Phytochemistry, Vol. 20, No. 8, pp. 2003–2007, 1981.
Printed in Great Britain.

0031–9422/81/082003–05 $02.00/0

ALKALOIDS OF *CRINUM PRATENSE**

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(*Received* 24 *October* 1980)

Key Word Index—*Crinum pratense*; Amaryllidaceae; bulbs; alkaloids; lycorine; 1,2-diacetyllycorine; ambelline; narcissidine; phenanthridone alkaloids; hippadine; pratorinine; anhydrolycorin-7-one.

Abstract—From the bulbs of *Crinum pratense*, collected at flowering, lycorine, 1,2-diacetyllycorine, ambelline, narcissidine, and three phenanthridone alkaloids, viz. hippadine, pratorinine and anhydrolycorin-7-one, were isolated and characterized on the basis of comprehensive spectral analyses (UV, IR, ^{1}H NMR, ^{13}C NMR, MS, $[\alpha]_D$) and chemical evidence. Among the phenanthridone alkaloids (**1**–**3**), only the natural occurrence of hippadine was previously known. Pratorinine is a new phenanthridone alkaloid and anhydrolycorin-7-one was known before only as a synthetic compound. The physiological significance of hippadine is appraised.

INTRODUCTION

Crinum pratense (syn. *C. longifolium*) is cultivated in the upper Gangetic plains in India as a garden flower. An extract of its bulbs is used in popular medicine as a bitter tonic, a laxative and in chest ailments. The species was previously reported [1] to contain only lycorine, the widespread alkaloid of family Amaryllidaceae. We now wish to report the isolation and characterization of seven alkaloids from the bulbs of the title species, collected at flowering.

RESULTS AND DISCUSSION

Extensive column and prep. TLC of the crude alkaloid fractions from petrol and EtOH extracts of dried and powdered bulbs afforded lycorine, 1,2-diacetyllycorine, ambelline, narcissidine, and three phenanthridone alkaloids, viz. hippadine, pratorinine and anhydrolycorin-7-one. Complete characterization of hippadine and pratorinine only is described here.

Hippadine

This alkaloid was previously reported from *Hippeastrum vittatum* [2] and *Crinum bulbispermum* [3] but remained uncharacterized until this investigation. It was obtained from *Crinum pratense* in appreciable yield (*ca* 0.05%), when the bulbs were collected at flowering time. The identity of the latter compound with hippadine was established by direct comparison. Hippadine, mp 209–210° (from *Crinum pratense*) (lit. [2, 3] mp 213–215°), $C_{16}H_9NO_3$ (by combustion analyses and MS), was assigned structure **1** on the basis of comprehensive spectral analyses and chemical evidence. Detailed interpretation of the ^{1}H NMR spectrum of hippadine was provided (Table 1). The ^{13}C NMR spectrum of hippadine is reported for the first time (Table 2). Chemical proof in favour of structure **1** for hippadine was provided by two crucial chemical transformations. Reduction of hippadine by $LiAlH_4$ in ether–tetrahydrofuran afforded anhydrolycorine. Dehydrogenation, on the other hand, of a synthetic sample of anhydrolycorin-7-one by DDQ, in anhydrous benzene under reflux, afforded hippadine in 75% yield.

Takagi and Yamaki some years ago reported [4] a phenanthridone alkaloid, 'N-3', mp 235°, from *Lycoris sanguinea*, to which they assigned structure **1** on the basis of UV, IR, ^{1}H NMR data and limited chemical evidence. Although the properties of 'N-3' [4] are comparable to those reported here for hippadine, there are certain differences, e.g. in the mp and UV data of the two (see Experimental). Direct comparison was not possible due to non-availability of 'N-3'.

