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OCCURRENCE OF TWO EPIMERIC ALKALOIDS AND METABOLISM COMPARED WITH LYCORINE IN *CRINUM LATIFOLIUM**

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Key Word Index—*Crinum latifolium*; Amaryllidaceae; epimeric alkaloids; 2-epilycorine; 2-epipancrassidine; metabolic pathways; biochemical significance.

Abstract—Two new epimeric pyrrolophenanthridine alkaloids, 2-epilyocorine and 2-epipancrassidine, were isolated from the flower-stem fluid of *Crinum latifolium*. The structures of the two alkaloids were established by spectroscopic analyses, crucial chemical transformation, and synthesis in case of the former. The differences in the metabolic pathways of lycorine and 2-epilycorine were studied under simulated physiological conditions and their biochemical significance was appraised.

INTRODUCTION

The literature dealing with the chemistry and biological activity of Amaryllidaceae alkaloids shows no sign of abatement during the past 15 years [1–4] but there has been dearth of information on the accumulation, transformation and metabolism of alkaloids in the different organs of Amaryllidaceae species. Investigation of the regulation of alkaloid metabolism has been carried out mainly with relatively simple systems, e.g. with microorganisms and plant cells cultivated *in vitro* [5]. In earlier parts of this series, we reported a state of relentless catabolic reactions of Amaryllidaceae alkaloids *in vivo* [2]. From each 'fundamental' ring system, e.g. the pyrrolophenanthridine (lycorine) and 5,10b-ethanophenanthridine [vittatine/(−)-crinine], a number of alkaloids were considered to be derived by different catabolic pathways. However, despite the structural diversity of the alkaloids, the expression of biosynthesis of the various Amaryllidaceae alkaloids seems to be an integrated process, i.e. involved as a whole in the developmental programmes of the producer plants. This contention is supported by the co-occurrence of a number of same or very similar alkaloids in several genera of the Amaryllidaceae at comparable periods of their vegetation/ontogeny. In this paper, we report for the first time the natural occurrence of 2-epilycorine (**1**) and 2-epipancrassidine (**7**), in the flower-stem fluid of *Crinum latifolium* L. Additionally, we have studied the metabolic pathways of lycorine (**2**) and 2-epilycorine, under simulated physiological conditions, to evaluate their biochemical significance.

