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ALKALOIDS OF *CRINUM LATIFOLIUM**

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Key Word Index—*Crinum latifolium*; Amaryllidaceae; flowering bulbs; alkaloids; lycorine; ambelline; pyrrolophenanthridone alkaloids; hippadine; pratorinine; pratorimine; pratosine.

Abstract—From the bulbs of *Crinum latifolium*, collected at flowering, two new pyrrolophenanthridone alkaloids, pratorimine and pratosine, have been isolated and characterized on the basis of comprehensive spectral analyses, chemical transformation and synthesis. Additionally, four known alkaloids, hippadine, pratorinine, ambelline and lycorine, encountered before in other Amaryllidaceous species, have now been isolated and characterized from *C. latifolium*.

INTRODUCTION

In connection with our work on the extractives of Amaryllidaceae [1–3], we investigated the alkaloidal constituents of *Crinum latifolium* (sub-family: Amaryllidoideae). The plant grows abundantly in the upper Gangetic Plain and is also cultivated as a garden flower. Extracts of different parts of this species are used in popular medicine for a variety of purposes, e.g. a rubefacient in rheumatism and piles, and for abcess treatment to promote suppuration. No previous phytochemical investigation has been reported on *C. latifolium*. The present paper describes the isolation and characterization of the alkaloidal constituents of the bulbs of this plant.

RESULTS AND DISCUSSION

Extensive column and thick layer chromatography of the crude alkaloid fractions from petrol and ethanol extracts of dried and powdered bulbs, collected at flowering, afforded lycorine [2], ambelline [2], hippadine [2], pratorinine [2] and two new pyrrolophenanthridone alkaloids which we named pratorimine and pratosine. Complete characterization of the two new alkaloids is described in the present paper.

Pratorimine (1)

This alkaloid, mp 263–265°, $C_{16}H_{11}NO_3$ (M^+ and elemental analyses), responded to the ferric test for phenols. It exhibited UV maxima, in methanol, closely similar to those of pratorinine (3) [2]. However, that this alkaloid was different from pratorinine was evident from analytical TLC, the former being less polar, and from ^1H NMR data (Table 1). Also, unlike that of 3, the UV A of pratorimine was significantly modified in the short-wave region, in the presence of sodium acetate, and strong bathochromic shifts of the UV maxima were observed in the presence of sodium methoxide. The IR spectrum of pratorimine exhibited significant bands assignable to a hydroxyl function, a substituted lactam and a strongly conjugated system. On treatment with ethereal diazomethane, pratorimine formed a monomethyl ether, mp 230–232°, $C_{17}H_{13}NO_3$, and on acetylation with acetic anhydride and pyridine, an *O*-acetyl derivative, mp 211–212°, $C_{18}H_{13}NO_4$. In locating the hydroxyl group at C-9 and the methoxyl at C-8 of the pyrrolophenanthridone ring, the ^1H NMR data of the alkaloid and its derivatives (see Table 1) were quite informative. Special mention may be made of the following points in support of the assignments: (1) the H-1 and H-10 resonances in pratorimine were assigned on the basis of the observed H-4 and H-5 resonances in phenanthrene derivatives [4] which are strongly deshielded; (2) a line broadening effect of H-1 and H-10 resonances was observed due to their long range coupling; (3) in the presence of NaOD–D_2O, the maximum upfield shift (δ 0.12) was exhibited by H-10 (the location of the hydroxyl group in ring A of cherylline was made on the basis of similar evidence [5]); (4) in *O*-acetylpratorimine, only the H-10 experienced an appreciable downfield shift (δ 0.37 from the corresponding hydrogen resonance in the parent compound and δ 0.25 from that of the permethyl ether); and (5) irradiation of the methoxyl signal of pratorimine and of *O*-acetylpratorimine caused, 22% and 18% area enhancements of the H-7 signal, respectively. Similar area enhancements were observed in polyoxygenated xanthones which formed a basis for determining the positions of their hydroxyl–methoxyl functions [6, 7]. Pratorimine and pratorinine (3) are, therefore, isomeric with respect to their hydroxyl–methoxyl groups. Hence, pratorimine was identified as 4,5-etheno-8-methoxy-9-hydroxy-6-phenanthridone (1).

