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GLUCOSYLOXY ALKALOIDS FROM *PANCRATIUM BIFLORUM**

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Key Word Index—*Pancratium biflorum*; Amaryllidaceae; proto alkaloids; β-phenethylamine; tyramine; hordenine; alkaloids; lycorine; pseudolycorine; pretazettine; tazettine; glucosyloxy alkaloids; hordenine-4-*O*-β-D-glucoside; lycorine-1-*O*-β-D-glucoside; pseudolycorine-1-*O*-β-D-glucoside; ontogenic variation; alkaloid–metal ion complex; alkaloid–sterol complex; root growth promoter.

Abstract—From fluids of flower stems and bulbs, and from extracts of roots of *Pancratium biflorum*, collected at different stages of growth, three new glucosyloxy alkaloids, viz. hordenine-4-*O*-β-D-glucoside, lycorine-1-*O*-β-D-glucoside and pseudolycorine-1-*O*-β-D-glucoside, have been isolated and characterized. Additionally, three proto alkaloids, β-phenethylamine, tyramine and hordenine, together with four true alkaloids, lycorine, pseudolycorine, pretazettine and tazettine, encountered before in other memebrs of the Amaryllidaceae, have now been isolated also from this species. Ontogenic variations of alkaloidal constituents have been observed. The ability of the alkaloidal constituents to complex with divalent metal ions and phytosterols has been examined with a view to evaluating their significance in plant biochemistry.

INTRODUCTION

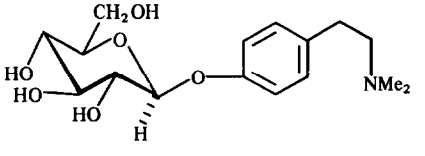
The isolation and characterization of three chromone aglucones, one glucosyl and one glucosyloxy chromones from *Pancratium biflorum* Roxb. (Amaryllidaceae) were reported from this laboratory recently [1, 2]. However, no phytochemical investigation was reported on the alkaloidal constituents of this species. The present paper describes the isolation and characterization of free and glucosyloxy alkaloids from different parts and at different stages of growth of *P. biflorum*. Additionally, the ability of the alkaloids to form complexes with divalent metal ions and phytosterols was examined since involvement and significance of such complexes in plant biochemistry have literature precedents [3, 4].

RESULTS AND DISCUSSION

Sap fluids of flower stems and bulbs of *P. biflorum*, collected daily and at short intervals (2–12 hr), over a period of 9 days (entire period of blooming of a flower stick), showed on prep. PC, TLC and HPLC different amounts of free and glyco alkaloids as well as two free sugars, glucose and rhamnose. Solvent extracts of roots and bulbs at this time contained larger quantities of these constituents. Extensive column and layer chromatography of the gradient-solvent extractives of the roots afforded three alkamines, β-phenethylamine, tyramine and hordenine; four true alkaloids, lycorine, pseudolycorine, pretazettine and tazettine; and three new glyco alkaloids (compounds **1–3**), in quantities sufficient for their complete characterization. Structure elucidation of the three new glyco alkaloids only is described here.

Compound **1**, $C_{16}H_{25}NO_6 \cdot H_2O$, obtained as a hygroscopic solid, was optically active. It responded to Dragendorff's reagent for alkaloids and benzidine metaperiodate reagent for polyols. The UV spectrum of the compound was similar to those of *p*-oxygenated-β-phenethylamines [5]. The UV maxima remained unaffected on addition of sodium acetate and sodium methoxide. The Fe^{3+} test was also negative. In its IR spectrum, the major bands were for $\geq$NMe, *p*-substituted aromatic nucleus and a sugar moiety. The EI-

*Part 7 in the series "Chemical Constituents of Amaryllidaceae". For part 6 see ref. [19].



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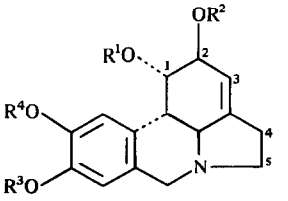

2 $R^1 = \beta$-D-glc; $R^2 = H$; $R^3 + R^4 = -CH_2-$
3 $R^1 = \beta$-D-glc; $R^2 = R^4 = H$; $R^3 = Me$
4 $R^1 = Ac$; $R^2 = Me$; $R^3 + R^4 = -CH_2-$

mass spectrum of the compound did not exhibit any $[M]^+$ peak but appreciable fragment-ion peaks appeared due to the aglycone (*m/z* 165) and a C_6-sugar moiety (*m/z* 162, $[M-18]^+$). Acetylation of the compound formed a tetraacetate, mp 101–103°, $C_{24}H_{33}NO_{10}$ ($[M]^+$, *m/z* 495). The ^1H NMR spectrum of the tetraacetate suggested that all the acetyl functions were attached to the sugar moiety. The anomeric proton signal suggested a β-glucosidic linkage. Hydrolysis of **1** with emulsion gave hordenine and D-glucose. The above data suggested hordenine-4-*O*-β-D-glucoside (**1**) as its structure.

