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CATHARINENSINE, AN OXINDOLE ALKALOID FROM *PESCHIERA CATHARINENSIS*

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Key Word Index—*Peschiera catharinensis*, Apocynaceae, alkaloids, catharinensine, coronaridine, isovoacangine, heyneanine, 16-epiaffinine, decarbomethoxyvoacamine, conodurine

Abstract—The structure of catharinensine isolated from *Peschiera catharinensis* was deduced from a detailed analysis of its ^{1}H NMR, ^{13}C NMR and mass spectra The synthesis of 17-demethoxyrhynchophylline confirmed its structure, and led us to suggest the configuration allo A for catharinensine Coronaridine, isovoacangine, heyneanine, 16-epiaffinine, decarbomethoxyvoacamine and conodurine were also isolated

INTRODUCTION

The presence of toxic plants in pastures is responsible for 5% of the cattle deaths in the State of Paraná, Brazil Giovannoni *et al* [1] identified 54 toxic species and confirmed the presence of toxic principles in 12 Of these, *Peschiera catharinensis* (DC) Miers (Apocynaceae, subtribe Tabernamontaninae) was the only alkaloid-containing species not previously studied, *Peschiera* represents an exclusively American genus and has not been fully investigated [2]

RESULTS AND DISCUSSION

From the methanolic extract of the bark, we isolated a new alkaloid, catharinensine (**1**), together with coronarine (**2**), isovoacangine (**3**), heyneanine (**4**), 16-epiaffinine (**5**), decarbomethoxyvoacamine (**6**) and conodurine (**7**)

Catharinensine (**1**), $C_{21}H_{26}N_2O_3$, a light yellow, oily compound had $[\alpha]_D^{25} - 194°$, and a UV absorption spectrum analogous to that of isorhynchophylline (**8**) [3,4] suggesting that these two compounds possessed similar chromophores The ^{1}H NMR assignments were based on comparison with isorhynchophylline (**8**), rhynchophylline (**9**), corynoxine (**10**) and isocorynoxine (**11**) [3,4] The low-field doublet at δ 7 56 (1H, J = 7 Hz) was indicative of a C-9 H and N-4 lone-pair interaction [3,4] This and consideration of the most probable conformations of the eight possible diastereoisomers of the rhynchophyllinoid type [4] suggested that **1** possessed either the configuration normal A, allo A, pseudo B or epiallo B The presence of two singlets at δ 5 46 and 6 46 and the lack of a peak attributable to a vinyl methyl ether group indicated a C-16 to C-17 terminal double bond

The mass spectrum of **1** displayed ions typical of a tetracyclic oxindole of the rhynchophylline type [5] (354 $[M]^+$, 209 (ring D), 194 (ring D – Me), 180 (ring D – Et), 178 (ring D – OMe)) but these peaks were shifted to lower mass by 30 mu, thus indicating a modification in ring D of **1** compared with that of **8** Similarly, the differences in the ^{13}C NMR spectra of **1** and **8** were in the δ-values of the ring D carbons and in the replacement of the olefinic quaternary and methine carbon resonances at 113 0 and 150 5 ppm assigned to C-16 and C-17, respectively, in **8** [6,7] by the olefinic quaternary and methylene carbon resonances at 142 7 (C-16) and 125 2 (C-17) ppm, respectively All these properties are consistent with structure **1** for catharinensine As this structure represents a novel oxindole alkaloid, the synthesis of **16** was undertaken to provide final confirmation and rhynchophylline (**9**) was taken as a starting material (Scheme 1) Only one of the two possible C-16 epimers of **12** was obtained in 45% yield, by treatment of **9** with $HgSO_4$–H_2SO_4 [8] followed by $NaBH_4$–EtOH reduction Treatment of **12** with MsCl–pyridine in CH_2Cl_2 produced two mesylates, **13** and **14**, in a ratio of 2 3 Because of the known C-7 isomerization of oxindole alkaloids in the presence of pyridine [3,4] and the presence of a low-field doublet at δ_H 7 80 together with four singlets at δ_H 3 80 (–COOMe), 3 60 (–COOMe), 3 00 (–SO_2Me), 2 84 (–SO_2Me), it was concluded that the mesylates were C-7 epimers Elimination of the mesyl group with DBN–CH_2Cl_2 at room temperature† afforded 17-demethoxyrhynchophylline (**15**) and 17-demethoxyisorhynchophylline (**16**) with the configuration normal B and normal A, respectively (taking into consideration that rhynchophylline (**9**), normal B [9], was our starting material and that the reactions should not alter the asymmetric centres, except for C-7) The mass spectra of **15**, **16** and **1** had fragment ions with equal m/z values but different relative abundances This fact and the nearly superimposable ^{1}H NMR spectra of **15**, **16** and **1** led us to the conclusion that these compounds were diastereoisomers Thus the synthesis of **15** and **16** confirmed the proposed structure for **1** but not its configuration

