Studies on the Constituents of *Aconitum* Species. XII. Syntheses of Jesaconitine Derivatives and Their Analgesic and Toxic Activities

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The role of the substituents at C3 and C8 of jesaconitine (**1**) on jesaconitine-induced analgesia and toxicity was examined. 3-*O*-Acetyljesaconitine (**2**), 3-*O*-anisoyljesaconitine (**3**), and 3-deoxyjesaconitine (**6**) showed dose-dependent analgesic action, and the potency of their compound-induced analgesia and toxicity was lower than those of **1**. The most remarkable difference was found in the toxicity. The results indicate that the C3 hydroxy function of **1** participate in the induction of toxicity rather than of analgesia. 8-*O*-Linoleoyl-14-anisoylaconine (**5**), 8-*O*-methyl-14-anisoylaconine (**7**), 8-*O*-ethyl-14-anisoylaconine (**8**), 14-anisoylaconine (**4**) and 8-deoxy-14-anisoylaconine (**9**) showed lower activities than jesaconitine-induced analgesia and toxicity. The analgesic activity of **7** was almost the same as that of **8**, but the toxicity of **7** was lower than that of **8**. The analgesic activity of **9** was lower than that of **4**, but the toxicities of both derivatives were not apparent. These facts indicate that the C8 function of **1** is important to the induction of analgesia and toxicity, and also that this function participates differently in the induction of the analgesia and toxicity. Subsequently, it was suggested that substituents at C3 and C8 of **1** played important roles of the induction of the analgesia and toxicity, and that the modes of this participation were not the same in analgesia and toxicity.

Keywords *Aconitum*; jesaconitine; analgesia; toxicity

The crude drug "bushi," prepared from roots of certain *Aconitum* spp. (Ranunculaceae) of Chinese and Japanese origin, is an indispensable material in oriental medicine. Traditionally, "bushi" had been thought to restore low metabolism in feeble patients and had been employed for such symptoms as pain, paralysis, atonia and coldness of the extremities. However, in modern clinical practice, processed aconite ("Kako-bushi" in Japanese), which is made by steaming raw aconite root under high pressure, is generally used, since the raw root has very strong toxicity. The toxicity has been found to be due to aconitine alkaloids, and the main alkaloids in *Aconitum* plants are aconitine, mesaconitine, hypaconitine and jesaconitine. These alkaloids not only have strong toxicity, but also potent analgesic action.^{1,2)} Accordingly, the pharmacological action of "bushi" can not be discussed without discussing the pharmacological action of aconitine alkaloids.

In some pharmacological studies on aconitine alkaloids, it has been reported that the mechanism of analgesic action

of mesaconitine is related to the function of the nor-adrenergic neuron in the central nervous system²⁾; that the sites of the analgesic action of mesaconitine are in the central nervous system; and that the nucleus reticularis gigantocellularis, the nucleus reticularis paragigantocellularis, the periaqueductal gray and the lumbar enlargement are involved in the sites of the analgesic action of mesaconitine.³⁾ Regarding toxicity, aconitine causes arrhythmia when a toxic dose is administered and is used as a tool for the induction of arrhythmia in experimental animals.^{4,5)} A consistent structure-activity relationship of aconitine alkaloids has not been reported. Investigation of the structure-activity relationship of aconitine alkaloids is important to elucidate the relation between its analgesic properties and its toxicity. In our preliminary experiment,⁶⁾ it was found that jesaconitine (**1**) among aconitine alkaloids has the most potent analgesic and toxic activities, and is one of the main alkaloids isolated from *Aconitum japonicum* native to Otaru, Hokkaido, Japan.⁷⁾ Accordingly, we concentrated on **1** among the aconitine alkaloids and studied the role of the oxygen-function attached to positions C3 and C8 of **1** and its derivatives in connection with the induction of toxicity and analgesia (Fig. 1). Regarding the method of measurement of analgesic activity, the acetic acid-induced writhing method was used.^{2,8-10)}

