CONTINUING INVESTIGATION ON THE CONSTITUENTS FROM ACONITUM FLAVUM

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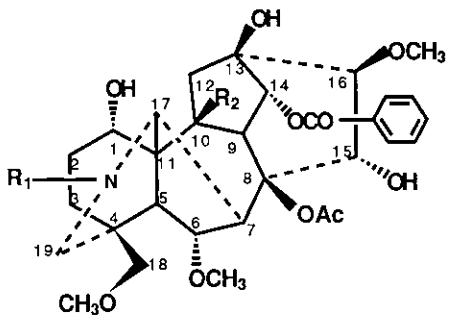
Abstract - The structures of three new C_{19} diterpenoid alkaloids, flavaconidine (1), N-acetylflavaconitine (2), and flavaconijine (3), isolated in the continuing investigation of Aconitum flavum Hand-Mazz, were determined on the basis of spectroscopic evidence and chemical correlations. They are the first examples of natural diterpenoid alkaloids bearing an acyl instead of usual alkyl group on the nitrogen.

3-Acetylaconitine, a major constituent responsible for analgesic and antiphlogistic effects of the roots of Aconitum flavum Hand-Mazz, has now been employed in China for the treatment of periomethritis, rheumatic and rheumatoid arthritis, sciatica, lumbago, dorsalgia, waist and joint sprain, etc[1]. In the works on the searching for pharmacological active components from the plant, we previously reported the isolation of a number of diterpenoid alkaloids[1]. Further investigation led to the isolation of other three new compounds named flavaconidine (1), N-acetylflavaconitine (2), and flavaconijine (3). These new diterpenoid alkaloids are characteristic with the presence of acyl instead of usual alkyl group on nitrogen. This paper deals with the structural elucidation of these compounds.



(1) R_1=CHO, R_2=OH flavaconidine
(2) R_1=$COCH_3$, R_2=OH N-acetylflavaconitine
(3) R_1=$COCH_3$, R_2=H flavaconijine
(4) R_1=H, R_2=OH

Flavaconidine (1) had mp 186.5-189.0°C, $[\alpha]_D^9$ -49.0°(c=0.568, DMF) and gave a molecular formula corresponding to $C_{32}H_{41}NO_{12}$ by the investigation of ms fragment at m/z 571.2433 ($C_{30}H_{37}NO_{10}$, Calcd 571.2449). This ion appeared as base peak in the lower resolution ms and was resulted from the loss of acetic acid from the molecu-

lar ion. The ir spectrum exhibited absorptions at 3400 (hydroxyl), 2820, 1633 (formamide), 1720, 1295, 1100 (ester), and 1604, 1594, 1485, 713 cm^{-1} (aromatic ring). The ^{1}H-nmr in pyridine-d_5 showed the following signals, δ1.37 (3H, s, $OCOCH_3$), 3.06, 3.27, 3.78 (each 3H, s, OCH_3), and 7.40-8.26 (5H, m, $OCOC_6H_5$). From the molecular formula and the above spectral data, it was suggested that this compound belonged to C_{19} diterpenoid alkaloids[2]. The unusual downfield proton signal at δ6.29 (1H, d, J=5.0 Hz, βH-14) and upfield signal of $OCOCH_3$ at δ1.37 (3H, s) suggested that the acetoxy and benzoyloxy groups should be assigned to C-8 and C-14 or C-14 and C-8, respectively, and the one at C-14 should be in α-orientation so that the two substituents were close each other spatially and $OCOCH_3$ shifted upfield by the shield effect of benzoyloxy group[3,4]. Here, the acetoxy group was assigned to C-8 and the benzoyloxy was assigned to C-14 according to chemical shift of $OCOCH_3$ which would be in the range of δ1.25-1.45 if acetoxy group was linked to C-8 and benzoyloxy to C-14 or about δ1.76 if the two substituents switched over as in ezochasmaconitine[3]. The assignment of $OCOCH_3$ to C-8 was also confirmed by the ms fragment at m/z 571 (M^+-CH_3COOH, 100%) which was resulted from elimination of acetoxy group at C-8 due to spatial interaction between 1,3-syn-axial substituents, acetoxy and benzoyloxy groups[3]. The three methoxys were assigned to C-18, C-6, and C-16, respectively, because the ether proton signals on these carbons were observed at δ3.28, 3.77 (each 1H, ABq, J=8.4 Hz, 2H-18), 4.35 (1H, d, J=7.7 Hz, βH-6), and 3.90 (1H, d, J=5.2 Hz, αH-16). The assignments were confirmed by the carbon signals at δ80.4 (CH_2, C-18), 84.0 (CH, C-6), and 92.1 (CH, C-16). The orientation of methoxys at C-6 and 16 was assigned to be α and β respectively, based on the carbon shifts of C-6, δ84.0, and C-16, δ92.1, which were identical with nomal chemical shifts of those carbons among aconitine-type compounds with αCH_3O-6 and βCH_3O-16[4]. The proton chemical shift of methoxy group at C-16, δ3.78, indicated the presence of hydroxyls at C-13 and 15[5]. Irradiation of signal at δ 5.10 (1H, dd, J=5.2, 2.9 Hz, βH-15) collapsed signals at δ3.90 (1H, d, J=5.2 Hz, αH-16) and 5.32 (1H, d, J=2.9 Hz, OH), providing evidence for the presence of OH-15. An α-configuration was assigned to OH-15 (with C-15 chemical shift, δ79.8) according to observing the carbon chemical shift of C-15, δ78.5-79.0 with αOH-15 and 68.1 with βOH-15 in nagarine[6]. The ^{1}H-nmr spectrum showed that the methylene protons of C-12 appeared as ABq peaks at δ2.95, 4.34 (each 1H, J=15.5 Hz), indicating both hydrogens at C-10 and C-13 were substituted by hydroxyls, which were confirmed by quaternary carbon signals at δ78.5 (C-10) and 76.0 (C-13). The presence of hydroxy group at C-10

