Bannucine – A New Dihydroindole Alkaloid from *Catharanthus roseus* (L) G.Don

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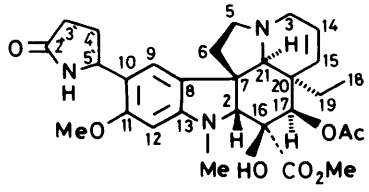
A new dihydroindole alkaloid, 'bannucine,' has been isolated from the leaves of *Catharanthus roseus* (L) G.Don, and has been found to have a five-membered lactam ring attached to the vindoline moiety.

Catharanthus roseus (L) G.Don (Apocynaceae) is widely distributed throughout Pakistan. It is used in the Unani system of medicine as a hypoglycaemic agent. The plant has been extensively studied during the last two decades, primarily on account of its antitumour properties. A large number of alkaloids have previously been reported from this plant of which vinblastine and vincristine are widely used in the chemotherapeutic management of a wide variety of human neoplasms.

We have previously reported several new alkaloids, from the leaves of *C. roseus*.[1–4] Our continuing studies on the plant have resulted in the isolation of a novel Aspidosperma alkaloid, which we call bannucine, to which structure (1) has been assigned.

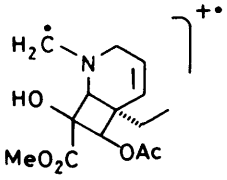


(1)

Results and Discussion

The compound afforded a dihydroindole u.v. spectrum, $\lambda_{max.}$ (MeOH) 238 and 280 nm, showing a slight bathochromic shift from the normal dihydroindole system.[5] The i.r. spectrum showed absorptions at 3 400 (NH), 3 200 (OH), 1 710 (ester C=O), and 1 690 cm^{-1} (amide C=O).

The mass spectrum proved to be highly instructive in determining the structure of bannucine. The molecular ion was observed at *m/z* 539, with other important peaks at *m/z* 479 (3%), 420 (2), 392 (4), 379 (18), 350 (9), 282 (16), 271 (30), 257 (10), 135 (100), 122 (33), 121 (36), and 107 (17). The overall fragmentation pattern was very similar to that of vindoline. High-resolution mass measurement on the molecular ion showed the exact mass to be *m/z* 539.2612, which was consistent with the formula $C_{29}H_{37}N_3O_7$ (539.2631), differing from vindoline by C_4H_5NO, *i.e.* 83 mass units (m.u.). Linked scan measurements on the molecular ion showed that the ions at *m/z* 479, 379, and 282 arise directly from it. The ion at *m/z* 479.2483 (calc. for $C_{27}H_{34}N_3O_5$: 479.2420) resulted from the loss of a methoxycarbonyl group from the molecular ion. An examination of the fragmentation pathway of the ion at *m/z* 479 by linked scan measurements showed that the following ions arise directly from it; *m/z* 420.2277 (calc. for $C_{25}H_{30}N_3O_3$: 420.2287, $M^+ - CH_3CO_2H - CO_2CH_3$), 379.2259 (calc. for $C_{23}H_{29}N_3O_2$: 379.2260, $M^+ - CO_2CH_3 - C_4H_5O_3$), and 271.1437 (calc. for $C_{16}H_{19}H_2O_2$: 271.1447). The fragment at *m/z* 379 was also seen to arise by loss of 160 m.u. from the ion at *m/z* 539. Linked scan measurements showed that the ion at *m/z* 379 fragments to daughter ions at *m/z* 271, 135, 122, and 107 which are characteristic fragments of vindoline and other related Aspidosperma alkaloids. This fragmentation is shown in the Scheme. Linked scan measurement of *m/z* 379 showed another daughter ion at *m/z* 257.1280 (calc. for $C_{15}H_{17}N_2O_2$: 257.1290, $M^+ - C_6H_8O_5 - C_8H_{12}N$). The fragment at *m/z* 271.1437 was also seen to be formed from the ion at *m/z* 479 by the loss of 209 m.u. ($M^+ - CO_2CH_3 - C_{11}H_{15}NO_3$). This further strengthened the suggestion that a substituent of 83 m.u. was attached to the dihydroindole ring system rather than to the remaining part of the vindoline structure. The formula of the fragment at *m/z* 271 ($C_{16}H_{19}N_2O_2$) was consistent with a unit comprising the indole ring system with a C-2 side-chain (*m/z* 188, $C_{12}H_{14}NO$) along with the C_4H_5NO (*m/z* 83) substituent. The molecular ion at *m/z* 539 showed another important fragment at *m/z* 282.1327 (calc. for $C_{14}H_{20}NO_5$: 282.1341). This agreed with structure (2), a common fragment formed from vindoline.[6] The fragment at *m/z* 84 arose by the loss of the vindolinyl moiety from bannucine. High-resolution mass measurement of the ion at *m/z* 84 afforded the exact mass as 84.0454 m.u. (calc. for C_4H_6NO: 84.0449).

