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PARASITISM OF *IMPERATA CYLINDRICA* ON *PANCRATIUM BIFLORUM* AND THE CONCOMITANT CHEMICAL CHANGES IN THE HOST SPECIES*

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Key Word Index—*Imperata cylindrica*; Gramineae; phanerogamic parasite; oligosaccharides; *Pancratium biflorum*; Amaryllidaceae; phytoalexins; flavans; chalcone; quinone methide; alkaloids.

Abstract—A rare incidence of phanerogamic parasitism of *Imperata cylindrica* on *Pancratium biflorum* and the concomitant changes in the chemical constituents, from the hypersensitive responses in the host species, are reported.

INTRODUCTION

Previously, the isolation and characterization of three chromone aglucones, one glucosyl and one glucosyloxy chromone, three glucosyloxy alkaloids, and several proto and true alkaloids of the Amaryllidaceae were reported from the different parts of *Pancratium biflorum* Roxb. [1–3]. In this paper, a rare incidence of phanerogamic parasitism by *Imperata cylindrica* Beauv. on *P. biflorum* and the concomitant chemical changes from the hypersensitive responses in the host species are reported.

RESULTS AND DISCUSSION

Imperata cylindrica (synonymous with *I. arundinaceae* Cyrill.) is a common grass widely distributed in the hotter parts of India from the Punjab southwards and eastwards to Malacca and Ceylon. Its roots are used in traditional medicine as an emollient, an anti-febrile agent, in fumigation of haemorrhoids and as a restorative tonic [4]. In the present study, *I. cylindrica* was found to grow abundantly in the vicinity of the bulbed plant, *P. biflorum*, widely distributed in the upper Gangetic plain. The main roots of the grass were often found to penetrate the bulbs of *P. biflorum*, sometimes two in succession (Fig. 1). The penetration, mediated by an haustoria, was restricted to the peripheral region (fleshy leaves) of the bulbs. The tip of the haustoria was dome-shaped with a sharply pointed beak. The internodes of the haustoria, below the dome, were closely spaced to provide strong mechanical pressure during the penetration. An abundant growth of adventitious roots of the parasite, near the points of entry to and emergence from the bulbs was noticed. There was no ramification of roots of the parasite inside the bulbs of the host species. A transverse section of the invaded bulbs showed a dark lesion along the course of the traversing parasite root (Fig. 2). Microscopic analysis further revealed that the host cells, in contact with the root, were necrotic and formed a thick crust. The cells in proximity to this region were thin-walled, inflated and filled with a red substance. The red substance was unevenly distributed downward for up to 3 to 4 cell layers and mainly accumulated at the angular positions of the intercellular space. When a section of the red zone was placed in lactophenol solution, it immediately became yellow.

The above observations and the fact that there are only a few seed plants (phanerogams), compared to the innumerable fungi, bacteria and nematodes, which are parasitic on living plants [5], prompted us to examine the chemical constituents of the affected tissue and to evaluate their biological significance. In particular we sought to answer the following question: (i) are the chemical constituents in the red necrotic zone present in diffusible forms? (ii) do they function in disease development/prevention? (iii) how do they function? (iv) why has *I. cylindrica*, which is capable of independent existence, opted to become partially parasitic on *P. biflorum*?

Trituration of the red necrotic zone with methanol afforded a reddish-purple solution, which on fractionation afforded one known and one new flavan unsubstituted in the heterocyclic ring [6], one new chalcone, one new quinone methide (anhydrobase) flavan, named bifloridin, three known Amaryllidaceae alkaloids, i.e. hippadine (**6**) [7], lycorine (**7**) [7], ungeremine (**10**) [8] and two new alkaloids, 4,5-dehydroanhydrolycorine (**5**) and pancrassidine (**11**). Characterization of the new compounds only is described here.

Polyphenolics

Aside from a known flavan, 7-hydroxy-4′-methoxyflavan (**1**) [6], three new polyphenolics (compounds **2**–**4**) were isolated and characterized.