Using the general procedure for configurational analysis of rhynchophylline-type alkaloids [4], we submitted a small sample of **1** to isomerization in pyridine After 48 hr,

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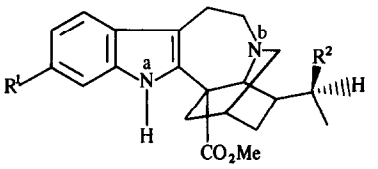
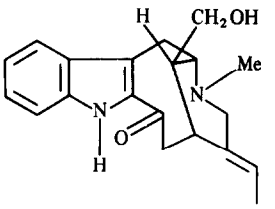
†3-Mesyl-ethylbutanoate was taken as a model compound for the mesyl group elimination reaction Among several bases (OEt^-, py, DBU, DABCO and DBN), the best results were obtained using DBN



2 $R^1 = H, R^2 = H$
3 $R^1 = OMe, R^2 = H$
4 $R^1 = H, R^2 = OH$



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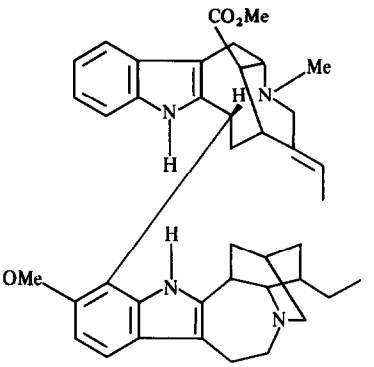

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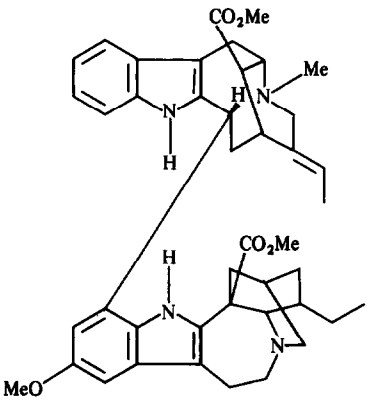

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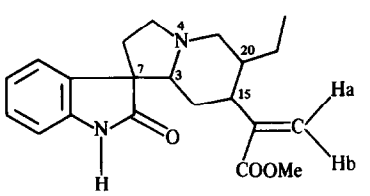
the original alkaloid was still present in the equilibrium mixture thus indicating that compound **1** belongs either to the normal or the allo series

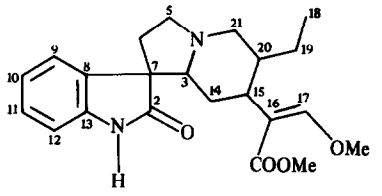
The non-symmetrical appearance of the C-18 methyl triplet signal in the ^{1}H NMR spectrum of **1** could have been indicative of a normal configuration [4] but this suggestion was eliminated by comparison with the ^{1}H NMR spectra of **15** and **16** Hence we concluded that catharinensine (**1**) possessed the configuration allo A On the basis of this conclusion and also of the fact that the CD curves of corynoxine (**10**) (allo A) [4] and **1** are mirror images of each other (Fig 1), we suggest that these alkaloids have opposite absolute configuration and that catharinensine (**1**) is a 7*R*,3*R*,4*S*,15*R*,20*R*-oxindole alkaloid Semisynthesis using corynoxine or corynantheidine as starting material would certainly support our configurational suggestion, but unfortunately no samples of corynoxine or corynantheidine were available

EXPERIMENTAL

^{1}H NMR spectra were determined at 60 or 100 MHz using $CDCl_3$ solns (unless indicated otherwise) and TMS as internal standard ^{13}C NMR spectra were recorded in $CDCl_3$ at 25 2 MHz in the Fourier transform mode, the values are in ppm downfield from TMS [$\delta(Me_4Si)$ = ($CDCl_3$) + 76 9] All mps are uncorr IR were recorded using thin films or KBr pellets Silica gel HF was utilized for TLC and spots were visualized by spraying

