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ROYLEININE, A NEW NORDITERPENOID ALKALOID FROM *DELPHINIUM ROYLEI*

Ayhan Ulubelen[*,a], Ali H. Meriçli[a], Filiz Meriçli[a], Ufuk Kolak[a], Muhammed Arfan[b], Manzoor Ahmad[b], and Habib Ahmad[c]

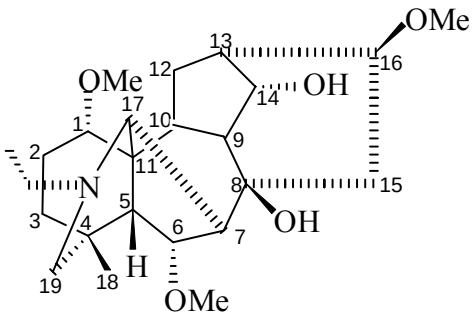
[a]Faculty of Pharmacy, University of Istanbul, 34452 Istanbul, Turkey,
[b]Department of Chemistry, University of Peshawer, Peshawer, Pakistan,
[c]Department of Botany, Postgraduate Jahan Zeb College, Saida Sharif, Swat, NWFP, Pakistan

Abstract – A new norditerpenoid alkaloid, royleinine (**1**) has been isolated from the aerial parts of *Delphinium roylei* Munz, along with three more norditerpenoid alkaloids, isotalitazidine, condelphine and senbusine-C. The structure of **1** has been established on the basis of its spectroscopic data (IR, HRMS, ^{1}H, ^{13}C, ^{1}H - ^{1}H and ^{1}H - ^{13}C COSY and NOESY spectra).

In continuation of our investigations[1-6] of *Delphinium* species from Turkey and Pakistan for their diterpene alkaloids, we have now studied *Delphinium roylei* Munz. We have isolated four norditerpenoid alkaloids, one new, royleinine (**1**), and three known, isotalitazidine (**2**),[7] condelphine (**3**) [8] and senbusine-C (**4**),[9] from the aerial parts of *Delphinium roylei,* a northern Pakistan plant.[4-6] A literature survey revealed that no previous study is present with *Delphinium roylei* and no biological activities were attributed to the plant, however it is used as an ornimental plant due to its colorful flowers. The crude alkaloid fraction extracted from the plant material at pH 8-10 was purified on a silica gel column. The combined fractions I-V were cleaned on preparative TLC plates. Fraction I has yielded isotalitazidine. Fraction II, including very small amounts of an alkaloidal mixture, was not worked. Fraction III yielded condelphine and senbusine-C. Fraction IV yielded royleinine (**1**), while fraction V did not yield any alkaloid.

Royleinine (**1**) was obtained as an amorphous, homogenous compound. The HRMS of **1** indicated the molecular formula $C_{24}H_{39}NO_5$ giving the molecular ion at *m/z* 421.2828. The NMR spectra of royleinine (**1**) showed the presence of a methyl group at C-18 [δ_C 26.8 q, δ_H 0.88 (3H, s)], N-ethyl group was at [δ_C 13.0 q, δ_H 1.07 (3H, t, J = 7 Hz); δ_C 49.2 t, δ_H 2.40 and 2.65 (2H, m)], as well as three methoxy groups [δ_C 56.5 q, δ_H 3.33 (3H, s); δ_C 56.0 q, δ_H 3.36 (3H, s); δ_C54.5 q, δ_H 3.38 (3H, s)]. These signals and biogenetic considerations indicated that royleinine (**1**) is a norditerpenoid alkaloid. The HRMS indicated six degrees of unsaturation accounted for the hexacyclic skeleton. The ^{13}C NMR (APT) spectrum indicated the

presence of five methyl, six methylene, ten methine and three quaternary carbons for 24 carbon atoms of royleinine (**1**). The alkaloid contains three methoxy and two hydroxy groups, as decided from the NMR



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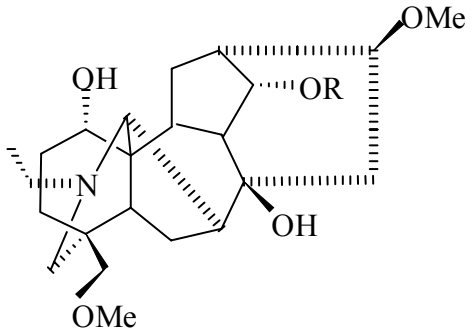
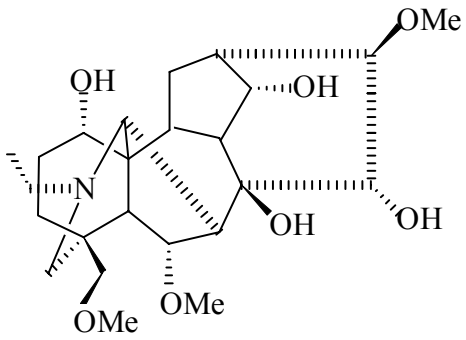


