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Studies on the Constituents of *Aconitum* Species. IV.¹⁾ On the Components of *Aconitum japonicum* THUNB.²⁾

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Two new alkaloids, aljesaconitine A and aljesaconitine B, and six known alkaloids, aconitine, jesaconitine, mesaconitine, neoline, hokbusine A, and delcosine, were isolated from the roots of *Aconitum japonicum* THUNB. (formerly known as *Aconitum subcuneatum* NAKAI). The structures of aljesaconitines A and B were determined on the basis of spectral data and derivation from jesaconitine. An acetoxy group at C-8 of an aconitine-type alkaloid was found to be easily changed to an alkoxy group by the use of alcohols such as methanol and ethanol in the extraction procedure. Quantitative determination and the LD₅₀ values of these 8-*O*-alkyl derivatives are also described.

Keywords—aljesaconitine A; aljesaconitine B; hokbusine A; delcosine; *Aconitum japonicum*; quantitative determination; acute toxicity

The isolation and structural elucidation of ten alkaloids from *Aconitum japonicum* THUNB. (earlier reported as *Aconitum subcuneatum* NAKAI) were reported in our previous paper.^{1,2)} In that paper, we dealt with fraction A among the chromatographic fractions A through E. In this work, fraction B was chromatographed over alumina and on silica gel to give two new alkaloids named aljesaconitine A (**7**) and aljesaconitine B (**8**), together with six known alkaloids, aconitine (**1**), jesaconitine (**2**), mesaconitine (**3**), neoline (**4**), hokbusine A (**5**),³⁾ and delcosine (**6**).⁴⁾ Compounds **1**—**4** have been reported^{1,5)} but **5** and **6** have not previously been found in this plant.

In this paper, we wish to report the structural elucidation of **7** and **8** separated from fraction B, as well as the quantitative determination of the main components (**1**, **2**, and **3**) and 8-*O*-methyl derivatives (**5** and **7**), and the acute toxicity of the 8-*O*-alkyl derivatives.

Structure Determination

Compound **7** showed the following properties; amorphous powder, $[\alpha]_D^{20} = +7.5^\circ$ ($c=0.93$, EtOH), C₃₄H₄₉NO₁₁. The proton nuclear magnetic resonance (¹H-NMR) spectrum of **7** was very similar to that of **2** having an anisoyloxy group at C-14, but did not show the signal of a shielded acetoxy group at C-8 which appeared near δ 1.36 in the case of **2** as well as **1**.⁶⁾ Six methoxy groups were apparent from the spectrum and one of them was at δ 3.15, suggesting a shielded methoxy group. The methoxy group was assigned to be at C-8 in place of an acetoxy group, because a methoxy group was seen at δ 3.13 in the spectrum of hokbusine A (**5**), which was the first 8-*O*-alkyl aconitine-type alkaloid isolated from *A. carmichaeli*.³⁾ The spectrum of **7** suggested that its structure was 8-*O*-methyl-14-anisoylaconine. This structure was supported by the carbon-13 (¹³C-) NMR spectrum (Table I). Compound **7** was readily formed when **2** was stirred in methanol.

Compound **8** showed the following properties; amorphous powder, $[\alpha]_D^{20} = +5.8^\circ$ ($c=1.18$, EtOH), C₃₅H₅₁NO₁₁. The ¹H-NMR spectrum of **8** was very similar to that of **7** except for the shielded methoxy group. A methyl group was seen at δ 0.64 (3H, t, $J=6.0$ Hz)

and was assigned as the methyl group of an ethoxy group located at C-8 in place of the methoxy group of **7**. The structure of **8**, therefore, was assigned as 8-*O*-ethyl-14-anisoyleaconine. This was confirmed by the ^{13}C -NMR spectrum and the transformation of **2** into **8** by the use of ethanol.

