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Original article

Pyrrolizidine alkaloids from *Heliotropium transoxanum* Bunge

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Abstract

Background and objectives: The plants belonging to the genus *Heliotropium* L. (Boraginaceae) are the main sources of toxic pyrrolizidine alkaloids (PAs). In the present study, we have investigated the PAs of the aerial parts of *Heliotropium transoxanum* Bunge, a perennial species native to Iran. **Methods:** Silica gel column chromatography and silica gel PTLC were applied for the isolation of PAs present in the total methanol extract of *H. transoxanum*. The structures of the isolated compounds were identified using ^{1}H-NMR, ^{13}C-NMR and EIMS spectral analyses. **Results:** Three PAs, heliotrine (**1**), lasiocarpine (**2**) and heliotrine N-oxide (**3**), with known mutagenic and genotoxic properties, were isolated from the aerial parts of *H. transoxanum*. **Conclusion:** The results of this study on the presence of toxic PAs in *H. transoxanum* introduce this herb as a poisonous species and also suggest it as an appropriate source for the isolation of heliotrine and lasiocarpine for further toxicological and pharmacological studies.

Keywords: heliotrine, heliotrine N-oxide, *Heliotropium transoxanum* Bunge, lasiocarpine, pyrrolizidine alkaloids

Introduction

The genus *Heliotropium* L. (Boraginaceae) with about 300 species all over the world is one of the main sources of pyrrolizidine alkaloids (PAs) [1,2]. PAs-containing plants such as *Senecio*, *Heliotropium*, *Crotalaria* and *Amsinckia* species have been recognized responsible for poisoning of livestock and other ruminants [3,4]. In humans, Pas-induced liver and pulmonary damages can also arise via consumption of contaminated foods, honey, milk, medicinal plants and herbal medicines [4,5]. Toxicological researches have revealed that this toxicity is derived through the alkylation of biological nucleophiles such as nucleic acids and proteins (DNA, RNA and enzymes) by metabolites of 1,2-unsaturated PAs [4,5]. It has been believed that PAs produced by plants protect them against insects and herbivores [6]. Beside well known toxicity of 1,2-unsaturated PAs, these compounds have been considered for their antitumor, antimicrobial, antiviral and antifeedant effects [1,2].

Heliotropium transoxanum Bunge (Syn: *Heliotropium dasycarpum* subsp. *transoxanum* (Bunge) Akhani & Forther), is one of the twenty five *Heliotropium* species represented in flora of Iran [7]. This species grows as a perennial plant in arid and semi-arid habitats of the east and

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center of Iran, also in Afghanistan, Pakistan and central Asia [7]. Previous phytochemical investigation of *H. transoxanum* by Akramov *et al*. led to the isolation of heliotrine from this species [8]. In the present study, in continue of our previous studies on *Heliotropium* species of Iran [9-11], we report the isolation and structure elucidation of alkaloids from the aerial parts of *H. transoxanum* Bunge.

Experimental

Plant material

The aerial parts of *Heliotropium transoxanum* Bunge were collected and identified by Prof. H. Akhani (School of Biology, College of Science, University of Tehran, Tehran, Iran) in July 2010 from Sarakhs region, north-Khorasan province, north-east of Iran.

Extraction procedure

The air-dried and powdered plant sample (1.4 kg) was macerated with methanol (5×8 L) at room temperature to get total extract. The obtained extract was concentrated using a rotary evaporator under reduced pressure at 40 °C.

Isolation of crude alkaloid fraction

The total extract (170 g) was suspended in 1% HCl in water (400 mL) and partitioned by the addition of $CHCl_3$ (300 mL). The aqueous phase was separated and washed with ethyl acetate (300 mL) to remove any semi-polar impurities. To obtain the salt form of alkaloids, the pH of aqueous phase (600 mL) was adjusted to 2 by addition of 2 N HCl and NaCl (20.0 g). The filtrate of the above mixture was basified to pH 10 with 25% NH_4OH (200 mL) and extracted with $CHCl_3$ to get crude alkaloid fraction.

Isolation and purification of alkaloids

The crude alkaloid fraction (3.85 g) was divided to seven subfractions (A-G) on a silica gel (Mesh 35-70, Merck) column, eluted with petroleum ether and a gradient mixture of $CHCl_3$:MeOH (100:0→50:50), respectively. Subfraction B yielded a pure alkaloid as compound **1** (720 mg). Subfraction D was suspected to contain N-oxide PAs. For reduction of N-oxide PAs present in the subfraction D, the aqueous solution of this fraction was acidified with 2N H_2SO_4 to pH 2 and stirred for 24 h in the presence of Zn dust. The reduced PAs were then extracted as described above for crude alkaloid fraction. Preparative thin layer chromatography (PTLC) of the reduced PAs on handmade silica gel (Kieselgel 60 HF 254+366, Merck) plates using $CHCl_3$:MeOH:NH_4OH (17:3.8:0.25) resulted in the isolation of compound **2** (65 mg). Compound **3** (80 mg) was also isolated from the subfraction F on PTLC with $CHCl_3$:MeOH:NH_4OH (17:3.8:0.25) as eluent.