Pratosine (2)

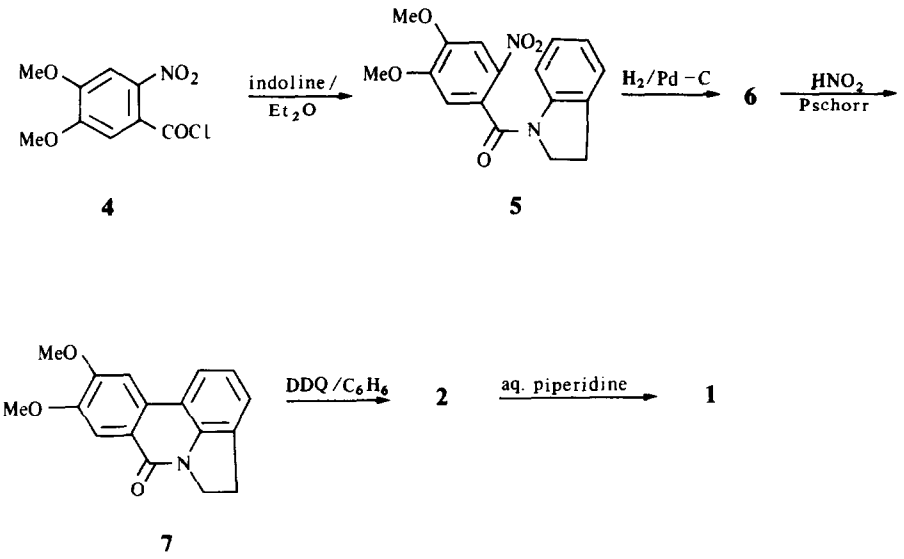
This alkaloid, mp 232–233°, $C_{17}H_{13}NO_3$ (M^+ and elemental analyses) showed physical and spectral pro-

*Part 3 in the series "Chemical Constituents of Amaryllidaceae". For Part 2 see ref. [1].

Table 1. ^{1}H NMR data of pyrrolophenanthridone alkaloids*

Alkaloid	Solvent	H-1	H-2	H-3	H-4	H-5	H-7	H-10	OMe	OH/OAc
1	DMSO-d_6	8.29 *dd*† ($J_{1,3}$ 1.0 Hz, $J_{1,2}$ 7.6 Hz)	7.52 *dd* ($J_{1,2}$ 7.6 Hz, $J_{2,3}$ 7.5 Hz)	7.82 *dd* ($J_{1,3}$ 1.0 Hz, $J_{2,3}$ 7.5 Hz)	7.06 *d* ($J_{4,5}$ 3.6 Hz)	8.09 *d* ($J_{4,5}$ 3.6 Hz)	7.75 *s*	7.92 *s*†	4.11	9.95‡
1	DMSO-d_6–D_2O–NaOD	8.23	7.52	7.82	7.05	8.08	7.69	7.80	4.11	—
1§	$CDCl_3$	7.99 *dd*	7.48 *dd*	7.77 *dd*	6.78 *d*	7.93 *d*	7.70 *s*	7.90 *s*	4.14	7.5–7.8‡‖
2	$CDCl_3$	8.04 *dd* ($J_{1,3}$ 1.0 Hz, $J_{1,2}$ 7.6 Hz)	7.54 *dd* ($J_{1,2} = J_{2,3}$ 7.6 Hz)	7.82 *dd* ($J_{1,3}$ 1.0 Hz, $J_{2,3}$ 7.6 Hz)	6.94 *d* ($J_{4,5}$ 3.7 Hz)	8.10 *d* ($J_{4,5}$ 3.7 Hz)	7.72 *s*	8.02 *s*	4.0, 4.1	—
3	$CDCl_3$	7.95 *dd* ($J_{1,3}$ 1.0 Hz, $J_{1,2}$ 7.6 Hz)	7.45 *dd* ($J_{1,2} = J_{2,3}$ 7.6 Hz)	7.73 *dd* ($J_{1,3}$ 1.0 Hz, $J_{2,3}$ 7.6 Hz)	6.90 *d* ($J_{4,5}$ 3.7 Hz)	8.06 *d* ($J_{4,5}$ 3.7 Hz)	7.67 *s*	8.10 *s*	4.11	—
8	$CDCl_3$	8.01 *dd* ($J_{1,3}$ 1.0 Hz, $J_{1,2}$ 7.6 Hz)	7.49 *dd* ($J_{1,2} = J_{2,3}$ 7.6 Hz)	7.78 *dd* ($J_{1,3}$ 1.0 Hz, $J_{2,3}$ 7.6 Hz)	6.91 *d* ($J_{4,5}$ 3.7 Hz)	8.07 *d* ($J_{4,5}$ 3.7 Hz)	7.75 *s*	8.27 *s*	4.07	2.40

