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ALKALOIDS OF *ZEPHYRANTHES FLAVA**

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Key Word Index—*Zephyranthes flava*; Amaryllidaceae; seeds; alkaloids; known alkaloids; new 2-oxyphenanthridinium alkaloids; zefbetaine; zeflabetaine; developmental changes; biological activity.

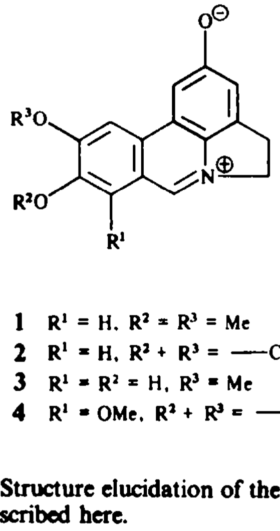
Abstract—From fresh, mature seeds of *Zephyranthes flava*, two new 2-oxyphenanthridinium alkaloids, zefbetaine and zeflabetaine along with 15 known free and glucosyloxy alkaloids have been isolated by gradient solvent extraction, chromatography and derivatization. The new compounds have been characterized by comprehensive spectroscopic analyses, chemical transformations and synthesis. Additionally, changes in the major alkaloids during development of the fruits have been analysed and their biological significance appraised.

INTRODUCTION

The isolation and characterization of the phenolic constituents of the bulbs of *Zephyranthes flava* were reported recently [1]. The alkaloidal constituents of the bulbs, fruits and seeds of this species have now been investigated. *Zephyranthes flava* is one of a few species of Amaryllidaceae that produces fruits and viable seeds. The earlier studies on the chemical constituents of Amaryllidaceae species were mainly restricted to bulbs. The other parts, such as fruits and seeds, received only cursory attention by phytochemists [2]. In this paper, the isolation and characterization of the alkaloids of fresh, mature seeds of *Z. flava* are described. Additionally, information on the turn over of the major pyrrolinophenanthridine alkaloids and their advanced catabolic products (2-oxyphenanthridinium betaines), in the developing fruits, has been obtained. The biological significance of the changes in alkaloidal patterns is appraised.

RESULTS AND DISCUSSION

Extensive CC and prep. TLC of the gradient solvent extracts of the fruits, seeds and bulbs of *Z. flava* were separately carried out for their contained alkaloids. The fruits and seeds afforded qualitatively the same alkaloids, the amounts being more in the seeds. There are eight tertiary alkaloids, crinamine [3], haemanthamine [4], lycorine [5], maritidine [3], methylpseudolycorine [5], pretazettine [5], haemanthidine [4] and pseudolycorine [5]; two lactam alkaloids, narciclassine [4] and pratorimine [6]; three glucosyloxy alkaloids, kalbreclassine [4], lycorine-1-*O*-β-D-glucoside [5] and pseudolycorine-1-*O*-β-D-glucoside; and four 2-oxyphenanthridinium betaines, criasbetaine (**2**) [7], ungeremine (**1**) [8], zefbetaine (**3**) and zeflabetaine (**4**). Among these, the two last named compounds are new alkaloids. The flowering bulbs of *Z. flava* also yielded the same alkaloids except the 2-oxyphenanthridinium betaines which were obtained only in trace amounts. Structure elucidation of the two new alkaloids only is described here.



1 $R^1 = H,\ R^2 = R^3 = Me$
2 $R^1 = H,\ R^2 + R^3 = -CH_2-$
3 $R^1 = R^2 = H,\ R^3 = Me$
4 $R^1 = OMe,\ R^2 + R^3 = -CH_2-$

Zefbetaine, $C_{16}H_{13}NO_3$ (by combustion analyses and $[M]^+$), was obtained as a yellow amorphous solid and was optically inactive. It exhibited UV maxima in methanol and in presence of the usual basic and acidic shift reagents [2] which were characteristic of 2-oxyphenanthridinium betaines [7, 9]. It formed a hydrated hydrochloride and a mono-*O*-acetyl derivative. The 90 MHz ^1H NMR spectrum of the parent compound, in CD_3OD, showed signals for four aromatic and one highly deshielded olefinic protons (all singlets), one Ar–OMe and a $-CH_2CH_2-$ grouping, the chemical shifts and coupling constants of the protons suggested the substitution pattern as in **3**. In locating the methoxyl group at C-9 and the hydroxyl group at C-10, the ^1H NMR data of the acetate derivative were compared with those of the parent compound. In the acetate derivative, it was only the H-11 which experienced a downfield shift (*ca* 0.15 ppm) from the corresponding resonance of H-11 in the parent compound. The ^1H NMR assignments were based on earlier generalizations [6, 7]. The chemical proof in support of the assigned structure (**3**) was obtained by oxidizing pseudolycorine with SeO_2 according to a previously described procedure [7] when

