Phytochemistry, Vol. 26, No. 3, pp. 823–828, 1987.
Printed in Great Britain.

0031-9422/87 $3.00 + 0.00
Pergamon Journals Ltd.

PHOSPHATIDYLPYRROLOPHENANTHRIDINE ALKALOIDS FROM *ZEPHYRANTHES FLAVA**

SHIBNATH GHOSAL, SUSHIL K. SINGH and SANKARA G. UNNIKRISHNAN

Pharmaceutical Chemistry Research Laboratory, Department of Pharmaceutics, Banaras Hindu University, Varanasi 221005, India

(*Received* 14 *May* 1986)

Key Word Index *Zephyranthes flava*; Amaryllidaceae; flowers; phosphatidylpyrrolophenanthridines; 2-*O*-glycerophosphoryllycorine, phosphatidyllycorines, phosphatidylpseudolycorines, phosphatidyllycorinium methocation; biological activity.

Abstract -Four new alkaloidal phospholipids, 2-*O*-glycerophosphoryllycorine, phosphatidyllycorines, phosphatidylpseudolycorines and phosphatidyllycorinium methocation, were isolated from the flowers of *Zephyranthes flava*. The structures of these compounds were established by comprehensive spectral analyses, chemical transformations and synthesis, where possible. The biological profile of this novel group of alkaloidal conjugates is appraised.

INTRODUCTION

The flowers of Amaryllidaceae species have not so far attracted the notice of phytochemists although over 100 distinctly different alkaloids have been reported from the roots, bulbs, leaves, fruits and seeds of about 150 species belonging to 36 genera of this family [1]. We were interested in the alkaloidal constituents of flowers of Amaryllidaceae species for several reasons. (i) Very few Amaryllidaceae plants bear fruits and viable seeds; they propagate mainly through offsets of bulbs. The physiological purpose of flower bearing is not clear. (ii) Initiation of floral differentiation is conceivably an important biochemical event and in many plant families alkaloid synthesis is either inhibited, stopped or significantly modified at the commencement of flowering [2, 3]. It is to be seen if either of these phenomena is operative at the time of flowering of Amaryllidaceae plants. (iii) Preliminary screening of flowers of Amaryllidaceae species, in our laboratory, indicated that this organ was rich in free and conjugated alkaloids. These compounds may perform certain functions that cannot be performed by alkaloids contained in other parts. For instance, during off years of flowering the total weights of the propagated bulbs are significantly reduced due, presumably, to impaired synthesis of the cell wall-forming glycans. (iv) 2-Oxyphenanthridiniums, the quaternary alkaloids characteristic of the seeds of *Crinum asiaticum* [4], *C. latifolium* [4] and *Z. flava* [5], consisted of phosphatidic acids as their anionic part. The ease with which known phosphatides (lecithins, cephalins) are hydrolysed by native phospholipases, at the time of their extraction from plant sources, would seem to suggest the natural occurrence of phosphatidylpyrrolophenanthridinium beatines (fully aromatic oxide betaines) as a distinct possibility. The pyrrolophenanthridinium alkaloids of *Z. flava* occur in varying amounts in the different parts of this plant and also exhibit seasonal variations. These are (a) trace components in the roots only at the time of flowering; (b) a minor entity in young and growing bulbs; (c) in traces in flower-stem and fleshy-scale fluids; (d) a major entity in fruits, the concentration increasing with maturity; and (e) dominant in seeds at all stages. These compounds strongly prevent seed germination, root and stem growth in both producer and non-producer plants [4, 5]. (v) Phosphatidyllycorines have recently been encountered in the flower-stem and fleshy-scale fluids of *C. asiaticum* [6] and *Pancratium biflorum* [7] although their complete characterization has not been reported before. The possibility of the widespread natural occurrence of these alkaloidal conjugates and their potential biological significance warrant further and more detailed studies of flowers and other parts of flower-bearing Amaryllidaceae plants. In this paper, we report the isolation, characterization and biological profile of four new alkaloidal phospholipids from the flowers of *Z. flava*.

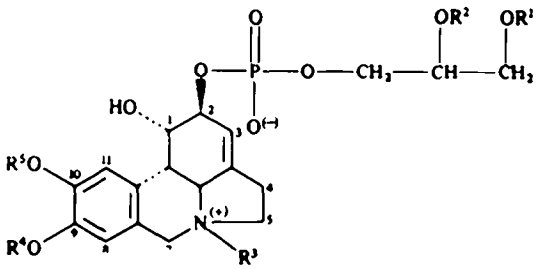
RESULTS AND DISCUSSION

Extensive solvent extraction, column (CC) and preparative TLC and HPLC of the polar constituents from $CHCl_3$–MeOH extracts of flowers of *Z. flava* afforded four alkaloidal phospholipid fractions (**1**–**4**). Complete characterization of these compounds is described below.

