Pergamon 0031-9422(95)00660-S

Phytochemistry, Vol. 41, No. 3, pp. 963–967, 1996

0031-9422/96 $15.00 + 0.00

INDOLE ALKALOIDS FROM *ASPIDOSPERMA RAMIFLORUM*

M. FÁTIMA S. MARQUES, LUCÍLIA KATO, HERMÓGENES F. LEITÃO FILHO and FRANCISO DE A. M. REIS*

Instituto de Química, Universidade Estadual de Campinas, C. P. 6154, Campinas 13083-970, São Paulo, S.P. Brazil

(*Received in revised form* 31 *July* 1995)

Key Word Index—*Aspidosperma ramiflorum*; Apocynaceae; bis-indole alkaloids; ramiflorines A and B; ^{13}C NMR.

Abstract—Two new bis-indole alkaloids, ramiflorines A and B, were isolated, together with the known compounds, β-yohimbine and 10-methoxygeissoschizol, from the bark and seeds of *Aspidosperma ramiflorum*. 2D-NMR analysis, together with CD measurements, established the absolute configuration of both stereogenic centres in the ramiflorines.

INTRODUCTION

Aspidosperma ramiflorum commonly known as yellow 'guatambu', is a tree native to the Mata Altântica in the southeast of Brazil. The genus *Aspidosperma* has been classified by Woodson [1] into nine differents series, all of which have been subjected to chemical investigation, except for *Stegomeria* (three species) and *Ramiflora* (one species, *A. ramiflorum*). Few modifications have been proposed [2] for this classification, but the series Ramiflora remained unchanged. Although widely distributed *A. ramiflorum*, has never been chemically studied and, consequently, this is the first phytochemical report on this species or series.

RESULTS AND DISCUSSION

We have isolated β-yohimbine (**1**) and 10-methoxygeissoschizol (**2**) from the ethanolic extract of the seeds of *A. ramiflorum*. The chloroform extract of the ground bark led to the isolation of **2** and two new bis-indole alkaloids, ramiflorines A and B.

The IR spectrum of ramiflorine A (**3**) showed absorptions at 3400 cm^{-1} (NH) and 1210 cm^{-1} (C–O–C). The UV absorptions λ_{max}^{EtOH} nm (log ε) 224 (4.9); 280 (4.4) and 288 (4.3) were characteristic of a 10-methoxyindole chromophore. The EI mass spectrum showed a $[M]^+$ at *m/z* 466.2730 corresponding to the molecular formula $C_{30}H_{34}N_4O$. The ions at *m/z* 282 and 185 clearly indicated fragmentation between C-15 and C-16, yielding two fragments representing the two indole moieties of this compound. Fragment *m/z* 282 corresponds to a quinolizidine including a methoxyl group, thus confirming the UV data. Fragment *m/z* 185 is characteristic of a tetrahydroharmane moiety. Such fragmentation is typical of 'quasidimeric' alkaloids [3].

The IR spectrum of ramiflorine B (**4**) showed absorptions at 3420 and 1220 cm^{-1}, corresponding to an amine and an ether group, respectively. The UV absorptions at λ_{max}^{EtOH} nm (log ε) 224 (4.9); 280 (4.4) and 288 (4.3) corresponded to a 10-methoxyindole chromophore. The EI mass spectrum showed a $[M]^+$ at *m/z* 466.2730 corresponding to $C_{30}H_{34}N_4O$. Other ions in this spectrum were similar to those observed for **3**.

The above data strongly suggested that **3** and **4** were bis-indole alkaloid isomers, as was confirmed by NMR spectra. Acetylation of **3** and **4** gave the corresponding acetamides **5** and **6**, respectively, thus confirming a secondary amine function in both compounds. The ^{1}H NMR (Table 1) indicated methoxyl groups at δ3.87 (**3**) and 3.92 (**4**), two indolic NH at δ8.5 and 7.4 (**3**) and 8.45 and 7.15 (**4**), a trisubstituted double bond at H-18 δ1.66 (3H, J = 6.9 Hz for **3**) and 1.59 (3H, *d*, J = 6.3 Hz for **4**) and H-19 at δ5.55 (1H, *q*, J = 6.9 Hz for **3**) and 5.36 (1H, *q*, J = 6.3 Hz for **4**), together with seven aromatic protons, including an ABX-system in both compounds.

