THE STRUCTURES OF FOUR NEW C_{19}-DITERPENOID ALKALOIDS FROM *ACONITUM FORRESTII* STAPF

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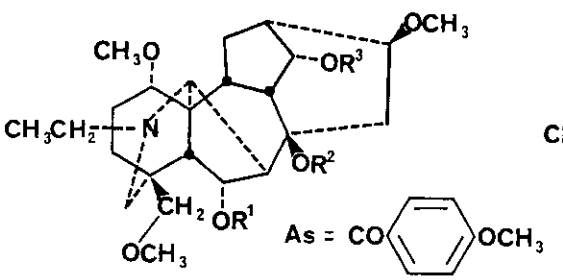
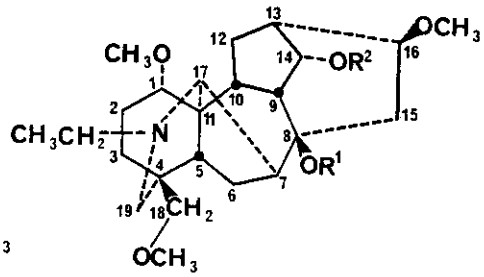
Abstract - From the root extracts of Aconitum forrestii Stapf, we have isolated four new alkaloids: acoforine (6), acoforesticine (8), acoforestine (10) and acoforestinine (14), besides crassicauline A, forestine and yunaconitine. The structures of these alkaloids were determined on the basis of spectral data and correlation with alkaloids of established structures. The structure and stereochemistry of acoforestine (10) was confirmed by an X-ray crystal structure determination.

In an earlier investigation of the alkaloids of *Aconitum forrestii* Stapf[1] we had reported the isolation of chasmanine (1), talatizamine (2), yunaconitine (3), forestine (4), and foresticine (5), from the crude alkaloid fraction obtained at pH 8. In this communication we wish to record the results of our investigation on the constituents isolated from the alkaloidal fraction taken at pH 5-6. The roots of *A. forrestii* Stapf were extracted with boiling 95% ethanol and the crude alkaloid mixture was obtained by acidic extraction, basification with ammonium hydroxide to pH 5-6 and extraction with chloroform. Extensive chromatographic separation (see experimental) afforded seven alkaloids of which four have not been reported previously.

The first alkaloid to elute was obtained as an amorphous compound, $[\alpha]_D$ -35.3°C (c, 0.57, EtOH), and was designated *acoforine*. The molecular formula, $C_{28}H_{45}NO_6$, for this alkaloid was derived from the proton and ^{13}C-nmr spectral data. The ^1H-nmr spectrum showed two triplets at δ 1.07 (J = 7.5 Hz) and 1.09 (J= 7.5 Hz) assigned to primary methyl groups. The spectrum also exhibited signals at δ 2.97 (3H, s, $OCOCH_3$), 3.23, 3.24, 3.26 (each 3H, s, OCH_3) and 4.65 (1H, t, J = 4.5 Hz C(14)-α-H), indicating that acaforine is a C_{19}-diterpenoid alkaloid having an *N*-ethyl, an *O*-ethyl, three methoxyls and one acetyl group. The ^{13}C nmr spectrum showed 27 lines corresponding to 28 carbon atoms of the molecule and the pattern of the chemical shifts (Table 1) indicated that the structure of acoforine is **6**. We have recently reported the isolation and structure of columbidine (**7**) from *Aconitum columbianum* Nutt.[2] Acoforine was shown to be identical in its tlc, ir, ^1H-nmr and ^{13}C-nmr spectra with those of 14-acetylcolumbidine. The structure of columbidine had earlier been established by a correlation with talatizamine.[2] As the structure of talatizamine has been proven by synthesis[3] and also by correlation with

isotalatizidine and condelphine (X-ray structure)[4,5] the structure of acoforine is secure.



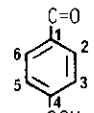


1 CHASMANINE $R^1 = CH_3$; $R^2 = R^3 = H$
2 TALATIZAMINE $R^1 = R^2 = H$
5 FORESTICINE $R^1 = R^2 = R^3 = H$
6 ACOFORINE $R^1 = C_2H_5$; $R^2 = Ac$
7 COLUMBIDINE $R^1 = C_2H_5$; $R^2 = H$
8 ACOFORESTICINE $R^1 = CH_3$; $R^2 = H$; $R^3 = As$
9 FORESACONITINE $R^1 = CH_3$; $R^2 = Ac$; $R^3 = As$

The fraction which eluted next, designated as *acoforesticine* was obtained as an amorphous compound, $[\alpha]_D$ +40.5° (c, 0.92, EtOH). The molecular formula, $C_{33}H_{47}NO_8$, was determined from the mass spectrum m/z, 570 (M^+ -15) and the proton and ^{13}C-nmr spectra. The ^1H-nmr spectrum showed the following signals: δ 1.08 (3H, t, J = 7.5 Hz, $NCH_2\underline{CH_3}$), 3.2, 3.26, 3.29, 3.3 (each 3H, s, $OC\underline{H_3}$), 3.84 (3H, s, aromatic $C\underline{H_3}$), 4.14 (1H, d, J = 6.5 Hz, C(6)-β-H), 5.11 (1H, t, J = 5 Hz, C(14)-α-H), 6.92, 7.95 (each 2H, d, J = 9 Hz, AB q, aromatic H). The loss of 31 mass units (m/z 554) from the molecular ion to give the base peak suggested a methoxyl group at C(1) of a C_{19}-diterpenoid alkaloid.[6] The ^{13}C-nmr spectrum (Table 1) is in accordance with structure **8** for acoforesticine. The spectrum closely resembles that of foresaconitine (**9**) which has also been isolated from *A. forrestii* Stapf var. *albo-villosum* (Chen et Liu) W. T. Wang[7] and *A. forrestii* Diels.[8,9] Acetylation of acoforesticine afforded foresaconitine (**9**) identified by comparison with an authentic sample.

