ALKALOIDS OF ACONITUM BALFOURII STAPF.

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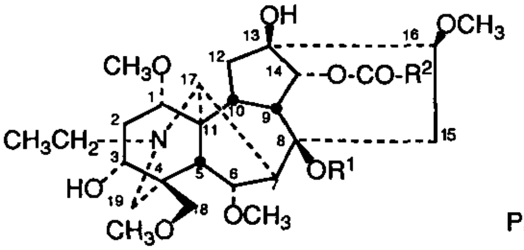
Abstract – Three new norditerpenoid alkaloids, 8-*O*-methylveratroylpseudaconine (**9**), veratroylbikhaconine (**10**), and balfourine (**11**), have been isolated from Aconitum balfourii, together with eight known alkaloids, pseudaconitine, veratroylpseudaconine, indaconitine, ludaconitine, 8-deacetylyunaconitine, bikhaconitine, neoline, and chasmanine. The new structures were derived from physical and spectroscopic data and chemical correlations with alkaloids of established structures.

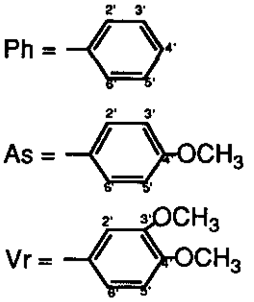
The roots of Aconitum balfourii Stapf. are reported to be highly poisonous and are found in the subalpine and alpine Himalayas from Garhwal to Nepal between 7500 and 14,000 ft.[1] There is one previous publication on the chemical investigation of this plant in which, the isolation of pseudaconitine has been reported.[2] This alkaloid was first isolated in 1877 from the roots of Aconitum ferox Wall.[3] and after a series of investigations by Marion and other workers, the structure (**1**) for pseudaconitine was established in 1963 by Tsuda and Marion.[4]

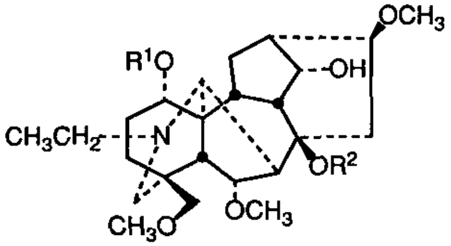
In the present investigation, a detailed study of the basic fraction of the roots of A. balfourii has resulted in the isolation and structure elucidation of three new and eight previously known norditerpenoid alkaloids. Isolation of the pure alkaloids was carried out by vacuum liquid chromatography[5] and by centrifugally accelerated, radial, thin layer chromatography on alumina.[6] The norditerpenoid alkaloids of known structures are: a) pseudaconitine[7,8] (**1**), b) veratroylpseudaconine[7,8] (**2**), c) indaconitine[7,8,9] (**3**), d) ludaconitine[10] (**4**), e) 8-deacetylyunaconitine[11] (**5**), f) bikhaconitine[12] (**6**), g) neoline[13] (**7**) and h) chasmanine[13] (**8**). The three new alkaloids isolated from this plant are: i) 8-*O*-methylveratroylpseudaconine (**9**), j) veratroylbikhaconine (**10**) and k) balfourine (**11**).

The identification of the alkaloids a to h is based on their physical constants, and infrared, mass, proton and carbon-13 nmr spectral data. Comparison of tlc, infrared and ^{13}C nmr spectral data with those of authentic samples confirmed their identity. The ^{13}C nmr spectral data for the alkaloids (**1** – **11**) are given in Table 1. The assignments for certain of the carbon atoms given in the literature[14] have been revised and the revised values are indicated by an asterisk. The ^{13}C nmr data for ludaconitine (**4**) are new and were not recorded in the literature cited.[10] When C-8 carries an acetoxyl group, this carbon appears downfield by ~9–10 ppm when compared with the alkaloid having a C-8 hydroxyl group. Also, the adjacent carbons C-7, C-9 and C-15 are shifted upfield by about 3, 5 and 2–3 ppm, respectively, when C-8 bears an acetoxyl group.

