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THE STRUCTURES OF FORESTINE AND FORESTICINE, TWO NEW C_{19}-DITERPENOID ALKALOIDS FROM *ACONITUM FORRESTII* STAPF

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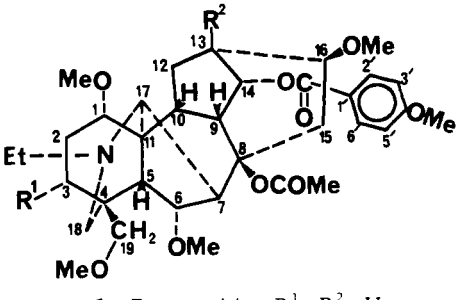
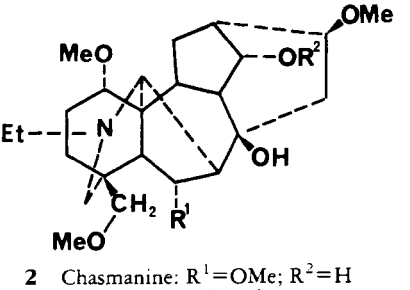
ABSTRACT.—Chemical investigation of the roots of *Aconitum forrestii* Stapf resulted in the isolation of two novel C_{19}-diterpenoid alkaloids, forestine (**6**) and foresticine (**7**), together with three known alkaloids, chasmanine (**2**), talatizamine (**3**), and yunaconitine (**4**). The structure derivation of the new alkaloids is based mainly on spectroscopic evidence and correlation of foresticine with chasmanine (**2**).

The roots of *Aconitum forrestii* Stapf are used in the Chinese traditional medicine for the treatment of rheumatism. Chen and Breitmaier have reported the isolation of a "new" alkaloid, foresaconitine (**1** actually, vilmorrianine C.), from *Aconitum forrestii* Stapf var. *albo-villosum* (Chen et Liu) W.T. Wang (1). As part of a program to investigate the crude drugs of China (2-5), a careful examination of the roots of *A. forrestii* Stapf has shown that this plant contains five diterpenoid alkaloids. These were isolated from the 90% EtOH extract by a combination of pH gradient separation, column and thick-layer chromatographic techniques. Four of the compounds were isolated from the crude alkaloid fraction (pH-8; 0.9% of the dry roots), of which three were identified as chasmanine (**2**) (6), talatizamine (**3**) (7), and yunaconitine (**4**) (8-9) by direct comparison with authentic samples. A new alkaloid designated as forestine (**6**) was obtained in an amorphous form, and its molecular formula $C_{33}H_{47}NO_9$ was derived from the mass-spectral (M^+ 601) and cmr data. In the infrared absorption spectrum, forestine shows hydroxyl (3460 cm^{-1}), aromatic (1610, 1580 cm^{-1}), and ester carbonyl (1715 cm^{-1}) bands. The pmr spectrum of the base shows that it contains a methyl group at δ 1.1 (t, J=7 Hz, N-CH_2-CH_3), four methoxyl singlets at δ 3.3, 3.32, 3.33, and 3.4. The spectrum also exhibits one-proton doublets at δ 4.06 (J=6 Hz) and 5.12 (J=5 Hz) attributed to C-6 and C-14 β-protons, respectively. Aromatic protons with A_2B_2 pattern at δ 6.95 (2H, J=9 Hz), 8.05 (2H, J=9 Hz) together with an aromatic methoxyl at δ 3.86 and a uv maximum at λ 255 nm (log ε, 4.04) indicated the presence of an anisoyl group (10). Considering a total of 14 carbon atoms accounted so far, forestine should be a C_{19}-diterpenoid alkaloid. Biogenetic considerations and the oxygenation pattern of alkaloids belonging to this group lead to the partial structure (**5**). Cmr spectral values

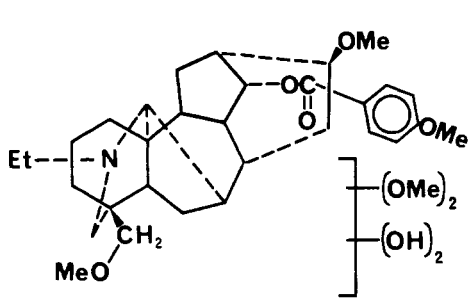


1 Foresaconitine: R^1=R^2=H
4 Yunaconitine: R^1=R^2=OH



2 Chasmanine: R^1=OMe; R^2=H
3 Talatizamine: R^1=R^2=H
7 Foresticine: R^1=OH; R^2=H
8 R^1=OAc; R^2=Ac
9 R^1=OMe; R^2=Me

[1]On leave from the Kunming Institute of Botany, Kunming, Yunnan, China.