The structure of **5** was determined by comparison of the spectral data with the literature values³⁾ and transformation of **3** into **5** by methanol treatment. The identification of **6** was carried out by direct comparison with an authentic sample.⁴⁾

Compounds **5**, **7**, and **8**, the aconitine-type alkaloids having an alkoxy group at C-8, were probably artifacts formed in the extraction procedure with methanol or ethanol. Recently, 8-*O*-ethyl-14-benzoylmesaconine (**9**) was isolated from *A. ibukiense* and derived from mesaconitine (**3**) by Sakai *et al.*⁷⁾ and 8-*O*-methyl-14-benzoylaconine (**10**) was derived from **1** by Kitagawa *et al.*⁸⁾ These two groups suggested that the 8-*O*-alkyl derivatives were formed during extraction with alcohols.^{7,8)} Furthermore, the two groups suggested their respective mechanisms that an acetoxy group at C-8 was transformed into the alkoxy group. We concluded that **5** and **7** were natural products, since **5** and **7** were found in both the chloroform and the ether extracts by means of high-performance liquid chromatography (HPLC), as described below. With respect to the mechanism, we support the proposal of Sakai *et al.*,⁷⁾ because when mesaconitine *N*-oxide was allowed to stand in methanol at 60 °C for 7 d, no transformation into the 8-*O*-methyl derivative occurred. The above result favors the mechanism involving the lone-pair electrons on the nitrogen atom.

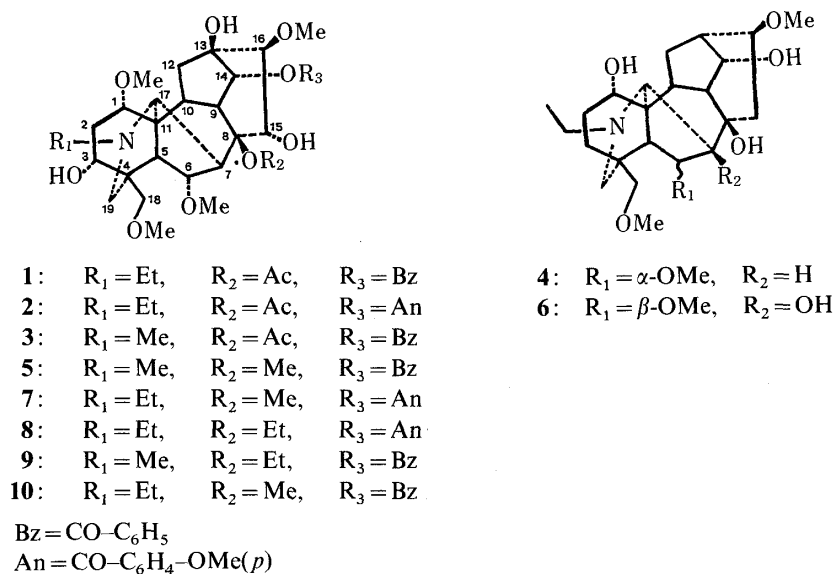


Chart 1

Quantitative Determination

Highly toxic compounds **1**—**3** were the main components in the extract of this plant. To determine whether **5** and **7** were natural products or artifacts, we evaluated the contents of the three main components and two C-8 methoxy derivatives **5** and **7** by means of HPLC, since HPLC is an important tool in the quantitative determination of diterpene alkaloids.⁹⁾

Three kinds of extracts, methanol, chloroform, and ether extracts, were prepared according to Hikino *et al.* (Chart 2).¹⁰⁾ The chromatogram of the chloroform extract is shown in Fig. 1. Aljesaconitine A (**7**) appeared as a shoulder peak (20 min) and its content was too small to determine. However, it was found that **7** was not an artifact since the peak appeared in the chromatograms of the methanol, chloroform, and ether extracts. Hokbusine A (**5**) was also concluded to be a natural product on the same basis. The absence of methanol in the

TABLE I. ^{13}C -Chemical Shifts and Assignments for Jesaconitine (2),⁵⁾
 Aljesaconitine A (7) and Aljesaconitine B (8)