*δ-Values (ppm) from TMS at zero. Decoupling experiments substantiated the assignments.
†Line broadening (long range coupling of H-1/H-10).
‡Exchangeable with D_2O.
§Data obtained from the spectrum of a mixture (1:2) of **1** and **8**; pure **1** was practically insoluble in $CDCl_3$.
‖Obscured by Ar-H signals.

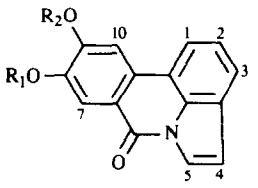


Scheme 1. Synthesis of pratorimine (**1**) and pratosine (**2**).

perties indistinguishable from those of *O*-methylpratorimine and was, therefore, assigned structure **2**.

Finally, chemical proof for the structures of **1** and **2** was obtained by their synthesis (Scheme 1). The synthesis of **7** from **4** was accomplished by a modified procedure of Humber *et al.* [8] used for the synthesis of anhydrolycorine. In the present synthesis, *N*-acylation of indoline with 2-nitro-4,5-dimethoxybenzoyl chloride (**4**) afforded 1-(2-nitro-4,5-dimethoxybenzoyl)-indoline (**5**). Catalytic reduction, in the presence of Pd–C, provided the aminoamide (**6**) which, on Pschorr cyclization, gave 4,5-ethano-8,9-dimethoxy-6-phenanthridone (**7**). Dehydrogenation of **7** with DDQ, in dry benzene, according to a published procedure [2], afforded pratosine (**2**). 9-*O*-Demethylation of pratosine with aqueous piperidine, according to a standard method [9], gave pratorimine (**1**). The yield of the Pschorr cyclization was only *ca* 10%, all other steps provided 70–80% yields. Pratorimine (**1**) and pratosine (**2**) have not seen encountered before naturally nor have they been synthesized.

The pyrrolophenanthridone alkaloids are native to *C. latifolium* and *C. pratense* Herb. [2, 3]. This was established by direct analytical TLC of the respective exudates of flowering bulbs. The concentrations of pratorimine (**1**), pratosine (**2**), pratorinine (**3**) and hippadine (**9**) were maximum during the pre- and post-flowering stages



1 R_1 = Me; R_2 = H
2 R_1 = R_2 = Me
3 R_1 = H; R_2 = Me
8 R_1 = Me; R_2 = Ac
9 R_1 + R_2 = —CH_2—

of *C. latifolium*, covering a period of *ca* 40 days. In the resting bulbs, these alkaloids were present only in traces. Similar observations were made with the alkaloids of *C. pratense* [2, 3]. It is also noteworthy that the flowers and flowering stems of both the species were devoid of any pyrrolophenanthridone alkaloids.

EXPERIMENTAL

All mps were taken on a Kofler block in open capillaries and are uncorr. UV spectra were recorded in MeOH; the UV shift reagents were prepared and used according to ref. [10]. IR spectra were recorded in KBr, unless otherwise stated, and only the major bands are quoted. EIMS were recorded at 70 eV. 90 MHz ^{1}H NMR spectra were determined using $CDCl_3$ and/or DMSO-d_6 as solvents. Separation by CC was carried out with Si gel (BDH, 60–120 mesh). Analytical TLC was conducted with Si gel G (plate 1) or with Sil G/UV_{254} (plate 2) and thick layer chromatography with Si gel G (layer thickness, 2 mm). Three solvent systems were used, C_6H_6–HOAc (50:1, solvent 1), $CHCl_3$–HOAc (50:1, solvent 2) and $CHCl_3$–MeOH (90:10, solvent 3). UV (254 μm), I_2 vapour, $FeCl_3$ and Dragendorff reagents were used for visualization.

Extraction. Dried and powdered bulbs of *C. latifolium* L. (105 g) were successively extracted in a Soxhlet with petrol (60–80°) (fraction A) and then with EtOH (fraction B) (40 hr each). The plant species, cultivated in Varanasi, was identified by Dr. S. K. Roy, Department of Botany, Faculty of Science, Banaras Hindu University. The bulbs were collected several times during the pre- and post-flowering stages (July–September, 1979–1980) and were separately extracted for chemical constituents. A typical experimental procedure with bulbs, collected at flowering, is described.

