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Diterpenoid alkaloids from *Delphinium crispulum*

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Abstract

From the aerial parts of *Delphinium crispulum* five known norditerpenoid alkaloids, deltatsine, browniine, karakoline, ezochasmanine and isotalatizidine, and the C_{20} alkaloid hetisine as well as two new compounds, crispulidine and delphicrispuline, were isolated. Structures of the newly isolated alkaloids were established by spectroscopic methods. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: *Delphinium crispulum*; Ranunculaceae; Diterpenoid alkaloids; Crispulidine; Delphicrispuline

1. Introduction

As a part of our continuing investigations of Turkish *Delphinium* species (Ulubelen, Desai, Joshi, Venkateswarlu, Pelletier, Meriçli, et al., 1995, 1996; Meriçli, Meriçli, Becker, Ulubelen, Özden, Dürüst, et al., 1996), we have now studied the aerial parts of *D. crispulum* Rupr. (syn. *D. speciosum* Bieb var. *linearilobum* Trautv.) and isolated a diterpenoid and seven norditerpenoid alkaloids, of which two are new compounds.

The known compounds were established as hetisine (Pelletier, Glinski, Joshi, & Chen, 1983; Glinski, Joshi, Jiang, & Pelletier, 1988), deltatsine (Joshi, Glinsky, Chokshi, Chen, Srivastava, & Pelletier, 1984), browniine (Pelletier, Mody, Sawhney, & Bhattacharyya, 1977), karakoline (Yang, Hao, & Chow, 1979; Konno, Shirasaka, & Hikino, 1982), ezochasmanine (Takayama, Ito, Koga, Sakai, & Okamoto, 1981; Takayama, Tokita, Ito, Sakai, Kurosaki, & Okamoto, 1982; Desai, Joshi, & Pelletier, 1986) and isotalatizidine (Pelletier & Djarmati, 1976; Pelletier, Mody, Venkov, & Jones, 1979) on the basis of comparison of their spectral data (^{1}H and ^{13}C NMR) with those of literature values. The structures of the new compounds, crispulidine (**1**) and delphicrispuline (**2**) were derived from 1D and 2D NMR spectral techniques.

2. Results and discussion

The HRMS mass spectrum (*m*/*z* 407.2650, calc. 407.2672) indicated the molecular formula $C_{23}H_{37}NO_5$ for crispulidine (**1**). The ^{1}H and ^{13}C NMR spectra showed the presence of an *N*-ethyl group (δ_C 13.0 *q*; δ_H 1.12, 3 H, *t*, $J = 7$ Hz; δ_C 46.5 *t*; δ_H 2.50 *m*, 2.58 *m*, 2H) and two methoxy groups (δ_C 57.2 *q*; δ_H 3.38, 3H, *s*; δ_C 48.2 *q*; δ_H 3.35, 3H, *s*). The molecular formula as well as the biogenetic considerations showed that crispulidine (**1**) is a norditerpenoid alkaloid. The signals at δ_C 27.2 (*q*) and δ_H 0.90, (3H, *s*) indicated the presence of a methyl group at C-4. Study of the ^{1}H NMR and IR spectra showed the absence of carbonyl, ether oxygen and methylene dioxy groups. Crispulidine should therefore contain two methoxy and three hydroxy groups. The location of these groups was accomplished by ^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY, ^{1}H–^{13}C HETCOR, and as well as COLOC spectra. The stereochemistry was decided by NOESY experiment.

Twenty three carbons were observed using the 1D, ^{1}H-decoupled ^{13}C (APT) spectrum, and the chemical shift patterns indicated two methyl, two methoxyl, six

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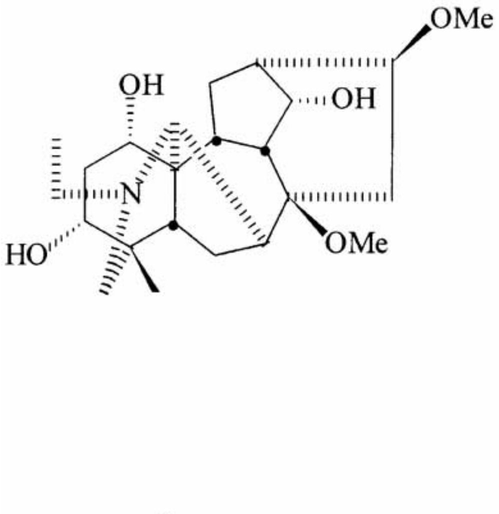

