Indian Journal of Chemistry
Vol. 46B, August 2007, pp. 1364-1366

Note

Constituents of high altitude Himalayan herbs. part XX. A C-19 diterpenoid alkaloid from *Aconitum balfourii*

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Received 12 March 2006; accepted (revised) 25 May 2007

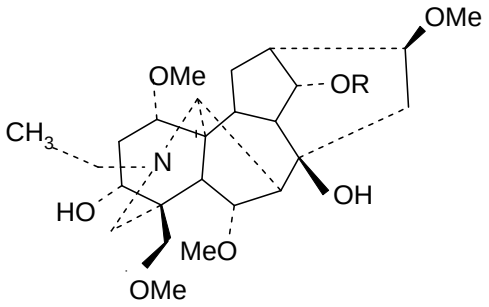
A C-19 Diterpenoid alkaloid, 14-benzoylpseudaconine **1** has been isolated from the aerial parts of *Aconitum balfourii* and identified by MS, ^{1}H ,^{13}C NMR (SFORD and DEPT) as well as by chemical analysis.

Keywords: Norditerpenoid alkaloid, *Aconitum balfourii*, 14-benzoylpseudaconine

Aconitm balfourii (Renunculaceae) is an important highly prized herb[1] found in the Uttaranchal Himalaya in India. It has been frequently used in Ayurvedic systems of medicines since long time but unfortunately it is not taken up of for its systematic chemical investigation after 1928 when an alkaloid[2] was reported to be present in its roots. In our program to search useful high altitude Himalayan herbs we collected this plant from an altitude of 5100 m and identified the alkaloids[3-5] viz: 8-*O*-methyl-veratroylpseudaconine, veratroylpseudaconine, veratroylbikhaconine, balfourine. Laudaconitine pseudaconitine indaconitine, 8-deacetylynaconitine bikhaconitine, neoline, chasmanine, and 9-hydroxysenbushine from its roots 2,3 and condilphine, 1-*O*-methyldelphisine, isotalazidine, bulletin C, yunaconitine indaconitine, pseudaconitine and neoline from its aerial, as reported earlier. Working further on the minor constituents of its aerial parts, an additional C-19 diterpenoid alkaloid 14-benzoylpseudaconine **1** has been isolated and identified by spectral and chemical methods.

Results and Discussion

The molecular formula of the alkaloid $C_{32}H_{45}NO_8$ (**Figure 1**) obtained as an amorphous solid is based on its MS (M^+ 587) and ^{13}C NMR spectral data. It showed prominent absorption bands in its IR spectra at 3420 (OH), 1736 (C=O) and 1610, 1462, 1495, 1452, 3040, and 3090 cm^{-1} (aromatic ring). The ^{1}H NMR spectra showed absorptions for an N-CH_2-CH_3 group (t, 3H, *J*=7.5 Hz) centered at δ 1.16 four aliphatic methoxyls (s, 3H, 3.28, 3.31, 3.36 and 3.38) and five aromatic protons in the region δ 7.6-8.0. The spectrum also exhibited a one proton doublet at δ 5.1 (*J*=4.5 Hz) and 4.0 due to β-protons attached to C-14 and C-6 carbons respectively.



R= COC_6H_5
R= H
Figure 1

The ^{13}C NMR spectra of the alkaloid showed 32 signals in the noise decoupled spectrum and DEPT experiments carried out at 45°, 90°, 135° led us to assign the nature of each carbon atom. It showed five quartets, six triplets, fifteen doublets and six singlets suggesting the presence of five methyl groups, six methylenes, fifteen methine and six quarternary carbons in the molecule (**Table I**).

The resonances of five aromatic protons (δ 6.8-8) in its ^{1}H NMR and low field signal at δ 166.8 ppm in ^{13}C NMR spectra clearly indicated the presence of benzoyl ester group in the molecule.

SFORD experiments showed the alkaloid has eight oxygenated carbons. Out of these four were due to four methoxyl groups as evidenced by four methoxyl proton signals in its ^{1}H NMR spectra, one due to the attachment of benzoyl ester group and the remaining three were accounted for three hydroxyls groups.

Since the alkaloid does not contain any signal in the region δ 87.5-89.0 ppm for C-7 oxygenated carbon atom, the alkaloid is aconitine type[6,7]. Thus the point of substitution in the alkaloid may be C-1, C-3, C-6, C-8, C-13, C-14, C-16 and C-18. The signal at 43 ppm is inferred for C-4 when C-3 is substituted with OH group appearing as a doublet at δ 71.9 ppm. The