Pratorinine

This alkaloid, mp 265–267°, $C_{16}H_{11}NO_3$, exhibited UV maxima closely similar to those of hippadine. In the ^{1}H NMR spectrum (Table 1), one OH and one OMe function appeared in lieu of the methylenedioxy group of hippadine. The chemical shift values of the aromatic H-8 and H-11, and the maximum upfield shift (*ca* 0.4 ppm) experienced by the former proton in the presence of NaOD–D_2O, located the OH at C-9 and therefore the OMe must be at the C-10 position. Hence pratorinine is assigned structure **2**. Chemical proof in favour of this structure was obtained by opening of the methylenedioxy ring of hippadine into OH–OMe groups by heating with NaOMe in DMSO. Similar opening of the methylenedioxy ring is known in acridone [5] and in piperonal and nitro derivatives [6]. Hippadine on heating with NaOMe in DMSO, at 150°, afforded pratorinine in 12% yield. A concomitant opening of the lactam ring of hippadine was expected and realized leading to indolopiperonylic acid (**4**). Pratorinine (**2**) has not been encountered before in nature nor has it been prepared synthetically.

*Part I in the series: "Chemical Constituents of Amaryllidaceae". Presented at the IVth Asian Symposium on Medicinal Plants and Spices, Mahidol University, Bangkok, Thailand. 15–19 September, 1980.

Table 1. ^{1}H NMR Data* of Hippadine (**1**) and Pratorinine (**2**)

Alkaloid	Protons†							
	H_A	H_B	H_C	H_D	H_E	H_F	H_G	OCH_2O/OMe
1	6.88 *d* (*J* 3.66)	8.03 *d* (*J* 3.66)‡	7.61 *s*	7.95 *s*	7.33 *dd* (J_{EF} 1.0, J_{EG} 7.6)	7.87 *dd* (J_{FG} 7.6, J_{EF} 1.0)	7.44 *t* (J_{EG} 7.6, J_{FG} 7.55)	6.15 *s* (2H)
2	6.90 *d* (*J* 3.70)	8.06 *d* (*J* 3.70)	7.67 *s*	8.1 *s*	7.73 *dd* (J_{EG} 7.6, J_{EF} 1.0)	7.95 *dd* (J_{FG} 7.6, J_{EF} 1.0)	7.45 *dd* ($J_{EG} = J_{FG}$ 7.6)	4.11 *s* (3H)

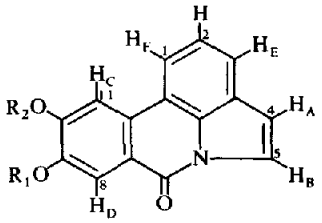
*In $CDCl_3$, ppm from TMS at zero.
†Decoupling experiments substantiated the assignments.
‡Line broadening.

Table 2. ^{13}C NMR Data of Hippadine (1)

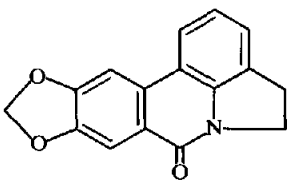
Carbon assignment	δ_C	Multiplicity	J(Hz)
C-11	101.55	*d*	164.5
OCH_2O	102.11	*t*	174.7
C-8	107.84	*d*	168.8
C-4	110.59	*ddd*	175.8, 8.1, 2.3
C-11a	116.56	*s*	
C-1	118.20	*dd*	159.3, 7.5
C-11a	119.58	*s*	
C-3	122.42	*dd*	162.3, 8.3
C-5	123.34	*dd*	194.6, 7.7
C-2	123.82	*d*	161.0
C-3a	128.28	*s*	
C-11c	130.85	*s*	
C-7a	131.51	*sd*	4.4
C-9	148.37	*sdd*	2.1, 2.4
C-10	152.45	*sd*	1.8
C-7	157.95	*s*	

The concentration of hippadine in *C. pratense* was maximal during pre- and post-flowering periods covering a span of *ca* 40 days. Subsequently, a rapid catabolism of hippadine was observed. In the resting bulbs hippadine was present only in traces. That hippadine is not an artefact is evident from the fact that anhydrolycorin-7-one, which could be formed from lycorine by ready dehydration followed by oxidation at C-6, remained unaffected even on long exposure to air. Pratorinine (2) and anhydrolycorin-7-one (3) were minor alkaloids of *C. pratense* and were only obtained during flowering.

