DELBRUNINOL, A NEW NORDITERPENOID ALKALOID FROM *DELPHINIUM BRUNONIANUM* ROYLE

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Abstract – A new norditerpenoid alkaloid, delbruninol (**1**), has been isolated from the aerial parts of *Delphinium brunonianum* Royle Its structure has been established on the basis of its spectroscopic data (IR, HRFABMS, ^{1}H, ^{13}C,^{1}H-^{1}H and ^{1}H-^{13}C COSY, ROESY, TOCSY, HMQC-TOCSY and HMBC NMR spectra). The known alkaloids delcosine, browniine, blacknidine, delbrunine, 18-methoxygadesine and nudicaulamine were also isolated and identified. The roots of *D. brunonianum* Royle furnished the known alkaloids methyllycaconitine and lycoctonine.

In previous reported studies on *Delphinium brunonianum* Royle (whole plant), collected in the southwestern parts of People's Republic of China, a new diterpenoid alkaloid brunonine[1] and three new norditerpenoid alkaloids, delbruline (**2**) delbrunine (**3**), and delbrusine (**4**) were isolated along with four known norditerpenoid alkaloids delcosine, browniine, lycoctonine and septentriodine.[1,2] This plant is used in Tibet as folk medicine for influenza, itchy rash, and snake-bite. In the present study this plant was collected from Northern parts of Pakistan (Peshawar). The aerial parts (810 g, dry weight) and roots (300 g) were examined separately. From the aerial parts we report the isolation and characterization of a new norditerpenoid alkaloid, delbruninol (**1**, 3 mg), along with the previously reported delbrunine (**3**, 6 mg), browniine (25 mg) and delcosine (150 mg). The known norditerpenoid alkaloids blacknidine (5 mg),[5] 18-methoxygadesine (3.1 mg), and neudicaulamine (3.2 mg), isolated from the aerial

parts, are new for this plant. Besides these, the previously unreported known alkaloid methyllycaconitine (99.8 mg) and lycoctonine (149.5 mg) were isolated as the major alkaloids from the roots of this plant. All the known compounds isolated from this plant were identified through the study of their ^{1}H, ^{13}C, DEPT NMR and ESIMS spectra as compared with those reported for the authentic samples.[3-5]

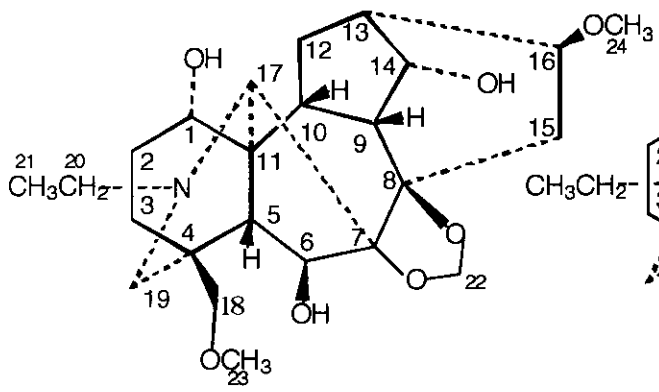
The molecular formula of the new alkaloid, $C_{24}H_{37}NO_7$, was established by HRFABMS which showed a $[M+H]^+$ at *m/z* 452.2648; calculated for $C_{24}H_{38}NO_7$, $[M+H]^+$, *m/z* 452.2648. ESIMS also gave a molecular ion peak at *m/z* 452.2 $[M+H]^+$; $[\alpha]^{25}_D$ -3.6° (c=0.01, $CHCl_3$); IR ν_{max} 3565, 3451, 3298, and 851 cm^{-1}. A completely decoupled ^{13}C NMR spectrum showed 24 signals for the 24 carbon atoms of the molecule. The DEPT spectra showed four signals for quaternary carbons at δ 36.8, 50.7, 84.1, 92.4; nine signals for methines at δ 38.6, 42.1, 45.5, 47.9, 65.7, 71.9, 74.6, 78.6, 81.3; eight signals for methylenes at δ 27.3, 28.6, 29.2, 34.2, 50.2, 57.9, 78.5, 93.3; and three signals for methyls at δ 13.6, 56.4, 59.5. The ^{1}H NMR spectrum showed the presence of two methoxyl groups at δ 3.36 and 3.40 (each 3H, s), an *N*-Et group at δ 1.12 (3H, t, *J* =7.1Hz, *Me of N* Et), a 1H triplet at δ 4.19 (*J* = 4.5 Hz) for the C(14) β H, two singlets (1H, each) at δ 5.19 and 5.29 along with a methylene carbon at δ 93.3 for the methylenedioxy group. On the basis of the following 2D NMR results stucture (**1**) was assigned for delbruninol. The stereochemistry of the -OH group on C(6) was assigned a β configuration by comparing the ^{13}C chemical shifts reported for the alkaloids delcorine[3] (δ 78.9), delpheline[6] (δ 79.3), delelatine[7] (δ 78.8), 6-acetyldelpheline[4] (δ 78.4) and dictyocarpinine[3] (δ 77.3). All these alkaloids have a methylenedioxy group attached to C(7) and C(8).

