STUDIES ON ACONITUM SPECIES. VIII.[1]
COMPONENTS OF "KAKO-BUSHI-MATSU"

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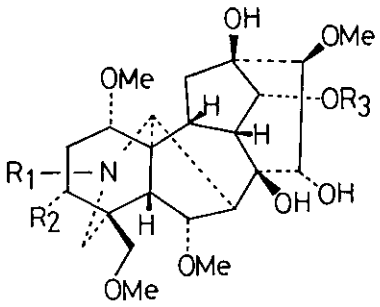
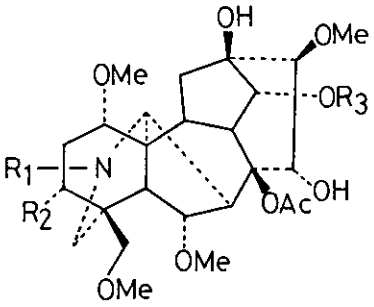
Abstracts --- Seventeen C_{19}-diterpenoid alkaloids were isolated from the processed aconite, "Kako-bushi-matsu". Structures of those compounds were determined on the basis of their spectral and chemical data. It was demonstrated that 15-keto-pyro type compounds 10, 11, and 15-20 were induced by heating of aconitine mesaconitine, jesaconitine, and hypaconitine present in the raw aconite roots.

The crude drug "bushi" obtained from the roots of some Aconitum species has been used in Japan and China as an indispensable oriental medicine on the therapy for the rheumatism and improvement of hypometabolism. However, it has been known that the raw roots have a very strong toxicity. On practice, the processed aconite called as "Kako-bushi-matsu", which was produced by autoclaving of the raw roots at 110°C, 40 min, to decrease the toxicity, has been used for the medical treatment for rheumatism, neulalgia, climacteric disorder and so on.[2-6] The present work was initiated as a part of the investigation of the effective components present in the processed aconite which was commercially available. We report in this paper on the isolation and structure determination of seventeen diterpenoid alkaloids from "Kako-bushi-matsu". "Kako-bushi-matsu" was extracted with MeOH. The MeOH extract was treated as shown in Experiment to give fractions





1: R_1= Me, R_2= OH, R_3= Bz

2: R_1= Et, R_2= OH, R_3= Bz

3: R_1= Me, R_2= H, R_3= Bz

4: R_1= Et, R_2= OH, R_3= As

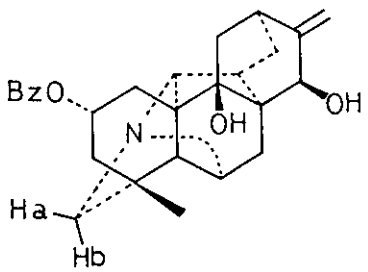
5: R_1= Me, R_2= OH, R_3= Bz

6: R_1= Me, R_2= H, R_3= Bz

7: R_1= Et, R_2= OH, R_3= Bz

8: R_1= Et, R_2= OH, R_3= As

Bz= benzoyl, As= anisoyl



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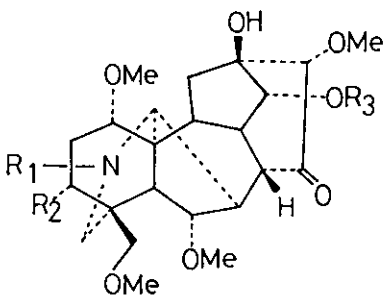

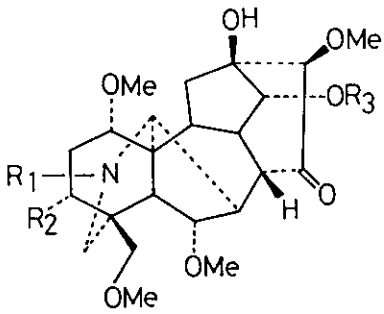

10: R_1= Et, R_2= OH, R_3= Bz

14: R_1= Et, R_2= OH, R_3= H

15: R_1= Me, R_2= OH, R_3= Bz

17: R_1= Et, R_2= OH, R_3= As

19: R_1= Me, R_2= H, R_3= Bz

11: R_1= Et, R_2= OH, R_3= Bz

12: R_1= Me, R_2= OBz, R_3= Bz

13: R_1= Et, R_2= OH, R_3= H

16: R_1= Me, R_2= OH, R_3= Bz

18: R_1= Et, R_2= OH, R_3= As

20: R_1= Me, R_2= H, R_3= Bz

A, B, and C. From the fraction A, seventeen alkaloids were isolated by high performance liquid chromatography and silica gel chromatography and were listed in Table I. Four known alkaloids among them, mesaconitine (5), hypaconitine (6), aconitine (7), and jesaconitine (8), were identified by comparison of their melting points and spectral data with each authentic sample.[7] Benzoylmesaconine (1), benzoylaconine (2), benzoylhypaconine (3), and 14-anisoylaconine (4) were identical to partial hydrolysis products of compounds 5, 7, 6, and 8, respectively.