Compound **2**, $C_{22}H_{27}NO_9 \cdot H_2O$, obtained as an amorphous solid, was also optically active. It responded to both Dragendorff's test for alkaloids and the benzidine metaperiodate test for polyols. The Fe^{3+} test was negative. The UV and IR spectra of the compound showed close similarities to those of lycorine. However, unlike lycorine the compound was freely soluble in water from which it could be re-extracted with *n*-BuOH. It fragmented before showing any $[M]^+$ peak in its EI-mass spectrum. The identifiable fragment-ion peaks were due to the aglycone (*m/z* 287) and a C_6-sugar moiety (*m/z* 162, $[M-18]^+$). Hydrolysis with emulsin afforded lycorine and D-glucose. The point of attachment of the glucosyl moiety to lycorine was determined as follows. The compound formed a pentaacetate which exhibited an identifiable $[M]^+$ peak at *m/z* 659. Additionally, significant fragment-ion peaks appeared at *m/z* 617 (from the loss of a ketene moiety from an acetoxy), 331, 271, 169 (glucosyl tetraacetate), 287, 227 and 226 (lycorine). In the ^1H NMR spectrum of the pentaacetate in $CDCl_3$, the signal of the acetoxy attached to the aglucone appeared at δ2.10 which was the normal position for C-2 acetate in lycorine. 1,2-Diacetyllycorine, prepared from lycorine, exhibited acetoxy signals at δ2.08 and 1.93 due to C-2 acetate and C-1 acetate, respectively. The upfield shift in the C-1 acetate resonance was due to shielding by the aromatic ring [6]. The glucosyl moiety is therefore attached at the C-1 hydroxyl of lycorine. The above conclusion finds further support from the fact that the C-2′ acetate signal (δ1.8) of the pentaacetate showed an upfield shift (by *ca* Δδ0.2) from the normal acetoxy signals (δ2.1–2.0) in aliphatic glycosides [7]. A concomitant shielding of the H-11 resonance was also observed. Methylation of **2** with sodium hydride and methyl iodide, in tetrahydrofuran, according to ref. [8], gave the permethyl ether. Cautious hydrolysis of this product followed by acetylation of the aglucone afforded a monoacetylmonomethyl derivative, mp 198–202°, which exhibited the acetoxy signal at δ1.92 (C-1 OAc), H-1 (*br*) signal at δ5.72 and H-11 (line broadening due to long range coupling with H-1) at δ6.70. Irradiation of the H-11 signal caused NOE at H-1 by *ca* 16% and attenuation of the broad signal. The C-2 methoxyl signal appeared at δ3.4 which is the normal position for an aliphatic methoxyl in the Amaryllidaceae alkaloids [9]. Hence, compound **2** was assigned the structure lycorine-1-*O*-β-D-glucoside.

Compound **3**, $C_{22}H_{29}NO_9 \cdot H_2O$, obtained as an amorphous solid, was also optically active. The Fe^{3+} test was positive. It exhibited UV maxima in methanol, and in the presence of the usual basic shift reagents, characteristic of pseudolycorine. The IR spectrum showed major bands due to hydroxyl, ArOMe and sugar moieties. On hydrolysis with emulsin, as also with dil. hydrochloric acid, it gave pseudolycorine and D-glucose. The compound formed a hexaacetate ($[M]^+$, *m/z* 703), mp 103–107°, which showed fragment-ion peaks in its mass spectrum, due to pseudolycorine (after elimination of the acetoxy) and glucose tetraacetyl fragments. The assignments of the signals in the ^1H NMR spectrum of the hexaacetate were made as described for the pentaacetate of **2**. Thus, the hexaacetate showed two acetoxy signals at δ1.78 (C-2′ OAc) and 2.08 (C-2 OAc) suggesting the attachment of the glycosyl at C-1 hydroxyl. Hence, compound **3** was assigned the structure pseudolycorine-1-*O*-β-D-glucoside.

Compounds **1–3** have not been encountered before in nature nor was any one of them prepared before synthetically. In fact, this is the first report of natural occurrence of any glucosyloxy alkaloid in the family Amaryllidaceae.

