Me₂CO–petrol, mp 118°. Yield 0.002%. TLC on silica gel (C₆H₆–EtOAc (9:1), Rₖ = 0.25) (Found: C, 70.20; H, 7.2; N, 5.4. Calc. for C₁₅H₁₇N0₃: C, 70.31; H, 7.01; N, 5.12%).

Cyclisation of glycolone. Glycolene (200 mg) was refluxed with 6 N HCl (50 ml) for 6 hr. The reaction product was cooled, 10% aq NaOH added in excess and the mixture extracted with EtOAc. On evapn of solvent a solid was obtained which was crystallised from Me₂O–petrol, mp 131°. Yield 90 mg. TLC on silica gel (C₆H₆–EtOAc (9:1), Rₖ = 0.38). (Found: C, 69.31; H, 6.72; N, 5.5. Calc. for C₁₅H₁₇N0₃: C, 69.48; H, 6.61; N, 5.40%).

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REFERENCES

INTRODUCTION
In connection with our work on the reactive intermediates of Amaryllidaceae alkaloids [1–4], we have investigated the alkaloidal constituents of the fresh bulbs of Zephyranthes rosea, collected during flowering. The species grows abundantly in the upper Gangetic plain as well as in the Sikkim region of the Eastern Himalayas up to 1000 m, and is also grown in gardens as an ornamental flowering plant and for medicinal purposes. Extracts of its flowers and bulbs are used for a variety of therapeutic purposes which can be described in modern terms as immunomodulators. The species, of European origin, was previously reported [5] to contain only galanthamine. We report the isolation and characterisation of four alkaloids from methanol extracts of fresh bulbs of this species. Additionally, a facile transformation of maritidine to (+)-epimaritidine is described and the mechanism of the epimerisation is appraised.

RESULTS AND DISCUSSION
Column chromatography of the chloroform-soluble fraction of the residue from methanol extracts of fresh bulbs of Z. rosea, collected during the first onset of flowers, afforded one new (compound 1), and three known alkaloids, crinamine, haemanthamine and maritidine, in quantities sufficient for their complete characterisation. Only the structural elucidation of the new alkaloid is described below.

The new compound, C₁₅H₂₁O₃N (by accurate mass measurement), mp 214–215°, exhibited UV, IR and mass spectra similar to those of maritidine. The splitting pattern of the olefinic hydrogens in the 90 MHz ¹H NMR spectrum of the compound was, however, different from that of maritidine [4, 6]. It had the same HPLC Rₛ as a reference sample of (+)-epimaritidine. Maritidine, isolated from Z. flav a Roem & Schult. [4], on oxidation with active manganese dioxide, in chloroform, gave (+)-oxomaritidine [6], which on reduction with sodium borohydride, in methanol, gave (+)-epimaritidine, referred to here as the reference sample. The UV, IR,
1H NMR and mass spectra of compound 1 were indistinguishable from those of the reference sample. The CD spectra of compound 1 and of the reference sample were also identical and matched, in shape and sign, those of maritidine [7]. Furthermore, as expected for a C-3 epimeric 5,10b-ethanophenanthridine alkaloid, the dihroism in the case of compound 1 was of decreased magnitude compared with that of maritidine. On the basis of the above data, (+)-epimaritidine structure 1 was assigned to this compound. This is the first report of the natural occurrence of (+)-epimaritidine. A biogenetic-type synthesis of (+)-epimaritidine was reported earlier [8].

Another noteworthy observation was the facile conversion of maritidine (2) to (+)-epimaritidine (1), in the presence of aqueous hydrochloric acid. Schwartz and Holton previously reported [8] that (+)-epimaritidine, on heating with aqueous hydrochloric acid, was partially converted into (+)-maritidine (ca 29%), an appreciable amount (ca 33%) of the starting material (racemic epimaritidine) was recovered unchanged. These authors designed the experiment on the basis of literature precedents [9, 10] that (i) cyclohexenyl cations exhibit a pronounced tendency to pick up nucleophiles in a quasi-axial manner [9] and (ii) since the C-ring of 5,10b-ethanophenanthridine alkaloids is sterically unexceptional [10], they would predominantly produce epimers, under aqueous acidic conditions, with quasi-axial C-3-OH by collapse of the cyclohexenyl cation (of type 3) when captured by water. Thus, 'steric approach control' was expected to be the predominant phenomenon in this equilibration reaction. However, contrary to this expectation, the actual conversion of (+)-epimaritidine to (+)-maritidine was only partial. We thought it worthwhile to attempt this epimerization in the reverse direction (2 → 1), on prolonged heating with aqueous hydrochloric acid. The rationale of this approach was as follows. Construction of a Dreeiding model suggested that epimaritidine (C-3-3-OH quasi-equatorial), unlike maritidine, is free from 1,3-diauxial interaction at this centre and would therefore be thermodynamically more stable. This is exactly what happened during the 'product development' from prolonged heating of maritidine in the presence of aqueous hydrochloric acid. Thus, (+)-epimaritidine was obtained as the major product (ca 85%) from the equilibration of maritidine (+)-epimaritidine.