Compound 9, mp 214-215°C, $C_{27}H_{31}NO_4$ (M^+ 433.2259, calcd for 433.2253), showed following properties. The uv absorption of 9 showed a maximum at 230 nm (log ε =4.1), indicating the presence of a benzoyl group. The ir absorption at 1717 cm^{-1} suggested the presence of a carbonyl group. The ^{1}H-nmr spectrum of 9 revealed the presence of a benzoyl group [δ 7.43-8.03 (5H, m)], an angular methyl group at δ 1.06 (s), and an carbinyl methine at δ 4.12 (s). The remaining carbon number of the molecule led the compound to C_{20}-diterpenoid alkaloid with an exo-methylene group, of which protons were observed at δ 5.00 (1H, s) and 4.97 (1H, s) in the ^{1}H-nmr and 155.2 and 109.6 ppm in the ^{13}C-nmr spectra. The deshielded chemical shift values of C(19)-Ha (δ 3.04) and C(20)-H (δ 3.31) were explained by the anisotropic effect of an axial α-oxygen atom at the C(2) position.[8] In the ms spectrum, the base peak was observed at m/z 416 (M^+-OH). This finding suggested that compound 9 had a hydroxy group at C(9) position.[9] Consequently, compound 9 was determined to be ryosenamine by comparison of the spectral data reported by Sakai et al.[9]

Compound 10 was obtained as colorless needles, mp 166-167°C, $[\alpha]_D$ = -64.3°, $C_{32}H_{43}NO_9$ (M^+ 585.2925, calcd for 585.2938). The ^{1}H-nmr spectrum showed a benzoyl group [δ 7.44-7.60 (3H, m), 7.96-7.98 (2H, m)], four methoxy groups [δ 3.26 (6H, s), 3.29 (3H, s), 3.81 (3H, s)], a methine assignable to C(14)-βH [δ 5.43 (1H, d, J=5.0 Hz)] and an N-ethyl group [δ 1.07 (3H, t, J=7.3 Hz)]. The ^{13}C-nmr signal at 211.7 ppm and ir absorption at 1717 cm^{-1} showed a carbonyl group. Based on the results of the ^{1}H- and ^{13}C-nmr spectra of 10, it was suggested that both of the acetoxy and the hydroxy groups attached to C(8) and C(15) of aconitine (7), respectively, were not present. These data suggested that compound 10 was a pyro-type alkaloid, and also were very similar to those of compound 11 as follows. The ir, uv, and ^{1}H- and ^{13}C-nmr spectra of compound 11, $C_{32}H_{43}NO_9$ (M^+ 585.2954, calcd for 585.2938), were almost the same as those of

compound 10, with the exception of the chemical shift assignable to the methoxy group at C(16); δ 3.81 and 3.64 in the ^{1}H-nmr spectra of 10 and 11, respectively. The ^{1}H- and ^{13}C-nmr signals at C(16) of 11 were shifted 0.17 ppm upfield and 3.2 ppm downfield in comparison with those of 10, respectively. On the basis of the ^{1}H-nmr spectrum and consideration of the stereochemistry in the molecular model, the methoxy protons at C(16) in 11 were affected by the shielding effect of the aromatic ring but the C(16) methoxy group in 10 was not. These data suggested that compound 11 was an epimer to compound 10 at C(16) position. According to the report of Sakai et al.[10], the configuration of C(16) methoxy group in aconitine alkaloids was changed by epimerization in case of C(15) ketone type, and the signal at δ 3.77 for C(16)β-methoxy group was shifted at δ 3.80 by the epimerization in 3-benzoylpyromesaconitine (12). Katz has also reported that the signal for C(16)β-methoxy group in desbenzoylpyroaconitine (13) was shifted downfield at δ 3.88 assigned as C(16)α-methoxy group by the epimerization under alkaline condition.[11] In order to confirm their structures of 10 and 11, aconitine (7) was heated at 200°C for 30 min under reduced pressure. The pyrolysis of aconitine (7) gave two compounds A and B (in 38.5 and 55.8% yields, respectively), which were identical to compounds 10 and 11 in terms of the ^{1}H- and ^{13}C-nmr, ms, and ir spectra and tlc behavior, respectively. During the purification of 11, it was found that a part of 11 was transformed into 10 by the epimerization at C(16). Hydrolysis product (14) of 10 was identified to 16-epi-desbenzoylpyroaconitine with C(16)α-methoxy group in terms of the ^{1}H-nmr, ir, and ms spectra and melting point (perchlorate) described in literature.[11] Consequently, both compounds 10 and 11 were epimers on the stereochemistry at C(16) and were determined to be 16-epi-pyroaconitine and pyroaconitine, respectively.