Fraction 1

This compound, $C_{19}H_{24}NO_9P$ (elemental analyses and FAB-MS), responded to Dragendorff's test for alkaloids and the ammonium molybdate test for phosphate esters. It exhibited UV maxima, in MeOH, similar to those of protonated lycorine [1]. The IR spectrum showed significant bands due to ammonium, hydroxyl, methylenedioxy and phosphoryl groups. The 90 MHz ^{1}H NMR spectrum of the compound suggested the structural features of a glycerophosphoryllycorine derivative. It formed a tri-*O*-acetyl derivative, mp 232–234°, $C_{25}H_{30}NO_{12}P$, which, in the ^{1}H NMR spectrum, exhibited a downfield shift ($\Delta\delta$ 1.3) in the H-1 signal from that of the parent

*Part 25 in the series "Chemical Constituents of Amaryllidaceae". For Part 24 see ref. [20].



1 $R^1 = R^2 = R^3 = H$, $R^4 + R^5 = -CH_2-$
2a R^1 = palmitoyl, R^2 = stearoyl, $R^3 = H$, $R^4 + R^5 = -CH_2-$
2b R^1 = palmitoyl, R^2 = oleoyl, $R^3 = H$, $R^4 + R^5 = -CH_2-$
3a R^1 = palmitoyl, R^2 = stearoyl, $R^3 = H$, $R^4 = Me$, $R^5 = H$
3b R^1 = palmitoyl, R^2 = oleoyl, $R^3 = H$, $R^4 = Me$, $R^5 = H$
4 R^1 = palmitoyl, R^2 = stearoyl, $R^3 = Me$ (α- and β-), $R^4 + R^5 = -CH_2-$

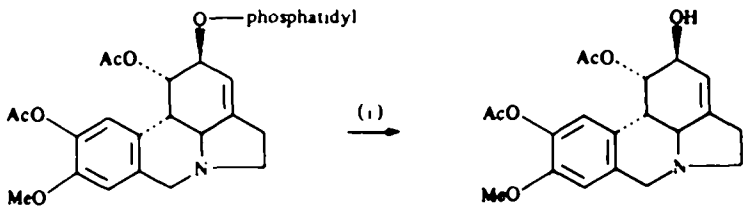
compound thereby locating one acetyl group at C-1 of the lycorine moiety. The other two acetyl groups were attached to the glycerol moiety. On hydrolysis with aq. HCl, in a sealed tube at 100°, compound **1** afforded glycerol-3-phosphoric acid and lycorine. Hence, the 2-*O*-glycerophosphoryllycorine structure (**1**) was assigned to this compound. To our knowledge, this is the first report of the natural occurrence and structure of an alkaloidal phospholipid.

Fraction **2**

This material, consisting of a mixture of two closely related entities, $C_{53}H_{98}NO_{11}P$ (M^+, 100%) and $C_{53}H_{86}NO_{11}P$ (M^+, 22%), exhibited UV, IR, and ^1H NMR spectra characteristic of fatty acid esters of 2-*O*-glycerophosphoryllycorine. On acetylation with acetic anhydride–pyridine at 95°C, it afforded a monoacetyl derivative, mp 228–232° (dec). The acetylation caused a downfield shift ($\Delta\delta$ 1.28) of the H-1 signal from that of the parent compound; the ^1H NMR spectrum being taken in the same solvent. Hydrolysis of compound **2** with phospholipase D gave lycorine and a phosphatidic acid. The latter on graded hydrolysis (see Experimental) afforded glycerol, three fatty acids (palmitic, stearic and oleic acids in a *ca* 1.2:1:0.2 ratio), and phosphoric acid. Selective deacylation at the C-2 position of the glycerol moiety, with phospholipase A_2, gave stearic and oleic acids. On the basis of these observations, compound **2** was assigned the 2-*O*-acylglycerophosphoryllycorine structures (**2a–b**). Compounds **2a–b** have not been encountered before in nature nor have they been prepared before synthetically.