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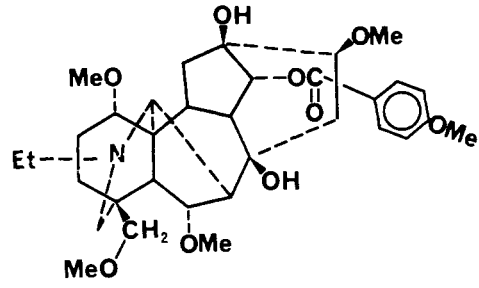

6 Forestine

(cf. **1,2,4,9** in Table 1) and the normal oxygenation of this class of alkaloids indicate that C-1 and C-6 are substiuted by methoxyl groups (11). The C-6 methoxyl is α-oriented, as seen from the pmr signal at δ 4.06, which appears as a broad doublet (J=6Hz, ½ w 1.5 Hz) showing maximum coupling with the C-5 proton and a negligible coupling with the C-7 proton (~92° dihedral angle). In all probability, the methoxyl at C-1 is present in the α-configuration as in the case of a large number of C_{19}-diterpenoid alkaloids (12). The cmr spectrum of forestine showed 31 signals corresponding to 33 carbon atoms of the molecule (Table 1). The signals at 131.8 and 113.8 ppm represent two carbons each and are assigned to the 2′,6′- and 3′, 5′-carbons, respectively, of the anisoyl group. According to the known substitution patterns, the remaining hydroxyl groups in the partial structure (**5**) can be assigned two of the five possible sites, *viz*: C-7, C-8, C-9, C-10, or C-13, to accomodate the observed singlets at 73.7 and 76.1 ppm. Chemical shifts due to C-7, C-9, and C-10 bearing a hydroxyl group appear significantly downfield in the region 78-88 ppm. The two hydroxyls can therefore be placed at C-8 and C-13, leading to the assignment of structure (**6**) for forestine. The structure appears to show consistent cmr signals when compared with the related alkaloids yunaconitine (**4**) (8-9) and foresaconitine (vilmorrianine C) (**1**) (1,8,9,13).

The alkaloid extracted at pH 10 (0.05%) after column and thick-layer chromatography on alumina afforded foresticine (**7**), mp 79-80°, $[\alpha]^{21}D$ −1.9° (c 1%, $CHCl_3$) as colorless plates. The high resolution ms, 437.2785, indicated a molecular formula $C_{24}H_{39}NO_6$ for the compound. The ir spectrum showed a hydroxyl band and no peaks in the carbonyl region. The pmr spectrum (90 MHz; $CDCl_3$) exhibited the following signals: δ 1.12 (3H, t, J=7 Hz; N-CH_2-CH_3), 3.32, 3.4, 3.42 (3H each, s, OCH_3), 4.2 (1H, t, J=4.5 Hz; H-14β), 4.9 (1H, d, J=7 Hz, H-6β). These data suggested that foresticine is also a C_{19}-diterpenoid alkaloid containing an *N*-ethyl group, three aliphatic methoxyls, and three hydroxyl groups. The noise decoupled cmr spectrum exhibited 23 signals for the 24 carbon atoms of the molecule (Table 1). The modulated off-resonance decoupled spectrum showed three singlets at 39.1, 50.6, and 74.0 ppm, which can be attributed to the quaternary carbon atoms C-4, C-11, and C-8 (bearing an oxygen function), respectively. The methoxyls whose signals appear at 56.1, 56.4, and 59.2 ppm can be assigned to carbons C-1, C-16 and C-18, respectively, on the basis of the known substitution pattern in this class of alkaloids (11). Two of the remaining three hydroxyls should be placed at C-8 and C-14 on biogenetic considerations, consistent with the ^{13}C-chemical shift assignments. The third hydroxyl group can occupy either the C-3 or C-6 position in conformity with the aconitine class, although the former alternative is less likely from the observed coupling constant of 7 Hz at δ 4.9.