Compound **2**. This compound, $C_{17}H_{18}O_4$ (M^+ and elemental analyses), was optically active and exhibited UV and IR spectra characteristic of naturally occurring flavans [6]. The EIMS of the compound was also typical of a flavan consisting of a hydroxymethoxy A-ring and a methoxy-containing B-ring [6]. The chemical shifts, coupling patterns and constants in the ^1H NMR spectrum

*Part 20 in the Series "Chemical Constituents of Amaryllidaceae". For Part 19 see ref. [22].

Fig. 1. *Imperata cylindrica* penetrating two bulbs of *P. biflorum* in succession. Note the abundant growth of roots of *I. cylindrica* near the points of entry to and emergence from the bulb. Note also the dark scales at the points of puncture of the bulb.

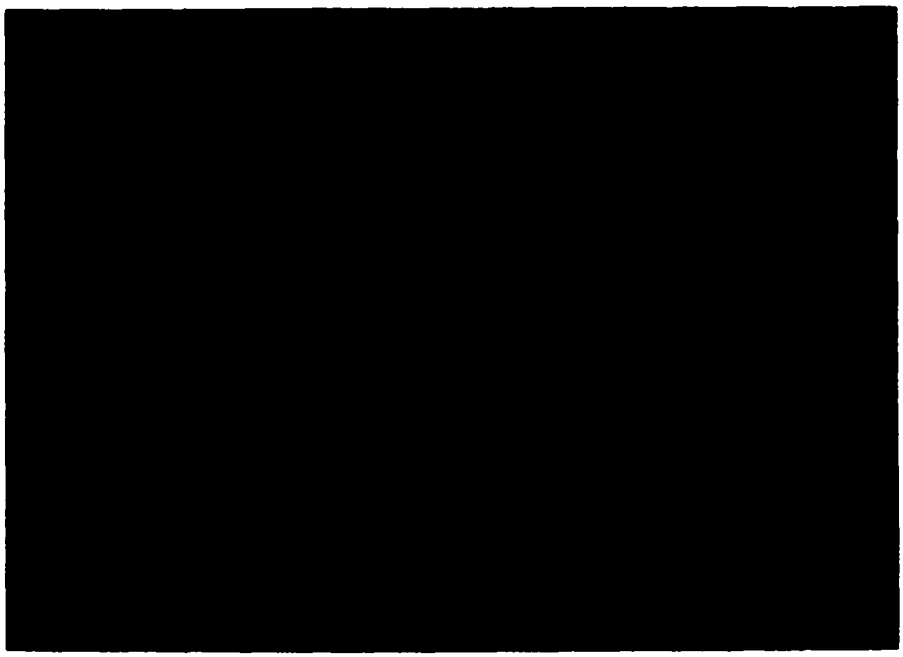


Fig. 2. Transverse section of a bulb of *P. biflorum* invaded with *I. cylindrica*. Note the absence of ramification of the parasite roots inside the bulb. Note also the dark scale (red) associated with the traversing root inside the bulb.

of the compound suggested the 5,4'-dimethoxy-7-hydroxyflavan structure (**2**) for this compound.

Compound **3**. This compound, $C_{17}H_{16}O_4$ (M^+ and elemental analysis), was obtained as a yellow powder and was optically inactive. It exhibited UV and mass spectra characteristic of a dihydroxy-dimethoxychalcone. The location of the hydroxy-methoxy functions (as in **3**) was established by the ^1H NMR spectral data of the compound and from its conversion into **2** and **4**.

Compound **4** (*bifloridin*). This compound, $C_{17}H_{14}O_4$, was obtained as a reddish-purple powder. The spectral (UV, IR, ^1H NMR, mass) properties of the compound were typical of quinone methide flavans of the nor-dracorhodin type [9]. It was unstable in solution. Its UV

spectrum in methanol exhibited a growing absorption with a maximum around 385 nm indicating its transformation into a polyoxygenated chalcone [10]. Co-HPLC with 3 suggested it to be identical with this chalcone. Likewise, when a solution of the chalcone 3, in chloroform, was kept at ordinary temperature, a mixture resulted from which bifloridin was obtained by prep. TLC. The flavan 2, on oxidation with DDQ, in dioxan, was converted into bifloridin which on reduction with sodium borohydride in methanol, regenerated 2. Thus structure 4 was assigned to bifloridin.