Fraction A. Concd (*ca* 200 ml) and kept overnight at room temp. when a light brown solid (fraction A_1, 80 mg) separated. The solid was collected and the petrol mother liquor evaporated to give a brown gummy material (fraction A_2, 0.57 g).

Separation of alkaloids from fraction A_1. The solid was triturated with Me_2CO. The Me_2CO-soluble fraction showed three major components on analytical TLC, R_f 0.2, 0.35 and 0.42 (plate 1, solvent 1). The solvent was evaporated and the residue

dissolved in C_6H_6. It was chromatographed on a Si gel column (20 × 1.5 cm). Elution was carried out with petrol (500 ml), petrol–C_6H_6 (1.2 l.), C_6H_6 (1.5 l.) and C_6H_6–$CHCl_3$ (1:1, 500 ml). Fractions (100 ml) were collected and monitored by TLC.

Hippadine (**9**). Fractions 12–17 were combined and concd when the alkaloid was obtained as colourless flakes (37 mg), mp 207–209°; mp remained undepressed on admixture with a reference sample of hippadine [2, 3], mp 209–210°; their co-TLC behaviour, R_f 0.42 (plate 1, solvent 1) and ^1H NMR spectra were also identical.

Pratosine (**2**). Fractions 21–25 afforded a solid which crystallized from Et_2O–MeOH as colourless fine needles (4 mg), mp 232–233°; R_f 0.35 (plate 1, solvent 1); UV λ_{max} nm (log ε): 228 (4.18), 237 (4.20), 248 (4.39), 254 (4.12), 305 sh (3.99), 335 (3.88), 350 (3.72); IR ν_{max} cm^{-1}: 1675, 1622, 1612, 1595; MS: *m/z* 279 $[M]^+$ (100%), 264 (18), 250 (7), 249 (12), 236 (14), 221 (9), 208 (5), 193 (4); (*m/z* 279 → 264 transition, *m** 250; 264 → 236 transition, *m** 211). (Found: C, 73.00; H, 4.44; N, 4.91. $C_{17}H_{13}NO_3$ requires: C, 73.11, H, 4.65; N, 5.01).

Pratorinine (**3**). Fractions 27–33, which were $FeCl_3$ positive, were combined and evaporated. The residue crystallized from $CHCl_3$–EtOH as light brown microcrystals (5 mg), mp 266–267°; mp remained undepressed on admixture with an authentic sample of pratorinine [2, 3], mp 265–267°; their co-TLC behaviour R_f 0.22 (plate 1, solvent 1), 0.45 (plate 1, solvent 2) and IR spectra were also identical.

Separation of alkaloids from fraction A_2. A portion (0.25 g) was dissolved in C_6H_6 and chromatographed on a Si gel column (24 × 2 cm). Elution was carried out with petrol (2 l.), C_6H_6 (3 l.) and C_6H_6–$CHCl_3$ (1:1, 500 ml). Fractions (100 ml) were collected and monitored by TLC. The middle C_6H_6 eluates afforded a further quantity of hippadine (11 mg), while the later C_6H_6 eluates gave additional pratosine (3 mg).

Pratorimine (**1**). The C_6H_6–$CHCl_3$ eluates were combined and concd when pratorimine was obtained as a light brown amorphous solid (19 mg). It crystallized from $CHCl_3$–EtOH as light brown microcrystals (14 mg); R_f 0.27 (plate 1, solvent 1), 0.50 (plate 1, solvent 2); UV λ_{max} nm (log ε): 226 (4.18), 235 (4.21), 248 (4.37), 255 (4.34), 285 (3.87), 295 (4.12), 337 (3.26), 348 (3.59), 362 (3.51); $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 226 (4.60), 236 (4.51), 250 (4.79), 256 (4.71), 285 (4.11), 295 (4.43), 337 (3.55), 348 (3.66), 365 (3.59); $\lambda_{max}^{MeOH-NaOMe}$ nm: 222, 236, 245 sh, 274, 292, 304, 395; IR ν_{max} cm^{-1}: 3400 (broad, OH), 1672 (>NCO), 1620 (>C=C<), 1040 (OH); MS: *m/z* 265 $[M]^+$ (100%), 250 (28), 236 (6), 222 (30), 194 (5) (*m/z* 265 → 250 transition, *m** 236; 250 → 222 transition, *m** 197.5; 222 → 194 transition, *m** 170.) (Found: C, 72.12; H, 4.30; N, 5.08. $C_{16}H_{11}NO_3$ requires: C, 72.45; H, 4.15; N, 5.19.)