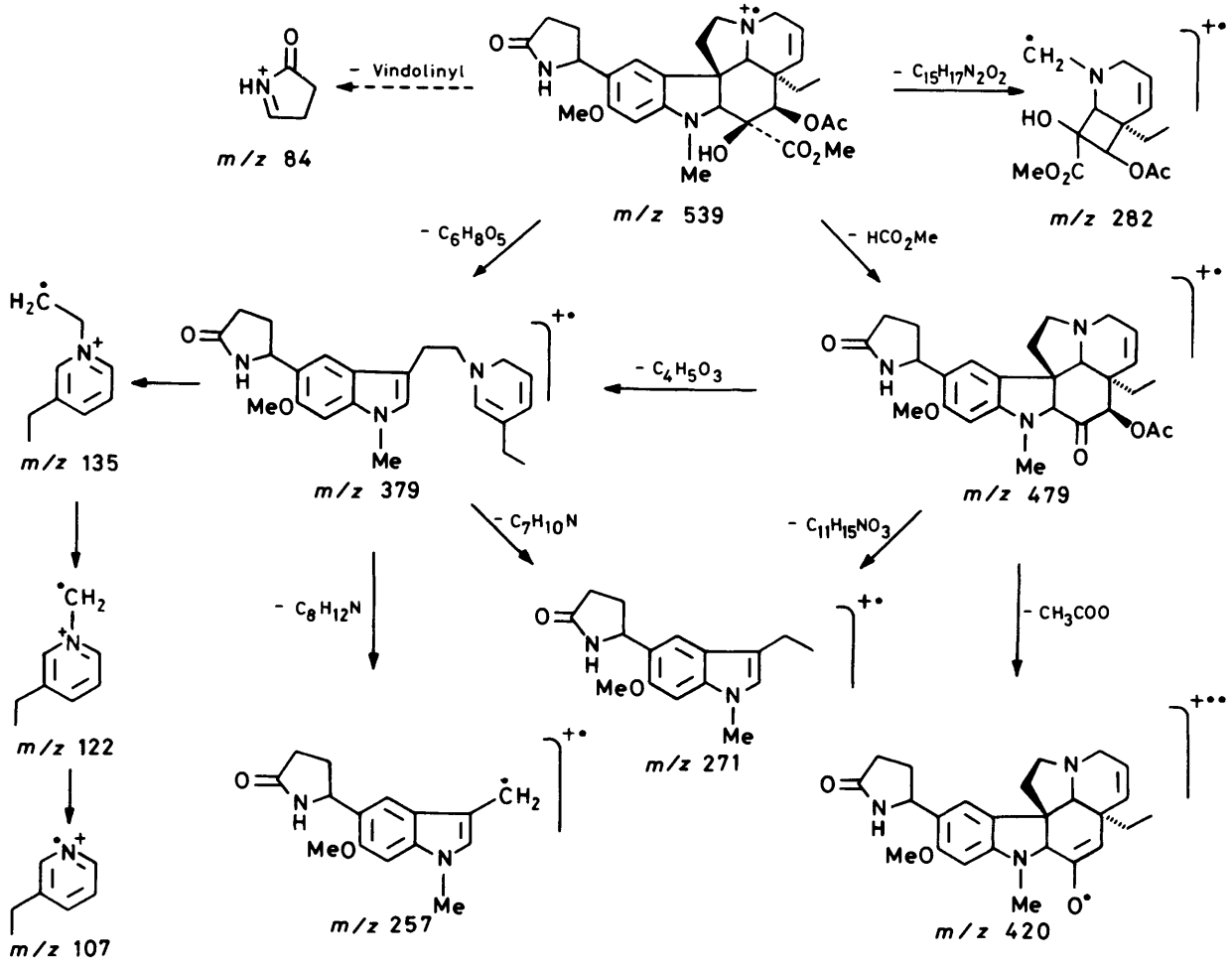


m/z 282

(2)

