Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Phytochemistry 62 (2003) 543–550
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Norditerpenoid alkaloids from *Delphinium* species

Nyamdari Batbayar[a], Shiiter Enkhzaya[a], Jigjidsuren Tunsag[a], Dulamjav Batsuren[a], David S. Rycroft[b], Susanne Sproll[c], Franz Bracher[c,*]

[a]*Institute of Chemistry and Chemical Technology, Mongolian Academy of Science, Ulaanbaatar, Mongolia*
[b]*NMR Spectroscopy Laboratory, Department of Chemistry, Joseph Black Building, University of Glasgow, Glasgow G12 8QQ, UK*
[c]*Department für Pharmazie, Zentrum für Pharmaforschung, Ludwig-Maximilians-Universität, Butenandtstr. 5-13, D-81377 Munich, Germany*

Received 14 August 2002; received in revised form 4 October 2002

Abstract

From the aerial parts of four *Delphinium* species 11 known and 3 new norditerpenoid alkaloids have been isolated: from *D. dissectum* Huth: delavaine A/B, deoxylycoctonine, methyllycaconitine; new: 10-hydroxymethyllycaconitine; from *D. excelsum* Reichenb.: delcaroline, delectinine, delterine, methyllycaconitine; new: 10-hydroxymethyllycaconitine, 18-*O*-methyldelterine and 10-hydroxynudicaulidine; from *D. grandiflorum* L.: delcosine, deltatsine, grandiflorine, methyllycaconitine; from *D. triste* Fisch.: delcosine, macrocentridine, 14-dehydrodelcosine. The structures of the new alkaloids were established on the basis of MS, ^{1}H, ^{13}C, DEPT, homonuclear COSY, HMQC and HMBC NMR spectroscopic techniques.

Keywords: *Delphinium dissectum*; *Delphinium excelsum*; *Delphinium grandiflorum*; *Delphinium triste*; Ranunculaceae; Norditerpenoid alkaloids; 10-Hydroxymethyllycaconitine; 18-*O*-Methyldelterine; 10-Hydroxynudicaulidine

1. Introduction

The majority of phytochemical studies on *Delphinium* species (Ranunculaceae), the main sources of diterpenoid alkaloids, have been carried out with species from Asia, Europe and North America. *Delphinium dissectum, D. excelsum, D. grandiflorum* and *D. triste* are widely distributed in the north west and central part of Mongolia. They are used in Mongolian traditional medicine. In folk medicine, decoctions from herbs of some *Delphinium* species have been used as antipyretics in cases of infectious fever, binding in case of diarrhoea caused by bilious disorder and to relieve toothache (Ligaa, 1996). No phytochemical investigations of *D. excelsum* and *D. dissectum* have yet been reported. From *D. triste* the alkaloid methyllycaconitine (**4**) was isolated (Mats, 1972). Numerous norditerpenoid alkaloids have been isolated from *D. grandiflorum* (Li and Chen, 1993).

In this paper, we report on the isolation and structural elucidation of three new norditerpenoid alkaloids, 10-hydroxymethyllycaconitine (**1**), 18-*O*-methyldelterine (**2**) and 10-hydroxynudicaulidine (**3**) from aerial parts of these plants (Fig. 1). In addition we isolated known alkaloids methyllycaconitine (**4**), deoxylycoctonine (**8**) (Pelletier et al., 1984) and delavaine A/B (**7a**/**7b**) (Pelletier and Joshi, 1991) from *D. dissectum*, delcosine (**11**), 14-dehydrodelcosine (**15**) (Pelletier et al., 1984) and macrocentridine (**14**) (Benn et al., 1989) from *D. triste* and methyllycaconitine (**4**), delcaroline (**9**), delectinine (**10**) (Pelletier et al., 1984) and delterine (**5**) (Narzullaev et al., 1986) from *D. excelsum*. *D. grandiflorum* yielded the known constituents methyllycaconitine (**4**) and grandiflorine (**13**) (Manners et al., 1998), as well as the alkaloids deltatsine (**12**) (Pelletier and Joshi, 1991) and delcosine (**11**), that have not yet been found in this species. All known alkaloids were identified by comparison of their spectroscopic data (NMR, MS) with literature values.

2. Results and discussion

The new alkaloid 10-hydroxymethyllycaconitine (**1**) is an amorphous substance with $[\alpha]_D^{20}$ +51.0° ($CHCl_3$). The molecular formula $C_{37}H_{50}N_2O_{11}$ was derived from the HREI and FAB mass spectra ($[M]^+$ 698.3416), and its ^{13}C NMR spectral data. Its IR spectrum showed

* Corresponding author. Fax: +49-89-21807802.
E-mail address: franz.bracher@cup.uni-muenchen.de (F. Bracher).

0031-9422/03/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0031-9422(02)00514-9

absorptions at 3442 (OH), 1717 (CO), 1618 and 1490 (aromatic system) cm^{-1}.

The DEPT studies of **1** indicated ten quarternary carbon singlets, thirteen methine carbon doublets, eight methylene carbon triplets and six methyl quartets (see Table 1). The ^1H NMR spectrum indicated the presence of an *N*-CH_2–CH_3 group (H-20a: δ 2.82, *m*; H-20b: δ 2.96, *m*; H-21: δ 1.07, 3H, *t*, $J=7.5$ Hz), four methoxy groups (δ 3.21, 3.34, 3.37, 3.43, each 3H, *s*), a 14-β proton (δ 4.12, *t*, $J=4.6$ Hz), a 6-α proton (δ 3.91, *br. s*), and resonances typical of a C-18 ester residue such as is found in methyllycaconitine (**4**) (Pelletier et al., 1984).

