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Studies on the Constituents of Aconitum Species. II.[1] Structure of Deoxyjesaconitine[2]

TAKAO MORI, HIDEO BANDO, YOSHIO KANAIWA, KOJI WADA, and TAKASHI AMIYA*

Hokkaido Institute of Pharmaceutical Sciences, 7–1 Katsuraoka-cho, Otaru 047–02, Japan

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A new diterpene alkaloid, deoxyjesaconitine, was isolated from the rhizoma of *Aconitum subcuneatum* NAKAI (Ranunculaceae) and its structure was established by analysis of the spectral data and by conversion of jesaconitine into deoxyjesaconitine. Deoxyaconitine, hypaconitine, jesaconitine, aconitine, and mesaconitine were also isolated from the same plant.

Keywords——deoxyjesaconitine; deoxyaconitine; hypaconitine; jesaconitine; aconitine; mesaconitine; anhydrojesaconitine; *Aconitum subcuneatum*; ^{13}C-NMR

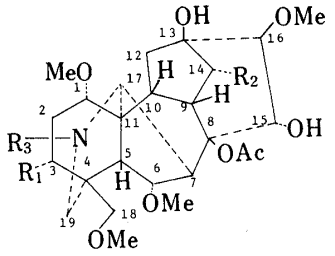
The constituents of *Aconitum subcuneatum* NAKAI were investigated many years ago, and the isolation of jesaconitine was reported by Majima *et al.*[3] We reinvestigated the constituents of the same plant by using modern separation techniques.

Crude alkaloids (5.7 g) were obtained from the dried rhizoma (1.31 kg) according to the general procedure for alkaloid extraction. The alkaloid extract was subjected to silica gel column chromatography and high performance liquid chromatography (HPLC) to give a new diterpene alkaloid, deoxyjesaconitine (I), together with five known alkaloids,[4a,b] deoxyaconitine (II), hypaconitine (III), jesaconitine (IV), aconitine (V), and mesaconitine (VI).

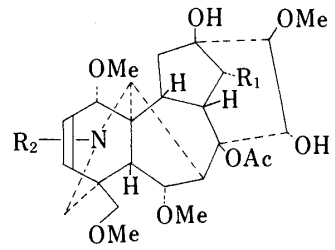
The new alkaloid, deoxyjesaconitine (I), showed the following properties; mp 174—176 °C, $[\alpha]_D = +52°$ ($c = 0.065$), $C_{35}H_{49}NO_{11}$, m/z 659 (M^+). The ultraviolet (UV) spectrum of I showed an absorption maximum at 257 nm ($\log \varepsilon = 4.23$) indicating the presence of an anisoyl group. The 100 MHz ^{1}H-nuclear magnetic resonance (NMR) spectrum showed a methyl group, an acetyl group, four methoxyl groups, an aromatic methoxyl group, a methine attached to an ester group at δ 4.83 (d, $J = 5.0$ Hz), assigned to C-14, and aromatic protons with an A_2B_2 pattern. These spectral data suggested that I was an aconitine-type alkaloid having two ester groups. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of I was related to those of II and IV as regards the carbon atoms of the skeleton and ester groups.

Furthermore, the structure of I was confirmed by derivation of I from IV through dehydration and catalytic hydrogenation. Compound IV was treated in an atmosphere of nitrogen with thionyl chloride to give a good yield of anhydrojesaconitine (VII). The NMR spectrum of VII showed two olefinic protons at δ 6.07 (d d, $J = 9.7$, 3.2 Hz, C_2-H) and δ 5.79 (d, $J = 9.7$ Hz, C_3-H). The NMR spectrum was very similar to those of anhydroaconitine (VIII) and anhydromesaconitine (IX).

