-*nor*-echitamine, 12-methoxy-tubotaiwine, angustilobine B and 6,7-*seco*-angustilobine B

Echitamine **2** ^{1}H NMR (300 MHz $CDCl_3$, CH_3OD) 7 6 (*d*, *J* = 7 Hz, H-9), 6.95 (*t*, *J* = 7 Hz, H-11), 6 65 (*m*, H-10 + H-12), 5 62 (*br q*, *J* = 7 Hz, H-19), 4 45 (*br d*, *J* = 14 Hz, H-21), 4 35 (*dd*, *J* = 11, 6 Hz, H-3), 3 87 (*br d*, H-15), 3 68 (*s*, OMe), 3 24 (*s*, N-Me), 2 55 (*ddd*, *J* = 7, 11, 16 Hz, H-14), 2 32 (*dt*, *J* = 7, 10 Hz, H-6), 2.05 (*dd*, *J* = 7, 10 Hz, H-6), 1 69 (*dd*, *J* = 1 5, 7 Hz, Me-18), 1 46 (*dd*, *J* = 4, 11 Hz, H-14)

12-Methoxy-N(4)-methylakuammicine **7** (CR blue), $[\alpha]_D$ = +70° ($CHCl_3$, *c* 0 15), UV λ_{max}^{MeOH} nm 225, 282, 289, 333; IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3350, 1700, 1660, 1580, 1400, MS *m/z* (rel. int) 367 [M]$^+$, 5), 366 (10), 352 (15), 322 (10), 294 (25), 277 (15), 264 (20), 188 (20), 122 (100), 121 (70), ^{1}H NMR (300 MHz, $CDCl_3$) 8,8 (*br s*), 8.68 (*br s*), 7.95 (*d*, *J* = 7 Hz), 7 55 (*d*, *J* = 7 Hz), 7 2 (*t*, *J* = 7 Hz), 6 95 (*m*, 2H), 6 8 (*m*, 2H), 5.8 (*q*, *J* = 7 Hz, H-13), 5.05 (*br s*, H-3), 4 5 (*m*, H-5), 4.32 (*d*, *J* = 13 Hz, H-21), 4.12 (*m*, H-5 + H-15), 3 93 (*d*, *J* = 13 Hz, H-21), 3.87 (*s*, Ar OMe), 3 8 (*s*, CO_2Me), 3 76 (*s*, N-Me), 2 9 (*dt*, *J* = 7, 13 Hz, H-6), 2.45 (*br d*, *J* = 12 Hz, H-14), 2.25 (*dd*, *J* = 7, 13 Hz, H-6), 1 8 (*d*, *J* = 7 Hz, Me-18), 1.6 (*br d*, *J* = 12 Hz, H-14)

12-Methoxytubotaiwine **9**. (CR blue); $[\alpha]_D$ = +305 ($CHCl_3$, *c* 0 15); UV λ_{max}^{MeOH} nm· 210, 291, 333; IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3370, 2930, 1730, 1675, 1610, 1490, 1460, 1430, 1265, 1230, 1160, 1100, 1030; MS *m/z* (rel int) 355 (90), 354 ([M]$^+$, 100), 339 (10), 324 (20), 297 (30), 259 (80), 135 (60), 124 (100), 122 (90), 107 (30), 98 (35), 95 (40), 71 (95), ^{1}H NMR (300 MHz, $CDCl_3$)· 8.75 (*s*, NH), 6 85 (*m*, 2H), 6 72 (*m*, 1H), 3.85 (*s*, Ar OMe), 3.78 (*s*, CO_2Me), 3 15 (*m*, 2H), 2.85 (*m*, 2H), 2 52 (*dt*, *J* = 7, 11 Hz, H-3), 2 0 (*m*, H-20), 1.8 (*m*, 2H), 0.85 (*m*, 2H-19), 0 7 (*t*, *J* = 7 Hz, Me-18).

6,7-Secoangustilobine A **11**. (CR grey turning to pink after 2 days), $[\alpha]_D$ = +78° ($CHCl_3$, *c* 0 3)· UV λ_{max}^{MeOH} nm 225, 274, 282, 290, IR $\nu_{max}^{CHCl_3}$ cm^{-1}· 3350, 1730, 1450, 1240, 760; MS *m/z* (rel. int.): 340 ([M]$^+$, 60), 281 (20), 201 (70), 154 (20), 122 (100); ^{1}H NMR (300 MHz, $CDCl_3$), 8 4 (*s*, N–H), 7 55 (*d*, *J* = 8 Hz, H-9), 7.3 (*d*, *J* = 8 Hz, H-12), 7.15 (*t*, *J* = 8 Hz, H-11), 7 08 (*t*, *J* = 8 Hz, H-10), 6 4 (*br s*, H-7), 5 65 (*dd*, *J* = 10, 17 Hz, H-19), 5 45 (*dd*, *J* = 17, 1.8 Hz, H-18), 5 15 (*dd*, *J* = 10, 1 8 Hz, H-18), 4 95 (*d*, *J* = 9 Hz, H-17), 4 62 (*d*, *J* = 9 Hz, H-17), 3.6 (*s*, CO_2Me), 2 9 (*dd*, *J* = 12, 6 Hz, H-15), 2.8 (*br d*, *J* = 13 Hz, H-21), 2.7 (*br d*, *J* = 11 Hz, H-3), 2.23 (*s*, N–Me), 2 01 (*d*, *J* = 13 Hz, H-21), 1.85 (*dt*, *J* = 1.8, 10 1 Hz, H-3), 1 4 (*m*, H-14), 1 2 (*m*, H-14).