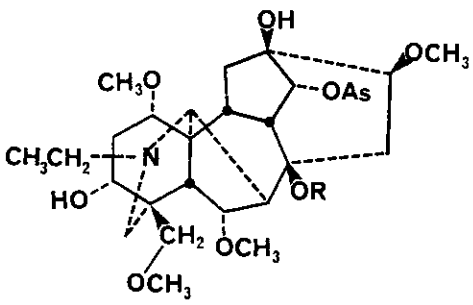
The next fraction obtained in the chromatographic separation gave colorless crystals, mp 203-204°C, $[\alpha]_D$ +23.4° (c, 0.56, EtOH), designated as *acoforestine*. The alkaloid, $C_{35}H_{51}NO_9$, showed in the mass spectrum the molecular ion peak at m/z 629 and the base peak at m/z 598 owing to the loss of a methoxyl (M^+-31). The ^1H nmr spectrum exhibited the following signals: δ 0.6 (3H, t, J = 7.5 Hz, $OCH_2\underline{CH_3}$), 1.09 (3H, t, J = 7.5 Hz, $NCH_2\underline{CH_3}$), 3.26 (6H, s, OCH_3), 3.29, 3.5 (each 3H, s, $OC\underline{H_3}$), 3.84 (3H, s aromatic $OC\underline{H_3}$), 4.1 (1H, d, J = 7 Hz, C(6)-β-H), 4.81 (1H, d, J = 5 Hz, C(14)-α-H), 6.88, 8.0 (4H, AB q, J = 9 Hz, aromatic H). The proton and ^{13}C-nmr spectra suggested that acoforestine is a C_{19}-diterpenoid alkaloid and the assignments of the carbon atoms (Table 1) are in conformity with structure **10**. This structure was confirmed by synthesis of acoforestine by heating an ethanolic solution of crassicauline A (**11**) in a sealed tube at 130-135°C. Crassicauline A (**11**) has been isolated from *Aconitum crassicaule* W.T. Wang[10] and *A. forrestii* Diels.[8] Although the structure of the alkaloid was based on its transformation to triacetylbikhaconine, no direct comparison appears to have been made. In order to establish the structure (**10**) proposed for acoforestine and also its stereochemistry, we undertook an X-ray crystal structure determination (see below).

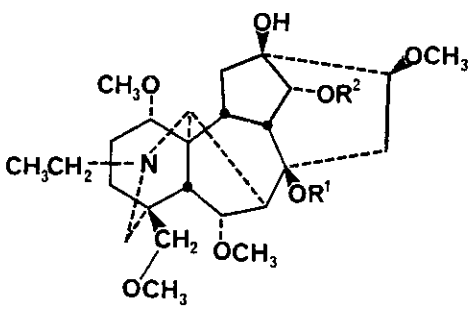
Table 1. ^{13}C nmr chemical shifts and assignments for acoforine (**6**), columbidine (**7**), acoforesticine (**8**), foresaconitine (**9**)[7], acoforestine (**10**), crassicauline A (**11**), acoforestinine (**14**), yunaconitine (**3**) and 8-deacetyl-8-*O*-propylyunaconitine (**15**).

Carbons	6	7	8	9	10	11	14	3	15
C(1)	85.5	85.8	85.4	85.1	85.4	84.9	85.8	83.2	82.7
C(2)	26.3	26.1	26.2	26.4	26.4	26.3	33.9	33.8	33.3
C(3)	32.4	32.6	34.8	34.9	34.8a	34.8a	71.5	71.0	71.8
C(4)	38.1	38.5	39.2	39.1	39.3	39.1	43.5	43.3	43.1
C(5)	40.9	40.7b	49.1	49.2	48.7	49.1	47.8	47.3	47.6
C(6)	24.0	23.9	82.7	82.9a	84.5	83.0a	83.5	82.3a	83.2
C(7)	43.1	46.2a ,b	46.9	44.9	46.7	45.1	46.5	44.8a	45.9
C(8)	77.5	78.1	73.8	85.9	78.3	85.5	78.5	85.6	78.2
C(9)	45.7a	45.5a	49.1	49.3	49.1	49.1	48.7	48.9	48.6
C(10)	45.1a	45.8a	45.1	43.9	41.7	41.0	41.6	40.8	41.4
C(11)	49.0	48.8	50.3	50.3	50.9	50.1	50.9	50.2	50.8
C(12)	28.9	28.7	29.1	29.0	37.3	35.8a	36.2	35.3	35.6
C(13)	38.3	38.7	41.3	39.1	75.6	74.8	75.4	74.8	75.2
C(14)	75.8	75.1	76.7	75.4	79.3	78.5	79.3	78.6	79.1
C(15)	36.3	34.7	36.9	37.9	36.8a	39.1	37.6	39.6	37.8
C(16)	83.4	82.5	81.7a	83.5a	83.3	83.6a	84.3	83.6s	83.9
C(17)	61.7	62.5	61.9	61.7	61.3	62.0	61.0	61.7	61.0
C(18)	79.7	79.6	80.7	80.4	80.4	80.3	76.7	76.5	77.0
C(19)	53.1	53.1	54.0	53.8	54.2	53.6	49.2	48.9	43.1
N-CH_2	49.3	49.3	49.6	49.0	49.6	49.1	46.5	47.3	45.7
CH_3	13.5	13.6	13.5	13.4	13.5	13.4	13.4	13.4	13.3
C(1)'	56.2	56.1	56.2	56.6	56.1	56.1	55.8	55.9	55.9
C(6)'	-	-	57.6	57.8	58.8	57.8	58.8	58.8	58.7
C(16)'	56.2	56.3	56.0	56.0	58.8	58.7	58.8	57.8	58.7
C(18)'	59.4	59.4	59.2	59.1	59.1	59.1	59.1	59.1	59.1
C(8)-OCH_2	55.6	55.8	-	-	55.9	-	55.8	-	-
CH_3	16.3	16.2	-	-	15.3	-	15.3	-	-
C(8)-OCH_2	-	-	-	-	-	-	-	-	62.4
CH_2	-	-	-	-	-	-	-	-	23.2
CH_3	-	-	-	-	-	-	-	-	10.5
C=O	171.4		-	169.8	-	169.8	-	169.8	-
CH_3	21.4		-	21.8	-	21.6	-	21.6	-
			166.2	166.2	166.4	166.0	166.2	166.0	166.2
		1	122.8	123.0	123.8	122.7	123.6	122.6	123.2
		2,6	131.6	131.8	131.9	131.7	131.8	131.7	131.8
		3,5	113.7	113.7	113.6	113.7	113.6	113.8	113.4
		4	163.3	163.5	163.3	163.3	163.4	163.5	163.1
			55.4	55.4	55.4	55.4	55.4	55.4	55.4

a The assignments may be interchanged in any vertical column.

b Literature values have been reworked.