Carbon	2	7	8	Carbon	2	7	8
1	83.3	82.2	82.5	N-CH ₂	48.9	48.8	48.9
2	33.6	32.8	33.2	CH ₃	13.3	13.0	13.2
3	70.9	71.5	71.6	1'	55.8	55.6	55.8
4	43.1	43.0	42.9	6'	57.9	58.4	58.5
5	46.6	45.4	45.5	16'	61.1	62.3	62.3
6	82.3	83.1	83.4	18'	59.0	59.0	59.0
7	44.6 ^{a)}	45.0	45.2	O=C	165.7	165.7	165.8
8	91.9	82.2	82.1	C ₆ H ₅	122.1	122.4	122.7
9	44.2 ^{a)}	42.4	42.9		131.6	131.6	131.6
10	40.8	41.4	41.3		113.8	113.5	113.6
11	49.9	50.3	50.5		163.5	163.2	163.1
12	35.8	36.1	36.2	OCH ₃ (<i>p</i>)	55.4	55.3	55.3
13	74.0	74.7	74.2	O=C	172.4	—	—
14	78.6 ^{b)}	79.4	79.2	CH ₃	21.5	—	—
15	78.8 ^{b)}	76.9	77.3	8-O-CH ₃	—	49.8	—
16	90.0	93.3	93.3	8-O-CH ₂	—	—	57.1
17	60.9	61.3	61.1	CH ₃	—	—	15.3
18	75.8	77.4	76.8				
19	46.9	47.6	47.3				

δ (ppm) downfield from TMS in CDCl₃. Assignments marked *a*) or *b*) may be interchanged in each column.

 TABLE II. Quantitative Determination of Diterpene Alkaloids
 in Dried Powder (mg/kg)

Alkaloid	MeOH	Ether	CHCl ₃
Aconitine (1)	1.61×10^3	2.02×10^3	1.71×10^3
Jesaconitine (2)	2.66×10^3	3.21×10^3	2.55×10^3
Mesaconitine (3)	9.45×10^2	1.31×10^3	1.04×10^3
Hokbusine A (5)	1.37×10^2	1.23×10^2	1.39×10^2

dried powder (6 g)
 ↓ solvent (60 ml)
 28% NH₄OH (2.5 ml)
 ↓
 stirred for 30 min
 ↓ H₂O (2.5 ml)
 filtered
 ↓ evaporated
 extract

Chart 2. Extraction Procedure for the Quantitative Determination of Diterpene Alkaloids in Dried Powder of *Aconitum japonicum* THUNB.

 TABLE III. Acute Toxicities of 8-O-Alkyl
 Derivatives in Mice

Alkaloid	LD ₅₀ (mg/kg)	
	<i>s.c.</i>	<i>p.o.</i>
Hokbusine A (5)	21	207
Aljesaconitine A (7)	5.2	56.5
Aljesaconitine B (8)	1.9	43.8
8-O-Ethyl-14-benzoylmesaconine (9)	3.6	45.9
<i>A. japonicum</i>		
MeOH extr.	9.1	48.6
H ₂ O extr.	20.5	101.4

chloroform and ether used for extraction was confirmed by gas chromatography.

Quantitative determination of 1, 2, 3, and 5 was based on calibration curves, and the results are shown in Table II. The content of jesaconitine (2) was the highest.