Fraction **3**

This material, like fraction **2**, also consisted of a mixture of two closely related entities, $C_{53}H_{90}NO_{11}P$ (M^+, 100%) and $C_{53}H_{88}NO_{11}P$ (M^+, 7%), and showed UV and IR spectra similar to those of compounds **2a–b**. However, it formed a diacetyl derivative which in the ^1H NMR spectrum showed one Ar–OAc and one Ar–OMe in lieu of the methylenedioxy signal in the acetate derivative of compound **2**. Additionally, it showed an aliphatic acetoxyl signal and a downfield shift ($\Delta\delta$ 1.3) in the H-1 signal from that of the parent compound. Hydrolysis of fraction **3** with phospholipase D gave pseudolycorine and a phosphatidic acid, the latter compound was identical with that obtained from the enzymic hydrolysis of compound **2**. On the basis of these observations, compound **3** was assigned the 2-*O*-acylglycerophosphorylpseudolycorine structures (**3a–b**). In order to provide chemical evidence in support of the location of the acylglycerophosphoryl group at the allylic position of the pseudolycorine moiety, the diacetyl derivative (**5**), in tetrahydrofuran (THF), was treated with NaOMe in presence of a Pd(O) catalyst when 1,10-di-*O*-acetylpseudolycorine (**6**) was obtained. The identity of the product was established by its synthesis from 1,2,10-tri-*O*-acetylpseudolycorine following a similar route. The methanolysis reaction (Scheme 1) with π-allyl palladium inter-



5 **6**

(i) NaOMe, THF, $Pd(PPh_3)_4$, at room temp, stirring for 30 min.

Scheme. 1. Methanolysis of π-allylpalladium intermediate of di-*O*-acetylphosphatidylpseudolycorine.

mediates had literature precedents [8]. Compounds **2** and **3** thus differ in the alkaloidal moiety, their phosphatidyl component being the same. The difference in the 'basic head' (lycorine/pseudolycorine) of the phosphatidylpyrrolophenanthridines would seem to suggest that the conjugation of the Amaryllidaceae alkaloids with the phospholipid moiety is not a freak (*lusus naturae*). This is further manifested in the compound **4**.

Fraction **4**

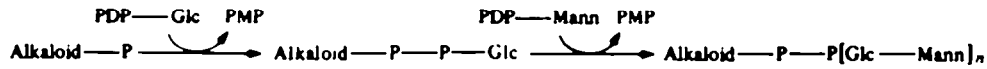
This material, obtained as a hygroscopic solid, exhibited a UV spectrum similar to that of compound **1**. It formed a monoacetyl derivative with acetic anhydride–pyridine; the acetyl derivative showed ^{1}H NMR signals characteristic of phosphatidyllycorine methocation. Mild hydrolysis of the parent compound, with dilute HCl, afforded lycorine α- and β-methocations and phosphatidic acid. The latter compound on graded deacylation afforded palmitic acid and stearic acid from the C-1 and C-2 positions, respectively, of the glycerol moiety. On complete hydrolysis, the phosphatidic acid gave glycerol, the two fatty acids and phosphoric acid in almost equal ratios. Hence structure **4** was assigned to this compound.

In addition to the above mentioned fatty acids, a number of other saturated and unsaturated (0:1–3) fatty acids (C_{16}-C_{24}) were detected in the total phospholipids. Further characterization of these compounds is currently underway.

The phosphatidylpyrrolophenanthridines seem to produce a broad range of biological actions in different organs of the producer plants. Common phosphatides (lecithins and cephalins), in combination with proteins, induce different types of stimuli to enzymic activity within the cell, e.g. in the release of arachidonic acid (AA) from phosphatidylinositol and other AA-containing phospholipids. One of the mechanisms considered for the release of AA from phospholipids is the activation of phospholipase A_2. This enzyme is Ca^{2+} ion dependent. The release of AA from phospholipids has been shown to be stimulated by Ca^{2+} [9]. The influx of Ca^{2+}, needed for the optimal enzyme activity, is promoted by an increase in *N*-methylation of the phosphatides. According to this model, ligand-receptor binding activates a series of enzymes that cause *N*-methylation of phosphatidylethanolamine to form phosphatidylcholine. As the phospholipid is methylated, the reaction being coupled to Ca^{2+} influx, the product is translocated from the inside to the outside of the lipid bilayer. A similar sequence of reaction can be envisaged for the *N*-methylation of phosphatidyllycorine to phosphatidyllycorine methocation (**4**) in *Z. flava*. We have acquired evidence that phosphatidyllycorines (**2a–b**) cause an influx of Ca^{2+} within the cell. Thus, in doses of 25 μg/ml and above, (**2a–b**) caused extensive degranulation of rat mesenteric mast cells *in vitro*. The degranulation was mediated by Ca^{2+} influx [10]. Several independent lines of evidence also suggested that increased levels of Ca^{2+} in mast cells can trigger the release of inflammatory mediators in mast cells which cause their degranulation [11]. The *trans*-acylation reaction recognized in the Amaryllidaceae, in the form of co-occurrence of the lysophosphatidyllycorine (**1**) and palmilycorine (= 1-*O*-palmitoyllycorine) [6], is strong circumstantial evidence in favour of the acyl release reaction mediated by phosphatidylpyrrolophenanthridines. Furthermore, the phosphatidyllycorine methocation (**4**), formed from the *N*-methylation of phosphatidyllycorine (**2a**), is conceivably converted into hippeastrine *in vivo*. The rapid turnover of lycorine and hippeastrine in a number of Amaryllidaceae plants [4, 12] suggests their dynamic state of metabolism to meet the physiological demand of the producer plants.