Table 1
^{13}C NMR spectral data of **1**, **2, 3**, **4**, **5,** and **6** (δ, ppm)

Carbon number	Compound					
	1	**4**	**2**	**5**	**3**	**6**
1	77.2 *d*	83.9 *d*	77.5 *d*	78.0 *d*	77.7 *d*	85.5 *d*
2	26.0 *t*	26.0 *t*	26.1 *t*	26.9 *t*	26.3 *t*	25.9 *t*
3	31.7 *t*	32.0 *t*	32.1 *t*	37.3 *t*	37.3 *t*	37.3 *t*
4	37.3 *s*	37.6 *s*	37.9 *s*	34.1 *s*	34.5 *s*	34.3 *s*
5	46.0 *d*	50.3 *d*[a]	45.6 *d*	51.1 *d*	51.4 *d*	55.0 *d*
6	91.4 *d*	90.8 *d*	91.1 *d*	92.2 *d*	91.8 *d*	90.8 *d*
7	87.8 *s*	88.5 *s*	87.6 *s*	88.1 *s*	88.6 *s*	89.2 *s*
8	75.8 *s*	77.4 *s*	75.8 *s*	76.2 *s*	75.5 *s*	*
9	53.5 *d*	43.2 *d*[a]	53.8 *d*	53.9 *d*	54.6 *d*	45.1 *d*
10	81.2 *s*	46.1 *d*[b]	81.3 *s*	81.6 *s*	80.7 *s*	46.0 *d*
11	54.5 *s*	49.0 *s*	54.5 *s*	55.0 *s*	54.4 *s*	48.5 *s*
12	39.7 *t*	28.7 *t*	39.2 *t*	40.0 *t*	38.3 *t*	27.5 *t*
13	38.2 *d*	38.0 *d*[b]	38.2 *d*	38.6 *d*	37.4 *d*	36.5 *d*
14	82.4 *d*	83.9 *d*	82.4 *d*	82.8 *d*	74.0 *d*	75.3 *d*
15	34.8 *t*	33.6 *t*	34.5 *t*	34.9 *t*	34.3 *t*	33.1 *t*
16	82.0 *d*	82.5 *d*	82.0 *d*	82.4 *d*	81.6 *d*	81.7 *d*
17	64.8 *d*	64.5 *d*	65.1 *d*	65.1 *d*	66.1 *d*	65.0 *d*
18	69.3 *t*	69.5 *t*	78.0 *t*	27.1 *q*	27.1 *q*	26.8 *q*
19	52.4 *t*	52.3 *t*	52.7 *t*	56.8 *t*	56.7 *t*	56.6 *t*
20	51.0 *t*	50.9 *t*	51.1 *t*	51.3 *t*	51.5 *t*	51.2 *t*
21	14.0 *q*	14.0 *q*	14.1 *q*	14.4 *q*	14.7 *q*	14.3 *q*
1-OCH_3	55.6 *q*	55.7 *q*	55.5 *q*	55.8 *q*	56.0 *q*	56.0 *q*
6-OCH_3	58.5 *q*	58.2 *q*[c]	57.6 *q*	58.7 *q*	59.0 *q*	58.5 *q*
14-OCH_3	57.9 *q*	57.8 *q*[c]	57.9 *q*	58.8 *q*		
16-OCH_3	56.3 *q*	56.3 *q*	56.2 *q*	56.5 *q*	56.8 *q*	56.5 *q*
18-OCH_3			59.1 *q*			
C=O	164.2 *s*	164.1 *s*				
1′	127.1 *s*	127.1 *s*				
2′	132.9 *s*	133.1 *s*				
3′	130.0 *d*	130.0 *d*[d]				
4′	133.7 *d*	133.6 *d*				
5′	129.5 *d*	129.4 *d*[d]				
6′	131.1 *d*	131.0 *d*[d]				
1″	176.0 *s*	175.8 *s*				
2″	35.2 *d*	35.3 *d*				
3″	37.0 *t*	37.0 *t*				
4″	180.0 *s*	179.8 *s*				
5″	16.2 *q*	16.4 *q*				

*Signal obscured by solvent peaks.
[a] The literature assignments have been reversed.
[b] The literature assignments have been reversed.
[c] The literature assignments have been reversed.
[d] The literature assignments have been reversed.

The presence of two nitrogens in the molecular formula suggested that **1** might be related to **4** and contains a α-*N*-(2-methylsuccinimido)anthranoyl unit. This would account for the *m*/*z* 482 and 216 ions, the latter being the [*N*-(2-methylsuccinimido)anthranoyl] fragment corresponding to **4**. Support for this conclusion was provided by the ^1H NMR spectrum of **1** in $CDCl_3$ (see Table 2), which contained a set of four one-proton resonances in the aromatic region, corresponding to an *ortho*-disubstituted benzene, and a three-proton resonance at δ 1.45 (*d*, $J=7$ Hz) corresponding to the methyl group of the succinimidoyl unit of **4** in which this signal is seen at δ 1.47 (*d*, $J=6$ Hz). Also, the ^{13}C NMR spectrum of **1** contained a set of resonances very similar to those described for the *N*-(2-methylsuccinimidoyl)anthranoyl structural fragment of **4** (see Table 1). These spectral data suggested that **1** is a norditerpenoid alkaloid, $C_{19}H_{20}(OCH_3)_4(OH)_3$(*N*-$CH_2$–$CH_3$) [$OCOC_6H_4N(CO)_2CH(CH_3)CH_2$].

Consistent with this, a comparison of the ^{13}C NMR spectra of **1** and **4** (see Table 1) revealed only significant differences involving ring ''C'', and carbon atoms C-1, C-5 and C-11. In our experiments, methyllycaconitine (**4**) has been isolated from *D. excelsum* along with **1**.

The main difference between **1** and **4** in their DEPT spectra was that the methine carbon (δ_C 46.1, *d*) in **4** was replaced by a quaternary carbon (δ_C 81.2, *s*) bearing oxygen in **1**. Also, by comparing the ^{13}C NMR spectral data of **1** and **4**, the assignments of the signals for C-9, C-10, C-11 and C-12 in **1** were found to have downfield shifts of 10.3, 35.1, 5.5 and 11.0 ppm respectively. This indicated that one of the C-9, C-10 and C-13 positions was substituted. The signal at δ_H 4.12 (*t*, $J=4.6$ Hz) attributable to H-14 indicated that the C-9 and C-13 sites were not oxygenated (De La Fuente and Ruiz-Mesia, 1994).

