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Norditerpenoid alkaloids from *Delphinium kohatense* Munz

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Abstract: A new aconitine-type norditerpenoid alkaloid, kohatenine (**1**) [10*β*-hydroxy-1*α*,8*β*,16*β*-trimethoxy-6*α*,14*α*-diacetyl-*N*-ethyl aconitane], along with four known alkaloids, condelphine (**2**), talatisamine (**3**), peregrine (**4**), and 14-*O*-acetyltalatisamine (**5**), was isolated from the aerial parts of *Delphinium kohatense* Munz. The structure of the new compound was deduced on the basis of combined MS (EI and FAB) and NMR (1D and 2D) spectroscopic techniques. The known compounds were confirmed by comparison of the physical and spectroscopic data with those reported in literature.

Keywords: Norditerpenoid alkaloids; Kohatenine; *Delphinium kohatense*; Ranunculaceae.

1. Introduction

Delphinium (Larkspur), an important genus of the family Ranunculaceae, is well known for its potential uses in medicine [1]. The genus is recognized as a rich source of biologically active and structurally complex diterpenoid and norditerpenoid alkaloids with febrifuge, sedative, cardiotonic and analgesic activities [2-5]. *D. kohatense* Munz is a 15-30 cm high perennial herb found at an elevation of 1800-2300 m in the dry places of India and northern areas of Pakistan and Jammu & Kashmir [6]. To the best of our knowledge no phytochemical work has been reported on this plant. Previously, we have reported many diterpenoid and norditerpenoid alkaloids from *Aconitum* and *Delphinium* species [7-10]. In the present paper, we describe the isolation, characterization and structure elucidation of new aconitine-type norditerpenoid alkaloid, kohatenine (**1**), along with four known compounds; condelphine (**2**) [11], talatisamine (**3**) [11], peregrine (**4**) [12], and 14-*O*-acetyltalatisamine (**5**) [13] (Figure 1). The structure of compound **1** was elucidated on the basis of spectroscopic techniques.

2. Material and Methods

2.1. General

Optical rotations were measured on a JASCO DIP 360 polarimeter. IR Spectra were recorded on a Bruker, VECTOR 22 spectrophotometer. EI-MS and HREI-MS were recorded on mass spectrometers JEOL JMS HX 110. The ^{1}H and ^{13}C NMR Spectra were recorded on Bruker NMR spectrometers

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operating at 400 MHz (100 MHz for ^{13}C). The chemical shift values are reported in ppm (δ) and the coupling constants (*J*) are given in Hz. For TLC, precoated plates (silica gel 60F-254, E. Merck) were used. The TLC plates were viewed under UV at 254 and 366 nm and by spraying with Dragendorff's reagent. Solvent system, *n*-hexane: acetone: diethylamine (90:9.5:0.5), was used in chromatographic separations.

2.2. Plant Material

The plant, *Delphinium kohatense*, was collected from Swat, KPK, Pakistan, in May 2004, and was identified by Prof. Dr. Habib Ahmad, Chairman, Department of Botany, Hazara University Dhodial. A voucher specimen (KD-03) was deposited in the herbarium of the Department.

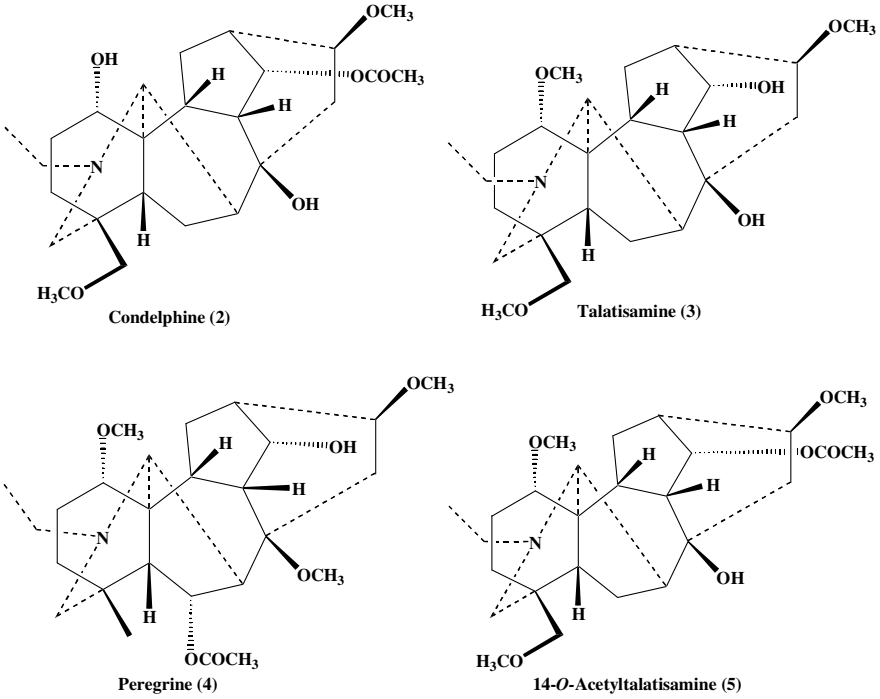

Figure 1. Structures of compounds **2-5**

2.3. Extraction and Isolation

Dried and powdered aerial parts (4 kg) of the plant were extracted exhaustively with 80% ethanol at room temperature. The filtrate was evaporated in vacuum to yield 200 g of the residue. The residue was partitioned in different solvents on the basis of increasing polarity to get *n*-hexane (8.5 g), chloroform (13.4 g), ethyl acetate (12.8 g), and *n*-butanol (48.6 g).