Another effect of the phosphatidylpyrrolophenanthridine-protein combination could be in the control of cell division and cell differentiation. This suggestion stems from the observed immuno-suppressive action of the phosphatidyllycorines (**2a b**). These compounds, in low doses (1 μg/ml), *in vitro*, provided significant protection to sensitized rat mesenteric mast cells from degranulation. The protective effect was found to be due to suppression of the initial process of sensitization *in vivo*. The manifestation of this property in plants could be suppression of cell division in members of the Amaryllidaceae, at the dormant stage, when the phosphatidylpyrrolophenanthridines are present in appreciable amounts.

The phosphatidylpyrrolophenanthridines may also participate in the active deposition of acylated sugars in producer plants. The bulbs of many Amaryllidaceae species, *viz. Crinum latifolium* L., *Narcissus tazetta* L. (var. *chinensis* Roemer), *Lycoris radiata* Herbert and *L. squamigera* Maxim., synthesize highly acylated glycans (glucans and glucomannans [1, 13]); the concentrations of these polysaccharides increase considerably during resting time of the plants. There exist a number of enzymes whose substrates are covalently bound to a carrier lipid molecule. The members of this group which have been studied in detail are those involved in the polysaccharide synthesis in bacteria. The cycle of reactions involved in the formation of lipopolysaccharides in bacteria has been well documented [14]. Glycans of the bulbs of Amaryllidaceae plants may conceivably be synthesized by a similar sequence (Scheme 2), involving the phosphomonoesters of lycorine/pseudolycorine, where the alkaloid moiety acts as the lipid carrier molecule. It is interesting to note that in off-years of flowering, the proliferated bulbs, through off-sets, remain emaciated, presumably due to impaired synthesis of glycans. This phenomenon may be



Alkaloid - P, phosphomonoester of lycorine / pseudolycorine; PDP and PMP (purines: guanosine, thymidine, uridine) diphosphate and monophosphate; Glc, glucose; Mann, mannose

Scheme. 2. Hypothetical role of the carrier alkaloid lipids in the biosynthesis of the glycans of Amaryllidaceae plants.

correlated with the decline in the concentration of the phosphatidylpyrrolophenanthridines during off-years of flowering.

Finally, a dynamic state of the phosphatidylpyrrolophenanthridines in *Z. flava* was recognized by a transphosphatidylation reaction *in vitro*. When an enriched phospholipase–transphosphatidylase enzyme preparation, from *Z. flava*, was used to equilibrate phosphatidylcholine (dipalmitoyl) and lycorine, phosphatidyllycorine was obtained (in about 18% yield) among other products. This experiment supports the contention that the phosphatidylpyrrolophenanthridines are not a freak and by controlling the experimental conditions many more can be isolated from members of the Amaryllidaceae.

EXPERIMENTAL

General. Mps were uncorr. UV spectra were recorded in MeOH. IR spectra were determined in KBr, unless otherwise stated, and only the major bands are quoted. 90 MHz ^{1}H NMR spectra were obtained using TMS as an internal standard. In FAB-MS, argon was used for the primary beam; the spectra were obtained at an accelerating voltage of 3 kV. The ion source was maintained at ambient temperature. The samples were taken in $CHCl_3$–MeOH and applied to a stainless steel probe tip with an appropriate amount of glycerol. The probe was immediately inserted into the mass spectrometer. Analytical and semi-preparative HPLC were carried out on a Waters Associates assembly fitted with a M-6000 pump, a U 6K injector, UV detector, a C_{18} μ-Bondapak column (30 cm × 4 mm i.d. for analytical and 30 cm × 8 mm for semi-preparative analyses); MeOH–H_2O–MeCN (90:7:3) was used as developer at a flow rate of 1.5 ml/min for analytical and 4 ml/min for the semi-preparative runs. For GC analyses of the methyl esters of fatty acids the following conditions were used (10% diethyleneglycol succinate on 100–120 mesh, 3 m × 2 mm i.d., Gaschrom Q, programmed from 70–200°, 40 ml/min N_2). For preparative TLC, silica gel 60 F_{254} plates were used; the plates were developed with $CHCl_3$–MeOH–7 M NH_3 (69:27:4). For analytical TLC, silica gel G (E. Merck), plate thickness 0.2 mm, was used. H_2SO_4 satd. aq. $K_2Cr_2O_7$ (7:3), ammonium molybdate–perchloric acid and modified Dragendorff's (according to Munier and Machaeboeuf) reagents were used for detection.