All of the aforementioned evidence supported structure of **1** having a hydroxyl group at the C-10 position. Four methoxy groups were placed at the C-1, C-6, C-14 and C-16 positions based on the long-range connectivities of H-1 (δ 3.59, 1H, *t*, $J=7.8$ Hz) with 1-OCH_3, H-6 (δ 3.91, 1H, *br. s*) with 6-OCH_3, H-14 (δ 4.12, 1H, *t*, $J=4.6$ Hz) with 14-OCH_3, and H-16 (δ 3.18, 1H, *t*, $J=8$ Hz) with 16-OCH_3 respectively, in HMBC experiments (Table 2). Finally, alkaloid **1** is 10-hydroxymethyllycaconitine as confirmed by 1D and 2D NMR spectral data (Table 2).

The stereochemistry of **1** was determined as follows: absence of correlation between H-13 and H-16 as shown by COSY indicates that ring ''D'' is in boat conformation and consequently 16-OCH_3 is β (Xu et al., 1996).

The HREI mass spectrum of compound **2** ($[M]^+$ 497.2984) suggested the molecular formula $C_{26}H_{43}NO_8$. The IR spectrum of **2** exhibited an OH absorption band at 3432 cm^{-1} with no carbonyl absorption band observed. The ^1H NMR spectrum of **2** indicated the

presence of N-CH_2–CH_3 (H-20a: δ 2.77, *dq*, $J=12.8$ and 7.1 Hz; H-20b: δ 2.90, *dq*, $J=12.8$ and 7.1 Hz; H-21: δ 1.05, 3H, *t*, $J=7.1$ Hz) and five methoxy groups (δ 3.25, 3.30, 3.33, 3.42 and 3.44, each 3 H, *s*). The geminal protons' absorptions at δ 3.57 (1H, *dd*, $J=7.6$ and 10.0 Hz, C-1-β-H), 3.91 (1H, *br. s*, C-6-α-H), 4.10 (1H, *t*, $J=4.6$ Hz, C-14-β-H), and 3.17 (1H, *dd*, $J=5.8$ and 10.1 Hz, C-16-α-H) were observed.

The ^{13}C NMR spectrum of **2** gave 26 carbon signals. The DEPT spectrum revealed five quarternary carbon singlets at δ 37.9, 54.5, 75.8, 81.3, and 87.6, eight doublets at δ 38.2, 45.6, 53.8, 65.1, 77.5, 82.0, 82.4, and 91.1, seven triplets at δ 26.1, 32.1, 34.5, 39.2, 51.1, 52.7, and 78.0, and six quartets at δ 14.1, 55.5, 56.2, 57.6, 57.9, and 59.1. In the DEPT spectrum a methylene triplet at δ 78.0 and a quaternary carbon singlet at 37.9 ppm indicate that a CH_2–OR group is attached to C-4 of **2** (Pelletier et al., 1984). The mass spectrum base peak M^+-31 of **2** indicates that C-1 is α-methoxylated. As well, M^+-15 and M^+-33 peaks present in the mass spectrum of **2** show that C-6 is β-methoxylated and an OH group is attached to C-7. The above mentioned spectral data suggest that **2** is a norditerpenoid alkaloid having C-1-α-methoxyl, C-6-β-methoxyl, C-7-hydroxy, C-4-CH_2–OR and C-14-α-OR.

From detailed combined study of ^{1}H, ^{13}C, DEPT spectral data in addition to the mass spectrum of **2**, the presence of three OH groups in the molecule can be suggested. For the confirmation of the suggested structure, further COSY and HMBC spectra have been used. COSY experiment revealed correlations of ''A'' ring protons H-1 with H-2a and H-2b (δ_H 2.08 and 2.11, 2H, *m*), H-2a, b with H-3a, b (δ_H 1.58 and 1.60, 2H, *m*). To reveal correlations of ''C'' ring protons H-14 signal was used as the key. As indicated in HMBC spectrum, H-14 has a coupling with a quartet at 57.9 ppm suggesting that α-OCH_3 is located at C-14.

Also as shown by COSY, H-14 correlates with H-13 (δ_H 2.48 *dd*, $J=4.6$ and 8.3 Hz, δ_C 38.2, *d*) and H-9 (δ_H 2.86, 1H, *d*, $J=4.6$ Hz, δ_C 53.8, *d*).

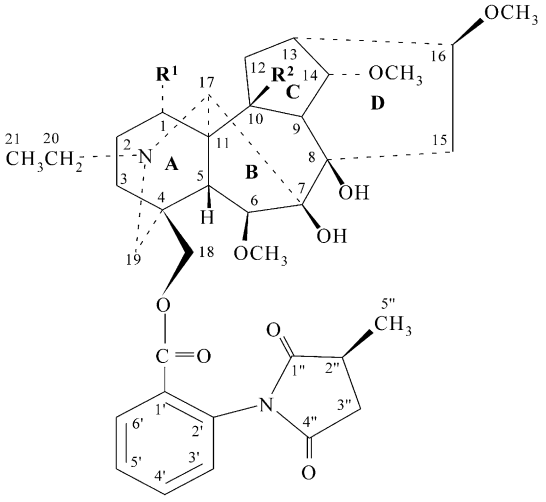
In the ^{13}C NMR spectrum, a singlet of C-11 was observed at δ 54.5 or 3–7 ppm downfield of its normal