The crude chloroform fraction (13.4g) was subjected to column chromatography and eluted with *n*-hexane with gradient of chloroform upto 100% and methanol upto 20%. Five fractions were obtained. On repeated flash column chromatography using solvent system *n*-hexane-acetone-diethylamine (90:9.5:0.5), four diterpenoid alkaloids; condelphine (**2**) (4.2 mg), isotalatizidine (**3**) (3.9 mg), peregrine (**4**) (5.1 mg), and 14-*O*-acetyltalatisamine (**5**) (4.8 mg)] were obtained. Kohatenine (**1**) [10β-hydroxy-1α, 6β, 16β-trimethoxy-6β, 14α-diacetyl-*N*-ethyl aconitane] (4.5 mg) was obtained from sub-fraction (F-3) by repeated flash column chromatography using solvent system *n*-hexane-acetone-diethylamine (85:14.5:0.5).

2.4. 10β-hydroxy-1α,8β-trimethoxy-6α,14α-diacetyl-N-ethylaconitane (Kohatenine)

White amorphous powder (4.5 mg): $[\alpha]_D^{30}$ +14 (*c* 0.34, C_2H_5OH); IR ($CHCl_3$): ν_{max} 3500, 3406, 2927, 1732, 1246, 1092 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz)**:** see Table 1; EI-MS (70 eV): *m/z* (rel. int.) 521 $[M]^+$ (5), 490 $[M^+$-OMe] (11), 478 $[M^+$-Ac] (9), 475 $[M^+$-OMe-Me] (17), 462 $[M^+$-OAc] (22), 416[M^+-OMe-OAc-Me] (9), 404 (6), 368 (6), 85 (66), 83 (100), 58 (17); (-)FAB-MS: *m/z* 520.3 $[M^+ - 1]$; HR-EI-MS *m/z* 521.2927 (calculated for $C_{28}H_{43}NO_8$ 521.2932, $[M]^+$)

3. Results and Discussion

Compound **1** was isolated from the crude 80% ethanolic extract of *D. kohatense* Munz as an amorphous powder. The EI-MS showed the molecular ion at *m/z* 521. The negative FAB-MS showed the quasimolecular ion $[M-1]^+$ at *m/z* 520.3 consistent with the formula, $C_{28}H_{42}NO_8$, for the deprotonated ion. The molecular formula, $C_{28}H_{43}NO_8$, of compound **1** was further confirmed through HR-EI-MS showing the molecular ion at *m/z* 521.2927 (calculated for $C_{28}H_{43}NO_8$ 521.2932). The other prominent fragments in the mass spectrum were observed at *m/z* 490 $[M^+$-OMe], 478 $[M^+$-Ac], 475 $[M^+$-OMe-Me], 462 $[M^+$-OAc], 416 $[M^+$-OMe-OAc-Me], 404, 368, 85, 83, 58, characteristics of alkaloids with an aconitine type skeleton (Figure 2) [14]. The IR spectrum of compound **1** showed absorption bands at 3500, and 3406 cm^{-1} (OH group), 1732, and 1246 cm^{-1} (COO), 1092 cm^{-1} (C-O), and 2927 cm^{-1} (C═C).

