

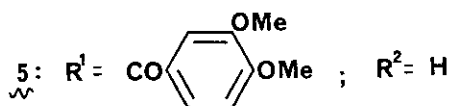
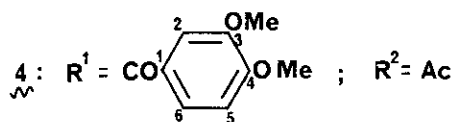
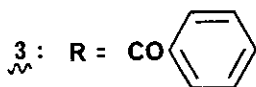
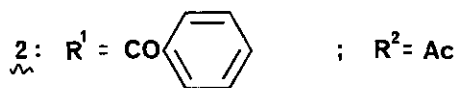
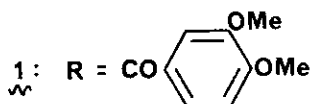
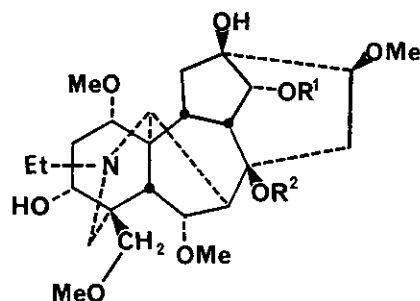
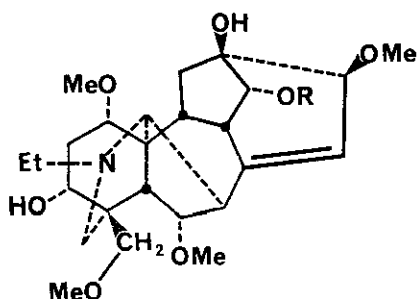
STRUCTURES OF FALCONERINE AND FALCONERINE 8-ACETATE, TWO NEW C<sub>19</sub>-DITERPENOID ALKALOIDS

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**Abstract** - Two new alkaloids designated as falconerine (6) and falconerine 8-acetate (8) have been isolated from the Indian crude drug *Mitha telia* (Bish or Bikh), identified as *Aconitum falconeri* Stapf. The structures of these C<sub>19</sub>-diterpenoid alkaloids were derived from their spectral data and by correlation with ezochasmanine.

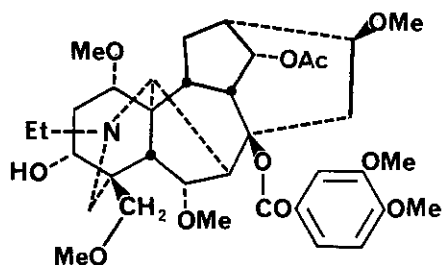
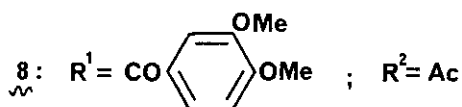
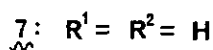
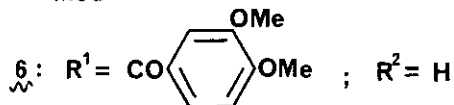
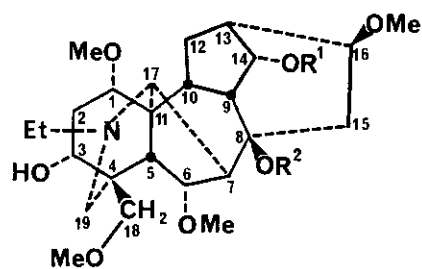
In earlier studies from our laboratories, we had reported the isolation and structure of falconitine (1), indaconitine (2), mithaconitine (3), pseudaconitine (4) and veratroyl-pseudaconine (5)<sup>1,2</sup> from the roots of *A. falconeri* Stapf.



