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NORDITERPENOID ALKALOIDS FROM *DELPHINIUM PYRIMADALE*

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Key Word Index—*Delphinium pyrimadale*; Ranunculaceae; norditerpenoid alkaloids; 8-acetylcondelphine.

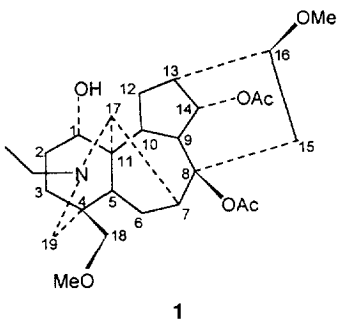
Abstract—From the aerial parts of *Delphinium pyrimadale* a new norditerpenoid alkaloid, 8-acetylcondelphine, has been isolated, together with the known alkaloids isotalatizidine, condelphine and senbusine C. The structure of the new alkaloid was established on the basis of ^{1}H, ^{13}C, APT, DEPT homonuclear COSY, HETCOR and COLOC NMR spectral studies and by chemical reactions.

INTRODUCTION

In continuation of our studies on indigeneous Pakistan *Delphinium* species [1], we have investigated the aerial parts of *D. pyrimadale* from the Swat district of Pakistan. No phytochemical work appears to have been carried out on this species until now. The present study led to the isolation of a novel norditerpenoid alkaloid, 8-acetylcondelphine (**1**), together with three known alkaloids, isotalatizidine [2], condelphine [3] and senbusine C [4]. The plant is considered to be poisonous by local people (pers. comm.).

RESULTS AND DISCUSSION

The crude alkaloidal mixture obtained from the aerial parts of *D. pyrimadale* at pH 10 was separated on a neutral Al_2O_3 rotor of a Chromatotron apparatus without primary separation. Elution started with petrol and then a gradient of CH_2Cl_2 was used up to 100%, followed by EtOH. The combined fractions were purified by preparative TLC to yield 8-acetylcondelphine (**1**) isotalatizine, condelphine and senbusine C.



1

The molecular formula $C_{27}H_{41}NO_7$ was derived from the HRMS of **1** (*m/z* 491.2878, calcd 491.2884), which was consistent with its ^{13}C NMR spectrum. The IR spectrum showed the presence of hydroxyl (3446 cm^{-1}) and acetyl (1732, 1725 and 1245 cm^{-1}) absorbancies. The ^{1}H NMR spectrum showed that **1** contains two acetyl groups at δ 1.96 and 2.02 (each 3H, *s*), two methoxyls at δ 3.27 and 3.30 (each 3H, *s*) and an *N*-ethyl group at δ 1.10 (3H, *t*, J = 7 Hz) and 2.48 (2H, *m*).

Biogenetic considerations indicated that **1** is a norditerpenoid alkaloid. The ^{1}H and ^{13}C spectra of the new alkaloid **1** excluded the presence of a methylenedioxy function or other ethers, the remaining oxygen atom being ascribed to the hydroxyl group previously inferred from the IR spectrum. The ^{13}C NMR (APT, DEPT 135°) spectra showed the presence of five methyl, eight methylene, eight methine (for nine methine carbons) and five quaternary carbon signals for 27 C atoms in compound **1**. The signal at δ 41.1 was the most intense and indicated two methine carbons for C-7 and C-13. The number of methylene and methine groups showed that **1** is an aconitine-type alkaloid, rather than a lycoctonine-type. The spectral data indicated that its structure is quite similar to that of condelphine, except for the presence of a second acetyl group in **1**. The signal at δ_H 4.80 (1H, *t*, J = 4.5

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Hz) and δ_C 77.1 clearly showed that C-14 had an α-acetyl group and that there were no substituents on the adjacent carbons, C-9 and C-13, as observed in various other examples [5–7]. The second acetyl group could be placed either at C-1 or C-8. If it is placed at C-1 the NMR signal should be at δ_H 4.8–5.1 [9] and δ_C 74.0–77.0 [9], while the δ_C signal for C-8 having an acetyl group should be at δ 80.0–85.0 [6, 8, 9], as observed in the present case. The mono and diacetyl derivatives of condelphine were prepared by Pelletier *et al.* [2, 3, 9], these derivatives were 1-acetylcondelphine and 1,8-diacetylcondelphine, none of them had the same spectral data, thus indicating that the second acetyl group of **1** should be placed at C-8. The only hydroxyl group was therefore assigned to C-1 (which corresponds to resonances δ_C 72.1; δ_H 3.72, 1H, *brs* $W_{1/2} = 4.0$ Hz). The two methoxyl groups were assigned to C-16 (δ_C 82.0; δ_H 3.25 *dd*, $J = 4$ and 11 Hz) and to C-18 (δ_C 78.9; δ_H 3.0, *d*, $J = 9$ Hz; and 3.15, *d*, $J = 9$ Hz). COSY and HETCOR experiments led to the assignments of all the vicinal protons and proton-bearing carbons of the molecule (Table 1). Conclusive evidence for the structure of compound **1** was obtained by a long-range ^{13}C-^1H NMR shift-correlated spectrum (COLOC, 10 Hz, Table 1), which displayed cross-peaks between the protons and carbons three bonds away and rarely two and four bonds away. In order to verify the presence of the acetyl groups at C-8 and C-14, basic hydrolysis of compound **1** was performed. The spectral data of the resulting product **1a** was identical to those given for isotalatizine [2] (Table 2). Acetylation of **1a** for 36 hr yielded 1,8-diacetylcondelphine as shown by its ^1H NMR and HR mass spectra. Both 1D and 2D NMR spectral data clearly indicated that the structure of compound **1** was 8-acetylcondelphine.