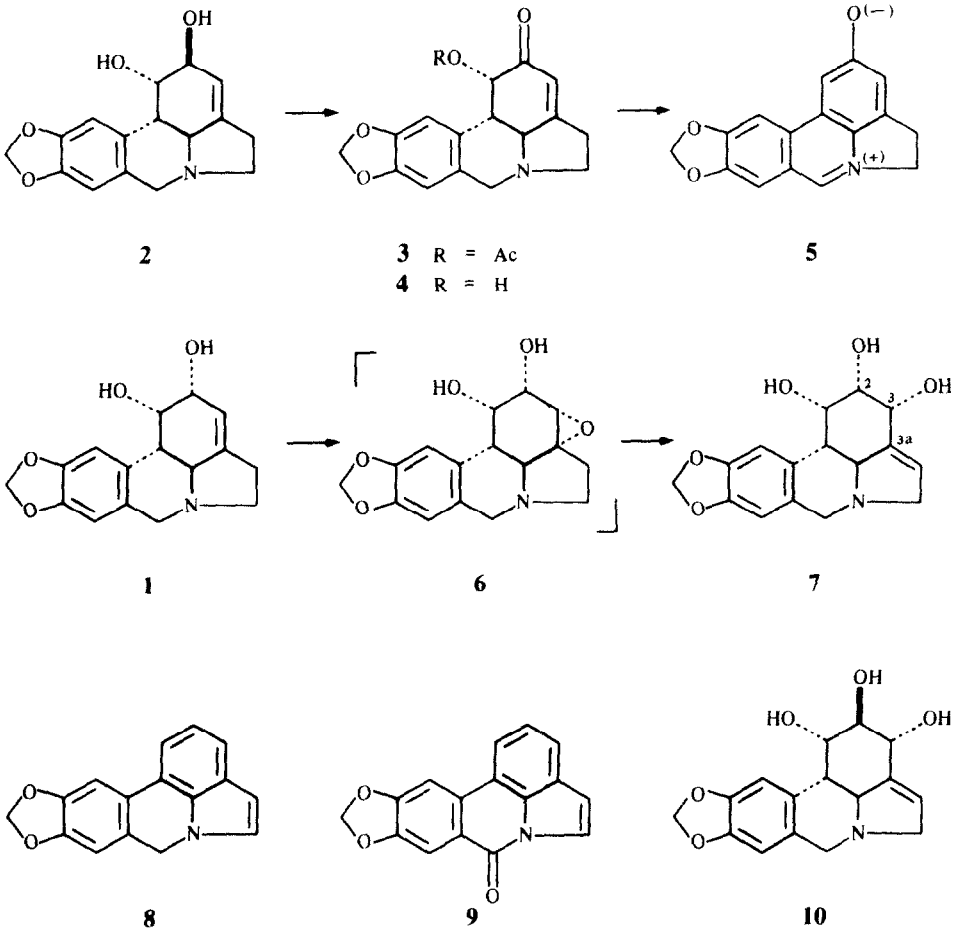
RESULTS AND DISCUSSION

Semi-preparative HPLC of the residue from the flower-stem fluid of *C. latifolium* yielded 2-epilycorine along with other products. The identity of 2-epilycorine was established by direct comparison with a synthetic sample prepared from 1-*O*-acetyllycorine, according to a previously described procedure [6]. 1-*O*-Acetyllycorine [7], on oxidation with active manganese dioxide, in chloroform, afforded 1-*O*-acetyllycorin-2-one (**3**). The product on stereospecific reduction with lithium aluminium hydride, in tetrahydrofuran, gave 2-epilycorine.

The metabolism of the two epimeric alkaloids (**1, 2**) in the flower-stem fluid of *C. latifolium* was investigated under simulated physiological conditions. The two alkaloids were added separately, in excess (*ca* 10 times than that was ordinarily present in the natural fluid) to phosphate buffer solution of the flower-stem fluid. The mixture was incubated at 21° for 14 hr and the metabolism of the two alkaloids was monitored at 1 hr intervals by HPLC. The fluid containing lycorine produced, in succession, lycorine-2-one (**4**) and 2-oxyphenanthridinium betaine (= ungeremine, **5**) as the major metabolites which persisted till the end of the 14 hr period. The concentration of lycorine in the fluid gradually decreased in concert. 2-Epilycorine, under similar conditions, produced 2-epipancrassidine (**7**), presumably via the 3,3a-epoxide (**6**), followed by dehydroanhydrolycorine (**8**) and hippadine (**9**). The structure of 2-epipancrassidine was established from its differences to pancrassidine (**10**) [8] in respect of the C_2-OH configuration (α, *quasi*-axial). The α-configuration of the C_2-OH function was established by the ready formation of an isopropylidene derivative of epipancrassidine. 2-Epipancrassidine was subsequently detected in the flower-stem fluid of *C. latifolium* by analytical HPLC and spectroscopy (UV, MS). This represents the first account of 2-epipancrassidine and its formation as a metabolic product of 2-epilycorine. The above led us to postulate the metabolic sequence of 2-epilycorine and lycorine given in Scheme 1.

The following were obtained in support of the sequences described in Scheme 1: vanadium-catalysed epoxidation/oxidation reaction of allylic alcohols [9] was carried out separately with 1-*O*-acetyllycorine and 1-*O*-

*Part 30 in the series 'Chemical Constituents of Amaryllidaceae'. For Part 29 see ref. [11].



