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^{13}C NMR ANALYSIS OF ALKALOIDS FROM *PESCHIERA FUCHSIAEFOLIA*

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Key Word Index—*Peschiera fuchsiaefolia*, Apocynaceae, bisindole alkaloids, voacamine, decarbomethoxyvoacamine, demethylvoacamine, dihydrovoacamine, voacamidine, affinisine, 16-epiaffinine, perivine, voachalotine, voacangine, voacanginehydroxyindolenine, ^{13}C NMR

Abstract—Fractionation of an ethereal extract of *Peschiera fuchsiaefolia* resulted in the isolation of decarbomethoxyvoacamine, demethylvoacamine, voacamidine, perivine, 16-epiaffinine and voacanginehydroxyindolenine, together with the previously reported alkaloids voacamine, voacangine, voachalotine and affinisine Analysis of the ^{13}C NMR spectra of the bisindole alkaloids and of 16-epiaffinine is reported

INTRODUCTION

Reports on the anticancer activity of bisindole alkaloids of the voacamine type [1] led us to re-investigate the bark of *Peschiera fuchsiaefolia* [2, 3], directing our research on the isolation of these compounds

RESULTS AND DISCUSSION

After alkalinization the ground bark of *P fuchsiaefolia* was extracted with ether The crude extract was dispersed in 10% acetic acid and the aqueous solution was extracted with chloroform and ether at different pHs, yielding fractions A, B and C These fractions were monitored by mass spectrometry, which revealed that B was rich in dimeric compounds This fraction was then submitted to Sephadex LH 20 column chromatography allowing a good MW separation, followed by preparative TLC purification leading to the isolation of the bisindoles voacamine (**1a**), decarbomethoxyvoacamine (**1b**), demethylvoacamine (**1c**) and voacamidine (**1d**), and the monomeric indoles affinisine (**2**) and voachalotine (**3**) Pure voacangine (**4**) voacanginehydroxyindolenine (**5**) and voacamine (**1a**) were obtained from fraction A, while fraction C furnished two α-acylindoles, perivine (**6**) and 16-epiaffinine (**7**), which were not reported previously [2, 3]

The ^{13}C NMR spectra were interpreted on the basis of standard chemical shift theory, comparison with reference compounds and mainly by analysis of the SFORD and fully coupled spectral data

Table 1 presents the ^{13}C NMR data of compounds **1a–1f** The shift assignments were made in conformity with the published data of voacangine (**4**) [4, 5] vobasinol (**8**) [6] and ibogaine (**9**) [4, 5] The replacement of the C-3 hydroxyl group of **8** by a voacangine unit in voacamine (**1a**) produced the expected shielding at C-3 ($\Delta\delta$ 29 4) and deshielding at C-14 and C-15 ($\Delta\delta$ 1 1 and 4 1, respectively) Analogous effects were observed in the tabernaelegantines [7] On the other hand, in the voacangine moiety the replacement of the hydrogen at C-11 by a vobasine unit induced a deshielding of the *ipso* ($\Delta\delta$ 15 7) and shielding of the *ortho* carbons ($\Delta\delta_{C\text{-}10}$ 3 0 and $\Delta\delta_{C\text{-}12}$ 1 4) The remaining carbons showed little or no modification

The *R* configuration at C-20 of dihydrovoacamine (**1e**), the sole product of the catalytic hydrogenation of **1a**, was deduced from the chemical shifts of C-14 and C-16 C-14 at δ 31 4 was shielded ($\Delta\delta$ 5 2) in **1e** by comparison with the same site of **1a** due to a γ-interaction with C-19, while C-16 was deshielded ($\Delta\delta$ 3 7) (The differences produced by the α or β ethyl group orientation were previously discussed for tabernaelegantines A and B [7], dregamine and tabernamontanine [6])