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*Part 22 in the series: "Chemical Constituents of Amaryllidaceae". For Part 21 see ref. [7].

zefbetaine was obtained in a high yield (*ca* 78%). The oxide betaine has not been encountered before in nature or has it been chemically synthesized.

Zeflabetaine, $C_{17}H_{13}NO_4$, is an A-ring-methoxy-methylenedioxy-2-oxyphenanthridinium betaine as was indicated by (i) its UV maxima in methanol and in the presence of basic and acidic shift reagents, (ii) a sharp band in its IR spectrum at ν 1618 cm^{-1} (due to Ar–OMe) [10], (iii) the chemical shift of the lone A-ring proton at δ7.24 (H-11), (iv) area enhancement (NOE) of the H-7 signal by *ca* 18% by irradiation of the methoxyl signal (similar area enhancements were observed in pyrrolophenanthridone alkaloids which formed a basis for determining the position of their hydroxyl–methoxyl functions [6], (v) loss of 17 mu from the $[M]^+$ in the EI mass spectrum due to a methoxyl group *peri* to a $>C=N^+<$ [11]. The aromatic methoxyl group of zeflabetaine was removed by treatment with sodium and isoamyl alcohol according to a published procedure [10], when ungeremine, a known alkaloid, was produced. Thus, the 8-methoxy-9,10-methylenedioxy-2-oxyphenanthridinium betaine structure (**4**) was assigned to zeflabetaine. This alkaloid also has not been encountered before in nature or by synthesis. Zefbetaine (**3**) and zeflabetaine (**4**) are the third and fourth examples of naturally occurring 2-oxyphenanthridinium betaines, the first two in this series being criasbetaine (**1**) [7] and ungeremine (**2**) [7, 8].

The 90 MHz ^1H NMR spectral data of haemanthamine and orinamine, obtained in this study, are noteworthy. A sharp difference in the splitting patterns of the olefinic protons (H-1,2) was observed when the spectra were determined in two different solvents, $CDCl_3$ and deuteriodimethyl sulphoxide (DMSO-d_6). The results are given in Table 1. Haugwitz *et al.* [12], in their paper on ^1H NMR spectra of Amaryllidaceae alkaloids, reported that the olefinic hydrogen pattern in the crinamine series showed marked differences from those of their haemanthamine analogues. Thus, crinamine itself, in $CDCl_3$, exhibited a broadened singlet for its H-1,2 (half-height band width 1.0 Hz), while haemanthamine exhibited a 4-line doublet (with a shift difference of less than 5 Hz). A clear distinction in the splitting patterns of the olefinic protons of these two alkaloids, when the spectrum was taken in DMSO-d_6, was observed (Table 1). The general applicability of this solvent-induced shift in olefinic protons in other epimeric pairs of the 5,10b-ethanophenanthridine alkaloids is currently being investigated.

Another noteworthy observation was the rapid turnover of certain alkaloids in the developing fruits of *Z. flava*. Analyses of the methanol extracts of fresh developing fruits by TLC, prep. TLC/UV and HPLC showed a gradual increase in the amounts of the 2-oxyphenanthridinium betaines (**1–3**) with a concomitant decrease in the amounts of the corresponding pyrrolinophenanthridine precursors (Table 2).

The free alkaloids, lycorine, pseudolycorine and methylpseudolycorine were found to inhibit, germination of seeds and growth of seedlings, to both producer and non-producer plants. The corresponding 1-*O*-glucosides (1–5 × 10^{-4} M) promoted seed germination and also growth of the seedlings. Seeds of *Z. flava* treated separately with **1–4** (1–3 × 10^{-8} M) were then germinated. The rate of germination and subsequent growth of the seedlings were both considerably reduced compared to a distilled water control. However, plants from the treated seeds produced larger flowers with a larger number of viable seeds. Alkaloids **1–4**, when tested according to published procedures [13–15], exhibited significant anti-tumour activities in the *in vitro* P-388 (**1**, 1.02; **2**, 0.82; **3**, 0.64; **4**, 0.77 μg/ml) and KB test systems (ED_{50}: **1**, 2.4; **2**, 1.2; **3**, 1.8; **4**, 2.5 μg/ml) and caused cytolysis of Sarcoma 180 ascites tumour cells (SCL: **1**, 25.32 ± 2.04; **2**, 20.48 ± 1.14; **3**, 18.44 ± 1.82; **4**, 24.44 ± 2.74; normal spleen cells, SCL: 7.04 ± 0.78; *n* = 5–8). The details of the biological screening will be reported elsewhere.