EXPERIMENTAL

General

Optical rotations were measured in $CHCl_3$. IR spectra were recorded in $CHCl_3$. HRMS were at 70 eV. ^1H

Table 1. NMR data of 8-acetylcondelphine (**1**)

Position	^1H	^{13}C	COSY	COLOC
1	3.72 *brs*	72.1	2β	C-2, C-5, C-10
2α	2.55 *m*	29.1	1β,2β	C-11, C-17
2β	1.28 *dd* (5,12)*		2α	
3α	1.60 *dd* (4,12)	29.6		
3β	2.20 *m*		3α	
4	—	37.1		
5	1.85 *m*	41.3	6α	C-7, C-17, C-19
6α	1.68 *dd* (4,14)	24.9	5,6α,7β	
6β	1.95 *m*		6α,7β	C-7, C-11, C-17
7β	2.07 *m*	45.5	6α,6β	C-5, C-6
8	—	84.5		
9	2.62 *t* (6)	36.6	10,14	C-7, C-8, C-11
10	2.15 *dd* (3,13)	44.6	9,12	C-13, C-17
11	—	49.0		
12α	1.58 *dd* (4,11)	26.5	10,12β	
12β	1.94 *m*		12α	
13	1.97 *m*	43.2	12,14	
14	4.84 *t* (4.5)	77.1	9,13	C-8, C-16
15α	1.92 *m*	42.5	15β,16	C-8, C-9
15β	2.25 *dd* (5,10)		15α,16	
16	3.25 *dd* (4,11)	82.0	15α,15β	C-8, C-14
17	2.74 *brs*	63.6		C-1, C-5, C-6, C-11
18α	3.00 *d* (9)	78.9	18β	
18β	3.15 *d* (9)		18α	
19α	2.32 *m*	56.5	19β	
19β	1.98 *m*		19α	
N—CH_2	2.48 *m*	48.4		
CH_3	1.10 *t* (7)	12.9		
OMe-16	3.27 *s*	56.5		
OMe-18	3.30 *s*	59.5		
CO	—	170.1		
CH_3	2.05 *s*	21.3		
CO	—	169.7		
CH_3	2.03 *s*	22.1		

* *J* values given in Hz.

Table 2. NMR data of compounds **1a**, **2**, **1b** and **3**

Position	**1a**		**2**		**1b**		**3**
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	3.72 *brs*	72.3	3.70 *m*	72.7	4.88 *q* (5)[a]	77.0	4.88 *q*
2α	2.52 *m*	28.4		28.8	2.65 *m*	29.0	
2β	1.20 *m*						
3α	1.60 *m*	30.7		30.6		29.5	
3β	2.22 *m*						
4	—	37.6		37.8		37.4	
5	1.85 *m*	41.9		42.2	1.90 *m*	41.6	
6α	1.65 *dd* (3,13)	24.8		25.6		25.2	
6β	1.95 *m*						
7β	2.10 *m*	45.2		45.5		45.5	
8	—	74.2		74.2		77.8	
9	2.58 *t* (7)	46.9		47.4		45.8	
10	2.22 *m*	44.9		44.8		44.0	
11	—	49.1		49.1		49.0	
12α	1.65 *m*	27.7		27.8		27.4	
12β	2.00 *m*						
13	2.05 *m*	39.9		40.7		38.8	
14	4.20 *t* (4.6)	76.3	4.19 *t* (4.5)	76.3	4.78 *t* (4.5)	76.4	4.76 *t*
15α	1.90 *m*	43.3		43.1		43.0	
15β	2.50 *m*						
16	3.22 *dd* (4,12)	82.6		82.6		82.3	
17	2.80 *brs*	64.1		64.1		64.0	
18α	3.00 *d* (8.5)	79.0		79.3	3.15 *d* (9)	79.1	3.03 *m*
18β	3.17 *d* (8.5)						
19α	2.25 *m*	56.5		56.9	3.20 *d* (9)	56.8	
19β	2.08 *m*				2.50 *m*		
N—CH_2	2.50 *m*	48.5		48.6		48.5	
CH_3	1.11 *t* (7)	13.1	1.11 *t* (7)	13.2	1.07 *t* (7)	13.0	1.08 *t*
OMe-16	3.32 *s*	56.0	3.32 *s*	55.9	3.26 *s*	55.8	3.28 *s*
OMe-18	3.30 *s*	59.5	3.29 *s*	59.1	3.24 *s*	59.3	3.25 *s*
C=O						169.8	
CH_3					2.00 *s*	21.2	2.00 *s*
C=O						170.3	
CH_3					2.04 *s*	22.1	2.03 *s*
C=O						170.8	
CH_3					1.98 *s*	22.4	1.93 *s*