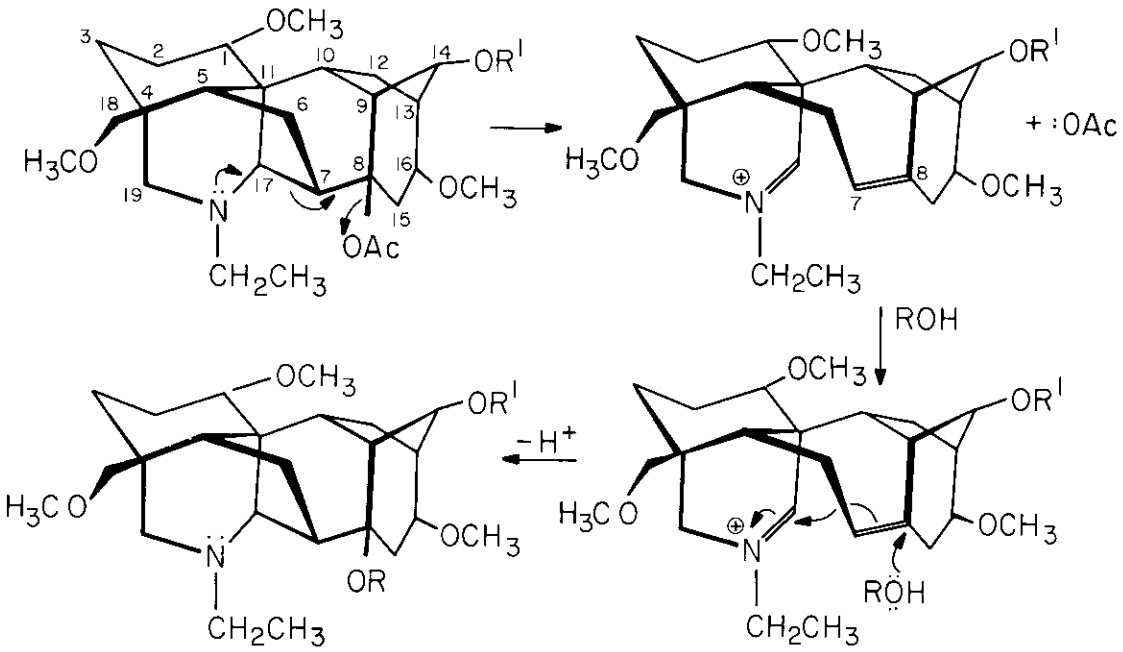


3 YUNACONITINE R=Ac
14 ACOFORESTININE R=C_2H_5
15 R=$CH_2CH_2CH_3$

4 FORESTINE R^1=H; R^2=As
10 ACOFORESTINE R^1=C_2H_5; R^2=As
11 CRASSICAULINE A R^1=Ac; R^2=As
12 CHASMACONITINE R^1=Ac; R^2=COPh
13 LIWACONITINE R^1=R^2=As

The next two fractions from the chromatogram were identified as crassicauline A (**11**) and forestine (**4**) by comparison with authentic samples. Two closely related alkaloids, chasmaconitine (**12**) and liwaconitine (**13**) have been isolated from *A. forrestii* Diels[8] and *A. forrestii* Stapf.[9] The sixth alkaloid to be isolated in the eluting order was an amorphous compound designated as *acoforestinine*, $C_{35}H_{51}NO_{10}$, M^+ m/z 645, $[\alpha]_D$ +27.3° (c, 0.71, EtOH). Major fragments in the mass spectrum appeared at m/z 614 (M^+-31) indicating a methoxyl group at C(1) of a C_{19}-diterpenoid alkaloid, and at m/z 135 suggestive of an anisoyl group ($C_8H_7O_2$). The ^{1}H-nmr spectrum showed the following signals: δ 0.6 (3H, t, J = 7.5 Hz, OCH_2-CH_3), 1.08 (3H, t, J = 7.5 Hz, NCH_2-CH_3), 3.26, 3.28, 3.32, 3.53 (each 3H, s, OCH_3), 3.84 (3H, s, aromatic OCH_3), 4.03 (1H, d, J= 5 Hz, C(6)-β-H), 4.82 (1H, d, J= 5 Hz, C(14)-α-H), 6.84, 7.97 (4H, AB q, J= 9 Hz, aromatic-H). On the basis of the above data and the ^{13}C-nmr assignments (see Table 1), structure **14** was proposed for acoforestinine. The structure has been confirmed by synthesis of **14**, by heating a solution of yunaconitine (**3**) in ethanol in a sealed tube at 130-135°C.

The last alkaloid isolated from the chromatographic separation was identified as yunaconitine (**3**). Heating yunaconitine with *n*-propanol at 130-135°C gave by replacement of the 8-acetoxyl group, the corresponding 8-propylether (**15**). The facile conversion of the 8-acetoxy group in some C_{19}-diterpenoid alkaloids to the 8-alkoxy compounds can be considered as a synchronous fragmentation process such as described by Grob.[11] The free electron pair of the nitrogen atom is oriented anti and parallel (anti-periplanar) with respect to the C C bond which undergoes cleavage as shown:



R = alkyl; R^1 = alkyl or ester group

X-Ray crystal structure determination of acoforestine. Crystals of acoforestine suitable for X-ray analysis were obtained by slow crystallization of a dilute solution in methanol. A crystal of size 0.18 x 0.18 x 0.65 mm mounted on a glass fiber was used to determine the cell dimensions and intensity data collection. Acoforestine crystallises in the monoclinic space group $P2_1$ with a unit cell of dimensions a = 10.300(2), b = 11.899(5), c = 13.817(2)Å and β = 90.75(2)°, volume V = 1693Å^3. Calculated density for two formula units in the unit cell is 1.235 g cm^{-3}. Intensity data were collected on an Enraf-Nonius CAD-4 automated diffractometer using CuKα (λ = 1.5405Å) radiation. Out of 3740 unique reflections measured using ω-2θ scan technique, 1875 reflections had intensities greater than 2σ (I) and hence were used in structure determination and refinement. Intensities were corrected for Lorentz polarisation effects in an empirical absorption correction[17] was also applied.