Comparison of the ^{13}C chemical shifts of demethylvoacamine (**1c**) and voacamine (**1a**) revealed some interesting conformational aspects concerning the N_b-methyl group Introduction of the *N*-methyl group into **1c** induced simultaneous shielding at C-6 ($\Delta\delta$ 4 8) and C-16 ($\Delta\delta$ 6 3) These facts can be explained by taking the inversion of N_b into consideration Thus the rapid interconversion between the equatorial and axial N_b-methyl group leads to the observed shieldings Analogous effects were observed at C-6 ($\Delta\delta$ 6 3) and C-16 ($\Delta\delta$ 2 6) of **1e** for the introduction of the *N*-methyl group in demethyldihydrovoacamine (**1f**) (going from **1f** to **1e**) The larger $\Delta\delta$ value at C-6 suggests a preferential equatorial position for the N_b-methyl group in the dihydro compound, thus avoiding an additional methyl H_{20} 1,3-diaxial interaction

An interesting feature revealed in our work was the isolation of demethylvoacamine (**1c**) together with voacangine (**4**) and the α-acylindole perivine (**6**), implying that both monomers **4** and **6** are precursors in the biosynthetic pathway of **1c** Though *in vitro* data [8] support the above suggestion, rigorous *in vivo* experiments would provide final confirmation

EXPERIMENTAL

Mps are uncorr Specific rotations were measured in $CHCl_3$, UV spectra in EtOH and IR spectra in $CHCl_3$ ^{1}H NMR spectra

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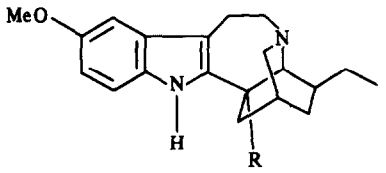
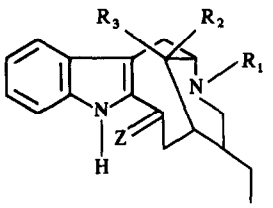
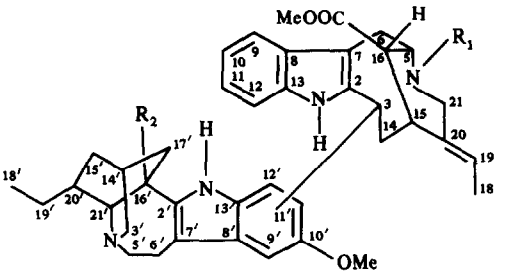


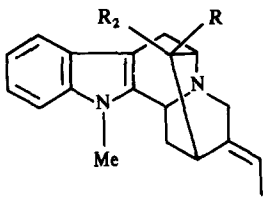
4 R = COOMe
9 R = H



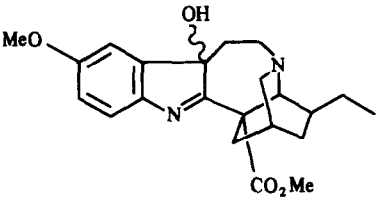
6 R_1 = H, Z = O, R_2 = H, R_3 = COOMe
7 R_1 = Me, Z = O, R_2 = CH_2OH, R_3 = H
8 R_1 = Me, Z = αH, βOH, R_2 = H, R_3 = CO_2Me



1a R_1 = Me, R_2 = COOMe, LINKAGE at 11′
1b R_1 = Me, R_2 = H, " " 11′
1c R_1 = H, R_2 = COOMe, " " 11′
1d R_1 = Me, R_2 = COOMe, " " 9′
1e R_1 = Me, R_2 = COOMe, 19–20 DIHYDRO, " " 11′
1f R_1 = H, R_2 = COOMe, 19–20 DIHYDRO, " " 11′



2 R_1 = CH_2OH, R_2 = H
3 R_1 = COOMe, R_2 = CH_2OH



5

at 60 and 100 MHz were obtained using TMS as int standard ^{13}C NMR spectra were recorded at 25 2 MHz with Fourier transform using $CDCl_3$ as solvent and TMS as int standard MS were determined at 70 eV Silica gel 0 05–0 25 mesh (Carlo Erba) and silica gel $HF_{254-366\,nm}$ (Merck) were used for CC and TLC, respectively Detection of components was made by UV (254 and 305 nm) and 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 *P fuchsiaefolia* (DC) Miers was collected in the Zeferino Vaz University City The air-dried bark (2 929 g) was moistened with a saturated $NaHCO_3$ soln and extracted in a Soxhlet with Et_2O On concn, the Et_2O extract gave a viscous oil which was added to a 10% HOAc soln and kept at 5° overnight After filtration the aq phase was extracted with Et_2O (extract A 6 65 g) and $CHCl_3$ (extract B 18 14 g) The pH was then raised to 8 with a saturated $NaHCO_3$ soln and extracted with Et_2O (extract C 8 52 g) and $CHCl_3$ (extract D 0 25 g)