The anhydro compound gave a main product, mp 175—176 °C, together with a minor product on hydrogenation in the presence of platinic oxide. The main product was identical with I on the basis of infrared (IR), ^{1}H and ^{13}C-NMR and mass spectral (MS) comparisons and mixed melting point determination. The minor product seemed to be the 1-demethoxy-3-deoxy derivative but further investigation was not attempted because of the small amount of the sample.[4b]



I: R_1=H, R_2=OAs, R_3=Et
II: R_1=H, R_2=OBz, R_3=Et
III: R_1=H, R_2=OBz, R_3=Me
IV: R_1=OH, R_2=OAs, R_3=Et
V: R_1=OH, R_2=OBz, R_3=Et
VI: R_1=OH, R_2=OBz, R_3=Me



VII: R_1=OAs, R_2=Et
VIII: R_1=OBz, R_2=Et
IX: R_1=OBz, R_2=Me

Bz=CO–C_6H_5
As=CO–C_6H_4–OMe(*p*)
Fig. 1

TABLE I. ^{13}C-Chemical Shift Assignments for Deoxyjesaconitine (I), Deoxyaconitine (II),[5] Jesaconitine (IV), and Aconitine (V)

Carbon atom	I	II	IV	V	Carbon atom	I	II	IV	V
1	85.2	85.0	82.3	82.3	19	53.2	53.3	46.9	46.9
2	26.4	26.3	33.6	34.0	N–CH_2	49.1	49.1	48.9	48.8
3	35.2	35.2	70.6	70.4	CH_3	13.4	13.4	13.3	13.3
4	39.0	39.0	43.1	43.2	1′	56.2	56.0	55.8	55.7
5	49.2	49.1	46.6	46.6	6′	58.0	57.9	57.9	57.9
6	83.3	83.3	83.3	83.4	16′	61.1	60.9	61.1	61.0
7	45.2[b]	45.1[b]	44.6[b]	44.8	18′	59.1	59.0	59.0	58.9
8	92.1	92.0	91.1	92.0	O=C	172.4	172.2	172.4	172.2
9	44.1[b]	44.6[b]	44.2[b]	44.2	CH_3	21.5	21.3	21.5	21.3
10	41.0	41.0	40.8	40.8	O=C	165.8	165.9	165.7	165.9
11	49.9	49.9	49.9	49.8	C_6H_5	122.3	129.9	122.1	129.8
12	36.6	36.7	35.8	36.0		131.6	129.9	131.6	129.6
13	74.2	74.0	74.0	74.0		113.8	128.5	113.8	128.6
14	78.7[c]	78.8[c]	78.6[c]	78.9		163.4	133.1	163.5	133.2
15	78.8[c]	79.0[c]	78.8[c]	78.9	OCH_3(*p*)	55.5		55.4	
16	90.2	90.2	90.0	90.1					
17	61.4	61.2	60.9	60.7					
18	80.3	80.2	75.8	75.6					

a) δ (ppm) downfield from TMS in $CDCl_3$.
b, *c*) Each column may be reversed.

During our structural investigation, the relaxation times (T_1) of carbons of aconitine were measured. The T_1 values of C-19 and the methylene of the *N*-ethyl group were 0.14 and 0.22 s, respectively, and the original assignments[5] must be reversed, in view of the general consideration that a skeleton carbon possesses shorter T_1.[6]

Experimental

All melting points are uncorrected. IR spectra were taken with a JASCO IRA-2 spectrometer. NMR spectra were taken in chloroform-*d* solution with a JEOL FX-100 spectrometer using tetramethylsilane as an internal standard; abbreviations used are: s, singlet; d, doublet; d d, double doublet; t, triplet; q, quartet; m, multiplet. T_1

values were measured by the inversion recovery method under the following conditions at 23 °C: concentration, 50 mg/0.2 ml; double pulse, 90 ° and 180 °; pulse repetition, 3.0 s; pulse interval, 0.01, 0.06, 0.18, 0.24, 0.30, 0.50 and 0.80 s. MS were measured with a Shimadzu LKB-9000B mass spectrometer. Ultraviolet (UV) spectra were measured in EtOH solution with a Shimadzu D-300 spectrometer. Optical rotation was measured with a JASCO DIP-4 polarimeter. HPLC was carried out using a Nucleosil 10-C_{18} column (8 × 300 mm) with a mixed solution (CH_3CN : H_2O : MeOH : AcOH : NH_4OH = 30 : 20 : 5 : 2 : 1) as the mobile phase. Column chromatography and thin-layer chromatography (TLC) were performed on Silica gel 60 (Merck) and HF_{254} (type 60, Merck) respectively. The developing solvent for silica gel chromatography was a solution of hexane and ether or chloroform saturated with 28% NH_4OH.