8-*O*-Methylveratroylpseudaconine (**9**); $C_{35}H_{51}NO_{11}$; ms: m/z 661 (M^+, 0.5%), 630 (M^+ -OCH_3, 100) was obtained as an amorphous compound. The ^1H nmr spectrum showed δ: 1.15 (3H, t, J = 7 Hz, N-$CH_2C\underline{H}_3$), 3.00, 3.25, 3.26, 3.28, 3.51 (each 3H, s, aliphatic OCH_3), 3.91, 3.92 (each 3H, s, Ar-OCH_3), 4.85 (1H, d, J = 4.5 Hz, C(14)-β-H), 6.88 (1H, d, J = 9 Hz, H-5'), 7.60 (1H, d, J = 2 Hz, H-2'), 7.70 (1H, dd, J = 9, 2 Hz, H-6'). The C(8)-methoxyl group is shielded due to the ring current of the 14-α-veratroyl group and appears at δ 3.00. Also, in the ^{13}C nmr spectrum, the C(8)-methoxyl carbon is observed upfield (from the usual methoxyl position) at 48.7 ppm as in the case of other alkaloids having a methoxyl group at the C(8) position.[15,16]



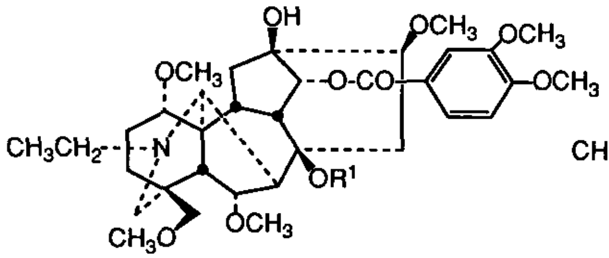




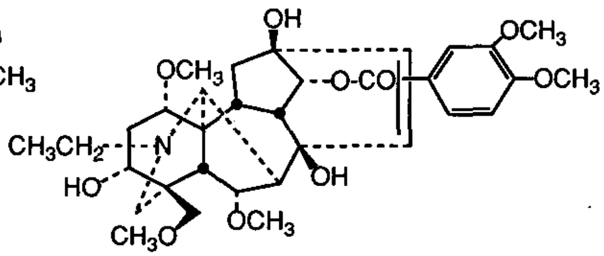
7 Neoline $R^1, R^2 = H$
8 Chasmanine $R^1 = CH_3, R^2 = H$

1 Pseudaconitine $R^1 = Ac, R^2 = Vr$
2 Veratroylpseudaconine $R^1 = H, R^2 = Vr$
3 Indaconitine $R^1 = Ac, R^2 = Ph$

4 Ludaconitine $R^1 = H, R^2 = Ph$
5 8-Deacetylyunaconitine $R^1 = H, R^2 = As$
9 8-*O*-Methylveratroylpseudaconine $R^1 = CH_3, R^2 = Vr$



6 Bikhaconitine $R^1 = Ac$
10 Veratroylbikhaconine $R^1 = H$



11 Balfourine

A crystalline compound, mp 90–92°C, isolated by chromatographic separation, $[\alpha]_D$ +46.5°, was identified as veratroylbikhaconine (**10**), $C_{34}H_{49}NO_{10}$, ms: m/z 631 (M^+, 2.5%), 600 (M^+ -OCH_3, 100); ^1H nmr δ: 1.09 (3H, t, J = 7.5 Hz, N-$CH_2C\underline{H}_3$), 3.25, 3.26, 3.29, 3.39 (each 3H, s, OCH_3), 3.92, 3.93 (each 3H, s, Ar-OCH_3), 5.12 (1H, d, J = 4.5 Hz, C(14)-β-H), 6.88 (1H, d, J = 10 Hz, H-5'), 7.58 (1H, d, J = 2 Hz, H-2'), 7.65 (1H, dd, J = 10, 2 Hz, H-6'). The structural assignment was confirmed by partial hydrolysis of bikhaconitine in refluxing dioxane-water (1:1) for 3 days to afford **10**, identical (tlc, ir) with the alkaloid isolated from A. balfourii. The sluggish hydrolysis is probably due to steric hindrance of the veratroyl group. In comparison, the 8-acetoxyl group in aconitine possessing a benzoate group at C(14), is hydrolyzed in 1 h.[17]