Large amounts of free sugars (15–20%) were previously reported in *Amaryllis* bulbs [10]. The source was, however, commercially not viable because of the pronounced toxicity of its extracts to man and cattle alike. From the coocurrence of alkaloids and sugars, often in large quantities, the presence of glycoalkaloids in Amaryllidaceae species should be anticipated, but this has neither been considered nor been tested before experimentally. Preliminary screening in our laboratory with three other Amaryllidaceae species, viz. *Crinum latifolium*, *Haemanthus kalbreyeri* and *Zephyranthes flava*, has shown that occurrence of glycoalkaloids in the Amaryllidaceae is a common phenomenon. It is therefore a little surprising that glycoalkaloids of the Amaryllidaceae have, until this investigation, eluded the notice of phytochemists. Two factors may have contributed to this omission: (i) the glycoalkaloids are produced and present, in substantial amounts, only for a limited period of time during ontogeny of the producer organisms; (ii) strongly polar extractives (e.g. *n*-BuOH), which contain mainly glycoalkaloids, have not been examined.

The other noteworthy observations made during this investigation were: (i) the transitory existence of certain advanced alkaloidal intermediates; (ii) rapid gain and loss of certain alkaloids during intense active growth of the producer organism. Sap fluid samples of flower stems of *P. biflorum* were initially collected, at daily intervals, for 9 days and were subjected to PPC and HPLC when the presence of alkaloids and free sugars was detected. A gradual increase in the contents of the glycoalkaloids and sugars was observed up to day 3 of flowering which was followed by a sharp fall in their concentrations as the flowers started wilting (days 5–8). When the experiment was conducted at short (2-hourly) intervals, quite surprising results were obtained. Rapid changes in the alkaloidal patterns were discernible; many minor alkaloids and glycoalkaloids (which are yet to be fully characterized) appeared and quickly disappeared. There has been observed gain and loss, in inverse proportions, in respect of lycorine, pseudolycorine and the corresponding glycosides (**2** and **3**), respectively. This observation seems to suggest that a reversible reaction—alkaloid aglucone + glucose ⇌ alkaloid glucoside—might be operative during the intense cellular activity of *P. biflorum*. The glucoalkaloids were subsequently found to be accumulated in the roots (*ca* 0.3%) and bulbs (*ca* 0.1%), at the post-flowering stage (covering a period of *ca* 8 weeks). During the resting period, these constituents were present only in traces.

Although the biochemical significance of the above

observations has not yet been entirely elucidated but the following observations might be of relevance. (i) The glucosyloxy alkaloids (**1–3**) and their aglucones formed stable complexes with divalent metal ions (e.g. Cu^{2+}, Zn^{2+}, Fe^{2+}) and were able to translocate them from the rhizosphere to aerial parts. Alkaloids are known to elicit their activities by modulating the transport of metal ions across biological membranes [3]. The glucosyloxy alkaloid (**2**)-Cu^{2+} complex was found to produce significant root growth in *P. biflorum* as also in other Amaryllidaceae species, e.g. *Z. flava*. Lycorine–Cu^{2+} complex, on the other hand, was a root growth inhibitor. These observations are not unprecedented because a number of alkaloids were found to be toxic when applied as extraneous agents to plants that normally produce them [11]. However, the difference in activity between the glucosyloxy alkaloid–metal ion and the aglucone–metal ion complex is noteworthy. (ii) The glucosyloxy alkaloids (**1–3**) formed stable complexes with phytosterols, e.g. sitosterol, stigmasterol and campesterol, when examined according to ref. [4]. Complexation of glucosyloxy alkaloids of the Solanaceae with phytosterols is well documented [4, 12]. One of the biochemical effects of this complexation was considered to be cell binding [12]. The aglucones, corresponding to **1–3**, formed only weak complexes with the above sterols. The glucosyloxy alkaloid (**1–3**)–sterol complexes also promoted root growth in the above mentioned species.