Compound 15 was obtained as amorphous powder, $[\alpha]_D$ = -79.5°, $C_{31}H_{41}NO_9$ (M^+ 571.2799, calcd for 571.2781). The ^{1}H-nmr spectrum showed a benzoyl group [δ 7.40-7.61 (3H, m), 7.97-8.00 (2H, m)], four methoxy groups [δ 3.26 (3H, s), 3.27 (3H, s), 3.30 (3H, s), 3.80 (3H, s)], three methines assignable to C(14)-βH [δ 5.43 (1H, d, J=5.0 Hz)], C(6)β-H [δ 3.91 (1H, d, J=6.8 Hz)], and C(16)β-H [δ 3.89 (1H, s)], and an N-methyl group [δ 2.30 (3H, s)]. The ^{13}C-nmr signal at 211.5 ppm and ir absorption at 1717 cm^{-1} showed a carbonyl group. No acetyl group showed in the ^{1}H-nmr spectrum but other resonances were similar to those of mesaconitine (5). Those spectral data suggested that compound 15 was a pyro-

Table I. Alkaloids Isolated from Fraction A.

Alkaloids	mp (°C)	Yield (mg)
Benzoylmesaconine (1)	amorphous	5811
Benzoylaconine (2)	amorphous	4723
Benzoylhypaconine (3)	amorphous	932
14-Anisoylaconine (4)	183-184	11651
Mesaconitine (5)	207-209	347
Hypaconitine (6)	189-190	54
Aconitine (7)	202-205	187
Jesaconitine (8)	amorphous	192
Ryosenamine (9)	213-214	246
16-Epi-pyroaconitine (10)	166-167	43
Pyroaconitine (11)	amorphous	17
16-Epi-pyromesaconitine (15)	amorphous	78
Pyromesaconitine (16)	amorphous	36
16-Epi-pyrojesaconitine (17)	181-182	83
Pyrojesaconitine (18)	amorphous	46
16-Epi-pyrohypaconitine (19)	amorphous	24
Pyrohypaconitine (20)	amorphous	8

type of mesaconitine. On the other hand, the ir and ^{1}H-nmr spectra of compound 16, amorphous, $C_{31}H_{41}NO_9$ (M^+ 571.2777, calcd for 571.2781), were almost the same as those of 15 with exception of the chemical shift assignable to methoxy group at C(16) in the nmr spectrum; δ 3.80 and 3.63 in the spectra of 15 and 16, respectively. Both compounds 15 and 16 were a pyro-type of mesaconitine and the stereochemistry of methoxy group at C(16) was determined as α and β configuration in 15 and 16, respectively, on the basis of shielding effect of the aromatic ring as mentioned above. The stereochemistry was also supported by means of high resolutional nmr measurement. The ^{1}H-nmr (400 MHz) spectrum of 15 showed the signal at δ 3.89 assignable to C(16)-H as triplet. On the other hand that of 16 showed the signal at δ 3.91 as singlet. Those assignments were confirmed by 2D nmr measurement. The C(16)-H in 15 was found to be coupled to C(8)-H and C(12)-βH with W-type long range coupling and the stereochemistry of the proton was determined as β-position. The stereochemistry at C(16) agreed with that of 15 isolated from the processed aconite roots by Niitsu, et al.[12] Pyrolysis of mesaconitine (5) gave two compounds C and D, which were identical to 15 and 16 in terms of the ^{1}H- and ^{13}C-nmr, ir, and ms spectra and tlc behavior. Compound 15

was also yielded during purification of 16. These results showed that 15 and 16 were 16-epi-pyromesaconitine and pyromesaconitine, respectively.