EXPERIMENTAL

General procedures were the same as those reported recently [5]. Plant material was collected during July–August, every year for four consecutive years (1981–1984), from the Banaras Hindu University Campus and were identified by Professor S. K. Roy, Department of Botany, Faculty of Science. Voucher specimens have been preserved at the Department of Pharmaceutics, Banaras Hindu University, Varanasi.

Isolation procedure. In a typical expt, fresh mature seeds of *Z. flava* (Roem. and Schult) (*ca* 200 g) were macerated in aq. MeOH in a high-speed blender, filtered and the filtrate evapd under red. pres. to give a viscous brown slurry (22.5 g). This was triturated with hot petrol (60–80°) to remove fatty materials and weakly polar alkaloids as the petrol-soluble fraction (fraction A). The petrol-insoluble fraction was treated with aq. HOAc (4%,

Table 1. Solvent-induced ^1H NMR shifts of olefinic protons of crinamine and haemanthamine

Solvent	Assignment of proton	Crinamine	Haemanthamine
		Chemical shifts*	
$CDCl_3$	H-1	6.22 (*br s*)	6.42†
	H-2	6.22 (*br s*)	6.38†
	H-3	4.0 (*J* = 9.4, 7.0, 2.0 Hz; partially obscured by H-11)	3.85 (*m*)
DMSO-d_6	H-1	6.27 (*dd*, *J* = 10.5, 2.0 Hz)	6.46 (*d*, *J* = 10 Hz)
	H-2	5.93 (*dd*, *J* = 10.5, 5 Hz; line broadening)	6.13 (*dd*, *J* = 10, 5 Hz)
	H-3	3.9 (*m*)	3.75 (*dd*, *J* = 4.5, 3.6, 2.5 Hz).

*Chemical shifts in δ ppm from TMS at zero.

†H-1 and H-2 form the AB part of an ABX (where X = H-3) pattern with only 4 lines discernible; H-1,2 exhibited a shift difference of less than 5 Hz [12].

Table 2. Changes in the concentration of pyrrolinophenanthridine and 2-oxyphenanthridinium alkaloids in developing fruits of *Zephyranthes flava*

Alkaloid	μg/g of fruit†					
Day of collection:	1	3	5	7	9	11
Lycorine	110 ± 12	80 ± 10	44 ± 5	32 ± 5	18 ± 3	15 ± 3
Lycorine-1-*O*-glucoside	202 ± 28	74 ± 11	28 ± 5	26 ± 3	17 ± 4	12 ± 2
Ungeremine (2)	55 ± 7	114 ± 10	164 ± 18	178 ± 22	182 ± 14	194 ± 20
Methylpseudolycorine	52 ± 5	35 ± 4	17 ± 4	12 ± 2	+	+
Criasbetaine (1)	18 ± 2.5	27 ± 7	28 ± 5	28 ± 7	30 ± 4.5	32 ± 6
Pseudolycorine	68 ± 9	55 ± 9	34 ± 6	16 ± 4	8 ± 2	+
Pseudolycorine-1-*O*-glucoside	114 ± 14	88 ± 12	75 ± 10	68 ± 8	52 ± 7	20 ± 5
Zefbetaine (3)	22 ± 4	37 ± 7	40 ± 6	45 ± 5	48 ± 8	50 ± 10

*In samples of developing fruits, collected from the day of withering of flowers for *ca* 2 weeks.

†Mean of 6–9 replicates; values (obtained from UV of prep. TLC scrapings and analytical HPLC) represent mean ± s.e.; +, traces.

150 ml) and a brown solid that separated was collected by filtration (fraction B, 0.78 g). The clarified aq. HOAc soln was extracted with Et_2O to give the Et_2O-soluble acetates (fraction C) and then basified (NH_4OH). The liberated bases were extracted, in succession, with $CHCl_3$ (fraction D), EtOAc (fraction E) and *n*-BuOH (fraction F). The aq. mother liquor was discarded.