EXPERIMENTAL

General. All mps are uncorr. UV spectra were recorded in MeOH; UV shift reagents were prepared and used according to ref. [7]. IR spectra were recorded in Nujol or KBr and only the major bands are quoted. EIMS were recorded at 70 eV; the samples were directly inserted by probe. 90 MHz ^{1}H NMR spectra were determined in $CDCl_3$ and/or DMSO-d_6 using TMS as an int. standard. Separation by CC was carried out with silica gel (BDH, 60–120 mesh). Analytical TLC was done with silica gel G (Merck, plate thickness, 0.2 mm, plate 1) or with silica gel G/UV_{254} (Machery-Nagel precoated, plate 2). Three solvent systems, viz. C_6H_6–HOAc (50:1, solvent 1), $CHCl_3$–HOAc (50:1, solvent 2), $CHCl_3$–MeOH–Et_2NH (90:5:5, solvent 3) were used. UV (λ_{254}), I_2 vapour, $FeCl_3$, Dragendorff (D), benzidine metaperiodate (BMP) and tetrazotized-*o*-anisidine (TDA) reagents were used for visualization. For PC of sugars, Whatman No. 1 was used; EtOAc–pyridine–H_2O (13:5:4) was used as developer and $AgNO_3$ or aniline oxalate for vizualization. For analytical HPLC, a 440/254 nm detector was used.

Extraction. In a typical expt, fresh roots* (*ca* 5 kg), collected soon after flowering, were macerated with MeOH and the extract was kept for 3 days at room temp. After filtration (Buchner) over a little celite, the solvent was removed at ~ 40°, when a brown viscous residue was obtained. It 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 portion was treated with aq. HOAc (4%, 400 ml), a brown solid that separated was collected by filtration (fraction B). The clarified aq. acidic soln was extracted with Et_2O (Et_2O-soluble acetates, fraction C) and then basified with NH_4OH. The liberated bases were extracted, in succession, with Et_2O (fraction D), EtOAc (fraction E), and *n*-BuOH (fraction F). The aq. mother liquor was preserved for further treatment with NH_4^+ Reineckate.

Treatment of fraction A. This fraction was further extracted with aq. citric acid (2 N, 100 ml). The aq. acidic extract was basified with NH_4OH and the liberated bases were extracted with Et_2O. The organic layer was worked up in the usual way to give a brown residue.

β-Phenethylamine. The basic brown gummy material was triturated with *n*-hexane. The hexane-soluble fraction gave β-phenethylamine as a viscous liquid (0.11 g); yellow picrate from EtOH, mp and mmp (with picrate of β-phenethylamine) 171–173°; the identity of the compound was established by direct comparison (co-TLC, UV, ^{1}H NMR) of the base with an authentic sample of β-phenethylamine [5].

Treatment of fraction B. This fraction afforded the polyoxygenated chromones as described before [1, 2].

Treatment of fraction C. The residue from this fraction was triturated with dil HCl (1 N, 50 ml). The aq. acidic soln was extracted with $CHCl_3$ to separate the $CHCl_3$-soluble (fraction c_1) from $CHCl_3$-insoluble (fraction c_2) alkaloid hydrochlorides.

Treatment of fraction c_1. This fraction on usual work up afforded a brown amorphous solid (78 mg) which showed two major spots on analytical TLC (plate 1, solvent 2): R_f 0.55 (I_2 and Fe^{3+} positive; TDA, yellowish-orange); 0.42 (I_2 and D, positive; TDA, brown). The mixture was dissolved in MeOH (5 ml) and applied to a column of Amberlite IRA-400 (HO^-) (20 × 1 cm). The column was eluted with aq. MeOH (30%) to give the non-phenolic alkaloid, R_f 0.42, first. Subsequent washing with MeOH–HOAc–H_2O (6:3:1) afforded the phenolic compound, R_f 0.55.

Pretazettine. The non-phenolic alkaloid was further purified by passing its concd $CHCl_3$ soln through a short column of silica gel G. Elution was carried out, under a small pressure, with C_6H_6–EtOAc (9:1) as eluant. Fractions (25 ml) were collected and monitored by TLC. Fractions 8–11 were combined and concd to give pretazettine as a straw coloured solid (17 mg), mp 170–180° (with prior softening around 120°); UV λ_{max} nm (log ε): 240 sh (3.64), 288 (3.55); the MeOH soln was unstable in the presence of alkali; IR ν_{max} cm^{-1}: 3450 (broad), 2830, 1618, 1595, 1480, 940; MS *m/z* (rel. int.): 331 $[M]^+$ (62%), 316 (14), 315 (9), 314 (7), 300 (9), 247 (100); ^{1}H NMR ($CDCl_3$): δ6.82 (1H, *s*, Ar–H), 6.51 (1H, *s*, Ar–H), 6.17 (1H, *m*, H-1), 6.08 (1H, *s*, H-8; in lieu of the characteristic AB pattern at δ4.8 due to the two benzylic protons of tazettine) [14, 15], 5.92 (2H, *s*, OCH_2O), 5.84 (1H, *br*, H-2), 3.40 (3H, *s*, OMe), 2.42 (3H, *s*, NMe). An aq. EtOH soln of the alkaloid on heating for 4 hr at 100° was converted to tazettine, mp and mmp 206–208° (co-TLC, ^{1}H NMR).