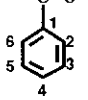
Table I. ^{1}H-Nmr Data (400 MHz, δ, pyridine-d_5) for the Alkaloids

Proton	1	2	3	4
H-1	4.95, m	4.96, m	3.85, m	4.77, br s
2H-2	2.04, 2.11, m	2.09, m	1.98, m	1.98, m
2H-3	1.92, m	1.92, m	1.78, 1.86, m	1.98, 2.10, m
H-5	3.28, d, J=7.3 Hz	3.24, d, J=6.7 Hz	2.31, d, J=6.8 Hz	3.23, d, J=7.0 Hz
H-6	4.35, d, J=7.7 Hz	4.33, d, J=5.8 Hz	4.19, d, J=6.9 Hz	4.29, d, J=6.4 Hz
H-7	3.21, s	3.18, s	3.05, s	3.22, s
H-9	3.38, d, J=5.0 Hz	3.39, d, J=5.0 Hz	3.12, m	3.34, d, J=3.9 Hz
H-10			2.28, m	
2H-12	2.95, 4.34, ABq, J=15.5 Hz	2.97, 4.35, ABq, J=15.6 Hz	3.77, m, 2.63, t, J=14.0 Hz	2.94, 3.45, ABq, J=14.8 Hz
H-14	6.29, d, J=5.0 Hz	6.29, d, J=5.1 Hz	5.49, d, J=4.6 Hz	6.24, d, J=4.8 Hz
H-15	5.10, dd, J=5.2, 2.9 Hz	5.09, dd, J=5.5, 3.4 Hz	4.96, m	5.00, m
H-16	3.90, d, J=5.2 Hz	3.83, d, J=5.6 Hz	3.77, m	3.77, d, J=5.7 Hz
H-17	4.60, s	4.65, s	4.65, s	3.56, s
2H-18	3.28, 3.77, ABq, J=8.4 Hz	3.30, 3.80, ABq, J=8.3 Hz	3.44, 3.69, ABq, J=8.1 Hz	3.12, 3.73, ABq, J=8.1 Hz
2H-19	3.55, 4.37, ABq, J=13.3 Hz	3.51, 4.32, ABq, J=13.3 Hz	3.48, 4.62, ABq, J=13.2 Hz	2.42, 3.52, ABq, J=10.8 Hz
CH_3O-18	3.06, s	3.06, s	3.12, s	3.08, s
CH_3O-6	3.27, s	3.26, s	3.29, s	3.28, s
CH_3O-16	3.78, s	3.81, s	3.78, s	3.75, s
AcO-8	1.37, s	1.38, s	1.44, s	1.41, s
BzO-14	7.39, 2H, t, J=7.3 Hz 7.52, 1H, t, J=7.4 Hz 8.26, 2H, d, J=7.9 Hz	7.38, 2H, t, J=7.7 Hz 7.51, 1H, t, J=7.3 Hz 8.25, 2H, d, J=7.7 Hz	7.39, 2H, t, J=7.5 Hz 7.70, 1H, t, J=7.6 Hz 8.25, 2H, d, J=8.3 Hz	7.35, 2H, t, J=7.8 Hz 7.48, 1H, t, J=7.3 Hz 8.23, 2H, d, J=7.7 Hz
OH-1	6.55, d, J=3.9 Hz	6.57, d, J=3.9 Hz	6.52, d, J=3.8 Hz	6.52, br s
OH-15	5.32, d, J=2.9 Hz	5.33, d, J=3.2 Hz	5.30, d, J=3.0 Hz	5.18, d, J=2.4 Hz
OH-10/13	6.62, 7.16, s	6.60, 7.12, s		
N-CHO	8.87, s			
N-$COCH_3$		2.90, s	2.80, s	

