



DITERPENOID ALKALOIDS FROM *DELPHINIUM UNCINATUM*

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Abstract—From the aerial parts of *Delphinium uncinatum* a new diterpenoid uncinatine and four known norditerpenoid alkaloids have been isolated. By spectral data (^1H and ^{13}C NMR) the known compounds were established as 14-acetylperegrine, 14-acetylvirescenine, condelphine and delbrusine. The structure of the new alkaloid was deduced by 1D and 2D NMR techniques. © 1998 Elsevier Science Ltd. All rights reserved

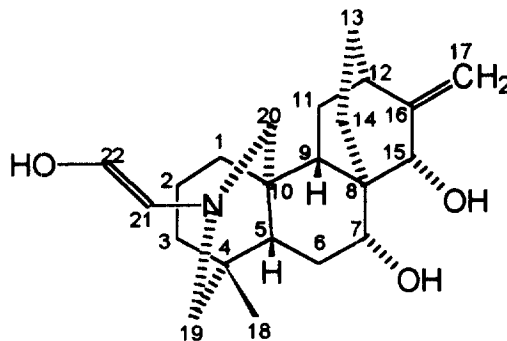
INTRODUCTION

In continuation of our studies on the diterpenoid alkaloids from the Ranunculaceae, we have investigated *Delphinium* [1–10], *Consolida* [11] and *Aconitum* [12–14] species, all native to Turkey. We also initiated a collaborative study on the constituents of *Delphinium* species indigenous to Pakistan. We report here the isolation and structural determination of the C_{19} and C_{20} diterpenoid alkaloids of *D. uncinatum*. The crushed plant parts of this species are used in the treatment of acne and wounds as folk medicine in Pakistan (information obtained by M.A. from local people).

RESULTS AND DISCUSSION

Aerial parts of *D. uncinatum* were extracted to yield a crude alkaloidal mixture, which was subjected to vacuum liquid chromatography (VLC) to afford three major fractions A–C. Fraction A consisted of one main compound which was purified by preparative TLC to yield the new C_{20} diterpenoid alkaloid, designated as uncinatine (**1**). Fractions B and C were separated on neutral Al_2O_3 rotors of a Chromatotron. Fraction B yielded 14-acetylperegrine [15] and 14-acetylvirescenine [16], and fraction C gave condelphine [16] and delbrusine [17].

Uncinatine (**1**), an amorphous compound, was homogeneous on TLC. Its structure was derived on the basis of detailed spectroscopic data. The molecular formula $\text{C}_{22}\text{H}_{33}\text{NO}_3$ was derived from its HR mass spectrum (m/z 359.2445, calcd 359.2460) and from



^{13}C NMR spectra. The IR spectrum showed hydroxyl (3300 cm^{-1}) and unsaturation ($1600, 1570\text{ cm}^{-1}$). UV absorption at 224 nm confirmed the unsaturation. Biogenetic considerations and the lack of methoxyl group(s) and the presence of an exocyclic methylene group signals in the ^1H NMR spectrum indicated that **1** must belong to the atisan-type diterpenoid alkaloid [18]. The ^{13}C NMR spectra (APT, DEPT, 90° and 135°) revealed the presence of a methyl quartet, 10 methylene triplets, seven methine doublets and four carbon singlet signals for 22 carbon atoms (Table 1). The ^1H NMR spectrum exhibited characteristic signals for an exocyclic methylene group at δ 5.12 and 5.02 (each 1H, *br s*) and, in the ^{13}C NMR spectrum, this group was observed at δ 155.8 s (C-16) and 110.5 t (C-17). Two high-field methine signals at (δ_{H} 7.08, *d*, $J = 8.5\text{ Hz}$; δ_{C} 132.4 *d*) and (δ_{H} 6.75, *d*, $J = 8.5\text{ Hz}$; δ_{C} 116.7 *d*) indicated unsaturation in the molecule. Two other low-field methine signals were at (δ_{H} 4.2, *d*, $J = 3\text{ Hz}$; δ_{C} 70.8 *d*) and (δ_{H} 3.9, *br d*, $J = 5\text{ Hz}$; δ_{C} 68.8 *d*) showing the presence of two oxygenated carbon

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Table 1. NMR data of uncinatine (**1**)