Results and Discussion

Chemistry 3-*O*-Acetyljesaconitine (**2**): Compound **1** was converted to **2** by treatment with acetic anhydride in a 92.5% yield. The ¹H-nuclear magnetic resonance (¹H-NMR) spectrum of **2** revealed the presence of two acetoxy groups. One of them at δ 1.43 was assigned to be in the C8 acetoxy group, which was shielded by the C14 anisoyl group. Another acetoxy group found a δ 2.06 was assigned to be at C3. The double doublet signal at δ 4.91 (1H, $J=12.2$, 5.3 Hz) was assigned to be C3- β -H. All of the signals in the ¹³C-nuclear magnetic resonance (¹³C-NMR) spectrum (Table I) of **2** are in agreement with the assigned structure.

3-*O*-Anisoyljesaconitine (**3**): Compound **1** was converted

1 : R ₁ = OH, R ₂ = OAc	: jesaconitine
2 : R ₁ = OAc, R ₂ = OAc	: 3- <i>O</i> -acetyljesaconitine
3 : R ₁ = OAs, R ₂ = OAc	: 3- <i>O</i> -anisoyljesaconitine
4 : R ₁ = OH, R ₂ = OH	: 14-anisoylaconine
5 : R ₁ = OH, R ₂ = <i>O</i> -linoleoyl	: 8- <i>O</i> -linoleoyl-14-anisoylaconine
6 : R ₁ = H, R ₂ = OAc	: 3-deoxyjesaconitine
7 : R ₁ = OH, R ₂ = OCH ₃	: 8- <i>O</i> -methyl-14-anisoylaconine
8 : R ₁ = OH, R ₂ = OCH ₂ CH ₃	: 8- <i>O</i> -ethyl-14-anisoylaconine
9 : R ₁ = OH, R ₂ = H	: 8-deoxy-14-anisoylaconine
- Ac = COCH₃, As = COC₆H₄ (*p*-OCH₃)

Fig. 1. Structures of Jesaconitine and Its Derivatives

to **3** by treatment with *p*-anisoyl chloride in a 27.6% yield. The ^1H -NMR spectrum of **3** revealed the presence of two anisoyl groups found at δ 7.99 (4H, d, $J=8.9$ Hz) and δ 6.93 (4H, d, $J=8.9$ Hz). The double doublet signal at δ 5.17 (1H, $J=12.1$, 5.4 Hz) was assigned to be C3- β -H. This structure was supported by the ^{13}C -NMR spectrum (Table I).

3-Deoxyjesaconitine (**6**): Compound **6** was synthesized in accordance with the method of a previous paper.¹¹⁾

14-Anisoylaconine (**4**): Compound **4** was also synthesized in accordance with the method of a previous paper.¹²⁾

8-*O*-Linoleoyl-14-anisoylaconine (**5**): Treatment of **1** with linoleic acid in pyridine yielded **5** in a 79.9% yield. The ^1H -NMR spectrum was similar to that of **1** except for the

disappearance of the acetyl group. A methyl group of linoleic acid residue attached at position C8 was seen at δ 0.97 (3H, t, $J=7.6$ Hz). The olefinic protons of linoleic acid residue at C8 were seen at δ 5.36 (4H, m). This structure was confirmed by the ^{13}C -NMR spectrum (Table I).

8-*O*-Methyl-14-anisoylaconine (**7**): Compound **7** was synthesized in accordance with the method of a previous paper.⁷⁾

8-*O*-Ethyl-14-anisoylaconine (**8**): Compound **8** was synthesized in accordance with the method of a previous paper.⁷⁾

8-Deoxy-14-anisoylaconine (**9**): Compound **1** was converted to pyrojesaconitine by heating at 200 °C under reduced pressure in a 65% yield.¹²⁾ Pyrojesaconitine was

TABLE I. ^{13}C -Chemical Shifts and Assignments for Jesaconitine (**1**), 3-*O*-Acetyljesaconitine (**2**), 3-*O*-Anisoyljesaconitine (**3**), 3-Deoxyjesaconitine (**6**), 14-Anisoylaconine (**4**), 8-*O*-Linoleoyl-14-anisoylaconine (**5**), 8-*O*-Methyl-14-anisoylaconine (**7**), 8-*O*-Ethyl-14-anisoylaconine (**8**) and 8-Deoxy-14-anisoylaconine (**9**)