Tyramine. The phenolic compound, from the resin column, was obtained as a snuff coloured solid which crystallized from EtOH–Me_2CO as light brown microcrystals (22 mg), mp and mmp (with tyramine) 158–161°. Its identity was established by comparison of its spectral properties (UV, ^{1}H NMR, MS) with those of a reference sample of tyramine [5, 13].

Processing of fraction c_2, in the usual way, afforded lycorine (92 mg), mp and mmp 256–258° (co-TLC, UV, IR).

Treatment of fraction D. The residue (0.22 g) from this fraction showed 4 major Dragendorff-positive spots on analytical TLC. The mixture was triturated with hot Me_2CO and the Me_2CO-soluble (fraction d_1) and -insoluble (fraction d_2) fractions were separated by filtration.

Treatment of fraction d_1. This fraction afforded a light yellow

*The plant species, cultivated in Varanasi, was identified by Dr. S. K. Roy, Reader, Department of Botany, Faculty of Science, Banaras Hindu University. The plants used for larger extractions were collected for 3 consecutive years during the flowering season (August to October, 1979–1981) and at post-flowering (November–March) stages. The different parts were separately processed for chemical constituents.

solid, mp 90–170°, and was clearly a mixture. It showed two major Dragendorff-positive spots on TLC (plate 2, solvent 3), R_f 0.54 and 0.65.

Tazettine. Repeated crystallization of the above mixture from C_6H_6–Me_2CO afforded the less polar component as shining needles (31 mg), mp 202–204°; $[\alpha]_D^{20}$ +160.5° (*c* 0.68, $CHCl_3$); MS *m/z* (rel. int.): 331 $[M]^+$ (22%), 316 (20), 298 (18), 260 (9), 247 (100), 219 (7), 201 (12); ^{1}H NMR ($CDCl_3$): δ6.85 (1H, *s*, H-12), 6.50 (1H, *br s*. line broadening due to coupling with H-8, H-9), 6.15 (1H, *ddd*, *J* = 10.5, 2.0, 1.2 Hz, H-2), 5.90 (2H, *s*, OCH_2O), 5.60 (1H, *ddd*, *J* = 10.5, 2.0, 1.5 Hz, H-1), 4.95 (1H, *dd*, *J* = 14.7, 0.5 Hz, H_{eq}-8), 4.65 (1H, *dd*, *J* = 14.7 Hz, H_{ax}-8), 4.13 (1H, *m*, H_{ax}-3), 3.45 (3H, *s*, C_3-OMe), 3.30 (1H, *d*, *J* = 10.5 Hz, H-6), 2.83 (1H, *m*, H_{eq}-4a), 2.65 (1H, *d*, *J* = 10.5 Hz, H-6), 2.50 (1H, OH, exchangeable with D_2O), 2.40 (3H, *s*, NMe), 2.20 (1H, *m*, H_{eq}-4α), 1.60 (1H, *m*, H_{ax}-4β). The physical and spectral properties of the alkaloid were indistinguishable from those of tazettine reported in lit. [7, 16].

The C_6H_6–Me_2CO mother liquor, after separation of tazettine, was chromatographed over a column of silica gel (24 × 3 cm). Elution was carried out with C_6H_6, $CHCl_3$, MeOH and different proportions of mixtures therof. Fractions (100 ml) were collected and monitored by TLC. The $CHCl_3$ eluates afforded a further crop of tazettine (14 mg).

Hordenine. The $CHCl_3$–MeOH (99 : 1) eluates were combined and evapd to give an amorphous solid which crystallized from *n*-hexane–C_6H_6 as light brown needles (28 mg), mp and mmp (with hordenine) 116–118°; direct comparison (co-TLC, UV, ^{1}H NMR, MS) with an authentic sample of hordenine [5] established that they were identical.

Treatment of fraction d_2. This fraction crystallized from EtOH as colourless prisms (42 mg), mp and mmp (with lycorine) 257–259°; $[\alpha]_D^{20}$ −78.8° (*c* 0.82, EtOH); direct comparison (co-TLC, UV, IR, ^{1}H NMR, MS) with an authentic sample of lycorine [17] established that they were identical.