Table 2
^{1}H NMR spectral data and 2D NMR correlations of **1**

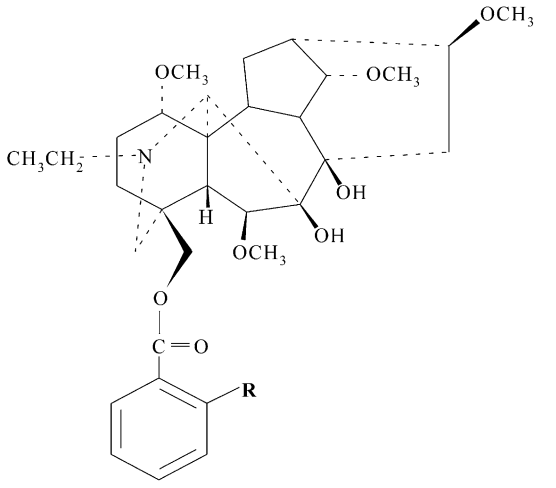
Proton number	^{1}H NMR (δ, ppm; *J*, Hz)	COSY (^{1}H)	HMBC
1	3.59 *t* (7.8)	2a, 2b	C-2, C-10, C-17, C-1-OCH_3
2a	2.12 *m*	1, 2b	
2b	2.19 *m*	1, 2a	
3a	1.54 *m*	2a, 2b, 3b	
3b	1.75 *m*	2a, 2b, 3a	
5	1.99 *br. s*	17	C-4, C-6, C-17, C-19
6	3.91 *br. s*		C-4, C-7, C-8, C-11, C-6-OCH_3
9	2.89 *d* (4.6)	14	C-8, C-10, C-12, C-13, C-15
12a	1.70 *dd* (8.0, 15.6)	12b, 13	C-8, C-16
12b	3.09 *d* (15.6)	12a	C-14, C-16
13	2.49 *dd* (4.8, 7.2)	12a, 14	C-9,C-10, C-12, C-15
14	4.12 *t* (4.6)	9, 13, 16	C-8, C-9, C-13, C-16, C-14-OCH_3
15a	1.72 *dd* (8.0, 15.0)	15b, 16	C-7, C-8, C-16
15b	2.67 *dd* (8.0, 15.0)	15a, 16	C-7, C-8, C-9, C-13, C-16
16	3.18 *t* (8.0)	15a, 15b	C-12, C-14, C-16-OCH_3
17	2.86 *br. s*	5	C-5, C-6, C-19
18a	4.00 *d* (11.2)	18b	C=O, C-5
18b	4.20 *d* (11.2)	18a	C-3, C-19
19a	2.48 *d* (12.6)	19b	C-3, C-18
19b	2.70 *d* (12.6)	19a	C-3, C-4, C-5, C-17
20a	2.82 *m*	20b, 21	C-19
20b	2.96 *m*	20a, 21	C-19, C-21, C-17
21	1.07 *t* (7.5)	20a, 20b	C-20
1-OCH_3	3.21 *s*		C-1
6-OCH_3	3.37 *s*		C-6
14-OCH_3	3.43 *s*		C-14
16-OCH_3	3.34 *s*		C-16
3′	7.27 *dd* (1.0, 7.6)	4′, 5′	C-1′, C-2′, C-5′
4′	7.68 *dt* (1.0, 7.6)	3′, 5′, 6′	C-2′, C-6′
5′	7.54 *dt* (1.0, 7.6)	4′, 3′, 6′	C-1′, C-3′
6′	8.04 *dd* (1.0, 7.6)	4′, 5′	C-4′, C=O
2″	3.05 *m*	3″a, 5″	
3a″	2.53 *m*	3″b, 2″	
3b″	3.11 *m*	3″a, 2″	
5″	1.45 *d* (7.0)	2″, 3″a	C-1″, C-3″

shift (Pelletier et al., 1980) and ^{1}H NMR resonance for H-14 has been observed at δ 4.10, shifted downfield, as 1H triplet with $J=4.6$ Hz indicating location of hydroxyl at C-10. The HMBC spectral data supported these NMR spectra data. The HMBC spectrum of **2** revealed correlations H-1 with C-10 (δ 81.3, *s*), H-1 with 1-OCH_3 (δ 55.5, *q*), H-6 with 6-OCH_3 (δ 57.6, *q*), H-14 with 14-OCH_3 (δ 57.9, *q*), H-16 with 16-OCH_3 (δ 56.2, *q*), and H-18a with 18-OCH_3 (δ 59.1, *q*). Thus, the remaining singlet at δ 87.6, which correlates with H-6 and H-15a and b could be assigned to C-7.

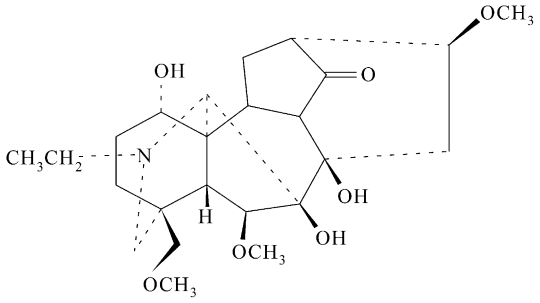
HMBC spectrum revealed the following correlations: H-18a proton correlates with carbons resonating at δ 45.6 (*d*) and δ 52.7 (*t*) indicating that those were carbons located at 5 and 19 positions respectively. H-19b correlates with the doublet at δ 65.1 (C-17). H-17 correlates with the doublet at δ 91.1 (C-6). H-9 correlates with triplets at δ 39.2 and 34.5, which are attributable to C-12 and C-15. H-14 correlates with C-16 (δ 82.0, *d*). To assign triplets at δ 39.2 and 34.5 we used COSY spectrum data according to which H-16 couples with protons H-15a and b which are attached to the carbon



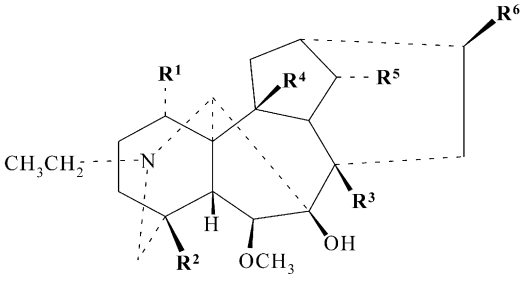
	R^1	R^2
methyllycaconitine (**4**)	OCH_3	H
10-hydroxymethyllycaconitine (**1**)	OCH_3	OH
grandiflorine (**13**)	OH	H



delavaine A (**7a**) **R** = NH-CO-CH(CH_3)-CH_2-CO_2CH_3
delavaine B (**7b**) **R** = NH-CO-CH_2-CH(CH_3)-CO_2CH_3



14-dehydrodelcosine (**15**)



	R^1	R^2	R^3	R^4	R^5	R^6
delterine (**5**)	OCH_3	CH_3	OH	OH	OCH_3	OCH_3
18-*0*-methyldelterine (**2**)	OCH_3	CH_2-OCH_3	OH	OH	OCH_3	OCH_3
nudicaulidine (**6**)	OCH_3	CH_3	OH	H	OH	OCH_3
10-hydroxynudicaulidine (**3**)	OCH_3	CH_3	OH	OH	OH	OCH_3
delcosine (**11**)	OH	CH_2-OCH_3	OH	H	OH	OCH_3
delcaroline (**9**)	OCH_3	CH_2-OCH_3	OH	OH	OH	OCH_3
delectinine (**10**)	OCH_3	CH_2-OH	OH	H	OH	OCH_3
deltatsine (**12**)	OH	CH_2-OCH_3	OCH_3	H	OH	OCH_3
deoxylycoctonine (**8**)	OCH_3	CH_3	OH	H	OCH_3	OCH_3
macrocentridine (**14**)	OH	CH_2-OCH_3	OH	H	OH	OH

Fig. 1. Structures of the alkaloids isolated from *Delphinium* species.

appearing as triplet at δ 34.5 as revealed by HMQC spectrum. Thus, the other triplet at δ 39.2 should be assigned to C-12.