Compound 17, mp 181-182°C, $[\alpha]_D$ = -58.9°, showed the following properties. The hr-ms (M^+ 615.3050, calcd for 615.3043) indicated the molecular formula, $C_{33}H_{45}NO_{10}$. The uv absorption at 257 nm (log ε= 4.02) indicated the presence of an anisoyl group. The ir absorption at 1717 cm^{-1} and ^{13}C-nmr signal at 211.6 ppm suggested the presence of a carbonyl group. The ^1H-nmr spectrum of 17 revealed the presence of an anisoyl group [δ 7.93 (2H, d, J= 8.9 Hz), 6.94 (2H, d, J=8.9 Hz)], four methoxy groups [δ 3.26 (6H, s), 3.30 (3H, s), 3.80 (3H, s)], a methoxy of the anisoyl group [δ 3.87 (3H s)], an carbinyl methine assignable to C(14)-βH [δ 5.39 (1H, d, J=5.0 Hz)], and an N-ethyl group [δ 1.05 (3H, t, J=7.3 Hz)]. These spectra suggested that 17 was a pyro-type of jesaconitine like the pyro-type of aconitine and mesaconitine mentioned above. On the other hand, the ir, uv, and ^1H- and ^{13}C-nmr spectra of compound 18, $C_{33}H_{45}NO_{10}$ (M^+ 615.3042, calcd for 615.3043), were very similar to those of compound 17 with the exception of the chemical shift assignable to the methoxy group at C(16); δ 3.80 and 3.63 in the ^1H-nmr spectra of 17 and 18, respectively. The signals at δ 3.80 and 3.63, therefore, could be assigned to C(16)α- and C(16)β-methoxy groups, respectively, according to the assignment for C(16)-methoxy group in the pyro-type of aconitine and mesaconitine. On the basis of these findings, compounds 17 and 18 were epimers on the stereochemistry at C(16) and were determined as 16-epi-pyrojesaconitine and pyrojesaconitine[13], respectively.

Keith and Pelletier reported on pyrolysis of jesaconitine (8) to yield pyrojesaconitine (18).[14] However, they did not mention the stereochemistry at C(16). Pyrojesaconitine (18) obtained by them was possibly epimerized on configration at C(16) but currently the possibility could not be investigated.

In order to confirm their structures of 17 and 18, pyrolysis of jesaconitine (8) gave two compounds E and F, which were identical to 17 and 18 in terms of the ^1H- and ^{13}C-nmr, ms, and ir spectra and tlc behavior, respectively. A part of compound 18 was transformed into 17 during the purification as the case of that in 11 and 16 described as above.

Compound 19 was obtained as amorphous powder, $[\alpha]_D$ = -65.0°, $C_{30}H_{38}NO_7$ (M^+-OCH_3 524.2625, calcd for 524.2648). The ^1H-nmr spectrum showed a benzoyl group [δ 7.44-7.60 (3H, m), 7.96-8.00 (2H, m)], four methoxy groups [δ 3.25 (3H, s), 3.27 (3H, s), 3.28 (3H, s), 3.80 (3H, s)], a methine assignable to C(14)-βH [δ 5.44

Table II. ^{13}C-Chemical Shifts and Assignments for Desbenzoylpyroaconitine (13), 16-Epi-pyroaconitine (10), Pyroaconitine (11), 16-Epi-pyromesaconitine (15), Pyromesaconitine (16), 16-Epi-pyrojesaconitine (17), Pyrojesaconitine (18), 16-Epi-pyrohypaconitine (19), Pyrohypaconitine (20).

Carbon	13[11]	10	11	15	16	17	18	19	20
1	84.1	83.4	83.5	83.6	83.5	83.6	83.5	86.2	86.4
2	34.2	32.5	34.1	32.7	34.2	32.9	34.2	26.0	26.2
3	71.8	71.6	71.6	71.6	71.2	71.9	71.6	35.2	35.1
4	43.6	43.6	43.5	43.9	43.7	43.6	43.1	39.8	39.6
5	47.1	48.0	48.1	47.8	47.4	48.6	48.1	47.5	48.5
6	83.7	84.0	84.0	84.0	83.9	84.1	84.1	84.2	84.2
7	42.3	41.9	42.5	40.7	41.5	41.7	42.5	41.1	41.8
8	43.3	49.0	48.5	49.3	48.3	49.4	48.5	49.4	48.9
9	48.5	38.5	38.6	38.7	38.6	38.8	38.6	38.9	38.9
10	41.2	44.6	43.1	44.7	43.0	44.8	43.5	45.2	43.5
11	51.0	51.2	50.9	51.3	51.0	51.3	50.9	51.4	51.1
12	35.2	33.8	36.1	34.1	35.9	34.1	36.1	33.1	36.3
13	76.0	77.4	76.6	77.4	76.1	77.4	76.6	76.6	76.3
14	78.5	78.5	79.5	78.3	79.4	78.3	79.2	78.6	79.7
15	210.6	211.7	211.8	211.5	211.6	211.6	211.9	211.7	211.9
16	89.1	86.0	89.2	86.1	89.1	86.1	89.2	86.2	89.3
17	61.8	61.6	61.7	62.8	63.0	61.7	61.7	63.1	63.4
18	77.0	76.6	76.2	76.5	76.1	76.8	76.2	80.3	80.3
19	47.6*	47.6	47.4	49.7	49.7	47.3	47.4	56.4	56.3
N-CH_2	49.1*	49.4	49.1	-	-	49.1	49.1	-	-
CH_3	13.4	13.2	13.3	42.1	42.0	13.4	13.3	42.3	42.3
1'	56.1	56.0	56.1	56.4	56.4	56.1	56.1	56.5	56.5
6'	57.8	57.9	57.9	57.8	57.9	57.8	57.9	58.0	58.0
16'	60.9	62.3	61.9	62.3	61.8	62.3	61.9	62.3	61.9
18'	59.3	59.2	59.2	59.2	59.1	59.2	59.2	59.2	59.2
C=O		166.0	167.1	166.0	167.0	165.8	166.8	166.1	167.3
$C_6H_{4(5)}$		133.6	133.2	133.5	133.2	121.6	122.0	133.6	133.2
		129.6	129.8	129.6	129.8	131.8	131.9	129.7	129.9
		129.3	129.6	129.3	129.5	113.8	113.7	128.7	128.4
		128.6	128.4	128.6	128.3	164.0	163.6		
p(OMe)		-	-	-	-	55.5	55.4	-	-