Carbon	^{13}C -Chemical shifts (ppm)								
	1	2	3	6	4	5	7	8	9
1	82.3	81.9	83.5	85.2	82.4	82.3	82.2	82.5	83.7
2	33.6	31.9	32.1	26.4	33.0	33.4	32.8	33.2	33.8
3	70.6	71.4	71.6	35.2	71.9	71.5	71.5	71.6	72.0
4	43.1	42.2	42.7	39.4	43.0	43.0	43.0	42.9	43.2
5	46.6	45.9	45.9	49.2	47.6	46.4	45.4	45.5	47.2
6	83.3	83.5	82.0	83.3	83.5	83.4	83.1	83.4	85.2
7	44.6	45.2	44.7	45.2	46.2	44.7	45.0	45.2	39.2
8	91.1	91.8	91.9	92.1	78.6	91.7	82.2	82.1	40.7
9	44.2	44.6	45.3	44.1	46.0	44.3	42.2	42.9	37.5
10	40.8	40.5	40.5	41.0	42.0	40.9	41.4	41.3	41.6
11	49.9	47.1	49.7	49.9	50.4	50.1	50.3	50.5	50.6
12	35.8	36.3	36.5	36.6	36.0	35.7	36.1	36.2	36.6
13	74.0	74.1	74.1	74.2	74.8	74.0	74.7	74.2	75.1
14	78.6	78.5	78.5	78.7	75.9	73.6	79.4	79.2	80.2
15	78.8	78.7	78.7	78.8	81.9	78.9	76.9	77.3	72.4
16	90.0	90.2	90.1	90.2	90.7	90.0	93.3	93.3	94.3
17	60.9	61.0	60.7	61.4	61.7	61.3	61.3	61.1	62.4
18	75.8	71.4	71.6	80.3	77.3	75.7	77.4	76.8	77.4
19	46.9	49.7	47.4	53.2	48.9	47.0	47.6	43.7	47.3
N-CH ₂	48.9	49.0	49.0	49.1	48.4	48.8	48.8	48.9	49.1
CH ₃	13.3	13.4	13.4	13.4	13.5	13.3	13.0	13.2	13.4
1'	55.8	56.3	56.4	56.2	55.7	55.9	55.6	55.8	56.1
6'	57.9	58.2	58.3	58.0	58.0	58.1	58.8	58.5	57.9
16'	61.1	60.7	61.0	61.1	60.9	60.9	62.3	62.3	61.8
18'	59.0	58.7	58.7	59.1	59.1	59.0	59.0	59.0	59.1
C=O(C14)	165.7	165.7	165.6	165.8	165.9	165.6	165.7	165.8	165.9
C ₆ H ₄	122.1	122.3	122.3	122.3	122.1	122.2	122.4	122.7	122.2
	131.6	131.6	131.6	131.2	131.8	131.7	131.6	131.6	131.8
	113.8	113.8	113.8	113.8	113.6	113.8	113.5	113.6	113.7
	163.5	163.4	163.4	163.4	163.5	163.5	163.2	163.1	163.6
<i>p</i> (OMe)	55.4	55.4	55.4	55.5	55.4	55.4	55.3	55.3	55.4
C=O(C8)	172.4	172.3	172.3	172.4	—	—	—	—	—
CH ₃	21.5	21.4	21.5	21.5	—	—	—	—	—
8-O-CH ₂ (3)	—	—	—	—	—	—	49.8	57.1	—
CH ₃	—	—	—	—	—	—	—	15.3	—
C=O(C3)	—	170.2	—	—	—	—	—	—	—
CH ₃	—	21.1	—	—	—	—	—	—	—
C=O(C3)	—	—	165.2	—	—	—	—	—	—
C ₆ H ₄	—	—	123.1	—	—	—	—	—	—
	—	—	113.5	—	—	—	—	—	—
	—	—	131.7	—	—	—	—	—	—
	—	—	163.2	—	—	—	—	—	—
<i>p</i> (OMe)	—	—	55.4	—	—	—	—	—	—
C=O(C8)	—	—	—	—	—	175.1	—	—	—
lipo	—	—	—	—	—	—	—	—	—