From the HMBC spectrum, the H-14 proton correlates with the singlet at δ 75.8, which is assigned to C-8.

Lack of correlation of H-13 with H-16, as revealed by a COSY experiment suggests boat conformation of ring D and consequently 16-OCH_3 is β. Finally, on the basis of the aforementioned spectral data and by comparison of ^{13}C NMR spectrum shifts of **2** with those of delterine **5** (which has been isolated along with **2** from *D. excelsum*) (Table 1), the suggested formula for **2** has been confirmed.

Compound **3** is an amorphous substance with $[\alpha]_D^{20}+26.3^\circ$ ($CHCl_3$). The HREI mass spectrum ($[M]^+$ 453.2729) suggested the molecular formula $C_{24}H_{39}NO_7$ for the alkaloid **3**. The NMR spectra showed that the alkaloid **3** contains a *N*-ethyl group (δ_C 14.7, *q*, δ_H 1.05, 3H, *t*, $J=7.1$ Hz; δ_C 51.5, *t*, δ_H 2.81, 2.90 each 1H, *dq*, $J=6.8$, 12.8 Hz) and three methoxy groups (δ_C 56.0, *q*, δ_H 3.24, 3H, *s*; δ_C 56.8, *q*, δ_H 3.35, 3H, *s*; δ_C 59.0, *q*, δ_H 3.42, 3H, *s*). Biogenetic considerations and the molecular formula $C_{24}H_{39}NO_7$ indicated that **3** is a norditerpenoid alkaloid. As there are no carbonyl functionalities or methylenedioxy groups, the alkaloid should contain four hydroxyl groups and three methoxy groups. The ^1H (Table 4) and ^{13}C NMR spectra (Table 1) indicated the presence of a methyl group (δ_C 27.1, *q*; δ_H 0.99, 3H, *s*) at C-4. As no other functional groups are discernible in the IR or the NMR spectra, a partial structure (Fig. 2) can be written for **3**.

The quarternary carbon signals at δ_C 34.5, 54.4, and 75.5 can be readily assigned to C-4, C-11, and C-8 respectively.

Downfield shift of C-11 (δ_C 54.4, *s*) resonance by 6 ppm from normal (δ_C ~48.0) suggests that a OH group is attached to C-10 (Pelletier et al., 1980). The carbon signal at δ_C 74.0 *d* (δ_H 4.61, 1H, *t*, $J=4.9$ Hz) is clearly assigned to C-14 bearing an α-hydroxyl group and indicated a hydroxyl group at C-10, and there are no substituents on the adjacent carbons C-9 and C-13 (De La Fuente and Ruiz-Mesia, 1994).

By COSY spectrum for H–H correlations and following application of HMQC technique, couplings between H-14 and H-9 (δ_H 2.97, 1H, *d*, $J=4.9$ Hz; δ_C 54.6, *d*), H-14 and H-13 (δ_H 2.53, 1H, *dd*, $J=4.9$ and 8.6 Hz; δ_C 37.4, *d*) have been revealed. Further, H-13 couples with the proton at 1.70 ppm (1H, *dd*, $J=8.6$ and 16.0 Hz), correlating with carbon at δ_C 38.3 ppm, *t*, which can be assigned only as H-12a. Thus assignments of all the protons of the "C" ring with their corresponding carbons have been made.

^1H–^{13}C correlation method HMBC technique revealed couplings of H-13 with carbons at 80.7, 81.6,

Table 3
^1H NMR spectral data and 2D NMR correlations of **2**

Proton number	^1H NMR (δ, ppm; *J*, Hz)	COSY (^1H)	HMBC
1	3.57 *dd* (7.6, 10.0)	2a, 2b	C-10, C-17, C-1-OCH_3
2a	2.08 *m*	1, 2b, 3a, 3b	
2b	2.11 *m*	1, 2a, 3a, 3b	
3a	1.58 *m*	2a, 2b, 3b	C-5
3b	1.60 *m*	2a, 2b, 3a	
5	1.94 *br. s*	17	C-1, C-4, C-6, C-10, C-17, C-18, C-19
6	3.91 *br. s*		C-4, C-7, C-8, C-11, C-6-OCH_3
9	2.86 *d* (4.6)	14	C-8, C-10, C-12, C-13, C-15
12a	1.69 *dd* (8.3, 15.8)	12b, 13	C-13, C-14, C-16
12b	3.05 *d* (15.8)	12a	C-13, C-14, C-16
13	2.48 *dd* (4.6, 8.3)	12a, 14	C-9,C-14, C-15, C-16
14	4.10 *t* (4.6)	9, 13	C-8, C-16, C-14-OCH_3
15a	1.71 *dd* (5.8, 15.3)	15b, 16	C-7, C-8, C-13, C-16
15b	2.64 *dd* (9.0, 15.3)	15a, 16	C-7, C-8, C-13, C-16
16	3.17 *dd* (5.8, 10.1)	15a, 15b	C-12, C-14, C-16-OCH_3
17	2.82 *br. s*	5	C-1, C-5, C-6, C-19
18a	2.98 *d* (9.0)	18b	C-3, C-4, C-5, C-19, C-18-OCH_3
18b	3.40 *d* (9.0)	18a	C-3, C-4
19a	2.59 *d* (11.6)	19b	C-3, C-4, C-18
19b	2.84 *d* (12.0)	19a	C-3, C-4, C-5, C-17
20a	2.77 *dq* (7.1, 12.8)	20b, 21	C-17, C-19, C-21
20b	2.90 *dq* (7.1, 12.8)	20a, 21	C-17, C-19, C-21
21	1.05 *t* (7.1)	20a, 20b	C-20
1-OCH_3	3.25 *s*		C-1
6-OCH_3	3.42 *s*		C-6
14-OCH_3	3.44 *s*		C-14
16-OCH_3	3.33 *s*		C-16
18-OCH_3	3.30 *s*		C-18

