Norditerpenoid Alkaloids from *Delphinium flexuosum* Bieb.

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Delphiniflexine, a new norditerpenoid alkaloid, together with the three known norditerpenoid alkaloids methyllycaconitine, ajadine, and acoseptrigenine were isolated from the aerial parts of *Delphinium flexuosum*. The structure of delphiniflexine was established on the basis of ^{1}H, ^{13}C, DEPT, homonuclear ^{1}H COSY, NOESY, HSQC, and HMBC NMR studies.

Key words: Norditerpenoid Alkaloids, *Delphinium flexuosum*, Delphiniflexine

Introduction

Delphinium (larkspur) species are very toxic plants due to their content of diterpenoid alkaloids. These alkaloids are neurotoxic agents, causing bradycardy, muscle system spasms, hypotension, and death by arrest of respiration (Bisset, 1981; Benn and Jacyno, 1983; Meriçli *et al.*, 2004). In continuation of our investigations on Turkish *Delphinium* species (Meriçli *et al.*, 1999, 2001, 2007; Süzgeç *et al.*, 2006) we now report the alkaloid contents of *Delphinium flexuosum* Bieb. No previous work has been done on this species with respect to its diterpenoid alkaloid constituents. The chemical investigation of the aerial parts of *D. flexuosum* has led to the isolation of a new diterpenoid alkaloid, delphiniflexine (**1**), together with together with the known methyllycaconitine (**2**), ajadine (**3**), and acoseptrigenine (**4**) (Meriçli *et al.*, 2006; Pelletier and Sawhney, 1978). This paper describes the isolation and structural elucidation of **1**.

Material and Methods

General

Optical rotations were measured on a Perkin Elmer Model 241 polarimeter (Foster City, CA, USA). NMR spectra were recorded on a Bruker 500 MHz spectrometer (Bremen, Germany). LC-MS was done on a Finnigan MAT 90 spectrometer (San Jose, CA, USA). Vacuum liquid chromatography (VLC) was carried out with Merck (Darmstadt, Germany) Al_2O_3 (EM 1085) and SiO_2 60 G (7731). Chromatographic separations on a chromatotron were carried out on rotors coated with an 1-mm thick layer of Al_2O_3 60 GF-254 (1092) or SiO_2 60 PF-254 (7749) from Merck. Thin-layer chromatograms were run using the solvent systems toluene/EtOAC/diethylamine (7:2:1, 7:4:1, or 7:4:2, v/v/v) and $CHCl_3$/MeOH/NH_4OH (5:3:1).

Plant material

The aerial parts (400 g) of *Delphinium flexuosum* Bieb. (Ranunculaceae) were collected on the Baykent Plateau, Artvin-Posof, Turkey, at an altitude of 2100 m, in October 2007 and identified by one of us (M. K.). A voucher specimen has been deposited in the herbarium of the Faculty of Pharmacy, Istanbul University, Istanbul, Turkey (No. ISTE 84195).

Extraction and isolation

The crude alkaloidal extract (1.2 g) obtained from 400 g of aerial parts was first separated by VLC on a neutral Al_2O_3 column eluted with petroleum ether/$CHCl_3$/MeOH mixtures. VLC fractions 46 and 47, eluted with $CHCl_3$/MeOH (90:10, v/v) (390 mg), were combined and chromatographed on a SiO_2 rotor with petroleum ether/

$CHCl_3$/MeOH mixtures to give methyllycaconitine (**2**, 62 mg) and ajadine (**3**, 38 mg). VLC fractions 48–50, eluted with $CHCl_3$/MeOH (85:15) (130 mg), were combined and chromatographed on a SiO_2 rotor with petroleum ether/$CHCl_3$/MeOH mixtures to give acoseptrigenine (**4**, 52 mg). VLC fractions 51–54, eluted with $CHCl_3$/MeOH (80:20) (110 mg), were combined and chromatographed on a SiO_2 rotor with petroleum ether/$CHCl_3$/MeOH mixtures to give delphiniflexine (**1**, 13 mg). All known compounds were identified by comparison of their ^{1}H and ^{13}C NMR data and thin-layer co-chromatography with authentic samples of **2** and **4**.