We note finally that: (i) (+)-epimaritidine is the second example of a 5,10b-ethanophenanthridine alkaloid (maritidine being the first) to contain aryl-dimethoxy substituents in place of the methylenedioxy commonly found in the congener alkaloids; (ii) it (1) comprises a missing link in the naturally occurring C-3-epimeric pairs of 5,10b-ethanophenanthridine alkaloids of the vittatine–haemanthamine type (C-11 to C-12 bridge-head below), e.g. crinamine–haemanthamine, (+)epicrinine–vittatine, hamayne-11-hydroxyvittatine, (+)-epimaritidine–maritidine, (+)-epibuphaninone–complementary pair still missing; (iii) in the case of the naturally occurring (−)-crinine–powelline type alkaloids (C-11 to C-12 bridge-head above), the orientation of the C-3 oxygen substituent is invariably quasi-axial and these alkaloids generally co-occur with their corresponding 1,2-β-epoxy analogues (in place of the olefinic unsaturation). The biochemical significance of these findings is currently being studied by trapping reactive intermediates of alkaloids at the time of intense active growth of amarilloidaceus plants.

**EXPERIMENTAL**

The general procedures were the same as those reported recently [3]. The plant material was collected from the Banaras Hindu University Campus and was identified by Professor S. K. Roy, Department of Botany, Banaras Hindu University. A voucher specimen of the plant has been preserved at the Department of Pharmaceutics, Banaras Hindu University, Varanasi.

**Isolation procedure.** Fresh bulbs of *Z. rosea* Lindl. (ca 1 kg) were macerated in ac. MeOH in a high-speed blender, filtered, and the filtrate evaporated *in vacuo* to give a viscous residue (4.3 g). A portion (0.5 g) of the residue was triturated with CHCl₃ (containing traces of MeOH) and the CHCl₃ concentrate chromatographed over a column of Florisil (20 x 2 cm). Elution was carried out with CH₃OH (1 : 1), CH₃OH–CHCl₃ (1 : 1, 1 : 1.5, 1 : 1), CHCl₃ (1 : 1), and CHCl₃–MeOH (99 : 1, 95 : 5, 1 : 1 each). Fractions (100 ml) were collected and monitored by analytical TLC using CHCl₃–MeOH (99 : 1) as developer. The later CHCl₃ and early CHCl₃–MeOH (99 : 1) eluates were combined and the solvent was evaporated to give a colourless solid consisting of a mixture of two major alkaloids plus traces of minor bases.

**Crinamine.** The solid was triturated with dry Me₂CO to give crinamine (11 mg) as the sparingly soluble component, mp 193–195°; [α]₀°²⁸ + 157.4° (c 0.31; CHCl₃); 1H NMR (DMSO-d₆): 6.68 (1H, s, H-10), 6.55 (1H, s, H-7), 6.27 (1H, dd, J = 10.5, 2 Hz, H-1), 5.98 (2H, s, OCH₂O), 5.93 (1H, dd, line broadening, J = 10.5, 5 Hz, H-2), 4.1 (1H, d, J = 17 Hz, H-6o), 3.9 (1H, m, overlapping by H-11, H-3), 3.7 (1H, d, J = 17 Hz, H-6b), 3.4–2.8 (5H, m, CotMe, H-4a, H-12), 2.1–1.7 (2H, m, H-4a, 4f); CIMS m/z (rel. int.): 302 [M + H]⁺ (100).

