Diterpenoid Alkaloids of *Delphinium buschianum* GROSSH.

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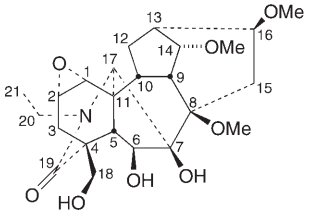
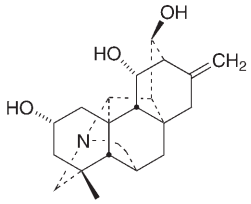
From the aerial parts of *Delphinium buschianum* GROSSH., collected in Turkey, a new diterpenoid alkaloid **1**, named budelphine, was isolated along with the known diterpenoid alkaloids karakoline (**2**), 18-hydroxy-14-*O*-methylgadesine (**3**), delsoline (**4**), lapaconidine (**5**), columbianine (**6**), 14-benzoylneoline (**7**), and hetisine (**9**). The structure of **1** was established on the basis of ^{1}H-, ^{13}C-, DEPT, ^{1}H,^{1}H-COSY, NOESY, HSQC, and HMBC NMR studies.

Introduction. – *Delphinium* (larkspur) species are very toxic plants due to their diterpenoid alkaloid content. These alkaloids are neurotoxic agents, causing bradycardy, muscle-system spasms, hypotension, and death by arrest of respiration [1–3]. In continuation of our investigations on Turkish *Delphinium* species [4–8], we now report the alkaloid contents of *Delphinium buschianum* GROSSH. No previous work had been done on this species for its diterpenoid alkaloid constituents, except for the identification of methyllycaconitine obtained from this plant [9]. The chemical investigation of the aerial parts of *D. buschianum* led to the isolation of budelphine (**1**), a new diterpenoid alkaloid, together with karakoline (**2**), 18-hydroxy-14-*O*-methylgadesine (**3**), delsoline (**4**), lapaconidine (**5**), columbianine (**6**), 14-benzoylneoline (**7**), dihydrogadesine (**8**), and hetisine (**9**) [10–17].

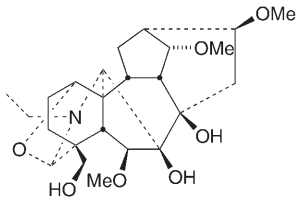
Results and Discussion. – A novel diterpenoid alkaloid, designated as budelphine (**1**), was isolated from the aerial parts of *D. buschianum* collected at an altitude of 2100 m on Kizir Mountain, Kars-Arpaçay, Turkey. The molecular formula of **1**, $C_{24}H_{35}NO_8$ (EI-MS, M^+ at m/z 465), was derived by HR-MS (M^+ at m/z 465.54120; calc. 465.53950) and confirmed by the ^{1}H- and ^{13}C-NMR and DEPT data. The IR spectrum showed absorptions of OH groups and a lactam moiety at 3380 and 1656 cm^{-1}, respectively, but no aromatic absorptions. The structure of **1** was established by its ^{1}H- and ^{13}C-NMR, DEPT, ^{1}H,^{1}H-COSY, NOESY, HSQC, and HMBC data (*Tables 1* and *2*).



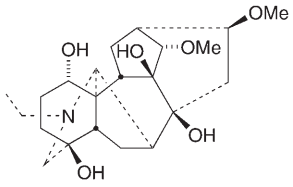
1 Budelphine



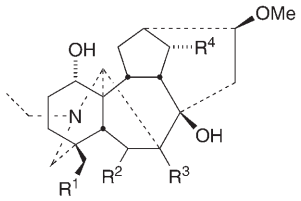
9 Hetisine



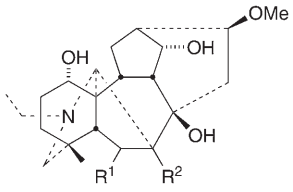
3 18-Hydroxy-14-*O*-methylgadesine



5 Lapaconidine



	R^1	R^2	R^3	R^4
4 Delsoline	MeO	β-MeO	β-OH	MeO
7 14-Benzoylneoline	MeO	α-MeO	H	BzO
6 Columbianine	OH	H	H	OH



