Phytochemistry, Vol. 26, No. 3, pp. 833–836, 1987.
Printed in Great Britain.

0031-9422/87 $3.00 + 0.00

QUATERNARY ALKALOIDS FROM *PESCHIERA FUCHSIAEFOLIA*

RAQUEL M. BRAGA and FRANCISCO DE A. M. REIS*

Instituto de Química, Universidade Estadual de Campinas C.P. 6154, Campinas 13100, São Paulo, Brazil

(*Received* 20 *March* 1986)

Key Word Index—*Peschiera fuchsiaefolia*; Apocynaceae; quaternary alkaloids; 12-methoxy-4-methylvoachalotine; 12-methoxy-4-methylvoachalotine ethyl ester; fuchsiaefoline; 4-methylvoachalotine; 4-methylaffinisine; ^{13}C NMR.

Abstract—From an ethanolic extract of *Peschiera fuchsiaefolia* three quaternary alkaloids have been isolated and their structures determined from their spectral data as 12-methoxy-4-methylvoachalotine, 12-methoxy-4-methylvoachalotine ethyl ester and fuchsiaefoline. Analyses of the ^{13}C NMR spectra of some sarpagine alkaloids were carried out to confirm the structures of these compounds.

INTRODUCTION

Reports of the *in vitro* anticancer activity of *bis*-indole alkaloids [1] led us to reinvestigate the bark of *Peschiera fuschsiaefolia* [2–5], directing our research to the isolation of active compounds. Different extracts of *P. fuchsiaefolia* which were submitted to *in vivo* test against lymphocytic leukaemia (P 388) showed biological activity [6]. Fractionation of the extract led to the isolation of several indolic bases [5] and to three new quaternary salts.

RESULTS AND DISCUSSION

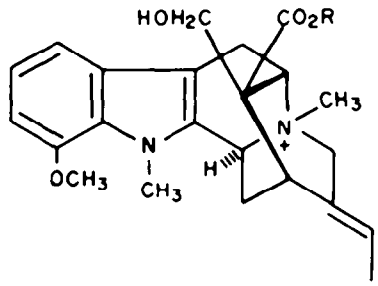
The quaternary alkaloids were separated on a silica gel CC and further purified by TLC yielding 12-methoxy-4-methylvoachalotine **1**, 12-methoxy-4-methylvoachalotine ethyl ester **2** and fuchsiaefoline **3**.

12-Methoxy-4-methylvoachalotine **1**, $C_{24}H_{31}N_2O_4$, is an amorphous solid, mp 221–223 and $[\alpha]_D^{25} = -106.19°$ (*c* 1.0; MeOH). Its spectroscopic properties closely resemble those of voachalotine. The mass spectrum reveals a $[M-1]^+$ ion at *m/z* 410, $[M-15]^+$ at 396.2028 (100%, $C_{23}H_{28}N_2O_4$ requires 396.2048), and ions at *m/z* 381, 365, 293, 213 and 212, similar to those of voachalotine [4]. The UV spectrum presents absorptions at λ_{max}^{EtOH} nm (log ε) 268 (3.78), 283 (3.67) and 293 (3.60) which indicates the presence of a methoxy substituent in the indole ring. The IR spectrum shows absorptions for hydroxy and ester groups at 3300 and 1720 cm^{-1}, respectively. The ^{1}H NMR spectrum showed the presence of five methyl groups, C_{18} (δ1.55, 3H, *d*, *J* = 6 Hz), N_4–CH_3 (δ3.13, 3H, *s*), CO_2CH_3 (δ3.75, 3H, *s*), and N_1–CH_3 and –OCH_3 (δ3.92, 6H, *s*), a trisubstituted double bond C19-H (δ5.4, 1H, *m*) and aromatic protons (δ6.2–7.1, 3H, *m*). The ^{13}C NMR spectrum (Table 1) confirms the presence of a C_{24} compound and clearly shows the presence of a N_4–CH_3 (δ48.8, *q*), a carbomethoxy group [δ172.7 (*s*) and 52.4 (*q*)], methoxy group (δ55.4, *q*), C19-H (δ120.4, *d*), C_{20} (δ132.4, *s*) and eight indole carbons. The above spectral features confirm the structure of a quaternary sarpagine type of compound as depicted in **1**.