**Haemanthamine.** The Me₂CO mother liquor, after separation of crinamine, was passed through a short column of Florisil and washing was done with CHCl₃–MeOH (99.5 : 0.5) (200 ml). The combined eluates were evaporated and the residue was crystallized from Me₂CO–petrol to give haemanthamine as colourless crystals (24 mg), mp 201–203°; [α]₀°²⁸ + 38.3° (c 0.45; CHCl₃).

1H NMR: the complex splitting patterns of H-1 and H-2, as depicted in an earlier paper [11], were not discernible when the
90 MHz spectrum was taken in CDCl₃, instead only the AB part (4-line) of the ABX (X, H-3) multiplicities was observed. However, when the spectrum was taken in DMSO-d₆, the expected multiplicities appeared: 6.95 (1H, s, H-10), 6.57 (1H, s, H-7), 6.46 (1H, d, J = 10 Hz, H-1), 6.13 (1H, dd, J = 10.5, 5 Hz, H-2), 5.90 (2H, s, CH₂O), 4.95 (line width of co 8 Hz, OH), 4.16 (1H, d, J = 17 Hz, H-1'), 3.70 (1H, d, J = 17 Hz, H-6'), 3.50 (1H, d, J = 17 Hz, H-6A), 3.2 (3H, s, C₆H₃OMe), 2.95 (3H, m, H-4a, 12°129°), 2.0 (1H, ddd, J = 13.5, 13, 3.6 Hz, H-5); MS m/z (rel. int.): 301 [M+] (100). The needles CHCl₃-MeOH (99: 1) eluates afforded a straw-coloured solid consisting of a mixture of two alkaloids, R, 0.48 (major) and 0.4 (minor).

(+)-Epinaritidine (1). The solid was repeatedly crystallized from CHCl₃ (containing traces of MeOH) to give pure (+)-epinaritidine as colourless micro-crystals (27 mg), mp 214-215°; [α]D +83.2° (c 0.47; MeOH); IR νKBr cm⁻¹: 3600, 1618, 1038, 1005; ¹H NMR (CDCl₃): δ 6.86 (1H, s, H-10), 6.55 (1H, dd, J₁₂ = 10 2 Hz, H-1; irradiation of H-2 and H-3, in succession, resulted in the collapse of the multiplicities into a doublet in each case: J₁₂ = 10 Hz, and J₁₂ = 2 Hz), 6.50 (1H, s, H-7), 5.92 (1H, d, J₁₂ = 10 Hz, H-1), 4.25 (1H, ddd, J₂₁ = 2 Hz, J₁₂ = 10 Hz, H-2), 3.89 (3H, s, Ar-GMe), 3.82 (3H, s, Ar-GMe); MS m/z (rel. int.): 287 [M+] (100), 270 (42), 269 (19), 262 (12), 253 (17), 252 (11), 197 (7) [M⁺]⁺ by accurate mass measurement: 287.150 (M⁺). Maritidine (2). The combined CHCl₃-MeOH (95: 5) eluates on evap gave maritidine as colourless crystals (31 mg), mp 250-252°; [α]D +26.8° (c 0.78; MeOH); +22.4° (CHCl₃). ¹H NMR (CDCl₃): δ 6.80 (1H, s, H-10), 6.66 (1H, d, J = 10 Hz, H-11), 6.01 (1H, dd, J = 10.2 Hz, H-21), 4.31 (1H, m, H-3), 3.92 (3H, s, Ar-GMe), 3.88 (3H, s, Ar-GMe); MS m/z (rel. int.): 287 [M⁺] (100).

Oxidation of maritidine (31 mg) with active MnO₂ in CHCl₃, according to ref. [12], gave oxomaritidine (17 mg), mp 140-142° (UV, IR, ¹H NMR, MS) [6]. Reduction of oxomaritidine (12 mg), with NaBH₄ (22 mg) in MeOH afforded (+)-epinaritidine (reference sample) (10 mg), mp 214-216° (mp, co-TLC, ¹H NMR).

Epimerization of maritidine to (+)-epinaritidine. A solution of maritidine (22 mg) in aq. HCl (10%, 10 ml) was heated under reflux for 4 hr. The soln was cooled, basified (NaHCO₃) and extracted with CHCl₃ (3 × 50 ml). The combined CHCl₃ extracts were processed in the usual way to give a brown dry powder. This was re-dissolved in CHCl₃ (5 ml) and chromatographed over a column of Florisil (20 g), as described above, to give (+)-epinaritidine (17 mg), followed by maritidine (ca 2 mg).

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REFERENCES