Treatment of fraction A. The brown gummy material (0.82 g) on analytical TLC (solvent 1, plate 1) [5], showed one major and several minor Dragendorff-positive spots. A portion (0.2 g) was dissolved in C_6H_6 and chromatographed over a column of silica gel (18 × 2 cm). Elution was carried out with petrol (0.5 l.), C_6H_6 (1.2 l.), C_6H_6–$CHCl_3$ (1.5 l.) and $CHCl_3$ (0.7 l.). Fractions (100 ml) were collected and monitored by TLC.

Pratorimine. Fractions 28–37 were combined and evapd. The residue crystallized from $CHCl_3$–EtOH as light brown needles (8 mg), mp and mmp 263–265°. Direct comparison (co-TLC, UV, IR) with a ref. sample of pratorimine [6] established that they were identical.

Treatment of fraction B. This fraction afforded the flavans [1] and phosphatidyllycorine [13] as before.

Treatment of fraction C. This fraction was triturated with dil HCl (1 N, 20 ml). The aq. acidic soln was extracted with $CHCl_3$ to separate the $CHCl_3$-soluble (fraction c_1) from the $CHCl_3$-insoluble (fraction c_2) alkaloid hydrochlorides.

Treatment of fraction c_1. This fraction on usual work up afforded a brown powder (0.14 g) which showed two major Dragendorff-positive spots on analytical TLC (plate 1, solvent 2) R_f 0.32 (I_2, D and Fe^{3+}, positive; TDA, yellowish-orange) [5]; 0.45 (I_2, D, positive; TDA, brown). The mixture was dissolved in MeOH (10 ml) and applied to a column of Amberlite IRA-400 (HO^-) (30 × 1 cm). The elution was performed with aq. MeOH (30%) to give the non-phenolic alkaloid, R_f 0.45, first. Subsequent washing with MeOH–HOAc–H_2O (6:3:1) gave the phenolic alkaloid, R_f 0.32.

Pretazettine. The non-phenolic alkaloid was further purified by passing its concd $CHCl_3$ soln through a short column (10 × 1.5 cm) of silica gel G (E. Merck). Elution was carried out, under low pressure, with C_6H_6–EtOAc (9:1). Fractions (25 ml) were collected and monitored by TLC. Fractions 8–17 were combined and concd in a stream of N_2 when pretazettine was obtained as an amorphous powder (41 mg) (co-TLC, UV, ^1H NMR) [5].

Pseudolycorine. The phenolic alkaloid from the resin column was obtained as a gummy material. It crystallized from $CHCl_3$–Me_2CO as straw coloured micro-crystals (35 mg), mp and mmp 247–249°. Direct comparison (co-TLC, UV, IR) with a ref. sample of pseudolycorine [5] established that they were identical.

Processing of fraction c_2 in the usual way [5] afforded haemanthidine (14 mg), mp and mmp 189–191° (co-TLC, MS); lycorine (27 mg), mp and mmp 255–257° (co-TLC, MS) and methylpseudolycorine (17 mg), mp and mmp 238–241° (co-TLC, MS, ^1H NMR) [5].

Treatment of fraction D. The $CHCl_3$ extract, on concn, gave a further crop of lycorine (55 mg). The $CHCl_3$ mother liquor showed four major Dragendorff-positive spots on TLC. It was chromatographed over a column of Florisil (20 × 2 cm). Elution was carried out with CH_2Cl_2, $CHCl_3$, MeOH and different proportions of $CHCl_3$–MeOH. The eluates were monitored by TLC. Prep. TLC of the concentrates, using $CHCl_3$–MeOH (9:1), yielded crinamine (15 mg) [3], haemanthamine (21 mg), haemanthidine (5 mg) [4] and maritidine (27 mg) [3].

Treatment of fraction E. The residue from this fraction was dissolved in MeOH–dioxane (4:1, 25 ml) and filtered. The filtrate, exhibiting a violet fluorescence, was kept at room temp. for 18 hr when a light brown solid separated (98 mg). This was a mixture of two major and two minor alkaloids, all of which responded to the benzidine–metaperiodate test for polyols. The solid was redissolved in MeOH, combined with silica gel (12 g), dried under vacuum and loaded onto a silica gel column (30 × 2.5 cm). Elution was carried out with EtOAc (1.8 l.), EtOAc–MeOH (99:1, 2.4 l.) and MeOH (0.5 l.). Fractions (100 ml) were collected and monitored by analytical TLC.