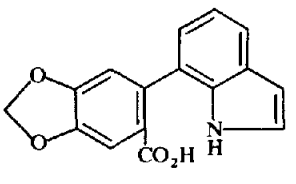
Physiological actions of hippadine in laboratory animals have been studied [7]. Hippadine-treated albino rats (3 mg/rat/day, in saline, ip, for 3 days) produced the following physiological changes. The DNA content of testis was depleted; loss of tissue wt of testis was observed; steroidogenic cells were active and wt of ventral prostate was increased which suggested growing hormonal activity in the animals; protein concentration of testis was marginally but consistently increased suggesting a continued cellular activity. Hippadine did not produce any anti-mitotic activity. The above observations suggest that hippadine exerts its effects at the genetic level and may prove to be a useful agent in fertility control.



1 $R_1 + R_2 = -CH_2-$
2 $R_1 = H$, $R_2 = Me$



3



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EXPERIMENTAL

All mps were taken on a Kofler block in open capillaries and are uncorr. UV spectra were recorded in MeOH. IR spectra were recorded in KBr, unless otherwise stated, and only the major bands are quoted. MS were determined at 70 eV. Separation by column chromatography was carried out with Si gel (BDH, 60–120 mesh) and TLC with Si gel G (E. Merck, Darmstadt). Three solvent systems, viz. C_6H_6–HOAc (50:1, solvent 1), $CHCl_3$–HOAc (50:1, solvent 2) and $CHCl_3$–MeOH–HOAc (10:1:1, solvent 3), were used as developers. I_2, $FeCl_3$, Dragendorff and Ehrlich reagents were used for visualization.

Extraction. Dried and powdered bulbs of *C. pratense* Herb.* (1.1 kg) were successively extracted in a Soxhlet with petrol (60–80°) (fraction A) and then with EtOH (fraction B) (40 hr, each). The two extracts (fractions A and B) were separately processed.

Treatment of Fraction A. The petrol extract was concd (*ca* 200 ml) and kept overnight at room temp., when a light brown solid (fraction A_1, 0.51 g) separated. This was collected by filtration and the petrol mother liquor evapd to give a brown gummy material (fraction A_2, 3.8 g).

Separation of alkaloids from fraction A_1. The solid showed 3 major blue fluorescent spots, under short-wave UV light, R_f 0.18, 0.3, 0.4 (solvent 1). A portion of the solid (255 mg) was dissolved in C_6H_6 and chromatographed over a Si gel column (20 × 2 cm). Elution was carried out with petrol (300 ml), petrol–C_6H_6 (1:1, 1.5 l), C_6H_6 (1.7 l), C_6H_6–$CHCl_3$ (1:1, 500 ml). Fractions (100 ml) were collected and monitored by analytical TLC.

Hippadine (1). Fractions 17–22 were combined and concd when the alkaloid was obtained as colourless flakes (180 mg), mp 209–210°; mp remained undepressed on admixture with a ref. sample of hippadine, mp 213–215°, their ^1H NMR data were also identical (see Table 1); R_f 0.38 (solvent 1); UV λ_{max} nm (log ε): 230 (4.25), 238 (4.29), 248 (4.42), 255 (4.26), 300 (4.21), 308 sh (4.03), 335 (3.97), 350 (3.99), 368 (3.87)†; IR ν_{max} cm^{-1}: 1675 (>NCO), 1620 (>C=C<), 1035, 938 (OCH_2O); MS *m/z*: 263 (M^+, 100%), 235 (3), 234 (4), 207 (6), 206 (5), 205 (8), 178 (10), 177 (30), 150 (2), 133 (14), 132 (17), 131.5 (M^{2+}). (Found: C, 72.83; H, 3.40; N, 5.19. $C_{16}H_9NO_3$ requires: C, 73.00; H, 3.45; N, 5.32).

Anhydrolycorin-7-one (3). Fractions 25–27 were combined, concd and subjected to TLC (solvent 1). TLC scrapings of the R_f zone 0.3 were eluted with $CHCl_3$. The solvent was removed from the $CHCl_3$ soln and the residue crystallized from C_6H_6–EtOH as microcrystals (5 mg), mp 228–230°; the physical and spectral

*The plant species, cultivated in Varanasi, was identified by Dr. S. K. Roy, Department of Botany, Faculty of Science, Banaras Hindu University, Varanasi-5, India. The bulbs were collected during flowering time (September 1976).