The structure was solved using the direct methods program MULTAN 80.[18] Using 308 reflections with E>1.575, 48 phase sets were generated. An E-map calculated using the phases from the set with the highest combined figure of merit gave a 42 atom fragment. The structure was further developed by successive weighted fourier maps. The asymmetric unit contains one molecule of acoforestine, a total of 45 non-hydrogen atoms. The structure was refined by full matrix least squares technique. All the atoms were treated isotropically in the inital stages and changed to anisotropic thermal parameters in the final stages of the refinement. The factor minimised in the least squares refinement was $\Sigma |F_o-F_c|^2/w$ where $w = 1/\sigma^2(F)$. The final R-factor based on 1875 observed reflection is 0.059 (R_w = 0.057).

Table 2 gives the final atomic parameters while bond lengths and angles are given in Tables 3 and 4. The average estimated standard deviation in bond lengths and angles involving non hydrogen atoms are 0.009Å and 0.7°, respectively. The C(2)-C(3) bond distance is 1.452(6)Å which indicates a partial double bond character. The N-C bond lengths vary from 1.450(5)Å to 1.468Å but agree well with the expected bond lengths. Other bond distances compare well with the values reported for other diterpenoid alkaloids.[19]

The conformation of acoforestine is shown in the perspective drawing (Figure 1).[20] The molecule contains four six membered and two five membered ring systems fused together. Three of the four six membered rings are in a chair conformation while the fourth ring D has a boat conformation. Ring A [C(1), C(2), C(3), C(4), C(5), C(11)] is a flattened distorted chair (Table 5) with C(1) and C(4) below (0.4Å) and above (0.7Å), respectively, the plane through the atoms C(2), C(3), C(5) and C(11). The ring system B [C(4), C(5), C(11), C(17), N, C(19)] has a chair conformation with C(4) 0.7Å below and C(17) 0.8Å above the plane through C(5), C(11), N and C(19). The six membered ring C [C(7), C(8), C(9), C(10), C(11), C(17)] is also a distorted chair with C(7) 0.8Å below and C(10) 0.5Å above the plane through C(8), C(9), C(11), and C(17). Ring D [C(8), C(9), C(14), C(13), C(16), C(15)] has a half-boat conformation with C(14) and C(15) forming the end atoms above the plane through C(8), C(9), C(13) and C(16) by 0.86Å and 0.26Å, respectively. The five membered ring systems E [C(5), C(6), C(7), C(17), C(11)] and F [C(9), C(10), C(12), C(13), C(14)] have a distorted half chair conformation as indicated by the parameter[21] ϕ = 301°, and τ_m = 48.6° for E and ϕ = 114° and τ_m = 53.7° for F.

There is no good inter or intramolecular hydrogen bond formation in acoforestine. The shortest contact is 3.168Å between N and O(1) which is too long to form a hydrogen bond. Unlike pyroneoline-like structures that have an intramolecular hydrogen bond to stabilise the boat conformation for ring A, acoforestine assumes a chair conformation. Although ring A in acoforestine and pyrodelphinine[19] have a chair conformation, the ring D conformation is different, apparently due to a double bond between C(8)-C(15) in pyrodelphinine in contrast to a single bond in acoforestine.

