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PALMILYCORINE AND LYCORISIDE: ACYLOXY AND ACYLGLUCOSYLOXY ALKALOIDS FROM *CRINUM ASIATICUM**

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Key Word Index—*Crinum asiaticum*; Amaryllidaceae; fruits; alkaloids; alkaloidal conjugates; palmilycorine, 1-*O*-palmitoyllycorine; lycoriside, lycorine-1-*O*-(6′-*O*-palmitoyl-β-D-glucopyranoside); lycorine-1-*O*-β-D-glucoside; promoters of cell viability, cell growth and root growth.

Abstract—Two new types of alkaloidal conjugates, a C_{16}-acyloxy derivative, named palmilycorine, and an acylglucosyloxy derivative, named lycoriside, were isolated from the fruits of *Crinum asiaticum*. The presence of these compounds was also detected in the fleshy scale leaves and in roots of this species. The structures of the two compounds were established as 1-*O*-palmitoyllycorine (**1**) and lycorine-1-*O*-(6′-*O*-palmitoyl-β-D-glucopyranoside) (**2**), respectively, on the basis of chemical transformation and comprehensive spectral evidence. The biological effects of the alkaloids were evaluated.

INTRODUCTION

Acyloxy, glucosyloxy and acylglucosyloxy alkaloids have now been found to commonly co-occur with the corresponding free bases in several members of the family Amaryllidaceae. The glycosyloxy alkaloids commonly contain glucose as the sugar moiety although rhamnose and galactose have also been found [1–3]. The frequent co-occurrence of the alkaloidal conjugates with their free bases seems to be significant from the point of view of plant biochemistry and warrants detail study. The present paper describes the isolation and characterization of a new acyloxy alkaloid, which has been named palmilycorine, and an acylglucosyloxy alkaloid, named lycoriside, from the fruits of *Crinum asiaticum* L. Additionally, the biological profile of the new compounds is evaluated.

RESULTS AND DISCUSSION

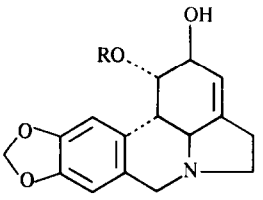
Solvent and pH-gradient extractions followed by a combination of CC, prep. TLC and HPLC of the fruit extracts of *C. asiaticum* afforded the two new compounds in addition to the previously reported seven alkaloids, ambelline [4], crinamine [5], crinasiatine [5], lycorine [4], 1,2-β-epoxyambelline [6], hippadine [4] and lycorine-1-*O*-β-D-glucoside [3]. Details of characterization of the two new compounds only is described here.

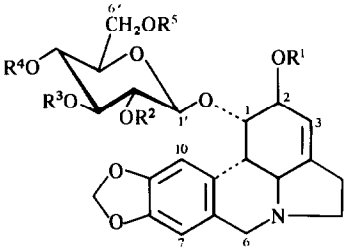
Palmilycorine, $C_{32}H_{47}NO_5$, obtained as a cream coloured amorphous solid, was optically active. It showed UV maxima, in methanol, typical of pyrrolinophenanthridine alkaloids [7]. The IR and ^{1}H NMR spectra of the compound suggested the structural features of a fatty ester of lycorine. The compound fragmented before exhibiting an $[M]^+$ peak in its EI mass spectrum. The fragment ion peaks, however, suggested the presence of lycorine and a C_{16}-acyl moiety. The CI mass spectrum provided an identifiable $[M]^+$ peak at *m/z* 525. Attempts to crystallize the compound from common organic solvents resulted in partial degradation of the molecule into anhydrolycorinium cation and palmitic acid. Sublimation of the compound *in vacuo* also caused similar degradation. Deacylation with sodium methoxide–methanol, according to a published procedure [8], produced lycorine (**3**) and methyl palmitate. Methylation of palmilycorine with MeI–NaH, in tetrahydrofuran, followed by deacylation and *O*-acetylation, in succession, afforded 1-*O*-acetyl-2-*O*-methyllycorine [3]. Hence the 1-*O*-palmitoyllycorine structure (**1**) was assigned to this compound.