Table 4
^{1}H NMR spectral data and 2D NMR correlations of **3**

Proton number	^{1}H NMR (δ, ppm; J, Hz)	COSY (^{1}H)	HMB*c*
1	3.68 *dd* (7.4, 10.2)	2a, 2b	C-10, C-17, C-1-OCH_3
2a	2.02 *m*	1, 2b, 3a, 3b	C-4
2b	2.15 *m*	1, 2a, 3a, 3b	C-1
3a	1.22 *m*	2a, 2b, 3b	C-18, C-19
3b	1.60 *m*	2a, 2b, 3a	C-4, C-5
5	1.65 *br. s*	17, 6	C-4, C-6, C-7, C-17, C-18, C-19
6	3.89 *s*	5	C-4, C-7, C-8, C-11, C-6-OCH_3
9	2.97 *d* (4.9)	14	C-8, C-10, C-12, C-13, C-15
12a	1.70 *dd* (8.6, 16.0)	12b, 13	C-13, C-16
12b	2.52 *d* (16.0)	12a	C-9, C-14, C-16
13	2.53 *dd* (4.9, 8.6)	12a, 14	C-9, C-10, C-14, C-15, C-16
14	4.61 *t* (4.9)	9, 13	C-8, C-16
15a	1.75 *dd* (2.1, 17.4)	15b, 16	C-8, C-16
15b	2.62 *dd* (8.7, 17.4)	15a, 16	C-7, C-8, C-13, C-16
16	3.43 *dd* (3.2, 8.7)	15a, 15b	C-8, C-14, C-16-OCH_3
17	3.05 *br. s*	5	C-5, C-6, C-8, C-10, C-19, C-20
18	0.99 *s*		C-3, C-4, C-5, C-19
19a	2.46 *d* (12.0)	19b	C-3, C-18
19b	2.65 *d* (12.0)	19a	C-3, C-4, C-17, C-20
20a	2.81 *dq* (6.8, 12.8)	20b, 21	C-17, C-19, C-21
20b	2.90 *dq* (6.8, 12.8)	20a, 21	C-17, C-19, C-21
21	1.05 *t* (7.1)	20a, 20b	C-20
1-OCH_3	3.24 *s*		C-1
6-OCH_3	3.42 *s*		C-6
16-OCH_3	3.35 *s*		C-16

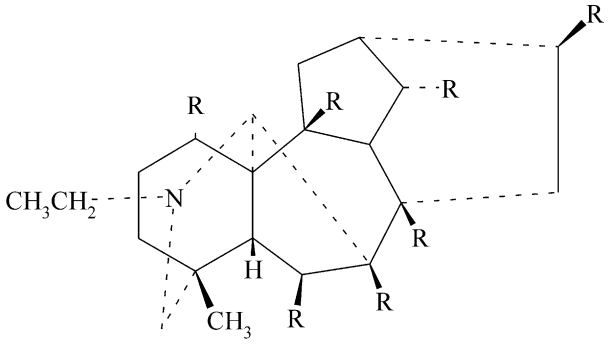
and 34.3 ppm, and those were assigned as C-10, C-16, and C-15. H-15b has been selected as a key signal for further assignments. H-15b correlates with C-8, C-13, and C-16 and with carbon at 88.6 ppm, assigned to C-7, which has no long-range or three-bond coupling with methoxy protons suggesting that an OH group is attached to this site.

M^+-31 peak (100%) in the EI-mass spectrum and 77.7 ppm doublet (δ_H 3.68, 1H, *dd*, $J = 7.4$ and 10.2 Hz) in ^{13}C NMR spectrum of **3** confirms α configuration of the methoxy group at C-1 and that C-10 is bearing a hydroxy group (Joshi and Pelletier, 1990).

With H-1 proton resonance at 3.68 ppm in the COSY spectrum H-2a and H-2b protons and the other protons in the molecule of **3** have been derived sequentially. Application of HMQC spectrum allowed to assign carbons corresponding to each proton. By HMBC spectrum, H-3a couples with the triplet at 56.7 ppm, and the quartet at 27.1 ppm, H-3b couples with the doublet at 51.4 ppm, which have been assigned to C-19, C-18 and C-5 respectively. H-19b couples with methine carbon at 66.1 ppm assigned as C-17. Methine carbon at 91.8 ppm which couples with both H-17 and H-5 has been assigned as C-6. In the HMBC spectrum, methoxy protons at 3.42 ppm and 3.35 ppm couple with C-6 and C-16 indicating those carbons were methoxylated. In the ^{1}H NMR spectrum, H-6 proton appears as singlet at 3.89 ppm indicating that C-6-OMe is β (Pelletier et al., 1983).

Lack of coupling between H-16 and H-13 as shown by COSY spectrum indicates that ring ''D'' of the molecule has boat conformation and β-configuration of the methoxy group at C-16 (Bando et al., 1989). Thus 3 is 1α,6β,16β-trimethoxy-4β-methyl-7β,8β,10β,14α-tetrahydroxy-*N*-ethyl-aconitane [= 10-hydroxynudicaulidine (**3**)].

