Me_2CO–petrol, mp 118°. Yield 0.002%. TLC on silica gel (C_6H_6–EtOAc (9:1), R_f = 0.25) (Found: C, 70.20; H, 7.2; N, 5.4. Calc. for $C_{16}H_{19}NO_3$: C, 70.31; H, 7.01; N, 5.12%.)

Cyclisation of glycolone. Glycolone (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 Me_2CO–petrol, mp 131°. Yield 90 mg. TLC on silica gel (C_6H_6–EtOAc (9.1), R_f = 0.38). (Found: C, 69.31; H, 6.72; N, 5.5. Calc. for $C_{15}H_{17}NO_3$: C, 69.48; H, 6.61; N, 5.40%)

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REFERENCES

1. Bhattacharyya, P. and Chowdhury, B. K. (1984) *Phytochemistry* **23**, 1825.
2. Das, B. P. and Chowdhury, D. N. (1978) *Chem. Ind* 272
3. Rapoport, H. and Holden, K. G (1960) *J. Am. Chem. Soc.* **82**, 4393.
4. Goodwin, S and Horning, E. C. (1959) *J. Am. Chem. Soc.* **81**, 1908.
5. Openshaw, H. T (1967) *The Alkaloids* (Manske, R H. F., ed.), Vol. IX, p. 223.

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(+)-EPIMARITIDINE, AN ALKALOID FROM *ZEPHYRANTHES ROSEA**

SHIBNATH GHOSAL, ASHUTOSH and SUSHMA RAZDAN

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

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Key Word Index—*Zephyranthes rosea*; Amaryllidaceae; 5,10b-ethanophenanthridine alkaloid; (+)-epimaritidine; C-3 epimerization.

Abstract—The isolation and determination, by spectroscopic analyses and chemical correlation, of the structure and stereochemistry of (+)-epimaritidine, a new alkaloid from *Zephyranthes rosea*, is reported. A facile transformation of maritidine to (+)-epimaritidine is described and the mechanism is appraised in the light of the thermodynamic stability of the latter epimer. (+)-Epimaritidine comprises a missing link in the C-3 epimeric pairs of 5,10b-ethanophenanthridine alkaloids of the vittatine–haemanthamine type.

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 characterization 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 epimerization is appraised.

*Part 9 in the series "Chemical Constituents of Amaryllidaceae" For Part 8 see ref. [1].

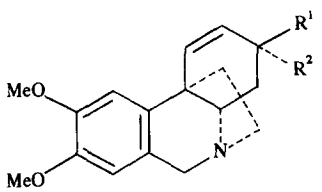
RESULTS AND DISCUSSION

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 characterization. Only the structural elucidation of the new alkaloid is described below.

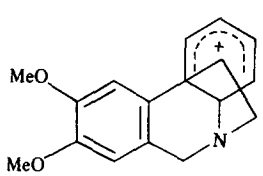
The new compound, $C_{17}H_{21}O_3N$ (by accurate mass measurement), mp 214–215°, exhibited UV, IR and mass spectra similar to those of maritidine. The splitting pattern of the olefinic hydrogens in the 90 MHz ^1H NMR spectrum of the compound was, however, different from that of maritidine [4, 6]. It had the same HPLC R_t as a reference sample of (+)-epimaritidine. Maritidine, isolated from *Z. flava* 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,

^{1}H 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 dichroism 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 Dreiding model suggested that epimaritidine (C-3-OH quasi-equatorial), unlike maritidine, is free from 1,3-diaxial 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.