Extract A (2 96 g) was fractionated on a silica gel column eluting with $CHCl_3$, and $CHCl_3$ with increasing amounts of MeOH, yielding voacamine (**1a**) (0 076 g) [9], voacangine (**4**) (0 41 g) [10] and voacangine hydroxyindolenine (**5**) (0 1388 g) [11] TLC also indicated the presence of demethylvoacamine (**1c**) [12] and affinisine (**2**) [13]

Extract B A Sephadex LH 20 column (6 g) eluted with $CHCl_3$–MeOH (9 1) permitted a crude separation of the dimeric from the monomeric compounds The combined fractions were further purified using a silica gel column and/or prep TLC, yielding the bisindoles voacamine (**1a**) (0 196 g) [9], demethylvoacamine (**1c**) (0 232 g) [12], decarbomethoxyvoacamine (**1b**) (0 178 g) [14] and voacamidine (**1d**) (0 206 g) [12], and the indoles vonchapotine (**3**) (0 221 g) [13] and affinisine (**2**) (0 329 g) [13]

Extract C (3 g) was fractionated on a silica gel column eluting with $CHCl_3$, and $CHCl_3$ with increasing amounts of MeOH, leading to the isolation of perivine (**6**) (0 1399 g) [8, 10], 16-epiaffinine (**7**) (0 2043 g) [15], affinisine (**2**) (0 2167 g) [13] and decarbomethoxyvoacamine (**1b**) (0 1295 g) [14]

Dihydrovoacamine (**1e**) An EtOH soln of voacamine (**1a**) (0 206 g) with a catalytic amount of PtO_2 was submitted to hydrogenation (45 psi H_2) in a Parr apparatus for 30 min Filtration through a Celite pad and evapn of solvent furnished 0 198 g of dihydrovoacamine, mp 210–212° (MeOH), $[\alpha]_D^{25}$ +40 9° (0 010 g/ml $CHCl_3$), UV $\lambda_{max}^{CHCl_3}$ nm (log ε) 226 2 (4 75), 286 2 (4 28), 293 7 (4 28), IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3450, 1710; ^1H NMR δ 0 93 (*m*), 2 47 (3H, *s*), 2 60 (3*H*, *s*), 3 63 (3H, *s*), 3 93 (3H, *s*), 7 48 (1H, *s*), 7 60 (1H, *s*), MS *m/z* (rel int) 720 (55), 706 $[M]^+$ (31), 511 (100)

Demethyldihydrovoacamine (**1f**) An EtOH soln of demethylvoacamine (**1c**) (0 206 g) was hydrogenated as above to furnish 0 149 g **1f** Mp 210° (MeOH), UV $\lambda_{max}^{CHCl_3}$ nm (log ε) 226 8 (4 68), 285 (4 23), 293 (4 22), IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3460, 1720; ^1H NMR δ 0 93 (*t*), 2 47 (3H, *s*), 3 67 (3H, *s*), 3 97 (3H, *s*), MS *m/z* (rel int) 720 (62), 706 $[M]^+$ (44), 136 (100)

Acknowledgements—We wish to express our appreciation to Professor Anita J Marsaioli for helpful suggestions, Dr Norbert