The ^{13}C NMR (Table 2) corroborated the presence of C_{30} compounds with 18 sp^2 and 12 sp^3 carbons; 2D-NMR led to the assignment of all carbons in both compounds. Also, the carbon shifts of the indolquinolizidine moieties of **3** and **4** were very similar to those observed [4] for **2**. The signals of C-3 (53.0 ppm), C-6 (18.0 ppm) and C-21 (52.9 ppm) for **3**, and C-3 (52.9 ppm), C-6 (17.7 ppm) and C-21 (52.7 ppm) for **4**, revealed a *cis*-C/D ring-junction [5]. The aromatic carbon shifts of **3** and **4** were similar, but differences were observed in the terpenic part of these alkaloids. Carbons C-14 (33.0 ppm), C-15 (30.9 ppm), C-17 (51.5 ppm) and C-19 (121.6 ppm) in **3** were deshielded when compared to the same carbons (C-14, 29.6 ppm; C-15, 29.6 ppm; C-17, 50.2 ppm and C-19, 119.1 ppm) in **4**. Such differences have been observed previously [3, 6] with the 4′,17-dihydrotchibangensines, **7** and **8**. Furthermore, comparison of

*Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR spectral data for ramiflorines and their acetyl derivatives

H	3	4	5	6
3	4.21 *bs*	4.25 *bs*	4.3 *bs*	4.38 *bs*
5	3.15–3.3 *m*	3.0–3.3 *m*	—	3.2–3.4 *m*
6	2.5–2.7 *m*	2.57 *m*	2.7–2.85 *m*	2.45–2.6 *m*
	3.1 *m*	3.0 *m*	2.5–2.7 *m*	3.05–3.2 *m*
9	6.95 *d*, $J = 2.3$	7.0 *d*, $J = 2.4$	6.93 *d*, $J = 2.4$	7.03 *d*, $J = 1.8$
11	6.7 *dd*, $J = 8.7$; 2.3	6.91 *dd*, $J = 8.7$; 2.4	6.78 *dd*, $J = 8.5$; 2.4	6.95–7.05 *m*
12	7.12 *d*, $J = 8.7$	7.16 *d*, $J = 8.7$	7.23 *d*, $J = 8.5$	7.35 *d*, $J = 8.6$
14β	2.07 *ddd*, $J_{gem} = 14$, $J_{14-3} = 4$, $J_{14-15} = 3.5$	2.2 *m*	2.2–2.4 *m*	2.7–2.9 *m*
14α	2.25 *ddd*, $J_{gem} = 14$, $J_{14-15} = 6.0$, $J_{14-3} = 6.5$	2.1 *m*		2.1–2.2 *m*
15	3.08 *m*	3.2 *m*	3.00–3.15 *m*	2.6–2.75 *m*
16a	1.67 *ddd*, $J_{gem} = 15$, $J_{16-15} = 11$, $J_{16-17} = 4$	1.67 *dd*, $J_{gem} = 12.5$ $J_{16-15} = 12.5$, $J_{16-17} = 0$	1.75 *dd*, $J_{gem} = 12$ $J_{16-17} = 12$	1.56 *dd*, $J_{gem} = 13$ $J_{16-15} = 13$
2				
16b	1.46 *ddd*, $J_{gem} = 15$, $J_{16-17} = 10$, $J_{16-15} = 6$	1.19 *dd*, $J_{gem} = 12.5$ $J_{16-17} = 12.5$, $J_{16-15} = 0$	1.56 *dd*, $J_{gem} = 12$ $J_{16-15} = 12$	1.35 *dd*, $J_{gem} = 13$ $J_{16-17} = 13$
17	3.76 *dd*, $J = 10.4$	4.14 *bd*	5.82 *bd*	6.0 *bd*, $J_{17-16} = 13$
18	1.66 *d*, $J = 6.9$	1.59 *d*, $J = 6.3$	1.4 *d*, $J = 6.7$	1.53 *d*, $J = 6.3$
19	5.55 *q*, $J = 6.9$	5.36 *q*, $J = 6.3$	5.35 *q*, $J = 6.7$	5.33 *q*, $J = 6.3$
21β	2.96 *bd*, $J_{gem} = 12$	2.92 *d*, $J = 12$	2.92 *d*, $J = 11$	2.9 *d*, $J = 12$
21α	3.66 *bd*, $J_{gem} = 12$	3.6 *d*, $J = 12$	3.8 *m*	3.62 *bd*, $J = 12$
5′	3.05 *m*	3.0–3.3 *m*	—	3.35–3.45 *m*
	3.2 *m*			3.9–4.0 *m*
6′	2.5–2.7 *m*	2.57 *m*	2.58 *m*	2.6–2.9 *m*
		3.0 *m*	2.8 *m*	
9′	7.39 *bd*, $J = 7.7$	7.38 *dd*, $J = 6.7$, 18	7.3 *m*	7.31 *bd*, $J = 7.3$
10′	6.9–7.1 *m*	6.98–7.09 *m*	7.06 *t*, $J = 7.8$	6.95–7.05 *m*
11′	6.9–7.1 *m*	6.98–7.09 *m*	7.14 *t*, $J = 7.8$	
12′	6.9–7.1 *m*	6.86 *dd*, $J = 6.2$, 1.6	7.3 *m*	6.78 *d*, $J = 7.8$
$-OCH_3$	3.87 *s*	3.92 *s*	3.87 *s*	3.92 *s*
$CH_3-\overset{O}{\overset{\|}{C}}-$			2.34 *s*	2.26 *s*
$N_{(1)}-H$	8.5	8.45	8.7	9.8
$N_{(1')}-H$	7.4	7.15	9.5	7.15
$N_{(4')}-H$	2.4	2.3		