During our efforts to obtain the previously reported alkaloid bishatisine,<sup>3</sup> we have isolated from the roots of the Indian crude drug *Mitha telia*<sup>4</sup> two new alkaloids designated as falconerine and falconerine 8-acetate. The crude base extracted by the procedure described by Singh *et*

at<sup>3</sup> was dissolved in dilute sulfuric acid and precipitated as Meyer's complex.<sup>6</sup> The alkaloids were liberated by passing the methanolic solution of the Meyer's complex through Amberlite IRA-400 (OH form) and were separated by vacuum liquid chromatography<sup>7</sup> on alumina and eluted with chloroform and methanol. The chloroform fraction was separated on a "Chromatotron"<sup>8</sup> with a 2 mm thick alumina rotor. The chromatographic separation was monitored by a uv lamp.

Falconerine, C<sub>34</sub>H<sub>49</sub>NO<sub>10</sub>, is amorphous but homogeneous by t.l.c. (R<sub>f</sub> 0.5, Al<sub>2</sub>O<sub>3</sub>; Et<sub>2</sub>O:3% MeOH), ir: (nujol)  $\nu_{\max}$  3470 (OH), 1725 (ester carbonyl), 1600 (aromatic) cm<sup>-1</sup>; <sup>1</sup>H-nmr (CDCl<sub>3</sub>):  $\delta$  1.09 (3H, t, J = 7 Hz, N-CH<sub>2</sub>-CH<sub>3</sub>), 3.22, 3.26, 3.31, 3.34 (each 3H, s, aliphatic OCH<sub>3</sub>), 3.92 (6H, s, aromatic OCH<sub>3</sub>), 5.15 (1H, t, J = 4.5 Hz, C(14)- $\beta$ -H), 6.83 (1H, d, J = 9 Hz, H-5 of veratroyl), 7.59 (1H, d, H-2 of veratroyl), 7.65 (1H, dd, J = 9, 2 Hz, H-6 of veratroyl). Four methoxys, an ethyl and a veratroyl group in the molecule (15 carbons) indicated that falconerine belongs to the C<sub>19</sub>-diterpenoid class of alkaloids. No other alkaloid has been reported earlier with this molecular formula.<sup>9</sup> The fully decoupled <sup>13</sup>C-nmr spectrum showed 33 lines for 34 carbon atoms of the molecule and the SFORD spectrum showed 7 singlets, 14 doublets, 6 triplets and 7 quartets (Table 1). The singlets at 43.2 and 50.5 ppm can be assigned to C(4) and C(11), respectively. The signals in the downfield region beyond 110 ppm are due to the veratroyl group. The doublets appearing at 82.9, 82.7 and 81.9 ppm have been assigned to C(1), C(16) and C(6) bearing methoxyl groups. The fourth methoxyl is present as a methoxymethylene group at C(18) which is seen as a triplet at 77.5 ppm. The remaining two oxygen functions are present as hydroxyls located at C(3) and C(8) positions. The veratroyl group is present at C(14) and not at C(8) as indicated by the singlet appearing at 74.1 ppm. The C(8) bearing an ester function as in anisoezochasmanitine appears around 85.9 ppm.<sup>10</sup> The above evidence leads to the unique structure (6) for falconerine.



9

In order to correlate falconerine with an alkaloid of established structure, it was hydrolysed with methanolic potassium hydroxide to give a compound, mp 116-118°C, identical with ezochasmanine (7).<sup>10,11</sup>