Fig. 2. Analgesic Effect of Jesaconitine and Its Derivatives

$n = 7, 8$. Substances were administered s.c. 30 min before the injection i.p. of 0.7% acetic acid. Significantly different from the control, a) $p < 0.01$ or b) $p < 0.05$.

subsequently hydrogenated with $\text{LiAl(OMe)}_3\text{H}$ in tetrahydrofuran (THF) solution at -70°C to yield 8-deoxyaconine in a 96.8% yield. Next, treatment of 8-deoxyaconine with *p*-anisoyl chloride in a pyridine-dichloromethane solution at -70°C yielded **9** in a 64.3% yield. This structure was supported by both the $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra (Table I).

Analgesic Activity To investigate the participation of the C3-hydroxy function in jesaconitine-induced analgesia and toxicity, the toxic and analgesic activities of **1** were compared with those of **2**, **3** and **6**. The compounds of **1**, **2**, **3** and **6** showed significant analgesic activity in a dose-dependent manner (Fig. 2). The toxic and analgesic activities of **6** were a little lower than those of **1**, and the decrease of the toxicity of **6** was found more marked than that of its analgesic activity (Table II). This result indicates that the hydroxy function at position C3 of **1** relates more closely to the induction of toxicity. Both the toxic and analgesic activities of **2** and **3** were lower than those of **1**. In particular, a more remarkable difference was found in the toxicity. The differences in the physical and chemical properties of the structures of **1** and **2**, **3** or **6** are present in the presence or absence of the substituents at position C3. From the stereo-models of **1**, **2**, **3** and **6**, the hydroxy function at position C3 of **1** may have hydrogen bonding or other binding to the active site of **1**, but the substituents at position C3 of **2**, **3** and **6** were not apt to cause such binding. Accordingly, it was considered that the hydroxy function at position C3 of **1** participates in the induction of toxicity. However, this participation seems only to assist in the induction of toxicity, since **6** still has the strong toxicity.

Next, to investigate the participation of the C8 O-function of **1** in toxicity and analgesia, the toxic and analgesic activities of **1** were compared with those of **4**, **5**, **7**, **8** and **9**. The compounds of **4**, **5**, **7**, **8** and **9** showed significant analgesic activity in a dose-dependent manner (Fig. 2). First, the analgesic activities of these compounds were observed. Compound **5** showed an analgesic activity about 1/15 lower

TABLE II. The Analgesic and Toxic Activity of Jesaconitine and Its Derivatives at the C3 Position in Mice

Substance	ED ₅₀ value in mg/kg, s.c. (95% confidence limits)	LD ₅₀ value in mg/kg, s.c. (95% confidence limits)
1	0.035 (0.019—0.064)	0.25 (0.19—0.33)
6	0.056 (0.039—0.080)	0.70 (0.63—0.78)
2	0.050 (0.027—0.093)	0.87 (0.78—0.97)
3	0.060 (0.022—0.164)	0.65 (0.44—0.96)

$n = 7, 8$. Substances were administered s.c. 30 min before the injection i.p. of 0.7% acetic acid.

TABLE III. The Analgesic and Toxic Activity of Jesaconitine and Its Derivatives at the C8 Position in Mice

Substance	ED ₅₀ value in mg/kg, s.c. (95% confidence limits)	LD ₅₀ value in mg/kg, s.c. (95% confidence limits)
1	0.038 (0.018—0.080)	0.23 (0.18—0.29)
4	0.665 (0.388—1.140)	> 100
5	0.580 (0.220—1.540)	> 100
7	0.160 (0.039—0.653)	9.90 (9.11—10.76)
8	0.130 (0.058—0.269)	1.60 (1.26—2.04)
9	1.180 (0.468—2.974)	> 100