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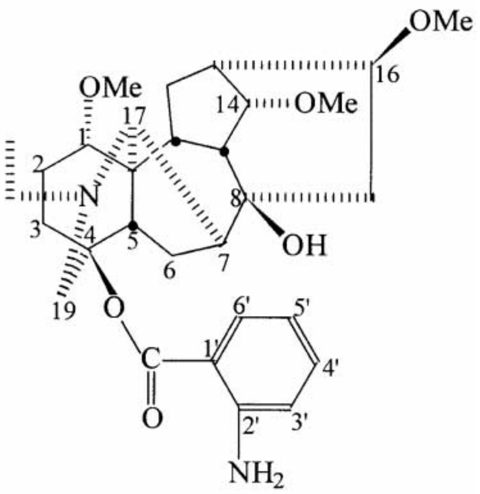

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methylene, ten methine and three quaternary carbons. The methoxy groups were located at C-16 (δ 82.5 *d*, 57.2 *q*) and C-8 (δ 78.9 *s*, 48.2 *q*); there are other examples of compounds having the C-8 OMe at around 48.0 ppm (Bai, Benn, & Majak, 1989; Desai, Joshi, Ross, & Pelletier, 1989). One of the three hydroxy groups was assigned to C-14 from the typical signals at δ_C 75.7 (*d*) and δ_H 4.22 (*t*, $J = 4.5$ Hz). The other two hydroxy groups were assigned to C-1 (δ_C 72.6 *d*; δ_H 3.72, *t*, $J = 3.5$ Hz) and C-3 (δ_C 72.2 *d*; δ_H 3.68, *dd*, $J = 4$ and 11 Hz). Both H-1 and H-3 showed correlation with the protons at C-2 in the COSY spectrum. The proton at C-1 showed correlations with C-3 (δ 72.2) and C-10 (δ 43.9) in the COLOC spectrum

Table 1
NMR data crispulidine (**1**)

	^{1}H	COSY ^{1}H–^{1}H	^{13}C	COLOC ^{13}C–^{1}H	NOESY
1β	3.72 *t* (3.5)[a]	H-2α, H-2β	72.6 *d*	H-3, H-5, H-9	H-3β, H-10
2α	1.74 *m*	H-1β, H-2β, H-3β	33.6 *t*		
2β	1.80 *m*	H-1β, H-2α			
3β	3.68 dd (4,11)	H-2β	72.2 *d*	H-1, H-5	H-1β
4	–	–	39.1 *s*		
5	1.90 *d* (8)	H-6a, H-17	41.6 *d*	H-9, H-17, H-18	H-2β, H-6
6a	1.5 *dd* (8,14)	H-5	24.8 *t*	H-7	H-5
6b	1.8 *m*	H-7		H-7	H-5
7	2.4 *d* (8)	H-6b	39.5 *d*	H-6a, H-6b	H-6a
8	–	–	78.9 *s*		
9	2.10 *dd* (5,11)	H-10, H-14	45.2 *d*	H-5, H-10, H-14	H-5
10	1.76 *m*	H-9, H-12b	43.9 *d*	H-9, H-17	
11	–	–	49.0 *s*		
12a	1.60 *m*	H-12b, H-13	29.6 *t*	H-14	
12b	2.0 *d* (9)	H-12a, H-10			
13	2.32 *m*	H-12a, H-14	43.0 *d*		
14	4.22 *t* (4.5)	H-9, H-13	75.7 *d*	H-9, H-16	
15	2.25 *m*	H-16	40.0 *t*	H-16	
16	3.25 *t* (9.0)	H-15	82.5 *d*	H-14, H-15	
17	2.20 *s*	H-5	64.5 *d*	H-5	
18	0.90 *s*		27.2 *q*		
19a	2.30 *m*		55.0 *t*		
19b	1.95 *d* (11)				
N–CH_2–CH$_3$	2.50 *m*, 2.58 *m*		46.5 *t*		
N–CH$_2$–CH_3	1.12 *t* (7)		13.0 *q*		
OMe-8	3.35 *s*		48.2 *q*		
OMe-16	3.38 *s*		57.2 *q*		

[a] In Hz.

