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TWO NEW DITERPENE ALKALOIDS, 10-HYDROXYISOTALATIZIDINE AND 10-HYDROXYTALATIZAMINE

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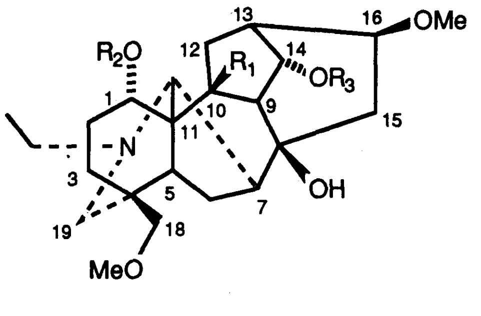
Two new C_{19} diterpene alkaloids **1** and **2** were isolated from *Aconitum sanyoense* Nakai and their novel structures with a hydroxy function at C_{10} position were elucidated.

KEYWORDS *Aconitum sanyoense*; diterpene alkaloid; 10-hydroxyisotalatizidine; 10-hydroxytalatizamine; ^{13}C-NMR; X-ray analysis

From the aerial parts of *Aconitum sanyoense* Nakai var. *tonenze* Nakai, a plant native to Kuzure-Sawa, Nagano Prefecture, two new C_{19} diterpene alkaloids were isolated along with the five known diterpene alkaloids, isotalatizidine (**3**), talatizamine (**5**), 14-O-acetyltalatizamine,1) condelphine2) and sanyonamine,3) and the two aporphine alkaloids, N-methyllaurotetanine4) and isoboldine.5)

The new alkaloid (**1**) was isolated as an amorphous solid, $[\alpha]_D^{14}$ +7.0° (c=0.28, $CHCl_3$). The high resolution mass spectrum of **1** presents the molecular ion m/z 423.2636, corresponding to the formula $C_{23}H_{37}O_6N$. The intensive IR absorption at 3650 cm^{-1} indicated the presence of hydroxy groups. The ^1H-NMR spectrum showed the characteristic signals of C_{19} type diterpene alkaloid due to $N\text{-}CH_2C\underline{H_3}$ (δ 1.11, 3H, t, J=7Hz), OMe x 2 (δ 3.32, 6H, s) and C_{14}-H (δ 4,67, 1H, t, J=5Hz). The base peak at m/z 406 (M^+-OH) in the mass spectrum strongly indicated that a hydroxy group was located at C_1 position.6) Treatment of **1** with acetic anhydride in pyridine at room temperature afforded diacetate (**4**) as a crystalline compound, mp. 156-158.5°C (from acetone-isopropylether). In the ^1H-NMR spectrum of **4**, two characteristic signals were observed at δ 5.29 (1H, t, J=5Hz) and δ 5.44 (1H, dd, J=10 and 7Hz), which appeared at δ 4.67 (1H, t, J=5Hz) and δ 4.07 (1H, br s) in the spectrum of **1**, respectively. This indicates that two secondary hydroxy groups exist at C_1 and C_{14} position in **1**. The ^{13}C-NMR spectrum of **1** resembled that of isotalatizidine (**3**)2) except for a few changes (Table). The appearance of an extra singlet at δ82.3 in the spectrum of **1** afforded evidence for the presence of an additional tertiary hydroxyl group in **1** compared with **3**. The chemical shifts of C_9, C_{11} and C_{12} in **1** appeared at δ56.1, 53.3 and 39.1, respectively, which were significantly shifted downfield compared with those of **3**. This indicated that the neighboring



1: R_1=OH, R_2=R_3=H
2: R_1=OH, R_2=Me, R_3=H
3: R_1=R_2=R_3=H
4: R_1=OH, R_2=R_3=Ac
5: R_1=R_3=H, R_2=Me
6: R_1=OH, R_2=Me, R_3=Ac