$n = 7, 8$. Substances were administered s.c. 30 min before the injection i.p. of 0.7% acetic acid.

than **1** (Table III). This result indicates that the 8-*O*-acetyl function of **1** participates in the induction of the analgesia. Compounds **7** and **8** showed lower activities than **1**, although differences between the activities of **7** and **8** were not apparent. The analgesic activity of **4** was lower than that of either **7** or **8** (Table III). These results demonstrate that the carbon atom of the carbonyl function at position C8 is important in inducing the analgesia by **1**. In addition, judging from the fact that the analgesic activity of **9** was lower than that of **4** (Table III), it was suggested that the oxygen atom binding to position C8 of **1** also participated in the induction of analgesia.

Secondly, the toxic activities of these compounds were observed. Compound **5** showed much lower toxicity than **1** (Table III). This fact indicates that the C8-acetyl function is important in inducing the toxicity by **1** and that the difference between the toxic activities of **5** and **1** may be due to the difference in size of the substituents at position C8. Compounds **4**, **7** and **8** showed lower toxicities than **1**; compound **8** had more potent toxicity than **7**, and compound **4** showed no toxicity (Table III). These results suggest that the two carbons and ketone of the acetyl function at position C8 of **1** more closely participate in the induction of toxicity. Additionally, from the fact that the toxicities of both **4** and **9** were not apparent, it was considered that the oxygen atom binding to position C8 is not essential in inducing toxicity. These facts indicate that for the induction of analgesia by **1**, the presence of a

C8-acetyl function is important, that two carbons in the C8-acetyl function participate in the induction of toxicity, and that the carbonyl carbon more closely participates in the induction of analgesia.

On the basis of the above evidence, it was demonstrated that the substituents at C3 and C8 of **1** play important roles in the induction of toxicity and analgesia. In addition, it was suggested that the C3 hydroxy function of **1** assists in the induction of toxicity rather than analgesia, that the acetyl function at position C8 plays a more important role in inducing toxicity and analgesia, and that the oxygen atom and carbonyl carbon at position C8 are more closely related to the induction of analgesia. Therefore, it was considered that the C3 and C8 substituents of **1** had participated differently in the induction of analgesia and toxicity.

Experimental

Optical rotations were measured with a JASCO DIP-4 polarimeter. Infrared (IR) spectra in KBr disks were taken with a JASCO IRA-2 spectrometer, and ultraviolet (UV) spectra were measured in an ethanol solution with a Shimadzu UV-240 spectrometer. Nuclear magnetic resonance (NMR) spectra were measured in a CDCl₃ solution with JEOL FX-100 and JEOL GX-270 spectrometers using tetramethylsilane as an internal standard. Mass spectra (MS) were measured with Shimadzu-LKB 9000B and Hitachi M-2000 spectrometers. Column chromatography was performed on Silica gel 60 (0.06–0.200 mm, Merck). Thin layer chromatography (TLC) was performed on Silica gel 60 F₂₅₄ (Merck).

Jesaconitine (1) Compound **1** was isolated in accordance with the method of Mori *et al.*¹¹⁾ Briefly, the dried aconite roots (1.31 kg) were extracted with ethanol (18 l). A suspension of ethanol extract (80 g) in water was partitioned with petroleum ether, and the water layer was adjusted to pH 9 with NH₄OH. 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 B was subjected to column chromatography to give **1** (2.5 g). *Anal.* Calcd for C₃₅H₄₉NO₁₂: C, 62.21; H, 7.31; N, 2.07. Found: C, 62.16; H, 7.46; N, 1.98.