Table 2
NMR data of delphicrispuline (**2**)

	^1H	COSY ^1H–^1H	^{13}C	COLOC ^{13}C–^1H
1β	3.20 *d* (9)[a]	H-2α, H-2β	82.5 *d*	H-5, H-9, H-17
2α	1.50 *m*	H-1β, H-2β, H-3	24.4 *t*	
2β	1.65 *m*	H-1β, H-2α		
3	1.62 *m*	H-2α	34.6 *t*	
4	–	–	83.2 *s*	H-6
5	1.75 *d* (8)	H-6a	49.0 *d*	H-1, H-9
6a	1.60 *m*	H-5	26.2 *t*	H-7, H-17
6b	1.90 *m*	H-7		H-7
7	2.45 *d* (7.5)	H-6b	47.2 *d*	H-5
8	–	–	75.4 *s*	
9	2.0 *dd* (4,10)	H-14	43.2 *d*	H-5, H-17
10	1.85 *m*	H-12b	38.4 *d*	
11	–	–	47.2 s	
12a	1.60 *m*	H-10	24.0 *t*	H-14, H-16
12b	2.05 *dd* (4,8)			
13	2.30 *m*	H-14	48.2 *d*	
14	3.45 *t* (4.5)	H-9, H-13	83.4 *d*	H-9, H-10, H-16
15	2.05 *m*	H-16	42.2 *t*	H-16
16	3.17 *d* (8)	H-15	83.8 *d*	H-14, H-15
17	3.02 *s*		62.0 *d*	H-5
18				
19a	2.65 *d* (11)		56.2 *t*	
19b	3.60 *d* (11)			
N–*C*H_2–CH_3	2.45 *m*, 2.74 *m*		50.1 *t*	
N–CH_2–*C*H_3	1.07 *t* (7)		11.2 *q*	
OMe-1	3.32 *s*		56.2 *q*	
OMe-14	3.30 *s*		57.3 *q*	
OMe-16	3.40 *s*		59.0 *q*	
Ar-C=O	–		168.0 *s*	
C-1′			110.7 *s*	
C-2′			151.1 *s*	
C-3′	6.67 *d* (8)		116.3 *d*	
C-4′	7.28 *dd* (1.5, 8)		135.6 *d*	
C-5′	6.63 *dd* (1.5, 8)		116.8 *d*	
C-6′	7.80 *dd* (1.5, 8)		130.6 *d*	
NH_2	5.72 *brs*			

[a] In Hz.

while the one at C-3 showed correlations with C-1 (δ 72.6) and C-5 (δ 41.6). Table 1 shows the ^1H and ^{13}C NMR data of compound **1**. The results of COSY and COLOC experiments are also given in Table 1. These data clearly indicated that the three hydroxy groups are located at C-1, C-3 and C-14 and the methoxy groups at C-8 and C-16. The NOESY experiment indicated the presence of α hydroxy groups at C-1 and C-3 (Table 1). Thus crispulidine was assigned structure **1**.

The second new compound delphicrispuline (**2**) has a molecular formula $C_{30}H_{42}N_2O_6$ (*m/z* 526.2961, cal. 526.3040). The NMR spectrum of **2** indicated the presence of an aromatic ring system (δ_C 130.6 *d*, C-6′; δ_H 7.8, 1H, *dd*, $J = 1.5$ and 8 Hz, H-6′; δ_C 135.6 *d*, C-4′; δ_H 7.28, 1H, *dd*, $J = 1.5$ and 8 Hz, H-4′; δ_C 116.3 *d*, C-3′; δ_H 6.67, 1H, *brd*, $J = 8$ Hz, H-3′; δ_C 116.8 *d*, C-5′; δ_H 6.63, 1H, *dd*, $J = 1.5$ and 8 Hz, H-5′ and δ_C 151.1 *s*, C-2′; 110.7 *s*, C-1′; 168.0 *s*, ArCO). The broad singlet signal at δ 5.72 (2H) (disappeared with D_2O) indicated the presence of an amine group attached to the aromatic ring, thus showing the anthranilic acid moiety of the molecule. Other ^1H NMR signals showed the presence of three methoxy groups at δ 3.40, 3.32, 3.30 (each 3H, *s*), an *N*-ethyl group at δ 1.07 (3H, *t*, $J = 7$ Hz). Thirty carbons were observed in the ^1H-decoupled ^{13}C (APT) spectrum, which showed one methyl, three methoxyl, seven methylene, thirteen methine and six quaternary carbons. The assignments of the methoxy groups to C-1, C-14 and C-16 were decided from the ^1H and ^{13}C chemical shifts. The signals at δ_H 3.20, (1H, *d*, $J = 9$ Hz, H-1) and δ_C 82.5 (*d*, C-1), δ_H 3.17 (1H, *d*, $J = 8$ Hz, H-16) and δ_C 83.8 (*d*, C-16), and δ_H 3.45, (*t*, $J = 4.5$ Hz, H-14) and δ_C 83.4 (*d*, C-14) clearly indicated the placements of the three methoxy groups. The anthranoyl moiety could be placed at C-18 or C-8. The signal at δ_C 75.4 indicated that there should be a hydroxy group present at C-8. The lack of a methyl signal at