Z. flava Roem. and Schult. cultivated in the Banaras Hindu University Campus, was identified by Professor S. K. Roy, Department of Botany, Faculty of Science, Banaras Hindu University. The flowers were collected in three consecutive years (1982–1984) during June–August and were immediately processed for chemical constituents.

Extraction. In a typical experiment, fresh flowers (*ca* 1 kg) were macerated with $CHCl_3$–MeOH (2:1) in a high-speed blender. The extract was warmed at 60° for 15 min; EDTA (0.1 M) was added to retard the phospholipase activity, and the mixture was kept at room temp. overnight. It was filtered and the filtrate was concd (*ca* 150 ml) when a cream coloured solid was pptd which was collected by filtration (fraction A, 132 mg). The mother liquor was further concd (*ca* 20 ml) and preserved (fraction B).

Treatment of fraction A. The solid was triturated with hot hexane (fraction A_1), $CHCl_3$ (fraction A_2) and MeOH (fraction A_3), in succession, when glycerophosphoryllycorine was obtained as the MeOH-insoluble solid.

Glycerophosphoryllycorine (1). This compound (112 mg) did not melt up to 280° but decomposed > 300°, R_f (analytical HPLC) = 17.82 min; UV λ_{max} nm (log ε) 236–238 (3.62), 285–286 (3.70), 325–330 sh (2.93) [lycorine: λ_{max}^{MeOH} 230–232 (3.55), 290–292 (3.66); UV $\lambda_{max}^{MeOH\text{-}0.1\ N\text{-}HCl}$ nm (log ε) 237–239 (3.53), 285–286 (3.68), 235–330 sh]; IR ν_{max} cm^{-1}: 3350 (*br*, OH), 2550 (*br*, NH$^+$), 1600 (*br*), 1240 (P=O), 1045, 940 (OCH_2O); ^{1}H NMR (CD_3OD–CD_3COOD): δ6.84 (1H, *s*, H-11), 6.65 (1H, *s*, H-8), 6.02 (2H, *s*, OCH_2O), 5.67 (1H, *br*, H-3), 5.35 (1H, *br*, H-2), 4.65 (2H, *d*, *J* = 7 Hz, P–O–CH_2–CHOH–), 4.38 (1H, *br*, H-1); MS *m/z* (rel. int.): 441 [M]$^+$ (100) (found: C, 51.3; H, 5.7; N, 2.8; P, 7.5; $C_{19}H_{24}NO_9P$ requires C, 51.7; H, 5.4; N, 3.1; P, 7.0); treatment of (1, 12 mg) with Ac_2O (0.5 ml) and C_5H_5N (0.2 ml) at 95° for 1 hr afforded the tri-*O*-acetyl derivative as colourless crystals (8 mg) (from MeOH), mp 232–234° (dec); IR ν_{max} cm^{-1}: 1740 (ester CO), 1242, 1204; ^{1}H NMR: δ6.84 (1H, *s*), 6.68 (1H, *s*), 6.02 (2H, *s*), 5.84 (2H, *m*, $W_{1/2}$ = 10 Hz, H-1, 3), 5.28 (1H, *m*, $W_{1/2}$ = 10 Hz, H-2), 5.0–4.2 (glyceryl methine and methylene H), 2.14 (3H, *s*, OAc), 2.10 (3H, *s*, OAc), 2.04 (3H, *s*, OAc); MS *m/z* (rel int): 567 [M]$^+$ (100). Hydrolysis of 1 (22 mg) with 1 N HCl (10 ml) at 100° for 15 min in a sealed tube gave glycero-3-phosphoric acid (3 mg) (co-TLC, co-HPLC, MS of TMS ether derivative) and lycorine (7 mg), mp 248–253° (mmp, co-HPLC, MS).

Treatment of fraction B. This fraction was mixed with HCl-washed florisil (4 g), dried *in vacuo*, and charged onto a column of HCl-washed florisil (20 × 1.5 cm). Elution was carried out with C_6H_6 (500 ml, fraction B_1), $CHCl_3$ (500 ml, fraction B_2), $CHCl_3$–MeOH (95:5, 200 ml; 9:1, 100 ml; 3:1, 100 ml; 1:1, 200 ml) (fractions B_3–B_6), and MeOH (200 ml, fraction B_7). BHT (3,5-di-*tert*-butyl-4-hydroxytoluene) (0.1 mg), in hexane, was added to each of the seven fractions to prevent autoxidation of the lipids. The solvents were evapd with N_2. The neutral lipids and the free alkaloids were eluted out in fractions B_1–B_4. Fractions B_5–B_7 showed the presence of similar phosphatidylpyrrolophenanthridines, on analytical TLC, and were combined (fraction C).