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



3

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, (+)-epibuphanisine–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 amaryllidaceous 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 aq. MeOH in a high-speed blender, filtered, and the filtrate evapd *in vacuo* to give a viscous residue (4.3 g). A portion (0.5 g) of the residue was triturated with $CHCl_3$ (containing traces of MeOH) and the $CHCl_3$ concentrate chromatographed over a column of Florisil (20 × 2 cm). Elution was carried out with C_6H_6 (1 l.), C_6H_6–$CHCl_3$ (1:1, 1.5 l.), $CHCl_3$ (1 l.), and $CHCl_3$–MeOH (99:1, 95:5, 1 l. each). Fractions (100 ml) were collected and monitored by analytical TLC using $CHCl_3$–MeOH (9:1) as developer. The later $CHCl_3$ and early $CHCl_3$–MeOH (99:1) eluates were combined and the solvent was evapd 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 Me_2CO to give crinamine (11 mg) as the sparingly soluble component, mp 193–195°; $[\alpha]_D^{22}$ +157.4° (*c* 0.31; $CHCl_3$); ^{1}H NMR (DMSO-d_6): δ6.88 (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*, OCH_2O), 5.93 (1H, *dd*, line broadening, J = 10.5, 5 Hz, H-2), 4.1 (1H, *d*, J = 17 Hz, H-6α), 3.9 (1H, *m*, overlapping by H-11, H-3), 3.7 (1H, *d*, J = 17 Hz, H-6β), 3.4–2.8 (5H, *m*, C_3-OMe, H-4a, H-12), 2.1–1.7 (2H, *m*, H-4α,4β); CIMS *m/z* (rel. int.): 302 $[M+H]^+$ (100).

Haemanthamine. The Me_2CO mother liquor, after separation of crinamine, was passed through a short column of Florisil and washing was done with $CHCl_3$–MeOH (99.5:0.5) (200 ml). The combined eluates were evapd and the residue was crystallized from Me_2CO–petrol to give haemanthamine as colourless crystals (24 mg), mp 201–203°; $[\alpha]_D^{22}$ +38.3° (*c* 0.45; $CHCl_3$), ^{1}H 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 $CDCl_3$, instead only the AB part (4-line) of the ABX (X, H-3) multiplicities was observed. However, when the spectrum was taken in DMSO-d_6 the expected multiplicities appeared: δ6.95 (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*, OCH_2O), 4.95 (line width of *ca* 8 Hz, OH), 4.16 (1H, *d*, J = 17 Hz, H-6α), 3.70 (1H, *ddd*, J = 4.5, 3.6, 2.5 Hz, H-3), 3.50 (1H, *d*, J = 17 Hz, H-6β), 3.2 (3H, *s*, C_3-OMe), 2.95 (3H, *m*, H-4a, 12α,12β), 2.0 (1H, *ddd*, J = 13.5, 13, 3.6 Hz, H-4α), 1.8 (1H, *ddd*, J = 13.5, 3.8, 2.5 Hz, H-4β); MS *m/z* (rel. int.): 301 $[M]^+$ (100).

The middle $CHCl_3$–MeOH (99:1) eluates afforded a straw-coloured solid consisting of a mixture of two alkaloids, R_f 0.48 (major) and 0.4 (minor).

(+)-*Epimaritidine* (1). The solid was repeatedly crystallized from $CHCl_3$ (containing traces of MeOH) to give pure (+)-epimaritidine as colourless micro-crystals (27 mg), mp 214–215°; $[\alpha]_D^{22}$ +83.2° (*c* 0.47; MeOH); IR ν_{max}^{KBr} cm^{-1}: 3600, 1618, 1038, 1005; ^{1}H NMR ($CDCl_3$): δ6.86 (1H, *s*, H-10), 6.55 (1H, *dd*, $J_{1,2}$ = 10 Hz, $J_{1,3}$ = 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: $J_{1,2}$ = 10 Hz, and $J_{1,3}$ = 2 Hz), 6.50 (1H, *s*, H-7), 5.92 (1H, *d*, $J_{2,1}$ = 10 Hz, H-2), 4.25 (1H, *ddd*, $J_{3,1}$ = 2 Hz, $J_{3,4\beta}$ = 7 Hz, $J_{3,4\alpha}$ = 9.4 Hz, H-3), 3.89 (3H, *s*, Ar–OMe), 3.82 (3H, *s*, Ar–OMe); MS *m/z* (rel. int.): 287 $[M]^+$ (100), 270 (42), 269 (4), 258 (19), 244 (22), 232 (12), 215 (72), 203 (11), 189 (7) ($[M]^+$ by accurate mass measurement: 287.150. $C_{17}H_{21}NO_3$ requires: $[M]^+$ 287.150); $\Delta\varepsilon_{294}$ +1.98, $\Delta\varepsilon_{285}$ +2.46, $\Delta\varepsilon_{243}$ −1.02 (maritidine: $\Delta\varepsilon_{294}$ +2.08, $\Delta\varepsilon_{286.5}$ +2.58, $\Delta\varepsilon_{243.5}$ −1.39).