The ^{1}H-NMR and ^{13}C-NMR spectra of the compounds were obtained on Varian 400 unity Plus Spectrometer and their EIMS spectra were recorded on a Finnigan TSQ system at 70 eV.

Results and Discussion

Phytochemical investigation of PAs present in the aerial parts of *H. transoxanum* led to the isolation of three PAs, heliotrine (**1**), lasiocarpine (**2**) and heliotrine N-oxide (**3**) (figure 1). The structures of the isolated compounds were elucidated using ^{1}H-NMR, ^{13}C-NMR and EIMS spectral analyses, as well as by comparing with data published in the literature [11,12].

Chromatographic and spectroscopic data of compounds

Compound **1** (heliotrine): R_f=0.52 ($CHCl_3$:MeOH:NH_4OH) (17:3.8:0.25); EIMS (70 eV), m/z (%): 314 $[M+H]^+$ (3.3), 254 (1), 214 (1), 197 (1.5), 156 (15), 138 (100), 120 (8), 93 (81), 59 (33); ^{1}H-NMR (see table 1); ^{13}C-NMR ($CDCl_3$, ppm); δ 136.14 (C-1), 126.74 (C-2), 62.12 (C-3), 54.08 (C-5), 34.16 (C-6), 75.38 (C-7), 79.83 (C-8), 62.63 (C-9), 174.37 (C-1'), 82.13 (C-2'), 78.92 (C-3'), 12.37 (C-4'), 31.85 (C-5'),17.08 (C-6'),16.51 (C-7'), 56.87 (C-8') [11].

Compound **2** (lasiocarpine): R_f=0.8

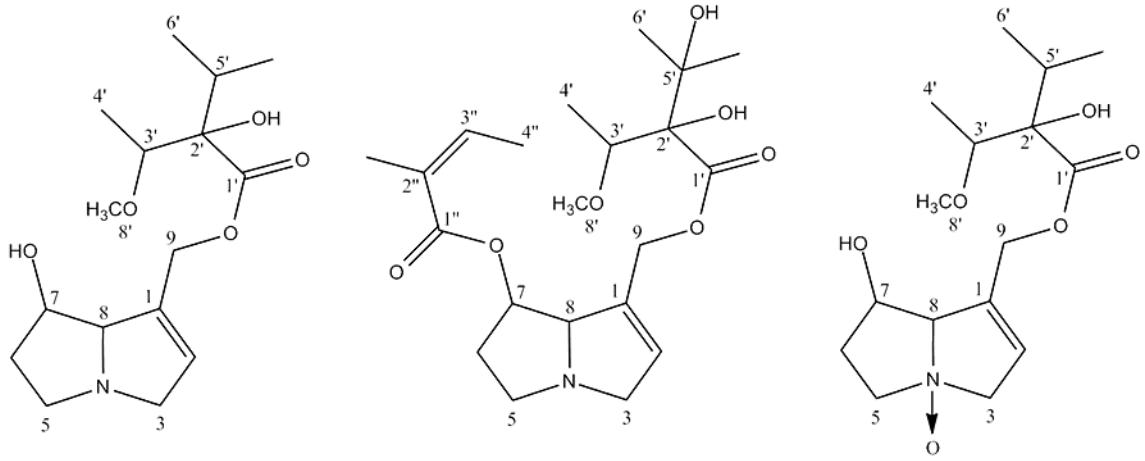

Figure 1. Structures of the isolated compounds from *H. transoxanum*

Table 1. ^{1}H-NMR (400 MHz, $CDCl_3$) data of the isolated compounds **1**-**3** from *H. transoxanum*

H no.	Compound 1	Compound 2	Compound 3
2	5.72 (*br s*)	5.65 (*br s*)	5.72 (*br s*)
3u[a]	3.37(*br d, J*=16.8 Hz)	3.45 (*d, J*= 16.0 Hz)	4.37 (*d, J*= 16.4 Hz)
3d[b]	3.91 (*br d, J*=16.8 Hz)	3.85 (*d, J*= 16.0 Hz)	4.50 (*d, J*= 16.4 Hz)
5u	2.62 (*m*)	2.85 (*m*)	3.60 (*m*)
5d	3.37 (*m*)	3.15 (*m*)	4.03 (*m*)
6u	1.94 (*m*)	1.81 (*m*)	2.11 (*m*)
6d	2.04 (*m*)	1.81 (*m*)	2.43 (*m*)
7	4.12 (*q, J*= 6.4 Hz)	5.13 (*m*)	4.31 (*m*)
8	3.97 (*br s*)	4.15 (*br s*)	4.77 (*br s*)
9u	4.65 (*d, J*= 13.2 Hz)	4.93 (*d, J*= 14.4 Hz)	4.78 (*d, J*= 14.0 Hz)
9d	5.05 (*d, J*= 13.2 Hz)	4.93 (*d, J*= 14.4 Hz)	4.89 (*d, J*= 14.0 Hz)
3'	3.59 (*q, J*= 6.4 Hz)	3.70 (*q, J*= 6.0 Hz)	3.70 (*q, J*= 6.0 Hz)
4'	1.13(*d, J*= 6.4 Hz)	1.21 (*d, J*= 6.0 Hz)	1.14 (*d, J*= 6.0 Hz)
5'	2.15 (*hept, J*= 6.8 Hz)	-	1.96 (*hept, J*= 6.8 Hz)
6'	0.93 (*d, J*= 6.8 Hz)	1.25 (*s*)	0.93 (*d, J*= 6.8 Hz)
7'	0.87 (*d, J*= 6.8 Hz)	1.11 (*s*)	0.91 (*d, J*= 6.8 Hz)
8'	3.4 (*s*)	3.26 (*s*)	3.29 (*s*)
3"	-	5.85 (*q, J*= 7.2 Hz)	-
4"	-	1.95 (*d, J*= 7.2 Hz)	-
5"	-	1.80 (*s*)	-