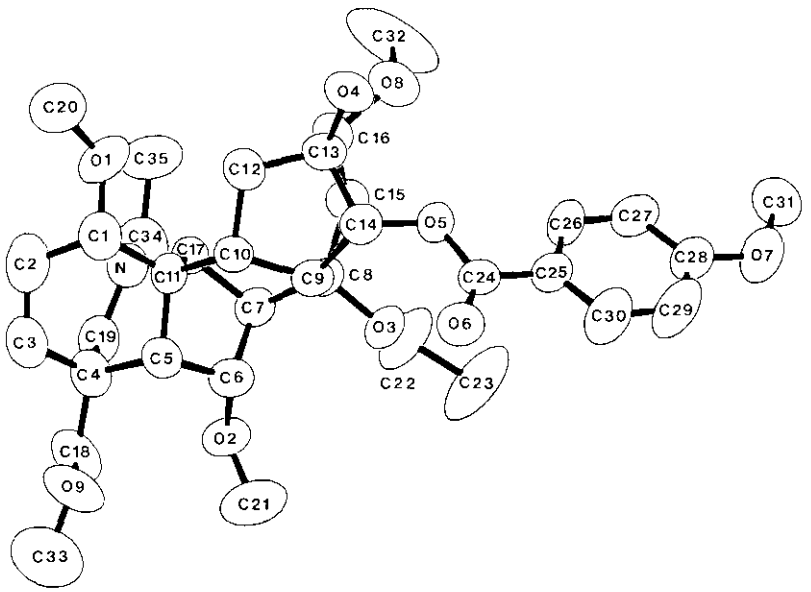


Table 2. Table of positional parameters and their estimated standard deviations

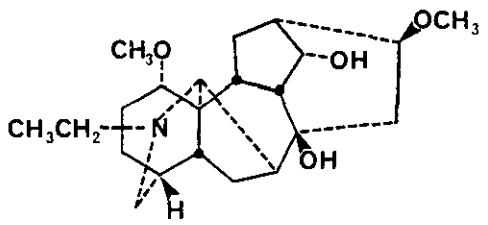
Atom	X	Y	Z	B(A2)
N	0.2649(5)	0.0126(5)	0.4497(4)	4.5(1)
O(1)	0.1021(4)	0.2206(5)	0.3723(4)	7.0(1)
O(2)	0.6060(4)	0.0500(4)	0.5164(3)	5.1(1)
O(3)	0.6631(4)	0.1107(4)	0.2661(3)	4.5(1)
O(4)	0.3207(5)	0.3548(4)	0.0806(3)	5.7(1)
O(5)	0.5943(4)	0.2784(4)	0.1200(3)	4.3(1)
O(6)	0.7537(4)	0.3753(4)	0.1920(4)	5.8(1)
O(7)	1.0396(5)	0.127	-0.1546(4)	8.3(2)
O(8)	0.3661(5)	0.1522(5)	0.0304(3)	6.7(1)
O(9)	0.4822(5)	0.2208(5)	0.7078(3)	7.7(2)
C(1)	0.1945(7)	0.2510(6)	0.4471(5)	5.4(2)
C(2)	0.1385(7)	0.2092(7)	0.5408(6)	6.9(2)
C(3)	0.2379(7)	0.1953(7)	0.6153(5)	6.2(3)
C(4)	0.3565(7)	0.1313(6)	0.5845(5)	4.8(2)
C(5)	0.4248(6)	0.1944(6)	0.5031(4)	4.3(2)
C(6)	0.5369(6)	0.1284(6)	0.4566(5)	4.5(2)
C(7)	0.4762(6)	0.0704(6)	0.3687(5)	3.9(1)
C(8)	0.5251(6)	0.1266(6)	0.2766(5)	3.9(1)
C(9)	0.5110(6)	0.2562(5)	0.2885(5)	4.0(2)
C(10)	0.3823(6)	0.2889(6)	0.3392(4)	3.7(1)
C(11)	0.3266(6)	0.2084(6)	0.4179(4)	3.9(1)
C(12)	0.2888(7)	0.3165(6)	0.2497(5)	4.9(2)
C(13)	0.3660(6)	0.2891(6)	0.1596(5)	4.3(2)
C(14)	0.5037(6)	0.3149(5)	0.1917(5)	4.1(2)
C(15)	0.4556(6)	0.0858(6)	0.1821(5)	4.3(2)
C(16)	0.3533(6)	0.1607(6)	0.1341(4)	4.7(2)
C(17)	0.3301(6)	0.0876(5)	0.3814(4)	3.8(1)
C(18)	0.4439(8)	0.1134(6)	0.6749(5)	6.5(2)
C(19)	0.3238(7)	0.0138(6)	0.5459(5)	5.0(2)
C(20)	0.9799(8)	0.2856(9)	0.3676(6)	8.9(3)
C(21)	0.7230(8)	0.0862(7)	0.5519(7)	7.7(2)
C(22)	0.2964(7)	0.4972(7)	0.7428(7)	7.5(2)
C(23)	0.8483(8)	-0.0011(9)	0.2320(9)	11.8(4)
C(24)	0.7167(6)	0.3093(6)	0.1307(5)	4.4(2)
C(25)	0.8000(6)	0.2585(6)	0.0587(5)	4.6(2)
C(26)	0.7561(7)	0.1786(8)	-0.0010(5)	6.1(2)
C(27)	0.8327(7)	0.1277(7)	-0.0721(5)	6.3(2)
C(28)	0.9575(7)	0.1658(7)	-0.0848(5)	6.2(2)
C(29)	0.0018(8)	0.2512(8)	0.9755(7)	8.8(3)
C(30)	0.9271(7)	0.2909(8)	0.0468(6)	8.1(2)
C(31)	0.9912(8)	0.0473(9)	-0.2191(5)	8.0(3)
C(32)	0.309(1)	0.0497(8)	0.9920(6)	13.5(4)
C(33)	0.535(1)	0.2192(9)	0.7977(7)	10.4(3)

Table 2 continued.

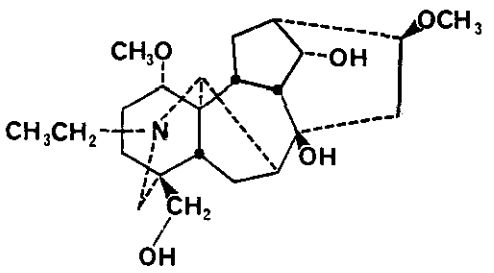
Atom	X	Y	Z	B(A2)
C(34)	0.2568(8)	-0.1024(6)	0.4153(6)	6.0(2)
C(35)	0.8420(8)	0.3856(8)	0.6736(7)	8.6(3)

Anisotropically refined atoms are given in the form of the isotropic equivalent thermal parameter defined as: (4/3) • [a2*β(1,1) + b2*β(2,2) + c2*β(3.3) + ab(cos γ)*β(1,2) + ac(cos β)*β(1,3) + bc(cos α)*β(2,3)]

A few C_{19}-diterpenoid alkaloids containing a methoxyl group at C(8), have been reported in the literature, e.g. alkaloid A[12], ambiguine[13], puberaconitidine[14], septentrionine[15] and 8-*O*-methyltalatizamine[16]. Columbidine is the only alkaloid reported with an ethoxyl group at C(8)[2]. The question as to whether the alkaloids containing a methoxyl or an ethoxyl group at C(8) are artefacts or are present in the plant cannot be answered unequivocally as pointed out earlier.[2] The three closely related alkaloids aconosine (**16**)[8,9] cammaconine (**17**)[8,9] and talatizamine (**2**)[1] known to be present in *A. forrestii* are unlikely to give acoforine (**6**). The precursor should be 8,14-diacetyltalatizamine, but this compound has not been isolated so far from this plant. Moreover, it is not clear why the 8-acetyl group of foresaconitine (**9**) present in this plant is not replaced by an ethoxy group during extraction of the plant with hot ethanol. Instead, acoforesticine (**8**), which is the deacetylation product of **9**, has been obtained during the isolation.