NMR SPECTRAL ASSIGNMENTS

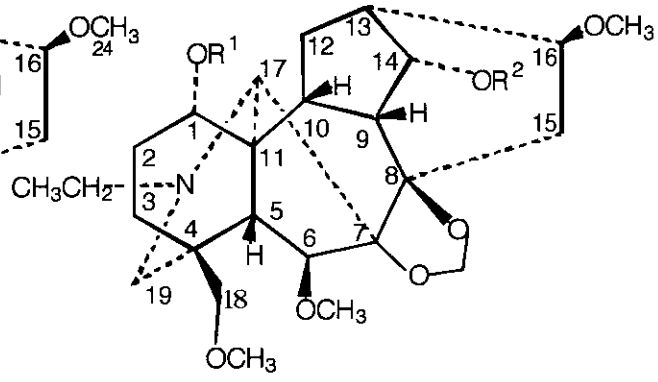
All NMR data were acquired on a Varian Inova-500 spectrometer (499.8 MHz). The assignments were obtained by studying 2D ^{1}H phase sensitive COSY,[8] clean TOCSY,[9] ROESY,[10] HMQC,[11] HMQC-clean TOCSY,[12] and HMBC[13] plots. Assignments of ^{1}H and ^{13}C chemical shifts, shown in Table 1, were made using a two-stage method.[14] Segments were established by spin systems identified on the basis of scalar coupling in TOCSY and HMQC-TOCSY spectra. Sequence-specific assignments of ^{1}H and ^{13}C within a segment were obtained by ^{1}H-^{1}H primary connectivities in COSY spectrum and ^{1}H-^{13}C correlations in HMQC spectrum. The overall structure was obtained by inter-segment ^{1}H-^{1}H NOE connectivities in ROESY spectrum and long-range ^{1}H-^{13}C coupling cross peaks between segments in HMBC spectrum, summarized in Table 2.

Twenty-four carbons were observed using 1D ^{1}H-decoupled ^{13}C, DEPT and 2D HMQC spectra, which were determined by their characteristic ^{1}H and ^{13}C chemical shift patterns as one methyl carbon, two methoxyl, eight methylene, nine methine and four quaternary carbons.

Inspection of HMQC-TOCSY spectrum, shown in Figure 1, reveals five segments. The primary ^{1}H-^{1}H and ^{1}H-^{13}C correlations within the above four spin systems were obtained using the COSY and HMQC spectra, respectively. The assignments for ^{1}H signals were initiated at the resolved ^{1}H resonances such as CH protons at δ 4.38, 4.19, 4.06, 3.82 and 3.65.



1 DELBRUNINOL



2 R^1=OCH_3, R^2=H **DELBRULINE**
3 R^1=R^2=H **DELBRUNINE**
4 R^1=R^2=OCH_3 **DELBRUSINE**

Starting at the methine resonance frequency at δ 4.19 (segment 2 shown in Figure 1), TOCSY and HMQC-TOCSY spectra showed that this methine proton shows cross peaks to five carbons in HMQC-TOCSY and five additional protons in TOCSY. The COSY coupling pattern indicates that the segment has a ring structure with sequence, at δ 4.19, 2.51, (2.18, 2.19), 2.25, and 3.65 assigned as H14, H13, H12b, H12a, H10 and H9, respectively, while HMQC yields ^{13}C chemical shifts for the sequence. The hydroxyl group on C(14) contributes the higher frequency shift of ^{1}H and ^{13}C chemical shifts. Using a similar analysis for assignment of segment 2, segments 1, 3, 4 and 5 in Figure 1 were assigned as C(5)-C(6), C(1)-C(2)-C(3), C(15)-C(16) and C(20)-C(21), respectively. ROESY connectivities from resonance at δ 4.38 (^{13}C, δ 65.7) to H12, H15 and H16 confirmed the assignment of H17. H19 methylene protons were assigned based on ROESY cross peaks to H17 and H6. C(16) was assigned a methoxyl group instead of hydroxyl group (δ 3.40) by the fact that the methoxyl group has NOE to H13 and H16. Similarly, the other methoxyl group (δ 3.36) is attached on C(18) because of the NOE from δ 3.36 to H18 and $H19_b$. Absence of coupling between H13 and H16 is caused by the perpendicular orientation between these two protons. The connection between the two segments is supported by ROESY and HMBC cross peaks among the inter-segment protons.