12-Methoxy-4-methylvoachalotine ethyl ester **2**, $C_{25}H_{33}N_2O_4$, is an amorphous solid, mp 211–214°, $[\alpha]_D^{25} = -55.94°$ (*c* 1.0; MeOH). The mass spectrum shows ions at *m/z* 424 $[M-1]^+$, 410.2199 $[M-15]^+$ (100%; $C_{24}H_{30}N_2O_4$ requires 410.22054), and ions at 379 $[M-31]^+$, 365 $[M-45]^+$ and 337 $[M-73]^+$ corresponding to the loss of –CH_2OH, CH_3CH_2O– and $CO_2C_2H_5$, respectively, the latter two peaks confirming the presence of the ethyl ester group. Further confirmation is given by the peak at δ1.33 (3H, *t*, *J* = 6 Hz) in the ^{1}H NMR spectrum and the absorption at 1720 cm^{-1} in the IR spectrum. The ^{13}C NMR spectrum shows 25 carbons with chemical shifts analogous to those of **1** except for those assigned to the ethyl group.

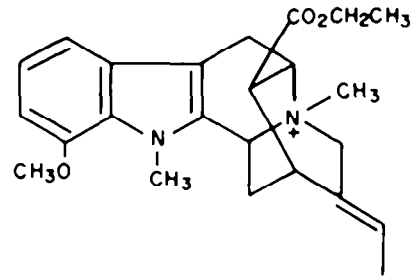
Fuchsiaefoline **3**, $C_{24}H_{31}N_2O_3$, is an oil, $[\alpha]_D^{25} = -55.73°$ (*c* 0.9; MeOH). The mass spectrum shows peaks at *m/z* 394 $[M-1]^+$, and at 380.20955 ($[M-15]^+$, 100%; $C_{23}H_{28}N_2O_3$ requires 380.20998) and a fragment at 307 $[M-73]^+$ which clearly indicates a loss of $CO_2C_2H_5$. The others fragments are related to those of affinisine. The UV absorptions at λ_{max}^{EtOH} nm (log ε) 269 (3.90), 284 (3.80) and 294 (3.73) indicate a 12-methoxy-1-methyl chromophore. In the IR spectrum, the absorption at 1720 cm^{-1} confirms the presence of an ester group. Signals at δ1.26 (3H, *t*, *J* = 6 Hz, CH_2CH_3), 1.65 (3H, *d*, *J* = 6 Hz, CH_3), 3.47 (3H, *s*, N_4–CH_3), 3.95 (3H, *s*, N_1–CH_3), and 4.07 (3H, *s*, OCH_3) were detected in the ^{1}H NMR spectrum. The ^{13}C NMR spectrum (Table 1) corroborates a C_{24} compound and clearly shows a N_4–CH_3 (δ46.9), a carbethoxy group (δ169.9, *s*; δ61.9, *t*; and δ13.9, *q*) and a methoxy group at δ55.4 as well as peaks at δ132.8 (C20) and δ121.2 (C_{19}), and eight indole carbons. The presence of a C–H at δ47.6 assigned to C_{16}, corroborate structure **3** for this compound. The isolation of an ethyl ester is unusual and this fact led us to suppose that compounds **2** and **3** are artefacts; probably due to the transesterification of the corresponding methyl ester or the esterification of a betaine type of compound during the ethanol extraction.

In order to confirm the above structures we carried out a ^{13}C NMR analysis of some sarpagine alkaloids and their corresponding *N*-methyl salts (see Table 1). The chemical shifts of normacusine B4, affinisine **5** and voachalotine **6** were assigned by comparison with other sarpagine derivatives [7, 8]. The assignments of 4-methylaffinisine **7** and 4-methylvoachalotine **8** were based on the known effects of quaternatization of tertiary alkaloids [9, 10]. Comparison of the chemical shifts of 12-methoxy-4-