Spectra obtained at 300 MHz in Fourier transform-mode in $CDCl_3$ using TMS as internal standard. J values in Hz.

compound **3** with **7**, and **4** with **8**, suggests that they have the same stereochemistry.

^{13}C NMR of ochrolifuanines A and B revealed [7] that the configuration at C-17 induces such changes. Of the arrangements around the C-15–C-16 bond, 17αH-compounds prefer the rotamer (**a**), in which C-17 and C-14 are *trans*-diaxially-oriented, and in 17βH compounds, the rotamer (**b**), where C-17 and C-14 are *gauche*. In ramiflorine B, the signal for C-14 at 29.6 ppm indicated a *gauche* interaction between C-17 and C-14 ($\Delta\delta = -3.4$ ppm) and that C-19 at 119.1 indicated the loss of a δ interaction between C-17 and C-20 ($\Delta\delta = -2.5$ ppm), when compared to **3**. The signal for H-3 at δ4.21 (**3**) and 4.26 (**4**) is characteristic of a *cis*-C/D ring-junction conformation in quinolizidines. Also, the broad singlet exhibited by these protons indicates that H-3 intersects the C-14 methylene protons with a small coupling constant. The J values (Table 1) could be measured only for **3**, since in **4**, the C-14 methylene protons showed second-order spectra.

It is well known that the circular dichroic (CD) spectra of 3αH-yohimbane and corynane compounds exhibit a positive Cotton effect, although the 3βH-compound shows a negative Cotton effect at 280 nm [8]. Previous studies on ochrolifuanines [9] and cinchophylines [10] have shown that compounds with 3αH- and 17αH-configurations give positive Cotton effects at 280 nm, which are more intense than those of compounds with a 3αH- and 17βH-stereochemistry. The above results and the CD spectra of **3** ($\Delta\varepsilon = +2.8$) and **4** ($\Delta\varepsilon = +1.6$) at 280 nm, together with the ^{13}C and ^{1}H NMR analysis, unambigously fixed their absolute configurations, as depicted in **3** and **4**, respectively. All of these data allowed us to suggest the conformer (**c**) for both compounds, with C-2 and C-16 axially oriented towards ring D, as previously observed for geissospermine [11].