gave the best explanation why βH-14 signal (δ6.29, 1H, d, J=5.0 Hz) was much lower than the usual one even considered effect of solvent shift of pyridine-d_5 (see Table I), which was due to spatial interaction between OH and H at C-10 and 14[5]. Decoupling experiments revealed that the signal at δ4.95 (1H, m, βH-1) was correlated with δ2.04, 2.11 (each 1H, m, 2H-2) which were associated with δ1.92 (2H, m, 2H-3), suggesting the presence of a hydroxy group at C-1 or C-3. As the carbon signal of C-4 appeared at δ38.6, the hydroxy group was assigned to C-1 because the carbon chemical shift of C-4 would be in the range of 43.0-44.0 ppm[4] if the hydroxy group was linked to C-3. Flavaconidine **(1)** had no alkaloid properties and did not react with Dragendorff's reagent. The ir spectral absorptions at 2820 and 1633 cm^{-1} suggested there was a formyl group attached to nitrogen, which was confirmed by proton signal at δ8.87 (1H, s, N-CHO) and carbon signal at δ163.3 (N-CHO). Compared the ^{1}H-nmr spectrum with that of flavaconitine **(4)** (see Table I), 1.04 and 0.99 ppm downfield of H-17 and 2H-19, respectively, provided evidence for the presence of N-formyl group which has strong electron drawing property. The spectroscopic studies above permitted depicting the structure of flavaconidine as **(1)**. Unfortunately, the configuration of OH at C-1 remained unknown due to influence of the substitution of formyl group at nitrogen and hydroxy group at C-10. Among the C_{19} diterpenoid alkaloids, there are few examples having a hydroxy group at C-1 in β-orientation[7]. So the most possible configuration for OH-1 was α. Confirmation of the structure **(1)** was obtained by partial synthesis. By reaction with N-formyloxysuccinimide at room temperature, flavaconitine **(4)** afforded N-formylflavaconitine[8] which was identical with flavaconidine **(1)** in all of spectra included ir, ^{1}H-nmr, ms, and tlc behaviour. Thus, the structure of flavaconidine **(1)** was established.

N-Acetylflavaconitine (2), $C_{33}H_{43}NO_{12}$ (m/z 645.2772, Calcd 645.2784), with mp 274.5-275.5°C and $[\alpha]_D^{13}$ -45.6° (c=1.54, CH_3OH), had similar property to flavaconidine **(1)**, i.e. it did not react with Dragendorff's reagent. The ir spectrum exhibited absorptions at 3500 (hydroxyl), 1620 (amide), and 1720, 1280, 1100, 718 cm^{-1} (benzoyloxy). The ^{1}H-nmr spectrum was very similar to that of flavaconidine **(1)** except the presence of δ2.90 (3H, s, N-$COCH_3$) and the absence of δ8.87 (1H, s, N-CHO) (see Table I), suggesting that an acetyl instead of a formyl group attached to the nitrogen. Therefore, the structure **(2)** was deduced to this compound. By the partial acetylation with acetic anhydride at room temperature, flavaconitine **(4)** furnished N-acetylflavaconitine[9] which showed ir, ^{1}H-nmr, ms, and tlc behaviour identical with the natural compound. Thus, the structure **(2)** was established.

Table II. ^{13}C-Nmr Data (100 MHz, δ, pyridine-d_5) for the Alkaloids

Carbon	1	2	3[a]	4[b]	Carbon	1	2	3[a]	4[b]
1	67.6	67.9	73.5	69.2	6'	58.0	58.1	57.9	59.1
2	31.9	31.9	31.5	28.7	16'	61.5	61.6	61.3	61.9
3	33.5	33.6	33.8	30.2	18'	59.1	59.0	59.2	59.5
4	38.6	38.4	38.3	38.3	C=O	172.2	172.2	172.1	173.4
5	54.9	54.2	51.1	53.3	CH_3	21.4	21.4	21.3	21.4
6	84.0	84.5	83.5	83.2	C=O	166.4	166.4	166.0	167.1
7	51.9	51.7	48.0	48.2	 1	130.9	130.8	130.1	130.7
8	89.6	89.9	91.3	88.9	2	130.1	130.0	129.7	130.7
9	44.9	44.2	43.7	40.5	3	129.0	129.0	128.7	129.9
10	78.5	78.6	41.1	78.9	4	133.6	133.5	133.3	134.8
11	55.9	56.7	50.4	54.9	5	129.0	129.0	128.7	129.9
12	45.3	45.9	36.6	48.5	6	130.1	130.0	129.7	130.7
13	76.0	76.0	74.4	76.0	N-CHO	163.3			
14	80.6	79.8	78.9	80.8	N-C=O		170.3	170.7	
15	79.8	80.6	78.9	79.4	CH_3		22.9	22.4	
16	92.1	92.1	90.5	91.4					
17	59.4	58.7	59.2	59.1					
18	80.4	80.5	79.8	79.8					
19	49.0	49.4	46.8	49.3					