That compounds 3 and 4 are not artefacts but native in the necrotic tissue of *P. biflorum* was established by analytical HPLC of a fresh methanolic extract without any processing. The complex nature of the reddish-purple powder, as revealed from its TLC and HPLC, suggested the presence of other quinone methide bases in addition to 4. Further studies to isolate and characterize these bases are currently in progress.

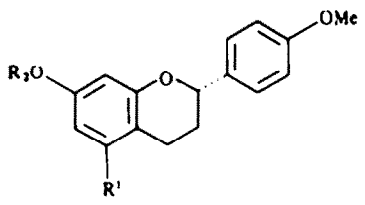
Alkaloids

4,5-Dehydroanhydrolycorine (5) was known before only as a synthetic substance [11]. This is the first report of its isolation from a natural source. We have obtained additional spectral data for this compound.

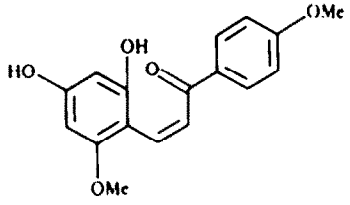
Pancrassidine (11). This compound, $C_{16}H_{17}NO_5$ (M^+ and elemental analyses), was optically active. Its UV spectrum was closely similar to that of narcissidine [12]. The maxima remained unchanged on addition of sodium acetate and sodium methoxide. The Fe^{3+} test was negative. The presence of a methylenedioxy group and three alcoholic hydroxyl groups in the molecule was suggested by its IR and ^1H NMR (D_2O exchange) spectra. The EIMS of the compound exhibited, aside from the molecular ion peak at m/z 303, a significant fragment-ion peak at m/z 243 $[M-60]^+$, formed by the loss of $C_2H_4O_2$. A similar fragmentation was observed in narciclasine, which contains three vicinal hydroxyl groups in the C-ring [13]. Acetylation (Ac_2O–pyridine) of pancrassidine, formed a triacetate, $C_{22}H_{23}NO_8$, mp 201–204°, in which all the acetyl functions were attached to the alcoholic hydroxyl groups (^1H NMR). When the alkaloid was heated above its mp, under N_2, aromatization of the C-ring occurred to give 4,5-dehydroanhydrolycorine (5). Thus structure 11 was assigned to pancrassidine.

Oligosaccharides

The methanol insoluble residue (fraction D), a straw coloured powder, softened at 208–215° but did not melt up to 300°. It was soluble in water and gave a positive benzidine–metaperiodate test for polyols. The substance was strongly polar in nature as was suggested from its analytical TLC. Attempts were made to purify the compound by fractional precipitation from its aqueous solution with ethanol. Its average M_r was determined as 10 500 by Sephadex G-50 Gel filtration chromatography [14]. Acid hydrolysis of the compound followed by the usual work up [15] indicated it to be composed of an oligosaccharide(s) (68.5%) [consisting of glucose and



1 $R^1 = R^2 = H$
2 $R^1 = OMe$, $R^2 = H$



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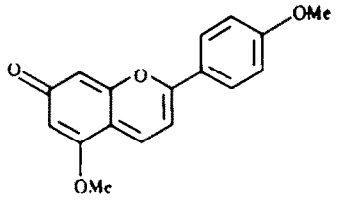

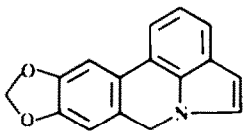

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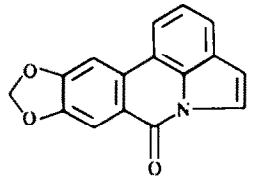

