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ALKALOIDS OF BORAGINACEAE II [1], PYRROLIZIDINE ALKALOIDS OF *HELIOTROPIUM EUROPAEUM* L. POPULATION GARMSAR

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Abstract

Helitropium europaeum L. population Garmsar was shown to contain three major alkaloids: heliotrine N-oxide (**2**), lasiocarpine (**4**) and lasiocarpine N-oxide (**5**); and four minor alkaloids: heliotrine (**1**), europine (**3**), acetyllasiocarpine (**6**) and a novel alkaloid acetyllasiocarpine N-oxide (**7**).

Introduction

Pyrrolizidine alkaloids (PAs) occur world-wide as tertiary bases and N-oxides in many genera of Boraginaceae, Compositae, and Leguminosae. Unsaturated PAs are toxic to man and animals because of the alkylating capacity of their pyrrolic derivatives [2,3].

Hepatotoxic, pulmotoxic, antimitotic, teratogenic, mutagenic and carcinogenic effects of PAs have been reported [2, 4]. Some PAs have revealed antitumor activity [5,6].

Heliotropium europaeum is a herbaceous plant which may grow as a weed among cultivated plants. There are some reports about the hepatotoxicity of *H. europaeum* to man due to the consumption of herbal tea in India, Sri Lanka and Hong Kong [2,7]. Isolation of PAs from this species has been reported previously [3,8,9]. Due to the variation of alkaloids because of different ecological and regional conditions [10], we considered this species as part of our research project to see whether the alkaloid content of *H. europaeum* of Iran is different from other reported regions.

Keywords: Acetyllasiocarpine N-oxide; Pyrrolizidine alkaloids *Heliotropium europaeum*

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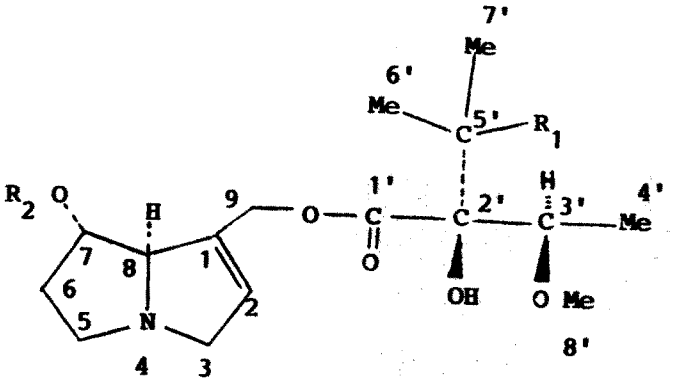
Results and Discussion

The following alkaloids were isolated from *H. europaeum* population Garmsar by chromatographic methods (Table 1, Fig. 1).

The spectral data and the m.p.s of alkaloids 1 to 6 were identical to those reported in the literature [3,11-13]. A novel alkaloid, acetyllasiocarpine N-oxide (**7**) was also identified. This alkaloid was obtained as a yellow gum and its structure was based on its spectra (IR, NMR, MS). ^{1}H-NMR spectrum of this alkaloid was very similar to lasiocarpine N-oxide (**5**) [12]. H_3, H_5 and H_8 were deshielded relative to lasiocarpine. In addition, 10'-CH_3(O-CO-CH_3) appeared as a singlet at 1.98, and 6' and 7' methyl appeared at 1.64 and 1.62 ppm respectively,

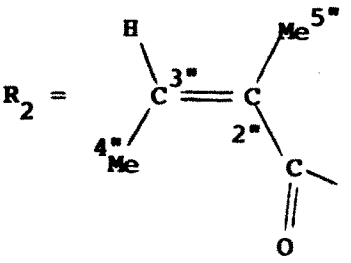


1-heliotrine $R_1=R_2=H$

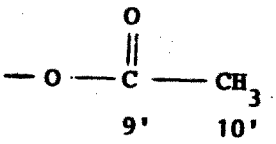
2-N-oxide of 1

3-europine $R_1=OH, R_2=H$

4-lasiocarpine $R_1=OH$



5-N-oxide of 4

6-acetyllasiocarpine $R_1=$  R_2=Angelyl

7-acetyllasiocarpine N-oxide

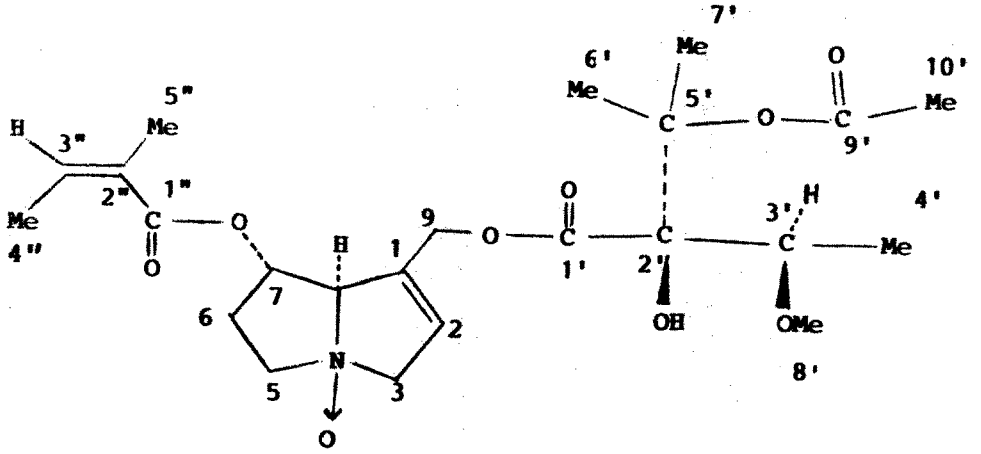


Figure 1

Table 1. TLC results of *H. europaeum*

Alkaloids	Rf[a]	% of PAs[b]
Heliotrine N-oxide(2)	0.26	0.08
Europine(3)	0.31	0.02
Lasiocarpine N-oxide(5)	0.35	0.22
Acetyllasiocarpine N-oxide(7)	0.47	0.05
Heliotrine(1)	0.50	0.02
Lasiocarpine(4)	0.78	0.09
Acetyllasiocarpine(6)	0.83	0.03