	R^1	R^2
2 Karakoline	H	H
8 Dihydrogadesine	β-MeO	β-OH

A broadband-decoupled ^{13}C-NMR spectrum of **1** confirmed the presence of 24 C-atoms in the molecule. The DEPT spectra showed five quaternary C-atoms at δ 179.8, 86.8, 76.9, 48.3, and 37.9, ten signals for CH groups at δ 87.5, 83.7 82.5, 80.4, 77.1, 67.0, 45.5, 44.6, 42.1, and 37.7, five signals for CH_2 groups at δ 65.9, 50.0, 32.9, 29.6, and 24.6, and four signals for Me groups at δ 57.9, 57.6, 56.2, and 13.2. Diterpenoid alkaloids usually belong to either of two main groups, those with a C_{19} lycoctonine/aconitine-type skeleton with characteristic MeO groups and those derived from a C_{20} atisine-type one with an exocyclic methylene group [18][1]). The ^{1}H-NMR spectrum of budelphine (**1**) suggested the presence of three MeO groups; therefore, it should be a C_{19} norditerpenoid alkaloid, and according to the NMR signals there is an Et group attached to the N-atom (δ(C) 13.2 (*q*) and 50.0 (*t*); δ(H) 1.07 (*t*, $J = 7$ Hz, *Me*CH_2N) and 2.44–2.46 and 2.58–2.61 (2*m*, MeC*H*$_2$N)) The lactam =O group should be placed at C(19) with the signal at δ(C) 179.8 (*s*) [19] and on the basis of the long-range ^{1}H,^{13}C-HMBC correlations

[1]) Lycoctonine = (1α,6β,14α,16β)-20-ethyl-4-(hydroxymethyl)-1,6,14,16-tetramethoxyaconitane-7,8-diol, aconitine = (1α,3α,6α,14α,15α,16β)-20-ethyl-1,6,16-trimethoxy-4-(methoxymethyl)aconitane-3,8,13,14,15-pentol 8-acetate 14-benzoate, and atisine = (6*R*,6a*R*,8a*S*,9*R*,11*S*,12a*R*,12b*S*,12c*S*)-decahydro-6-methyl-10-methylene-9*H*,12c*H*-8a,11-ethano-6,12b-propano-5*H*-benz[*h*]oxazolo[2,3-*a*]isoquinolin-9-ol.

Table 1. *^{1}H- and ^{13}C-NMR Data of Budelphine* (**1**). δ in ppm, J in Hz.

	δ (H)	δ (C)
H_β–C(1)	3.75 (*d*, *J* = 9)	87.5 (*d*)
H_β–C(2)	3.91–3.94 (*m*)	82.5 (*d*)
H_α–C(3)	1.73–1.77 (*m*)	24.6 (*t*)
H_β–C(3)	2.45 (*dd*, *J* = 13, 5)	
C(4)	–	37.9 (*s*)
H–C(5)	1.95–1.98 (*m*)	37.7 (*d*)
H_α–C(6)	4.40 (br. *s*)	77.1 (*d*)
C(7)	–	86.8 (*s*)
C(8)	–	76.9 (*s*)
H–C(9)	1.79–1.81 (*m*)	45.5 (*d*)
H–C(10)	1.61–1.63 (*m*)	42.1 (*d*)
C(11)	–	48.3 (*s*)
H_a–C(12)	2.29–2.31 (*m*)	29.6 (*t*)
H_b–C(12)	1.60–1.63 (*m*)	
H–C(13)	2.39–2.41 (*m*)	44.6 (*d*)
H–C(14)	3.59 (*t*, *J* = 5)	83.7 (*d*)
H_a–C(15)	1.68–1.71 (*m*)	32.9 (*t*)
H_b–C(15)	2.54 (*dd*, *J* = 12, 14)	
H–C(16)	3.75 (*dd*, *J* = 7, 12)	80.4 (*d*)
H–C(17)	2.88 (*s*)	67.0 (*d*)
H_a–C(18)	3.30 (*d*, *J* = 10)	65.9 (*t*)
H_b–C(18)	3.55 (*d*, *J* = 10)	
C(19)	–	179.8 (*s*)
H_a–C(20)	2.58–2.61 (*m*)	50.0 (*t*)
H_b–C(20)	2.44–2.46 (*m*)	
Me(21)	1.07 (*t*, *J* = 7)	13.2 (*q*)
MeO–C(8)	3.42 (*s*)	57.9 (*q*)
MeO–C(14)	3.35 (*s*)	57.6 (*q*)
MeO–C(16)	3.32 (*s*)	56.2 (*q*)

