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Diterpenoid alkaloids from *Delphinium virgatum* Poiret.

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From the aerial parts of *Delphinium virgatum* Poiret, which is an endemic plant growing wildly in Turkey, a new norditerpenoid alkaloid, *N*-deethylperegrine alcohol, has been isolated along with the known alkaloids peregrine, peregrine alcohol, davisinol, hetisine, hetisinone and atisine.

1. Introduction

From the time of Dioscorides *Delphinium* species (Ranunculaceae) have been used against lice and scorpions [1]. During the Waterloo war the British army used *Delphinium staphisagria* and *D. peregrinum* powder against lice. *Delphinium* species are also considered as an antiparasitic agent. In Turkish folk medicine, their extracts were used against epilepsy seizures and tetanus tremors as well as against rabies. Due to the high toxicity oral use of *Delphinium* extracts was abandoned, but it is still used against lice administered externally mixed with lanoline; however, even external use may lead to poisoning. Currently in the villages animal parasites are being controlled by hanging the plants in animal houses. Diterpenoid alkaloids in *Delphinium* species are neurotoxic agents, causing bradycardia, muscle failure, hypotension and death by the arrest of respiration [2–4].

2. Investigations, results and discussion

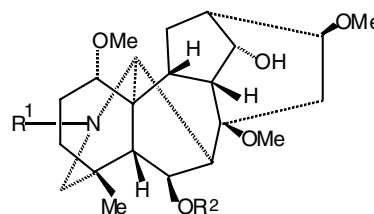
In continuation of our investigations on Turkish *Delphinium* species [5–7] we have now studied *Delphinium virgatum* Poiret. *D. virgatum* is an endemic plant growing only in Turkey. This is the first report on the investigation of alkaloids from this plant.

Dried and powdered aerial parts of *D. virgatum* (500 g) were extracted with 90% EtOH and the concentrated extract was processed [8] to give 1.80 g of the crude alkaloidal mixture. By a combination of vacuum liquid chromatography (VLC) [9], centrifugally accelerated radial thin layer chromatography (Chromototron) [10] and PTLC we report the isolation and identification of two known norditerpenoid alkaloids: peregrine (1), peregrine alcohol (2), and four known diterpenoid alkaloids: davisinol (3), hetisine (5), atisine (6) together with a new norditerpenoid alkaloid, *N*-deethyl-peregrine alcohol (7), from the aerial parts of this plant.

Peregrine alcohol (2) which is a relatively rare alkaloid [11–13] is the major alkaloid in the plant, but besides peregrine alcohol and peregrine (1) a third norditerpenoid alkaloid with a similar structure was also isolated from the plant. The optical rotation is $[\alpha]_D^{25} = 0.5 (c = 0.34, \text{CHCl}_3)$. The molecular formula $\text{C}_{22}\text{H}_{36}\text{NO}_5$ (ESIMS, $[\text{M}+1]^+$ m/z 394) for the new alkaloid 7 was derived by HRFABMS $[\text{M}+1]^+$, m/z 394.25130; calc. 394.25934) and confirmed by ^{13}C NMR and DEPT spectral data. The IR spectrum showed hydroxyl absorption at 3440 cm^{-1} , but no carbonyl and aromatic absorptions were observed.

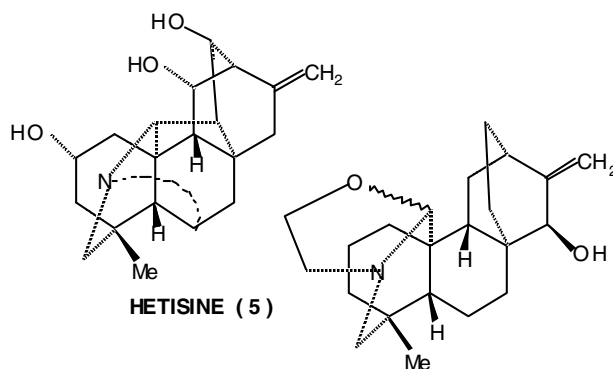
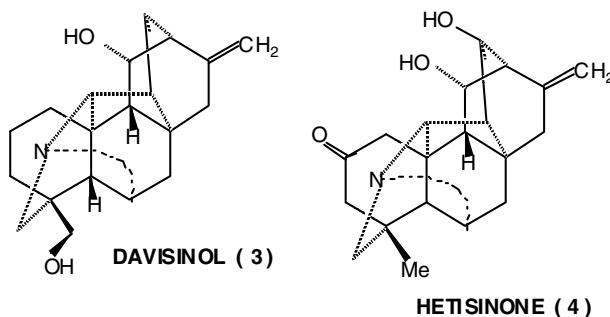
The NMR spectra of 7 gave signals at δ 0.98(3H, s) and δ 26.4q of an angular methyl group (Me-18), δ 3.27, 3.31, and 3.38 (3H each, s) δ 48.3q, 55.7q and 56.4q for

three methoxyl groups. The ^{13}C NMR spectrum (Table) contained only three singlets upfield from 80 ppm at δ 34.1 (C-4), 48.4 (C-11) and 77.3 (C-8) indicating that the compound is an aconitine-type norditerpenoid alkaloid possessing a tertiary methoxyl group at C-8 (δ 48.3q). The carbon resonances at δ 82.1 d and 56.4 q allow to locate one methoxyl group at C-16 β , the third methoxyl group according to the carbon resonances at 83.0 d and



R¹ R²

Et	Ac	PEREGRINE (1)
Et	H	PEREGRINE ALCOHOL (2)
H	H	<i>N</i> -DEETHYLPEREGRINE ALCOHOL (7)



ATISINE (6)

Table: ^{13}C NMR assignments of peregrine (1), peregrine alcohol (2) and *N*-deethylperegrine alcohol (7)

