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

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Abstract

Heliotropium 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.

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). ‘H-NMR spectrum of this alkaloid was very similar to lasiocarpine N-oxide (5) [12]. H₃, H₅ and H₆ were deshielded relative to lasiocarpine. In addition, 10' CH₃(O-CO-CH₃) appeared as a singlet at 1.98, and 6' and 7' methyl appeared at 1.64 and 1.62 ppm respectively.

Keywords: Acetyllasiocarpine N-oxide; Pyrrolizidine alkaloids Heliotropium europaeum

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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 \( R_1 = \bigg\| - O - C - CH_3 \), \( R_2 = \)Angelyl
7-acetyllasiocarpine N-oxide

Figure 1
Table 1. TLC results of H. europaeum

<table>
<thead>
<tr>
<th>Alkaloids</th>
<th>Rf</th>
<th>% of PAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heliotrine N-oxide(2)</td>
<td>0.26</td>
<td>0.08</td>
</tr>
<tr>
<td>Europine(3)</td>
<td>0.31</td>
<td>0.02</td>
</tr>
<tr>
<td>Lasiocarpine N-oxide(5)</td>
<td>0.35</td>
<td>0.22</td>
</tr>
<tr>
<td>Acetyllasiocarpine N-oxide(7)</td>
<td>0.47</td>
<td>0.05</td>
</tr>
<tr>
<td>Heliotrine(1)</td>
<td>0.50</td>
<td>0.02</td>
</tr>
<tr>
<td>Lasiocarpine(4)</td>
<td>0.78</td>
<td>0.09</td>
</tr>
<tr>
<td>Acetyllasiocarpine(6)</td>
<td>0.83</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*Solvent system: chloroform-methanol-25% ammonia (17:3.8:0.25)
*bIn 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 [(M+H)-16]. The fragmentation pattern was identical to acetyllasiocarpine. In addition, deoxygenation of this alkaloid with Zn dust and H₂SO₄ 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 ¹C-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 Garmshar 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 HF₅₄ (20x20 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 ν₅₃₄: 3450 (OH), 1733 cm⁻¹ (C=O), 1180-1280 cm⁻¹ (N=O);¹H-NMR: 1.26 (d, 3H, Jₓₓₓ=6 Hz, Hₓₓₓ), 1.62 (s, 3H, Hₓₓₓ), 1.64 (s, 3H, Hₓₓₓ), 1.92 (m, 3H, Hₓₓₓ), 1.98 (s, 3H, Hₓₓₓ), 2.02 (q, 3H, Jₓₓₓₓ=7.2 Hz, Hₓₓₓ), 2.75 (m, 1H, Hₓₓₓₓ), 3.27 (m, 1H, Hₓₓₓₓ), 3.83 (m, 1H, Hₓₓₓₓ), 3.85 (q, 1H, J=6.4 Hz, Hₓₓₓ), 3.95 (m, 1H, Hₓₓₓ), 4.48 (d, 1H, J=16 Hz, Hₓₓₓ), 4.59 (d, 1H, J=16 Hz, Hₓₓₓ), 4.65 (bs, 1H, Hₓₓₓ), 4.97 (ABq, 2H, J=14.8 Hz, Hₓₓₓ), 5.16 (bt, 1H, J=2.4 Hz, Hₓₓₓ), 5.89 (d, 1H, J=1.6 Hz, Hₓₓₓ), 6.21 (q, 1H, Jₓₓₓₓ=7.2, Jₓₓₓₓ=1.6 Hz, Hₓₓₓ). ¹C-NMR: 12.53 (C-4'), 15.98 (C-4''), 20.29 (C-5'''), 22.37 (C-6'), 22.51 (C-1''), 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 [(M+H)-16, 1.4], 394 [(M+H)-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 H₂SO₄ and stirred overnight with 500 mg of Zn dust [12]. After filtration, the solution was basified with ammonia (pH>9) and extracted with chloroform (3x20 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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References