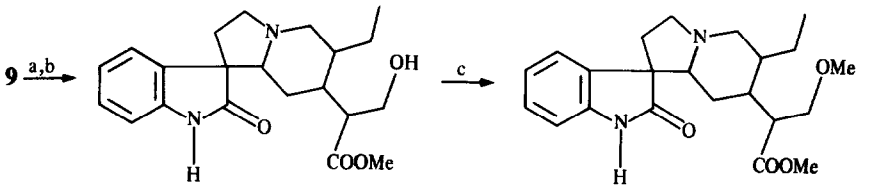


1 7*R*, 3*R*, 4*S*, 15*R*, 20*R*



	C_7	C_3–H	C_{15}–H	C_{20}–H	
8	A	α	α	β	(Normal)
9	B	α	α	β	(Normal)
10	A	α	α	α	(Allo)
11	B	α	α	α	(Allo)
	A or B	β	α	α	(Epiallo)
	A or B	β	α	β	(Pseudo)

A oxindole C=O below C/D plane
B oxindole C=O above C/D plane



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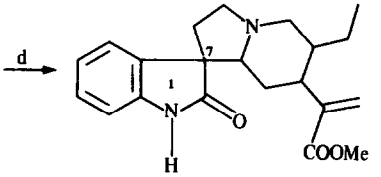
13 (Normal B)
14 (Normal A)



15 (Normal B)
16 (Normal A)

a $HgSO_4$ – aq H_2SO_4, b $NaBH_4$ – MOH, c MsCl – pyridine
d DBN – CH_2Cl_2

Scheme 1 Synthesis of **16**

Dragendorff soln followed by MeOH–H_2SO_4 and heating at 110° CD curves were measured in 1 cm cells using 1 mg of sample in 15 ml MeOH

P catharinensis (DC) Miers was collected in the State of Paraná, Brazil by Dr Gert Hatschbach A herbarium specimen has been deposited at the Museo Botânico Municipal, Curitiba, Paraná (UR 43868) The finely ground bark (1874 g) was extracted with MeOH, yielding 170 g crude extract This was absorbed on cellulose (200 g) and eluted with hexane (31 0 g), $CHCl_3$ (6 g) and MeOH (76 g) C on silica gel afforded the alkaloids

Catharinensine (**1**) 0 120 g, $[\alpha]_D^{25}$ −194° (*c* 0 5, $CHCl_3$), UV λ_{max}^{EtOH} nm (log ε) 250 (3 63), 282 (3 29), IR ν^{KBr} cm^{-1} 3430 (N–H), 1739 (COOR), 1635 (C=O), ^{1}H NMR δ 0 49 (asymmetric *t*, 3H, peaks 5 Hz apart, H-18), 3 72 (*s*, 3H, –COOMe), 5 46 (*s*, H-16a), 6 46 (*s*, H-16b), 6 8–7 56 (4H, aromatic), 7 56 (*br d*, *J* = 7 Hz, H-9), MS *m/z* (rel int) 354 $[M]^+$ (100), 339 (6 2), 325 (6 9), 323 (11 7), 209 (77), 208 (67), 194 (80), 180 (43 5), 159 (20), 146 (60), 144 (15 2), 130 (27), 69 (22), ^{13}C NMR δ 182 9 (C-2), 72 1 (C-3), 53 9 (C-5), 37 5 (C-6), 56 2 (C-7), 133 9 (C-8), 125 0 (C-9), 122 3 (C-10), 127 4 (C-11), 109 7 (C-12), 140 3 (C-13), 33 5 (C-14), 40 4 (C-15), 142 7 (C-16), 125 2 (C-17), 8-0 (C-18), 19 7 (C-19), 40 8 (C-20), 52 9 (C-21) (Found $[M]^+$ *m/z* 354 19468, $C_{21}H_{26}N_2O_3$ requires *m/z* 354 19433)

Coronaridine (**2**) 0 780 g, $[\alpha]_D^{25}$ −44° (*c* 2 0; $CHCl_3$), UV λ_{max}^{EtOH} nm (log ε) 225 (1 05), 284 (1 05), 292 (1 01), IR ν^{KBr} cm^{-1} 3380 (N–H), 1725 (C=O), ^{1}H NMR [10] δ 0 91 (*t*, *J* = 7 Hz, H-18), 3 71 (*s*, –COOMe), 6 86–7 50 (*m*, H-11, H-12, H-

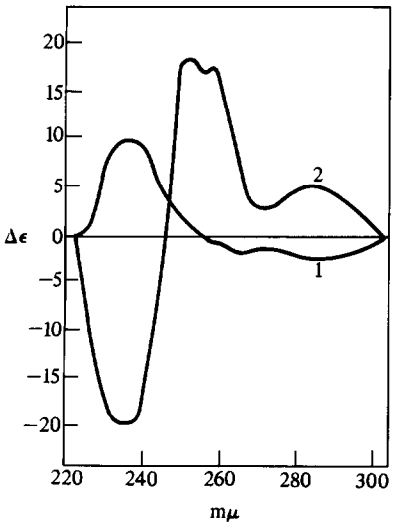


Fig 1 CD spectra of corynoxine (1) and catharinensine (2)