The second alkaloid (Rf 0.59 Al<sub>2</sub>O<sub>3</sub>; Ether:3 % MeOH), mp 162-163°C, has the molecular formula C<sub>36</sub>H<sub>51</sub>NO<sub>11</sub>; ir (nujol)  $\nu_{\max}$  3480 (OH), 1730, 1685 (ester carbonyl), 1600 (aromatic) cm<sup>-1</sup>; <sup>1</sup>H nmr (CDCl<sub>3</sub>):  $\delta$  1.08 (3H, t, J = 7 Hz, N-CH<sub>2</sub>-CH<sub>3</sub>), 1.37 (3H, s, OAc), 3.19, 3.25, 3.3, 3.4 (each 3H, s, aliphatic OCH<sub>3</sub>), 3.91, 3.94 (each 3H, s, aromatic OCH<sub>3</sub>), 5.02 (1H, t, C(14)- $\beta$ -H), 6.9 (1H, d, J = 9 Hz, H-5 of veratroyl), 7.65 (1H, d, H-2 of veratroyl), 7.71 (1H, dd, J = 9,2 Hz, H-6 of veratroyl). The methoxyls (4 C), an N-ethyl (2), an acetoxy (2), and a veratroyl (9) add to seventeen carbon atoms indicating that the compound is a C<sub>19</sub>-diterpenoid alkaloid. The completely decoupled <sup>13</sup>C nmr spectrum showed 35 lines corresponding to the 36 carbon atoms of the molecule and the SFORD spectrum indicated 8 singlets, 14 doublets, 6 triplets and 8 quartets (Table 1). Bikhacnitrine (10) is the only other known alkaloid having the same molecular formula as this alkaloid, but its physical properties are different.<sup>9</sup> Of the eleven oxygen functions in the new alkaloid, four are part of the veratroyl ester, two are present as acetoxy, four are methoxyl groups and the remaining oxygen should be present as a hydroxyl group. The singlets at 43.1 ppm and 50.4 ppm can be assigned to C(4) and C(11), respectively. The singlets downfield of 120 ppm are easily assigned (Table 1) to the acetoxy and the veratroyl groups. The singlet appearing at 85.8 ppm can only be ascribed to the C(8) carbon carrying an ester function which can be an acetoxy or a veratroyl group. The doublets appearing downfield of 75 ppm can be assigned to C(1), C(6), C(14) and C(16), all bearing oxygen functions. The doublet at 71.6 ppm can only be ascribed to C(3) carrying a hydroxyl group. The remaining triplet at 77.0 ppm is due to the C(18) methoxymethylene carbon. The above evidence leads to two alternative structures (8) or (9) for this alkaloid.<sup>12</sup> As anticipated, it afforded ezochasmanine (7) when subjected to alkaline hydrolysis. In order to choose between the two structures 8 and 9, the alkaloid was preferentially deacetylated at the C(8) position, as in the case of aconitine,<sup>13,14</sup> by gentle boiling with water to give falcone-rine (6). On the basis of the above data the second alkaloid has been constituted as falcone-rine 8-acetate (8).

The known alkaloid pseudaconitine (4)<sup>15</sup> was also isolated during the chromatographic separation.

#### EXPERIMENTAL

Mps are corrected. Spectra were recorded on the following instruments: Ir, Perkin-Elmer Model 1430; <sup>1</sup>H nmr, Perkin-Elmer EM-390, 90 MHz; <sup>13</sup>C nmr, JEOL FT Models FX-60 and FX-90Q; mass spectra, VG-Micromass ZAB2F, 70 e.v. Specific rotations were recorded on a Perkin-Elmer Polarimeter, Model 141.

Isolation and purification of crude bases — The powdered roots of the drug *Mitha telid*<sup>A</sup> (2.05 kg) were extracted at 50-60°C with 95% ethanol (3 x 8 l) and 75% ethanol (2 x 9 l). The combined extract was evaporated under vacuum to afford a dark viscous residue which was extracted with 10% HCl (200 ml). After removal of the neutral fraction by shaking with ether, the acidic layer was basified with ammonia and extracted with chloroform (20 x 150 ml). The chloroform layer was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to give the crude alkaloid mixture (51.8 g). Part of this fraction (5.0 g) was dissolved in 2% H<sub>2</sub>SO<sub>4</sub> (150 ml), filtered and Meyer's reagent was added to the acidic solution until precipitation was complete. The Meyer's complex was collected, washed with

water, dried under vacuum (5.86 g) and dissolved in methanol (2 l). The methanol solution was passed through a column of ion exchange resin (4 cm x 21 cm, Amberlite IRA-400, OH form). The eluted methanol on evaporation under vacuum gave a gum (3.01 g). A solution of the gum (2.7 g) in chloroform was passed through a vlc column<sup>7</sup> and eluted with chloroform to give a residue (2.56 g) (A).