Quaternary carbons were assigned by long-range ^{1}H-^{13}C coupling cross peaks from nearby protons in the HMBC spectrum. C(4) was assigned by ^{1}H-^{13}C coupling to H18 and H19, while C(11) has a coupling to H17. C(7) and C(8) carbons were located by the HMBC coupling to

several protons. However, the ^{13}C chemical shifts could not be distinguished by those HMBC couplings owing to the fact that C(7) and C(8) are adjacent to each other. Thus, the hydroxyl group on C(6) was used to make the assignment because it shifts the C(7) ^{13}C chemical shift value to higher frequency.

Stereochemistry of the compound was determined using the ROESY cross peaks from the protons of asymmetric carbons to other protons nearby in distance. The ROESY cross peak between H17 proton and H16 proton indicates that the methoxyl group at C(16) is in the β-configuration. The β-Configuration of H1 is supported by the ROESY cross peaks of H1 to H10 and to H5.

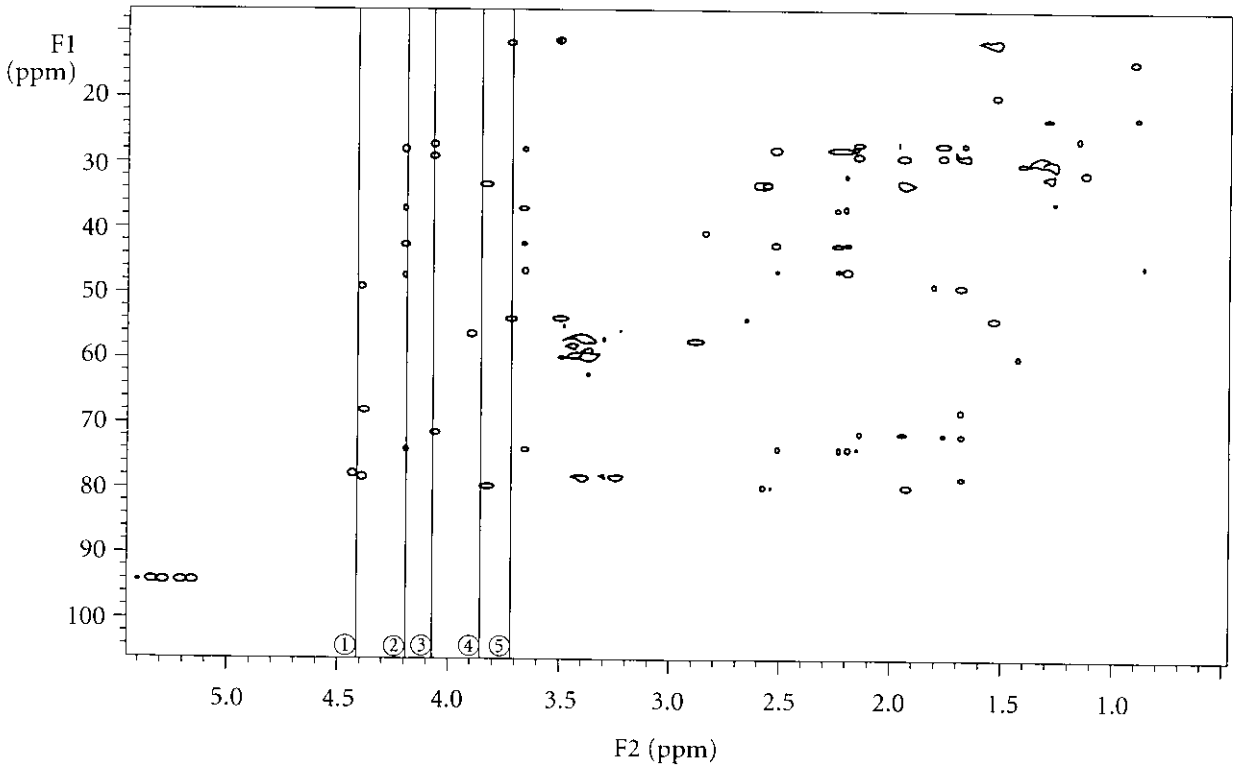


Figure 1. HMQC-clean-TOCSY spectrum of delbruninol (**1**).
The identified spin systems are labeled by vertical lines.

Table 1. ^{13}C and ^{1}H NMR Chemical Shifts Assignments of Delbruninol(**1**), in $CDCl_3$.