Position	¹³ C	¹ H	COSY ¹ H- ¹ H	COLOC ¹³ C- ¹ H	NOESY
1	35.4 <i>t</i>	α , 1.7 <i>m</i> β , 2.8 (<i>br d</i> , 14)	1 α , 2 α , 2 β 1 α , 2 α	C-5, C-9, C-20	
2	19.5 <i>t</i>	α , 1.8 (<i>ddd</i> , 3, 10, 12) β , 1.2 (<i>dd</i> , 3, 12)	1 α , 2 β 1 α , 1 β , 2 α		
3	41.6 <i>t</i>	α , 1.87 (<i>dd</i> , 5, 14) β , 2.1 (<i>ddd</i> , 5, 12, 14)	2 α , 2 β , 3 β 2 β , 3 α	C-19	
4	34.1 <i>s</i>	—	—		
5	44.2 <i>d</i>	β , 1.72 (<i>dd</i> , 2, 14)	6 α , 6 β	C-7, C-19, C-20	6 β , 9 β
6	20.0 <i>t</i>	α , 1.24 (<i>dd</i> , 2, 12) β , 1.6 (<i>m</i>)	5 α , 6 β , 7 β 6 α , 7 β		5 β , 7 β , 18 β 5 β , 7 β , 15 β
7	68.8 <i>d</i>	β , 3.9 (<i>d</i> , 5)	6 α , 6 β	C-5, C-15	
8	46.9 <i>s</i>	—	—		
9	40.4 <i>d</i>	β , 2.2 (<i>d</i> , 3)	11 α , 11 β	C-1, C-7, C-12, C-20	5 β , 6 β , 15 β
10	43.5 <i>s</i>	—	—		
11	28.9 <i>t</i>	α , 2.0 (<i>dd</i> , 3, 12) β , 1.5 (<i>m</i>)	9 α , 11 β 11 α		
12	36.7 <i>d</i>	α , 2.4 (<i>d</i> , 3)	11 α , 11 β		11 β , 13 α , 13 β
13	28.5 <i>t</i>	α , 1.8 (<i>m</i>) β , 2.05 (<i>m</i>)	13 β , 14 α , 14 β		
14	24.8 <i>t</i>	1.9 (<i>m</i>)	13 α , 13 β		
15	70.8 <i>d</i>	β , 4.2 (<i>d</i> , 3)	17a, 17b	C-7, C-9, C-16, C-17	7 β , 9 β
16	155.8 <i>s</i>	—	—		
17	110.5 <i>t</i>	a, 5.02 (<i>br s</i>) b, 5.12 (<i>br s</i>)	17b, 15 β 17a, 15 β	C-12, C-15, C-16	
18	25.8 <i>q</i>	1.07 (<i>s</i>)	—	C-3, C-5, C-10, C-19	
19	60.3 <i>t</i>	α , 3.60 (<i>d</i> , 10) β , 3.45 (<i>d</i> , 10)	19 β 19 α		
20	64.9 <i>t</i>	4.15 (<i>d</i> , 12)		C-19	
21	132.4 <i>d</i>	7.08 (<i>d</i> , 8.5)			
22	116.7 <i>d</i>	6.75 (<i>d</i> , 8.5)			

atoms. HETCOR and the COLOC experiments indicated that the unsaturated carbons were at N (CH=CH—OH) and the presence of this group was indicated by the mass spectral fragment at m/z 316 [M-43]⁺. One of the oxygenated carbons of **1** should be placed at C-15 due to the chemical shifts of C-16 (δ 155.8) in the ¹³C NMR and the H₂-17 protons at δ 5.12 and 5.02 in the ¹H NMR spectra. The COLOC experiment showed correlation between C-17 and H-15 (δ 4.2, *d*, J = 5 Hz) (Table 1). When there is no hydroxyl group at C-1, C-2 and C-3 in ring A, the signal for C-2 appears at *ca* δ 19–20 [19]. Since the most up-field ¹³C NMR signal was at δ 19.50 (*t*), it does not bear an OH in ring A. The remaining methylenes on which a second hydroxyl group may be located are C-6, C-7, C-11, C-13, C-14, C-19 and C-20. Two low-field methylene triplets occur at δ 60.3 (*t*) and 64.9 (*t*). The former signal can be assigned to C-19 (δ 3.60 and 3.45 AB, J = 10 Hz) based on the observed NOESY correlation between one of the protons on C-19 (δ 3.45) and C-20 (δ 4.15, *d*, J = 12) and the COLOC correlation between C-19 with H-20 and H₃-18 (Table 1). The second signal can be assigned to