16 ACONOSINE



17 CAMMACONINE

EXPERIMENTAL

Melting points were taken on a Thomas-Kofler hot stage equipped with a microscope and polarizer. Ir spectra were taken on Perkin-Elmer model 1420 ratio recording spectrometer and rotations were determined on Perkin-Elmer polarimeter model 141. ^{1}H-nmr spectra were taken on Varian EM-390 90MHz nmr spectrometer with TMS as an internal reference. ^{13}C-nmr spectra were run on JEOL-FX-60 and JEOL-FX-90Q nmr spectrometers in $CDCl_3$; chemical shifts are reported in ppm downfield from TMS. Mass spectra were determined on a Finnegan Quadrupole 4023 instrument.

Extraction and fractionation. Powdered roots of *A. forrestii* collected in Yunnan, China (3.2 kg) were extracted with boiling 95% ethanol (three times) at the Kunming Institute of Botany, Kunming, China. The residue obtained by evaporation of the solvent was processed at the University of Georgia. It was extracted with 2% HCl and the acidic extract made basic (pH 5-6) with ammonium hydroxide and extracted with chloroform to give a crude alkaloid mixture (4.09 g).

Isolation of acoforine (**6**), acoforesticine (**8**), acoforestine (**10**), crassicauline A (**11**), forestine (**4**), acoforestinine (**14**) and yunaconitine (**3**). A solution of the crude alkaloid (3.0 g) in methylene chloride was evaporated with 8 g of alumina. The mixture was placed on top of a column filled with 130 g of alumina (activity III) and eluted with a mixture of hexane and increasing amounts of acetone. The column was finally washed with 400 ml of a mixture of acetone: hexane:methanol (50:50:4). Two hundred and fifty fractions were collected and the fractions were pooled after monitoring by tlc as shown below.

No. of fractions	Eluting Solvent	Weight (mg)	No. of major tlc spots	Alkaloid isolated	Rf*
1-69 (1650 ml)	hexane:acetone (98:2)	-	-	-	-
70-94 (600 ml)	hexane:acetone (96:4)	33	two	6	0.7
95-105 (250 ml)	hexane:acetone (96:4)	56	three		
106-115 (250 ml)	hexane:acetone (92:8)	66	two	8	0.5
116-130 (350 ml)	hexane:acetone (92:8)	50	three		
131-150 (480 ml)	hexane:acetone (88:12)	336	two	10,11	0.45,0.4
151-170 (480 ml)	hexane:acetone (88:12)	330	two	4	0.3
171-195 (570 ml)	hexane:acetone (82:18)	110	two		
196-200 (100 ml)	hexane:acetone (82:18)	170	two	14	0.1
201-207 (150 ml)	hexane:acetone (73:27)	356	three		
208-225 (400 ml)	hexane:acetone (73:27)	610	one	3	0.08
226-250 (570 ml)	hexane:acetone (73:27)	180	four		

*Alumina plate, hexane:acetone 60:40, visualization by iodine vapors.

By preparative tlc on 0.25 mm alumina plates, fractions 70-94 and 106-115 afforded acoforine (**6**, 14 mg) and acoforesticine (**8**, 50 mg), respectively. Acoforestine (**10**, 32 mg) and crassicauline A (**11**, 120 mg) were isolated from fractions 131-150 by a combination of column chromatography and preparative tlc. Fractions 151-170 afforded after two preparative tlc separations forestine **4** (140 mg). Preparative tlc of fractions 196-200 afforded acoforestinine (**14**, 103 mg). Yunaconitine (**3**, 140 mg) was isolated by preparative tlc of fractions 208-225. The alkaloids **3**, **4** and **11** were identified by comparison of mp, mmp, ir, ^{1}H-nmr and ^{13}C-nmr spectra with those of authentic samples of yunaconitine, forestine and crassicauline A, respectively.

Preparation of acoforestine (**10**) from crassicauline A (**11**).

Crassicauline A (28 mg) was heated with absolute ethanol (5 ml) in a sealed tube at 130-135°C for 24 h. The crude product (29 mg) was chromatographed through a small column of alumina (activity

III, 2 g) using methylene chloride as eluent. The residue (21 mg) crystallized from methanol, mp 203°C, and was identical with those of acoforestine in its mmp, co-tlc and ir spectrum.

Preparation of foresaconitine (vilmorrianine C) (**9**) from acoforesticine (**8**).
Acoforesticine (22 mg) was heated at 95°C with acetic anhydride (10 ml) and *p*-toluenesulfonic acid (18 mg) for 4 h. The crude product (22 mg) was chromatographed on a short column of alumina and eluted sucessively with benzene (20 ml), methylene chloride (20 ml) and methylene chloride: 1% methanol (20 ml). The methylene chloride eluates afforded a solid (18 mg) which crystallized from ether, mp 154-156°C. Comparison of the mmp, ^{1}H-nmr, and ^{13}C-nmr spectra with those of an authentic sample of foresaconitine showed them to be identical.

Preparation of acoforestinine (**14**) from yunaconitine (**3**). Yunaconitine (81 mg) was heated at 130-135°C with absolute ethanol (5 ml) in a sealed tube for 24 h. The crude product was purified by vacuum liquid chromatography to afford acoforestinine **14** (67 mg). The identity was established by comparison of the tlc, ^{1}H-nmr and ^{13}C-nmr spectra with those of the sample isolated from *Aconitum forrestii*.

Preparation of 8-deacetyl-8-*O*-propylyunaconitine (**15**). Yunaconitine (30 mg) was heated in a sealed tube at 130-135°C for 24 h with dry n-propanol. The crude product was purified by vacuum liquid chromatography to afford **15** as an amorphous compound. ^{1}H-nmr:δ 0.53 (3H, t, J=7 hz, $OCH_2CH_2\underline{CH_3}$), 1.11 (5H, m, $OCH_2\underline{CH_2}CH_3$ and $N\text{-}CH_2\underline{CH_3}$), 3.24, 3.27, 3.31, 3.52 (each 3H, s, $O\underline{CH_3}$), 3.84 (3H, s, $Ar\text{-}O\underline{CH_3}$), 4.04 (1H, d, J=6 Hz, C(6)-β-H), 4.8 (1H, d, J=5 Hz, C(14)-α-H), 6.9, 8.01 (4H, ABq, J=9 Hz, aromatic-H).