Lycoriside, $C_{38}H_{57}NO_{10}$, a hygroscopic solid, was optically active. It responded to both Dragendorff's test for alkaloids and the benzidine–metaperiodate test for polyols. The UV spectrum of the compound was similar to that of **1**. The IR and 90 MHz ^{1}H NMR spectra of the compound suggested the structural features of an acylglucosyloxy alkaloid. Deacylation of the compound, as before, gave lycorine-1-*O*-β-D-glucoside and methyl palmitate (containing traces of methyl stearate, GC). The glucosyloxy alkaloid (**4**) has been encountered before in a number of Amaryllidaceae species [3, 9]. Lycorine-1-*O*-β-D-glucoside (**4**), on further hydrolysis with emulsin, gave lycorine and D-glucose (identified as the alditol acetate by GC). The permethyl ether of lycoriside, prepared as above, on hydrolysis with MeOH–HCl gave lycorine, palmitic acid and 2,3,4-tri-*O*-methylglucose (1 : 1 proportion with respect to the alkaloid aglucone) [8]. Hence, lycorine-1-*O*-(6′-*O*-palmitoyl-β-D-glucopyranoside) structure **2** was assigned to lycoriside.

The two new compounds (**1** and **2**) were also found in the fleshy scales of the bulbs and roots of *C. asiaticum*. In the scape portion of the plant, which contained a strongly acidic fluid, only unconjugated alkaloids, e.g. lycorine, ambelline and 1,2-β-epoxyambelline, were present. The relative proportion of **1** and **2**, as was determined by prep. TLC, UV and HPLC, was more in the mature green fruits

*Part 18 in the Series "Chemical Constituents of Amaryllidaceae". For Part 17 see ref. [15].





1 R = palmitoyl
3 R = H

2 $R^1 = R^2 = R^3 = R^4 = H$, R^5 = palmitoyl
4 $R^1 = R^2 = R^3 = R^4 = R^5 = H$
5 $R^1 = R^2 = R^3 = R^4$ = Me, R^5 = palmitoyl

than in the bulbs and roots. All these alkaloids (**1**–**4**) were also found in the bulbs and roots of *C. augustum* Roxb. at the time of flowering. To our knowledge, the acyloxy alkaloid of the type **1** and the acylglucosyloxy alkaloid of the type **2** have not been reported before nor have they been synthesized. It was thought worthwhile to examine the biological profile of the new compounds using the screen designed earlier for the unconjugated alkaloid, lycorine [1, 3, 9].

Lycorine, which occurs almost ubiquitously and exclusively in the Amaryllidaceae produced various inhibitory effects in plant and animal cells. These were manifested as anomalies in cell division, elongation and in synthetic processes (protein/DNA) [1, 7, 9]. Lycorine-1-*O*-β-D-glucoside (**4**), on the other hand, exhibited promotion of cell growth, seed germination, and rate of development of root and root hairs in higher plants [3]. Compound **4** was also found to activate blast formation in mouse spleen lymphocytes [9].

In the present study, bulbs of *Allium cepa*, *P. biflorum* and *Z. flava*, exposed to a solution of lycoriside (**2**) (10^{-4} M), were stimulated with regard to the formation of primary roots and in their linear growth. Palmilycorine (**1**) only marginally promoted the growth of root and root hairs in these plants. Interestingly, all the compounds (**1**–**4**) inhibited the emergence of leaves. Furthermore, **2** and **4** markedly potentiated the viability of ascites tumour cells, whereas **1** and **3** significantly inhibited it (Table 1). In the *in vivo* experiment, only **3** had a pronounced inhibitory effect on the growth of the tumour cells. The inhibitory effect of **1**, *in vivo*, was statistically insignificant, whereas **2** and **4** only marginally potentiated the growth (Table 1).

It is of interest that similar types of conjugates, the acyl, glycosyloxy and acylglycosyloxy derivatives, are frequently encountered among the phytosterols (sitosterol, stigmasterol) occurring in higher plants including the title species [8, 10–12]. The enzymatic machinery of *C. asiaticum* thus seems to be catholic to the extent that both sterols and alkaloids are converted to similar types of conjugates. While the biochemical significance of this transformation has not been entirely elucidated, the following observations would seem to be of relevance. (i) The formation of esters of sterols and alkaloids as transfer products between intracellular organelles would, in itself, constitute an important biochemical process [1, 10]. (ii) Certain steryl esters exhibit some special physical properties, e.g. formation of a well-defined cholesteric mesophase which would make them well suited for living systems [10, 12]. (iii) Acylsterylglycosides and the acylglucosyloxy alkaloid **2** exhibit membrane-stabilizing action in induced stomach ulceration of laboratory animals [8, 12]. (iv) Lycoriside **2** and lycorine-1-*O*-β-D-glucoside **4** are used by Amaryllidaceae plants for recognition (self from non-self) to reject the vast majority of micro-organism and phanerogamic parasites (e.g. *Imperata cylindrica*) with which they interact [Ghosal, S., unpublished].