[a]Solvent system: chloroform-methanol-25% ammonia (17: 3.8: 0.25)
[b]In dry plant

both these methyl were deshielded relative to lasiocarpine N-oxide [12]. In mass spectra, the compound loses oxygen and therefore molecular ions appeared at 454 [(MH^+)-16]. The fragmentation pattern was identical to acetyllasiocarpine. In addition, deoxygenation of this alkaloid with Zn dust and H_2SO_4 afforded acetyllasiocarpine.

Experimental Section

Melting points were taken on a Kofler hot stage apparatus and are uncorrected. The IR spectra were obtained using a Nicolet FT-IR 550 spectrograph. The UV spectra were obtained using a Shimadzu UV-160-A. The ^1H and ^{13}C-NMR spectra were recorded on a Bruker FT-80 or a Varian Unity 400 plus spectrometer and chemical shifts (δ) are in ppm relative to internal tetramethylsilane. The EI mass spectra were run on a Finigan TSQ-70 spectrometer at 70 eV.

Plant Material

The plant material was collected in August 1992 from a melon farm in the Garmsar area, 125 km east of Tehran. The plant was identified by the Department of Botany, Medical Sciences University of Tehran. A voucher specimen (No 6503) was deposited in the herbarium of the Faculty of Pharmacy. The entire plant was air dried in the shade, powdered and kept in tightened, light-protected containers.

Extraction Procedure

500 g of powdered plant material was perculated with methanol, and methanolic solution was evaporated under reduced pressure below 50°C. The alkaloids were extracted according to reported procedures [11,14] to give (0.6%) total crude alkaloids.

Isolation of Alkaloids

The alkaloids were separated by preparative TLC on silica gel HF_{254} (20×20 cm, 0.5 mm thickness) using solvent systems chloroform-methanol-25% ammonia (17:3.8:0.25) (Table 1). M.p. and spectral data of alkaloids **1** to **6** were identical to the reported ones [3, 11-13].

Acetyllasiocarpine N-oxide (7)

As a yellow gum, IR ν_{max}: 3450 (OH), 1733 cm^{-1} (C=O), 1180-1280 cm^{-1} (N^+-O^-); ^{1}H-NMR: 1.26 (d, 3H, $J_{4',3'}$= 6 Hz, $H_{4'}$), 1.62 (s, 3H, H_{7}), 1.64 (s, 3H, $H_{6'}$), 1.92 (m, 3H, $H_{5''}$), 1.98 (s, 3H, $H_{10'}$), 2.02 (q, 3H, $J_{4'',3''}$= 7.2 Hz, $J_{4'',5''}$= 1.2 Hz, $H_{4''}$), 2.23 (m, 1H, H_{6up}), 2.75 (m, 1H, H_{6down}), 3.27 (s, 3H, $H_{8'}$), 3.83 (m, 1H, H_{5up}), 3.85 (q, 1H, J= 6.4 Hz, $H_{3'}$), 3.95 (m, 1H, H_{5down}), 4.48 (d, 1H, J=16 Hz, H_{3up}), 4.59 (d, 1H, J=16 Hz, H_{3down}), 4.65 (bs, 1H, H_8), 4.97 (ABq, 2H, J=14.8 Hz, H_9), 5.16 (bt, 1H, J= 2.4 Hz, H_7), 5.89 (d, 1H, J=1.6 Hz, H_2), 6.21 (qq, 1H, $J_{3'',4''}$= 7.2, $J_{3'',5''}$= 1.6 Hz, $H_{3''}$). ^{13}C-NMR: 12.53 (C-4'), 15.98 (C-4"), 20.29 (C-5"), 22.37 (C-6'), 22.51 (C-10'), 27.16 (C-7'), 30.34 (C-6), 56.29 (C-8'), 60.90 (C-9), 67.24 (C-5), 72.75 (C-7), 77.32 (C-3), 78.36 (C-3'), 85.19 (C-2'), 85.57 (C-5'), 94.37 (C-8), 122.77 (C-2), 126.46 (C-2"), 132.76 (C-1), 141.09 (C-3"), 167.25 (C-1"), 170.50 (C-9'), 172.53 (C-1'), ms: m/z (%), 454 [(MH^+)-16, 1.4], 394 [(MH^+)-60, 1.4], 220 (71), 136 (33), 120 (84), 119 (100), 93 (29), 59 (41).

Deoxygenation of Acetyllasiocarpine N-oxide

5 mg of alkaloid (7) was dissolved in 20 ml of 2N H_2SO_4 and stirred overnight with 500 mg of Zn dust [12]. After filtration, the solution was basified with ammonia (pH>9) and extracted with chloroform (3 × 20 ml). After drying over anhydrous sodium sulfate, the solvent was removed under reduced pressure and a yellow gum was obtained, Rf value and spectral data were similar to acetyllasiocarpine.

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