Table 1. ^{13}C Nmr Chemical Shifts and assignments for Pseudaconitine (**1**), Veratroylpseudaconine (**2**), Indaconitine (**3**), Ludaconitine (**4**), 8-Deacetylyunaconitine (**5**), Bikhaconitine (**6**), Neoline (**7**), Chasmanine (**8**), 8-*O*-Methylveratroylpseudaconine (**9**), Veratroylbikhaconine (**10**), and Balfourine (**11**).

Carbon	1	2	3[9]	4	5	6	9	10	11	7	8
C-1	83.6d	83.2d	83.5d	83.1d	83.1*	83.8d	83.7d	83.3d	82.5d	72.2d	86.2d
C-2	35.0t	35.7t	35.2t	35.3t	35.9t	26.3t	35.4t	35.4t	34.0t	29.1t	26.0t
C-3	71.6d*	71.9d	71.3d	72.1d	72.1	35.1t	71.4d	34.9t	71.9d	29.9t[a]	35.3t
C-4	43.1s	43.2s	43.2s	43.3s	43.3s	39.1s	43.1s	39.3s	43.4s	38.1s	39.4s
C-5	48.7d	47.8d*	48.8d	48.0d	47.4d	49.5d	48.1d	49.6d	46.9d	44.9d	48.6d
C-6	82.1d[a]	82.4d[a]	82.3d	82.5d	82.6[a]	85.0d*	82.0d	85.4d	81.6d	83.1d	82.3d
C-7	44.6d*	47.8d*	48.8d	47.9d	48.1*	45.0d	45.6d	48.3d	48.0d	52.1d	52.6d
C-8	85.4s	73.8s	85.6s	73.8s	73.8	85.5s*	78.6s	76.1s	73.8s	74.2s	72.5s
C-9	47.0d	53.4d	47.3d	53.4d	53.3	49.5d	45.3d	53.7d	48.8d	48.3d	50.2d
C-10	40.7d	41.9d*	40.8d	42.0d	42.0	40.9d	41.2d	42.2d	42.5d	44.1d*	45.6d*
C-11	50.3s	50.2s	50.2s	50.3s	50.3	50.2s	50.8s	50.3s	50.2s	49.5s	50.3s
C-12	33.2t	33.6t	33.7t	33.6t	33.6*	35.7t	32.4t	36.4t	40.6t	29.8t[a]	28.3t
C-13	74.7s	75.8s	74.7s	76.0s	76.0	74.9s	75.2s	73.8s	76.0s	40.2d*	37.8d*
C-14	78.5d	79.8d	78.8d	80.2d	79.9	78.6d*	78.9d	80.0d	80.3d	76.1d	75.6d
C-15	39.8t	42.3t	39.5t	42.3t	42.2	39.5t	36.9t	42.1t	134.7d[a]	42.9t	38.7t
C-16	83.0d[a]	82.5d[a]	83.2d	82.6d	82.5[a]	83.0d	82.7d	82.5d	130.1d[a]	81.7d	82.0d
C-17	61.9d	61.9d	61.7d	61.9d	61.9	62.0d	61.7d	62.3d	62.4d	63.8d	62.7d
C-18	77.0t*	77.4t*	76.7d	77.5t	77.2	80.3t	76.9t	80.6t	77.2t	80.3t	80.8t
C-19	48.9t	48.9t	48.8t	49.0t	49.0*	53.6t	48.8t	53.8t	49.1t	57.0t	53.8t
N-CH$_2$	47.7t	47.4t*	47.3t	47.4t	47.9	49.1t	48.3t	49.3t	47.5t	48.3t	49.3t
CH$_3$	13.2q	13.5q	13.3q	13.6q	13.6	13.3q	12.8q	13.6q	13.4q	13.1q	13.8q
C-1'	55.9q	55.8q	55.9q	56.1q	56.1	55.8q	56.0q	56.4q	56.0q	–	56.2q
C-6'	57.9q	57.5q	57.8q	57.6q	58.4	57.8q	58.7q	57.6q	57.5q	57.9q	57.3q
C-8'	–	–	–	–	–	–	48.7q	–	–	–	–
C-16'	58.8q	58.4q	58.7q	58.3q	57.6	58.1q	58.9q	58.4q	–	56.3q	56.4q
C-18'	59.2q	59.1q	59.1q	59.2q	59.2	59.0q	59.1q	59.2q	59.2q	59.2q	59.3q
CO	170.0s	–	169.8s	–	–	169.9s	–	–	–	–	–
CH$_3$	21.6q	–	21.5q	–	–	21.7q	–	–	–	–	–
CO	166.0s	166.3s	166.2s	166.8s	166.5	166.1s	166.1s	166.5s	166.0s	–	–
1'	122.6s	122.5s	130.2s	130.0s	122.3	123.0s	122.9s	122.5s	121.6s	–	–
Ar 2'	110.4d*	110.3d*	129.7d*	129.7d	131.8	110.3d	110.2d	110.4d	110.4d	–	–
3'	148.4s*	148.6s*	128.5d*	128.6d	113.8	148.6s	148.4s	148.6s	148.6s	–	–
4'	153.0s*	153.0s*	133.2d	133.2d	163.6	153.0s	152.8s	153.1s	153.1s	–	–
5'	111.8d*	112.2d*	128.5d*	128.6d	113.8	111.9d	112.1d	112.1d	112.2d	–	–
6'	123.7d*	123.7d*	129.7d*	129.7d	131.8	123.7d	123.8d	123.7d	123.6d	–	–
Ar-OCH$_3$	56.0q	55.9q	–	–	55.5	56.2q	55.8q	56.1q	–	–	
Ar-OCH$_3$	56.0q	55.9q	–	–	–	56.0q	55.8q	56.1q	–	–	

