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Alkaloidal Constituents from *Aconitum jaluense*

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Aconitum jaluense Komar. (Ranunculaceae) is one of the *Aconitum* plants growing in Korean peninsula. An investigation of the alkaloidal constituents of this species led to the isolation of seven C_{19}-norditerpenoid and a C_{20}-diterpenoid alkaloid. Three of them have been identified as neoline, mesaconitine, and hypaconitine, which were isolated from this plant collected from Mt. Bultasan in the north part. The other five alkaloids were determined as lipomesaconitine, lipohypaconitine, 15α-hydroxyneoline, hokbusine A, and napelline, which have not been found in this plant. Structures of those alkaloids were determined on the basis of their spectral data. It is of interest to note that a comparison of the present work and the previous report showed some differences in the alkaloidal contents.

Key words: *Aconitum jaluense*, Ranunculaceae, Norditerpenoid alkaloid, Diterpenoid alkaloid

INTRODUCTION

The genus *Aconitum*, belonging to the family Ranunculaceae, comprises about twenty-three species of perennial herbs, particularly inhabiting mountainous regions (Lee, 1989) A limited number of alkaloids were reported from Korean *Aconitum* plants (Chung *et al.*, 1986; Chung and Lee, 1987; Chung *et al.*, 1988a; Chung and Lee, 1988b; Lee and Chung, 1989; Kim *et al.*, 1996; Kim *et al.*, 1998). In the course of our studies in *Aconitum* species, we have reported the isolation and structure elucidation of four new norditerpenoid alkaloids from the processed tubers of *A. carmichaeli* (Shim *et al.*, 2003). Continued investigation of our research on *Aconitum* species, the root of *A. jaluense* was conducted. *A. jaluense* is a perennial herb, 1 m in height, and is widespread in remote mountainous regions of the Korean peninsula. The root part of this plant has been used as a substitute for aconite in Korean folk medicine (Lee, 1989). Apart from a short report on the isolation of eight C_{19}-norditerpenoid alkaloids from this species, no other phytochemical information is available (Kim *et al.*, 1990). In this paper we describe the isolation and identification of seven known C_{19}-norditerpenoid alkaloids (**1**-**7**) and a napelline-type C_{20}-diterpenoid alkaloid (**8**) from the roots of *A. jaluense*.

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MATERIALS AND METHODS

General

Melting points were measured on a Mitamura-Riken apparatus, and are uncorrected. The optical rotations were determined on a JASCO P-1020 polarimeter. The IR spectra were obtained on a JASCO FT/IR-5300 spectrometer. EI mass spectra were obtained on a Hewlett-Packard 5989B spectrometer. The FAB mass spectra were obtained in a 3-nitrobenzyl alcohol as matrix in positive ion mode using a VG-VSEQ spectrometer. The NMR spectra were measured on a Varian Gemini 2000 instrument (300 MHz) or a Bruker AM-500 (500 MHz), and the chemical shifts were referenced to TMS. GC-MS analysis was performed as previously described (Shim *et al.*, 2003) using a Hewlett Packard 5989B mass spectrometer coupled to a 5890 Series II$^+$ gas chromatograph. TLC was performed on silica gel $60F_{254}$ (Merck).

Plant materials

The roots of *A. jaluense* were collected at Mt. Changbaek, Kangwon province, Korea in August 2000, and authenticated by one of authors (KHB). The voucher specimen (No. KSS 010811) was deposited in the Herbarium of the Natural Products Research Institute, Seoul National University.

Extraction and isolation

Powdered roots of *A. jaluense* (330 g) were extracted five times with MeOH at room temperature. The MeOH extracts were combined, and evaporated under reduced pressure to dryness (25 g). This was partitioned with 3% aqueous NH_4OH and $CHCl_3$. The $CHCl_3$ fraction (5.5 g) was separated into six fractions by chromatography on a silica gel column with a gradient of MeOH in $CHCl_3$ (1→5%). Each fraction was further separated by chromatography on a silica gel column using cyclohexane-EtOAc-Et_2NH (10:1:0.2) as the eluent to yield neoline (**1**, 25 mg), mesaconitine (**2**, 80 mg), lipomesaconitine (**4**, 20 mg) from subfraction **1** (0.7 g), hypaconitine (**3**, 30 mg) from subfraction **2** (0.5 g), lipohypaconitine (**5**, 7 mg) from subfraction **3** (0.5 g), 15α-hydroxyneoline (**6**, 10 mg) from subfraction **4** (0.1 g), hokbusine A (**7**, 20 mg) from subfraction **5** (0.1 g), and napelline (**8**, 5 mg) from subfraction **6** (0.1 g).