Table I — ^{13}C NMR chemical shift assignments of alkaloid **1**

C-1	83.1(d)	C-11	50.2(s)
C-2	35.9(t)	C-12	33.6(t)
C-4	71.9(d)	C-13	76.0(s)
C-4	43.3(s)	C-14	83.0(d)
C-5	48.0(d)	C-15	42.1(t)
C-6	82.5(d)	C-16	82.6(d)
C-7	47.9(d)	C-17	61.9(d)
C-8	73.8(s)	C-18	77.5(t)
C-9	53.3(d)	C-19	49.0(t)
C-10	42.0(d)	N-CH_2	47.5(t)
C-1′-OMe	56.1(q)	CH_3	57.6(q)
C-16′-OMe	59.2(q)		
C-18′-OMe	59. (q)	C-1″	167.7(s)
		C-2″	130.0(s)
		C-3″	129.7(d)
		C-4″	128.6(d)
		C-5″	133.2(d)
		C-6″	128.6(d)
		C-7″	129.7(d)

substitution of benzoyl ester group is accounted at C-13 as in case of other alkaloids existing in literature[7]. It appears as a doublet in ^{13}C NMR spectrum at 83 ppm. The ^{1}H NMR doublet at δ 5.1 (d, *J*=4.5 Hz) due to C-(14)-β-H indicates that C-13 is substituted with OH group. The remaining third OH is to be accounted for either C-6 or C-8. The carbon signal at C-6 appears around δ 72-73 ppm when substituted with OH. Since any signal in this region has not been observed in the ^{13}C NMR spectrum therefore OH is substituted to C-8 which appears as a singlet at δ 73.8. The doublet at δ 82.5 is accounted for the substitution of OMe at C-6.

The doublet at δ 83.1 ppm is accounted for the methoxyl group substituted at C-1. Further, the spectrum lacks any triplet signal at δ 64.5-68.5 ppm to account for OH group at C-18. It appears as a triplet at δ 77.5 indicating the substitution of C-18 with OMe group. Since the alkaloid is aconitine type[7], the only possible position for the fourth methoxyl group is C-16.

The four ^{13}C NMR aromatic signals at δ 130.0, 129.7, 128.6 and 133.2 ppm were assigned, respectively, on C-14 (1′), C-14 (2″, 6″), C-14(3″, 5″) C-14 (4″) whereas the methoxyl carbon signals appearing at δ 56.1 57.6, 58.3 and 59.2 ppm were assigned respectively to C-1′, C-6′ C-16′ and C-18′. The only remaining non-protonated signal appearing at δ 50.2 ppm was assigned to C-11. The signals appearing as doublets at δ 48.0, 82.5, 47.9, 53.3, 42.0 and 61.9 ppm were assigned to be carbons appearing correspondingly at C-5, C-6, C-7, C-9, C-10 and C-17. The three non-oxygenated carbons at C-2, C-12 and C-15 appeared as triplets at δ 35.9, 33.6 and 42.1 respectively. Whereas fourth methylene carbon due to N-CH_2-CH_3. On alkaline hydrolysis with 5% KOH the alkaloid **1** gave **2** which was exactly similar to pseudaconine, MF $C_{25}H_{41}NO_8$ in its m.p. (90-91°), (α) D + 49.4° ($CHCl_3$) and ^{13}C NMR values when compared with literature values[7]. Based on these evidences the alkaloid **1** can be assigned as 14-benzoylpseudaconine which is isolated for the first time from the aerial parts of *A. balfourii*.

Experimental Section

IR spectra were determined on a Perkin-Elmer model 298 spectrometer. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) were recorded on a Bruker AC-300 spectrometer. ^{13}C Chemical shift multiplicities were determined from DEPT spectra. Chromatographic separation in a chromatotron were carried out on rotors coated with 1 mm layer of Al_2O_3 60 PF-254-365 or SiO_2 60 vacuum liquid chromatography were carried out with Merc Al_2O_3 and SiO_2 60 H.

Plant Material. The plant was collected from an altitude of 5,100 m from Milam glaciers of Kumaon Himalaya Uttaranchal in the month of August and identified in the Department of Botany Kumaon University, Nainital where the voucher specimen is stored as *Aconitum* species.

Extraction. The shade dried aerial parts of *A. balfourii* were pulverized and extracted at room temperature with 70% EtOH. The extract was evaporated in vacuum until a residual mass obtained. The residue was defatted with *n*-hexane and the defatted portion was acidified with 2%H_2SO_4. on gradient *p*H fractionation with Na_2CO_3 an alkaloid rich fraction at *p*H 4.5 was obtained. The fraction was fractionated by vacuum liquid chromatography over SiO_2 with gradient elution in increasing polarity with hexane, ether and MeOH. The fraction eluted with hexane:ether (30:70, 40:60, 50:50) were pooled.

Isolation of Alkaloid 1. The fraction pooled as above was subjected to centrally accelerated radial thin layer chromatography[7,8] (CARTLC) by gradient elution with hexane and ether when fraction **1** was isolated which gave Dragendorff's positive test for alkaloids. Its purity was checked by HPLC using RI and variable wavelength (190-750 nm) UV detector.

The mass spectrum showed peaks at MS M^+ (587.4 m/z 57 (M-CH_3), m/z 556 (M-OCH_3), 538, 534, 390, 226, 149 $[\alpha]_D$ +28.50 ($CHCl_3$), IR (in Nujol): 3450 (OH) 1736 (ester str), 1615 (aromatic ring). ^{1}H NMR 1,16 (t, 3H, N-CH_2-CH_3) , δ 3.28, 3.31, 3.36, (s, 4 OMe), 4.0 (d, C-(6)- β-H), 5.1(d, C-(14)- β-H) and δ 7.6-8.0 (five aromatic protons). **Table I**.

Acknowledgements

The Department of Science and Technology, New Delhi is gratefully acknowledged for financial assistance and Central Drug Research Institute, Lucknow for spectral results.

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