

## Isolation and Characterization of Indole Alkaloids from the Flowers of *Ervatamia coronaria* (Syn: *Taberaemontana divaricata*)

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**Abstract** – From the flowers of *Ervatamia coronaria* four indole alkaloids have been isolated and characterized as harmine, heyneanine, voacristine and apparicine with the help of various spectral data. The alkaloids Harmine and Heyneanine are reported first time from the flowers of *Ervatamia coronaria*.

**Keywords** – *Ervatamia coronaria*, Apocynaceae, Indole alkaloids

### Introduction

*Ervatamia coronaria* (stapf.) (Syn: *Taberaemontana divaricata*) (Family, Apocynaceae) is a glabrous, evergreen, dichotomously branched shrub or small tree with milky juice, cultivated all over India primarily for the ornamental value of its fragrant white flowers and for medicinal uses. Various parts of the plant are used in the indigenous system of medicine for the treatment of ophthalmia, for application on wounds and inflamed parts of the body and as an anthelmintic etc. (The trees of Calcutta, 1946). Anticancer activity has also been reported from the crude extracts of the plant (Raj *et al.*, 1974). This species has been extensively investigated and a number of chemical constituents from the leaves, stems and roots of the plant have previously been reported in a number of instances (Van Beek *et al.*, 1984, 1985; Atta-ur-Rahman *et al.*, 1985, 1986; Danieli *et al.*, 1986; Sharma *et al.*, 1988; Kam *et al.*, 1992, 1993). The alkaloid metabolism in plant cell suspension culture of the species was also extensively investigated (Dagnino *et al.*, 1991, 1994). The present work deals with the isolation and characterization of four indole alkaloids from the flowers of *Ervatamia coronaria*.

### Experimental

**General experimental procedure** – Melting points (°C) were uncorrected. IR (KBr) spectra were recorded on a Perkin-Elmer Instrument. <sup>1</sup>HNMR spectra were recorded on the Bruker Avance DRX-300 using CDCl<sub>3</sub> as internal

standard. EIMS data 70 eV recorded on JOEL-JMSD spectrometer with direct inlet system and FAB-MS (positive) data as JOEL SX 102/DA-600. Silica gel (60-120 mesh) was used for column chromatography (CC) and thin layer chromatography (TLC) was run on silica gel coated plates.

**Plant material** – The flowers of *Ervatamia coronaria* were collected from Mangalore, Dakshina Karnataka, India. It was authenticated by Dr. Gopalakrishna Bhat, Department of Botany, Poomaprajna College, Udupi. A voucher specimen is deposited in the herbarium of our Institute.

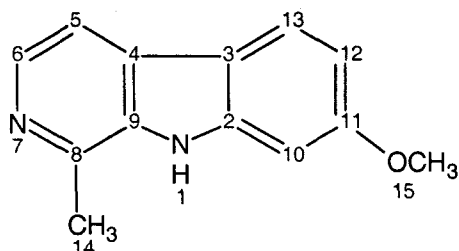
Fresh flowers were taken (15 kg) and dried by means of natural sun drying. The dried flowers were powdered (10 kg) and soaked with 25% aqueous ammonium hydroxide. It was then exhaustively extracted with chloroform in Soxhlet apparatus. The solvent from the extract was distilled off and the concentrate was evaporated on a water bath to give chloroform extract (50.5 gm). 50 gm of chloroform extract was taken and partitioned into dilute hydrochloric acid (0.1N) and the aqueous layer was alkalized with concentrated ammonium hydroxide. It was further partitioned with ether. The ether fraction was then concentrated to give an alkaloid fraction (10.2 gm).

10 gm of alkaloidal mixture was taken and subjected to column chromatography over silica gel eluting with chloroform : methanol (50:50). The elution was monitored by TLC (silica gel G, Visualization Dragendroffs reagent). Each time 10 ml were collected and identical eluates (TLC monitored) were combined and kept in a refrigerator. Eluates between fractions 40-100 resulted a mixture of four compounds after removing the solvent a residue (150 g) resulted. This residue was subjected to preparative TLC in solvent system Ethyl Acetate: Benzene (1:1). The four compounds

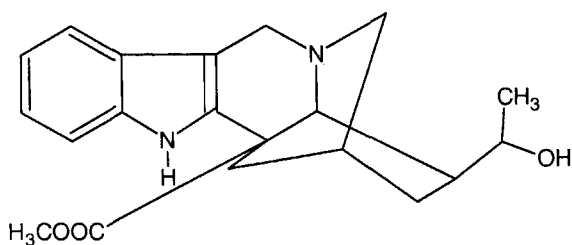
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were isolated as pure components. These were designated as compound A (20 mg) compound B (23 mg) compound C (32 mg) and compound D (36 mg).