Foresticine on acetylation with Ac_2O-pyridine afforded the 6,14-diacetate (**8**). In the pmr spectrum, **8** showed the following signals: δ 1.03 (3H, t, J=7.5 Hz, N-CH_2-CH_3), 2.03, 2.1 (each 3H, OAc), 3.29, 3.31 (9H, s, OCH_3), 4.85 (1H, t, J=4.5 Hz,

TABLE 1. Cmr Chemical Shifts and Assignments for Forestine (**6**), Foresaconitine (**1**), Yunaconitine (**4**), Foresticine (**7**), Chasmanine (**2**), Talatizamine (**3**), 6,14-Diacetylforesticine (**8**) and 6,14-Dimethoxyforesticine (**9**)

Atom	**6**	**1**(1)	**4**(8)	**7**	**2**(6)	**3**	**8**	**9**
C(1)	85.4	85.1	83.2	85.7	86.1	86.1(d)	84.4	85.4
C(2)	26.0	26.4	33.7	25.8	26.0	25.8(t)	26.2	26.5
C(3)	34.9	34.9	71.3	34.8	35.2	32.6(t)	34.9	35.0
C(4)	39.3(s)	39.1	43.2	39.1(s)	39.5	38.6(s)	38.8(s)	39.0(s)
C(5)	49.6[a]	49.2	47.4	49.3	48.8	37.9(d)	49.3	48.3
C(6)	82.5[b]	82.9[a]	82.3	71.9	82.5[a]	24.8(t)	74.7	84.7
C(7)	49.2[a]	44.9	44.8	54.3	52.8	46.1(d)[a]	55.0	52.8
C(8)	73.7(s)	85.9	85.6	74.0(s)	72.6	72.8(s)	73.8(s)	73.9(s)
C(9)	53.6	49.3	48.8	48.9	50.3	47.0(d)[a]	46.9	45.4
C(10)	42.3	43.9	40.8	38.7	38.4	45.9(d)[a]	37.6	39.0
C(11)	50.2(s)	50.3	50.3	50.6(s)	50.4	48.7(s)	49.0(s)	50.2(s)
C(12)	36.4	29.0	35.3	28.8	28.6	27.9(t)	29.0	30.1
C(13)	76.1(s)	39.1	74.8	45.6	45.7	47.0(d)[a]	45.3	41.9
C(14)	80.1	75.4	78.6	75.3	75.5	75.6(d)	76.3	83.6
C(15)	41.9	37.9	39.6	39.4	39.2	39.0(t)	39.7	37.2
C(16)	83.3[b]	83.5[a]	83.6	82.2	82.2[a]	82.2(d)	82.2	82.6
C(17)	62.2	61.7	61.6	62.6	62.4	62.7(d)	62.0	61.9
C(18)	80.6	80.4	76.6	80.8	80.8	79.5(t)	80.6	80.9
C(19)	53.6	53.8	48.8	54.3	54.0	53.2(t)	54.0	54.0
N-CH_2	48.3	49.0	47.4	50.4	49.3	49.4(t)	50.7	49.1
CH_3	13.6	13.4	13.3	13.5	13.6	13.6(q)	13.4	13.6
C(1)OCH_3	56.3	56.6	56.8	56.1	56.3	56.1(q)	56.0	56.4
C(6)OCH_3	58.3	57.8	58.8	—	57.2	—	—	57.6
C(16)OCH_3	57.5	56.0	57.8	56.4	55.9	56.4(q)	56.0	56.2
C(18)OCH_3	59.2	59.1	59.1	59.2	59.2	59.4(q)	59.3	59.2
C(14)OCH_3	—	—	—	—	—	—	—	57.6
-C=O	—	169.8	169.9	—	—	—	171.2(s)	—
CH_3	—	21.8	21.7	—	—	—	21.7, 21.4	—
-C=O	166.6(s)	166.2	166.1	—	—	—	—	—
	122.4(s)	123.0	122.6	—	—	—	—	—
	131.8	131.8	131.7	—	—	—	—	—
	113.8	113.7	113.8	—	—	—	—	—
	163.4(s)	163.5	163.5	—	—	—	—	—
OCH_3	55.4	55.4	55.4	—	—	—	—	—

[a, b]The assignments may be interchanged in any vertical column.

H-14β), 5.74 (1H, d, J=7 Hz, H-6β). The structure (**7**) assigned to foresticine was confirmed by methylation to give 6,14-dimethoxy foresticine (**9**) as an amorphous solid identical with the methylation product of chasmanine (**2**). The cmr spectra of the two methylation products were identical (Table 1).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were taken on a Thomas-Kofler hot stage equipped with a microscope and polarizer. Uv and ir spectra were determined on Perkin-Elmer Model 202 and Perkin-Elmer 1420 ratio recording spectrometers, respectively. Rotations were taken on a Perkin-Elmer polarimeter model 141. Pmr spectra were obtained with a Varian EM-390 90 MHz nmr spectrometer with TMS as an internal standard. Cmr spectra were run on a JEOL-FX-60 spectrometer in $CDCl_3$ at 15.03 MHz in Fourier mode and the chemical shifts are reported in ppm downfield from TMS. Mass-spectra were determined on a Finnegan Quadrupole 4023 instrument.