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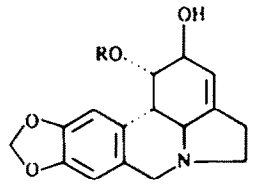
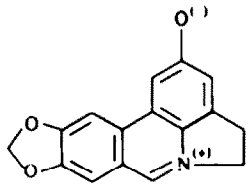


7 R = H
8 R = β-D-Glc
9 R = 6′-*O*-Palmitoyl-β-D-Glc



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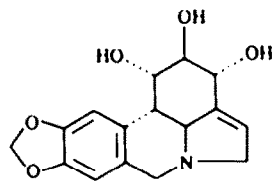

11

galactose (16:4.5)], traces of a polypeptide (*ca* 0.5%, consisting of the common α-amino acids), plus some unidentified substances (*ca* 0.8%). The oligosaccharide produced hypersensitive responses (HR) when administered to *P. biflorum*. Thus when the substance, in 10 μg/ml concentration, was administered to healthy bulbs of *P. biflorum*, by a wick arrangement, a reddish-purple zone appeared within a few hours. The control bulbs, which received only the vehicle (phosphate buffered saline, PBS, pH 7.2), did not show any colour. Subsequently, compounds **1**-**7**, **10** and **11**, which do not occur in healthy bulbs (in the fleshy leaves) were isolated from the reddish-purple zone. The toxic oligosaccharide(s) was finally extracted from the roots of *I. cylindrica* by using PBS.

HRS are shown by plants when they encounter potential pathogens [16]. In addition to the production of low-*M*, substances (phytoalexins, e.g. flavonoids) which are antagonistic to pathogens, other metabolic changes are triggered resulting in walling off and death of the affected tissue. The HR in *P. biflorum*, due to infestation with *I. cylindrica*, also exhibited this predicted pattern. Once contact between the pathogen and the host species occurred, the host cells repulsed the invasion by producing and accumulating substances that (a) restricted the invasion (compounds **1**–**4**) and (b) collapsed the cells thereby removing the components of nutrition and/or multiplication of the pathogen. The following observations are in harmony with this conclusion.

The healthy fleshy leaves of *P. biflorum* bulbs contained lycorine-1-*O*-β-D-glucopyranoside (**8**) [3], sterylacyl-glucosides [15] and lycorine-1-*O*-(6′-*O*-palmitoyl-β-D-glucopyranoside) (**9**) [17] as major entities. Lycorine itself was not present. It was present, in small amounts, in the form of a salt in the strongly acidic scape fluid. In the parasite-invaded red necrotic zone, however, free lycorine was present in abundant quantities (3 to 5-fold compared to that present in the scape fluid; estimated in the methanol extracts by UV spectrometry and HPLC). It would seem likely that secretion of the toxic oligosaccharide(s) by *I. cylindrica* resulted in the metabolism of the normal constituents (e.g. **8** and **9**) of the *P. biflorum* bulbs into **5**–**7**, **10** and **11**. Both **8** and **9** were found to promote root growth significantly in both producer and non-producer plants [3, 17] and would seem to be utilized by *I. cylindrica* for the same purpose (Fig. 1). In countering the ingress of the pathogen, *P. biflorum* not only produced large quantities of lycorine, a well documented cytotoxic compound, but also metabolized its potent growth promoters **8** and **9** into two other potent cytotoxic compounds **10** and **11**. Lycorine and ungeremine (**10**) were previously shown to inhibit the growth of S-180 ascites tumour cells, L1210 and P-388 systems and to decrease their viability. They also inhibit the germination of seeds and growth of stem and roots in both producer and non-producer plants [8, 13, 18, 19]. Pancrassidine (**11**), in 10^{-3} M concentration, has now been found to significantly inhibit the viability of S-180 ascites tumour cells *in vitro* (% viability of cells: mean ± s.d., 44.2 ± 2.88; control: 84.7 ± 7.02; $P < 0.01$) [Ghosal, S., unpublished].