of H–C(17) (δ(H) 2.88 (*s*)) and CH_2(18) (δ(H) 3.30 and 3.55 (each *d*, *J* = 10 Hz)). The three MeO groups (δ(H) 3.32, 3.35, and 3.42 (3*s*); δ(C) 56.2, 57.6, and 57.9 (3*q*)) could be positioned at CH(16) (δ(H) 3.75 (*dd*, *J* = 7, 12 Hz); δ(C) 80.4 (*d*)), CH(14) (δ(H) 3.59 (*t*, *J* = 5 Hz); δ(C) 83.7 (*d*)) and C(8) (δ(C) 76.9 (*s*)), respectively [20]. According to the NMR and MS data, budelphine (**1**) should contain nine O-bearing C-atoms as shown by the signals at δ(C) 179.8 (*s*), 87.5 (*d*), 86.8 (*s*), 83.7 (*d*), 82.5 (*d*), 80.4 (*d*), 77.1 (*d*), 76.9 (*s*), and 65.9 (*t*), but only eight O-atoms are present. Thus, besides the lactam group and three MeO groups, among the remaining five O-bearing C-atoms, three of them should carry OH groups, and an epoxy group should be placed between two C-atoms. The first two OH groups should be placed at C(7) (δ(C) 86.8 (*s*)) and CH_2(18) (δ(H) 3.30 and 3.58 (2*d*, *J* = 10 Hz); δ(C) 65.9 (*t*)) [21], and the place of the third OH group should be at CH(6) in β-position (δ(H) 4.40 (br. *s*); δ(C) 77.1 (*d*)) [22]. Some norditerpenoid alkaloids contain an epoxy group between C(1) and C(19), but in **1**, C(19) is part of a lactam group. The epoxy group should be placed between CH(1) (δ(H) 3.75 (*d*, *J* = 9 Hz); δ(C) 87.5 (*d*)) and another C-atom; on the basis of the long range ^{1}H,^{13}C-HMBC correlations of H–C(5) (δ(H) 1.95–1.98 (*m*)) and H–C(10) (δ(H) 1.61–1.63 (*m*)) (*Table 2*) and NOESY correlations of H–C(1) (δ(H) 3.20 (*d*, *J* = 9 Hz)) and H–C(5) (δ(H) 1.95–1.98 (*m*)) (*Table 2*), the second C-atom should be CH(2) (δ(H) 3.91–3.94 (*m*); δ(C) 82.5 (*d*)).

Table 2. *Summary of ^{1}H,^{1}H-COSY, NOESY, and HMBC Data of Budelphine* (**1**)

	^{1}H,^{1}H-COSY	NOESY	HMBC
H_β–C(1)	H_β–C(2)	H_β–C(2), H–C(10), H_b–C(12)	C(3), C(10)
H_β–C(2)	H_β–C(1), H_α–C(3)	H_β–C(1), H–C(5)	C(5), C(10)
H_α–C(3)	H_β–C(2), H_β–C(3)	–	–
H_β–C(3)	H_β–C(2), H_α–C(3)	H_b–C(18)	C(1), C(2), C(19)
H–C(5)	H–C(6), H–C(17)	H_β–C(2), H–C(6), H–C(9), H_a–C(18)	C(17), C(18), C(19)
H_α–C(6)	H–C(5)	H–C(5), H_a–C(18), H_b–C(18)	–
H–C(9)	H–C(10), H–C(14)	H–C(5), H–C(10), H_a–C(12), H–C(14)	C(7), C(12), C(13), C(14), C(16)
H–C(10)	H–C(9), H_b–C(12)	H_β–C(1), H–C(9), H_a–C(12), H–C(14), MeO–C(16)	–
H_a–C(12)	H_b–C(12), H–C(13)	H_b–C(12), H–C(13), H–C(14)	–
H_b–C(12)	H–C(10), H_a–C(12)	H_β–C(1), H_a–C(12), H–C(13), H–C(16), H–C(17)	C(14), C(16)
H–C(13)	H_a–C(12), H–C(14)	H_a–C(12), H_b–C(12), H–C(14), H–C(16), MeO–C(16)	C(14), C(15), C(16)
H–C(14)	H–C(9), H–C(13)	H–C(9), H–C(10), H_a–C(12), H–C(13)	C(16), MeO–C(14)
H_a–C(15)	H_b–C(15)	H–C(16), MeO–C(14)	C(7), C(16)
H_b–C(15)	H_a–C(15), H–C(16)	–	C(7), C(16)
H–C(16)	H_b–C(15)	H_b–C(12), H–C(13), H_a–C(15), MeO–C(16), H–C(17)	C(12), C(14), MeO–C(16)
H–C(17)	H–C(5)	H_b–C(12), H–C(16), H_a–C(20), H_b–C(20), Me (21)	C(5), C(7), C(10), C(19)
H_a–C(18)	H_b–C(18)	H–C(5), H–C(6), H_b–C(18)	C(3), C(19)
H_b–C(18)	H_a–C(18)	H_β–C(3), H–C(6), H_a–C(18)	C(3), C(5), C(19)
H_a–C(20)	H_b–C(20), Me(21)	H–C(17), H_b–C(20), Me (21)	C(17), C(19), C(21)
H_b–C(20)	H_a–C(20), Me(21)	H–C(17), H_a–C(20), Me (21)	C(17), C(19), C(21)
Me(21)	H_a–C(20), H_b–C(20)	H–C(17), H_a–C(20), H_b–C(20)	–
MeO–C(8)	–	–	C(8)
MeO–C(14)	–	H_a–C(15)	C(14)
MeO–C(16)	–	H–C(13), H–C(16)	C(16)