Results and Discussion

A novel diterpenoid alkaloid designated delphiniflexine (**1**), exhibiting $[\alpha]_D^{20}$ +2.31° (0.13, $CHCl_3$), has been isolated from the aerial parts of *D. flexuosum* collected at an altitude of 2100 m on the Baykent Plateau, Artvin-Posof, Turkey. The molecular formula, $C_{24}H_{40}NO_6$ (*m/z* = 438 $[M+H]^+$), was derived for the alkaloid by HRMS (*m/z* = 438.28482 $[M+H]^+$, calcd. 438.24042) and confirmed by the ^{1}H and ^{13}C NMR spectral and DEPT data, respectively. The IR spectrum showed hydroxy group absorptions at 3380 cm^{-1}, but no absorptions of aromatic groups. A completely decoupled ^{13}C NMR spectrum confirmed 24 carbon atoms of the molecule. The ^{13}C NMR and DEPT spectra showed three quaternary carbon atoms at δ_C 79.1, 49.6, and 38.2 ppm; ten signals for methane groups at δ_C 83.9, 82.6, 77.1, 71.7, 63.0, 52.0, 48.9, 45.2, 44.2, and 42.2 ppm; seven signals for methylene groups at δ_C 68.7, 56.9, 48.5, 40.7, 29.2, 29.0, and 27.2 ppm; and four signals for methyl groups at δ_C 57.9, 56.3, 48.3, and 13.3 ppm (Table I).

Table I. ^{1}H and ^{13}C NMR data of delphiniflexine (**1**) and ^{13}C NMR data of neoline (**5**) (δ in ppm, *J* in parentheses in Hz).

Position		**1**		**5**
		^{1}H	^{13}C	^{13}C
1	1β	3.38 dd (6, 9)	71.7 d	72.1 d
2	2α	1.79 m	27.2 t	29.5 t
	2β	1.47 m		
3	3α	1.82 m	29.2 t	29.9 t
	3β	2.42 m		
4		-	38.2 s	38.2 s
5		1.87 m	44.2 d	44.3 d
6		4.15 dd (1, 6)	83.9 d	83.3 d
7		2.14 d (1)	52.0 d	52.3 d
8		-	79.1 s	73.4 s
9		1.80 m	48.9 d	48.3 d
10		1.46 m	45.2 d	44.9 d
11		-	49.6 s	49.6 s
12	12a	2.42 m	29.0 t	29.8 t
	12b	1.50 m		
13		2.42 m	42.2 d	40.7 d
14		3.66 t (5)	77.1 d	75.9 d
15	15a	1.73 m	40.7 t	42.7 t
	15b	2.63 dd (12, 14)		
16		3.60 dd (7, 12)	82.6 d	82.3 d
17		2.87 s	63.0 d	63.3 d
18	18a	3.31 d (10)	68.7 t	80.3 t
	18b	3.66 d (10)		
19	19a	1.79 m	56.9 t	57.2 d
	19b	3.31 m		
20	20a	2.86 m	48.5 t	48.2 t
	20b	2.86 m		
Me-21		9.22 s	13.3 q	13.0 q
MeO-6		3.38 s	57.9 q	57.8 q
MeO-8		3.34 s	48.3 q	-
MeO-16		3.34 s	56.3 q	56.3 q
MeO-18		3.30 s	-	59.1 q

Diterpenoid alkaloids usually conform to two main groups, those with a C_{19} lycoctonine/aconitine-type skeleton with characteristic methoxy groups and those derived from a C_{20} atisine-type one with an exocyclic methylene group. The ^{1}H NMR spectrum of delphiniflexine (**1**) proved the presence of three methoxy groups, therefore it should be a C_{19} norditerpenoid alkaloid, and according to the NMR signals there is an ethyl group attached to the N atom (δ_C 13.3 ppm q, δ_H 1.07 ppm, 3H, t, *J* = 7 Hz, N-CH_2-CH_3; and δ_C 48.5 ppm, t, δ_H 2.45 ppm, 1H, m, and 2.60 ppm, 1H, m, N-CH_2-CH_3). Three methoxy groups (δ_H 3.34, 3.34, 3.38 ppm, each 3H, s; δ_C 48.3 ppm, q, 56.3 ppm, q, 57.9 ppm, q) could be assigned at C-8 (δ_C 79.1 ppm, s), C-16α (δ_H 3.60 ppm, dd, *J* = 7 and 12 Hz; δ_C 82.6 ppm, d), and C-6β (δ_H 4.15 ppm, dd, *J* = 1 and 6 Hz; δ_C 83.9 ppm, d), respectively (Pelletier *et al.*, 1985; Ross and Pelletier, 1988). According to the NMR and MS spectra, delphiniflexine (**1**) should contain six O-bearing C atoms. Three of them are methoxy groups, and the other three C atoms should carry OH groups. The OH groups should be placed on C-1β (δ_H 3.38 ppm, dd, *J* = 6 and 9 Hz; δ_C 71.7 ppm, d), C-14β (δ_H 3.66 ppm, t, *J* = 5.0 Hz; δ_C 77.1 ppm, d), and C-18 (δ_H 3.31 ppm, 1H, d, *J* = 10 Hz and 3.66 ppm, 1H, d, *J* = 10 Hz; δ_C 68.7 ppm, t) (Kulanthaivel *et al.*, 1986; Bitiş *et al.*, 2007), respectively. The chemical structure of delphiniflexine is very similar to that

Table II. Summary of COSY, NOESY, and HMBC correlation data of delphiniflexine (**1**).