Treatment of fraction C. Enrichment of the phosphatidylpyrrolophenanthridines in this fraction was accomplished by preparative TLC. The phospholipids were located by spraying the edge of the chromatograms with modified Dragendorff's reagent. The R_f zones 0.15 (fraction C_1), 0.3 (fraction C_2) and 0.55 (fraction C_3) were cut and the PLC scrapings were eluted with MeOH–H_2O–MeCN (90:7:3). The eluates were subjected to HPLC analyses. For analytical and semi-preparative HPLC, the residues in fractions C_1–C_3 were redissolved in MeOH–H_2O–MeCN (0.1–2 ml) and portions (10 μl to 1 ml containing 50 μg to 5 mg) were applied to HPLC columns. The elutions of the compounds, detected at λ 250 nm, were collected.

Reactions and transformations. A battery of techniques was adopted for the selective degradation and transformation of phospholipids [15, 16]. For micro-analysis of phosphorus, the phospholipids were digested with perchloric acid in a Technicon digester and analysed for inorganic phosphate by auto-analyser.

In a typical expt, the deacylation of the phospholipids (*ca* 5 mg) was carried out by treatment with an anhydrous methanolic soln (0.2 ml) of NaOMe (0.2 N). The mixture was kept at room temp. for 30 min. The solvent was evapd and the residue was extracted with pentane. The pentane-soluble fraction was passed through a short column of florisil (60–100 mesh) before being subjected to GC analyses for methyl esters of fatty acids. The residue left after the pentane extraction was acidified (HCl), cooled and made alkaline (NH_4OH). The liberated lyso-derivatives (glycerophosphorylpyrrolophenanthridines) were collected by centrifugation.

In another experiment, selective deacylation at the C_2-position of the glyceryl moiety in (2–4) was carried out by treating them, separately, with phospholipase A_2 (phosphatidyl-2-acylhydrolase, EC 3.1.1.4, Sigma Chemical Co.), at pH 8.9 and 25°, for 12 hr. The liberated acids extracted with Et_2O were converted into methyl esters by treatment with ethereal CH_2N_2.

The composition of the methyl esters of fatty acids was determined by GC-MS, according to published procedures [17].

For the separation and identification of the alkaloid moiety from the phosphatide, the following procedure was followed. A mixture (5 ml), containing the glycerophosphorylpyrrolophenanthridine (10 μM), acetate buffer (pH, 5.6, 800 μM), $CaCl_2$ (400 μM) and phospholipase D (EC 3.1.4.4, Sigma, 25 units), was shaken and incubated at 30° for 1 hr. At the end of the incubation, the reaction mixture was made alkaline (pH ~ 9) and extracted with Et_2O, AcOEt and *n*-BuOH, in succession. The extracts were worked up and the residues were combined. The pyrrolophenanthridine alkaloid in the residue was identified by TLC, HPLC (using reference samples) and MS. The phosphatidic acid, in the aq. mother liquor, was recovered by lyophilization. The residue was subjected to deacylation as before and the liberated *sn*-glycerol-3-phosphoric acid was identified by direct comparison TLC, HPLC, MS of diacetate with a reference sample (prepared from phosphatidylcholine).

Phosphatidyllycorines (**2a–b**). This compound was obtained from fraction C_3, by semi-preparative HPLC, as a straw coloured powder (64 mg from 1 kg flower); it did not melt but decomposed > 280°; R_f 12.2, 14.8; deacylation and GC-MS analyses established the fatty acid composition as $C_{16:0}/C_{18:0}$ (palmitic/stearic) in (**2a**); $C_{16:0}/C_{18:1}$ (palmitic/oleic) in (**2b**); UV (**2a–b**): λ_{max} nm (log ε): 222 sh (3.8), 235 sh, 282 (3.24); IR ν_{max} cm^{-1}: 3400 (*br*), 1735, 1600 (*br*), 1228, 1040, 942, 730; MS *m/z* (rel. int.): 945 [M]$^+$ (100) (**2a**), 943 [M]$^+$ (22) (**2b**); ^{1}H NMR (CD_3OD–CD_3COOD): δ6.98 (*s*, H-11), 6.80 (*s*, H-8), 6.0 (*s*, OCH_2O), 5.75 (*br s*, H-3), 5.3 (*m*, CH=CH), 5.0–4.2 (*m*, H-1, glycerol methine and methylene protons), 1.2 (fatty acyl methylenes), 0.9 (*s*, Me) (found: C, 67.5; H, 9.6; N, 1.2; P, 3.0; $C_{53}H_{88}NO_{11}P$ requires C, 67.3; H, 9.3; N, 1.5; P, 3.3%). Hydrolysis with phospholipase D afforded lycorine (co-TLC, co-HPLC, MS) [18]. Acetylation of the compound (7 mg) with Ac_2O (0.5 ml) and pyridine (0.2 ml), at 95°, afforded a monoacetyl derivative, glassy solid (5 mg), from AcMe–hexane, mp 228–232° (dec); MS *m/z*: 987/985 [M]$^+$; δ_H 5.78 (*br s*, H-3), 5.48 (*br s*, H-1), 2.04 (*s*, OAc).