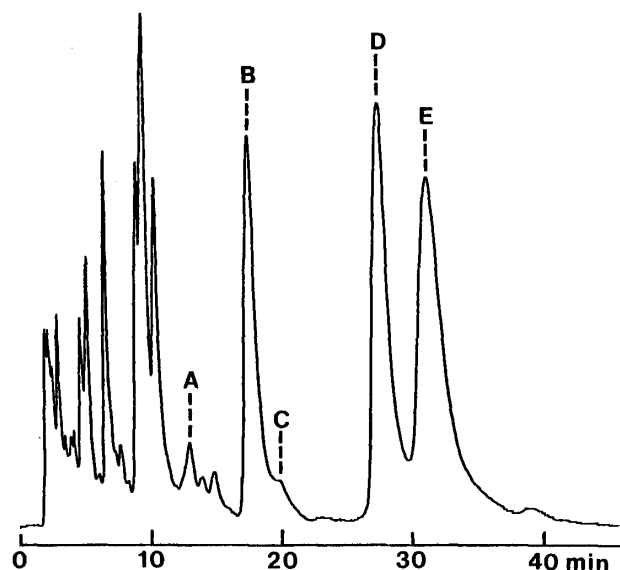


Fig. 1. High-Performance Liquid Chromatogram of Chloroform Extract

Column, Nucleosil 5-C₁₈ (300 × 5 mm, i.d.); mobile phase, 0.05 M phosphate buffer-acetonitrile (71:29) pH 2.5; flow rate, 1.1 ml/min; detector, UV (254 nm); sensitivity, 0.08 absorbance unit full scale (a.u.f.s.); temperature, 40 °C.

A, hokbusine A; B, mesaconitine; C, aljesaconitine A; D, aconitine; E, jesaconitine.

Acute Toxicity of 8-O-Alkyl Derivatives

Acute toxicity and the other biological activities of aconitine-type alkaloids have been reported by many investigators¹¹⁾ and it was suggested that the substituent at C-8 is important for the biological activities.^{11a)}

Table III shows oral and subcutaneous LD₅₀ values of some 8-O-alkyl derivatives in addition to the methanol and water extracts of this plant. In the toxicity experiment, characteristic aconitine syndromes^{11b)} were observed; the 8-O-alkyl derivatives still had high toxicities.

Experimental

All melting points are uncorrected. Optical rotations were measured with a JASCO DIP-4 polarimeter. Infrared (IR) spectra were taken with a JASCO IRA-2 spectrometer. Ultraviolet (UV) spectra were measured in EtOH solution with a Shimadzu D-300 spectrometer. NMR spectra were measured in CDCl₃ solution with a JEOL FX-100 spectrometer using tetramethylsilane as an internal standard, and the following abbreviations are used: s=singlet, d=doublet, t=triplet, m=multiplet. Mass spectra (MS) were measured with a Shimadzu LKB-9000B spectrometer. Column chromatography was performed on silica gel (0.063–0.200 mm, Merck) and alumina (activity II–III, 0.063–0.200 mm, Merck). Thin-layer chromatography (TLC) was performed on Silica gel 60 F₂₅₄ (Merck). Elemental analyses and high-resolution MS were performed by the Analytical Center, Hokkaido University. HPLC was carried out on a Shimadzu LC-3A equipped with an SPD-2A detector and a Chromatopac C-R1A recorder using a reverse-phase column of Nucleosil C₁₈ (Nagel Co.).

Isolation Procedure—Fraction B (91 g) obtained previously from the extract (234 g) of *A. japonicum* THUNB. was chromatographed over alumina (1.5 kg) to afford the following fractions: CHCl₃–hexane (4:1, 2l) gave Fr-B-1 (8.3 g), CHCl₃–hexane (1:1, 2l) gave Fr-B-2 (13.4 g), CHCl₃ (2l) gave Fr-B-3 (23.0 g), ethyl acetate (2l) gave Fr-B-4 (34.5 g), CHCl₃–MeOH (1:1, 2l) gave Fr-B-5 (8.2 g), and MeOH (2l) gave Fr-B-6 (2.4 g). Fr-B-1, 2, and 3 were combined and chromatographed on silica gel with a mixture of hexane and CHCl₃ saturated with 28% aqueous NH₃. The content of hexane was decreased gradually. Purification by repeated column chromatography gave **1** (20 mg), **2** (22 mg), **3** (234 mg), **4** (224 mg), **5** (105 mg), and **6** (124 mg, mp 205–207 °C). Compounds **7** (8 mg) and **8** (11 mg) were purified by HPLC under the following conditions: column, Nucleosil 5-C₁₈ (300 × 10 mm, i. d.); mobile phase, 0.05 M phosphate buffer-acetonitrile, pH 2.5 (70:30); flow rate, 3.0 ml/min; temperature, 40 °C.