Table 2. ^{13}C NMR spectral data for ramiflorines and acetyl derivatives

Carbon	3	4	5	6	7 [3]	8 [3]
2	135.4*	135.6*	134.6	134.1	136.0†	135.6†
3	53.0	52.9	52.7	52.3	53.3	53.3
5	51.3	51.4	50.0	51.2	51.3	51.0
6	18.0	17.7	18.6	17.0	18.3	17.9
7	107.3†	108.3†	107.2*	107.0*	108.2‡	108.1‡
8	127.5‡	127.5‡	127.5†	126.4†	127.4	127.9§
9	100.4	101.2	100.1	101.2	117.8*	118.0*
10	154.3	154.9	153.8	154.9	121.4[4]	121.8*
11	111.5	111.9	110.9	111.8	119.2*	119.8*
12	112.1	112.0	111.7	111.9	111.3	111.2
13	131.2	130.7	131.2	130.5	136.5†	137.5†
14	33.0	29.6	29.4	29.3	33.3	29.3
15	30.9	29.6	31.7	29.6	30.9	29.8
16	37.7	37.7	36.9	36.4	37.8	37.5
17	51.5	50.2	47.4	47.3	51.3	49.7
18	13.1	12.6	12.8	12.3	13.0	12.6
19	121.6	119.1	120.8	117.9	121.4*	119.8*
20	136.5§	136.5	136.5‡	135.8	134.5†	135.0†
21	52.9	52.7	52.7	51.2	54.0	53.3
2′	135.8*	138.2	133.6	138.7‡	137.5†	135.4†
5′	42.5	41.9	41.6	40.5	42.1	41.2
6′	22.4	22.8	21.5	22.0	22.4	22.5
7′	108.6†	108.8†	107.5*	108.0*	106.9‡	107.5‡
8′	128.0‡	128.9‡	126.5†	129.2†	127.4	127.1‡
9′	118.0	118.0	118.0	117.7	117.8	117.8
10′	119.4	119.4	119.5	119.5	121.4*	121.8*
11′	121.6	121.7	121.8	121.8	119.2*	119.8
12′	111.1	111.1	110.9	111.2	111.3	111.2
13′	136.0§	135.8*	137.5‡	137.4‡	136.1†	135.8†
$-O-CH_3$	56.0	56.2	56.0	56.1		
$CH_3-\overset{O}{\overset{\|}{C}}-$	—	—	170.0	171.0		
$CH_3-\overset{O}{\overset{\|}{C}}-$	—	—	22.3	22.0		

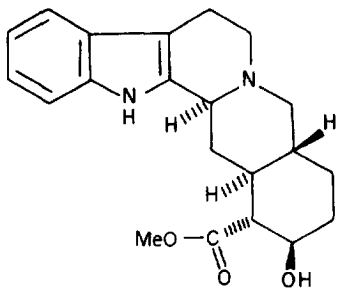
Spectra obtained at 75 MHz in $CDCl_3$ solution.
*†‡§Values within the same column may be interchanged.

EXPERIMENTAL

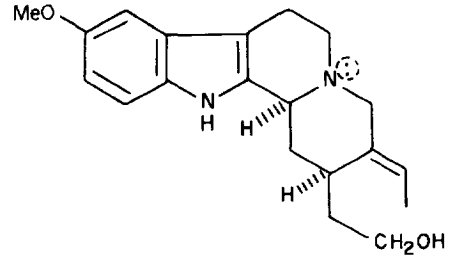
Mps are uncorr. Specific rotations were measured in $CHCl_3$, UV spectra in EtOH and IR spectra in $CHCl_3$. ^1H NMR were obtained at 300 MHz, ^{13}C NMR at 75.46 MHz, using $CDCl_3$ as solvent and TMS as int. standard. The DEPT pulse sequence to establish the numbers of attached protons, and the 2D ^1H–^1H COSY 90 and ^1H–^{13}C HETCOR expts., were also recorded under these conditions. EIMS were determined at 70 eV. CD were measured in EtOH soln. Silica gel 0.05–0.25 mesh and 254–366 HF were used for CC and TLC, respectively. Components were detected by UV (254 and 305 mm) and after spraying with Dragendorff's reagent, followed by MeOH–H_2SO_4 and heating the plates at 150° for 5 min.