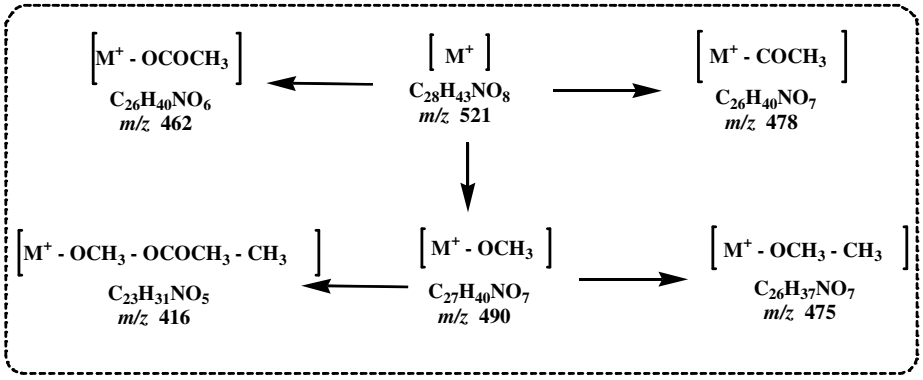

Figure 2. Major mass fragmentations in compound **1**

The ^{1}H NMR spectrum of compound **1** exhibited signals for *N*-ethyl group, three methoxy groups, two acetyl groups and nine methine protons. The overall spectral data of compound **1** was comparable to that of a known alkaloid, peregrine [15], with an additional acetyl substitution of OH group at C-14 position. Furthermore, C-10 methine of the known compound was replaced by hydroxyl substituted quaternary carbon in compound **1**. The signal for H-14*β* in the ^{1}H NMR spectrum of compound **1** was observed as triplet at δ 3.95, (*J* = 5.6 Hz), which indicated the acetyl substitution at C-14 and the presence of a hydroxyl group at C-10 position [16]. A triplet of three proton integration at δ 1.02 (*J*= 6.8 Hz) and a multiplet of two proton integration at δ 2.40 in the ^{1}H NMR spectrum indicated the presence of *N*-ethyl group. The ^{1}H NMR spectrum also displayed three singlets at δ 3.05, 3.22, and 3.32, corresponding to three methoxy groups at C-8, C-1, and C-16 positions respectively (Table 1).

The ^{1}H-^{13}C correlations were determined by HMQC spectrum, while the long-range ^{1}H-^{13}C connectivities were obtained through the HMBC technique (Figure 3). H-9 proton showed correlations with C-8 (δ 79.1), C-12 (δ 29.7), and C-13 (δ 38.5), whereas the H-7 proton showed interactions with C-6 (δ 73.4), and C-8 (δ 79.1). Similarly the HMBC interactions of H-17 were observed with C-6 (δ 73.4), and C-17 (δ 64.7).

The ^{1}H-^{1}H COSY experiment further confirmed the key positions in the structure (Figure 3). The H-9 (δ 2.95) proton showed coupling with H-14 (δ 3.95), which in turn showed coupling with H-13 (δ 2.3). This observation supported the presence of acetyl group at C-14 (δ 75.4). Similarly, H-15

proton (δ 2.0) showed coupling with H-16 proton (δ 3.33) which also showed coupling with H-13 (δ 2.3) indicating the position of methoxy group to be at C-16. The position of the second acetyl group at C-7 was confirmed by the COSY correlations between H-5 (δ 2.69) and H-6 (δ 5.21), and H-6 (δ 5.21) and H-7 (δ 1.43). Thus the structure of compound **1** was deduced as 10*β*-hydroxy-1*α*,8*β*,16*β*-trimethoxy-6*α*,14*α*-diacet-yl-*N*-ethyl aconitane (named kohatenine).

Table 1. NMR data of compound **1** (CD_3OD)

C. No.	*^1H (δ)*	*^{13}C (δ)*	*Multiplicity*
1	3.12, *m*	82.4	CH
2	2.1, *m*	28.6	CH_2
3		32.9	CH_2
4		34.5	C
5	2.69, d, *J* = 7.2 Hz	42.4	CH
6	5.21, d, *J* = 7.3 Hz	73.4	CH
7	1.43,	56.1	CH
8		79.1	C
9	2.95, d, *J* = 3.1 Hz	44.5	CH
10		84.6	C
11		48.2	C
12	1.65 and 1.72	29.7	CH_2
13	2.3, t. *J* = 5.6 Hz	38.5	CH
14	3.95, t, *J* = 5.6 Hz	75.4	CH
15	2.0	32.9	CH_2
16	3.33, d, *J* = 6.1 Hz	81.9	CH
17	3.15, d, *J* = 2.0 Hz	64.7	CH
18	0.799, s	25.8	CH_3
19		53.4	CH_2
$-N-CH_2$	2.40, m	49.2	CH_2
H_3C	1.02, t, *J* = 6.8 Hz	13.6	CH_3
OCH_3-1		57.5	OCH_3
OCH_3-8		48.3	OCH_3
OCH_3-16		56.4	OCH_3
$-O-CO$		170.01	C
CH_3	1.17, *s*	21.66	CH_3
$-O-CO$		170.02	C
CH_3	1.22, *s*	21.67	CH_3

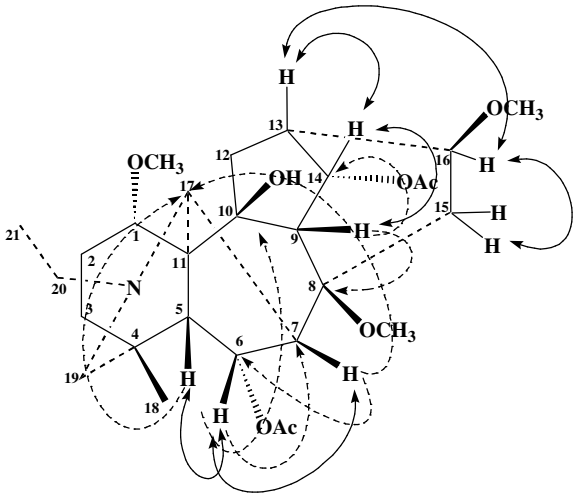

Figure 3. Key HMBC and COSY interactions in compound **1.**

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