

Alkaloids from the aerial parts of a yellowish-brown flowering *Delphinium peregrinum* L. sample

Sarı-kahverengi çiçekli *Delphinium peregrinum* örneği topraküstü kısımlarının alkaloitleri

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Abstract

In this work, the alkaloids of a yellowish-brown flowering *Delphinium peregrinum* sample growing in Turkey were investigated. The extraction was made by percolation with ethanol. The crude extract was fractionated first with VCL and then with chromatotron. The identified alkaloids from the aerial parts of the plant were 6 peregrine type norditerpenoid alkaloids (peregrine, 10-hydroxyperegrine, 14-O methylperegrine, 14-O acetylperegrine, 14-O-benzoylperegrine, delhiperegrine), 3 diterpenoid alkaloids (α -atisine, hetisine, chellespontine) and 1 phthalideisoquinoline alkaloid (corydecumbine). This is the first report on the isolation of chellespontine and corydecumbine from *Delphinium peregrinum*.

Key words: *Delphinium peregrinum*, diterpenoid alkaloids, chellespontine, corydecumbine.

Introduction

Delphinium (larkspur) species are very toxic plants due to their diterpenoid alkaloid content. These alkaloids are neurotoxic agents, causing bradycardia, muscle-system spasms, hypotension, and death by arrest of respiration (Benn and Jacyno 1983, Gessner and Orzechowski 1974, Olsen and Sisson 1991). There are 31 *Delphinium* species growing wildly in Turkey. The diterpenoid alkaloids of Turkish *Delphinium* species have been investigated in our laboratory (Bitiş et al.2007, Meriçli et al. 1999, Süzgeç et al. 2006, 2009).

Delphinium peregrinum is a very common plant growing in Turkey. This species is generally characterized with dark violet flowers. The diterpenoid alkaloids of dark violet flowering *Delphinium peregrinum* samples were investigated by our group and norditerpenoid alkaloids nudicaulidine, bicoloridine, peregrine, peregrine alcohol, delhiperegrine and pergilone were isolated by Ulubelen et al. (1992, 1994).

Delphinium peregrinum samples with yellowish-brown flowers grow on Taurus Mountains in Turkey, in a very small district.

In this work, the alkaloid content of *Delphinium peregrinum* samples with yellowish-brown flowers is reported.

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Experimental

Plant material

Aerial parts of yellowish-brown flowering *Delphinium peregrinum* L. (*Ranunculaceae*) were collected in July 2006 from fields in Ermenek-Turkey by F. Meriçli and A. H. Meriçli. Voucher specimens have been deposited in the herbarium of the Faculty of Pharmacy, Istanbul University (ISTE 85932).

Aparatus

Vacuum liquid chromatography (VLC): Al₂O₃ (EM 1085) and SiO₂ 60 G (7731) Merck. Chromatographic separations: chromatotron with rotors coated with a 1 mm thick layer of Merck Al₂O₃ 60 GF-254 (1092) or SiO₂ 60 PF-254 (7749). TLC: eluents toluene / AcOEt / Et₂NH 7:4:1 or 7:4:2 and CHCl₃ / MeOH / NH₄OH 5:3:1. ESI MS were recorded on a Perkin Elmer SCIEX API-1 mass spectrometer. NMR spectra were recorded on a Bruker 500-MHz spectrometer.

Extraction and isolation of the alkaloids

Dried and powdered aerial parts of the plants were extracted with 90 % EtOH by percolation at room temperature and the extracts obtained were evaporated to dryness *in vacuo*. The residues were treated with 0.5 N H₂SO₄ and extracted with CHCl₃. NaOH (5 %) was then added to the aqueous solutions (cooled in ice) to bring them to pH 10. The solutions were again extracted with CHCl₃. The CHCl₃ extracts were evaporated to dryness, yielding crude alkaloid mixtures.

5 g of the crude alkaloid extract (6.3 g) obtained from 2.9 kg aerial parts of *Delphinium peregrinum* was first separated by VLC column: neutral Al₂O₃, elution solvent: petroleum ether / CHCl₃ / MeOH mixtures.

The combined fractions 10-12 (petroleum ether/CHCl₃ 60:40 – 50:50, 520 mg) were subjected using chromatotron to a SiO₂ rotor (petroleum ether/CHCl₃ / MeOH mixtures) and 14-O- benzoylperegrine (9 mg), and delphiperegrine (9 mg) were obtained.

The VLC fractions 13-16 (petroleum ether/CHCl₃ 45:55-0:100; 720 mg) were subjected to a SiO₂ rotor (petroleum ether/CHCl₃/MeOH mixtures) and 14-O- acetylperegrine (17 mg), 14-O-methylperegrine (12 mg), peregrine (25 mg) and 10-hydroxyperegrine (13 mg) were obtained. The VLC fraction 17 (CHCl₃/MeOH 99:1, 730 mg) was subjected to a Al₂O₃ rotor (petroleum ether/CHCl₃/MeOH mixtures) and corydecumbine (11 mg) and α -atisine (16 mg) were obtained. VLC fraction 18 (CHCl₃/MeOH 98:2, 580 mg) was subjected to a Al₂O₃ rotor (petroleum ether/CHCl₃/MeOH mixtures) and hetisine (30 mg) and chellespontine (13 mg) were obtained.

All the alkaloids were identified by comparison of their ¹H and ¹³C, DEPT NMR data and except corydecumbine, by Co-TLC behavior with those of authentic samples.