Treatment of fraction E. This fraction was concd (*ca* 100 ml) and kept at room temp overnight when a further crop of lycorine (0.87 g) was obtained. The EtOAc mother liquor, after separation of lycorine, was evapd to give an amorphous solid which showed two major spots on TLC (plate 2, solvent 3): R_f 0.24 (D, positive; BMP, positive) and 0.32 (D, positive; BMP, negative). The solid was triturated with a large volume of Et_2O when Et_2O-soluble (fraction e_1) and -insoluble (fraction e_2) fractions were separated.

Treatment of fraction e_1. Evapn of the solvent from this fraction afforded the less polar component, R_f 0.32, as a brown solid.

Pseudolycorine. The above solid crystallized from $CHCl_3$–MeOH as light brown needles (12 mg), mp 247–249°; $[\alpha]_D^{20}$ −57.8° (*c* 0.3, EtOH); MS *m/z* (rel. int.): 289 $[M]^+$ (40%), 274 (9), 270 (5), 258 (6), 229 (42), 228 (100), 186 (14), 185 (7), 158 (4); ^{1}H NMR ($CDCl_3$–DMSO-d_6): δ6.78 (1H, *s*, H-11), 6.62 (1H, *s* with line broadening, H-8), 5.41 (1H, *m*, H-3), 4.8–4.7 (2H, *br*, exchangeable with D_2O, OH), 4.5–2.0 (10H, *m*); in presence of NaOD–D_2O, the maximum upfield shift was experienced by H-11 (δ6.78 → 6.64); tri-*O*-acetate, crystallized from MeOH–Me_2CO as colourless plates, mp 202–204°; MS *m/z* (rel. int.): 415 $[M]^+$ (28%), 400 (7), 373 (9), 358 (5), 356 (6), 355 (4), 314 (5), 272 (3), 229 (22), 228 (44), 43 (68), 42 (100). The physical and spectral properties of the alkaloid and its acetate derivative suggested its identity with pseudolycorine [18]. The alkaloid is of rare natural occurrence and its detail spectral properties are also reported here for the first time.

Treatment of fraction e_2. This fraction crystallized from EtOH–dioxane as a brown hygroscopic solid (37 mg) which was preserved in a desiccator.

Hordenine-4-O-β-D-glucoside (**1**). The above solid had mp 100–108° (dec); R_f 0.14 (plate 1, solvent 2), 0.24 (plate 2, solvent 3), D and BMP-positive; $[\alpha]_D^{20}$ −55.4° (*c* 0.48, EtOH); λ_{max} nm (log ε): 225 (3.92), 266 sh (3.61), 275 (3.52), 305 sh (2.98); no shift of UV maxima in presence of either NaOAc or NaOMe; IR: ν_{max} cm^{-1}: 3400 (*br*, OH), 2835 (NMe), 1650 (*br*, sugar moiety), 1600 (subst. Ar), 1040 (OH); MS *m/z* (rel. int.): 165 (14), 164 (9), 163 (8), 162 (5), 107 (22), 58 (100). (Found: C, 56.80; H, 7.55; N, 3.83. $C_{16}H_{25}NO_6 \cdot H_2O$ requires: C, 57.14; H, 7.73; N, 4.16.)

Hordenine-4-*O*-β-D-glucoside tetraacetate was prepared. Compound **1** (7 mg), Ac_2O (1 ml) and pyridine (0.2 ml) were mixed and kept at room temp overnight. This was followed by heating the mixture at 100° for 1 hr. The product was worked up in the usual way to give the tetraacetate as colourless needles from EtOH, mp 101–103°; IR ν_{max} cm^{-1}: 2832, 1750, 1612, 1208; MS *m/z* (rel. int.): 495 $[M]^+$ (2%), 331 (100), 271 (11), 169 (27), 165 (18), 164 (7), 58 (100); ^{1}H NMR ($CDCl_3$): δ7.16 (2H, *d*, *J* = 9.5 Hz, H-3, H-5), 6.80 (2H, *d*, *J* = 9.5 Hz, H-2, H-6), 5.28 (3H, *m*, H-1′, H-3′, H-4′), 4.0 (4H, *m*, H-2′, H-5′, H-6′), 3.0 (4H, methylene protons), 2.4 (6H, *s*, NMe_2), 2.0–1.8 (12H, 4 × OAc).

Treatment of fraction F. This fraction afforded a brown gummy material which showed several D- and BMP-positive spots on TLC. It was repeatedly extracted with hot Me_2CO and the Me_2CO-soluble (fraction f_1) and -insoluble (fraction f_2) fractions were separated.

Treatment of fraction f_1. This fraction on concn and standing gave a further crop of lycorine (0.21 g). The Me_2CO mother liquor contained a mixture of four alkaloids, as minor entities, which were separated by prep. TLC and one was identified as pseudolycorine (3 mg).