*: Those assignments should be reversed according to the preceeding paper.[7,15] The assignments for methine carbons, C(5), C(7), C(8), C(9), and C(10), were determined on the basis of the hetero-COSY spectrum of 15.

(1H, d, J=5.0 Hz], and an N-methyl group [δ 2.30 (3H, s)]. The ^{13}C-nmr signal at 211.7 ppm and ir absorption at 1717 cm^{-1} showed the carbonyl group. No acetyl group showed in the ^{1}H-nmr spectrum but other resonances were similar to those of hypaconitine (6). These data suggested that compound 19 was a pyro-type of hypaconitine. On the other hand, the ir and ^{1}H-nmr spectra of compound 20, amorphous, $C_{30}H_{38}NO_7$ (M^+-OCH_3 524.2653, calcd for 524.2648), were almost the same as those of 19 with exception of the chemical shift assignable to methoxy group at C(16) in the nmr spectrum; δ 3.80 and 3.62 in the spectra of 19 and 20, respectively. Pyrolysis of hypaconitine (6) gave two compounds 19 and 20 in terms of the ^{1}H- and ^{13}C-nmr, ir, and ms spectra and tlc behavior. Therefore, 19 and 20 were determined as 16-epi-pyrohypaconitine and pyrohypaconitine. Pyro-type of alkaloids (16, 18, 19, and 20) have not been reported so far. It was found that those alkaloids were produced by the processing procedure to prepare the commercial material, "Kako-bushi-matsu", since the pyro-type alkaloids (10, 11, 15-20) have not been isolated so far from raw roots of Aconitum species.

EXPERIMENTAL

"Kako-bushi-matsu" used is a commercial medicine produced by Sanwa Shoyaku Co. Ltd. All melting points are uncorrected. Optical rotations were measured with a JASCO DIP-4 polarimeter. Ir spectra in KBr disks were taken with a JASCO FT/7000 spectrophotometer, uv spectra were measured in EtOH solution with Shimadzu UV240 spectrophotometer. Nmr spectra were measured in $CDCl_3$ solution with a JOEL FX-100 and GX-270 and BRUKER MSL-400 spectrometers using TMS as an internal standard. Ms were measured with a Shimadzu LKB-9000B spectrometer, and hr-ms was measured with JMS-D300 mass spectrometer. HPLC was performed with Gasukuro Kogyo Model 572P and 502T.

Isolation procedure---"Kako-bushi-matsu" (5 kg) was extracted with MeOH (50 l) to afford the MeOH extracts (360 g). The extract was added to 5% aqueous HCl (1.5 l) and partitioned with hexane (1.5 l x 3). The aqueous layer was adjusted to pH 9 with 28% ammonia and extracted with $CHCl_3$ (1.5 l x 5) to afford $CHCl_3$ layer, fraction A (31.8 g). The hexane layer and the aqueous layer were evaporated to give fractions B and C, respectively. The fractions B and C were left for the future investigation. Separation of fraction A was performed by HPLC. The condition was as follows; column; Inertsil ODS (20 x 250 mm), mobile phase; 0.05 M