The structure of **3** resembles that of nudicaulidine (**6**) (Pelletier and Joshi, 1991). By comparison of ^{13}C NMR spectra of **3** and **6** (see Table 1) the assignments of the signals of C-10, C-12, C-9, and C-11 in **3** were found to have downfield shifts of 34.7, 10.8, 9.5 and 5.9 ppm



R = 4 OH, 3 OCH_3

Fig. 2. Partial structure for **3**.

respectively. This, in addition to the aforementioned evidence supported **3** having a hydroxyl group at C-10 position.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured in $CHCl_3$ or EtOH with a Perkin Elmer Polarimeter 241 MC. IR spectra were recorded as KBr discs on a JASCO FT/IR-410 spectrometer. MS were recorded on a Hewlett Packard 5989A mass spectrometer. HRMS was recorded on a Finnigan MAT 95 Q mass spectrometer. ^1H and ^{13}C and 2D NMR spectra were recorded on a Jeol GSX 400 or JNMR-GX 500 spectrometer in $CDCl_3$ with TMS as an internal standard. Silica (0.040–0.063 mm particle size) and Al_2O_3 (neutral, 200-300 mesh) was used for CC, silica 60 F_{254} plates (Merck) for analytical and preparative TLC (20×20 cm plates). Spots on chromatograms were detected under UV-light (254 nm) and by Dragendorff reagent. HPLC analyses were performed on a Merck LaChrom HPLC system using a diode array detector. All solvents used were of analytical grade.

3.2. Plant material

Aerial parts of *Delphinium triste* Fisch. were collected in Terelj, a location near Ulaanbaatar, in August 1998, aerial parts of *D. excelsum* Reichenb. were collected in Bulgan, Central Mongolia, in August 1997, aerial parts of *D. dissectum* Huth were collected in Bayan-Ovoo, Bayankhongor province, Mongolia in June 2000, aerial parts of *D. grandiflorum* L. in Bugat, Bulgan province, Mongolia in August 1997. Voucher specimens of the plants are deposited at the Institute of Chemistry, Mongolian Academy of Sciences, Mongolia.

3.3. Extraction of crude alkaloids

Dried and powdered aerial parts of the plants (*Delphinium triste* Fisch. 3 kg, *D. excelsum* Reichenb. 5 kg, *D. dissectum* Huth 27 kg, *D. grandiflorum* L. 7 kg) were exhaustively (5×) extracted by percolation with 80% ethanol at room temperature. After evaporation of combined extracts of each plant separately in vacuo concentrated extracts were acidified with 5% aq. H_2SO_4. Lipophilic impurities were removed from acidified extracts by extraction with Et_2O. The pH of the aqueous layers was adjusted to 4.5 with dry $NaHCO_3$, to pH 8.0 with Na_2CO_3 and to pH 10 with 40% aqueous NaOH. The three fractions were extracted with $CHCl_3$ and the solvent was removed by evaporation in vacuo yielding 3 fractions A, B, and C respectively. Yields were: 17 g of fraction A, 4 g of B, and 2 g of C of *D. excelsum*, 3 g of A, 8 g of B and 1.5 g of C from *D. triste*, 30 g of A, 120 g of B, 14 g of C from *D. dissectum* and 17 g of A, 15 g of B of *D. grandiflorum*. There was no fraction C obtained from the last plant.

3.4. Isolation of individual alkaloids

Fraction A of *D. excelsum* was subjected to CC on silica and eluted subsequently with a gradient of hexane, hexane–$CHCl_3$, $CHCl_3$, $CHCl_3$–MeOH. Methyllycaconitine (**4**) (800 mg) and 10-hydroxymethyllycaconitine (**1**) (400 mg) were purified from $CHCl_3$ and $CHCl_3$–MeOH eluates respectively, using Et_2O to precipitate the alkaloids as amorphous substances.

Fractions B and C were combined, as TLC of these fractions showed no significant difference, and submitted to VLC on Al_2O_3, and eluted with a gradient of $CHCl_3$–MeOH yielding fractions 1–5. 18-*O*-Methyldelterine (**2**) (5 mg) and delterine (**5**) (7 mg) were isolated by purification of fraction 1 on preparative TLC plates using the solvent system $CHCl_3$–MeOH 8:2. Combined fractions 3 and 4 were rechromatographed on preparative TLC, solvent system $CHCl_3$–MeOH 8:2, to yield 10-hydroxynudicaulidine (**3**) (15 mg), delcaroline (**9**) (5 mg) and delectinine (**10**) (10 mg).

Fraction B of *D. triste* was fractionated by VLC (Al_2O_3) eluted with petroleum ether, $CHCl_3$, EtOAc, gradient $CHCl_3$–MeOH subsequently. Delcosine (**11**) (250 mg), macrocentridine (**14**) (20 mg), and 14-dehydrodelcosine (**15**) were recrystallized with acetone from $CHCl_3$–MeOH 95:5, 90:10 and 80:20 eluates respectively.

Fraction C of *D. dissectum* was subjected to CC on silica and eluted subsequently with a gradient of hexane, hexane–$CHCl_3$, $CHCl_3$ and $CHCl_3$–MeOH. Five Fractions eluted with $CHCl_3$–MeOH were combined and submitted to CC on Al_2O_3 eluting with a $CHCl_3$, $CHCl_3$–MeOH gradient. Fractions of this column gave after another CC on Al_2O_3 using the same eluents 10 mg of methyllycaconitine (**4**).

Two fractions eluting after methyllycaconitine from the silica column were separated by preparative HPLC (Merck Hibar®, LiChrospher® 100 RP-18 (10 μm), 25×250 mm) using the following mobile phase at a flow rate of 30 ml min^{-1}: 0–8 min 50% $(NH_4)_2CO_3$ (2% aqueous), 25% MeOH, 25% acetonitril; then 30% $(NH_4)_2CO_3$ (2% aqueous), 25% MeOH, 45% acetonitril. The peak eluting at 14.0 min gave after extraction with chloroform, drying with Na_2SO_4 and evaporation of the solvent 10-hydroxymethyllycaconitine (**1**) (20 mg).

Seven grams of fraction B of *D. dissectum* were subjected to CC on silica and eluted subsequently with a gradient of $CHCl_3$ and $CHCl_3$–MeOH. A fraction eluted with $CHCl_3$–MeOH (99:1) was rechromatographed on a silica column using a gradient of EtOAc–MeOH. A fraction eluted with $CHCl_3$–MeOH (99:1)

was separated by preparative TLC using $CHCl_3$–MeOH, 8:2. One band gave again methyllycaconitine (**4**) (40 mg), another was deoxylycoctonine (**8**) (4 mg). The band in the middle (60 mg) was further purified by preparative HPLC (Merck Hibar®, LiChrosorb® Diol (7 μm) 25×250 mm), using pentane-EtOH (9:1) containing 0,05% diethylamine at a flow rate of 30 ml min^{-1}. After evaporation of the solvent a mixture of delavaine A and B (**7a**/**7b**) (31 mg) was obtained from one fraction.