Aljesaconitine A (7)—Amorphous powder, $[\alpha]_D^{20} = +7.5^\circ$ ($c=0.93$, EtOH). UV $\lambda_{\max}^{\text{EtOH}}$ nm (log ϵ): 255 (4.03). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3450, 1720, 1610, 1505, 1250, 1110. ¹H-NMR (δ): 1.13 (3H, t, $J=7.0$ Hz), 3.15 (3H, s), 3.28 (3H, s), 3.29 (3H, s), 3.31 (3H, s), 3.64 (3H, s), 3.87 (3H, s), 4.55 (1H, d, $J=6.0$ Hz, C₁₄ β –H), 6.92 (2H, d, $J=9.0$ Hz), 8.00 (2H, d, $J=9.0$ Hz). MS (m/z): 647 (M^+), 632 ($M^+ - \text{CH}_3$), 616 ($M^+ - \text{OCH}_3$, base peak), 135 (anisoyl cation). High-resolution MS Calcd for C₃₄H₄₉NO₁₁: 647.328. Found 647.330.

Aljesaconitine B (8)—Amorphous powder, $[\alpha]_D^{20} = +5.8^\circ$ ($c=1.18$, EtOH). UV $\lambda_{\max}^{\text{EtOH}}$ nm (log ϵ): 255 (4.02). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3450, 1720, 1610, 1515, 1260, 1100. ¹H-NMR (δ): 0.64 (3H, t, $J=6.0$ Hz), 1.11 (3H, t, $J=7.0$ Hz), 3.28

(3H, s), 3.33 (3H, s), 3.75 (3H, s), 3.88 (3H, s), 4.60 (1H, d, $J=6.0$ Hz, $C_{14\beta}$ -H), 6.93 (2H, d, $J=9.0$ Hz), 8.00 (2H, d, $J=9.0$ Hz). MS m/z : 661 (M^+), 646 ($M^+ - CH_3$), 630 ($M^+ - OCH_3$, base peak), 135 (anisoyl cation). High-resolution MS Calcd for $C_{35}H_{51}NO_{11}$: 661.346. Found 661.349.

Transformation of 2 into 7—i) A solution of **2** (100 mg) in MeOH (30 ml) was stirred at room temperature for 7 d. The solvent was evaporated off and the residue was purified by TLC to give **7** (10 mg, 10%). ii) At 60 °C for 3 d, the yield was increased to 82%. The products (i and ii) were identical with the natural product from *A. japonicum* in terms of the IR, NMR, and MS.

Transformation of 2 into 8—A solution of **2** (100 mg) in EtOH (30 ml) was stirred at 60 °C for 5 d. The solvent was evaporated off and the residue was purified by TLC to give **8** (72 mg, 72%). The derivative was identical with the natural product in terms of the IR, NMR, and MS.

Transformation of 3 into 5—A solution of **3** (300 mg) in MeOH (180 ml) was stirred at 60 °C for 7 d, then worked up as described above to give **5** (280 mg, 98%). The derivative was identical with hokbusine A based on a comparison of the IR, 1H - and ^{13}C -NMR, and MS with the literature data.³⁾ Hokbusine A perchlorate: mp 169–177 °C (dec.). Anal. Calcd for $C_{32}H_{45}NO_{11} \cdot HClO_4 \cdot H_2O$: C, 53.22; H, 6.70; N, 1.94. Found: C, 53.54; H, 6.62; N, 1.64.