C-4 as well as the lack of C-18 methylene group were indicated the attachment of the anthranoyl group at the C-4 hydroxyl to form an ester as in **2** (Pelletier & Djarmati, 1976; Pelletier et al., 1979; Dequan & Das, 1983; Desai et al., 1989). The mass spectrum had the base peak at *m/z* 406 indicating the splitting of the anthranoyl group $[M-C_7H_6ON]^+$. The unambiguous assignment of the signals were done by COSY, HETCOR and COLOC experiments. The data are given in Table 2.

3. Experimental

3.1. General

Opt. Rot. and IR: $CHCl_3$, ^1H (200 MHz and 400 MHz) and ^{13}C (50 and 100 MHz) NMR: $CDCl_3$; HRMS: 70 eV. Chromatographic separations were carried on a Chromatotron instrument using rotors coated with 1 mm thick layers of neutral Al_2O_3.

3.2. Plant material

Aerial parts of *D. crispulum* Rupr. were collected from eastern Turkey, Iğdir, (Small Ararat mountain) 1500–2000 m elevations in August 1984. A voucher specimen is deposited at the University of Ankara in the Herbarium of Faculty of Sciences: Ilarslan 1610.

3.3. Extraction of crude alkaloids

Dried and powdered aerial parts (250 g) of the plant were exhaustively extracted by percolation at room temp using MeOH. After evaporation in vacuo a residue (10 g) was obtained. The residue dissolved in MeOH, was brought to pH 1.5 (5% H_2SO_4) and extracted with CH_2Cl_2 to give a neutral fraction (7 g). The aq soln was basified (pH 8–10) and extracted with CH_2Cl_2 (20 × 100 ml) to yield 385 mg of a crude alkaloidal mixture. This was separated on a neutral Al_2O_3 rotor of a Chromatotron to yield seven alkaloids in the following order: deltatsine (5.8 mg), **1** (9.2 mg), **2** (8.5 mg), browniine (15 mg), karakoline (12 mg), ezochasmanine (16.4 mg) and isotalatizidine (24 mg).

3.4. Crispulidine (*1*)

$[\alpha]_D^{20} = +0^\circ$ ($CHCl_3$; *c* 0.9). IR $\nu_{max.}^{CHCl_3}$ (cm^{-1}): 3406, 2928, 1646, 1458, 1379, 1295, 1224, 1090, 1046, 974, 945, 876, 753; ^1H and ^{13}C NMR ($CDCl_3$): Table 1; HRMS *m/z* (rel. int.): 407.2650 $[M]^+$ (10), 390 $[M-OH]^+$ (32), 359 $[M-OMe-OH]^+$ (100), 344 (35), 328 (15), 306 (12), 206 (10), 122 (8), 98 (12), 71 (13), 58 (28).

3.5. Delphicrispuline (*2*)

$[\alpha]_D^{20} = +23.8^\circ$ ($CHCl_3$; *c* 0.8). IR $\nu_{max.}^{CHCl_3}$ (cm^{-1}): 3447, 2929, 1689, 1620, 1580, 1560, 1530, 1510, 1456, 1385, 1295, 1241, 1161, 1090, 880, 753; ^1H and ^{13}C NMR ($CDCl_3$): Table 2. HRMS *m/z* (rel. int.): 526.2961 $[M]^+$ (1), 434 $[M-C_6H_6N]^+$ (18), 406 $[M-C_7H_6ON]^+$ (100), 375 $[406-OMe]^+$ (75), 349 (50), 319 (35), 251 (10), 112 (18), 71 (23), 58 (22).

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