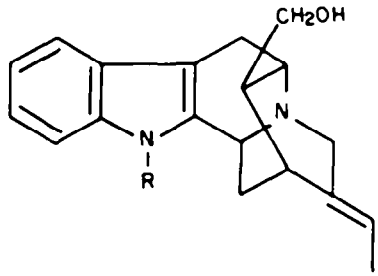


1 R = CH_3

2 R = CH_2CH_3

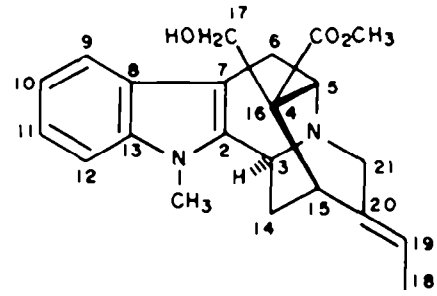


3

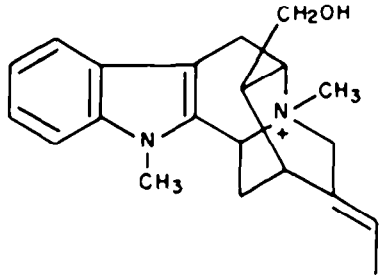


4 R = H

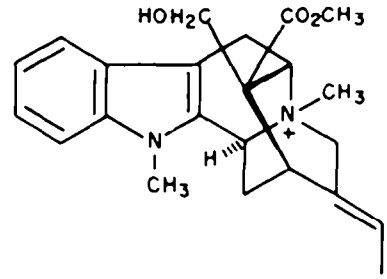
5 R = CH_3



6



7



8

methylvoachalotine 1 and voachalotine *N*-methosalt 8 reveals the similarity between the terpenic moiety of these compounds. Chemical shift calculations using empirical parameters, obtained by the comparison of indole, 7-methoxyindole [11] and cuanzine [12] applied to 8 led us to assign the methoxy group to C_{12} of 1. This was further supported by the deshielding effect observed on the N_1–CH_3 group when going from 8 to 1. The observed UV pattern of the 12-methoxy indole alkaloids may be characteristic and could be used to detect this chromophore. However, more examples are needed to confirm this observation. Nevertheless, the ^{13}C NMR does provide an alternative method.

The analysis of 12-methoxy-4-methylvoachalotine ethyl ester 2 was easily accomplished by comparison with 1. The chemical shift assignments of fuchsiaefoline 3 were based on those of 4-methylaffinisine 7.

It is clear from the above arguments that 1, 2, 6 and 8 have the same relative configuration. The structures 1 and 2 presented above depict the absolute configurations of 12-methoxy-4-methylvoachalotine 1 and its corresponding ethyl ester 2. This was deduced by comparing the optical rotations of 1 and 2 with that of 8 (obtained from 6) and taking into consideration the known absolute configuration of 6 [13].

EXPERIMENTAL

Mps are uncorr. Specific rotations were measured in MeOH, UV spectra in EtOH and IR spectra in $CHCl_3$. ^1H NMR spectra at 60 and 100 MHz were obtained using TMS as an int. std. ^{13}C NMR spectra were recorded at 25.2 MHz with Fourier transform in $CDCl_3$. MS were determined at 70 eV. Silica gel 0.05–0.25 mesh (Carlo Erba) and silica gel $HF_{254\ 366\ nm}$ (Merck)