Treatment of fraction f_2. This fraction was obtained as a straw coloured solid (1.8 g) which showed two major and five minor D- and BMP-positive spots on TLC. A portion (0.2 g) of the solid was dissolved in MeOH (10 ml) and chromatographed over a column of silica gel (30 × 3 cm). Elution was carried out with $CHCl_3$ (2 l.), $CHCl_3$–MeOH (9 : 1, 5 l; 3 : 1, 2 l; 1 : 1; 2 l.) and MeOH (5 l.). Fractions (500 ml) were collected and monitored by TLC.

Lycorine-1-O-β-D-glucoside (**2**). Fractions 9–14 were combined and the solvent was evapd under red. pres. to give a brown solid (55 mg); R_f 0.32 (plate 2, solvent 3); $[\alpha]_D^{20}$ −92.4° (*c* 0.53, MeOH); UV λ_{max} nm (log ε): 235 sh (3.5), 288 (3.61); IR ν_{max} cm^{-1}: 3450 (*br*), 1650–1600 (*br*), 1590, 1505, 1260, 1240, 1185, 1158, 1132, 940; MS *m/z* (rel. int.): 287 (14), 227 (95), 226 (100), 163 (5), 162 (4). (Found: C, 56.30; H, 5.82; N, 2.62. $C_{22}H_{27}NO_9 \cdot H_2O$ requires: C, 56.52; H, 6.21; N, 2.99.)

2-Pentaacetate (prepared by treatment with Ac_2O and pyridine in the usual way) crystallized from Et_2O–EtOH as platelets, mp 98–101°; MS *m/z* (rel. int.): 659 $[M]^+$ (2%), 617 (7), 331 (68), 287 (12), 271 (62), 227 (72), 226 (100), 169 (58); ^{1}H NMR ($CDCl_3$): δ6.68 (1H, *s*, H-11), 6.56 (1H, *s*, H-8), 5.94 (2H, *s*, OCH_2O), 5.75 (1H, *m*, H-1), 5.62 (1H, *m*, H-3), 5.30 (4H, *m*, H-2, H-1′, H-3′, H-4′), 4.15 (5H, *m*, H-7, H-2′, H-5′, H-6′), 3.50 (1H, *br*, H-7), 2.10 (3H, *s*, C-2 OAc), 2.02–1.98 (9H, glucosyl C-3′, C-4′, C-6′, OAc), 1.8 (3H, C-2′, OAc).

Pseudolycorine-1-O-β-D-glucoside (**3**). Fractions 22–27 were combined and evapd to give a brown hygroscopic solid (53 mg). It was repeatedly dissolved in MeOH and pptd with Et_2O when **3** was obtained as a light brown powder, R_f 0.12 (plate 2, solvent 3; D-, BMP- and Fe^{3+} test positive); $[\alpha]_D^{20}$ −105.5° (*c* 0.3, MeOH); UV λ_{max} nm (log ε): 238 sh (3.38), 288 (3.55); $\lambda_{max}^{MeOH-NaOMe}$ nm: 252 sh, 294; IR ν_{max} cm^{-1}: 3400 (br), 1650 (br), 1618, 1585, 1040, 835; MS: *m/z* 289 (18), 274 (11), 271 (7), 163 (5), 162 (4). (Found: C, 55.84; H, 5.50; N, 2.60. $C_{22}H_{29}NO_9 \cdot H_2O$ requires: C, 56.28; H, 6.61; N, 2.98). Hexaacetate crystallized from Me_2CO–MeOH as microcrystals, mp 103–107°; IR ν_{max} cm^{-1}: 1768 (Ar–OAc), 1750

(aliphatic OAc), 1628, 1595, 1208; MS *m/z* (rel. int.): 703 $[M]^+$ (1.5%), 631 (3), 619 (22), 577 (11), 331 (88), 289 (42), 271 (38), 229 (48), 169 (100); ^1H NMR ($CDCl_3$): δ6.71 (1H, *s*, H-11), 6.52 (1H, *s*, H-8), 3.98 (3H, *s*, Ar–OMe), 2.08 (3H, *s*, C-2 OAc), 2.0–1.78 (12H, sugar acetoxy).