3-O-Acetyljesaconitine (2) A mixture of **1** (100 mg, 0.15 mmol), pyridine (2 ml) and acetic anhydride (8 ml) was stirred for 2 h at room temperature. The usual work up and purification was performed by column chromatography on silica gel with Et₂O saturated with 28% ammonia water: hexane=3:2 as an eluting solvent to afford **2** (98 mg, 92.5%), amorphous powder, $[\alpha]_D^{25} + 27.7^\circ$ ($c=1.09$, CHCl₃). HR-MS: Calcd for C₃₆H₄₇NO₁₁ (M⁺–AcOH): 657.3149. Found: 657.3152 and EI-MS m/z : 717 (M⁺). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 258 (3.95). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{–1}: 1710 (C=O). ¹H-NMR (CDCl₃) δ : 7.89 (2H, d, $J=9.2$ Hz, anisoyl group), 6.93 (2H, d, $J=9.2$ Hz, anisoyl group), 3.73 (3H, s, OCH₃), 3.25 (3H, s, OCH₃), 3.20 (6H, s, OCH₃), 3.87 (3H, s, OCH₃ of anisoyl group), 4.84 (1H, d, $J=4.9$ Hz, C14- β -H), 4.46 (1H, dd, $J=5.3$, 3.0 Hz, C15- β -H), 4.08 (1H, d, $J=6.6$ Hz, C6- β -H), 1.43 (3H, s, CH₃ of acetoxy group at C8), 2.06 (3H, s, CH₃ of acetoxy group at C3), 1.10 (3H, t, $J=7.0$ Hz, CH₃ of C₂H₅ at nitrogen atom), 4.91 (1H, dd, $J=12.2$, 5.3 Hz, C3- β -H). MS m/z : 717 (M⁺), 686 (M⁺–OMe), 657 (M⁺–AcOH), 626 [M⁺–(OMe+AcOH)]. *Anal.* Calcd for C₃₇H₅₁NO₁₃: C, 61.91; H, 7.16; N, 1.95. Found: C, 62.18; H, 7.35; N, 2.08.

3-O-Anisoyljesaconitine (3) A mixture of **1** (150 mg, 0.22 mmol), pyridine (1 ml) and *p*-anisoyl chloride (40 mg, 0.24 mmol) was stirred for 1 h at 80 °C. After cooling, usual work up and purification by preparative TLC on SiO₂ with Et₂O saturated with 28% ammonia water: hexane=5:1 as a developing solvent afforded **3** (49.7 mg, 27.6%), amorphous powder, $[\alpha]_D^{18} + 24.0^\circ$ ($c=0.3$, CHCl₃). FD-MS m/z : 809 (M⁺). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 257 (4.49). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{–1}: 1705 (C=O). ¹H-NMR (CDCl₃) δ : 7.99 (4H, d, $J=8.9$ Hz, anisoyl group, A₂B₂ pattern), 6.93 (4H, d, $J=8.9$ Hz, anisoyl group, A₂B₂ pattern), 3.74 (3H, s, OCH₃), 3.26 (3H, s, OCH₃), 3.21 (3H, s, OCH₃), 3.12 (3H, s, OCH₃), 3.86 (6H, s, OCH₃ of anisoyl groups), 4.86 (1H, d, $J=5.3$ Hz, C14- β -H), 1.44 (3H, s, CH₃ of acetoxy group), 1.14 (3H, t, $J=7.0$ Hz, CH₃ of C₂H₅ at nitrogen atom), 5.17 (1H, dd, $J=12.1$, 5.4 Hz, C3- β -H). *Anal.* Calcd for C₄₃H₅₅NO₁₄: C, 63.77; H, 6.84; N, 1.73. Found: C, 64.01; H, 6.73; N, 1.74.

14-Anisoylaconine (4) A mixture of **1** (100 mg, 0.15 mmol), dioxane (10 ml) and water (10 ml) was refluxed for 4 h. After cooling, the solvent was evaporized and the residue was purified by preparative TLC on SiO₂, with CHCl₃ saturated with 28% ammonia water: MeOH=97:3 as a

developing solvent to give **4** (75 mg, 80%). NMR and MS spectra of **4** were identical to those of an authentic sample reported by Mori *et al.*¹²⁾ *Anal.* Calcd for C₃₃H₄₇NO₁₁: C, 62.54; H, 7.47; N, 2.21. Found: C, 62.35; H, 7.29; N, 2.12.