Plant material. Stem bark of *A. ramiflorum* Muell. Arg. was collected from a 30 m tree near Porto Ferreira, S.P. Air-dried bark (500 g) was extracted at room temp. with EtOH. After removal of EtOH, 61 g of crude extract was obtained, which was added to a 10% HOAc soln and kept at 5° overnight. The aq. phase obtained after filtration was first extracted with $CHCl_3$ (ext. 1, 0.77 g), then the pH raised to 7 and extraction with $CHCl_3$ repeated (ext. II, 6.14 g). The pH of the aq. phase remaining was raised to 10 and extracted with $CHCl_3$ (ext III, 1.63 g), Ext. II (1.5 g) was fractionated on a silica gel column, eluting with $CHCl_3$ followed by $CHCl_3$ with increasing amounts of MeOH. Further purification by silica gel prep. TLC led to the isolation of **2** (0.45 g), **4** (0.23 g) and **3** (0.19 g).

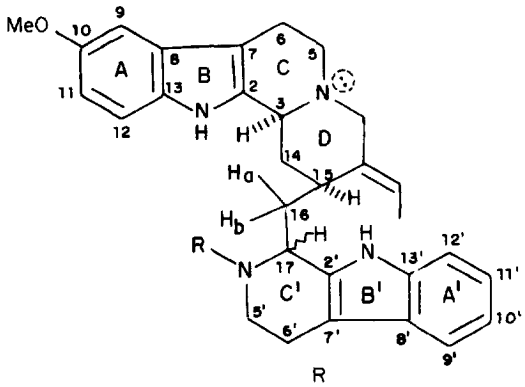
Seeds of *A. ramiflorum* (250 g) were submitted to the above fractionation scheme, giving ext. I (0.25 g), ext. II (2.48 g) and ext. III (0.77 g). Ext. II (1 g) was fractionated on an alumina column, eluting with $CHCl_3$ followed by $CHCl_3$ with increasing amounts of MeOH. Further



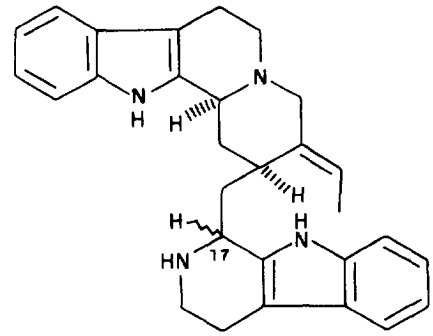
1



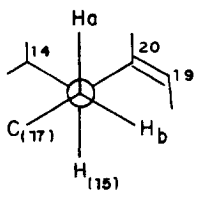
2



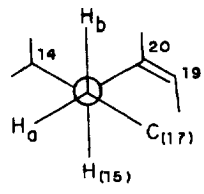
3 H , 17 α H
4 H , 17 β H
5 MeC(=O)–, 17 α H
6 MeC(=O)–, 17 β H



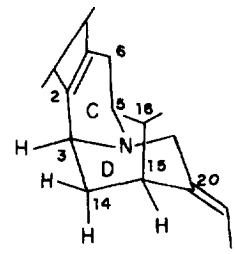
7 17 α H
8 17 β H



b



a



c

purification by silica gel prep. TLC led to the isolation of **1** (0.74 g) and **2** (0.060 g).

Ramiflorine A (**3**). Mp 159°. $[\alpha]_D^{25}$ + 23.52° (*c* 1.0; EtOH). CD nm (Δε) 281 (+ 2.8). UV λ_{max}^{EtOH} nm (log ε): 224 (4.9), 280 (4.4), 288 (4.3). IR ν^{CHCl_3} cm^{-1}: 3400 (N–H), 1210 (–C–O–C–). ^{1}H NMR: Table 1. ^{13}C NMR: Table 2. MS *m/z* (rel. int.) 466.2730 (23) ($C_{30}H_{34}N_4O$ requires 466.2735); 282 (77), 281 (35), 279 (33), 201 (25), 186 (27), 185 (100), 171 (38), 143 (49), 130 (33).