Table 1. Effects of lycorine and congeners on viability and growth of tumour cells

Treatment*	% viability of cells *in vitro* (mean ± s.d.)	Tumour cells *in vivo* × 10^7 (mean ± s.d.)
1	68.50 ± 5.22	12.84 ± 2.12
	$P < 0.1$	
2	98.03 ± 1.71	14.62 ± 1.40
	$P < 0.01$	
3	62.50 ± 4.31	7.25 ± 0.82
	$P < 0.05$	$P < 0.05$
4	95.64 ± 3.34	14.54 ± 1.37
	$P < 0.05$	
Control	80.85 ± 6.01	14.02 ± 1.43

*Concn of the test compound *in vitro* expt, 10^{-3} M; *in vivo* expt, 5 mg/100 g body wt (ip); $n = 5$; P, in relation to control.

EXPERIMENTAL

The general methods are the same as reported recently [12]. The statistical significance of the biological data (Table 1) was calculated by standard procedures [13].

C. asiaticum L., cultivated in the Banaras Hindu University Campus, was identified by Professor S. K. Roy, Department of Botany, Faculty of Science, Banaras Hindu University. The different parts of the plant, e.g. fruits, bulbs and roots, were collected on two consecutive years (1982–1983) during November–December and were separately processed for chemical constituents.

Extraction. In a typical expt, fresh mature fruits (55 g) were macerated with MeOH in a high-speed blender. The MeOH extract was warmed at 60° for 15 min, to inactivate glycosidase enzymes, and then kept at room temp. overnight. After filtration

(Buchner) on Celite, the soln was concd at 35° to one-third of its original vol. when a cream coloured solid separated which was then collected by filtration (Fraction A, 0.64 g). The MeOH mother liquor was evapd to give a light brown viscous residue. It was triturated with petrol (60–80°) to extract fatty materials and weakly polar alkaloids (Fraction B). The petrol-insoluble portion was treated with aq. HOAc (4%, 50 ml) when a brown solid that separated was collected by filtration (Fraction C, 0.38 g). The clarified aq. acidic soln was extracted with Et_2O (Et_2O-soluble acetates, Fraction D) and then basified with NH_4OH. The liberated bases were extracted, in succession, with Et_2O (Fraction E), EtOAc (Fraction F) and *n*-BuOH (Fraction G). The aq. mother liquor was discarded.

Fraction A. Solvent-gradient extraction followed by prep. PC, deacylation with NaOMe–MeOH (identification of Me esters of fatty acid by GC and MS) and determination of P [14] indicated it to be a mixture of phosphatidyllycorine and other bases. Further examination of this fraction is under way.

Fraction B. This fraction was further extracted with aq. citric acid (10%, 30 ml). The acid extract was basified ($NaHCO_3$) and the liberated bases extracted with Et_2O (fraction b_1) and EtOAc (fraction b_2) in succession.

Fraction b_1. The basic gummy material was dissolved in Et_2O–MeOH and repeatedly ppted with *n*-hexane to give palmilycorine (**1**) as a cream coloured amorphous powder (27 mg); R_f 0.37 ($CHCl_3$–MeOH, 9:1); R_t (HPLC) 418 sec (MeOH–H_2O, 4:1); $[\alpha]_D^{22}$ −58.5° (*c* 0.23, MeOH); UV λ_{max}^{MeOH} nm (log ε): 235 sh (3.02), 282 (3.33); IR ν_{max}^{Nujol} cm^{-1}: 3450, 1732, 1612, 1595, 1255, 1238, 944, 925, 825, 792, 755, 730; CIMS: *m/z* (rel. int.): 525 $[M]^+$ (1.8); EIMS *m/z* (rel. int.): 269 (7), 268 (11), 256 (5), 239 (17), 227 (95), 226 (100), 211 (7), 210 (15), 178 (7), 43 (45); ^1H NMR ($CDCl_3$–DMSO-d_6): δ6.70 (1H, *s*, H-10), 6.55 (1H, *s*, H-7), 5.94 (2H, *s*, OCH_2O), 5.72 (1H, *br s*, H-3), 5.58 (1H, *br s*, H-1), 5.27 (1H, *m*, H-2), 3.5–4.0 (2H, $H_{\alpha,\beta}$-6), 2.3–2.4 (8H, *m*, $-CH_2-$ and $-CH-$), 1.25 (26H, palmityl $-CH_2-$), 0.88 (3H, Me). (Found: C, 72.8; H, 8.8; N, 2.4. $C_{32}H_{47}NO_5$ requires C, 73.1; H, 8.9; N, 2.6.) Deacylation of **1**, according to ref. [8], gave lycorine, mp and mmp 256–258° (co-TLC, IR) and Me palmitate (GC, MS). Methylation of **1** (5 mg), in Na-dried THF (1 ml), with NaH (34 mg) and MeI (0.2 ml) under N_2, gave a syrupy material. This was hydrolysed with $NaHCO_3$–MeOH and the product acetylated (Ac_2O–pyridine) to give 1-*O*-acetyl-2-*O*-methyllycorine as colourless leaflets (from MeOH), mp and mmp 198–202° (co-TLC, co-HPLC) [3].