EXPERIMENTAL

The general procedures were the same as those reported for the isolation of the chemical constituents from healthy bulbs of *P. biflorum* [3]. For analytical HPLC the following conditions were used: μ-Bondapak C_{18} analytical column (30 cm × 4 mm i.d.), MeOH–H_2O (4:1) 1 ml/min, 440/250 nm. The R_ts of the individual compounds were recorded in min.

Isolation procedure. Bulbs of *P. biflorum*, invaded with *I. cylindrica*, were collected on several occasions from the Banaras Hindu University Campus in the periods May–July (flowering time of the Amaryllidaceae species), August–October and February–March for 3 consecutive years. On analytical TLC the MeOH extracts of the red necrotic zones from the different collections gave similar results. In a typical experiment, the thin slices of the red necrotic zone (14 g), collected in February–March 1982, were triturated with MeOH and the combined reddish-purple soln (100 ml) was evapd *in vacuo*. The residue was triturated in succession with petrol (60–80°) (fraction A), $CHCl_3$ (fraction B) and MeOH (fraction C); the MeOH-insoluble solid was also examined (fraction D).

Treatment of fraction A. This fraction was concd (*ca* 50 ml) and kept overnight at room temp. when a reddish-brown solid (fraction a_1, 68 mg) separated. This was collected by filtration and the mother liquor was further concd (10 ml, fraction a_2).

Separation of alkaloids from fraction a_1. The fraction gave two major and several minor Dragendorff-positive spots on analytical TLC, R_f 0.35 (major), 0.45 (major) (solvent 1). It was dissolved in C_6H_6–$CHCl_3$ (traces) and chromatographed on a silica gel column. Elution was carried out with petrol-C_6H_6 (1:1, 1.2 l.), C_6H_6 (1 l.) and C_6H_6–$CHCl_3$ (1:1, 1 l.). Fractions (100 ml) were collected and monitored by analytical TLC.

Hippadine (**6**). Fractions 12–20 were combined and concd when this alkaloid was obtained as colourless flakes (18 mg), mp and mmp with hippadine [20] 207–208°; R_f 7.8 (co-TLC, HPLC, MS).

4,5-Dehydroanhydrolycorine (**5**). Fractions 25–30 afforded a solid which was further purified by prep. TLC (silica gel G, $CHCl_3$–MeOH, 9:1). The R_f zone ~ 0.3 afforded **5** as microcrystals (14 mg), mp and mmp (with a reference prepared by a published procedure [11]) 154–156°; R_f 8.3; UV λ_{max}^{MeOH} nm (log ε): 225 sh (4.48), 235 (4.35), 285 sh (3.72), 305 sh (3.60), 335 (3.95), 355 (3.90); MS *m/z* (rel. int.): 249 $[M]^+$ (55), 248 (100), 221 (14), 220 (11), 192 (7), 175 (5). (Found: C, 77.2; H, 4.7; N, 5.4. $C_{16}H_{11}NO_2$ requires C, 77.1; H, 4.4; N, 5.4%.) The physical and UV data are indistinguishable from those reported for 4,5-dehydroanhydrolycorine [11].

Separation of flavans from fraction a_2. The petrol concentrate was passed through a column of alumina (Brockmann neutral, activity grade IV, 24 × 2 cm). Elution was carried out with petrol (1 l.), C_6H_6 (1 l.), C_6H_6–$CHCl_3$ (1:1, 1.5 l.) and $CHCl_3$ (500 ml). Fractions (100 ml) were collected and monitored by analytical TLC.

7-Hydroxy-4′-methoxyflavan (**1**). The middle C_6H_6–$CHCl_3$ eluates were combined and evapd and the residue crystallized from *n*-hexane–C_6H_6 to give **1** as colourless needles (9 mg), mp 118–120°; $[\alpha]_D^{22}$ −14.7° (0.34, $CHCl_3$); UV λ_{max}^{MeOH} nm (log ε): 227 sh (4.08), 277 (3.04); MS *m/z* (rel. int.): 256 $[M]^+$ (100), 135 (24), 134 (72), 123 (24), 122 (28), 91 (9). The physical and spectral properties of this compound were indistinguishable from those reported for 7-hydroxy-4′-methoxyflavan [6].