Three grams of fraction A of *D. grandiflorum* were subjected to CC on Al_2O_3 eluting with a $CHCl_3$, $CHCl_3$–MeOH gradient. One fraction eluted with $CHCl_3$ was further separated by preparative TLC ($CHCl_3$–MeOH, 8:2) and subsequently by HPLC (Merck, LiChrospher® 100 RP-18 (10 μm), 10×250 mm, flow rate 6 ml min^{-1}). The peak eluting at 9.8 min gave after extraction with chloroform, drying with Na_2SO_4 and evaporation of the solvent grandiflorine (**13**) (2.2 mg). Five fractions eluted with $CHCl_3$–MeOH (99:1) from the silica column were combined and separated by preparative TLC using $CHCl_3$–MeOH, 8:2. One band gave methyllycaconitine (**4**) (2 mg), the other deltatsine (**12**) (12 mg). Later fractions of the silica column yielded after recrystallization from acetone delcosine (**11**) (190 mg).

3.5. 10-Hydroxymethyllycaconitine (*1*)

$[\alpha]_D^{20} + 51.0°$ ($CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3442, 1717, 1618 and 1490. ^1H NMR and ^{13}C NMR ($CDCl_3$): Tables 1 and 2. HRMS *m/z* $[M]^+$ 698.3416 ($C_{37}H_{50}N_2O_{11}$). FAB$^+$ MS *m/z* (rel. int.): 699 [M + 1] (100), 681 (30), 667 (32), 482 (5), 452 (8), 216 (16).

3.6. 18-O-Methyldelterine (*2*)

IR ν_{max}^{KBr} cm^{-1}: 3432, 2925, 1630, 1460, 1384, 1203, 1090. ^1H NMR and ^{13}C NMR ($CDCl_3$): Tables 1 and 3. HRMS *m/z* $[M]^+$ 497.2984 ($C_{26}H_{43}NO_8$). EIMS *m/z* (rel. int.): 497 $[M]^+$ (4), 482 (17), 467 (28), 466 (100), 464 (10), 452 (13), 436 (13), 434 (15), 422 (11).

3.7. 10-Hydroxynudicaulidine (*3*)

$[\alpha]_D^{20} + 26.3°$ ($CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3444, 2929, 2822, 1090. ^1H NMR and ^{13}C NMR ($CDCl_3$): Tables 1 and 4. HRMS *m/z* $[M]^+$ 453.2729 ($C_{24}H_{39}NO_7$). EIMS *m/z* (rel. int.): 453 $[M]^+$ (10), 452 (25), 438 (16), 423 (26), 422 (100), 420 (18).

References

Bando, H., Wada, K., Tanaka, J., Kimura, S., Hasegawa, E., Amiya, T., 1989. Two new diterpenoid alkaloids from *Delphinium pacific giant* and revised ^{13}C-NMR assignment of delpheline. Heterocycles 29, 1293–1300.

Benn, M.H., Okanga, F.I., Manavu, R.M., 1989. The principal alkaloids of *Delphinium macrocentrum* from Mt. Kenya. Phytochemistry 28, 919–922.

De La Fuente, G., Ruiz-Mesia, L., 1994. Norditerpenoid alkaloids from *Aconitum vulparia* subsp. *neapolitanum*. Phytochemistry 37, 271–274.

Joshi, B.S., Pelletier, S.W., 1990. The structures of anwheidelphinine, bulleyanitines A-C, puberaconitine, and puberaconitidine. J. Nat. Prod. 53, 1028–1030.

Li, C., Chen, D., 1993. Alkaloidal constituents from aerial parts of *Delphinium grandiflorum* L. Zhiwu Xuebao 35, 80–83. (Chem. Abstr. 119:156226).

Ligaa, U., 1996. Medicinal Plants of Mongolia used in Mongolian Traditional Medicine. KCA Press, Seoul.

Manners, G.D., Panter, K.E., Pfister, J.A., Ralphs, M.H., James, L.F., 1998. The characterization and structure-activity evaluation of toxic norditerpenoid alkaloids from two *Delphinium* species. J. Nat. Prod. 61, 1086–1089.

Mats, M.N., 1972. New curarelike agents of plant origin. Rast. Resur. 8, 249–252. (Chem. Abstr. 77:79509).

Narzullaev, A.S., Matveev, V.M., Sabirov, S.S., Yunusov, M.S., 1986. Delterine, a new diterpene alkaloid from *Delphinium* ternatum. Khim. Prir. Soedin. 6, 802–803.

Pelletier, S.W., Mody, N.V., Joshi, B.S., Schramm, L.C., 1984. ^{13}C and proton NMR shift assignments and physical constants of C_{19}-diterpenoid alkaloids. In: Pelletier, S.W. (Ed.), Alkaloids: Chemical and Biological Perspectives, Vol. 2. John Wiley & Sons Inc, New York, pp. 205–462.

Pelletier, S.W., Mody, N.V., Dailey Jr., O.D., 1980. ^{13}C Nuclear magnetic resonance spectroscopy of methylenedioxy group-containing C_{19}-diterpenoid alkaloids and their derivatives. Can. J. Chem. 58, 1875–1879.

Pelletier, S.W., Glinski, J.A., Joshi, B.S., Szu-ying, Chen, 1983. The diterpenoid alkaloids of *Delphinium tatsienense* Franch. Heterocycles 20, 1347–1354.

Pelletier, S.W., Joshi, B.S., 1991. Carbon-13 and proton NMR shift assignments and physical constants of norditerpenoid alkaloids. In: Pelletier, S.W. (Ed.), Alkaloids: Chemical and Biological Perspectives, Vol. 7. Springer-Verlag New York Inc., pp. 297–564.

Xu, Q.-Y., Li, Z.-B., Wang, F.-P., Che, C.-T., 1996. Hemsleyanidine and isohemsleyanidine from *Aconitum hemsleyanum* var. *circinatum*. Heterocycles 43, 1243–1250.