The i.r. spectrum of bannucine contained an intense absorption at 1 690 cm^{-1} which was consistent with the presence of a 5-membered lactam in the molecule. The mass fragmentation pattern suggested that the C_4H_6NO substituent was a 5-membered lactam attached to the benzene ring of vindoline. The u.v. spectrum indicated that this attachment was through one of the saturated carbons of the lactam ring rather than through the nitrogen of the C_4H_5NO moiety, as in the latter case a more intense bathochromic shift would have been expected in the u.v. spectrum of bannucine.

The ^1H n.m.r. spectrum ($CDCl_3$; 300 MHz) of bannucine bore a striking similarity to that of vindoline.[7] Two three-proton singlets at δ_H 3.83 and δ_H 2.07 were assigned to the methoxycarbonyl and acetoxy methyl groups respectively. The OCH_3 group on the aromatic ring resonated as a three-proton singlet at δ_H 3.79, while the N–CH_3 protons appeared as another three-proton singlet at δ_H 2.65. The methylene protons of the *C*-ethyl group appeared as two multiplets centred at δ_H 1.22 (19-H_α) and δ_H 1.59 (19-H_β) indicating their non-equiva-



Scheme.

lence due to restricted rotation on account of steric hindrance,* each proton exhibiting both geminal and vicinal couplings. The C-18 methyl protons appeared as a triplet at δ_H 0.44 ($J_{18.19\alpha}$ = $J_{18.19\beta}$ = 7.3 Hz). Irradiation of one of the methylene protons (19-H_α) at δ_H 1.22 resulted in the methyl triplet collapsing to a doublet and a corresponding simplification of the multiplet for the other methylene proton, 19-H_β at δ_H 1.59, to a perturbed quartet. Similarly irradiation of the C-19 β-proton at δ_H 1.59 resulted in the C-18 methyl group signal at δ_H 0.44 collapsing into a doublet, while the multiplet at δ_H 1.22 for the C-19 α-proton became a perturbed quartet. Irradiation of the methyl protons at δ_H 0.44 on the other hand resulted in both the C-19 methylene proton signals at δ_H 1.22 and 1.59 collapsing into doublets, each showing geminal coupling only ($J_{19\alpha.19\beta}$ 13.5 Hz).

A doublet at δ_H 5.20 ($J_{15.14}$ 10.31 Hz) was assigned to the olefinic proton at C-15. The other olefinic proton at C-14 resonated at δ_H 5.82 and was seen to be coupled with the C-3 α- and β-methylene protons ($J_{14.3\alpha}$ 3.51, $J_{14.3\beta}$ 3.5 Hz). The C-3 α-proton resonated as a multiplet at δ_H 2.27, while the C-3 β-proton appeared at δ_H 2.91 ($J_{3\alpha.3\beta}$ 16.0 Hz). Irradiation of the C-3 β-proton at δ_H 2.91 resulted in a collapse of the multiplet of the C-3 α-protons at δ_H 2.27 into a doublet. Similarly irradiation at δ_H 2.27 also resulted in the C-3 α-proton signal at δ_H 2.91 collapsing into a doublet, while the multiplet of the C-14 olefinic proton at δ_H 5.82 collapsed into a symmetrical quartet ($J_{15.14}$ 10.31, $J_{14.3\beta}$ 3.51 Hz). Irradiation of 14-H at δ_H 5.82 on the other hand led to the collapse of the multiplets at δ_H 2.91 and 2.27 into simple doublets showing only geminal coupling between the C-3 α- and β-proton. A multiplet at δ_H 2.84 was assigned to the C-5 β-proton, while the C-5 α-proton appeared at δ_H 1.81 as another multiplet. Irradiation of the 5-H_β at δ_H 2.84 resulted in the C-5 α-proton signal at δ_H 1.81 collapsing into a perturbed double doublet showing coupling with the C-6 α- and β-protons which resonated as multiplets at δ_H 2.33 and 2.55 respectively. Similarly irradiation at δ_H 1.81 (5-H_α) resulted in the multiplet of the C-5 β-proton collapsing to a double doublet, while the multiplets for the C-6 α- and β-proton also collapsed into double doublets. Irradiation at δ_H 2.55 (6-H_β) caused the multiplet at δ_H 2.33 (6-H_α) to collapse to a double doublet while the multiplets of 5-H_β and proton resonances at δ_H 2.84 and 1.81 collapsed into corresponding double doublets.