Phosphatidylpseudolycorines (**3a b**). This compound was obtained from fraction C_2, by semi-preparative HPLC, as a light brown amorphous solid (27 mg); R_f 18.5, 19.3 min; deacylation followed by GC-MS of the methyl esters of fatty acids established their composition as $C_{16:0}/C_{18:0}$ (palmitic/stearic) in (**3a**); $C_{16:0}/C_{18:1}$ (palmitic/oleic) in (**3b**); UV (**3a–b**): UV λ_{max} nm (log ε): 220 sh, 230 sh, 250 sh, 285 (3.43); IR ν_{max} cm^{-1}: 3350, 2500–2350, 1740, 1600 (*br*), 1225, 730; ^{1}H NMR: δ6.88 (*s*, H-11), 6.68 (*s*, H-8), 5.78 (*br s*, H-3), 5.3 (*m*, CH=CH, H-2), 4.48 (*br*, H-1), 3.95 (*s*, OMe), 1.2 (CH_2), 0.88 (*s*, Me); MS *m/z*: 947 [M]$^+$ (100), in (**3a**); *m/z*: 945 [M]$^+$ (7) in (**3b**) (found: C, 66.8; H, 9.8; N, 1.4; P, 3.0; $C_{53}H_{90}NO_{11}P$ requires C, 67.1; H, 9.5; N, 1.5; P, 3.3%). 1,10-Di-*O*-acetylphosphatidylpseudolycorine, mp 238 242° (dec); MS *m/z*: 1031 [M]$^+$ (100); ^{1}H NMR: δ6.96 (*s*, H-11), 6.70 (*s*, H-8), 5.78 (*br s*, H-1), 3.98 (*s*, OMe at C-9), 2.34 (*s*, OAc at C-10), 2.04 (*s*, OAc at C-1). Hydrolysis of compound **3** with phospholipase D, as before, afforded pseudolycorine (co-TLC, co-HPLC, MS) [18] and phosphatidic acid; regioselective hydrolysis of the C_2-acyl group of the glycerol moiety, with phospholipase A_2 afforded a mixture of stearic and oleic acids (isolated as the methyl esters) (GC, MS); complete deacylation with NaOMe–MeOH gave *sn*-glycerol-3-phosphoric acid and a mixture of methylpalmitate, oleate and stearate (GC, MS).

Synthesis of 1,10-*di*-O-*acetylpseudolycorine* (**6**). To a stirred soln of 1,2,10-tri-*O*-acetylpseudolycorine (21 mg) [19], in THF (2 ml), $Pd(PPh_3)_4$ (27 mg) and NaOMe (11 mg) were added during 30 min. The stirring was continued for another 10 min and the solvent was removed with N_2. The residue was dissolved in MeOH and subjected to preparative TLC using $CHCl_3$–MeOH (95:5) as developer. The R_f zone 0.3 was eluted with $CHCl_3$, the solvent was removed and the residue crystallized from AcMe to give 1,10-di-*O*-acetylpseudolycorine as colourless needles (12 mg), mp 203–205°; MS *m/z* (rel. int.): 373 [M]$^+$ (100), 331 (64), 314 (12), 313 (34); ^{1}H NMR ($CDCl_3$): δ7.0 (1H, *s*, H-11), 6.75 (1H, *s*, H-8), 5.82 (1H, *m*, H-1), 3.98 (3H, *s*, OMe at C-9), 2.30 (3H, *s*, OAc at C-10), 2.05 (3H, *s*, OAc at C-1).

Transformation of 1,10-*di*-O-*acetylphosphatidylpseudolycorine into* (**6**). To a stirred soln of the diacetylphosphatidylpseudolycorine (74 mg) and $Pd(PPh_3)_4$ (85 mg), in dry THF (5 ml), NaOMe (42 mg) was gradually added during 30 min, at room temp. (22°). The product was worked up as before to give 1,10-di-*O*-acetylpseudolycorine (14 mg), mp and mmp 203–205°; co-HPLC, R_f 8.8, *m/z* 373 [M]$^+$.