Table 1. ^{13}C NMR data for sarpagine alkaloids

Carbon	4	5	6	7	8	1	2	3
2	132.8	135.6	136.9	125.3	124.4	126.4	126.5	125.6
3	50.3	49.3	47.7	59.2	57.0	56.6	56.6	58.4
5	55.0	54.4	53.5	65.4	64.9	64.2	64.3	62.3
6	26.5	27.1	22.2	24.3	19.5	19.2	18.7	24.4
7	103.8	103.4	104.8	100.0	100.9	100.9	100.8	99.0
8	127.1	127.1	125.9	126.6	126.0	127.1	127.2	127.5
9	117.9	117.9	118.0	118.1	118.7	111.3	111.3	110.6
10	119.2	118.5	118.6	119.7	120.1	119.3	119.6	120.6
11	121.5	120.6	120.7	120.6	120.1	103.7	103.8	103.8
12	111.1	108.4	108.5	109.1	109.6	147.9	147.7	147.7
13	136.8*	139.3	138.1	137.6	137.8	126.4	126.5	127.4
14	32.8	32.7	28.2	31.2	27.9	28.0	27.6	30.6
15	27.2	27.3	30.2	25.6	29.7	29.5	29.3	27.2
16	43.8	44.1	53.2	43.2	55.0	55.2	54.9	47.6
17	64.5	64.7	62.9	62.7	62.5	62.2	62.5	
18	12.7	12.6	12.7	12.3	12.5	12.3	12.0	12.7
19	117.9	116.2	115.7	122.4	123.0	120.4	120.6	121.2
20	136.5*	137.0	136.2	132.0	131.9	132.4	132.7	132.8
21	55.0	56.0	55.7	64.5	64.3	64.2	64.3	64.9
22			176.0		172.5	172.7	172.3	169.9
$CO_2\underline{C}H_3$			51.9		53.3	52.8		
N-CH_3		29.2	29.1	29.4	30.7	33.1	32.8	33.4
Ṅ-CH_3				47.1	49.4	48.8	48.7	46.9
$CO_2\underline{C}H_2CH_3$							62.5	61.9
$CO_2CH_2\underline{C}H_3$							13.4	13.9
OCH_3						55.4	55.5	55.4

Spectra were obtained 25.2 MHz in Fourier transform mode in $CDCl_3$ solutions. Chemical shifts are expressed on the TMS scale using δ76.9 for $CDCl_3$.

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

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 at Zeferino Vaz University City. After preliminary extraction with Et_2O, 2929 g of the ground bark was extracted in a Soxhlet with EtOH. After concn, the EtOH extract was added to a 10% HOAc soln and kept at 5° overnight. After filtration the aq. phase was extracted with Et_2O (extract I, 2.54 g) and $CHCl_3$ (extract II, 11.82 g). The pH was then raised to 8 with a satd $NaHCO_3$ soln and extracted with Et_2O (extract III, 0.14 g) and $CHCl_3$ (extract IV, 2.58 g). After silica gel CC followed by prep. TLC we isolated fuchsiaefoline 3 from extract II and 12-methoxy-4-methylvoachalotine 1 and 12-methoxy-4-methylvoachalotine ethyl ester 2 from extract IV.

12-Methoxy-4-methylvoachalotine 1. Mp 221–223°; $[\alpha]_D^{25}$ = −106.19° (*c* 1.0; MeOH); UV λ_{max}^{EtOH} nm (log ε): 225 (4.64), 268.5 (3.78), 283.5 (3.67), 293.0 (3.60); IR ν^{KBr} cm^{-1} 3300 (OH), 1720 (C=O); ^{1}H NMR: δ1.55 (3H, *d*, *J* = 6 Hz), 3.13 (3H, *s*), 3.75 (3H, *s*), 3.92 (6H, *s*), 4.69 (1H, *d*), 5.14–5.41 (2H, *m*); ^{13}C NMR see Table 1; MS *m/z* (rel. int.): 410 (4), 396.2028 (100) ($C_{23}H_{28}N_2O_4$ requires 396.2048), 395 (51), 381 (22), 379 (15), 365 (60), 337 (20), 293 (60), 213 (35), 212 (43).

12-Methoxy-4-methylvoachalotine ethyl ester 2. Mp 211–214°; $[\alpha]_D^{25}$ = −55.94° (*c* 1.0; MeOH); UV λ_{max}^{EtOH} nm (log ε): 225 (4.65), 270.5 (3.82), 284.5 (3.75), 294.0 (3.67); IR ν^{KBr} cm^{-1}: 3300 (OH), 1720 (C=O) ^{1}H NMR: δ1.32 (3H, *t*, *J* = 6 Hz), 1.57 (3H, *d*, *J* = 6 Hz), 3.10 (3H, *s*), 4.00 (6H, *s*); ^{13}C NMR see Table 1; MS *m/z* (rel. int.): 424 (3), 410.2199 (100) ($C_{24}H_{30}N_2O_4$ requires 410.2205), 409 (45), 379 (49), 365 (14), 337 (25), 293 (65), 280 (15), 213 (35), 212 (47).