On the grounds of mass fragmentation and high-resolution mass measurements of fragment peaks, it had been concluded that the C_4H_5NO substituent could conceivably be attached to C-9, C-10, or C-12 of the pentacyclic ring system. Examination of the aromatic signal region of bunnucine showed that only two aromatic protons were present, at δ_H 6.08 and 6.90, each resonating as a sharp singlet. The positions of these resonances as well as the lack of *ortho* or *meta* coupling agreed with their

* The presence of two different groups of peaks due to the CH_2 group of the ethyl side-chain, each integrating for 1 H, has been observed previously (B. K. Hunter, L. D. Hall, and J. K. M. Sanders, *J. Chem. Soc., Perkin Trans. 1*, 1983, 657).

being assigned to the C-9 and C-12 protons respectively, indicating that the lactam substituent was attached to C-10. Attachment at C-9 would have resulted in some *meta* coupling between the C-10 and C-12 protons, which was not discernible. Substitution at C-10 is also reasonable on mechanistic grounds on account of the high electron density of this carbon resulting in the expected preference of vindoline molecule to undergo nucleophilic substitution at this position (*e.g.* biosynthesis of vinblastine, vincristine, leurosine *etc.*).[8]

The protons of the five-membered lactam ring substituent at C-10 were readily recognized in the ^{1}H n.m.r. spectrum. The C-5′ proton appeared as a one-proton multiplet centred at δ_H 5.20, the rather low field position of this proton being consistent with its location α to the nitrogen and the aromatic ring. Homo-decoupling at this point resulted in a collapse of the signals at δ_H 1.78 and 2.56, which were therefore assigned to the C-4′ α- and β-proton respectively. Irradiation at δ_H 2.56 resulted in a simplification of the signal at δ_H 1.78, collapse of the multiplet at δ_H 5.20, and collapse of the multiplets at δ_H 2.24 and 2.77, the latter being attributed to the C-3′ α- and β-proton respectively. Similarly irradiation at δ_H 2.24 resulted in the collapse of the multiplet at δ_H 2.77 of the C-3′ β-proton into a perturbed double doublet and a simplification of the multiplets at δ_H 2.56 and 1.78.

Table. ^{13}C N.m.r. spectrum of bannucine[a]

Carbon	Chemical shift	Carbon	Chemical shift
2	83.60	18	7.63
3	51.06[a]	19	30.88
5	51.62[b]	20	42.80
6	44.00	21	66.96
7	53.24	CO_2CH_3	170.60
8	124.01	CO_2CH_3	52.05
9	119.01	$OCOCH_3$	172.00
10	121.20	$OCOCH_3$	52.00
11	158.10	CONH	178.50
12	93.60	3′	29.82
13	153.21	4′	20.93
14	124.40	5′	55.20
15	130.40	NCH_3	38.50
16	79.70		
17	76.68		

[a] The multiplicities were confirmed by off-resonance and DEPT experiments. [b] These assignments are interchangeable.

spectrum results agreed with those from the ^{1}H–^{1}H decoupling experiments. The NOESY spectrum (Figure 1) established the relative stereochemistry of several key functionalities in