phosphate buffer (pH 2.35):THF:CH_3CN (80:15:5 in volume ratio), flow rate; 10 ml/min, detection; uv 240 nm, each fraction and the retention time (min); fraction 1 (14.5-17.0): fraction 2 (17.5-20.0): fraction 3 (20.5-24.0): fraction 4 (24.5-27.0): fraction 5 (27.0-30.0): fraction 6 (30.0-33.0): fraction 7 (33.5-35.5): fraction 8 (38.0-42.0). Each fraction was made alkaline (pH 9) with 28% ammonia and extracted with $CHCl_3$. The $CHCl_3$ extracts from fractions 1 and 2 were chromatographed on silica gel (300 g, solvent: 30% MeOH in $CHCl_3$ and 25-30% MeOH in $CHCl_3$, respectively). Benzoylmesaconine (1; 5811 mg) was obtained from fraction 1. Benzoylaconine (2; 4723 mg) and benzoylhypaconine (3; 932 mg) were obtained from fraction 2. Fraction 3 was chromatographed on silica gel (250 g, 10-25% MeOH/$CHCl_3$) to afford 14-anisoylaconine (4; 11651 mg) and 16-epi-pyromesaconitine (15). Fraction 4 was separated with flash column chromatography (silica gel 20 g, 5-20% MeOH/$CHCl_3$) to give mesaconitine (5; 347 mg), pyromesaconitine (16; 36 mg), and 16-epi-pyrohypaconitine (19; 24 mg). Fractions 5, 6, and 7 were separated with flash column chromatography (silica gel 10 g, 2-20% MeOH/$CHCl_3$, 2-15% MeOH/$CHCl_3$, and 5% MeOH/$CHCl_3$, respectively). Hypaconitine (6; 54 mg), pyrohypaconitine (20; 8 mg), 16-epi-pyroaconitine (10; 43 mg), and ryosenamine (9; 246 mg) were obtained from fraction 5. Aconitine (7; 187 mg) and pyroaconitine (11; 8 mg) were obtained from fraction 6. From fraction 7, 16-epi-pyrojesaconitine (17; 83 mg) and pyrojesaconitine (18; 46 mg) were obtained. Fraction 8 was chromatographed on silica gel (10 g, ether saturated with 28% ammonia) to give jesaconitine (8; 192 mg). Purified compounds were listed in Table I and unidentified compounds are remained to investigate.

Benzoylmesaconine (1) -- Amorphous. Ir (ν, cm^{-1}): 3500, 1717, 1605, 1585, 1280, 1100. Ms (m/z): 589 (M^+), 574 (M^+-CH_3), 558 (M^+-OCH_3, base peak), 540 (558-H_2O), 105 (benzoyl cation). ^{1}H-Nmr (δ): 2.37 (3H, s), 3.28, 3.30, 3.31, 3.69 (each 3H, s), 4.09 (1H, d, J=6.9 Hz), 4.55 (1H, t, J=5.0 Hz), 5.01 (1H, d, J=5.0 Hz), 7.40-7.59 (3H, m), 8.01-8.04 (2H, m).

Benzoylaconine (2) -- Amorphous. Nmr and ms spectra of 3 were identical with those of authentic sample reported by Wang, et al.[16]

Benzoylhypaconine (3) -- Amorphous. Ir (ν, cm^{-1}): 3440, 1725, 1605, 1585, 1280, 1095. Ms (m/z): 573 (M^+), 556 (M^+-OH), 542 (M^+-OCH_3), 105 (benzoyl cation).

^{1}H-Nmr (δ): 2.37 (3H, s), 3.29, 3.30, 3.33, 3.69 (each 3H, s), 4.04 (1H, d, J=6.9 Hz), 4.54 (1H, d, J=5.3 Hz), 5.04 (1H, d, J=5.0 Hz), 7.42-7.60 (3H, m), 8.02-8.05 (2H, m).

14-Anisoylaconine (4) -- Amorphous. Ir (ν, cm-1): 3430, 1705, 1600, 1510, 1255, 1095. Ms (m/z): 633 (M^+), 618 (M^+-CH_3), 602 (M^+-OCH_3, base peak), 584 (602-H_2O), ^{1}H-nmr (δ): 1.09 (3H, t, J=7.0 Hz), 3.22, 3.29, 3.30, 3.70, 3.84 (each 3H, s), 4.08 (1H, d, J=6.4 Hz), 4.51 (1H, brs), 4.94 (1H, d, J=5.3 Hz), 6.86 (2H, d, J=8.9 Hz), 7.92 (2H, d, J=8.9 Hz).

Ryosenamine (9) -- Colorless prism, mp 213-214°C. Hr-ms: calcd for $C_{27}H_{31}NO_4$ 433.2253, found 433.2259. Uv (λ, nm): 230 (log ε= 4.10). Ir (ν, cm^{-1}): 3452, 1717. Ms (m/z): 433 (M^+), 416 (M^+-OH, base peak), 312 (M^+-OBz). ^{1}H-nmr (δ): 1.06 (3H, s), 2.62 (1H, d, J=13.1 Hz), 3.04 (1H, d, J=13.2 Hz), 3.31 (1H, brs), 3.33 (1H, brs), 4.12(1H, s), 4.97 (1H, s), 5.00 (1H, s), 5.54 (1H, m), 7.43-8.03 (5H, m).