Narciclassine. Fractions 10–17 were combined and evapd to give a cream coloured solid which crystallized from Me_2CO–MeOH as fine needles (27 mg), mp and mmp 250–253°. The identity was confirmed by direct comparison (co-TLC, UV, ^1H NMR) with a ref. sample of narciclassine [4].

Kalbreclassine. The later EtOAc–MeOH eluates, which showed a streak on TLC, were combined and evapd under red. pres. The residue was repeatedly dissolved in MeOH and pptd with Et_2O when a straw coloured hygroscopic solid (33 mg) was obtained. Direct comparison (co-TLC, IR) with a ref. sample of kalbraclassine and its acetate, mp and mmp 198–203° [4], established that they were identical.

Treatment of fraction F. This fraction afforded a brown gummy material which showed seven major Dragendorff and four benzidine–metaperiodate positive spots on TLC. The residue was repeatedly extracted with hot Me_2CO and the Me_2CO soluble (fraction f_1) and insoluble (fraction f_2) fractions separated by filtration.

Treatment of fraction f_1. This fraction, on concn, gave a further crop of methylpseudolycorine (7 mg). From the Me_2CO mother liquor, further crops of pseudolycorine (11 mg) and maritidine (17 mg) were obtained.

Treatment of fraction f_2. This fraction was treated with Me_2CO–MeOH (1:1) and the soluble fraction subjected to prep. TLC using $CHCl_3$–MeOH (3:1). The 2-oxyphenanthridinium alkaloids (1–4) exhibited blue and blue–green fluorescence under UV light (λ365 nm). The Me_2CO–MeOH insoluble fraction was a mixture (3:1) of the two glucosyloxy alkaloids, lycorine-1-*O*-β-D-glucoside (5 mg) and pseudolycorine-1-*O*-β-D-glucoside (29 mg), which were separated as before [5].

Ungeremine (**2**). Prep. TLC and removal of R_f zone 0.2 afforded ungeremine as a yellow powder (43 mg), mp > 270° (dec); HPLC R_t 27.0 min. The identity of this compound with ungeremine was established by direct comparison (co-TLC, HPLC, MS) [7].

Criasbetaine (**1**). Prep. TLC and removal of the R_f zone 0.4 afforded a light yellow powder (11 mg) (from EtOH), mp > 280° (dec); HPLC R_t 22.5 min. Co-TLC, HPLC, UV and MS of the alkaloid were identical with those of a ref. sample of criasbetaine [7].

Zefbetaine (**3**). Prep. TLC and removal of the R_f zone 0.12 afforded the alkaloid from EtOH as a brown powder (14 mg), mp > 300°; HPLC R_t 29.2 min; UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ε): 262 (4.70), 278 sh (4.59), 285 sh (4.55), 408 (3.78); $\lambda_{\max}^{\text{MeOH–0.01 N NaOMe}}$: 265, 280 sh, 300 sh, 458 nm; $\lambda_{\max}^{\text{MeOH–0.1 N HCl}}$: 263, 278, 283, 378 nm; IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3250 (br, OH), 1610, 1048, 1010; EIMS *m/z* (rel. int., %): 267 $[M]^+$ (92), 266 (100), 252 (32), 251 (32), 224 (22), 223 (28), 208 (12), 194 (14), 178 (10); ^1H NMR (CD_3OD): δ9.18 (1H, *s*, H-7), 7.80 (1H, *s*, H-8), 7.68 (1H, *s*, H-11), 7.60 (1H, *s*, H-1/3), 7.38 (1H, *s*, H-3/1), 5.20 (2H, *t*, 2H-5), 3.98 (3H, *s*, C-9 OMe), 3.72 (2H, *t*, 2H-4). The HCl of the alkaloid was pptd from an aq. soln of the base by addition of 10% aq. HCl as a pale yellow fluffy material, after drying at 120° over P_2O_5 *in vacuo*, mp > 300° (Found: C, 59.47; H, 4.48; N, 4.28. $C_{16}H_{13}NO_3 \cdot HCl \cdot H_2O$ requires C, 60.0; H, 4.9; N, 4.3). The parent alkaloid on treatment with Ac_2O–Et_3N, at room temp. for 18 hr gave the C-10 *O*-acetylzefbetaine, straw coloured solid from Me_2CO–petrol, mp 222–225°; $C_{18}H_{15}NO_4$ ($[M]^+$ accurate mass measurement, 309.0982); ^1H NMR (CD_3OD): δ9.20 (1H, *s*, H-7), 7.83 (1H, *s*, H-11), 7.81 (1H, *s*), 7.60 (1H, *s*), 7.40 (1H, *s*), 5.22 (2H, *t*), 3.98 (3H, *s*), 3.72 (2H, *t*), 2.32 (3H, *s*, C-10 OAc).