5,4′-Dimethoxy-7-hydroxyflavan (**2**). The combined $CHCl_3$ eluates was evapd and the residue crystallized from C_6H_6 to give **2** as colourless leaflets (16 mg), mp 101–103°; $[\alpha]_D^{22}$ −6.8° (*c* 0.47; $CHCl_3$); UV λ_{max}^{MeOH} nm (log ε): 232 sh (4.30), 278 (3.24), 282 (3.20); IR ν_{max}^{KBr} cm^{-1}: 3400 (broad), 1610, 1040, 995; MS *m/z* (rel. int.): 286 $[M]^+$ (100), 165 (22), 153 (17), 152 (42), 134 (88), 119 (14), 91 (11); ^1H NMR ($CDCl_3$): δ7.01 (4H, A_2B_2, H-2′, 3′, 5′, 6′), 6.18 (1H, *d*, *J* = 3 Hz, H-8), 6.08 (1H, *d*, *J* = 3 Hz, H-6), 4.9 (1H, X of ABX, H-2), 3.80 (3H, *s*, OMe), 3.77 (3H, *s*, OMe), 2.62 (2H, *m*,

H_2-4), 2.1 (2H, *m*, H_2-3). (Found: C, 71.2; H, 6.0. $C_{17}H_{18}O_4$ requires C, 71.3; H, 6.2%.)

Treatment of fraction B. The residue from this fraction showed two major Dragendorff(D)-positive and three Fe^{3+} (D-negative)-positive spots on analytical TLC. The mixture was triturated with hot Me_2CO and the Me_2CO-soluble (fraction b_1) and -insoluble (fraction b_2) fractions were separated by filtration. Processing of fraction b_1 in the usual way afforded a further crop of hippadine (**6**, 7 mg) and lycorine (7, 55 mg), R_f 11.7; mp and mmp 256–258° (co-TLC, co-HPLC, UV, IR) [7].

Treatment of fraction b_2. This fraction afforded a pink residue which showed several yellow and purple spots, on exposure to air, on analytical TLC ($CHCl_3$–MeOH, 9:1). Prep. TLC of the residue on silica gel G (E. Merck), using the same solvent system gave the yellow chalcone **3** and the reddish-purple bifloridin (**4**).

2,4-Dihydroxy-6,4'-dimethoxychalcone (**3**). This compound was obtained as a yellow powder (6 mg), R_f 7.55; UV λ_{max}^{MeOH} nm (log ε): 388 (4.11); MS *m/z* (rel. int.): 300 $[M]^+$ (65), 153 (22), 152 (11), 135 (100), 120 (7), 107 (14), 77 (9); ^{1}H NMR (CD_3OD): δ8.32 (1H, *d*, *J* = 16 Hz, α-H), 7.92 (1H, *d*, *J* = 16 Hz, β-H), 7.32 (4H, A_2B_2, H-2', 3', 5', 6'), 6.18 (1H, *d*, *J* = 2.5 Hz, H-3), 6.10 (1H, *d*, *J* = 2.5 Hz, H-5), 3.92 (6H, OMe). (Found: C, 68.1; H, 5.3. $C_{17}H_{16}O_5$ requires C, 68.0; H, 5.3%.)

Bifloridin (**4**). The lower R_f zone of the prep. TLC scraping afforded this compound as a reddish-purple powder (27 mg), R_f 8.2; UV λ_{max}^{MeOH} nm (log ε): 240 (4.0), 265 (3.82), 300 sh (3.08), 315 (3.36), 332 (3.24), 388 (3.02); IR ν_{max}^{Nujol} cm^{-1}: 1705, 1658, 1612 (br), 1595; MS *m/z* (rel. int.): 282 $[M]^+$ (2), 255 (7), 254 (6), 225 (5), 224 (7), 167 (11), 151 (8), 135 (17), 107 (9); ^{1}H NMR (CD_3OD): δ7.8–7.3 (5H, *m*, Ar–H), 6.84 (1H, *d*, *J* = 8 Hz, H-4), 6.25 (1H, *d*, *J* = 2.5 Hz, H-8), 6.02 (1H, *d*, *J* = 2.5 Hz, H-6), 3.8 (6H, OMe). (Found: C, 71.70; 71.51; H, 5.3, 5.0. $C_{17}H_{14}O_4$ requires C, 72.3; H, 4.9%.)