Table 3. Bond distances (Å) and their esd's in Acoforestine.

N -C(17)	1.468(5)	O(9)-C(18)	1.411(6)	C(9)-C(14)	1.511(6)
N -C(19)	1.455(5)	O(9)-C(33)	1.350(6)	C(10)-C(11)	1.564(5)
N -C(34)	1.450(5)	C(1)-C(2)	1.509(6)	C(10)-C(12)	1.591(5)
O(1)-C(1)	1.442(5)	C(1)-C(11)	1.512(6)	C(11)-C(17)	1.524(6)
O(1)-C(20)	1.478(6)	C(2)-C(3)	1.452(6)	C(12)-C(13)	1.522(6)
O(2)-C(6)	1.430(5)	C(3)-C(4)	1.506(6)	C(13)-C(14)	1.512(5)
O(2)-C(21)	1.365(5)	C(4)-C(5)	1.530(5)	C(13)-C(16)	1.573(6)
O(3)-C(8)	1.443(4)	C(4)-C(18)	1.546(6)	C(15)-C(16)	1.526(6)
O(3)-C(22)	1.419(6)	C(4)-C(19)	1.533(6)	C(22)-C(23)	1.535(7)
O(4)-C(13)	1.416(5)	C(5)-C(6)	1.543(5)	C(24)-C(25)	1.454(6)
O(5)-C(14)	1.437(5)	C(5)-C(11)	1.551(5)	C(25)-C(26)	1.333(6)
O(5)-C(24)	1.319(5)	C(6)-C(7)	1.524(6)	C(25)-C(30)	1.377(6)
O(6)-C(24)	1.214(5)	C(7)-C(8)	1.528(5)	C(26)-C(27)	1.406(6)
O(7)-C(28)	1.371(5)	C(7)-C(17)	1.531(5)	C(27)-C(28)	1.376(6)
O(7)-C(31)	1.390(6)	C(8)-C(9)	1.558(5)	C(28)-C(29)	1.387(7)
O(8)-C(16)	1.444(4)	C(8)-C(15)	1.558(5)	C(29)-C(30)	1.344(7)
O(8)-C(32)	1.452(6)	C(9)-C(10)	1.556(5)	C(34)-C(35)	1.592(7)

Table 4. Bond angles (°) and their esd's in Acoforestine.

C(17)-N -C(19)	113.1(3)	C(11)-C(10)-C(12)	116.3(3)
C(17)-N -C(34)	112.9(4)	C(1)-C(11)-C(5)	114.5(4)
C(19)-N -C(34)	109.3(4)	C(1)-C(11)-C(10)	108.6(4)
C(1)-O(1)-C(20)	117.0(4)	C(1)-C(11)-C(17)	115.4(4)
C(6)-O(2)-C(21)	115.6(4)	C(5)-C(11)-C(10)	110.7(3)
C(8)-O(3)-C(22)	115.1(3)	C(5)-C(11)-C(17)	97.6(3)
C(14)-O(5)-C(24)	117.8(3)	C(10)-C(11)-C(17)	109.7(3)
C(28)-O(7)-C(31)	117.5(4)	C(10)-C(12)-C(13)	105.9(3)
C(16)-O(8)-C(32)	112.4(4)	O(4)-C(13)-C(12)	110.0(3)
C(18)-O(9)-C(33)	113.1(4)	O(4)-C(13)-C(14)	114.3(4)
O(1)-C(1)-C(2)	106.0(4)	O(4)-C(13)-C(16)	109.8(3)
O(1)-C(1)-C(11)	108.3(4)	C(12)-C(13)-C(14)	102.4(3)
C(2)-C(1)-C(11)	118.3(4)	C(12)-C(13)-C(16)	110.4(4)
C(1)-C(2)-C(3)	111.9(4)	C(14)-C(13)-C(16)	109.7(4)
C(2)-C(3)-C(4)	115.1(4)	O(5)-C(14)-C(9)	116.5(3)
C(3)-C(4)-C(5)	110.0(4)	O(5)-C(14)-C(13)	110.6(3)
C(3)-C(4)-C(18)	107.9(4)	O(9)-C(14)-C(13)	101.6(3)
C(3)-C(4)-C(19)	112.6(4)	C(8)-C(15)-C(16)	119.3(3)
C(5)-C(4)-C(18)	113.1(4)	O(8)-C(16)-C(13)	106.4(3)
C(5)-C(4)-C(19)	107.0(3)	O(8)-C(16)-C(15)	108.6(4)
C(18)-C(4)-C(19)	106.1(4)	C(13)-C(16)-C(15)	114.6(4)
C(4)-C(5)-C(6)	114.2(4)	N -C(17)-C(7)	116.8(3)
C(4)-C(5)-C(11)	108.0(3)	N -C(17)-C(11)	110.4(3)
C(6)-C(5)-C(11)	102.8(3)	C(7)-C(17)-C(11)	101.0(3)
O(2)-C(6)-C(5)	117.4(3)	O(9)-C(18)-C(4)	107.0(4)
O(2)-C(6)-C(7)	111.2(4)	N -C(19)-C(4)	114.4(4)
C(5)-C(6)-C(7)	105.1(3)	O(3)-C(22)-C(23)	107.1(4)
C(6)-C(7)-C(8)	109.3(3)	O(5)-C(24)-O(6)	123.3(4)
C(6)-C(7)-C(17)	104.0(3)	O(5)-C(24)-C(25)	112.3(4)
C(8)-C(7)-C(17)	111.9(3)	O(6)-C(24)-C(25)	124.3(4)
O(3)-C(8)-C(7)	111.2(3)	C(24)-C(25)-C(26)	121.4(4)
O(3)-C(8)-C(9)	103.5(3)	C(24)-C(25)-C(30)	122.4(5)
O(3)-C(8)-C(15)	108.5(3)	C(26)-C(25)-C(30)	116.2(5)
C(7)-C(8)-C(9)	108.2(4)	C(25)-C(26)-C(27)	123.4(4)
C(7)-C(8)-C(15)	114.2(3)	C(26)-C(27)-C(28)	118.7(5)
C(9)-C(8)-C(15)	110.8(3)	O(7)-C(28)-C(27)	124.4(5)
C(8)-C(9)-C(10)	112.1(3)	O(7)-C(28)-C(29)	117.9(5)
C(8)-C(9)-C(14)	111.6(4)	C(27)-C(28)-C(29)	117.7(5)
C(10)-C(9)-C(14)	104.5(3)	C(28)-C(29)-C(30)	120.7(5)
C(9)-C(10)-C(11)	118.8(3)	C(25)-C(30)-C(29)	122.9(5)
C(9)-C(10)-C(12)	102.3(3)	N -C(34)-C(35)	111.7(4)

Table 5. Torsion angles (°) for Acoforestine.