Zeflabetaine (**4**). Prep TLC and removal of the R_f zone ~ 0.05 was subjected to further prep. TLC followed by crystallization from EtOH when zeflabetaine was obtained as a light brown powder (19 mg); HPLC R_t 33.8 min; UV $\lambda_{\text{nm}}^{\text{MeOH}}$ nm (log ε): 222 sh (4.02), 267 (4.68), 278 sh (4.15), 288 sh (4.08), 292 (3.90), 305 (3.98), 415 (3.55); $\lambda_{\max}^{\text{MeOH–0.1 N NaOMe}}$: 255 sh, 267, ~ 280, 435 nm; $\lambda_{\max}^{\text{MeOH–0.1 N HCl}}$: 267, 275, 282, 292, 300 sh, 368 nm; IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1600, 1618 (Ar–OMe), 938 (OCH_2O); EIMS *m/z* (rel. int. %): 295 $[M]^+$ (100), 294 (84), 278 (12), 267 (17), 266 (15), 250 (14), 206 (7), 205 (7); ^1H NMR (CD_3OD): δ9.15 (1H, *s*), 7.60 (1H, *s*), 7.42 (1H, *s*), 7.24 (1H, *s*), 6.28 (2H, *s*, OCH_2O), 5.18 (2H, *t*), 4.02 (3H, *s*, C-8 OMe), 3.70 (3H, *t*); zeflabetaine·HCl, yellow powder, mp > 300° (Found: C, 58.02; H, 4.33; N, 3.78. $C_{17}H_{13}NO_4 \cdot HCl \cdot H_2O$ requires C, 58.3; H, 4.5; N, 4.0.)

Transformation of zeflabetaine (**4**) *to ungeremine* (**2**). To a stirred suspension of molecularized Na (45 mg) in boiling xylene (3 ml) was added zeflabetaine (22 mg); redistilled isoamyl alcohol (0.3 ml in xylene 1 ml) was then added in one portion. After 3 min, an additional quantity of isoamyl alcohol (0.1 ml in 0.5 ml xylene) was added. The reaction mixture was stirred for 10 min under N_2, cooled and then extracted with dil HCl (10%, 3 × 10 ml). The aq. acidic soln was washed with C_6H_6 and Et_2O in succession and then basified (NH_4OH). The liberated bases were extracted with EtOAc (3 × 50 ml). The combined EtOAc extracts were worked up in the usual way to give a brown gummy material. This was dissolved in MeOH and subjected to prep. TLC when ungeremine (7 mg) was obtained (co-TLC, HPLC, UV, MS).

Ontogenic variation of alkaloids. In a typical expt, fresh developing fruits of *Z. flava* were macerated in MeOH in a high-speed blender and the mixture warmed at 60 ± 2° for 15 min (dry wt of marc *ca* 10 g). The MeOH extract was evapd *in vacuo* and the residue triturated with hot petrol (40–60°) to remove lipid materials. The petrol-insoluble solid was dissolved in MeOH and portions (containing 5–20 ng residue) were analysed by HPLC [UV 254 nm detector using a C_{18} μ-Bondapak analytical column, MeOH–H_2O (7:3) as eluant, flow rate 1 ml/min]. Lycorine R_t 10.8 min; pseudolycorine, 11.4 min; methylpseudolycorine, 12.7 min; criasbetaine, 22.5 min; ungeremine, 27.0 min; zefbetaine, 29.2 min; zeflabetaine, 33.8 min. The 1-*O*-β-D-glucosides of lycorine and pseudolycorine gave broad peaks having long R_t (50–70 min). These were separated, hydrolysed (with emulsin) and the aglycones (lycorine and pseudolycorine) estimated as before. Results are summarized in Table 2.

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