O-*Methylpratorimine* (= *pratosine* **2**). Pratorimine (7 mg) was dissolved in MeOH (5 ml) and treated with a large excess of Et_2O–CH_2N_2. After 24 hr, the soln was evaporated and the residue crystallized from Et_2O–MeOH as colourless crystals (5 mg), mp 232–234°; mp remained undepressed on admixture with pratosine; their co-TLC behaviour, in the two solvent systems, was also identical.

O-*Acetylpratorimine* (**8**). Pratorimine (12 mg) was dissolved in Ac_2O (1 ml) and pyridine (0.2 ml). After 72 hr, at room temp., the soln was evaporated to dryness *in vacuo*. The buff coloured solid was triturated with H_2O and extracted with $CHCl_3$. The solvent was removed from the organic layer and the residue crystallized from Me_2CO as flakes (9 mg), mp 211–212°; IR ν_{max}^{Nujol} cm^{-1}: 1762, 1668, 1620, 1595, 1228, MS: *m/z* 307 $[M]^+$ (44%), 265 (100), 264 (5), 250 (7), 249 (4), 237 (3), 222 (4).

Treatment of fraction B. This fraction was evaporated under red. pres. and the residue treated with aq. HOAc (4%, 100 ml). The HOAc soln was filtered and the residue collected (fraction B_1, 1.8 g). The aq. acidic soln was then extracted with Et_2O to obtain Et_2O-soluble alkaloid acetates (fraction B_2, 0.72 g) and then basified (NH_4OH). The liberated bases were successively extracted with Et_2O (fraction B_3), EtOAc (fraction B_4) and *n*-BuOH (fraction B_5). Only fraction B_3 was processed at this stage.

Separation of alkaloids from fraction B_3. During processing of this fraction, in the usual fashion, a solid separated at the interphase which was collected by filtration.

Lycorine. The solid crystallized from EtOH as colourless needles (0.26 g), mp 252–254°; R_f 0.3 (plate 2, solvent 3); $[\alpha]_D^{28}$ −81° (EtOH; *c* 1.2); MS: *m/z* 287 $[M]^+$ (70%), 286 (24), 268 (20), 227 (100), 226 (100), 211 (4), 147 (5); mp remained underpressed on admixture with an authentic sample of lycorine [2, 3], mp 257–258°; their co-TLC behaviour, in the three solvent systems, was also identical.

The solvent was removed from the Et_2O extract, after separation of lycorine, and the residue was triturated with hot petrol, C_6H_6 and Me_2CO in succession.

Ambelline. The Me_2CO-soluble fraction was concd and kept at room temp. overnight when a colourless solid separated (42 mg). It showed two major Dragendorff-positive spots on analytical TLC, R_f 0.52 and 0.45 (plate 1, solvent 3); R_f 0.48 and 0.42 (plate 2, solvent 3). The solid was dissolved in a small vol. of $CHCl_3$–MeOH (99:1) and the soln passed through a small column of Si gel G. The same solvent mixture was used for elution and each fraction (5 ml) was monitored by TLC. The residue from the enriched fractions of the more polar entity on crystallization from $CHCl_3$–MeOH afforded ambelline as shining needles (18 mg), mp 255–258°; $[\alpha]_D^{28}$ + 35.7° (MeOH; *c* 0.55); MS: *m/z* 331 $[M]^+$ (100%), 316 (10), 300 (35), 299 (21), 287 (25), 286 (27), 270 (14), 260 (18), 241 (16), 211 (12); mp remained undepressed on admixture with a reference sample of ambelline [2, 3], mp 255–257°; their co-TLC behaviour was also identical.

The less polar entity could not be separated from ambelline by the above method and was kept aside for further processing at a later date.