13, H-14), 7 6 (*s*, N_a–H) MS *m/z* $[M]^+$ 323

Isovoacangine (3) 0 028 g, $[\alpha]_D^{25}$ −40 8° (*c* 1 0, $CHCl_3$), UV λ_{max}^{EtOH} nm (log ε) 224 (3 7), 277 (3 09), 298 (3 13), IR ν^{KBr} cm^{-1} 3300 (N–H), 1720 (C=O), ^{1}H NMR [11] δ 0 93 (*t*, *J* = 6 Hz, H-18), 3 75 (*s*, –COOMe), 3 83 (*s*, –OMe), 6 46–7 4 (*m*, H-9, H-10, H-11, H-12), 7 66 (*s*, N_a–H), MS *m/z* (rel int) 368 $[M]^+$ (100)

Heyneanine (4) 0 130 g, mp 215 9–216 8° (MeOH), $[\alpha]_D^{25}$ −72 95° (*c* 2 2, $CHCl_3$) UV λ_{max}^{EtOH} nm (log ε) 220 (4 24), 284 (3 85), IR ν^{KBr} cm^{-1} 3280 (–NH, –OH), 1735 (–COOR), ^{1}H NMR [10], δ 1 13 (*d*, *J* = 6 Hz, H-18), 3 80 (*s*, –COOMe), 3 93 (*s*, 1H, OH), 4 20 (*q*, *J* = 6 Hz, H-19), 7 06–7 73 (*m*, H-9, H-10, H-11, H-12), 8 20 (*s*, N_a–H) MS *m/z* (rel int) 354 $[M]^+$ (100)

16-Epiaffinine (5) 0 110 g, $[\alpha]_D$ −150° (*c* 3 8, $CHCl_3$), UV λ_{max}^{EtOH} nm (log ε) 214 (4 16), 238 (4 07), 318 (4 28), IR ν^{KBr} cm^{-1} 3350 (–NH, –OH), 1620 (–C=O) cm^{-1}, ^{1}H NMR [12] δ 1 66 (*dd*, *J* = 1 5 and 6 Hz, H-18), 1 93 (*m*, H-16), 2 59 (*s*, N_b-Me), 3 56 (*d*, *J* = 5 Hz, H-17), 5 52 (*q*, *J* = 7 Hz, H-19), 7 00–7 83 (*m*, H-9, H-10, H-11, H-12), 9 51 (*s*, N_a-H) MS *m/z* (rel int) 324 $[M]^+$ (63) Acetyl derivative $[\alpha]_D$ −190° ($CHCl_3$), UV λ_{max}^{EtOH} nm (log ε) 2 15 (4 10), 237 (4 01), 318 (4 13), IR ν^{KBr} cm^{-1} 3300 (N–H), 1720 (–O–C(=O)–R), 1620 (–C=O) cm^{-1}, ^{1}H NMR δ 1 66 (*dd*, *J* = 2 and 7 Hz, H-18), 1 93 (*s*, –O–C(=O)–Me), 2 56 (*s*, N_b-Me), 5 6 (*q*, *J* = 7 Hz, H-19), 7 03–7 93 (*m*, H-9, H-10, H-11, H-12), 9 60 (*s*, N_a-H), MS *m/z* (rel int) 366 $[M]^+$ (65)

Decarbomethoxyvoacamine (6) 0 120 g, UV λ_{max}^{EtOH} nm (log ε) 233 (4 35), 288 (3 84), 295 (3 85), IR ν^{KBr} cm^{-1} 3350 (N–H), 1720 (–COOR), ^{1}H NMR [12] δ 0 92 (*t*, *J* = 6 Hz, H-18), 1 73 (*d*, *J* = 6 Hz, H-18), 2 51 (*s*, N_b-Me), 2 70 (*s*, –OMe), 3 73 (*s*, –COOMe), 5 46 (*q*, *J* = 6 Hz, H-19), 7 00–7 80 (*m*, H-9, H-10, H-11, H-12, H-9′, H-12′), 8 23 (*s*, N_a-H), MS *m/z* (rel int) 646 $[M]^+$ (3 4)

