

## PYRROLIZIDINE ALKALOIDS FROM *HELIOTROPIUM DISSITIFLORUM* BOISS.

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### ABSTRACT

*Heliotropium/dissitiflorum* Boiss. collected from Rud-e-Shur Tehran Province, Iran was investigated for Pyrrolizidine alkaloids (PAs). Four alkaloids namely: heliotrine **I**, heliotrine N-oxide **II**, europine **III** and 5'-deoxylasiocarpine **IV** were identified. The structures of all compounds were confirmed by spectroscopic methods.

**Key Words:** *Heliotropium dissitiflorum*, Heliotrine, Heliotrine N-oxide, Europine, 5'-Deoxylasiocarpine

### INTRODUCTION

Plant containing pyrrolizidine alkaloids (PAs) are widely distributed in the world (1). These alkaloids have been isolated from several plant families such as *Boraginaceae* including *Heliotropium* genus (2). The genus *Heliotropium* is among the genera of plants which are known to be rich in PAs (2,3). Numerous intoxications in animals and human which are caused by the consumption of certain plants containing PAs has promoted extensive studies on these alkaloids (1, 4). The source of human poisoning can be the contaminated food stuffs or traditional herbal medicine (4). The chemistry of these alkaloids in relation to their toxic and therapeutic effects has been the subject of several investigations (2,3). In continuation of our ongoing research on *heliotropium* species of Iran (5-7), the isolation and structural elucidation of PAs from *H. dissitiflorum* Boiss. is reported. Literature review showed that except a brief report by authors no detailed phytochemical studies on these species has been reported and this is the first report on the isolation of four PAs from this species (8). Of the four alkaloids, isolation of the alkaloid **IV** from this species is reported for the first time.

### MATERIALS AND METHODS

#### Plant material

Plant material was collected from Rud-e-Shur 60 Km of Southwest of Tehran in Jun 1998. The plant was identified by the Herbarium department of the Faculty of Pharmacy, Tehran University of Medical Sciences, where a voucher specimen is deposited under number 6525 TEH. The whole plant was air dried in the shade, powdered and kept in tightened light protected containers.

Instrumentation: FT-IR spectra were obtained on a Nicolet 550 spectrometer. EIMS spectra were measured by a Finnigan TSQ at 70 eV. <sup>1</sup>H NMR

(400 MHz) and <sup>13</sup>C NMR (100 MHz) Varian were used to record spectra. The optical rotation was measured on a Perkin-Elmer 241 polarimeter. Solvents and silica gel were purchased from Merck.

#### Extraction and Isolation Procedures

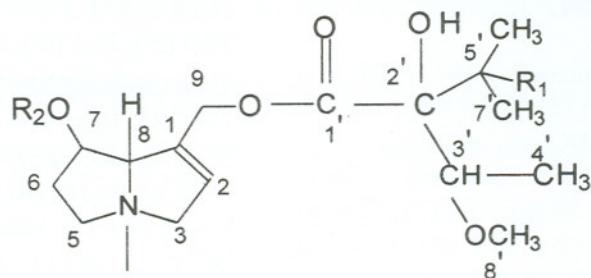
Powdered plant material (3600 g) was percolated with MeOH at room temperature and the extract was evaporated under reduced pressure below 50°C. The residue was partitioned between ethyl acetate and 2N HCl in water. The pH of the aqueous phase was adjusted to 2 by addition of 2N HCl and to it was added 0.5 g NaCl for every 15 ml of the solution. After 24 hr, the gummy non-alkaloid residue was filtered and the pH of the filtrate was adjusted to 10 by addition of 25% NH<sub>4</sub>OH and extracted with CHCl<sub>3</sub> (7×300 ml). The chloroform extract was evaporated to give 18 g crude alkaloid (part A).