PLANT MATERIAL.—Dried roots of *A. forrestii* Stapf were collected in Yunnan, China. The roots were authenticated by Dr. W.T. Wang, Beijing Institute of Botany, Academy of Sciences, Beijing, China. The voucher specimen is deposited in the Kunming Institute of Botany, Kunming, China.

EXTRACTION AND FRACTIONATION.—The dried and ground roots of *A. forrestii* (3.2 kg) were refluxed in 90% EtOH three times. The extract was evaporated to dryness *in vacuo* and the residue extracted with 2% HCl. The acidic extract was basified to pH 5-6 with NH_4OH and extracted with $CHCl_3$ to give extract A (4.09 g). The aqueous layer was basified with NH_4OH to pH 8 and extracted with $CHCl_3$ to give extract B (28.4 g). Further basification with NH_4OH to pH 10 and extraction with $CHCl_3$ afforded extract C (1.6 g).

ISOLATION OF FORESTINE (**6**), CHASMANINE (**2**), TALATIZAMINE (**3**), AND YUNACONITINE (**4**).—Extract B (18.57 g) was dissolved in a minimum volume of CH_2Cl_2 and chromatographed on neutral alumina (300 g; activity 3). Elution with hexane using increasing amounts of EtOAc gave the following fractions: hexane-10% EtOAc (6 liters), fraction I (3.2 g); hexane-15-40% EtOAc (3 liters), fraction II (5.9 g); hexane-50% EtOAc (1 liter), fraction III (2.2 g); EtOAc (1 liter), fraction IV (0.6 g).

Fraction I (1.15 g) was chromatographed on neutral alumina (40 g, activity 3) using 2-18% Me_2CO-hexane mixture and by thick-layer chromatography (alumina, PF 254, 20 cm×20 cm×3 mm, 45% Me_2CO in hexane) to give forestine (**6**, 260 mg) and chasmanine (**2**, 730 mg) as colorless amorphous compounds. Chasmanine was identified by its rotation $[\alpha]^{23}D$ +21.6° (c, 1% EtOH), ms M^+ *m/z* 451, pmr, cmr (see Table 1), and comparison wih an authentic sample. Fractions II and III were combined (8.1 g) and chromatographed on neutral alumina (200 g, activity 3) using 8-18% Me_2CO-hexane mixture as eluent to afford talatizamine (**3**, 240 mg), mp 143-144°, $[\alpha]^{23}D$ −4.4° (c, 1% $CHCl_3$), ms M^+ *m/z* 421; identical in tlc, mixture mp pmr, and cmr (see Table 1) with an authentic sample. The acetyl derivative of **3** was found to be identical with 14-acetyltalatizamine.

Fraction IV (0.24 g) was chromatographed on a thick layer plate (alumina PF-254, 20 cm×20 cm×3 mm, hexane-50% Me_2CO) to give yunaconitine (**4**, 120 mg), identical in its tlc behavior and cmr (see Table 1) spectra with those of an authentic sample.

ISOLATION OF FORESTICINE (**7**).—Extract C (1.6 g) was chromatographed on a thick-layer plate (alumina PF-254, 20 cm×20 cm×3 mm, CH_2Cl_2-10% MeOH) to give foresticine (**7**, 600 mg), mp 79-80°, $[\alpha]^{21}D$ −1.9° (c, 1%, $CHCl_3$). High resolution ms, found: 437.2785; calcd for $C_{24}H_{39}NO_6$: 437.2777.

METHYLATION OF FORESTICINE (**7**) AND CHASMANINE (**2**) TO GIVE 6,14-DI-METHOXYFORESTICINE (**9**).—A solution of **7** (60 mg) in *p*-dioxane was heated with sodium hydride (100 mg) and methyl iodide (0.5 ml) in a sealed tube at 110° for 40 h. The reaction mixture was diluted with H_2O, the solvent removed *in vacuo* and the residue extracted with CH_2Cl_2. The crude product showed two spots on tlc which were separated on thick-layer plates (alumina PF-254, 20 cm×20 cm×1 mm, hexane-40% Me_2CO). The major fraction (32 mg) was identified as **9** from its cmr spectrum (Table 1). A similar methylation of **2** afforded an identical product.

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