Treatment of fraction C. This fraction on prep. TLC, using $CHCl_3$–MeOH (19:3), afforded a further crop of bifloridin (11 mg) from the R_f zone 0.4. The lower R_f zone, 0.18, showed a bright yellow fluorescence under short-wave UV light. MeOH eluate of the PLC scraping afforded ungeremine.

Ungeremine (**10**). This compound crystallized from EtOH as a light yellow cluster of needles (18 mg), softening ~ 278–282° (dec); R_f 27.0; UV λ_{max}^{MeOH} nm (log ε): 262 (4.28), 285 sh (4.07), 300 sh (3.74), 418 (3.88); $\lambda_{max}^{MeOH-0.1\ M\ HCl}$ 261, 275, 368, 380 nm; $\lambda_{max}^{MeOH-0.01\ M\ NaOH}$ 265, 275, 323, 422 nm; MS *m/z* (rel. int.): 266 $[M+1]^+$ (100), 265 (88), 238 (28), 224 (16), 211 (14), 192 (12). Direct comparison with a synthetic sample of 2-oxyphenanthridinium betaine, obtained from lycorine according to a published procedure [12], established that the two compounds were identical.

The residue obtained from the prep. TLC scraping of the remaining layer was dissolved in AcOH (4%, 20 ml). The clarified aq. soln was basified (NH_4OH) and extracted with EtOAc (3 × 50 ml-portions). The combined EtOAc extracts was worked up in the usual way to give pancrassidine.

Pancrassidine (**11**). This compound crystallized from MeOH as straw coloured micro-crystals (26 mg), mp 206 208°; R_f 7.9; $[\alpha]_D^{22} - 28.2°$ (*c* 0.51; MeOH); UV λ_{max}^{MeOH} nm (log ε): 228 (3.96), 284 (3.53); IR ν_{max}^{KBr} cm^{-1}: 3500 (br, OH), 935 (OCH_2O), 825, 792 (>C=CH–); MS *m/z* (rel. int.): 303 $[M]^+$ (14), 285 (9), 267 (7), 243 (100), 242 (24), 214 (22), 185 (7), m* 195.5 ($243^2/303$); ^{1}H NMR (DMSO-d_6): δ6.84 (1H, *s*, H-7), 6.75 (1H, *s*, H-10), 6.04 (2H, *s*, OCH_2O), 5.75 (1H, *m* (*br*), H-4), 5.3–4.6 (3H, *br*, exchangeable with D_2O), 4.32–2.8 (9H, *m*); acetylation with Ac_2O–C_5H_5N afforded tri-*O*-acetylpancrassidine, mp 201–204°; MS *m/z*: 429 $[M]^+$ (42), 243 (100); ^{1}H NMR ($CDCl_3$): δ6.87 (1H, *s*, H-7), 6.70 (1H, *s*, H-10), 6.02 (2H, *s*, OCH_2O), 2.02–1.98 (9H, OAc). (Found: C, 61.3; H, 5.1; N, 3.1. $C_{22}H_{23}NO_8$ requires C, 61.5; H, 5.3; N, 3.2%.)

Treatment of fraction D. This fraction, a straw coloured amorphous powder (0.12 g), which softened at 208–215° but did not melt up to 300°, was dissolved in H_2O (2 ml) and precipitated with EtOH. The process was repeated three times and the residue was washed with absolute EtOH, dried in vacuo, and finally dissolved in a small amount of H_2O and lyophilized to give the oligosaccharide as a powder (88 mg).