Treatment of fraction b_2 with mixture of organic solvents and prep. TLC gave a further quantity of palmilycorine (11 mg) plus other minor products.

Fraction C. This fraction, when processed in the usual way [3], gave a further amount of phospholipids (0.21 g) along with the known alkaloids, lycorine (12 mg), mp 252–255° (mmp, co-TLC, IR) [4]; ambelline (7 mg), mp 248–250° (mmp, co-TLC, co-HPLC, MS) [4, 6]; 1,2-β-epoxyambelline (3 mg), mp 244–245° (mmp, co-HPLC, MS) [6]; crinamine (4 mg), mp 198–200° (mmp, co-TLC, co-HPLC, MS) [5]; and crinasiatine (2.5 mg), dec > 300° (co-HPLC, MS) [5].

Fraction D. This fraction was partitioned between Et_2O and H_2O (3:1) and the Et_2O extract further separated into neutral, basic and acidic fractions in the usual way. The basic fraction was dissolved in a soln of tartaric acid (4%) and the clarified acidic soln basified with NH_4OH. The liberated bases were extracted with Et_2O and EtOAc. The combined Et_2O–EtOAc extract was evapd to give a hygroscopic solid (0.14 g).

Lycoriside (**2**). Prep. TLC of the hygroscopic solid on precoated silica gel plates, using $CHCl_3$–MeOH (9:1), gave lycoriside as an amorphous powder (0.102 g); R_f 0.24; R_t 462 sec; $[\alpha]_D^{22}$ −34.4° (*c* 0.5, MeOH); UV λ_{max}^{MeOH} nm (log ε): 235 sh (3.30), 285 (3.54); IR ν_{max}^{nujol} cm^{-1}: 1730, 1600 (br), 1025, 942, 828, 790; MS *m/z* (rel. int. %): 268 (7), 256 (12), 239 (11), 227 (100), 226 (95), 144 (5), 73 (38), 60 (45), 57 (9), 43 (11); ^1H NMR ($CDCl_3$–DMSO-d_6): δ6.69 (1H, *s*, H-10), 6.54 (1H, *s*, H-7), 5.94 (2H, *s*, OCH_2O), 5.70 (1H, *br s*, H-3), 5.56 (1H, *br s*, H-1), 5.26 (1H, *m*, H-2), 5.0 (1H, *br s*, glucosyl H-1'), 3.55–4.0 (2H, $H_{\alpha,\beta}$-6), *ca* 3.4 (*br*, glucosyl 6H plus H_2O), 2.4 (6H, *m*), 2.3 (2H, *m*), 1.25 (26 H, palmityl $-CH_2-$), 0.90 (3H, Me). (Found: C, 64.3; H, 8.5; N, 1.8. $C_{38}H_{57}NO_{10}\cdot H_2O$ requires C, 64.7; H, 8.3; N, 1.9.)

Deacylation of **2**. Lycoriside (14 mg), MeOH (1 ml) and a freshly prepared soln (0.1 ml) of NaOMe (0.1 g Na dissolved in 100 ml of MeOH) were mixed and stirred for 30 min at room temp. under N_2. The mixture was dil with H_2O (2 ml) and Na^+ removed by treatment with Amberlite IR-120 (H^+). The aq. soln was processed in the usual way to give Me palmitate (GC, MS) together with traces of Me stearate (GC) [12] and lycorine-1-*O*-β-D-glucoside (co-TLC, IR) [3].