Synthesis of pratorimine (**1**). 1-(2-*Nitro*-4,5-*dimethoxybenzoyl*)-*indoline* (**5**). 2-Nitro-4,5-dimethoxybenzoyl chloride (**4**, 0.275 g, prepared from the corresponding benzoic acid and oxalyl chloride in the usual fashion [11]), in dry Et_2O (15 ml), was added with continuous stirring to a soln of dihydroindole (0.51 g), in dry Et_2O (50 ml). The cream coloured ppt was collected and crystallized from Et_2O–MeOH as a pale yellow solid (0.78 g), mp 187–189°; R_f 0.57 (plate 1, solvent 1); UV λ_{max} nm: 235, 285; IR ν_{max} cm^{-1}: 1695, 1678, 1660, 1642, 1570, 1090, 1000, 945; ^1H NMR ($CDCl_3$): δ 8.4 (1H, *m*, Ar-H), 7.5–7.1 (5H, *m*, Ar-H), 4.0 (3H, *s*, OMe), 3.98 (3H, *s*, OMe), 4.4–3.2 (4H, *m*, $-CH_2-$); MS *m/z* 328 $[M]^+$ (34%), 210 (100), 195 (7), 180 (11), 136 (14), 118 (48), 117 (38). (Found: C, 61.88; H, 4.60; N, 8.35. $C_{17}H_{16}N_2O_5$ requires: C, 62.2; H, 4.8; N, 8.5%.)

1-(2-*Amino*-4,5-*dimethyoxybenzoyl*)-*indoline* (**6**). Reduction of the nitroamide (**5**) (0.85 g), with H_2, in hot EtOH (10 ml), in the presence of Pd–C (10%, 0.55 g), for 4 hr, followed by the usual work-up afforded the aminoamide (**6**) as a gummy material. It crystallized from petrol–EtOAc as cream coloured flakes (79 mg), mp 127–129°; MS: *m/z* 298 $[M]^+$ (44%), 180 (100).

Ethanophenanthridone (**7**). To a cooled soln (0–5°) of **6** (72 mg), in a mixture of HOAc (5 ml) and H_2SO_4 (10%, 0.5 ml), an aq. soln of $NaNO_2$ (24 mg in 1.5 ml H_2O) added dropwise. After the addition was completed (starch–iodide paper), the mixture was allowed to come to room temp. and then heated at 100° for 4 hr. The solvent was removed *in vacuo*, the residue taken-up in H_2O (10 ml) and extracted with $CHCl_3$. The $CHCl_3$ layer was worked-up in the usual fashion when a basic gummy material was obtained. This was subjected to prep. TLC using solvent 3. The R_f 0.4 zone on elution with $CHCl_3$ and evaporation of the solvent

gave the ethanophenanthridone (7) as off-white crystals (7 mg), mp 229–232°; UV λ_{max} nm: 222, 245, 278, 282, 335; MS: *m/z* 281 $[M]^+$ (100%), 266 (14), 252 (7), 251 (11), 238 (5). (Found: N, 9.78. $C_{17}H_{15}NO_3$ requires: N, 9.96%.)

Dehydrogenation of 7 *with DDQ*. The ethanophenanthridone (7) (5 mg) was dissolved in dry C_6H_6 (25 ml) and refluxed (10 hr) with DDQ (15 mg). The solvent was evaporated, the residue dissolved in $CHCl_3$–MeOH (1:1) and subjected to prep. TLC using solvent 2. The Dragendorff-positive middle zone afforded pratosine as a colourless solid (3.5 mg), mp 228–230°. The synthetic compound was identical with the naturally occurring alkaloid in all respects (mp, mmp, co-TLC, UV, MS).

Conversion of pratosine into pratorimine. Pratosine (25 mg) in H_2O (1 ml) and piperidine (1 ml) was refluxed (15 hr). The solvent was removed *in vacuo*, the residue dissolved in $CHCl_3$ (5 ml) and chromatographed over a column of Si gel (12 × 1 cm). C_6H_6, C_6H_6–$CHCl_3$ (1:1) and $CHCl_3$–MeOH (99:1) were used as eluents. The early C_6H_6–$CHCl_3$ eluates afforded unreacted pratosine (6 mg). The later C_6H_6–$CHCl_3$ eluates and the early $CHCl_3$–MeOH fractions afforded pratorimine as light brown microcrystals (11 mg), mp and mmp 263–265°; the co-TLC behaviour of the synthetic and naturally occurring compounds and their UV and IR spectra were also identical. The later $CHCl_3$–MeOH washings gave a brown polymeric compound (5 mg).

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