Neoline (1): Colorless needles, mp 159~160°; $[\alpha]_D^{23}$ + 2.6° (*c* = 0.1, $CHCl_3$); IR, ν_{max} 3532, 3395 (OH), 1458, 1113, 1040 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.12 (3H, t, *J* = 6.8 Hz, *N*-$CH_2\underline{CH_3}$), 1.97 (1H, br s, H-7β), 2.03 (1H, dd, *J* = 6.0, 15.9 Hz, H-15), 2.34 (1H, dd, *J* = 9.3, 15.9 Hz, H-15), 2.15 (1H, br d, *J* = 6.6 Hz, H-5), 2.28, 2.68 (1H each, d, *J* = 10.8 Hz, H-19), 2.64 (1H, br s, H-17), 3.25, 3.61 (1H each, d, *J* = 8.1 Hz, H-18), 3.31 (3H, s, OCH_3), 3.32 (6H, s, 2×OCH_3), 3.34 (1H, overlap, H-16), 3.64 (1H, overlap, H-1), 4.16 (1H, br d, *J* = 6.6 Hz, H-6β), 4.17 (1H, t, *J* = 5.1 Hz, H-14β); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): Table I; EI-MS, *m/z* (rel. int., %) 437 $[M]^+$ (4.2), 420 $[M\text{-}OH]^+$ (17.2), 404 $[M\text{-}OH\text{-}O]^+$ (2.9), 388 $[M\text{-}OH\text{-}CH_3OH]^+$ (2.4), 372 $[M\text{-}OH\text{-}O\text{-}CH_3OH]^+$ (2.0), 85 (66.2), 83 (100).

Mesaconitine (2): Colorless needles, mp 189~190°; $[\alpha]_D^{23}$ + 16° (*c* = 0.1, $CHCl_3$); IR, ν_{max} 3507 (OH), 1713 (ester CO), 1638 (aromatic C=C), 1277 (OAc), 1098, 1028, 716 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.35 (3H, s, OAc), 2.32 (3H, s, *N*-CH_3), 3.04 (1H, br s, H-17), 3.08 (1H, dd, *J* = 6.3, 9.6 Hz, H-1), 3.14 (3H, s, 1-OCH_3), 3.26 (3H, s, 6-OCH_3), 3.27 (3H, s, 16-OCH_3), 3.30 (1H, br d, *J* = 4.8 Hz, H-16α), 3.53, 3.62 (1H each, d, *J* = 9.1 Hz, H-18), 3.71 (3H, s, 18-OCH_3), 3.72 (overlap, H-3), 3.93 (1H, br s, 13-OH), 4.01 (1H, br d, *J* = 6.3 Hz, H-6α), 4.33 (1H, d, *J* = 3.0 Hz, 15-OH), 4.44 (1H, dd, *J* = 3.0, 5.4 Hz, H-15β), 4.85 (1H, br d, *J* = 5.1 Hz, H-14α), 7.44 (2H, tt, *J* = 1.2, 7.2 Hz, H-3', 5'), 7.56 (1H, tt, *J* = 1.2, 7.2 Hz, H-4'), 8.01 (2H, dt, *J* = 1.2, 7.2 Hz, H-2', 6'); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): Table I; EI-MS, *m/z* (rel. int., %) 631 $[M]^+$ (0.1), 616 $[M\text{-}CH_3]^+$ (0.3), 614 $[M\text{-}OH]^+$ (0.3), 600 $[M\text{-}CH_3O]^+$ (17.1), 572 $[M\text{-}CH_3COO]^+$ (15.8), 556 $[M\text{-}(CH_3+CH_3COOH)]^+$ (55.3), 540 $[M\text{-}(CH_3O+CH_3COOH)]^+$ (100), 522 $[M\text{-}(CH_3O+CH_3COOH+H_2O)]^+$ (13.8), 418 $[M\text{-}(CH_3O+CH_3COOH+H_2O+C_6H_5CO)+H]^+$ (5.8), 252 (21.7), 178 (25.0), 105 $[C_6H_5CO]^+$ (19.1).