16-Epi-pyroaconitine (10) -- Colorless needles, mp 166-167°C. $[\alpha]_D$ = -64.3° (c=0.23, EtOH). Hr-ms: calcd for $C_{32}H_{43}NO_9$ 585.2938, found 585.2925. Uv (λ, nm): 230 (log ε= 4.15). Ir (ν, cm^{-1}): 3450, 1717. Ms (m/z): 585 (M^+), 554 (M^+-OMe, base peak). ^{1}H-Nmr (δ): 1.07 (3H, t, J=7.3 Hz), 3.26 (6H, s), 3.29 (3H, s), 3.81 (3H, s), 5.43 (1H, d, J=5.0 Hz), 7.44-7.60 (3H, m), 7.96-7.98 (2H, m). ^{13}C-Nmr spectrum was shown in Table II.

Pyroaconitine (11) -- Amorphous. $[\alpha]_D$ = +21.8° (c=0.22, EtOH). Hr-ms: calcd for $C_{32}H_{43}NO_9$ 585.2938, found 585.2954. Uv (λ, nm): 230 (log ε= 4.10). Ir (ν, cm^{-1}): 3450, 1717. Ms (m/z): 585 (M^+), 554 (M^+-OMe, base peak). ^{1}H-Nmr (δ): 1.03 (3H, t, J=7.0 Hz), 3.26 (3H, s), 3.29 (3H, s), 3.30 (3H, s), 3.64 (3H, s), 5.18 (1H, d, J=4.0 Hz), 7.30-7.53 (3H, m), 7.94-7.97 (2H, m). ^{13}C-Nmr spectrum was shown in Table II.

16-Epi-pyromesaconitine (15) -- Amorphous. $[\alpha]_D$ = -79.5° (c=0.44, EtOH). Hr-ms: calcd for $C_{31}H_{41}NO_9$ 571.2781, found 571.2799. Uv (λ, nm): 230 (log ε= 4.05). Ir (ν, cm^{-1}): 3450, 1717. Ms (m/z): 571 (M^+), 540 (M^+-OMe, base peak). ^{1}H-Nmr (δ): 2.30 (3H, s), 3.26 (3H, s), 3.27 (3H, s), 3.30 (3H, s), 3.80 (3H, s), 5.43

(1H, d, J=5.0 Hz), 7.40-7.61 (3H, m), 7.97-8.00 (2H, m). ^{13}C-Nmr spectrum was shown in Table II.

Pyromesaconitine (16) -- Amorphous. $[\alpha]_D$ = +15.4° (c=0.48, EtOH). Hr-ms: calcd for $C_{31}H_{41}NO_9$ 571.2781, found 571.2777. Uv (λ, nm): 230 (log ε= 4.05). Ir (ν, cm^{-1}): 3450, 1717. Ms (m/z): 571 (M^+), 540 (M^+-OMe, base peak). ^{1}H-Nmr (δ): 2.28 (3H, s), 3.29 (3H, s), 3.31 (6H, s), 3.63 (3H, s), 5.20 (1H, d, J=4.0 Hz), 7.40-7.57 (3H, m), 7.95-7.98 (2H, m). ^{13}C-Nmr spectrum was shown in Table II.

16-Epi-pyrojesaconitine (17) -- Colorless needles, mp 181-182°C. $[\alpha]_D$ = -58.9° (c=0.55, EtOH). Hr-ms: calcd for $C_{33}H_{45}NO_{10}$ 615.3043, found 615.3050. Uv (λ, nm): 257 (log ε= 4.02). Ir (ν, cm^{-1}): 3456, 1715. Ms (m/z): 615 (M^+), 584(M^+-OMe, base peak). ^{1}H-Nmr (δ): 1.05 (3H, t, J=7.3 Hz), 3.26 (6H, s), 3.30 (3H, s), 3.80 (3H, s), 3.87 (3H, s), 5.39 (1H, d, J=5.0 Hz), 6.94 (2H, d, J=8.9 Hz), 7.93 (2H, d, J=8.9 Hz). ^{13}C-Nmr spectrum was shown in Table II.

Pyrojesaconitine (18) -- Amorphous. $[\alpha]_D$ = +20.5° (c=0.43, EtOH). Hr-ms: calcd for $C_{33}H_{45}NO_{10}$ 615.3043, found 615.3042. Uv (λ, nm): 257 (log ε= 4.01). Ir (ν, cm^{-1}): 3484, 1711. Ms (m/z): 615 (M^+), 584 (M^+-OMe, base peak). ^{1}H-Nmr (δ): 1.03 (3H, t, J=7.3 Hz), 3.26 (3H, s), 3.29 (3H, s), 3.30 (3H, s), 3.63 (3H, s), 3.86 (3H, s), 5.14 (1H, d, J=4.0 Hz), 6.94 (2H, d, J=8.9 Hz), 7.92 (2H, d, J=8.9 Hz). ^{13}C-Nmr spectrum was shown in Table II.