Notes: [a] up, [b] down

($CHCl_3$:MeOH:NH_4OH) (17:3.8:0.25); EIMS (70 eV), m/z (%): 412 $[M+H]^+$ (2.8), 311 (1), 279 (1), 220 (23.8), 136 (50.4), 120 (100), 119 (71.4), 106 (6.6), 94 (30.4), 93 (28.5), 83 (4.2), 59 (36.1); ^{1}H-NMR (see table 1). ^{13}C-NMR ($CDCl_3$, ppm) 134.61 (C-1), 128.27 (C-2), 62.17 (C-3), 54.35 (C-5), 30.47 (C-6), 76.61 (C-7), 78.77 (C-8), 62.17 (C-9), 173.72 (C-1'), 83.61 (C-2'), 78.58 (C-3'), 12.83 (C-4'), 72.86 (C-5'), 26.42 (C-6'), 24.57 (C-7'), 56.41 (C-8'), 167.48 (C-1"), 127.42 (C-2"), 138.23 (C-3"), 15.91 (C-4"), 20.57 (C-5") [12].

Compound **3** (heliotrine N-oxide): R_f = 0.32 ($CHCl_3$:MeOH:NH_4OH) (17:3.8:0.25); EIMS (70 eV), m/z (%): 314 $[M+H]^+$-16 (39), 240 (1), 156 (5.7), 139 (22), 138 (100), 120 (13.3), 93 (46.6),

83 (4.7), 80 (15), 59 (28.5); ^{1}H-NMR (see table 1). ^{13}C-NMR ($CDCl_3$, ppm) 134.02 (C-1), 120.24 (C-2), 76.82 (C-3), 68.12 (C-5), 33.41 (C-6), 71.52 (C-7), 96.17 (C-8), 60.71 (C-9), 173.85 (C-1'), 83.07 (C-2'), 78.72 (C-3'), 11.51 (C-4'), 33.05 (C-5'), 17.12 (C-6'), 17.12 (C-7'), 56.53 (C-8') [11].

Among the isolated compounds, lasiocarpine (**2**) and heliotrine N-oxide (**3**) have been reported from this species for the first time. These compounds (**1**-**3**), however, have been previously reported from some other *Heliotropium* spp. such as *H. europaeum*, *H. disstiflorom*, *H. indicum*, *H. lasiocarpum*, etc. [2]. Heliotrine and lasiocarpine are classified as heliotridine-type PAs [5]. It has been shown that these two compounds (**1** and **2**) induce chromosome damage and mutagenicity via DHP (Didehydropyrrolizidine alkaloid)-derived DNA adducts [4,5]. Heliotrine and lasiocarpine have also been documented for their antimicrobial properties [13]. Heliotrine isolated from *H. subulatum* has exhibited a moderate antibacterial activity against *Escherichia coli*, *Streptococcus pneumoniae*, *Bacillus subtilis*, *Bacillus anthracis* and *Staphylococcus aureus* (Inhibition zones (IZ); 10-15 mm) and a weak antifungal activity against *Aspergillus niger*, *Aspergillus fumigates* and *Penicillium chrysogenum* (IZ; 8-10 mm) [13]. Satish *et al*. have also reported the antibacterial effects of lasiocarpine from *H. ellipticum* on *Escherichia coli* (IZ; 12.00 ± 0.34 mm) and *Escherichia cloacae* (IZ; 10.00 ± 0.41 mm) in disk diffusion assay [14]. Furthermore, heliotrine has been shown to possess antiviral activity (1×10^3 reduction factor) against *Poliomyelitis* and *Vesicular stomatitis* at a concentration of 10 µg /mL [15].

Considering the biological activities of heliotrine and lasiocarpine, the results of this study highlight *H. transoxanum* as a source of bioactive PAs which can be subjected to the isolation of heliotrine and lasiocarpine, in order to further evaluate their toxicological and pharmacological properties.

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Declaration of interest

The author declares that there is no conflict of interest. The author alone is responsible for the content of the paper.

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