Maritidine (2). The combined $CHCl_3$–MeOH (95:5) eluates on evapn gave maritidine as colourless crystals (31 mg), mp 250–252°; $[\alpha]_D^{22}$ +26.8° (*c* 0.78; MeOH), +22.4° ($CHCl_3$); ^{1}H NMR ($CDCl_3$): δ6.80 (1H, *s*, H-10), 6.66 (1H, *d*, J = 10 Hz, H-1), 6.01 (1H, *dd*, J = 10, 2 Hz, H-2), 4.3 (1H, *m*, H-3), 3.92 (3H, *s*, Ar–OMe), 3.88 (3H, *s*, Ar–OMe); MS *m/z* (rel. int.): 287 $[M]^+$ (100).

Oxidation of maritidine (23 mg) with active MnO_2 in $CHCl_3$, according to ref. [12], gave oxomaritidine (17 mg), mp 140–142° (UV, IR, ^{1}H NMR, MS) [6]. Reduction of oxomaritidine (12 mg), with $NaBH_4$ (22 mg) in MeOH afforded (+)-epimaritidine ('reference sample') (10 mg), mp 214–216° (mmp, co-TLC, ^{1}H NMR).

Epimerization of maritidine to (+)-epimaritidine. A soln of maritidine (22 mg) in aq. HCl (10%, 10 ml) was heated under reflux for 4 hr. The soln was cooled, basified ($NaHCO_3$) and extracted with $CHCl_3$ (3 × 50 ml). The combined $CHCl_3$ extracts were processed in the usual way to give a brown dry powder. This was re-dissolved in $CHCl_3$ (5 ml) and chromatographed over a column of Florisil (20 g), as described above, to give (+)-epimaritidine (17 mg), followed by maritidine (*ca* 2 mg).

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REFERENCES

1. Ghosal, S., Saini, K. S. and Arora, V. K. (1984) *J. Chem. Res.* (*S*) 232.
2. Ghosal, S., Saini, K. S. and Arora, V. K. (1983) *J. Chem. Res.* (*S*) 238.
3. Ghosal, S., Kumar, Y. and Singh, S. P. (1984) *Phytochemistry* **23**, 1167.
4. Ghosal, S. (1983) *Proc. Int. Symp. Medicinal Aromatic Plants*, pp. 13–14. CIMAP, Lucknow, India.
5. Boit, H. G., Doepke, W. and Stender, W. (1957) *Chem. Ber.* **90**, 2203.
6. Wildman, W. C. (1968) in *The Alkaloids* (Manske, R. H. F., ed.), Vol. XI. Academic Press, New York.
7. De Angelis, G. G. and Wildman, W. C. (1969) *Tetrahedron Letters* 729.
8. Schwartz, M. A. and Holton, R. A. (1970) *J. Am. Chem. Soc.* **92**, 1090.
9. Goering, H. L. (1960) *Rec. Chem. Progr.* **21**, 109.
10. Whitlock, H. W., Jr. and Smith, G. L. (1967) *J. Am. Chem. Soc.* **89**, 3600.
11. Haugwitz, R. D., Jeffs, P. W. and Wenkert, E. (1965) *J. Chem. Soc.* 2001.
12. Uyeo, S., Fales, H. M., Highet, R. J. and Wildman, W. C. (1960) *J. Am. Chem. Soc.* **82**, 2590.