Fuchsiaefoline 3. Oil; $[\alpha]_D^{25}$ = −55.73° (*c* 0.9; MeOH); UV λ_{max}^{EtOH} nm (log ε): 225.5 (4.66), 269.5 (3.90), 283.5 (3.80), 293.5 (3.73); IR ν^{KBr} cm^{-1} 3400 (OH), 1720 (C=O); ^{1}H NMR: δ1.26 (3H, *t*, *J* = 6 Hz), 1.65 (3H, *d*, *J* = 6 Hz), 3.45 (3H, *s*), 3.97 (3H, *s*), 4.07 (3H, *s*); ^{13}C NMR see Table 1; MS *m/z* (rel. int.): 395 (3), 394 (10), 381 (86), 380.20955 (100) ($C_{23}H_{28}N_2O_3$ requires 380.20998), 379 (92), 366 (61), 365 (72), 351 (55), 335 (31), 307 (82), 293 (37), 280 (18), 279 (19), 226 (19), 213 (87), 212 (97), 197 (59).

4-Methylaffinisine 7 and 4-methylvoachalotine 8 were prepared by stirring compounds 5 and 6, respectively, with MeI in MeOH [14].

4-Methylaffinisine 7. Mp 205–208°; UV λ_{max}^{EtOH} nm (log ε): 222 (4.53), 274.5 (3.79), 282 (3.79), 290.5 (3.69); IR ν^{KBr} cm^{-1} 3350 (OH); ^{1}H NMR: δ1.65 (3H, *d*, *J* = 6 Hz), 3.20 (*s*), 3.71 (3H, *s*), 4.31 (1H, *d*, *J* = 16 Hz), 4.70 (1H, *d*, *J* = 16 Hz); MS *m/z* (rel. int.): 332 (8), 308 (72), 307 (100), 293 (7), 291 (7), 277 (17), 183 (35), 182 (47).

Acknowledgements We thank Profs. Concetta Kascheres and Luzia Koike for the high resolution MS and ^{13}C NMR, respectively.

REFERENCES

1. Kingston, D. G. I. (1978) *J. Pharm. Sci.* **67**, 272.
2. *Peschiera* and *Tabernaemontana* have recently been considered as synonyms: Van Beek, T. A., Verpoorte, R.,

Svendsen, A. B., Leeuwemberg, A. J. M. and Bisset, N. G. (1984) *J. Ethnopharmacology* **10**, 1.
3. Fernandez, M. E., Albonico, S. M. and Ruveda, E. A. (1967) *An. Asoc. Quim. Argent.* **55**, 239.
4. Achenbach, H. (1966) *Tetrahedron Letters* 4405.
5. Braga, R. M., Leitão Filho, H. F. and Reis, F. de A. M. (1984) *Phytochemistry* **23**, 175.
6. Pure 12-methoxy-4-methylvoachalotine was revealed to be inactive. Gower, A. E., Periera, B. S. and Marsaioli, A. J. (1986) *Phytochemistry* **25**, 2908.
7. Bombardelli, E., Bonati, A., Gabetta, B., Martinelli, E., Mustich, G. and Danielli, B. (1976) *Phytochemistry* **15**, 2021.
8. Aimi, N., Yamaguchi, K., Sakai, S. I., Haginiwa, J. and Kubo, A. (1978) *Chem. Pharm. Bull.* **26**, 3444.
9. Marsaioli, A. J., Ruveda, E. A. and Reis, F. de A. M. (1978) *Phytochemistry* **17**, 1655.
10. Koike, L., Marsaioli, A. J. and Reis, F. de A. M. (1981) *J. Org. Chem.* **46**, 2385.
11. Rosemberg, E., Williamson, K. L. and Roberts, J. D. (1976) *Org. Magn. Reson.* **8**, 117.
12. Bombardelli, E., Bonati, A., Gabetta, B., Martinelli, E. M. and Mustich, G. (1974) *Tetrahedron* **30**, 4141.
13. Defay, N., Kaisin, M., Pecher, J. and Martin, R. H. (1961) *Bull. Soc. Chim. Belg.* **70**, 475.
14. Pecher, J., Defay, N., Gautier, M., Peeters, J., Martin, R. H. and Vandermeers, A. (1960) *Chem Ind.* 1481.