Angustilobine B-N-oxide 13. (CR grey); $[\alpha]_D$ = +97° ($CHCl_3$, *c* 0 2); UV λ_{max}^{MeOH} nm: 223, 285, 292; IR $\nu_{max}^{CHCl_3}$ cm^{-1}. 3400, 1730; MS *m/z* (rel int) 353 ([M]$^+$, 30), 338 (10), 307 (20), 294 (40), 279 (25), 265 (40), 263 (30), 251 (30), 122 (100), ^{1}H NMR (300 MHz, $CDCl_3$)· 7 5–7 1 (*m*, 4H), 5 5 (*br s*, H-19), 4.85 (*d*, *J* = 17 Hz, H-6), 4 53 (*br d*, *J* = 16 Hz, H-18), 4.56 (*d*, *J* = 13 Hz, H-17), 4.23 (*dd*, *J* = 16, 4 Hz, H-18), 4.0 (*d*, *J* = 17 Hz, H-6), 3.87 (*s*, CO_2Me), 2.05 (*m*, H-14), 1 72 (*m*, H-14)

Acknowledgements—Support of this work by 'Ministère de la coopération au développement' de Belgique (Etudes phytochimiques de flores africaines research project) is gratefully acknowledged The authors are indebted to Dr H Breyne for identification of vegetable material.

REFERENCES

1 Monachino, J (1949) *Pacific Sc* III, 133
2 Goodson, J. A. (1932) *J Chem. Soc.* 2627.
3 Monseur, X. and Van Bever, M. L (1955) *J. Pharm. Belg.* **10**, 93
4 Prista, L. N., Ferreira, M A, Alves, A. C and Roque, A S. (1965) *Garcia Orta* **13**, 571
5 Ferreira, M A., Alves, A. C, Prista, L N. and Cruz, M. A. (1968) *Garcia Orta* **16**, 31.
6. Banerji, A and Jana, S (1986) *J Indian Chem. Soc.* **63**, 449.
7 Legseir, B, Cherif, A, Richard, B, Pusset, J., Labarre, S., Massiot, G and Le Men-Olivier, L. (1986) *Phytochemistry* **25**, 1735
8 Boonchuay, W. and Court, W. E (1976) *Phytochemistry* **15**, 821
9 Ravao, T (1985). Ph.D thesis, University of Reims.
10 Hamilton, J A, Hamor, T. A., Robertson, J. M and Sim, G A (1961) *Proc. Chem Soc* 63
11 Conroy, H, Bernasconi, R., Brook, P R, Ikan, R., Kurtz, R. and Robinson, K W (1960) *Tetrahedron Letters* 1.
12 Zeches, M, Ravao, T, Richard, B, Massiot, G., Le Men-

Olivier, L, Guilhem, J and Pascard, C (1984) *Tetrahedron Letters* **25**, 659
13 Verpoorte, R, Van Beek, T A, Ruegman, B L M, Hyland, P I and Bisset, N G (1984) *Org Magn Reson* **22**, 328
14 Yagudaev, M R (1983) *Khim Prir Soedin* 210
15 Lounasmaa, M and Somersalo, P (1986) *Fortschr Chem Org Naturst* **50**, 27
16 Verpoorte, R and Baerheim Svendsen, (1976) *J Chromat* **124**, 152
17 Zèches, M, Ravao, T, Richard, B, Massiot, G, Le Men-Olivier, L and Verpoorte, R (1987) *J Nat Prod* **50**, 714
18 Atta-Ur-Rahman and Alvi, K A (1987) *Phytochemistry* **26**, 2139
19 Janot, M -M, Le Men, I, Gosset, I and Lévy, I (1962) *Bull Soc Chim Fr* 1079
20 Croquelois, G, Kunesch, N, Debray, M and Poisson, I (1972) *Plant Med Phyt* **6**, 122
21 Oguakwa, J U, Galeffi, C, Messana, I, Patamia, M, Nicoletti, M and Marini-Bettolo, G B (1983) *Gazz Chim It* **113**, 533
22 Oguakwa, J U (1984) *Phytochemistry* **23**, 2708