NMR in 200 MHz and ^{13}C NMR in 50 MHz using $CDCl_3$. Chromatographic separation was carried out on a Chromatotron using a rotor coated with 1 mm thick layer of neutral Al_2O_3.

Plant material

Aerial parts of *D. pyrimadale* Royle were collected from northern Pakistan, in the Swat district at an elevation of 1500 m, in June 1996. The species was identified by Prof. Dr Habib Ahmad (Government Degree College, Matta). A voucher specimen is deposited in the Herbarium of Department of Botany, Government Degree College Matta, Swat, Pakistan.

Extraction of crude alkaloids

Dried and powdered aerial parts (270 g) were exhaustively extracted by percolation at room temp. using hexane. The extracts were evapd *in vacuo* to yield a gummy residue (12 g), which contained no alkaloids. The defatted plant material was extracted with MeOH in a Soxhlet, the extract evapd to a small vol., acidified with 5% HCl to pH 3 and extracted with CH_2Cl_2 (10 × 250 ml). The remaining aq. soln was basified (pH 8–10) with cold aq. 10% NaOH and extracted with CH_2Cl_2 (15 × 250 ml). The extract was evapd to dryness *in vacuo* to yield 940 mg of crude alkaloid mixt. This was directly separated on a neutral Al_2O_3 rotor of a Chromatotron, eluting with petrol (40–60°) followed by gradients of CH_2Cl_2 and EtOH. The compounds obtained were **1** (19 mg), isotalatizidine (18 mg), condelphine (16 mg) and senbusine C (13 mg).

8-*Acetylcondelphine* (**1**)

$[\alpha]_D$ −28.5° ($CHCl_3$, *c*, 1.5). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3446, 2934, 2825, 1732, 1725, 1654, 1490, 1460, 1245, 1150,

1108, 1017, 950, 754. ^1H and ^{13}C NMR: Table 1. HRMS *m/z* (rel. int.): 491.2878 $[M]^+$ (47), 474 $[M-OH]^+$ (100), 432 $[M-OAc]^+$ (50), 414 $[474-HOAc]^+$ (20), 389 $[432-COMe]^+$ (15), 358 (10), 256 (5), 190 (5), 112 (7), 71 (20).

Hydrolysis of **1**

To a MeOH soln of **1** (10 mg), 5 ml KOH soln (2%) in EtOH was added. The mixt. was stirred at room temp. for 10 h, then extracted with CH_2Cl_2 (5 × 10 ml). The combined extracts were dried and evapd *in vacuo* to yield **1a** (7 mg). Its R_f, as well as ^1H and ^{13}C NMR spectra were identical to those of isotalatizidine (Table 2). The MS spectrum of **1a** was also similar to that of isotalatizidine (**2**). *m/z* (rel. int.): 407 $[M]^+$ $C_{23}H_{37}NO_5$ (84), 392 $[M-Me]^+$ (100), 374 $[392-H_2O]^+$ (90), 358 (50), 320 (55), 237 (10), 192 (10), 91 (23), 71 (22), 58 (47).

Acetylation of **1a**

Hydrolysis product (**1a**) (7 mg) dissolved in 1 ml of pyridine and 1 ml Ac_2O added to the soln. The mixt. was left at room temp. for 36 h, evapd to dryness and the residue subjected to prep. TLC using toluene–$CHCl_3$ (2:1). ^1H and ^{13}C NMR of the resulting compound **1b** (5 mg) indicated that it was identical to 1,8-diacetylcondelphine (**3**) [2] (Table 2). The MS of **1b** was in agreement with the given structure. *m/z* (rel. int.): 533 $[M]^+$ $C_{29}H_{43}NO_8$ (3), 490 $[M-COMe]^+$ (32), 431 $[490-OCOMe]^+$ (100), 416 $[431-Me]^+$ (65, 391 (45), 360 (25), 249 (7), 112 (13), 85 (15), 63 (17).

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