[a] These values may be interchanged in any vertical column.

The third new alkaloid designated as balfourine has been identified as 16-demethoxy-15,16-dehydroveratroylpseudaconine (**11**). This amorphous alkaloid, $C_{33}H_{45}NO_{10}$, showed ms: m/z 615 (M^+, 3%), 584 (M^+ -OCH_3, 100); ^1H nmr δ: 1.06 (3H, t, J = 8 Hz, N-$CH_2C\underline{H}_3$), 3.24 (3H, s, OCH_3), 3.32 (6H, s, 2-OCH_3), 3.92, 3.94 (each 3H, s, Ar-OCH_3), 4.22 (1H, d, J = 4.5 Hz, C(14)-β-H), 5.63, 5.90 (each 1H, dd, J = 5 Hz, CH=CH), 6.88 (1H, d, J = 9.5 Hz, H-5'), 7.48 (1H, d, J = 1.5 Hz, H-2'), 7.56 (1H, dd, J = 9.5, 1.5 Hz, H-6'). Falaconitine obtained by the pyrolysis of pseudaconitine (**1**), was treated with *p*-toluenesulfonic acid in benzene at room temperature for 4 days to afford balfourine

(**11**), in 90% yield, identical with the alkaloid isolated from A. balfourii. Balfourine (**11**) represents the first naturally occurring norditerpenoid alkaloid having an isopyro- structure.

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