Phosphatidylmethyllycorinium cations (**4**). This compound was obtained as a brown hygroscopic powder (23 mg) from semi-preparative HPLC of fraction C_1; R_f = 22.5 min; deacylation of the compound with NaOMe–MeOH afforded methyl esters of palmitic and stearic acids; UV λ_{max} nm (log ε): 222 sh, 250 (3.2), 290 (3.44); 1-*O*-acetyl derivative, mp > 250° (dec), softening 200–205°; ^{1}H NMR ($CDCl_3$): δ6.88 (*s*, H-11), 6.64 (*s*, H-8), 6.02 (*s*, OCH_2O), 5.84 (*br m*, H-1, 3), 5.28 (*br, m*, H-2), 4.23 (3H, *s*, N$^+$–Me), 2.02 (3H, *s*, OAc), 1.2 (–CH_2–), 0.9 (6H, Me); hydrolysis of compound **4** with phospholipase D gave lycorine α- and β-methocations; R_f = 16.5, 17.2 min. (Found: C, 65.8; H, 9.7; P, 2.8. $C_{54}H_{91}NO_{11}P \cdot H_2O$ requires C, 66.2; H, 9.5; P, 3.2%.) Compound **2** on treatment with MeI followed by crystallization from aq. MeOH afforded **4** (co-TLC, co-HPLC).

Transphosphatidylation in lycorine from phosphatidylcholine. A mixture of phosphatidylcholine (10 μM, Sigma Chemical Co.), in acetate buffer (pH, 5.6; 800 μM), $CaCl_2$ (40 μM), lycorine (40 μM) and enriched phospholipase D preparation (5–7 units), from flowers of *Z. flava* (total volume, 10 ml), was shaken at room temp. (22°) for 30 min. The product was lyophilized and the residue was taken in $CHCl_3$–MeOH (1:1) and subjected to preparative TLC when phosphatidyllycorine (dipalmitoyl) was obtained. The process was repeated several times to collect sufficient amount of the product for identification (HPLC, FAB-MS).

Acknowledgements We are indebted to the CDRI, Lucknow, and the Dabur Research Foundation, New Delhi, for providing analytical facilities. S.K.S. and S.G.U. are grateful to the University Grants Commission, New Delhi, for research fellowships.

REFERENCES

1. Ghosal, S., Saini, K. S. and Razdan, S. (1985) *Phytochemistry* **24**, 2141.
2. Mothes, K. (1960) in *The Alkaloids* (Manske, R. H. F., ed.) Vol. VI, ch. 1. Academic Press, New York.
3. Robinson, T. (1981) in *The Biochemistry of Alkaloids*, 2nd edn. Springer, New York.
4. Ghosal, S., Kumar, Y., Singh, S. K. and Kumar, A. (1986) *J. Chem. Res. S* 112.
5. Ghosal, S., Singh, S. K. and Srivastava, R. S. (1986) *Phytochemistry* **25**, 1975.
6. Ghosal, S., Shanthy, A., Kumar, A. and Kumar, Y. (1985) *Phytochemistry* **24**, 2703.
7. Ghosal, S., Kumar, Y., Chakrabarti, D. K., Lal, J. and Singh, S. K. (1986) *Phytochemistry* **25**, 1097.
8. Keinan, E., Sahai, M. and Roth, Z. (1985) *J. Org. Chem.* **50**, 3558.
9. Hirata, F. and Axelrod, J. (1980) *Science* **209**, 1082.

10. Ghosal, S., Shanthy, A., Mukhopadhyay, M., Sarkar, M. K. and Das, P. K. (1986) *Pharm. Res.* 3, 240.
11. Sullivan, T. J. and Brown, L. J. (1981) in *New Perspectives on Calcium Antagonists* (Weiss, G. B., ed.) pp. 159–168. American Physiological Society, Bethesda.
12. Suhadolnik, R. J. (1966) *Abh. dtsch. Akad. Wiss. Berlin, Kl. Chem. Geol. Biol.* 3, 369.
13. Tomada, M., Shimizu, N., Shimada, K. and Suga, M. (1985) *Chem. Pharm. Bull. (Tokyo)* 33, 16.
14. Bishop, D. G. (1971) in *Biochemistry and Methodology of Lipids* (Johnson, A. R. and Davenport, J. B., eds.) ch. 21. Wiley-Interscience, New York.
15. *Handbook of Lipids and Selected Carbohydrates*, 7th edn. (1981). Supelco, Bellefonte.
16. Dawson, R. M. C. (1967) in *Lipid Chromatographic Analysis* (Marinetti, G. V., ed.) Vol. I, p. 163. Dekker, New York.
17. Klopfenstein, W. E. (1971) *J. Lipid Res.* 12, 773.
18. Ghosal, S., Rao, P. H., Jaiswal, D. K., Kumar, Y. and Frahm, A. W. (1981) *Phytochemistry* **20**, 2003.
19. Ghosal, S., Kumar, Y. and Singh, S. P. (1984) *Phytochemistry* 23, 1167.
20. Ghosal, S. and Singh, S. K. (1986) *J. Chem. Res. S* 312.