Enzymatic hydrolysis of glucoalkaloids (**1–3**). Each compound (*ca* 10 mg) was dissolved in aq. NaOAc–HOAc buffer (pH ~ 5) (10 ml) and emulsin (12 mg) was added. The mixture was allowed to stand overnight at 37° and then filtered. The filtrate was extracted with EtOAc and the organic layer worked up in the usual way to give the respective aglucones (hordenine from **1**, lycorine from **2** and pseudolycorine from **3**). The identity of the hydrolysed products were established by direct comparison (mp, mmp, co-TLC, UV) with reference samples. The sugar component present in the aq. mother liquor was processed in the usual way and its identity with D-glucose was established by direct comparison (PC).

Permethylation of **2**. To a suspension of **2** (15 mg) in Na dried THF (5 ml), NaH (80 mg) was added and the mixture stirred under N_2 for 1 hr. It was cooled, MeI (1 ml) was added and the mixture was again stirred for 6 hr. The solvent was evapd under red. pres. and the residue was partitioned between Et_2O and H_2O (1:1). After further dilution with H_2O, the Et_2O layer was worked up to give a colourless gummy material (15 mg) which showed a greenish-blue fluorescence, R_f 0.38, (plate 2, solvent 3), under UV light. The compound was completely methylated as it did not exhibit any OH band in its IR spectrum.

Hydrolysis of per-Me ether of **2**. The per-Me ether was dissolved in dil HCl (1 N, 10 ml) and kept at room temp overnight. It was warmed at 60° for 30 min, cooled and basified (NH_4OH). The liberated base was extracted with Et_2O. The Et_2O extract was worked up in the usual way to give an amorphous solid, R_f 0.55 (plate 1, solvent 2), 0.48 (plate 2, solvent 2), D-positive; BMP-negative. The presence of 2,3,4,6-tetra-*O*-methylglucose in the aq. hydrolysate was detected by PPC and by GC of the corresponding alditol acetate prepared according to ref. [2].

1-O-*Acetyl*-2-O-*methyllycorine* (**4**). The above amorphous solid (9 mg) was acetylated with Ac_2O (1 ml) and pyridine (0.2 ml) at room temp overnight. The mixture was dried *in vacuo* and the residue crystallized from EtOH as colourless needles (7 mg), mp 198–202°; R_f 0.62 (plate 2, solvent 2); MS *m/z* (rel. int.): 343 $[M]^+$ (22%), 312 (5), 284 (4), 282 (3), 251 (4), 227 (100), 226 (100); ^1H NMR ($CDCl_3$): δ6.70 (1H, *s*, H-11), 6.64 (1H, *s*, H-8), 5.93 (2H, *s*, OCH_2O), 5.72 (1H, *br*, H-1), 5.50 (1H, *m*, H-3); 3.4 (3H, *s*, C-2 OMe), 1.92 (3H, *s*, C-1 OAc).

Ontogenic variations of alkaloids and sugars. The sap fluid samples of flower stem and bulbs collected by a hypodermic needle, at short (2 hr) and daily intervals, were dissolved in MeOH and lyophilized. Aliquots (10–30 μl) of the extractives and reference samples of free and the glucoalkaloids, in MeOH–H_2O (4:1), were introduced into a reversed phase C_{18} column (30 m × 3.8 mm) using the same solvent system as the eluant. The amounts of the individual samples were determined from the peak areas in the HLPC chromatogram.

Free glucose and rhamnose present in the sap fluid samples were detected by PC and TLC using respective reference samples. The amounts of the free sugars were estimated as their alditol acetates according to ref. [2].

Glucoalkaloid–metal ion complexes. All three glucoalkaloids (**1–3**) formed stable metal ion (Cu^{2+}, Zn^{2+}, Fe^{2+}) complexes when their aq. EtOH solns were mixed and stood at room temp overnight. The concn of metal ion in the respective complex was estimated by atomic absorption spectroscopy in respect of the metal ion. The uptake of the metal ions by the seedlings of *P. biflorum* was enhanced by the addition of the glucoalkaloids (**1–3**) in the nutrient soln in which the roots were submerged. Ashes obtained from weighed quantities of control and the glucoalkaloid-treated plants were leached with HCl. The metal ion concn of aliquots of the acidic solns were determined by atomic absorption spectroscopy. The mean number of roots in the treated plants was *ca* 5 times greater than that of the control.

Glucoalkaloid–phytosterol complexes. The glucoalkaloids (**2** and **3**) formed EtOH-soluble complexes with each of stigmasterol, campesterol and sitosterol. The unbound sterol in the EtOH soln was estimated by GC according to ref. [4]. The amount of the unbound sterol, in each case, was found to be inversely proportional to the amount of the glucoalkaloid used. The glucoalkaloid–phytosterol complexes also potentiated the root growth of *P. biflorum*, *Z. flava*, and *H. kalbreyeri* when tested as above.

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