Permethylation of **2**. To an ice-cooled soln of (**2**, 17 mg), in Na-dried THF (5 ml), NaH (82 mg) was added and the mixture stirred for 15 min. MeI (1 ml) was added and stirring was continued for a further 30 min under N_2. The product was filtered, the solvent evapd and the residue in $CHCl_3$ was subjected to prep. TLC ($CHCl_3$–MeOH, 9:1). The band at R_f 0.25 was eluted with $CHCl_3$–MeOH and the solvent evapd to give the perMe ether (**5**) as a syrupy liquid (14 mg). The IR spectrum of the derivative, in nujol, showed no OH absorption. Hydrolysis of the perMe ether with MeOH–HCl and acetylation (Ac_2O–pyridine) of the basic product gave 1-*O*-acetyl-2-*O*-methyllycorine, mp and mmp 195–198° (co-TLC, co-HPLC) [3].

Fractions E and F. Further quantities of lycorine (0.18 g), ambelline (19 mg), and 1,2-β-epoxyambelline (7 mg) were obtained when these two fractions were processed in the usual way [3].

Fraction G. This fraction afforded lycorine-1-*O*-β-D-glucoside (22 mg) [3] and a further amount of crinasiatine (3 mg) [5] when processed as before.

The existence of **1–4** in the MeOH extracts of bulbs and roots of *C. augustum* was established by analytical HPLC (MeOH–H_2O, 4:1) in presence of the ref. compounds obtained from *C. asiaticum*.

Effects of **1–4** *as plant growth regulators.* The effects on the development of roots and aerial parts of *A. cepa*, *P. biflorum* and *Z. flava* were determined. In a typical expt, 100 bulbs of *Z. flava* were surface-sterilized (0.1% NaOCl) and the bulbs divided into 10 groups of *ca* equal wt. Five groups were soaked (1 hr) in Pi buffered saline (PBS, 0.15 N NaCl, pH 7.2) soln of the test compound (10^{-4} M) and placed in a moist chamber at 27° under aseptic conditions. An equal number of bulbs treated with only the vehicle (PBS) were similarly kept as controls. Growth and elongation of the roots were recorded up to 144 hr at 24 hr intervals. The rate of growth was found to be maximal between 72 and 96 hr. At the end of this period, lycoriside (**2**) was found to promote the root growth (fr. wt 195% over control; $n = 5$; $P < 0.01$, χ^2 significance) and elongation (188% over the control; $P < 0.01$). The number of root hairs was also considerably higher (167%) in the lycoriside-treated group. Palmilycorine (**1**) only marginally potentiated the root growth and elongation during this period, whereas lycorine (**3**) significantly inhibited both (*ca* 30–35% in relation to the control group). The root-growth promoting effect of **4** has been previously reported [3]. All compounds (**1–4**) completely prevented the emergence of leaves in *P. biflorum* and *Z. flava* up to 120 hr and, thereafter, continued to inhibit the growth and development of leaves, when compared with controls, for several weeks.

Effects of **1–4** *on ascites tumour cells.* Test compounds were dissolved in aq. HOAc (4%) and the solvent evapd *in vacuo*. The

residue, in each case, was re-dissolved in PBS at a suitable concn, membrane (0.45 μm)-filtered and stored at 0° in small portions. A transplantable S-180 tumour of Swiss mice was used for the test tumour cells. The tumour was maintained by serial passage of tumour cells (1×10^6) by intraperitoneal (ip) inoculation.

Viability of tumour cells in vitro. Tumour cells were harvested on day 6 following tumour inoculation. The cells were washed and suspended in RPMI-1640 medium with 20 mM HEPES and fetal calf serum (FCS, 10%) [9], at a concn of 1×10^6 viable cells/medium. To portions (1 ml) of the tumour cell suspension, the test compound in PBS was added. The final concn of test compound in the tumour cell suspension was 10^{-3} M. Cell suspensions were incubated at 39° for 2 hr. As control, 1 ml portions of tumour cell suspension in PBS were incubated. For each sample, 5 sets of assays were made. At the end of incubation, the viability of the tumour cells were determined by trypan blue exclusion criteria as described before [9].

Growth of S-180 tumour cells in vivo. Tumour cells (1×10^6, all viable), suspended in PBS (0.5 ml), were inoculated (ip) into each of five groups of 12-week-old Swiss mice (five in each group). After 2 hr, test compound (5 mg/100 g body wt) was administered (ip). A control group was maintained by administering only PBS. On day 7, following inoculation, tumour cells were harvested from the peritoneal cavity of each mouse (the test compound-treated and the control groups) and the total number of viable cells in each case determined. The data are recorded in Table 1.

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