16-Epi-pyrohypaconitine (19) -- Amorphous. $[\alpha]_D$ = -65.0° (c=0.18, EtOH). Hr-ms: calcd for $C_{30}H_{38}NO_7$ (M^+-OCH_3) 524.2648, found 524.2653. Uv (λ, nm): 230 (log ε= 4.10). Ir (ν, cm^{-1}): 3450, 1717. Ms (m/z): 555 (M^+), 524 (M^+-OMe, base peak). ^{1}H-Nmr (δ): 2.30 (3H, s), 3.25 (3H, s), 3.27 (3H, s), 3.28 (3H), 3.80 (3H, s), 5.44 (1H, d, J=5.0 Hz), 7.44-7.60 (3H, m), 7.96-8.00 (2H, m). ^{13}C-Nmr spectrum was shown in Table II.

Pyrohypaconitine (20) -- Amorphous. $[\alpha]_D$ = +15.6° (c=0.18, EtOH). Hr-ms: calcd for $C_{30}H_{38}NO_7$ (M^+-OCH_3) 524.2648, found 524.2625. Uv (λ, nm): 230 (log ε= 4.07). Ir (ν, cm^{-1}): 3450, 1717. Ms (m/z): 555 (M^+), 524 (M^+-OMe, base peak). ^{1}H-Nmr (δ): 2.28 (3H, s), 3.25 (3H, s), 3.28 (6H, s), 3.62 (3H), 5.20 (1H, d, J=4.0 Hz), 7.40-7.65 (3H, m), 7.96-8.00 (2H, m). ^{13}C-Nmr spectrum was shown in Table II.

Partial hydrolysis of mesaconitine (5), hypaconitine (6), aconitine (7), and jesaconitine (8) -- Each sample (50 mg) was dissolved in a mixture (10 ml) of dioxane and water (1:1) and the each solution was refluxed for 4 h. The reaction mixture was evaporated and purified with tlc to give benzoylmesaconine (1, 40 mg, 84.8%), benzoylhypaconine (3, 40 mg, 86.5%), benzoylaconine (2, 39 mg, 82.4%), and 14-anisoylaconine (4, 40 mg, 85.3%) from 5, 6, 7, and 8, respectively.

Hydrolysis of 16-epi-pyraconitine (10) -- Compound 10 (23.9 mg) and K_2CO_3 (5.2 mg) were added to 90% aqueous MeOH (1.8 ml) and the solution was stirred for 40h at room temperature. The reaction mixture was poured into water (5 ml) and was extracted with $CHCl_3$ (10 ml x 3). The organic layer was washed with water, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give amorphous powder (15.3 mg, 77.9%). Mp 208-224 °C (perchlorate, decomp). Ir (ν, cm^{-1}): 3426, 1711, 1102. Ms (m/z): 481 (M^+), 466 (M^+-CH_3), 450 (M^+-OCH_3, base peak), 432 (450-H_2O). ^{1}H-Nmr (δ): 1.07 (3H, t, J=7.1 Hz), 3.23 (3H, s), 3.28 (3H, s), 3.31 (3H, s), 3.76 (3H, s), 3.88 (1H, brs), 3.97 (1H, d, J=6.3 Hz), 4.25 (1H, d, J=5.0 Hz). Those spectral data were identical with those of 16-epi-desbenzoyl-pyroaconitine (14) reported by Katz et al.[11]

Pyrolysis of mesaconitine (5), hypaconitine (6), aconitine (7), and jesaconitine (8) -- Each sample (100 mg) was heated at 200 °C for 30 min under reduced pressure (1.5-2 mmHg), respectively. The each reaction mixture was separated with flash column chromatography (silica gel 5 g, 5% MeOH/$CHCl_3$) to give 16-epi-pyroaconitine (10; 35 mg, 38.5%) and pyroaconitine (11; 52.8 mg, 55.8%) from 7, 16-epi-pyromesaconitine (15; 29.7 mg, 32.8%) and pyromesaconitine (16; 56.2 mg, 62.1%) from 5, 16-epi-pyrojesaconitine (17; 32 mg, 35.2%) and pyrojesaconitine (18; 53 mg, 58.2%) from 8, and 16-epi-pyrohypaconitine (19; 30.8 mg, 34.0%) and pyrohypaconitine (20; 53.2 mg, 58.9%) from 6.

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