Results and Discussion

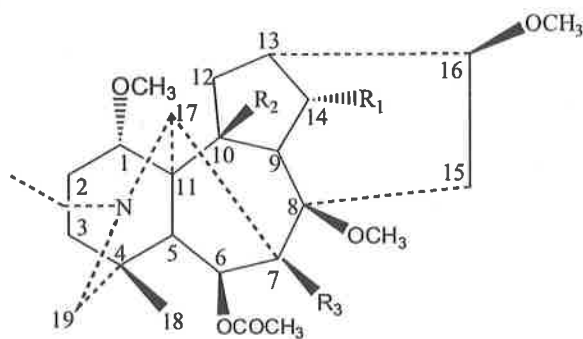
From the aerial parts of yellowish-brown flowering *Delphinium peregrinum* sample 6 peregrine type norditerpenoid alkaloids (peregrine, 10-hydroxyperegrine, 14-O methylperegrine, 14-O-acetylperegrine, 14-O-benzoylperegrine, delphiperegrine), 3 diterpenoid alkaloids (α -atisine, hetisine, chellespontine) and 1 phthalideisoquinoline alkaloid (corydecumbine) were isolated.

The alkaloids of the yellowish-brown flowering samples are similar to the dark violet flowering samples. Peregrine-type norditerpenoid alkaloids are characteristic alkaloids for *Delphinium peregrinum*. Nevertheless chellespontine, a diterpenoid alkaloid containing an aldehyde group,

which was isolated from *Consolida hellespontica* and *Delphinium staphisagria* before (Desai et al. 1993, Ulubelen et al. 1999), has also been found now in *Delphinium peregrinum*.

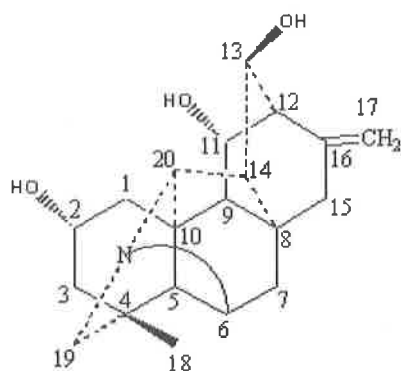
On the other hand, corydecumbine is a phthalideisoquinoline alkaloid and was isolated from *Corydalis decumbens* before (Basnet et al. 1993). Sometimes species containing diterpenoid alkaloids can also contain isoquinoline alkaloids (Desai et al. 1993, Zhang et al. 1999). In the first time we found corydecumbine in *Ranunculaceae* family.

The ^{13}C NMR data of the rare compounds chellespontine and corydecumbine, are given below in Table 1 and the structure of alkaloid isolated in Figure 1.

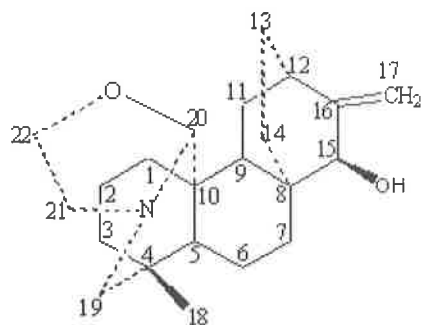


	R ₁	R ₂	R ₃
Peregrine	OH	H	H
14-O-acetylperegrine	OCOCH ₃	H	H
14-O-benzoylperegrine		OH	H
10-hydroxyperegrine	OH	OH	H
Delphiperegrine		H	OCH ₃
14-O-methylperegrine	OCH ₃	H	H

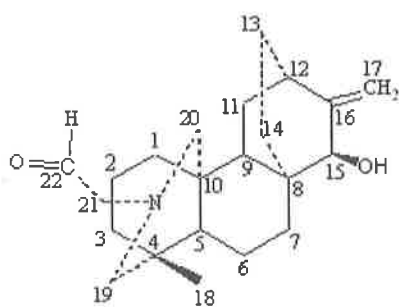
Figure 1. The structure of isolated alkaloids from *Delphinium peregrinum*.



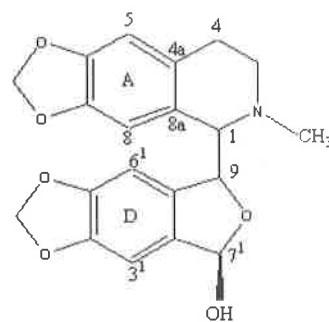
Hetisine



α - atisine



Chellespontine



Corydecumbine

Table 1. ^{13}C NMR (500 MHz) data of chellespontine and corydecumbine in CDCl_3

Position	Chellespontine	Position	Corydecumbine
1	26.2 t	1	64.4 d
2	19.5 t	3	58.1 t
3	41.2 t	4	33.9 t
4	33.5 s	4a	130.1 s
5	45.0 d	5	120.2 d
6	19.2 t	6	148.0 s
7	35.0 t	7	147.6 s
8	38.7 s	8	111.6 d
9	39.8 d	8a	118.6 d
10	46.2 s	9	83.8 d
11	31.7 t	1'	136.8 s
12	36.5 d	2'	132.5 s
13	26.1 t	3'	110.2 d
14	27.8 t	4'	145.6 s
15	74.2 d	5'	143.8 s
16	155.8 s	6'	107.0 d
17	110.2 t	7'	89.3 d
18	24.8 q	NCH ₃	45.1 q
19	60.3 t	OCH ₂ O (RingA)	101.1 t
20	58.4 t	OCH ₂ O (RingD)	101.8 t
21	65.2 t		
22	182.9d		

Acknowledgements

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Özet

Türkiye’de yetişen sarı-kahverengi çiçekli *Delphinium peregrinum* örneklerinden 6 peregrin tipi norditerpenoid alkaloid (peregrin, 10-hidroksiperegrin, 14-O metillperegrin, 14-O-asetilperegrin, 14-O-benzoilperegrin, delfiperegrin), 3 diterpenoid alkaloid (α -atisin, hetisin, kellespontin) ve 1 flalilizokinolin alkaloid (koridekumbin) izole edilerek yapıları açıklanmıştır.

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