Ramiflorine B (**4**). Mp 194°. $[\alpha]_D^{25}$ + 59.2° (*c* 1.0; EtOH). CD nm (Δε) 281 (+ 1.6). UV λ_{max}^{EtOH} nm (log ε):224 (4.9), 280 (4.4), 288 (4.3). IR ν^{CHCl_3} cm^{-1}: 3420 (N–H), 1220 (–C–O–C–). ^{1}H NMR: Table 1. ^{13}C NMR: Table 2. MS *m/z* (rel. int.) 466.2730 (25) ($C_{30}H_{34}N_4O$ requires 466.2735); 284 (58), 283 (80), 282 (77), 279 (70), 253 (48), 201 (35), 186 (58), 185 (100), 184 (62), 183 (65), 171 (80), 143 (49), 130 (33).

Acetylation of ramiflorines was carried out under usual conditions with Ac_2O–pyridine, 0.05 g

3 yielding 0.051 g of **5**, and 0.05 g **4** yielding 0.055 g **6**.

Acetylramiflorine A (**5**). Mp 214°. $[\alpha]_D^{25}$ + 17.7° (*c* 0.05; EtOH). UV λ_{max}^{EtOH} nm (log ε): 226 (4.65), 280 (4.08), 289 (4.02). IR ν^{CHCl_3} cm^{-1}: 3400 (N–H), 1630 (–CO–N–). ^1H NMR: Table 1. ^{13}C NMR: Table 2. MS *m/z* (rel. int.): 508 (31), 295 (29), 283 (21), 282 (100), 281 (29).

Acetylramiflorine B (**6**). Mp 253°. $[\alpha]_D^{25}$ + 12.24° (*c* 0.08; $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 224 (4.7), 280 (4.14), 289 (4.08). IR ν^{CHCl_3} cm^{-1}: 3410 (NH), 1640 (–CO–N–). ^1H NMR: Table 1. ^{13}C NMR: Table 2. MS *m/z* (rel. int.): 508 (88), 308 (74), 296 (20), 295 (89), 283 (24), 282 (100), 281 (79), 279 (31), 253 (23), 213 (31), 201 (26), 200 (31), 187 (26), 71 (22).

Acknowledgements—We thank Dr Laurence T. Nielsen from Union Camp Corp., Princeton, U.S.A., for the high-resolution MS. We are also grateful to Prof. Dr Fred Fujiwara of the Instituto de Quimica, UNICAMP, for carring out NMR experiments.

REFERENCES

1. Woodson, R. R. (1951) *Ann. Missouri Bot. Garden* **38**, 119.
2. Marcondes-Ferreira Neto, W. (1988) PhD thesis. Instituto de Biologia, Universidade Estadual de Campinas, Brazil.
3. Massiot, G., Thepenier, P., Jacquier, M.-I., Lounkokobi, J., Mirand, C., Zeches, M., Le Men-Olivier, L. and Delaude, C. (1983) *Tetrahedron* **39**, 3645.
4. Nunes, D. S., Koike, L., Taveira, J. J. and Reis, F. de A. M. (1992) *Phytochemistry* **31**, 2507.
5. Wenkert, E., Bindra, J. S., Chang, C. J. J., Cochran, D. W. and Shell, F. M. (1974) *Acc. Chem. Res.* **7**, 46.
6. Robert, G. M. T., Ahond, A., Poupat, C., Potier, P. and Jacquemin, H. (1983) *J. Nat. Prod.* **46**, 694.
7. Koch, M. C., Plat, M. M., Preaux, N., Gottlieb, H. E., Hagaman, E. W., Shell, F. M. and Wenkert, E. (1975) *J. Org. Chem.* **40**, 2836.
8. Klyne, W., Swan, R. J., Dastoor, N. J., Gorman, A. A. and Schmid, H. (1966) *Helv. Chim. Acta* **50**, 115.
9. Koch, M., Plat, M. and Préaux, N. (1973) *Bull. Soc. Chim. Fr.* 2868.
10. Zeches, M., Sigaut, F., Le Men-Olivier, L., Lévy, J. and Le Men, J. (1981) *Bull. Soc. Chim Fr.* 75.
11. Goutarel, R., Païs, M., Gottlieb, H. E. and Wenkert, W. (1978) *Tetrahedron Letters* 1235.