†('N-3'), mp 235°, was reported to exhibit UV λ_{max} nm (log ε): 247 (4.22), 295 (4.14), 330 (3.85), 340 (3.85), 360 (3.77) [4].

(UV, IR, MS) properties observed for this compound were consistent with those reported for anhydrolycorin-7-one in the lit. [8, 9]. Direct comparison (mp, mmp, co-TLC, UV) with a synthetic sample of anhydrolycorin-7-one showed that the two compounds were identical.

Pratorinine (**2**). Fractions 33–38 were combined and evapd. The residue crystallized from $CHCl_3$–EtOH as flakes (16 mg), mp 265–267°; R_f 0.18 (solvent 1), 0.45 (solvent 2); UV λ_{max} nm (log ε) 230 (4.08), 236 (4.02), 252 (4.37), 260 (4.28), 296 (3.99), 350 (3.55), 362 (3.02); IR ν_{max} cm^{-1}: 1672 (>NCO), 1622 (>C=C<), 3450 (OH); MS: *m/z* 265 (M^+, 100%), 250 (15), 236 (4), 222 (30), 206 (3), 194 (3). (Found: C, 72.02; H, 4.33; N, 5.01. $C_{16}H_{11}NO_3$ requires: C, 72.45; H, 4.15; N, 5.19).

Separation of alkaloids from fraction A_2. A portion of this fraction (0.35 g) was dissolved in C_6H_6 and chromatographed on a Si gel column (24 × 2 cm). Elution was carried out with petrol, C_6H_6, $CHCl_3$ and different proportions of mixtures thereof. Fractions were collected and monitored by analytical TLC.

1,2-Diacetyllycorine. The early C_6H_6 eluates afforded a solid which crystallized from Et_2O–MeOH as colourless platelets (14 mg), mp 212–213°; R_f 0.65 (solvent 2). The physical and spectral (IR, ^1H NMR, MS) properties observed for this compound were consistent with those reported for 1,2-diacetyllycorine in the lit. [10–12]. Direct comparison (mp, mmp, co-TLC, IR) with an authentic sample of 1,2-diacetyllycorine, prepared from lycorine, established that the two compounds were identical.

The later C_6H_6 and $CHCl_3$ eluates afforded further crops of hippadine (22 mg) and pratorinine (3 mg), respectively.

Treatment of fraction B. The EtOH extract was evapd under red. pres. and the residue treated with aq. HOAc (4%, 200 ml). The HOAc soln was filtered and the residue collected (fraction B_1). The aq. acidic soln was then extracted with Et_2O to obtain Et_2O-soluble alkaloid acetates (fraction B_2) 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. The solvent was evapd and the residue 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 (9 mg). It showed two Dragendorff-positive spots, R_f 0.4 and 0.5 (solvent 3). Crystallization of the mixture from EtOH afforded ambelline as shining needles (7 mg), mp 258–260°; R_f 0.5 (solvent 3); $[\alpha]_D^{28}$ +30.8° ($CHCl_3$, *c* 0.41); (Found: C, 65.17; H, 6.08; N, 4.11. (Calc. for $C_{18}H_{21}NO_5$: C, 65.24; H, 6.39; N, 4.23.); ambelline methiodide crystallized from EtOH as colourless needles, mp 295–297° (decomp.). The physical and spectral properties (UV, IR, MS, $[\alpha]_D$) observed for this compound were consistent with those reported for ambelline in the lit. [13, 14].

Lycorine. The Me_2CO-insoluble solid (from fraction B_3) was boiled with $CHCl_3$. From the $CHCl_3$-insoluble fraction lycorine was obtained as colourless crystals (224 mg) which was further crystallized from EtOH as needles, mp 257–258°: R_f 0.32 (solvent 3); $[\alpha]_D^{28}$ −78.5° (EtOH, *c* 0.82). (Found: C, 66.44; H, 6.16; N, 4.55. Calc. for $C_{16}H_{17}NO_4$: C, 66.89; H, 5.92; N, 4.87.) The physical and spectral (UV, IR, ^1H NMR and ^{13}C NMR, MS, $[\alpha]_D$) properties observed for this compound were consistent with those reported for lycorine in the lit. [10–12, 15]. It may be mentioned that lycorine was previously reported [1] from this species.