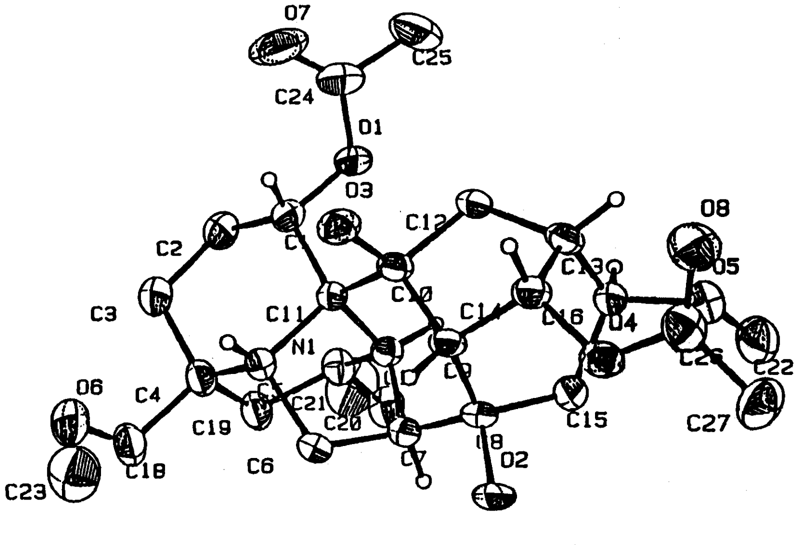


Fig. 1. ORTEP Drawing of 4

C_{10} carbon was substituted by a hydroxy group. The structure of **1** proposed by the spectroscopic analysis was confirmed by X-ray analysis of the diacetate derivative (**4**). The crystal of **4** belongs to a orthorhombic space group, $P2_12_12_1$, with the cell parameters of a=13.694(4), b=14.160(3), c=13.143(2)Å, Z=4, Cell volume=2548.6Å^3, and Dx=1.32g/cm^3. The structure was solved by the direct method MULTAN and the result was refined by a block diagonal least squares procedure to R=0.066 for 4433 unique reflections with $Fo>3\sigma(Fo)$ measured on a Rigaku AFC-5 diffractometer with CuKα radiation. The ORTEP drawing of the structure of 1,14-diacetyl-10-hydroxyisotalatizidine (**4**) is shown in Fig. 1.

Table. ^{13}C Chemical Shifts and Assignments of **1**, **3**, **2**, **5**, **6** and **7**

Carbon	(1)	(3)$^{2)}$	(2)	(5)$^{7)}$	(6)	(7)
1	69.2	72.2	78.5	86.1	78.6	79.6
2	26.6	28.7	25.7	25.7	26.0	26.3
3	30.8	29.7	32.5	32.6	32.3	32.4
4	36.9	37.2	38.5	38.6	38.3	38.0
5	40.5	41.6	42.0	45.7	41.7	42.9
6	25.0	24.9	25.4	24.8	25.4	24.0
7	44.7	45.1	45.2	45.7	45.6	42.9
8	73.4	74.3	72.1	72.7	72.8	77.2
9	56.1	46.6	56.0	46.9	55.0	47.4
10	82.3	43.9*	81.1	45.7*	80.6	86.9
11	53.3	48.6	54.0	48.6	54.1	56.0
12	39.1	26.7	37.6	28.6	39.1	36.8
13	37.5	40.1*	37.7	37.7*	35.5	37.5
14	74.3	75.7	74.1	75.7	76.3	83.3
15	43.4	42.4	39.5	39.2	41.9	39.2
16	81.3	82.0	81.7	82.2	81.2	82.5
17	64.8	64.0	63.9	62.8	63.1	62.7
18	78.9	79.0	79.4	79.4	79.4	79.9
19	56.6	56.5	52.9	53.1	53.1	53.6
N-CH_2	48.5	48.5	49.5	49.4	49.4	49.2
CH_3	13.0	13.1	13.7	13.6	13.5	13.4
1'OMe	-	-	56.0	56.1	56.0	56.0
8'OMe	-	-	-	-	-	48.0
10'OMe	-	-	-	-	-	53.2
14'OMe	-	-	-	-	-	57.8
16'OMe	56.3	56.3	56.4	56.3	56.1	56.3
18'OMe	59.4	59.4	59.5	59.3	59.5	59.5
C=O	-	-	-	-	170.7	-
CH_3	-	-	-	-	21.4	-

Chemical Shifts in δ downfield from TMS. Solvent; $CDCl_3$.

* The assignments of the resonance of C_{10} and C_{13} in **3** and **5** in the literature were reversed.