To investigate the presence of the N-oxide of PA, the pH of the aqueous layer was adjusted to 2 with H<sub>2</sub>SO<sub>4</sub>, and after addition of Zn dust (10 g) to the solution, the mixture was stirred for 48 h. The mixture was filtered, the solution was made alkaline and treated as above (part B). The crude extract of part A was applied to column chromatography over silica gel (Merck), eluted consecutively with following solvents in the order of increase in polarity: petroleum-ether, CHCl<sub>3</sub> and MeOH (1%). The obtained fractions were then applied to thick layer chromatography for further purification, using CHCl<sub>3</sub>, MeOH; NH<sub>4</sub>OH (17; 3.8; 0.25) as eluent. Part B was purified similarly.

### RESULTS AND DISCUSSION

The total alkaloid content of the dried plant was 0.5%. The whole plant of *H. dissitiflorum* furnished four PAs, namely heliotrine **I**, heliotrine N N-oxide **II**, europine **III**, 5'-deoxylasiocarpine **IV**.





(1)  $R_1=R_2=R_3=H$  (Heliotrine)

(2)  $R_1=R_2=H$ ,  $R_3=O$  (Heliotrine N - oxide)

(3)  $R_2=R_3=H$ ,  $R_1=OH$  (Europine)

(4)  $R_1=R_3=H$ ,  $R_2=$  (5'' - deoxylasiocarpine)

Fig 1.

Table 1.  $^1H$ NMR assignments of compound I-IV

Carbon	I	II	III	IV
2	5.65 (bs)	5.70 (bs)	5.78 (bs)	5.8 (bs)
3u	3.27(d)(J=16Hz)	4.37 (d)(J=16.4 Hz)	3.63 (d)	3.5 (d)
3d	3.8 (d)	4.88 (d)	4.2 (d)	4.18 (d)
5u	2.6(m)(J=12Hz)	3.6 (m)	2.81 (m)	3.00 (m)(J=6 Hz)
5d	3.3(m)	4.00 (m)	3.28 (m)	3.4 (m)
6u	1.87 (m)	2.079 (m)	1.92 (m)	1.8 (m)
6u	1.98 (m)	2.37 (m)	2.02 (m)	1.8 (m)
6d	4.09 (m)(J=6 Hz)	4.28 (m)	4.34 (m)	5.12 (m)
7	3.99 (bs)	4.73 (bs)	4.47 (s)	4.37 (bs)
8	4.63 (d)(J=13.2 Hz)	4.75 (d)(J=14.4)	4.75 (d) (J=14 Hz)	4.88 (d) (J=14.4 Hz)
9u	4.92 (d)	4.86 (d)	4.97 (d)	4.88 (d)
9d	3.57 (q)(J=6.4 Hz)	3.67 (q)(J=6Hz)	3.8 (q)(J=6 Hz)	3.7 (q) (J=6 Hz)
3'	1.08(d)	1.11 (d)	1.24 (d)	1.21 (d)
4'	0.87 (d) (J=6.8 Hz)	0.90 (d) (J=6.4 Hz)	1.26 (d)	0.95 (d)
6'	0.84 (d)	0.88 (d)	1.24 (d)	0.94 (d)
7'	3.27 (s)	3.24 (s)	3.33 (s)	3.22 (s)
7'	2.04 (hept)	1.92 (hept)	-	2.2 (hept)
8'				6.13(q)(J=7.2 Hz)
5'				1.95(d)(J=7.2 Hz)
3''				1.82(s)
4''				
5''				

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**Heliotrine I:** mp: 122-124°C;  $[\alpha]_D = 63.8$  (CHCl<sub>3</sub>);  $R_f = 0.5$  (CHCl<sub>3</sub>; MeOH; NH<sub>4</sub>OH) (17; 3.8; 0.25); IR (CHCl<sub>3</sub>):  $\nu$  cm<sup>-1</sup> 3377 (OH), 1736 (C=O), 1097 (C-O), 1280 (C-O)

EIMS m/z (% relative intensity) 314 [(MH<sup>+</sup>, 3)], 255 (1.4), 197 (4), 156 (12), 138 (100), 120 (7), 93 (100), 80 (25); <sup>1</sup>H NMR (see Table 1).