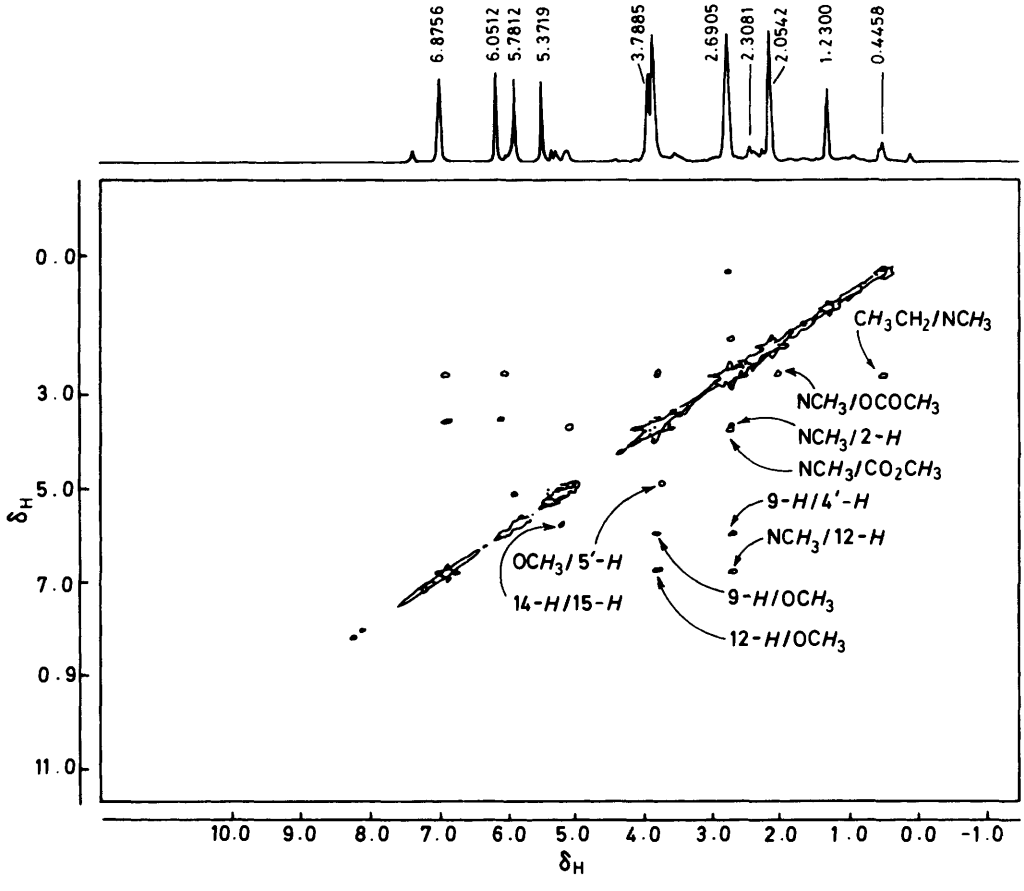


Figure 1. NOESY spectrum of bannucine

Two-dimensional n.m.r. measurements (2D *J*-resolved,[9] NOESY,[10,11] and COSY-45°)[12,13] fully agreed with the proposed structure (1) for bannucine. The multiplicity of the proton signals could be unambiguously established from the 2D*J*-resolved spectrum of bannucine, while the COSY-45° bannucine. Strong cross-peaks were observed corresponding to the nuclear Overhauser effect (n.O.e.) interactions between the *C*-ethyl and *N*-methyl groups. This supported the earlier observation that the ethyl group was sterically locked in a conformation which resulted in a differentiation betwen the two

methylene protons, and showed that the methyl of the ethyl group was situated in spatial proximity to the *N*-methyl group. The *N*-methyl signal also showed n.O.e. interaction with the ester methyl protons at δ_H 3.83 as well as with the methyl protons of the acetyl group at δ_H 2.07 and the C-2 proton at δ_H 3.71. This established that the *O*-acetyl and ester groups are orientated below the plane of molecule where they come close to the N–CH_3 group. The n.O.e. interaction between the C-12 aromatic proton at δ_H 6.90 and the OCH_3 group at δ_H 3.76 was consistent with the positioning of the OMe group at C-11. Similarly a strong n.O.e. interaction was seen between 12-H and the N–Me signal. The n.O.e. interaction between the C-5′ proton at δ_H 5.20 and the OCH_3 group established the β-orientation of 5′-H. The C-9 aromatic proton signal at δ_H 6.08 also showed n.O.e. cross-peaks with the C-4′ proton at δ_H 2.56. The olefinic protons at C-14 and C-15 showed n.O.e. interactions with each other. These results are consistent with the relative stereochemistry shown in Figure 2 for 'bannucine.'