8-O-Linoleoyl-14-anisoylaconine (5) A mixture of **1** (25 mg, 0.04 mmol), pyridine (0.09 ml) and linoleic acid (300 mg, 1.07 mmol) was stirred for 4 h at 70 °C. After cooling, ethyl acetate was added to the solution and washed with water, then dried over anhydrous sodium sulfate. The solvent was evaporized and the residue was purified by column chromatography on alumina with benzene:CHCl₃=3:1 as an eluting solvent to afford **5** (26.5 mg, 79.9%), oil, $[\alpha]_D^{25} + 9.24^\circ$ ($c=1.32$, CHCl₃). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 258 (4.00). FD-MS m/z : 895 (M⁺). MS m/z : 615 (M⁺–C₁₈H₃₂O₂, 6%), 584 (M⁺–C₁₈H₃₂O₂–CH₃O, base peak). ¹H-NMR (CDCl₃) δ : 7.97 (2H, d, $J=8.9$ Hz, anisoyl group), 6.92 (2H, d, $J=8.9$ Hz, anisoyl group), 3.75 (3H, s, OCH₃), 3.30 (3H, s, OCH₃), 3.26 (3H, s, OCH₃), 3.15 (3H, s, OCH₃), 3.85 (3H, s, OCH₃ of anisoyl groups), 4.06 (1H, d, $J=6.6$ Hz, C6- β -H), 1.10 (3H, t, $J=7.0$ Hz, CH₃ of C₂H₅ at nitrogen atom), 0.97 (3H, t, $J=7.6$ Hz, CH₃ of a fatty acid residue), 5.36 (4H, m, olefinic protons of linoleic acid residue). *Anal.* Calcd for C₅₁H₇₇NO₁₂: C, 68.35; H, 8.66; N, 1.56. Found: C, 68.06; H, 8.37; N, 1.53.

3-Deoxyjesaconitine (6) A mixture of **1** (100 mg, 0.15 mmol) 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 2N sodium carbonate. The alkaline solution was extracted with CHCl₃. The CHCl₃ extract was purified by preparative TLC on SiO₂ with Et₂O saturated with 28% ammonia water: hexane=4:1 as a developing solvent to yield a colorless amorphous powder (anhydrojesaconitine, 55 mg, 56%). Next, the solution of anhydrojesaconitine (54 mg, 0.08 mmol) in ethanol (5 ml) was subjected to hydrogen in the presence of platinum oxide (28 mg, 0.12 mmol) for 1.5 h. The solution was filtered and concentrated *in vacuo*. The resulting mixture was chromatographed on silica gel with Et₂O saturated with 28% ammonia water: hexane=4:1 as an eluting solvent and crystallized from acetone to give **6** (25 mg, 45%). NMR and MS spectra of **6** were identical to those of an authentic sample reported by Mori *et al.*¹¹⁾ *Anal.* Calcd for C₃₆H₄₉NO₁₁: C, 63.71; H, 7.49; N, 2.12. Found: C, 63.66; H, 7.49; N, 2.44.

8-O-Methyl-14-anisoylaconine (7) The solution of **1** (100 mg, 0.15 mmol) in methanol (30 ml) was stirred at 60 °C for 3 d. The solvent was evaporized and the residue was purified by preparative TLC on SiO₂ with CHCl₃ saturated with 28% ammonia water as a developing solvent to give **7** (83.3 mg, 82%). NMR and MS spectra of **7** were identical to those of an authentic sample reported by Bando *et al.*⁷⁾ *Anal.* Calcd for C₃₄H₄₉NO₁₁: C, 63.04; H, 7.62; N, 2.16. Found: C, 62.86; H, 7.47; N, 2.19.

8-O-Ethyl-14-anisoylaconine (8) The solution of **1** (100 mg, 0.15 mmol) in ethanol (30 ml) was stirred at 60 °C for 5 d. The solvent was evaporized and the residue was purified by preparative TLC on SiO₂ with CHCl₃ saturated with 28% ammonia water as a developing solvent to give **8** (72 mg, 72%). NMR and MS spectra of **8** were identical to those of an authentic sample reported by Bando *et al.*⁷⁾ *Anal.* Calcd for C₃₅H₅₁NO₁₁: C, 63.52; H, 7.76; N, 2.12. Found: C, 63.38; H, 7.95; N, 2.42.