The new alkaloid (**2**) was obtained as an amorphous solid, $[\alpha]_D^{20}$ +6.4° (c=0.33, $CHCl_3$), whose high resolution mass spectrum showed the M^+437.2799 ($C_{24}H_{39}O_6N$), which is 14 a.m.u. higher than the corresponding peak in the spectrum of 10-hydroxyisotalatizidine (**1**). The base peak at m/z 406 (M^+-OMe) suggested the presence of a methoxyl group at the C_1 position.$^{6)}$ Furthermore, the orientation of C_1-methoxyl group was α-equatorial as indicated by the coupling pattern of C_1-H (dd, J=10.2 and 6.6Hz). On acetylation with acetic anhydride in pyridine, **2** afforded monoacetate (**6**). The major diffeence in the ^1H-NMR spectrum between the acetate (**6**) and the original alcohol (**2**) was the downfield shift (Δ δ0.61) of the signal at δ4.72 (1H, t, J=5.1Hz) in **2**. This indicated the presence of a hydroxy group at C_{14} in **2**. Next, as in the case of isotalatizidine (**3**) and 10-hydroxyisotalatizidine (**1**), we compared the ^{13}C-NMR spectra of (**2**) and talatizamine (**5**).$^{7)}$ The presence of a tertiary hydroxy group at C_{10} was confirmed by a singlet at δ81.1 in the spectrum of **2** and by the downfield shift of the C_9 (Δ δ9.1), C_{11} (Δ δ5.4) and C_{12}(Δ δ9.0) in comparison with the spectrum of talatizamine (**5**). Treatment of **2** with NaH/CH_3I in DMF gave trimethylated product (**7**), $[\alpha]_D^{24}$ -11.8° (c=0.13, $CHCl_3$), in 74% yield (Chart 1). In the ^1H-NMR spectrum, there were six singlet signals (δ3.39, 3.37, 3.35, 3.29, 3.28 and 3.15) due to OMe groups. Tetramethylated compound (**7**), $[\alpha]_D^{23}$ -12.1° (c=0.13, $CHCl_3$), was obtained from **1** under the above methylation condition. The TLC behavior, IR, ^1H-NMR and ^{13}C-NMR spectra of both hexamethylethers (**7**) from **1** and from **2** were superimposable. Therefore, the structure of the new alkaloid (**2**) was concluded to be 10-hydroxytalatizamine.

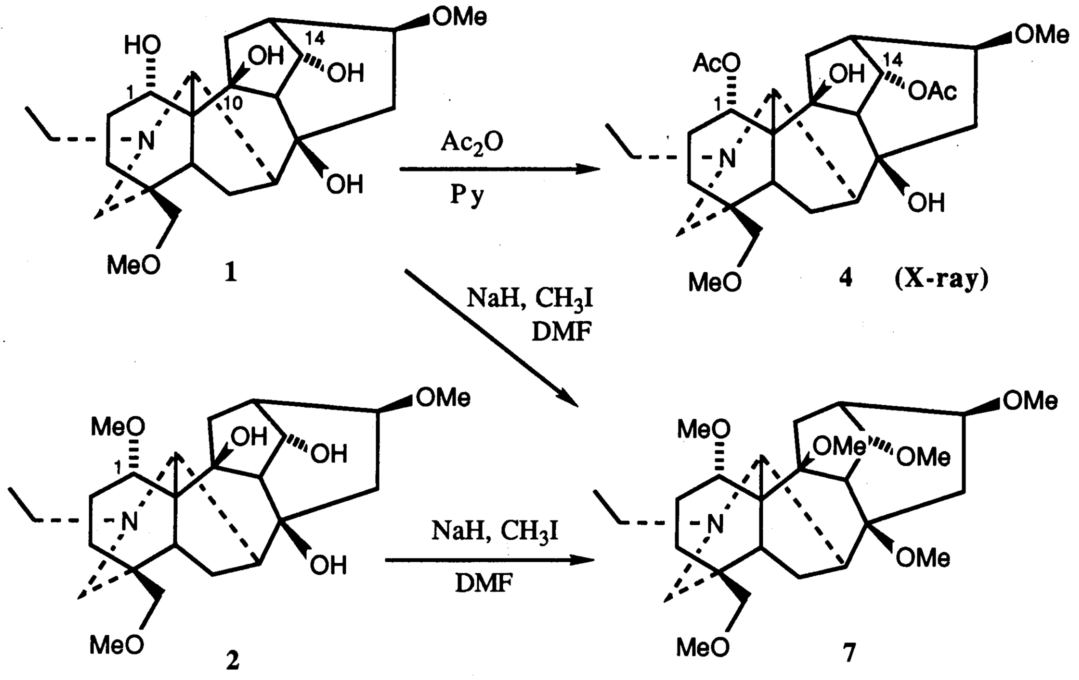


Chart 1

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