<sup>13</sup>CNMR (CDCl<sub>3</sub>)(carbon's number) 136(1), 127.2 (2), 62 (3), 54 (5), 34 (6), 75.4 (7), 78.4 (8), 62.6 (9), 175 (1'), 82.4 (2'), 79.9 (3'), 12.4 (4'), 31.7 (5'), 17 (6'), 16.4 (7'), 57 (8').

**Heliotrine N-oxide II:** oil  $[\alpha]_D = 26.6$  (EtOH);  $R_f = 0.26$  (CHCl<sub>3</sub>; MeOH; NH<sub>4</sub>OH)(17; 38,0.25); IR  $\nu$  cm<sup>-1</sup> (CHCl<sub>3</sub>): 3300 (OH), 2389 (N-O), 1727 (C=O), 1229 (C-O)

EIMS m/z (% relative intensity): 314[(MH<sup>+</sup>-16)], 4.7), 240 (5), 138 (100), 120 (5), 93 (40), 83 (12), 59 (14); <sup>1</sup>H NMR (see Table 1). <sup>13</sup>CNMR (CDCl<sub>3</sub>)(carbon's number) 133.7 (1), 120.7 (2), 76.6 (3), 68 (5), 33 (6), 71.6 (7), 96.2 (8), 60.6 (9), 173.9 (1'), 83.1 (2'), 78.7 (3'), 11.4 (4'), 32.9 (5'), 16.9 (6'), 16.9 (7'), 56.5 (8').

**Europine III:** oil;  $[\alpha]_D = +19.9^\circ$  (C=2.5 mg/cc in EtOH);  $R_f = 0.31$  (CHCl<sub>3</sub>; MeOH; NH<sub>4</sub>OH)(17; 3.8; 0.25); IR  $\nu$  cm<sup>-1</sup> (CHCl<sub>3</sub>): 3427 (OH), 1729 (C=O), 1991 (C-O), 1280 (C-O); EIMS m/z (% relative intensity): 329 (M<sup>+</sup>, 2.5), 240 (2), 138 (100), 120 (29), 93 (75), 80 (25); <sup>1</sup>H NMR (see Table 1).

<sup>13</sup>C NMR (CDCl<sub>3</sub>) (carbon's number) 135 (1), 125 (2), 61.1 (3), 54.5 (5), 33.1 (6), 73.7 (7), 79.6 (8), 61.3 (9), 174 (1'), 82.4 (2'), 79.1 (3'), 13 (4'), 72.6 (5'), 26.1 (6'), 24.8 (7')

**5'-Deoxylasiocarpine IV:** yellow oil; picrate mp: 190-198;  $[\alpha]_D = -13.7$  (EtOH);  $R_f = 0.8$  (CHCl<sub>3</sub>; MeOH; NH<sub>4</sub>OH)(17; 3.8; 0.25); IR  $\nu$  cm<sup>-1</sup> (CHCl<sub>3</sub>): 3414 (OH), 1722 (C=O), 1991 (C-O)

EIMS m/z (% relative intensity) 396 (MH<sup>+</sup>, 2), 295 (5.5), 263 (1.1), 220 (80), 120 (83), 93 (68), 83 (32); <sup>1</sup>H NMR (see Table 1).

The structures of compound I-III were identified by comparison of their spectral data with those reported in the literatures (9,10). Compound IV showed a carboxyl absorption at 1722. The EIMS showed a molecular ion at m/z 396 (M<sup>+</sup>+1) which corresponds to an empirical formula of C<sub>21</sub>H<sub>33</sub>NO<sub>6</sub>. The molecular formula was 16 mass unit lower than lasiocarpine. Furthermore, the <sup>1</sup>H NMR (table1) was very similar to the spectra of lasiocarpine except that H-5' appeared as heptate at  $\delta = 2.2$  which is in support of 5'-deoxylasiocarpine. The fragmentation pattern in the mass spectrum of compound IV likewise support this conclusion. Compound IV has been isolated from *Heliotropium* genus (11).

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