Scheme 1. Metabolic sequence of lycorine and 2-epilycorine in *C. latifolium*

acetyl-2-epilycorine. Vanadium-catalysed epoxidation of conformationally 'fixed' allylic alcohols showed preference for *cis*-stereoselectivity and *quasi*-axial group in the transition state. Hence vanadium-catalysed transformation of 1-*O*-acetyl-2-epilycorine was expected to proceed, preferentially, through the epoxide (**6**). Consistent with this expectation, the products (**7**–**9**) obtained from the vanadium-catalysed transformation were found to be identical with the metabolic products of 2-epilycorine (Scheme 1). When the allylic alcohol function is *quasi*-equatorial, as in lycorine (**2**), dehydrogenation competes with epoxidation and enone [e.g. (**4**)] becomes the main product. Lycorine thus produced, the enone (**4**) and, subsequently by autooxidation, compound **5**.

Earlier, we reported the formation of pancrassidine (*O*-demethylungiminorine) (**10**) in *Pancratium biflorum* Roxb. in response to hypersensitive reaction with the parasite, *Imperata cylindrica* [8]. The transformation: lycorine → pancrassidine seems to be due to predominance of the 3,3a-epoxidation of the former, under stress conditions, leading to the product **10**. Compound **10** is a growth-promoter to both the host and the pathogen, whilst the usual product (**5**) of the hypersensitive reaction is cytotoxic. Certain bacterial isolates, viz. *Staphylococcus aureus* and *Pseudomonas aeruginosa*, were found to transform lycorine into **10** which annuls the cytotoxic effect of the normal metabolite of lycorine, 2-oxyphenanthridinium betaine (ungeremine) (**5**) [10]. Pancrassidine (**10**), in small doses (2.5–12.5 μg/ml), augmented the growth of the two bacteria and also encouraged adventitious root growth in a number of Amaryllidaceae species. It therefore seems likely that the two epimeric alkaloids, lycorine and 2-epilycorine, under normal conditions of metabolism are transformed into metabolites which, respectively repress and promote the growth of the producer plant. Under adverse circumstances, an entirely different route of metabolism was discernible.

EXPERIMENTAL

Isolation procedure. C. latifolium L., cultivated in the gardens of the Banaras Hindu University Campus, comes into flower during August–September, every year. Ten plants were selected for the investigation. The fluids of 1- to 4-day-old flower stems were collected by a hypodermic syringe and dissolved in MeOH. The MeOH soln was lyophilized to give a white amorphous residue (1.94 g) which showed four major Dragendorff- (alkaloids) and three benzidine metaperiodate- (polyols) spots on analytical TLC [Sil G/UV_{254}, Macherey–Nagel, using $CHCl_3$–MeOH (19:1) as developer].

2-Epilycorine (**1**). A portion of the residue (0.2 g) was dissolved in MeOH–H_2O (4:1) and subjected to semi-prep. HPLC [on a

Spectra Physics assembly, fitted with a UV detector 440/254 nm and RP-8 column; MeOH–H_2O (4:1) as eluant at a flow-rate of 3 ml/min]. The eluates at 3–3.5 min were collected and again subjected to semi-prep. HPLC when lycorine (18 mg) and 2-epilycorine (7 mg) were obtained in succession. The whole process was repeated five times to collect *ca* 30 mg of 2-epilycorine, mp 168–170°; $[\alpha]_D^{28} - 212.8°$ (MeOH; *c* 0.6); MS: *m/z* (rel. int. %) 287 (M^+, 27), 227 (100), 226 (95); molecular formula, $C_{16}H_{17}NO_4$ by high resolution MS (M, observed: 287.1139; required 287.1153); the isopropylidene derivative was prepared by treatment with dry MeOAc, dry $ZnCl_2$ and H_3PO_4, as an amorphous powder which crystallized from alcohol as colourless needles, mp 182–184° (lit. [6] mp 184–186°); *m/z* 327 (M^+, 64).