Table 1 ^{13}C NMR data for voacamine alkaloids and their derivatives

Carbon	4	8	9	7	1a	1b	1c	1e	1d	1f
2		135 4		134 4	135 5	135 5	135 5	135 6	134 9	135 7
3		66 8		189 5	37 4	37 6	37 1	37 2	37 1	37 3
5		59 4		56 5	59 9	59 7	53 3	59 2	59 8	53 0
6		19 6		19 3	19 8	19 4	24 6	19 2	19 0	25 5
7		107 3		120 4	109 6	110 0	110 2	109 8	110 8	110 9
8		128 7		128 1	129 4	129 4	129 7	129 6	129 9	129 5
9		117 6		121 2	117 2	117 0	117 1	117 2	116 9	117 3
10		118 6		120 2	119 8*	118 6	118 7	118 6	118 5	118 7
11		121 4		126 5	121 3	121 2	121 3	121 3	120 5	121 3
12		110 0		112 1	110 1	110 0	110 6	110 5	109 4	110 4
13		136 7		136 3	136 2	137 5	137 1	136 9	137 4	137 1
14		35 5		43 4	36 6	36 1	36 3	31 4	37 1	31 6
15		29 2		31 2	33 3	33 4	34 1	32 8	32 1	33 4
16		47 1		38 5	46 0	46 7	52 3	49 7	46 9	52 3
18		12 2		12 0	12 3	12 2	12 0	11 4	12 2	11 1
19		118 6		120 2	118 7*	118 6	117 3	23 5	118 5	23 5
20		136 5		135 2	137 7	137 8	140 1	43 7	137 9	45 9
21		53 9		51 9	52 3	52 2	44 1	51 9	52 3	40 8
COOMe		174 3		—	170 7	171 1	171 0	171 5	171 4	171 3
$\underline{COOCH_3}$		50 3		—	49 8	49 7	49 8	49 5	49 8	49 7
NMe		42 1		41 7	41 9	42 1	—	42 4	42 2	—
CH_2OH				66 7						
2′	137 3		142 9		136 9	142 1	137 6	137 5	138 8	137 5
3′	51 7		50 0		52 0	49 7	51 8	52 3	51 0	51 9
5′	53 1		54 2		53 0	54 1	53 0	53 0	53 9	53 0
6′	22 2		20 7		22 1	20 6	22 2	22 2	24 9	22 2
7′	110 0		109 1		109 6	108 4	109 6	110 3	109 0	109 8
8′	129 1		129 7		129 4	128 4	129 7	130 0	126 2*	130 1
9′	100 7		100 3		99 1	98 5	99 1	99 0	126 4*	99 2
10′	154 0		153 9		150 6	150 7	150 7	150 8	152 4	150 9
11′	111 9		110 8		127 1	127 9	127 1	127 0	112 5	127 1
12′	111 1		110 6		109 5	109 6	109 7	109 6	109 4	109 6
13′	130 6		130 0		130 0	129 0	130 1	130 3	131 8	130 2
14′	27 3		26 5		27 2	26 1	27 3	27 3	27 2	27 3
15′	32 0		32 0		31 8	31 7	31 9	31 9	31 8	31 9
16′	55 0		42 0		54 8	40 9	54 8	54 8	55 8	54 9
17′	36 5		34 2		36 2	33 9	36 3	36 4	33 5	36 4
18′	11 7		11 9		11 5	11 8	11 6	11 6	11 6	11 5
19′	26 7		27 8		26 6	27 6	26 7	26 7	26 7	26 7
20′	39 1		41 5		38 8	41 7	38 9	38 9	38 9	38 9
21′	57 6		57 5		56 9	57 5	57 0	57 0	58 2	57 0
COOMe	175 6		—		174 9	—	174 9	175 0	175 5	174 9
$\underline{COOCH_3}$	52 7		—		51 8	—	50 0	49 7	52 5	50 6
OMe	55 7		56 0		56 0	55 9	56 0	56 0	57 8	56 0

Spectra were obtained at 25 2 MHz in Fourier transform mode in $CDCl_3$ solutions Chemical shifts are expressed on the TMS scale according to δ TMS = δ $CDCl_3$ + 76 9 ppm

*Assignments for these signals within a vertical column may be reversed

Neuss of Lilly Research Laboratories for providing an authentic sample of perivine and the Conselho Nacional de Desenvolvimento Científico a Technológico—CNPq—for financial support

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