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Experimental Part

General. Vacuum liquid chromatography (VLC): *Merck* Al_2O_3 (*EM 1085*) and SiO_2 *60 G* (*7731*). Chromatographic separations: chromatotron with rotors coated with a 1 mm thick layer of *Merck* Al_2O_3 *60 GF-254* (*1092*) or SiO_2 *60 PF-254* (*7749*). TLC: eluents toluene/AcOEt/Et_2NH 7:4:1 or 7:4:2 and $CHCl_3$/MeOH/NH_4OH 5:3:1. Optical rotations: *Perkin-Elmer 241* polarimeter. NMR Spectra: *Bruker* 500-MHz spectrometer. MS: *Finnigan MAT-90* spectrometer.

Plant Material. The aerial parts (500 g) of *Delphinium buschianum* GROSSH. were collected on Kizir Mountain Kars-Arpaçay, Turkey, at an elevation of 2100 m, in June 2002, and identified by one of us (*H. Ö.*). A voucher specimen was deposited in the Herbarium of the Faculty of Science and Literature, Süleyman Demirel University (No. Özçelik 9623), Isparta, Turkey.

Extraction and Isolation. The crude alkaloid extract (3.7 g) obtained from 1750 g of aerial parts was first separated by VLC (neutral Al_2O_3, petroleum ether/$CHCl_3$/MeOH mixtures). The combined

Fractions 8 and *9* (with petroleum ether/$CHCl_3$ 60:40 → 55:45; 974 mg) were subjected to a SiO_2 rotor (petroleum ether/$CHCl_3$/MeOH mixtures): delsoline (**4**; 17 mg), 18-hydroxy-14-*O*-methylgadesine (**3**; 22 mg), and lapaconidine (**5**, 14 mg). *Fr. 10–13* (with petroleum ether/$CHCl_3$ 50:50 → 20:80; 437 mg) were subjected to a SiO_2 rotor (petroleum ether/$CHCl_3$/MeOH mixtures): columbianine (**6**; 10 mg). *Fr. 14* and *15* (with petroleum ether/$CHCl_3$ 10:90 → $CHCl_3$; 375 mg) were subjected to a SiO_2 rotor (petroleum ether/$CHCl_3$/MeOH mixtures): karakoline (**2**; 50 mg) and budelphine (**1**; 18 mg; $[\alpha]_D^{20} = +6.95$ ($c = 0.18$, $CHCl_3$). *Fr. 18–20* (with $CHCl_3$/MeOH 96:4 → 92:8; 736 mg) were subjected to a SiO_2 rotor (petroleum ether/$CHCl_3$/MeOH mixtures): 14-benzoylneoline (**7**; 11 mg) and dihydrogadesine (**8**; 48 mg). *Fr. 22–28* (with $CHCl_3$/MeOH 80:20 → MeOH; 303 mg) were subjected to a Al_2O_3 rotor (petroleum ether/$CHCl_3$/MeOH mixtures): hetisine (**9**; 25 mg). Delsoline (**4**), lapaconidine (**5**), karakoline (**2**), and hetisine (**9**) were obtained in pure state directly from chromatotron fractions.

Budelphine (=*(1α,2α,6β,14α,16β)*-1,2-Epoxy-20-ethyl-6,7-dihydroxy-4-(hydroxymethyl)-8,14,16-trimethoxyaconitan-19-one; **1**), **7**, **8**, and **3** were purified by prep. TLC (SiO_2, toluene/AcOEt/Et_2NH 7:4:1) and **6** by prep. TLC (SiO_2, toluene/AcOEt/Et_2NH 7:4:2). All known compounds were identified by comparison of their ^1H- and ^{13}C-NMR data and co-TLC behavior with those of authentic samples.

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