Transformation of 3 into 9—A solution of **3** (300 mg) in EtOH (180 ml) was stirred at 60 °C for 10 d, then worked up as described above to give **9** (285 mg, 97%). IR ν_{max}^{KBr} cm^{-1} : 3450, 1715, 1600, 1580, 1270, 1090. 1H -NMR (δ): 0.57 (3H, t, $J=6.6$ Hz), 2.34 (3H, s), 3.27 (3H, s), 3.28 (3H, s), 3.31 (3H, s), 3.73 (3H, s), 4.08 (1H, d, $J=7.6$ Hz, $C_{6\beta}$ -H), 4.54 (1H, d, $J=6.0$ Hz, $C_{15\beta}$ -H), 4.81 (1H, d, $J=4.7$ Hz, $C_{14\beta}$ -H), 7.27–8.08 (5H, m). ^{13}C -NMR (ppm, carbon atom): 15.3 (q, 8- $O-CH_2CH_3$), 34.1 (t, 2), 36.3 (t, 12), 41.3 (d, 10), 42.0 (d, 9), 42.5 (q, $N-CH_3$), 43.4 (s, 4), 45.3 (d, 7), 45.6 (d, 5), 49.5 (t, 19), 50.5 (s, 11), 56.3 (q, 1'), 57.1 (t, 8- $O-CH_2CH_3$), 58.6 (q, 6'), 59.1 (q, 18'), 62.3 (d, 17), 62.4 (q, 16'), 71.3 (d, 3), 74.7 (s, 13), 76.4 (t, 18), 78.3 (d, 15), 79.4 (d, 14), 82.0 (s, 8), 82.7 (d, 1), 83.3 (d, 6), 93.4 (d, 16), 128.3 (d, 3''), 129.6 (d, 2''), 130.3 (s, 1''), 132.8 (d, 4''), 166.1 (s, C_6H_5-CO). MS m/z : 617 (M^+), 602 ($M^+ - CH_3$), 586 ($M^+ - OCH_3$, base peak), 540 (586-EtOH), 105 (benzoyl cation). High-resolution MS Calcd for $C_{33}H_{47}NO_{11}$: 617.320. Found 617.321.

Treatment of Mesaconitine N-Oxide with MeOH—A solution of mesaconitine *N*-oxide (20 mg) in MeOH was stirred at 60 °C for 7 d. The mixture was worked up as above to afford only the starting material.

Conditions of HPLC for Quantitative Determination—See the legend to Fig. 1.

Preparation of Extract for Analysis—Chloroform extract was prepared according to the method reported by Hikino *et al.* (Chart 2).¹⁰⁾ The procedure shown in Chart 2 was repeated three times, but water was added only the first time.

Standard Sample—Compounds **1**–**3** isolated from this plant and **5** and **7** derived from **3** were used as standard samples.

Calibration Curve—About 1 mg of standard sample, accurately weighed, was dissolved in the same solvent as used for the mobile phase in HPLC and made up to exactly 1 ml. The area of the chromatogram was measured. The calibration curves were linear and the correlations (y , area; x , mg) were as follows ($N=5$ in all cases): aconitine, $y=9.17 \times 10^6 x - 8750$ ($r=0.992$); jesaconitine, $y=7.43 \times 10^6 x - 4610$ ($r=0.999$); mesaconitine, $y=1.09 \times 10^7 x - 6270$ ($r=0.999$); hokbusine A, $y=4.95 \times 10^6 x - 258$ ($r=0.999$).

Determination of Alkaloids in *A. japonicum* THUNB.—The content of each alkaloid is shown in Table II. The recovery of added standard mesaconitine was 96.0–96.5% in the MeOH, $CHCl_3$, and ether extracts.

Acute Toxicity in Mice—Male mice, weighing 27–30 g, were used to determine the subcutaneous and peroral LD_{50} s. Mortality was recorded 24 h after administration. LD_{50} s were calculated by the up-and-down method described in the textbook.

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