8-Deoxy-14-anisoylaconine (9) Compound **1** (100 mg, 0.15 mmol) was heated at 200 °C for 30 min under reduced pressure (1.5–2.0 mmHg). The reaction mixture was separated with flash column chromatography (silica gel 5 g, 5% methanol–chloroform) to give pyrojesaconitine (60 mg, 65.9%). A THF solution (5 ml) of pyrojesaconitine (60 mg, 0.098 mmol) was added to a stirred suspension of LiAl(OMe)₃H, prepared from a dry-THF solution (5 ml) of LiAlH₄ (72 mg, 1.89 mmol) and dry methanol (0.23 ml) at 0 °C, and the reaction mixture was stirred for 1 h at –70 °C, and for an additional 1 h at room temperature. This solution was added to a wet-THF solution at 0 °C, and was filtered off. The filtrate was evaporized to give 8-deoxyaconine (45.6 mg, 96.8%). Next, *p*-anisoyl chloride (15 μ l) was added to a mixture of 8-deoxyaconine (45 mg, 0.093 mmol), pyridine (5 ml) and dichloromethane (1 ml) at –70 °C. This reaction mixture was stirred and its temperature raised from –70 to –5 °C, which took 2 h. The reaction mixture was added to ice water and made alkaline with a solution of 5% sodium bicarbonate. The alkaline solution was extracted with chloroform, and the chloroform solution was washed with water, then dried over anhydrous sodium sulfate. The solvent was evaporized and the residue was purified by column chromatography on silica gel with Et₂O saturated with 28% ammonia water as an eluting solvent to yield **9** (37 mg, 64.3%), amorphous powder, $[\alpha]_D^{20} - 5.2^\circ$ ($c=1.46$, CHCl₃). MS m/z : 617 (M⁺). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 257 (4.02). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{–1}: 1711 (C=O). ¹H-NMR (CDCl₃) δ : 7.99 (2H, d, $J=8.9$ Hz, anisoyl group), 6.93 (2H, d, $J=8.9$ Hz, anisoyl group), 3.69 (3H, s, OCH₃), 3.30 (3H, s, OCH₃), 3.28 (3H, s, OCH₃), 3.25 (3H, s, OCH₃), 3.87 (3H, s, OCH₃ of anisoyl

group), 4.43 (1H, d, $J=5.0$ Hz, C14- β -H), 4.51 (1H, dd, $J=10.2$, 6.6 Hz, C15- β -H), 1.11 (3H, t, $J=6.9$ Hz, CH₃ of C₂H₅ at nitrogen atom). *Anal.* Calcd for C₃₃H₄₇NO₁₀: C, 64.16; H, 7.67; N, 2.27. Found: C, 63.81; H, 7.32; N, 2.02.

Animals Male mice of the Std:ddY strain, weighing 20–24 g, were used. All mice were maintained at 24–25°C on a 12 h light-dark cycle and were given food and water *ad libitum*.

Measurement of the Analgesic Activity of Jesaconitine (1) and Its Derivatives on the Acetic Acid-Induced Writhing in Mice Substances tested were used as 3% arabic gum suspensions and were administered s.c. 30 min before the i.p. injection of 0.7% acetic acid in a 0.9% physiological saline solution. The number of mice writhing for 10 min from 10 min after the injection of 0.7% acetic acid was counted. When the number of writhing mice was less than half the mean writhing-number of the control group, the analgesic effect of the substance was determined to be positive. The ED₅₀ value and 95% confidence limits were calculated according to the method of Litchfield and Wilcoxon.¹³⁾

Measurement of the Toxicity of Jesaconitine (1) and Its Derivatives in Mice Substances tested were administered s.c. and mortalities were observed 72 h after administration. The LD₅₀ value and 95% confidence limits were calculated according to the method of Litchfield and Wilcoxon.¹³⁾

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