Isolation of falconerine (6), falconerine 8-acetate (8) and pseudaconitine (4) — The residue A (0.54 g) in  $\text{CH}_2\text{Cl}_2$  was loaded on a 2 mm alumina rotor (tlc grade  $\text{Al}_2\text{O}_3$  60, type E, neutral, E. Merck 1092-1) on a "Chromatotron"<sup>8</sup> and eluted with hexane, hexane-ethyl ether, ether and ether-methanol mixtures according to the separation of the bands visualized under 254 nm uv light. In all, six fractions were collected, monitored by TLC separation. Two of the fractions (ether and ether: 0.3% methanol) (0.21 g, Fraction 2) and ether: 0.3% methanol and ether: 3% methanol (0.16 g, fraction 5) were further purified on the "Chromatotron" on a 1 mm alumina rotor.

Falconerine 8-acetate (8) obtained from fraction 2 gave colorless crystals from hexane (80 mg) mp 162-163°C,  $[\alpha]_D^{30} +13.7^\circ$  (c, 1.05,  $\text{CHCl}_3$ );  $M^+$  m/z 673, EI and CI; ir (nujol)  $\nu_{\max}$  3480, 2950, 2920, 2855, 1730, 1685, 1600, 1588, 1550, 1518, 1463, 1415, 1372, 1365, 1350, 1304, 1271, 1240, 1195, 1180, 1140, 1110, 1085, 1040, 1020, 983, 965, 770, 730  $\text{cm}^{-1}$ . (Found: C, 64.25; H, 7.64; N, 2.05;  $\text{C}_{36}\text{H}_{51}\text{NO}_{11}$  requires: C, 64.17; H, 7.63; N, 2.08%).

Falconerine (6) was isolated from fraction 5 as an amorphous solid (0.11 g);  $[\alpha]_D^{30} +40.3^\circ$  (c, 0.55  $\text{CHCl}_3$ );  $M^+$  m/z 631, EI, CI; ir (nujol)  $\nu_{\max}$  3470, 2960, 2927, 2860, 1715, 1705, 1700, 1600, 1590, 1515, 1465, 1417, 1378, 1350, 1299, 1272, 1223, 1180, 1130, 1100, 1045, 1026, 985, 930, 915, 880, 822, 765, 727  $\text{cm}^{-1}$ . (Found: C, 64.56; H, 7.84; N, 2.17;  $\text{C}_{34}\text{H}_{49}\text{NO}_{10}$  requires: 64.64; H, 7.82; N, 2.22%).

A band which eluted subsequent to the separation of falconerine afforded pseudaconitine (4) (19 mg), mp 204.5-206.5°C (lit.<sup>15</sup> mp 205-207°C),  $[\alpha]_D^{22} +26^\circ$  (c, 0.1  $\text{CHCl}_3$ ), identified (tlc, ir,  $^1\text{H}$  and  $^{13}\text{C}$  nmr) by comparison with an authentic sample.

Hydrolysis of falconerine 8-acetate and falconerine to ezochasmanine (7) — Falconerine 8-acetate (58 mg) was stirred at room temperature with 5% methanolic KOH (7.0 ml) for 42 h. Usual work up gave a gum (40 mg) which was purified on a "Chromatotron" using an alumina rotor and elution with ether. This gave ezochasmanine (7) (16 mg) as colorless crystals from benzene-hexane, mp 116-118°C,  $[\alpha]_D^{21} +40.1^\circ$  (c, 0.35,  $\text{CHCl}_3$ ), identified by comparison of the mmp, tlc, ir,  $^1\text{H}$  nmr and  $^{13}\text{C}$  nmr with that of an authentic sample.

Falconerine (40 mg) in 5% methanolic KOH (7.0 ml) was stirred at room temperature for 17 h. Usual work up and purification on a "Chromatotron" afforded ezochasmanine identified by comparison with an authentic sample (mp, mmp, tlc, ir,  $^1\text{H}$  and  $^{13}\text{C}$  nmr) as above.