C(11)-C(1)-C(2)-C(3)	36.8	C(15)-C(8)-C(9)-C(14)	-31.0
C(1)-C(2)-C(3)-C(4)	-48.7	C(8)-C(9)-C(14)-C(13)	76.2
C(2)-C(3)-C(4)-C(5)	62.3	C(9)-C(14)-C(13)-C(16)	-70.0
C(3)-C(4)-C(5)-C(11)	-58.4	C(14)-C(13)-C(16)-C(15)	22.8
C(4)-C(5)-C(11)-C(1)	47.6	C(13)-C(16)-C(15)-C(8)	23.2
C(5)-C(11)-C(1)-C(2)	-38.2	C(16)-C(15)-C(8)-C(9)	-19.0
C(19)-C(4)-C(5)-C(11)	64.3	C(11)-C(5)-C(6)-C(7)	-22.3
C(4)-C(5)-C(11)-C(17)	-74.8	C(5)-C(6)-C(7)-C(17)	-10.7
C(5)-C(11)-C(17)-N	71.2	C(6)-C(7)-C(17)-C(11)	40.4
C(11)-C(17)-N-C(19)	-59.5	C(7)-C(17)-C(11)-C(5)	-53.1
C(17)-N-C(19)-C(4)	44.7	C(17)-C(11)-C(5)-C(6)	46.3
N-C(19)-C(4)-C(5)	-46.7		
		C(14)-C(9)-C(10)-C(12)	24.7
C(17)-C(7)-C(8)-C(9)	66.3	C(9)-C(10)-C(12)-C(13)	4.3
C(7)-C(8)-C(9)-C(10)	-40.0	C(10)-C(12)-C(13)-C(14)	-31.6
C(8)-C(9)-C(10)-C(11)	33.4	C(12)-C(13)-C(14)-C(9)	47.3
C(9)-C(10)-C(11)-C(17)	-46.3	C(13)-C(14)-C(9)-C(10)	-45.2
C(10)-C(11)-C(17)-C(7)	62.1		
C(11)-C(17)-C(7)-C(8)	-77.4		

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REFERENCES

1. S.W. Pelletier, S.Y. Chen, B.S. Joshi, and H.K. Desai, *J. Nat. Prod.*, **47**, 474 (1984).
2. S.W. Pelletier, S.K. Srivastava, B.S. Joshi and J.D. Olsen, *Heterocycles*, **23**, 331 (1985).
3. K. Wiesner, T.Y.R. Tsai, K. Huber, S.E. Bolton and R. Vlahov, *J. Am. Chem. Soc.*, **96**, 4990 (1974).
4. S.W. Pelletier, L.H. Keith, and P.C. Parthasarathy, *J. Am. Chem. Soc.*, **89**, 4146 (1967).
5. S.W. Pelletier, W.H. DeCamp, D.L. Herald, S.W. Page and M.G. Newton, *Acta Cryst.*, B**33**, 716 (1977).
6. M.S. Yunusov, Y.V. Rashkes, V.A. Tel'nov and S.Y. Yunusov, *Khim. Prir. Soedin.*, 515 (1969).
7. W.S. Chen and E. Breitmaier, *Chem. Ber.*, **114**, 394 (1981).
8. C.H. Wang, D.H. Chen and W.L. Sung, *Chinese Traditional and Herbal Drugs*, **14**, 5 (1983).
9. C.H. Wang, D.H. Chen and W.L. Sung, *Planta Medica*, **48**, 55 (1983).
10. F.P. Wang and Q.C. Fang, *Planta Medica*, **42**, 375 (1981); *Acta Pharmaceutica Sinica*, **16**, 49 (1981).
11. C.A. Grob, H.R. Kiefer, H. Lutz and H. Wilkens, *Tetrahedron Letters*, 2901 (1964).
12. P.W. Codding, K.A. Kerr, M.H. Benn, A.J. Jones, S.W. Pelletier and N.V. Mody, *Tetrahedron Letters*, 127 (1980).
13. S.W. Pelletier, R.S. Sawhney and N.V. Mody, *Heterocycles*, **9**, 1241 (1978).
14. D. Yu and B.C. Das, *Planta Medica*, **49**, 85 (1983).
15. S.W. Pelletier, R.S. Sawhney and A.J. Aasen, *Heterocycles*, **12**, 377 (1979).
16. V. Boido, O.E. Edwards, K.L. Handa, R.J. Kolt, and K.K. Purushothaman, *Can. J. Chem.*, **62**, 778 (1984).
17. A.C.T. North, D.C. Phillips and F.S. Mathews, *Acta. Cryst.*, A**24**, 351 (1968).
18. G. Germain, P. Main and M.M. Woolfson, *Acta. Cryst.*, A**27**, (1971).
19. S.W. Pelletier, J. Finer-Moore, R.C. Desai, N.V. Mody and H.K. Desai, *J. Org. Chem.*, **47**, 5290 (1982).
20. C.K. Johnson, "ORTEP-II"; Report ORNL-3794: Oak Ridge National Laboratories: Oak Ridge, TN, 1971.
21. C. Altona, H.J. Geise and C. Romers, *Tetrahedron*, **24**, 13 (1968).

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