C-20 (δ 4.15, *d*, J = 12 Hz), as observed in COLOC correlations between C-20 and H-1 (δ 2.8, *br d*, J = 14 Hz) and H-5 (δ 1.72, *dd*, J = 2, 14 Hz), as well as H-9 (δ 2.20, *d*, J = 3 Hz). These results rule out the possibility of C-19 and C-20 as the position of the second hydroxyl group. The remaining methylene groups are C-6, C-7, C-13 and C-14. Among them C-13 and C-14 were observed at δ 28.5 (*t*) and 24.8 (*t*), quite similar to those observed in ajaconine [20]. From the two remaining positions the hydroxyl group was decided to be at C-7. COLOC correlations, showed three-bond correlation of H-5, H-9 and H-15 with C-7. The proton-bearing carbons were unambiguously assigned by a HETCOR experiment (Table 1). The COSY spectrum and extended spin-decoupling experiments showed the sequences from H₂-6 to H-7 β (δ 3.9), as well as between H-5 and H₂-6. COSY experiments showed the correlations, C-1–C-3, C-5–C-7, and C-12–C-14. The stereochemistry of both hydroxyl groups at C-7 and C-15 were decided as α by NOESY correlations between one of the C-19 protons (δ 3.45) and H-20 (δ 4.15) and between H-19 and H-6 α (δ 1.24) and between H-6 α and β and H-7 β

(Table 1). Conclusive evidence for the location of both hydroxyl groups was provided 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.

Consolida ambigua provided a similar compound dihydroajaconine (**2**), isolated by Pelletier *et al.* [21], the difference being the presence of a double bond between C-21 and C-22. In dihydroajaconine, both hydroxyl groups at C-15 and C-7 are α . Hydrogenation of **1** was performed using PtO_2/C (10%) as a catalyst. A tetrahydrogenated product was obtained, whose spectral data (see Experimental) indicated similar signals for an $\text{N-CH}_2\text{CH}_2\text{OH}$ group. The spectral data indicated that uncinatine (**1**) is a didehydro derivative of **2**.

EXPERIMENTAL

General. OR in CHCl_3 . IR were recorded in CHCl_3 . HRMS were measured at 70 eV. ^1H (200 MHz) and ^{13}C (50 MHz) NMR were recorded in CDCl_3 . Chromatographic sepns were carried by VLC and later on Chromatotron using rotors coated with 1 mm thick layers of neutral Al_2O_3 .

Plant material. Aerial parts of *D. uncinatum* Hook f. and Thomas were collected in northern Pakistan, Bamboley district Dir, N.W.F.P. at an altitude 1030 m in May 1996 and identified by Prof. J. Shah (University Peshawar). A voucher specimen is deposited in the Herbarium of the Faculty of Sciences, Department of Botany, Islamia College, University of Peshawar, Pakistan.

Extraction of crude alkaloids. Dried and powdered aerial parts (568 g) were exhaustively extracted by percolation at room temp., followed by Soxhlet extraction using hexane. Evapn *in vacuo* of the combined extracts gave a gummy residue (23 g) which contained no alkaloid. The plant material was basified (pH 8–10) and extracted with CH_2Cl_2 (10 $\times$ 250 ml) to yield 1.07 g of a crude alkaloid mixt. This was sepd in a VLC column using petrol, EtOAc and EtOH. The 253 frs collected were sepd into three groups according to their alkaloid contents, frs 1–143 (A), frs 144–151 (B) and frs 152–253 (C). Fr. A was purified by prep. TLC using petrol–EtOAc– Et_2NH (5:1:1). Frs B and C were sepd on a Chromatotron eluting with petrol followed by EtOAc and finally with MeOH. The yields of compounds were **1** (35 mg), 14-acetylperegrine (12 mg), 14-acetylvirescenine (20 mg), delbrusine (15 mg) and condelphine (13 mg).

Hydrogenation of (1). To an EtOH soln of **1** (10 mg), 25 mg PtO_2/C (10%) was added and H_2 passed into the soln for 18 hr. The resulting soln was filtered, evapd to dryness and purified by prep. TLC; 7 mg of an amorphous compound (**1a**) was obtained. In the ^1H NMR of **1a**, the signals of the unsaturated group (δ 7.08 and 6.75), as well as the exocyclic methylene group signals (δ 5.12 and 5.02) disappeared. A methyl doublet signal was present at δ 1.12, $J = 7$ Hz. The

rest of the signals were similar to those of **1**. The signals for C-16, C-17, C-21 and C-22 were changed to δ 49.5, 11.2, 58.2 and 60.6, respectively, in the ^{13}C NMR spectrum, δ C-1 36.5, C-2 20.2, C-3 41.4, C-4 34.1, C-5 45.0, C-6 20.1, C-7 69.4, C-8 46.0, C-9 40.0, C-10 42.9, C-11 28.9, C-12 36.6, C-13 28.4, C-14 25.0, C-15 70.9, C-16 49.5, C-17 11.2, C-18 25.6, C-19 60.3, C-20 64.2, C-21 58.2, C-22 60.6.

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