Hydrolysis of falconerine 8-acetate to falconerine — Falconerine 8-acetate (40 mg) was heated under reflux with water (7 ml) under nitrogen. The solution was basified with ammonium hydroxide

TABLE 1. Chemical shifts and assignments for falconerine (6), falconerine 8-acetate (8), ezochasmanine (7)<sup>10</sup>, pseudaconitine (4)<sup>16</sup>, veratroypseudaconine (5)<sup>16</sup>, and bikhaconitine (10).

Carbon	6	8	7	4	5	10
1	82.9 d (a)	83.5 d	83.2	83.6	83.4	84.0
2	33.5 t	33.4 t	33.9	35.1	35.8	26.4
3	72.0 d	71.6 d	72.2	70.9	71.3	35.0
4	43.2 s	43.1 s	43.5	43.1	43.3	39.2
5	47.7 d (b)	48.6 d	48.5	48.7	47.5	49.6 (a)
6	81.9 d	82.4 d (a)	82.2 (a)	82.1	82.5	85.1 (b)
7	53.6 d	44.8 d	52.4	48.7	47.5	49.3 (a)
8	74.1 s	85.8 s	72.5	85.3	73.6	85.6 (b)
9	47.5 d (b)	46.9 d	48.8	47.2	53.8	45.2
10	46.6 d	43.5 d	45.3*	40.7	41.9	41.1
11	50.5 s	50.4 s	50.2	50.1	50.2	50.3
12	28.7 t	28.2 t	28.1	33.7	33.7	35.7
13	37.4 d	39.3 d	38.1*	74.7	75.8	75.0
14	76.5 d	75.3 d	75.5	78.4	79.8	80.5
15	41.7 t	38.2 t	39.1	39.6	42.4	39.5
16	82.7 d (a)	82.8 d (a)	82.0 (a)	83.0	82.5	83.2
17	61.5 d	61.3 d	62.2	61.4	61.6	62.0
18	77.5 t	77.0 t	77.4	76.2	76.7	78.7
19	48.8 t	48.6 t	47.4	48.7	48.9	53.7
N-CH <sub>2</sub>	45.1 t	47.6 t	49.1	47.2	47.5	49.1
CH <sub>3</sub>	13.5 q	13.3 q	13.7	13.3	13.5	13.4
1'	56.1 q	55.6 q	56.4	55.7	55.8	56.1
6'	57.7 q	57.9 q	57.3	57.6	57.5	57.8
16'	56.0 q	56.6 q	56.0	58.7	58.3	58.8
18'	59.2 q	59.1 q	59.2	58.9	59.1	59.1
C=O	-	169.7 s	-	169.4	-	169.8
CH <sub>3</sub>	-	21.7 q	-	21.5	-	21.7
C=O	166.0 s	165.9 s	-	165.6	166.2	166.1
1	122.9 s	122.9 s	-	122.5	122.5	123.0
2	110.5 d (c)	110.4 d (b)	-	110.2 (a)	110.5 (a)	110.6 (c)
3	148.7 s	148.7 s	-	148.4	148.6	148.8
4	153.0 s	152.9 s	-	152.8	153.1	153.1
5	112.2 d (c)	112.0 d (b)	-	111.8 (a)	112.3 (a)	112.2 (c)
6	123.6 d	123.6 d	-	123.5	123.5	123.0
2-OMe	{ 55.8 q	55.9 q	-	55.7	55.8	55.9
	{ 55.8 q	56.0 q	-	55.7	55.8	56.1

In ppm downfield from TMS. Spectra were taken in CDCl<sub>3</sub>.

(a),(b),(c) These assignments may be interchanged in any vertical column.

\* The published assignments for C(10) and C(13) have been reversed.

Values given for primed carbons refer to chemical shifts for methoxyls.

to pH 9 and extracted with  $\text{CHCl}_3$  (5 x 30 ml), washed with water and the organic layer dried over anhydrous  $\text{Na}_2\text{SO}_4$  and evaporated under vacuum to give a gum (36.5 mg). Chromatographic separation on a short column of alumina gave falconerine (tlc, ir,  $^{13}\text{C}$  nmr).

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