**Compound A (Harmine)** – Compound A, crystallized from methanol as white crystals, m.p. 261°C. It gave positive reactions for Mayer's test, Dragendorff's test, Wagners test and Hagers test for alkaloids. It exhibited UV :  $\lambda_{\max}^{\text{(MeOH)}}$  240, 302, 338 nm. IR (KBr  $\text{cm}^{-1}$ )  $\lambda_{\max}$  3475.13 (N-H), 3141.68 (Ar C-H), 2956.43 (C-H), 1622.64 (C=N). Its molecular formula was found to be  $\text{C}_{13}\text{H}_{12}\text{N}_2\text{O}$  ( $\text{M}^+$  212), other peaks were at 197, 183, 169, 167, 140, 128, 115, and 108.  $^1\text{H}$ NMR ( $\text{CDCl}_3$ ):  $\delta$  8.34 (s, 1H, NH),  $\delta$  6.93 to 7.99 (m, 5H, ArH),  $\delta$  3.93 (s, 3H,  $\text{OCH}_3$ )  $^{13}\text{C}$  NMR: 136.5 (C-2), 119.9 (C-3), 102.1 (C-4), 120.5 (C-5), 149.4 (C-6), 158.6 (C-8), 124.1 (C-9), 96.5 (C-10), 153.1 (C-11), 107.3 (C-12), 121.5 (C-13), 16.1 (C-14), 56.0 (C-15). From the above evidence compound A was determined as **harmine**.

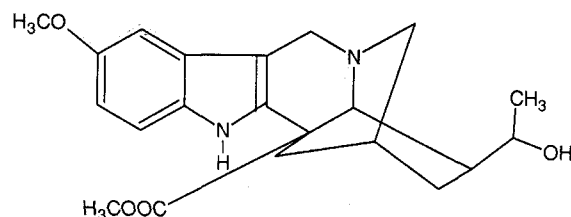


**Compound B (Heyneanine)** – Compound B, crystallized from methanol as white crystals, m.p. 157-160°C. It gave positive reactions for the general tests for alkaloids. IR (KBr  $\text{cm}^{-1}$ ):  $\lambda_{\max}$  3400 (OH), 3220 (Indole N-H), 1725 ( $\text{COOCH}_3$ ). Its molecular formula was found to be  $\text{C}_{21}\text{H}_{26}\text{N}_2\text{O}_3$  ( $\text{M}^+$  354), other peaks were at 253, 214, 195, 194, 154 and 130.  $^1\text{H}$ NMR ( $\text{CDCl}_3$ ):  $\delta$  7 to 7.5 (m, 4H, ArH),  $\delta$  8.3 (s, 1H, NH),  $\delta$  3.8 (s, 3H,  $\text{COOCH}_3$ ),  $\delta$  1.1 (d,  $J=6.5$  Hz, 3H,  $\text{CH}_3$ ). The signal due to other methine, methylene and the OH protons overlapped with each other and appeared as multiplets in the region of  $\delta$  1.5 to 4.4 integrating for 15 protons. From the above evidence, compound B was determined as **heyneanine**.

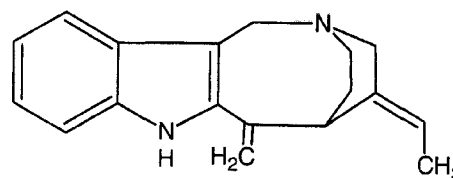


**Compound C (Voacristine)** – Compound C, crystallized from methanol as white crystals, m.p. 160-162°C. It gave positive reactions for the general tests for alkaloids. IR (KBr  $\text{cm}^{-1}$ ):  $\lambda_{\max}$  3400 (OH), 3220 (Indole NH), 1725 ( $\text{COOCH}_3$ ).

Its molecular formula was found to be  $\text{C}_{22}\text{H}_{28}\text{N}_2\text{O}_4$  ( $\text{M}^+$  384), other peaks were at 369, 366, 3512, 339, 325, 307, and 301.  $^1\text{H}$ NMR ( $\text{CDCl}_3$ ):  $\delta$  7.9 (s, 1H, NH),  $\delta$  6.75 to 7.35 (m, 3H, ArH),  $\delta$  3.85 (s, 3H,  $\text{OCH}_3$ ),  $\delta$  3.8 (s, 3H,  $\text{COCH}_3$ ),  $\delta$  1.1 (d,  $J = 6.5$  Hz 3H,  $\text{CH}_3$ ). The signals due to other methine, methylene and the OH proton overlapped with each other appeared as multiplet in the region of  $\delta$  1.5 to 4.4 integrating for 14 protons. From the above evidence compound B was determined as **voacristine**.



**Compound D (Apparicine)** – Compound D, crystallized from methanol as white crystals, m.p. 193°C. It gave positive reactions for the general test for alkaloids. IR (KBr  $\text{cm}^{-1}$ ):  $\lambda_{\max}$  3400 (NH). Its molecular formula was found to be  $\text{C}_{18}\text{H}_{20}\text{N}_2$  ( $\text{M}^+$  264), other peaks were at 249, 235, 222 and 208.  $^1\text{H}$ NMR ( $\text{CDCl}_3$ ):  $\delta$  7.9 (s, 1H, NH),  $\delta$  6.9 to 7.5 (m, 4H, Ar H),  $\delta$  5.24 and  $\delta$  5.37 (s, 2H, exocyclic  $\text{CH}_2$ ),  $\delta$  2.8 to 3.6 (s, 2H, C-7),  $\delta$  3.9 (s, 1H, C-2),  $\delta$  1.8 to 2.3 (m, 2H, C-3). From the above evidence compound D was determined as **apparicine**.



## Results and Discussion

Chromatographic resolution of the chloroform extract of the flowers of *Ervatamia coronaria* furnished four compound A, B, C, D which were characterized as harmine, heyneanine, voacristine and apparicine by detailed spectral analysis i.e. IR,  $^1\text{H}$ NMR and mass spectrum. Harmine and Heyneanine are being reported first time from the flowers of *Ervatamia coronaria*.

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