Narcissidine. The solvent was evapd from the above $CHCl_3$-soluble fraction. The residue crystallized from Me_2CO–MeOH as colourless prisms (6 mg), mp 203–205°, 216–218° (in an evacuated capillary); $[\alpha]_D^{28}$ −28.3° ($CHCl_3$, *c* 0.32); (Found: C, 64.44; H, 7.01; N, 4.11. Calc. for $C_{18}H_{23}NO_5$: C, 64.85; H, 6.95; N, 4.20.) The physical and spectral (UV, IR, MS, $[\alpha]_D$) properties observed for this compound were consistent with those reported for narcissidine in the lit. [11, 16].

$LiAlH_4$ *reduction of hippadine.* Hippadine (112 mg) was dissolved in a mixture of dry Et_2O and THF (1:1, 50 ml) and refluxed (14 hr) with $LiAlH_4$ (0.2 g). The product was worked up in the usual fashion. The solid was dissolved in EtOAc (20 ml) and passed through a short column of Si gel. C_6H_6 eluates provided a solid which crystallized from EtOH as colourless needles (21 mg), mp 111–112°; R_f 0.34 (solvent 2). (Found: C, 76.14; H, 5.31; N, 5.42. Calc. for $C_{16}H_{13}NO_2$: C, 76.49; H, 5.16; N, 5.56.) The mp, mmp, R_f, UV and MS spectra of the compound were identical with those of a synthetic sample of anhydrolycorine prepared according to a published procedure [8].

Synthesis of hippadine (**1**). Anhydrolycorin-7-one (26 mg) was dissolved in dry C_6H_6 (50 ml) and refluxed (10 hr) with DDQ (52 mg). The solvent was evapd and the residue dissolved in $CHCl_3$ (5 ml). Prep. TLC of the $CHCl_3$ soln (solvent 1) afforded hippadine as colourless flakes (19 mg), mp and mmp 208–209°. The synthetic compound was identical with hippadine in all respects (co-TLC, UV, IR).

Conversion of hippadine into pratorinine (**2**). Hippadine (24 mg), in dry DMSO (3 ml, distilled over Ca hydride) was treated with NaOMe (11 mg). The mixture was stirred at 150° (oil bath) for 140 sec when the reaction mixture became brown. The solvent was evaporated *in vacuo* and the residue treated with H_2O (10 ml). The aq. soln was extracted with Et_2O and the Et_2O layer worked up to give a mixture of pratorinine and unreacted hippadine. The two were separated by column chromatography, as described before, when pratorinine (2.5 mg), mp 265–267°, was obtained as colourless crystals.

Indolopiperonylic acid (**4**). The above aq. alkaline layer was acidified (HCl) when a red-brown solid (12 mg) separated. It crystallized from EtOH–$CHCl_3$ as brown microcrystals, mp 202–205°; R_f 0.22 (solvent 2). It did not respond to Dragendorff's reagent, but the Ehrlich test showed a purple colour suggesting the presence of an indole moiety; UV λ_{max}: nm 227, 235, 280, 288, 335; IR ν_{max}^{Nujol} cm^{-1}: 3400 (*br* OH), 1650 (COOH), 1612 (indole), 1030, 938 (O–CH_2–O); ^1H NMR: δ ($CDCl_3$/DMSO-d_6) ppm 7.53 (1 H, *s*, H_D), 6.91 (1 H, *s*, H_C), 6.60 (1 H, *dd*, *J* = 2, 3.5 Hz, H_B), 6.1 (2 H, *s*, O–CH_2–O); MS: *m/z* 281 (M^+, 42%), 263 (100), 235 (3), 210 (12); when heated above its mp, **4** produced hippadine (mp, mmp, co-TLC).

The aq. acidic layer, after separation of **4**, was extracted with Et_2O. The Et_2O layer, after usual work up, afforded a further crop of pratorinine (*ca* 1 mg).

Acknowledgements—The authors are indebted to Dr. B. C. Das, CNRS, Gif-Sur-Yvette, France, and the National Cancer Institute, Bethesda, U.S.A., for MS and anti-cancer screening of some of the *Crinum* alkaloids.

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