



NOTE

(+) Corydine from the Stems of *Tinospora cordifolia*

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The present work constitutes the systematic chemical screening of matured stems of *Tinospora cordifolia* as matured stems are preferred in commercial formulations for its efficacy. Earlier works on *T. cordifolia* collected from wild sources showed a lot of chemical variations with altogether different compounds which pose difficulties in its quality management as marker compounds. The stems of *T. cordifolia* were collected at matured stage from our gene bank and extracted with alcohol after its defatification with hexane. The isolated alkaloidal part on repeated Si-gel, flash and reversed phase C₁₈ column chromatography afforded four isoquinoline alkaloids palmatine, jatrorrhizine, magnoflorine and (+) corydine. The non-alkaloidal part gave columbin, a furano diterpenoid beside β -sitosterol. (+) Corydine, a rare alkaloid was reported first time from *T. cordifolia* and from the genus *Tinospora*. The structures of compounds were identified by 2D NMR and MS analyses.

Keywords: *T. cordifolia*, Stems, Isoquinoline alkaloids, Furano diterpenoid.

Tinospora cordifolia Meirs (Menispermaceae) is a perennial shrub and the whole plant parts are useful as medicine. *T. cordifolia* is used in Ayurvedic system of medicines to treat diabetes, skin diseases, jaundice, heart diseases, rheumatoid arthritis, urinary disorder and dysentery¹. The stems of *T. cordifolia* have been reported to exhibit hepatoprotective, antipyretic, cytotoxic, antidiabetic and immunomodulatory activities²⁻⁵. The alkaloidal fraction (palmatine, jatrorrhizine and magnoflorine) of stems of *T. cordifolia* reported to possess antihyperglycemic activity⁶. The free radical scavenging activity of *T. cordifolia* is reported due to the presence of alkaloids palmatine, tetrahydropalmatine, magnoflorine and tinosporin⁷. Corydine is reported to possess inhibitory activity against tumor cell lines, leukemia P388 and L1210, melanoma B16, bladder cancer MBC2 and colon cancer Colon 26⁸.

The chemical study on matured stems of *T. cordifolia* collected at different stages of growth from our gene bank maintained at Central Institute of Medicinal and Aromatic Plants, Lucknow, India showed the presence of alkaloids palmatine, jatrorrhizine, magnoflorine and a furano diterpenoid columbin with the isolation of (+) corydine first time from the genus *Tinospora*. The immature tender stems of *T. cordifolia* resulted in its low alcoholic extract (< 1 %; dw basis) with trace of alkaloid detected by Dragendorff reagent. The isolation of the above compounds are of chemotaxonomic significance as marker compounds and alkaloids perhaps play a major role

in the biological activity of the herbal extract of *T. cordifolia* in trade.

Plant material: The matured stems of *T. cordifolia* (9.6 kg, fresh weight) were collected from a cultivar of CIMAP, Gene bank, Lucknow, India, in December 2010 and dried at room temperature and ground into powder (2.5 kg).

Extraction and isolation of (+) corydine: The powdered stems were extracted with hexane and EtOH successively by cold percolation. The concentrated ethanol extract (126 g) after acidification with 5 % hydrochloric acid gave an acid insoluble residue and was filtered. The acidic filtrate was partitioned with chloroform at pH 2 and was extracted further to get CHCl₃ soluble (1.5 g) and *n*-BuOH soluble (1.3 g) alkaloids after basification with ammonia at pH 10. The CHCl₃ part (1.5 g) on chromatography over silica gel (100-200 mesh) afforded compound **1** (10 mg). Water part after *n*-BuOH extraction was neutralized and dried which on flash chromatography on Si gel and chromatography on RP C₁₈ (15 to 25 μ) successively, gave compounds palmatine (30 mg), jatrorrhizine (15 mg) and magnoflorine (35 mg), respectively from MeOH-water (60:40) as eluant.

Characterization of isolated compounds: ¹H NMR and ¹³C NMR spectra were recorded on Bruker Avance spectrometer in methanol-d₄. ESI-MS spectra were recorded on API 3000, Applied Biosystem spectrometer. Elemental analysis was done on Vario EL III.

(+) **Corydine 1**: R_f value: 0.5 (MeOH-CHCl₃, 1:19); m.p. 144-145 °C; $[\alpha]_D^{25} + 198^\circ$ (0.2, MeOH); ¹H NMR (CD₃OD, 300 MHz, δ ppm): 6.98 (1H, d, $J = 8.1$ Hz, H-9), 6.91 (1H, d, $J = 8.5$ Hz, H-8), 6.87 (1H, s, H-3), 3.94 (3H, s, 10-OCH₃), 3.86 (3H, s, 2-OCH₃), 3.65 (3H, s, 11-OCH₃), 3.03-3.17 (3H, m, H₂-5, H_a-6), 2.80 (2H, m, H₂-4), 2.54 (3H, s, N-CH₃), 2.51 (1H, m, H_a-7), 2.38 (1H, m, H_b-7); ESI-MS m/z (rel. int.): 342 $[M + H]^+$ (95) (calculated for C₂₀H₂₃NO₄); Elemental analysis found: %: C 70.43; H 6.77; N 4.10. C₂₀H₂₃NO₄, calculated %: C 70.38; H 6.75; N 4.11 (Fig. 1).

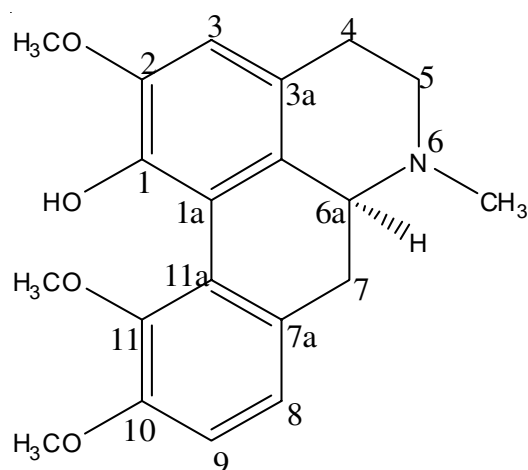


Fig. 1. Structure of (+) Corydine

Compound **1** in its ESI-MS showed its $[M + H]^+$ at m/z 342 which corresponded to its molecular formula C₂₀H₂₃NO₄. The ¹H NMR spectrum of compound **1** showed signals at δ 6.87 (H-3) and doublets at δ 6.98 (H-9) and 6.91 (H-8), respectively. Three methoxyl groups appeared at δ 3.94, 3.86 and

3.65, respectively. A singlet at δ 2.54 was assigned to N-methyl group. H-6a at δ 3.12 was determined by 2D-NOESY experiment as it showed strong NOE correlation to N-CH₃ (δ 2.54). On comparison of the physical and spectral data with those reported in literature, compound **1** was identified as (+) corydine^{9,10}.

The isolation of (+) corydine **1** constitutes its first report from *T. cordifolia* and genus *Tinospora*¹¹. The immature tender stems of *T. cordifolia* showed traces of alkaloid by Dragendorff reagent with low yield of extract.

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