Isolation Procedure——The rhizoma (1.31 kg) were collected at Zenibako-cho, in Otaru City, in 1978. The dried rhizoma were extracted with EtOH (18 l). A suspension of the EtOH extract (80 g) in H_2O was partitioned with petroleum ether, and the H_2O layer was adjusted to pH 9 with NH_4OH. The precipitate (5.7 g) was chromatographed over silica gel to give fractions A (170 mg), B (3.8 g), and C (1.2 g). Fraction A was subjected to TLC to afford two fractions (Fractions A-1 and A-2). Fractions A-1 and A-2 were crystallized from MeOH to give II (15 mg) and III (5 mg), respectively. The combined mother liquors were subjected to HPLC to afford a fraction containing I. The fraction was crystallized from acetone to give I (5 mg). Fraction B was subjected to column chromatography to give IV (2.5 g), V (570 mg), and VI (680 mg).

Deoxyjesaconitine (I)——Colorless needles, mp 174—176 °C, $[\alpha]_D = +52°$ ($c = 0.065$, MeOH), MS m/z: 659 (M^+). UV λ_{max}^{EtOH} nm (log ε); 257 (4.23). IR ν_{max}^{KBr} cm^{-1}: 3500, 1715, 1710, 1605. ^{1}H-NMR ($CDCl_3$) δ: 1.08 (t, 3H, J = 7.0 Hz), 1.43 (s, 3H), 3.17 (s, 3H), 3.28 (s, 3H), 3.30 (s, 3H), 3.74 (s, 3H), 3.88 (s, 3H), 4.83 (d, 1H, J = 5.0 Hz), 6.92 (d, 2H, J = 8.0 Hz), 7.97 (d, 2H, J = 8.0 Hz).

Anhydrojesaconitine (VII)——A mixture of IV (100 mg) and thionyl chloride (1 ml) was refluxed for 3 h in an atmosphere of nitrogen. The reaction mixture was concentrated *in vacuo*, then the residue was added to ice water, which was made alkaline with a solution of 2 N Na_2CO_3. The alkaline solution was extracted with $CHCl_3$. The $CHCl_3$ solution was purified by preparative TLC to afford a colorless amorphous powder (55 mg, 56%) and the starting material. IR ν_{max}^{KBr} cm^{-1}: 3500, 1715, 1705, 1605. MS m/z: 675 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 1.19 (t, 3H, N-$CH_2\underline{CH_3}$, J = 7.0 Hz), 1.45 (s, 3H, -OCO$\underline{CH_3}$), 3.18, 3.33, 3.77, 3.87 (s, each 3H, -OMe), 4.86 (d, 1H, J = 4.0 Hz, $C_{14}\beta$-H), 5.79 (d, 1H, J = 9.7 Hz, C_3-H), 6.07 (d d, 1H, J = 9.7 Hz, 3.2 Hz, C_2-H), 6.92 and 7.97 (4H, aromatic protons). *Anal.* Calcd for $C_{35}H_{47}NO_{11}$: C, 63.91; H, 7.20; N, 2.12. Found: C, 63.19; H, 7.16; N, 2.27.

Hydrogenation of Anhydrojesaconitine (VII)——A solution of VII (54 mg) in EtOH (5 ml) absorbed one mol of hydrogen in the presence of platinic oxide (28 mg) for 1.5 h. The solution was filtered and concentrated *in vacuo*. The resulting mixture was chromatographed on a silica gel plate and crystallized from acetone to give I as colorless needles (25 mg, 45%), mp 175—176 °C. This was identical with I obtained from the natural source on the basis of mixed melting point determination 174—176 °C and ^{1}H-NMR, ^{13}C-NMR, and IR spectral comparisons. *Anal.* Calcd for $C_{35}H_{49}NO_{11}$: C, 63.71; H, 7.49; N, 2.12. Found: C, 63.66; H, 7.46; N, 2.44.

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References and Notes

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