* The spectra of a) and b) were taked in $CDCl_3$ and methanol-d_4 respectively.

Flavaconijine (3) had mp 248.5-249.5°C, $[\alpha]_D^9$ -55.5°(c=0.492, DMF) and its molecular formula, $C_{33}H_{43}NO_{11}$, was established by high resolution ms, m/z 569.2646, $C_{31}H_{39}NO_9$, M^+-CH_3COOH, Calcd 569.2667. Like the compounds discussed above, it also had no basicity. The ir spectrum showed absorptions at 3500 (hydroxyl), 1620 (amide), and 1720, 1280, 1100, 710 cm^{-1} (benzoyloxy). Its ^{1}H-nmr in pyridine-d_5 exhibited signals of $OCOCH_3$-8 (δ1.44, 3H, s), $OCOC_6H_5$-14 (δ7.40-8.25, 5H, m, aromatic ring and δ5.49, 1H, d, J=4.6 Hz, βH-14), N-$COCH_3$ (δ2.80, 3H, s), and three methoxys, CH_3O- 18, 6, 16, (δ3.12, 3.29, 3.78, each 3H, s). The presence of OH at C-13 and 15 was suggested by proton chemical shift of methoxy group at C-16, δ3.78 (3H, s)[5], and was confirmed by decoupling correlation signals at δ4.96 (1H, m, βH-15) and δ3.77 (1H, d, αH-16) as well as δ5.30 (1H, d, J=2.5 Hz, OH). The orientation of substituents on C-6, 15, and 16 was assigned to be α, α, and β respectively according to carbon chemical shifts of those positions (see Table II). Compared with N-acetylflavaconitine **(2)**, 0.80 ppm upfield of H-14 (see Table I) indicated a hydrogen instead of a hydroxy group present at C-10. Irradiation of signal at δ2.28 (1H, m, H-10) collapsed the signals at δ2.63 (1H, t, J=14.0 Hz, βH-12), 3.77 (1H, m, αH-12), and 3.12 (1H, m, H-9), the last of which was associated with

βH-14 signal at δ5.49 (1H, d, J=4.6 Hz). This irradiation provided evidence for the presence of H-10. Decoupling experiments also revealed that the signal at δ3.85 (1H, m, βH-1) was associated with signal at δ1.98 (2H, m, 2H-2) which was correlated with δ1.78, 1.86 (each 1H, m, 2H-3), suggesting the presence of OH-1. Irradiation of H-1 signal at δ3.85 (1H, m) caused 10% enhancement of βH-10 signal at δ2.28 (1H, m) in n.O.e. difference and irradiation of βH-10 signal enhanced H-1 by 9%, indicating the H-1 was β-orientation. The n.O.e. between βH-1 and βH-10 gave a reasonable explaination for 1.11 ppm upfield of βH-1 compared with N-acetylflavaconitine **(2)** which had a hydroxy group instead of a hydrogen at C-10 to cause deshielding of the proton at 1,3-syn-axial position. All of assignment were agreed with the structure **(3)** (see Tables I, II).

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8. Partial synthetic procedure of flavaconidine **(1)** was as the following:
   To formic acid (0.03 ml) and N-hydroxysuccinimide (60 mg) in anhydrous dimethylformamide (1.5 ml) was added dicyclohexylcarbodiimide (100 mg). The mixture was stirred at 0°C for 2 h and then continuously stirred at room temperature for another 24 h. After removal of insoluble substances, the liquid part was added flavaconitine (60 mg) and the mixture was continuously stirred at room temperature for 24 h. Seperation of the reaction mixture with preparative tlc, SiO_2/CH_2Cl_2:CH_3OH (4:1), afforded flavaconidine (5 mg) with Rf 0.80.
9. N-Acetylflavaconitine **(2)** was prepared by direct reaction of flavaconitine with acetic anhydride at room temperature for 15 min. After evaporation in vacuo N-acetylflavaconitine was obtained in 100% yield.

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