C #	$\delta^{13}C$	H #	$\delta^{1}H$	J=Hz
1	71.9 (d)	1_β	4.06(t)	J = 3.1
2	27.3 (t)	2_α	2.14(m)	–
		2_β	1.75(m)	–
3	29.2 (t)	3_β	1.61(m)	–
		3_α	1.94(m)	
4	36.8 (s)	4	–	
5	47.9 (d)	5	1.68(d)	$J_{5,6\alpha}$ = 8.1
6	78.6 (d)	6_α	4.38(br s)	$1/2_W$ = 3.1
7	92.4 (s)	7	–	
8	84.1 (s)	8	–	
9	42.1 (d)	9	3.65(d,d)	$J_{9,10}$=10.4, $J_{9,14}$=4.7
10	45.5 (d)	10	2.25(m)	
11	50.7 (s)	11	–	
12	28.6 (t)	12_a	2.19(m)	Overlap
		12_b	2.18(m)	Overlap
13	38.6 (d)	13	2.51(m)	–
14	74.6 (d)	14	4.19 (br t)	J=4.5
15	34.2 (t)	15_a	2.57(m)	–
		15_b	1.90(m)	–
16	81.3 (d)	16	3.82(br d)	$J_{16,15}$ = 9.0
17	65.7(d)	17	4.38(br s)	$1/2_w$ = 3.1
18	78.5 (t)	18_a	3.45(AB)	J_{gem} = 10.4
		18_b	3.28(AB)	J_{gem} = 10.4
19	57.9 (t)	19_a	3.35(AB)	J_{gem} = 11.7
		19_b	2.88(AB)	J_{gem} = 11.7
20	50.2 (t)	20_a	3.70(octet)	–
		20_b	3.49(octet)	–
21	13.6 (q)	21	1.11(t)	J = 7.1
22	93.3 (t)	22_a	5.29(s)	–
		22_b	5.19(s)	–
23	59.5 (q)	23	3.36(s)	–
24	56.4 (q)	24	3.40(s)	–

Table 2. Summary of HMBC, ROESY and COSY Correlation Data of Delbruninol (**1**)

Obs. ^{1}H	HMBC	ROESY	COSY
H-1$_\beta$	C-2	H-2$_\alpha$, H-2$_\beta$, H-5, H-10, H-12$_b$, H-12$_a$, H-3$_\beta$(w)	H-2$_\alpha$, H-2$_\beta$
H-2$_\alpha$	–	–	H-1$_\beta$, H-3$_\alpha$, H-3$_\beta$
H-2$_\beta$	–	–5	H-2$_\alpha$, β
H-3$_\alpha$	–	–	H-2$_\alpha$, H-2$_\beta$, H-3$_\beta$
H-3$_\beta$	C-2	H-1$_\beta$	H-2$_\alpha$, H-2$_\beta$, H-3$_\alpha$
H-5	–	–	H-6$_\alpha$
H-6$_\alpha$	–	H-18$_b$, H-19$_a$, H-22$_a$, H-22$_b$	H-5
H-9	C-8	H-14$_\beta$	H-10, H-14$_\beta$
H-10	–	–	H-14$_\alpha$
H-12$_a$	–	H-1	H-10, H-13
H-12$_b$	–	H-1, H-14$_a$, H-16, H-17,	H-10, H-13
H-13	–	H-14, H-16, H-24	H-12$_a$, H-12$_b$, H-14
H-14	C-13	H-9, H-10, H-12$_b$, H-13, H-16	H-9, H-13
H-15$_a$	C-8, C-16	H-16, H-22$_b$	–
H-15$_b$	C-8, C-16	H-22$_b$	–
H-16	C-13, C-24	H-12$_b$, H-13, H-14, H-15$_a$ H-17, H-24	–
H-17	C-6, C-8, C-11	H-12$_b$, H-15$_a$, H-16, H-20	H-5
H-18$_a$	C-19	–	H-18$_b$
H-18$_b$	C-4, C-23	H-6$_\alpha$	H-18$_a$
H-19$_a$	–	H-6$_\alpha$,	H-19$_b$
H-19$_b$	C-4	H-17,	H-19$_a$
H-20$_a$	–	–	H-20$_b$, H-21
H-20$_b$	–	H-17	H-20$_a$, H-21
H-21	–	–	H-20$_a$, H-20$_b$
H-22$_a$	C-7, C-8	H-6$_\alpha$	–
H-22$_b$	C-7	H-15$_a$, H-15$_b$	–
H-23	C-18	H-18, H-19$_b$	–
H-24	C-16	H-13, H-16, H-10	–

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