Position	COSY	NOESY	HMBC
H-1β	H-2α, H-2β	H-2α, H-2β, H-10, H-12b	C-3, C-10
H-2α	H-1β, H-3α, H-3β	H-1β, H-3β	C-4, C-5, C-10
H-2β	H-1β, H-3α	H-1β, H-5	C-5, C-10
H-3α	H-2α, H-2β, H-3β	H-19a	C-19
H-3β	H-2α, H-2β, H-3α	H-2α, H-18b	C-1, C-2, C-19
H-5	H-6b	H-2β, H-6, H-9, C-18a, C-19b	C-17, C-18, C-19
H-6b	H-5	H-5, H-7, H-18a, H-18b, H-19b, OCH_3-6	OCH_3-6
H-7	H-6b, H-17	H-6, H-15b, H-17, H-19b, OCH_3-6	C-9, C-17
H-9	H-10, H-14	H-5, H-10, H-12a, H-14	C-7, C-12, C-13, C-14, C-16
H-10	H-9, H-12b	H-1β, H-9, H-12a, H-14, OCH_3-16	-
H-12a	H-12b, H-13	H-12b, H-13, H-14	-
H-12b	H-10, H-12a	H-1β, H-12a, H-13, H-16, H-17	C-14, C-16
H-13	H-12a, H-14	H-12a, H-12b, H-14, H-16, OCH_3-16	C-14, C-15, C-16
H-14	H-9, H-13	H-9, H-10, H-12a, H-13	C-16
H-15a	H-15b	H-16	C-7, C-16
H-15b	H-15a, H-16	-	C-7, C-16
H-16	H-15b	H-12b, H-13, H-15a, OCH_3-16, H-17	C-12, C-14, OCH_3-16
H-17	H-7	H-12b, H-16, H-20a, H-20b, CH_3-21	C-5, C-6, C-10, C-19
H-18a	H-18b	H-5, H-6b, H-18b, H-19b, OCH_3-6	C-3, C-19
H-18b	H-18a	H-3β, H-6b, H-18a, H-19a, H-19b	C-3, C-5, C-19
H-19a	-	H-3α, H-18b	C-3, C-18
H-19b	-	H-5, H-18a, H-18b	C-3, C-5, C-17
H-20a	H-20b, CH_3-21	H-17, H-20b, CH_3-21	C-17, C-19, C-21
H-20b	H-20a, CH_3-21	H-17, H-20a, CH_3-21	C-17, C-19, C-21
CH_3-21	H-20a, H-20b	H-17, H-20a, H-20b	-
OCH_3-6	-	H-18a	C-6
OCH_3-8	-	-	C-8
OCH_3-16	-	H-13, H-16	C-16

of the known norditerpenoid alkaloid neoline (**5**). Both compounds have two methoxy groups on C-6 and C-16 and two hydroxy groups on C-1 and C-14. The only difference is that delphiniflexine contains a hydroxy group on C-18 and a methoxy group on C-8, and neoline contains a methoxy group on C-18 and a hydroxy group on C-8 (Pelletier *et al.*, 1976; Ross and Pelletier, 1988).

The NMR data of delphiniflexine (**1**) are given in the Tables I and II. The structures of delphiniflexine (**1**) and neoline (**5**) are given in Fig. 1.

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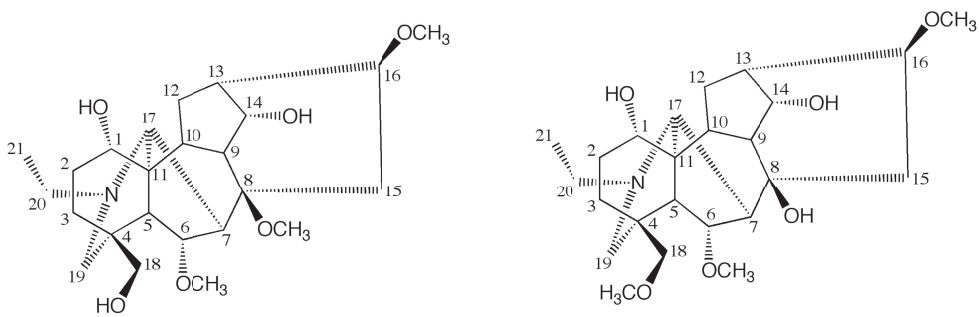

Fig. 1. Chemical structures of delphiniflexine (**1**) and neoline (**5**).

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