Acid hydrolysis of the oligosaccharide. A small amount (14 mg) of the lyophilized residue was dissolved in 2 M HCl (0.5 ml) and hydrolysed in a sealed tube at 100–105° (incubated) for 6 hr. The sugars present in the aq. hydrolysate were identified and estimated, as their alditol acetates by GLC following a published procedure [15]. Glucose and galactose (16:4.5) were identified as the only two sugar moieties in the oligosaccharide. The amino acids present in the aq. hydrolysate were separated by passing over a column of Dowex 50-X8. The identities of the amino acids in the effluent were established by PC, using authentic markers, as before [21]. Aspartic acid, alanine, glutamic acid, serine and threonine were detected.

Fresh roots of *I. cylindrica* were macerated in a high-speed blender using Pi buffered saline (PBS, pH 7.2) as the extracting solvent. The extract was centrifuged and the supernatant was processed as before to give the toxic oligosaccharide as an amorphous solid (yield, *ca* 0.3%).

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REFERENCES

1. Ghosal, S., Singh, S. P., Bhagat, M. P. and Kumar, Y. (1982) *Phytochemistry* **21**, 2943.
2. Ghosal, S., Kumar, Y., Singh, S. P. and Ahad, K. (1983) *Phytochemistry* **22**, 2591.
3. Ghosal, S., Kumar, Y. and Singh, S. P. (1984) *Phytochemistry* **23**, 1167.
4. Chopra, R. N., Nayar, S. L. and Chopra, I. C. (1956) *Glossary of Indian Medicinal Plants*, p. 140. C.S.I.R., New Delhi.
5. Singh, H. S. (1978) in *Plant Diseases*, IV edn, pp. 502–510. Oxford & I.B.H. Publishers, New Delhi.
6. Saini, K. S. and Ghosal, S. (1984) *Phytochemistry* **23**, 2415.
7. Ghosal, S., Rao, P. H., Jaiswal, D. K., Kumar, Y. and Frahm, A. W. (1981) *Phytochemistry* **20**, 2003.
8. Abduazimov, K. A. and Yunusov, S. Y. (1965) *Uzb. Khim. Zh.* **9**, 25.
9. Cardillo, G., Merlini, L., Nasini, G. and Salvadori, P. (1971) *J. Chem. Soc.* (*C*) 3967.
10. Ghosal, S., Saini, K. S. and Sinha, B. N. (1983) *J. Chem. Res.* (*S*) 330; (*M*) 2601.
11. Cook, J. W., Loudon, J. D. and McCloskey, P. (1954) *J. Chem. Soc.* 4176.
12. Fales, H. M. and Wildman, W. C. (1958) *J. Am. Chem. Soc.* **80**, 4395.
13. Ghosal, S., Saini, K. S. and Razdan, S. (1985) *Phytochemistry* **24**, 2141.
14. Granath, K. A. (1965) *Methods Carbohydr. Chem.* **5**, 20.
15. Ghosal, S. and Saini, K. S. (1984) *J. Chem. Res.* (*S*) 110; (*M*) 965.
16. Deverell, B. (1978) *Defence Mechanisms of Plants, Monographs in Experimental Biology*, Vol. 19, p. 110. Cambridge Press, Cambridge.
17. Ghosal, S., Shanthy, A., Kumar, A. and Kumar, Y. (1985) *Phytochemistry* **24**, 2703.

18. Zee Cheng, R. K.-Y., Yan, S.-J. and Cheng, C. C. (1978) *J. Med. Chem.* **21**, 199.
19. Chattopadhyay, U., Chaudhuri, L., Das, S., Kumar, Y. and Ghosal, S. (1984) *Pharmazie* **39**, 855.
20. Ghosal, S., Saini, K. S. and Frahm, A. W. (1983) *Phytochemistry* **22**, 2305.
21. Ghosal, S., Reddy, J. P. and Lal, V. K. (1976) *J. Pharm. Sci.* **65**, 772.
22. Ghosal, S., Kumar, Y., Singh, S. K. and Shanthy, A. (1986) *J. Chem. Res.* (*S*) (in press).