Metabolism of lycorine and 2-epilycorine. In a typical experiment, 2-epilycorine (11 mg) was added to Pi buffer saline (pH 7.2, 10 ml) soln of 1-day-old flower stem fluid (2.5 ml) of *C. latifolium*. The mixture was filtered through a 0.45 μ membrane and the filtrate was incubated at 21 ± 2° for 14 hr. The metabolic changes in the fluid were monitored by analytical HPLC [$C_{18}\mu$ Bondapak analytical column; UV 440/254 nm detector; MeOH–H_2O (7:3) as eluant; flow rate 1 ml/min] using reference samples as markers. R_t (the concn of the individual alkaloids at the end of the incubation period in parenthesis): 2-epipancrassidine (550 μg) 12.91; 2-epilycorine (3 mg) 9.40; 4,5-dehydroanhydrolycorine (1.1 mg) 8.22; hippadine (2.34 mg) 7.55. The concn of these alkaloids in the control flower stem fluid ranged from 10–20% of the respective amounts shown in parenthesis. Lycorine, under similar conditions, produced the following metabolic products: 2-oxyphenanthridinium betaine (ungeremine), R_t 17.7; lycorine-2-one, 3.8; and hippeastrine, 2.92.

Vanadium-catalysed transformation of 1-O-acetyl-2-epilycorine. To a soln of vanadyl acetylacetone (16 mg, Aldrich), in dry toluene (12 ml), 1-*O*-acetyl-2-epilycorine (0.11 g) was added under N_2. To this mixture, *t*-BuOOH (1.2 g, Aldrich), in dry toluene (5 ml), was gradually added (15 min). The mixture was stirred at 40° for 24 hr. The solvent was removed *in vacuo* and the residue was chromatographed on a column of silica gel H (Glaxo, 5 × 1 cm) using C_6H_6–EtOAc (9:1, 1:1) and EtOAc as eluants.

Hippadine (**9**). The middle C_6H_6–EtOAc (9:1) eluates gave hippadine (18 mg), mp 207–209° (mmp, co-HPLC, UV, MS) [7].

4,5-Dehydroanhydrolycorine (**8**). The later C_6H_6–EtOAc (9:1) and the early (1:1) fractions afforded this compound as an amorphous powder (7.5 mg). Direct comparison (co-HPLC, UV, MS) with an authentic sample [8] established that they were identical.

2-Epipancrassidine (**7**). The EtOAc eluates were combined and evapd and the residue crystallized from isopropyl alcohol to give 2-epipancrassidine as straw coloured micro-crystals (22 mg), mp 207–210° (dec); co-TLC with pancrassidine (**10**) [8] showed that they were different, 7 being more polar ($CHCl_3$–MeOH–HOAc, 18:1:1); $[\alpha]_D^{28} - 112.5°$ (MeOH; *c* 0.7); UV: λ_{max}^{MeOH} nm (log ε) 230 (sh), 284 (3.55); MS: *m/z* 303 (M^+, 17), 285 (9), 267 (5), 243 (100), 242 (18) (observed metastable peak at m* 195.0; $243^2/303$ requires: 194.88). The molecular formula $C_{16}H_{17}NO_5$ was established by high resolution MS (M^+ 303.1100); isopropylidene derivative, crystallized from Me_2CO as needles, mp 242–244°; MS: *m/z* 343 (M^+, 100); heating of (**7**) with MeOH–HCl gave 4,5-anhydro-dehydrolycorine by dehydration of ring C.

1-O-Acetyl-2-epilycorine. 1,2-Di-*O*-acetyl-2-epilycorine (0.34 g) was obtained from 2-epilycorine (0.52 g) by treatment with Ac_2O and pyridine, at room temp. for 24 hr. The solvent was removed by chasing with N_2 and the product crystallized from petrol–MeOAc as cluster of needles, mp 190–191°. This compound was treated with $CHCl_3$ (moist), saturated with HCl gas, at room temp. for 30 min when it was partially deacylated to 1-*O*-acetyl-2-epilycorine. CC of the product over florisil, using $CHCl_3$–MeOH (49:1, 19:1) as eluant afforded 1-*O*-acetyl-2-epilycorine (0.12 g) as a pure entity, mp 203–205°; MS: *m/z* 329 (M^+, 44), 270 (7), 269 (14); IR: ν_{max}^{KBr} 3550 (OH), 1735 (OAc), 938 (OCH_2O) cm^{-1}.

The products of vanadium-catalysed oxidation of 1-*O*-acetyllycorine were reported previously [10].

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