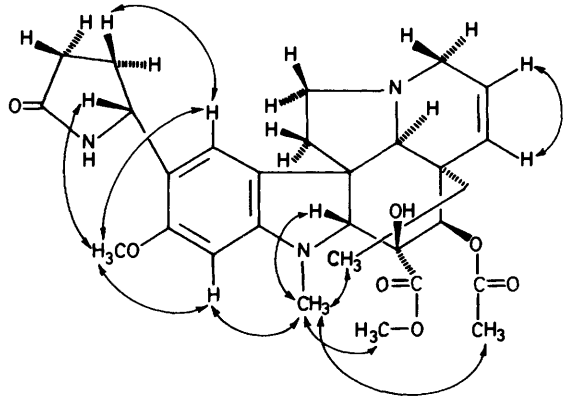


Figure 2. NOESY interactions in bannucine

The ^{13}C n.m.r. spectrum ($CDCl_3$) of 'bannucine' (broad band and DEPT)[14] showed interesting similarities to that reported for vindoline. The assignments of the ^{13}C resonance signals are shown in the Table. The ^{13}C resonances of most carbon atoms were close to those reported for vindoline itself or to corresponding carbons in those molecules in which vindoline is found bound to another alkaloidal moiety.[15–17] The C-10 carbon appeared at δ_C 121.20 and was shown by DEPT measurements to be non-proton-bearing, thus confirming that C-10 was the site of substitution. The presence of the C_4H_5NO substituent in the form of a 5-membered lactam ring was also substantiated by the ^{13}C n.m.r. spectrum. The lactam carbonyl carbon appeared at δ_C 178.5, while the C-3′ carbon afforded a signal at δ_C 29.85.[18] The secondary C-4′ carbon resonated at δ_C 20.93 while the C-5′ carbon appeared at δ_C 55.20. The multiplicity of each carbon was confirmed by DEPT spectra at different θ values (45, 90, and 135°).

A comparison of the chemical shift of the ^{13}C resonances of the asymmetric centres in the vindolinyl moiety of bannucine with corresponding resonances in vindoline showed there was a close correspondence between the two. Similarly the protonated asymmetric centres afforded very close chemical shifts to those found in vindoline in the ^1H n.m.r. spectrum. This suggests that the stereochemistry of the asymmetric centres in the vindolinyl moiety of bannucine is identical to that in vindoline.

In view of the above data, structure (**1**) is assigned to 'bannucine.' It is the first Aspidosperma alkaloid bearing a 5-membered lactam substituent.

Experimental

M.p.s were obtained on a Gallenkamp apparatus. ^1H N.m.r. spectra were run for $CDCl_3$ solutions on a Bruker WP-100 SY FT and Bruker AM 300 FT spectrometers, and ^{13}C n.m.r. spectra for $CDCl_3$ solutions on a Bruker AM 300 FT spectrometer, with $SiMe_4$ as internal reference. I.r. spectra were run on a Jasco-IR A-1 spectrophotometer for solutions in $CHCl_3$, and u.v. spectra on a Shimadzu U.V. 240 spectrophotometer for solutions in MeOH. Optical rotations were measured on a Schmidt and Haensch polartronic-D electronic polarimeter for solutions in $CHCl_3$. Mass spectra were obtained with Finnigan MAT 112 and Finnigan MAT 312 double focussing mass spectrometer.

Ethanolic extracts of the dried leaves (105 kg) of *Catharanthus roseus* (L) G.Don. were concentrated to afford a gum (183 g), which was dissolved in chloroform and the solution was extracted with pH-2 phosphate buffer solution (1 l). The pH of the aqueous layer was adjusted with aqueous ammonia to pH10 and the solution was extracted with chloroform (480 ml). The chloroform extracts were dried (anhydrous Na_2SO_4) and concentrated to 25% of their original volume. Light petroleum (b.p. 60—69 °C) (350 ml) was added to the chloroform solution, and this caused some of the alkaloids to precipitate out. These were filtered off and the filtrate was concentrated to afford a gum (46 g). The gummy alkaloidal material was subjected to flash chromatography over Al_2O_3 (1.5 kg), elution being carried out with increasing polarities of light petroleum, light petroleum–ethyl acetate, ethyl acetate, ethyl acetate–methanol, and methanol.

The fraction obtained on elution with 85% ethyl acetate–15% methanol was concentrated and separated on precoated t.l.c. plates [silica; acetone–light petroleum (3:2)] to afford a slower moving major band along with a number of faster moving minor alkaloids. The major band was repurified on alumina cards in ethyl acetate to afford the pure material (13 mg) as crystals, m.p. 152—154 °C, $[\alpha]_D$ −33° (*c* 0.26 in $CHCl_3$).

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