2 R = H

3 R = Ac



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spectra one hydroxy group placed at C-14 [δ_C 75.5 d, δ_H 4.20 (1H, t, J = 5.0 Hz)], the second at C-8 (δ_C 74.0 s). The first of the methoxy groups was placed at C-1 [δ_C 87.4 d, δ_H 3.80 (1H, m)], from the chemical shift and due to biogenetic considerations. The second methoxy group should be at C-16 [δ_C 81.9 d, δ_H 3.72 (1H, dd, J = 7 and 12 Hz)], since H-16 showed correlations with the protons at C-15 [δ_C 39.5 t, δ_H 2.70 (1H, dd, J = 7 and 14 Hz, H-15), 3.00 (1H, dd, J = 12 and 14 Hz, H-15')] in the COSY spectrum. The last methoxyl was placed at C-6 [δ_C 82.6 d, δ_H 4.05 (1H, dd, J = 1 and 7 Hz)].The structure of royleinine was established from its $^1H - ^1H$ COSY, $^1H - ^{13}C$ HETCOR and NOESY experiments. The stereochemistry of the methoxy group at C-6 was decided as α by studying a Dreiding model and from the NOESY experiment (Table 1). The proton at C-6 had NOE relation with H-5 and H-7 as well as with H-15' indicating that it is β stereochemistry therefore the methoxy group is α.

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

Position	^{1}H	^{13}C	COSY $^{1}H - ^{1}H$	NOESY
1β	3.80 m	87.4 d	2α, 2β	H-3β, Me-18
2α	1.80 m	26.9 t	2β, 3α	
2β	2.10 m		2α, 3α, 3β	
3α	1.60 m	31.5 t	2α, 2β, 3β	
3β	2.40 m		2β, 3α	H-1β
4	-	31.1 s		
5	2.00 m	40.1 d	6α	H-6β
6	4.05 dd (1;7 Hz)	82.6 d	7β	H-5β, H-7β, H-15’
7	2.50 d (1 Hz)	44.2 d	6α	H-6β
8	-	74.0 s		
9	1.80 m	45.8 d	10β	
10	1.75 m	36.9 d	9β	
11	-	43.1 t		
12α	1.45 m	29.4 t	12β, 13	
12β	2.50 m		12α, 13	
13	1.30 m	45.2 d	12α, 12β	
14	4.20 t (5 Hz)	75.5 d		H-9, H-10, H-12β
15	2.70 dd (7;14 Hz)	39.5 t	15’, 16	
15’	3.00 dd (12;14 Hz)		15 , 16	H-6β
16	3.72 dd (7;12Hz)	81.9 d	15 , 15’	
17	2.60 br s	62.2 d		
18	0.88 s	26.8 q		
19	1.80 m	48.2 t		
19’	3.15 m			
$N-CH_2$	2.40 m; 2.65 m	49.2 t		
CH_3	1.07 t (7 Hz)	13.0 q		
1 - OMe	3.38 s	54.5 q		
6 - OMe	3.36 s	56.0 q		
16 - OMe	3.33 s	56.5 q		

EXPERIMENTAL

General Experimental Procedures. – ^{1}H NMR spectra were recorded on 200 MHz and ^{13} C NMR spectra on 50.0 MHz spectrometer in $CDCl_3$. HRMS were measured at 70 eV. IR spectra were recorded in $CHCl_3$. Chromatographic separations were carried out on silica gel columns and on 1 mm thin layer of silica gel.

Plant material. – *Delphinium roylei* Munz was collected from Chitral Gol in Chitral, northern Pakistan in August 1998. A voucher specimen RD – 04 stored in the Herbarium at the Postgraduate Jahan Zeb College, Swat N.W.F.P. (Pakistan).

Extraction of Crude Alkaloids. – Dried and powdered aerial parts (250 g) of the plant were extracted exhaustively with n-hexane (3x250 mL), followed by MeOH (3x250 mL) extraction at rt for 24 h (3 times). The filtrate was evaporated *in vacuo* to yield 20 g of a residue. The residue was acidified to pH 2.0 by 5 % H_2SO_4 and extracted with CH_2Cl_2 to obtain non alkaloidal mixture (17 g). The acidic aqueous solution was basified (pH 8 –10) by using 10 % NaOH and extracted with CH_2Cl_2 (10x150 mL) to yield 180 mg of crude alkaloidal mixture, which was fractionated on silica gel column (2x50 cm) to obtain five combined fractions. Each fraction was separated on preparative TLC plates using toluene-ethyl acetate-diethylamine (6:2:0.5) and petroleum ether-toluene-ethyl acetate-diethylamine (4:3:1:0.5). The following compounds were obtained: isotalitazidine (**2**) (27 mg), condelphine (**3**) (15 mg), senbusine-C (**4**) (6.2 mg), royleinine (**1**) (12 mg).

Royleinine (**1**) – White, amorphous powder. $[\alpha]^{25}_{D} = +0^{o}$($CHCl_3$, 0.1). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3406, 2931, 1653, 1461, 1387, 1234, 1092, 881, 754. ^{1}H and ^{13}C NMR spectra ($CDCl_3$) are given in Table 1. HRMS *m/z* 421.2828 $[M]^+$ calcd. for $C_{24}H_{39}NO_5$ *m/z* 421.2828. MS *m/z* (rel.int.): 421 $[M]^+$ (6), 404 $[M- OH]^+$ (20), 390 $[M-OMe]^+$ (18), 375 $[M-OMe-Me]^+$ (100), 349 (62), 333 (28), 318 (35), 112 (17), 87 (7), 71 (11).

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