Conodurine (7) 0 011 g, mp 215 5–216 8° (MeOH), $[\alpha]_D^{25}$ −72 95° (*c* 1 0; $CHCl_3$), UV λ_{max}^{EtOH} nm (log ε) 220 (3 73), 284 (3 35), 290 (3 37), IR ν^{KBr} cm^{-1} 3360 (N–H), 1710 (–COOR), 1600 (–OMe), ^{1}H NMR [12] δ 0 76 (*t*, *J* = 8 Hz, H-18′), 1 63 (*dd*, *J* = 2 and 8 Hz, H-18), 2 51 (*s*, N_b-Me), 2 60 (*s*, –OMe), 3 68 (*s*, –COOMe), 5 26 (*m*, H-3′ and H-19), 7 66–6 74 (*m*, 6H, H-9, H-10, H-11, H-12, H-9′, H-10′), 7 56 (*s*, N_a-H), 7 66 (*s*, N_a-H) MS *m/z* (rel int) 704 $[M]^+$ (100)

17-Demethoxyrhynchophylline (15) *and 17-demethoxyisorhynchophylline* (16) Rhynchophylline (9) (0 150 g, 0 39 mmol) was treated with mercurous sulphate (9×10^{-3} mmol) and H_2SO_4–H_2O (1 1, 30 ml) overnight at room temp After filtration the soln was neutralized with a saturated soln of K_2CO_3 (150 ml) and extracted with $CHCl_3$ The $CHCl_3$ extracts were dried (Na_2SO_4) and evapd (0 134 g, 0 36 mmol) The residue was then treated with $NaBH_4$ (0 2 g) and EtOH (15 ml) After 30 min and usual work-up, an oily residue was obtained (0 11 g) Prep TLC and development with $CHCl_3$–MeOH (47 3) afforded **12**, 0 066 g (1 8 mmol, 50% yield) UV λ_{max}^{EtOH} nm 283, 250; IR ν^{film} cm^{-1} 3400 (NH, OH), 1700 (C=O), ^{1}H NMR δ 0 95 (*br s*, H-18), 3 59 (*s*, –COOMe), 6 80–7 20 (*m*, 4H aromatic), 9 60 (*s*, N_a-H), MS *m/z* (rel int) 372 $[M]^+$ (100)

A soln of **12** (0 066 g, 1 8 mmol) in CH_2Cl_2 (0 51 ml) and pyridine (0 21 ml) was added dropwise to a stirred soln of methanesulfonyl chloride (0 05 ml) and CH_2Cl_2 (0 51 ml) at 0° After 1 hr the mixture was poured into ice-H_2O, stirred for an additional 3 hr and then extracted with $CHCl_3$ (3 × 50 ml) The combined organic extracts were dried and evapd (0 044 g) NH_4OH was added to the aq phase until pH 9 and extracted once more The $CHCl_3$ extracts were dried (Na_2SO_4) and evapd, yielding an additional 0 066 g The crude residue (0 11 g) consisted of two mesylates, **13** and **14**, ^{1}H NMR δ 2 84 (*s*, –SO_2Me), 3 00 (*s*, –SO_2Me), 3 60 (*s*, –COOMe), 3 80 (*s*, –COOMe), 7 80 (*br d*)

To a soln of **13** and **14** (0 01 g), in CH_2Cl_2 (10 ml), was added diazobicyclo-[4 3 0]-non-5-ene (0 5 ml), the mixture stirred at room temp overnight, the CH_2Cl_2 evapd and the residue applied to a silica gel column and eluted with $CHCl_3$ to yield 0 0009 g of **15** [^{1}H NMR δ 0 82 (*t*, H-18), 3 70 (*s*, –COOMe), 5 62 (*s*, H-17), 6 14 (*s*, H-17), 6 7–7 2 (aromatic), 8 48 (*br s*, N–H), MS *m/z* (rel int) 354 $[M]^+$ (26), 339 (1), 337 (1), 325 (2), 323 (33), 209 (100), 159 (85), 146 (20), 144 (34), 130 (17), found high resolution MS $[M]^+$ *m/z* 354 97667, calc for $C_{21}H_{26}N_2O_3$ 354 194331] and 0 001 g of **16** [^{1}H NMR δ 0 86 (*t*, H-18), 3 70 (*s*, –COOMe), 5 42 (*s*, H-17), 6 14 (*s*, H-17), 6 80–7 20 (aromatic), 7 46 (*dd*, *J* = 7 and 2 Hz, H-9), 7 82 (*br s*, N_1-H), MS *m/z* (rel int) 354 $[M]^+$ (100), 339 (9), 337 (5), 325 (5), 323 (5), 209 (65), 159 (31), 146 (22), 144 (13), 130 (30)]

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