C	1	2	7
1	84.7 d	85.6 d	83.0 d
2	26.5 t	26.5 t	25.6 t
3	37.1 t	37.2 t	34.5 t
4	34.5 s	34.6 s	34.1 s
5	56.4 d	58.9 d	58.7 d
6	73.4 d	73.0 d	72.8 d
7	42.4 d	45.9 d	45.6 d
8	79.1 s	80.9 s	77.3 s
9	44.6 d	43.8 d	45.0 d
10	46.2 d	46.3 d	49.2 d
11	48.2 s	48.3 s	48.4 s
12	28.6 t	28.5 t	28.2 t
13	38.6 d	37.7 d	38.9 d
14	75.5 d	75.2 d	75.2 d
15	33.0 t	33.1 t	34.0 t
16	82.5 d	82.4 d	82.1 d
17	64.7 d	64.3 d	77.2 d
18	25.9 q	26.0 q	26.4 q
19	57.6 t	58.1 t	52.0 t
20	49.3 t	49.6 t	—
21	13.6 q	13.8 q	—
1'	56.0 q	56.3 q	55.7 q
8'	48.3 q	48.6 q	48.3 q
16'	56.4 q	56.5 q	56.4 q
CO	170.2 s	—	—
CH ₃	21.7 q	—	—

55.7 q should be located at C-1 α . The one proton signals at 4.58 (d, $J = 7$ Hz) and 4.10 (t, $J = 5$ Hz) and the methine carbon resonances at δ 72.8 and 75.2 suggest the presence of secondary hydroxyl groups at C-6 β and C-14 α , respectively. All these data are very close to those for peregrine alcohol (2) and peregrine (1) [11–15]. The differences are the absence of ^1H and ^{13}C signals for an *N*-ethyl group, an absorption at δ 3.24 (1H,s) for *N*-H and the δ differences at C-17 and C-19 which have relations with nitrogen in the structure (Table). Treatment of 2 with potassium permanganate in aqueous acetone gave *N*-deethylperegrine alcohol (7) [14], having identical TLC and ^1H NMR spectra as the compared with those of new compound.

3. Experimental

3.1. Equipment

IR spectra were recorded in CHCl_3 on a Perkin-Elmer Model 1420 spectrophotometer. Optical rotations were recorded on a Perkin-Elmer Model 141 polarimeter. ESIMS were recorded on a Perkin-Elmer SCIEX API-1 mass spectrometer. HRMS were determined on a Fisons Auto Spec ETOFFPD FAB⁺ mass spectrometer. The NMR spectra including DEPT were recorded on a Bruker AC-300 spectrometer. Chromatographic separations on a Chromatotron [10] were carried out on rotors coated with 1 mm thick layers of Merck Al_2O_3 60 PF 254, 365 (EM 1104) or 1 mm thick layers of SiO_2 (EM 7749).

3.2. Plant material

Aerial parts (500 g dry wt.) of *Delphinium virgatum* Poir. were collected and identified by one of us (R.I.) in Thermessos-Antalya, Turkey, at an elevation of 350 m, in July 1998. A voucher specimen is deposited in the Herbarium of the Faculty of Sciences, Ankara University (No. Ilarslan 1750), Ankara, Turkey.

3.3. Extraction and isolation

Extraction of the powdered plant material with EtOH and processing the

concentrated extract for the alkaloids yielded 1.80 g of the crude alkaloidal mixture. This mixture was first fractionated by VLC on a basic alumina column (90 g, Al_2O_3 EM 1085) eluting with a solvent gradient of hexane- CHCl_3 and MeOH. Twenty five fractions (100 ml each) were collected and the fractions thus obtained were combined on the basis of their TLC similarity. VLC fractions 7–11 (eluted with hexane - CHCl_3 , 40:60 to 10–90) were combined (106 mg) and chromatographed on a silica gel rotor of a Chromatotron, eluting with a gradient of hexane, CHCl_3 and MeOH to give peregrine (1, 25 mg), peregrine alcohol (2, 74 mg) and *N*-deethylperegrine alcohol (7, 5 mg).

VLC fractions 12–16 (CHCl_3 –MeOH, 99.5:0.5 to 96:4) were combined (100 mg) and chromatographed on a basic alumina rotor with hexane- CHCl_3 –MeOH mixtures to give davisinol (3, 6 mg) and hetisinone (4, 7 mg). VLC fractions 18–20 (CHCl_3 –MeOH, 90:10 to 70:30) were combined (250 mg) and chromatographed on a basic alumina rotor with hexane- CHCl_3 –MeOH mixtures to give hetisine (5, 50 mg) and a mixture of 20 α - and 20 β -atisine (6, 13 mg). All the known compounds were identified by comparison of their ^1H and ^{13}C , DEPT NMR data and co-TLC behavior with those of authentic samples.

3.4. *N*-Deethylperegrine alcohol (7) from peregrine alcohol (2)

To a solution of 2 (12 mg) in a mixture of Me_2CO (7.5 ml) and H_2O (0.5 ml) KMnO_4 (30 mg) dissolved in Me_2CO (13 ml) and H_2O (7 ml) was added and the reaction mixture was stirred at room temp. for 10 min. Excess KMnO_4 was destroyed with NH_4OH and the mixture was extracted with CHCl_3 . After solvent removal the reaction product was chromatographed on a silica gel plate with toluene-EtOAc-DEA (7; 2; 1) where two compounds were separated. The polar band corresponding to 7 was isolated and identified.

Acknowledgment: The authors thank NATO for a Collaborative Research Grant (CRG-931261). The Turkish authors thank the University of Istanbul Research Fund for the Grant No. UP-2–150197. F.M thanks also Fulbright Visiting Researcher Program .

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Received July 19, 2000
Accepted September 13, 2000

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