Table I. ^{13}C-NMR Data of Compounds **1**, **2**, **3**, **6**, **7**, and **8** (75.5 MHz, $CDCl_3$, δ)

Carbon no.	1	2	3	6	7	8
1	72.2	83.1	85.1	72.1	82.5	69.8
2	29.2*	35.7	26.4	29.3	33.8	30.7
3	29.8*	71.0	34.9	29.9	71.3	31.5
4	38.1	43.4	39.3	38.0	43.3	33.9
5	44.9	46.5	48.1	44.0	45.7	47.1
6	83.0	82.4	83.1	84.1	83.1	23.3
7	52.2	44.2	44.5	46.4	41.5	43.8
8	74.1	91.8	91.9	79.7	82.2	50.3
9	48.3	43.6	43.8	48.6	45.1	36.0
10	44.1	40.7	41.1	43.7	41.3	52.8
11	49.5	49.9	49.9	49.3	50.3	28.5
12	29.4*	34.1	36.2	30.5	36.2	76.0
13	40.2	74.0	74.1	40.6	74.7	48.2
14	75.9	78.8	78.8*	76.1	79.4	36.2
15	42.7	78.8	78.9*	78.8	77.3	76.5
16	81.7	89.9	90.1	90.1	93.4	159.1
17	63.9	62.1	62.2	62.7	62.5	108.5
18	80.2	76.2	80.1	80.1	76.5	26.3
19	57.0	49.4	56.0	56.7	49.7	57.7
20						66.2
N-$\underline{C}H_2CH_3$	48.4			48.5		51.6
N-$CH_2\underline{C}H_3$	12.9			13.1		13.3
N-$\underline{C}H_3$		42.6	42.6		42.4	
1-OCH_3		56.3	56.6		56.2	
6-OCH_3	57.8	57.9	58.0	57.3	58.5	
8-OCH_3					49.6	
16-OCH_3	56.3	61.0	61.0	58.0	62.2	
18-OCH_3	59.2	59.1	59.1	59.1	59.0	
$O\underline{C}OCH_3$		172.4	172.4			
$OCO\underline{C}H_3$		21.4	21.4			
$O\underline{C}OC_6H_5$		166.0	166.1		166.2	
C-1'		129.7	129.8		130.1	
C-2', 6'		129.6	129.6		129.6	
C-3', 5'		128.6	128.6		128.2	
C-4'		133.3	133.2		132.8	

*Maybe exchangeable.

Hypaconitine (3): White powder, mp 186~188°C; $[\alpha]_D^{17}$ + 2.3° (*c* = 0.026, $CHCl_3$); IR, ν_{max} 3507 (OH), 1713 (ester CO), 1638 (aromatic C=C), 1277 (OAc),

1098, 1028, 716 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.37 (3H, s, OAc), 2.34 (3H, s, *N*-CH_3), 3.16 (3H, s, 1-OCH_3) 3.27 (3H, s, 6-OCH_3), 3.28 (3H, s, 16-OCH_3), 3.33 (1H, br d, *J* = 5.4 Hz, H-16α), 3.11, 3.62 (1H each, d, *J* = 8.4 Hz, H-18), 3.73 (3H, s, 18-OCH_3), 3.91 (1H, br s, 13-OH), 3.98 (1H, br d, *J* = 6.3 Hz, H-6β), 4.35 (1H, d, *J* = 2.7 Hz, 15-OH), 4.46 (1H, dd, *J* = 3.0, 5.4 Hz, H-15β), 4.88 (1H, br d, *J* = 4.8 Hz, H-14β), 7.45 (2H, tt, *J* = 1.5, 7.5 Hz, H-3', 5') 7.57 (1H, tt, *J* = 1.5, 7.5 Hz, H-4'), 8.01 (2H, dt, *J* = 1.5, 7.8 Hz, H-2', 6'); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): Table I; EI-MS, *m/z* (rel. int., %) 584 [M-CH_3O]$^+$ (21.5), 524 [M-(CH_3O+CH_3COOH)]$^+$ (100), 402 [M-(CH_3O+CH_3COOH+H_2O+C_6H_5CO)+H]$^+$ (19.2), 298 (3.0), 178 (10.8).

Liponesaconitine (4): Colorless oil; $[\alpha]_D^{17}$ + 2.9° (*c* = 0.055 $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 0.85~0.90 (m, CH_3), 1.24 [br s, $(CH_2)_n$], 2.33 (3H, s, *N*-CH_3), 3.14 (3H, s, 1-OCH_3), 3.27 (3H, s, 6-OCH_3), 3.29 (3H, s, 16-OCH_3), 3.52, 3.62 (1H each, d, *J* = 9.0 Hz, H-18), 3.74 (3H, s, 18-OCH_3), 3.94 (1H, br s, 13-OH), 4.02 (1H, br d, *J* = 6.9 Hz, H-6β), 4.41~4.44 (2H, m, H-15β, 15-OH), 4.84 (1H, br d, *J* = 4.8 Hz, H-14β), 5.30~5.40 (2H, m, olefinic H), 7.44 (2H, br t, *J* = 7.5 Hz, H-3', 5'), 7.56 (1H, br t, *J* = 6.9 Hz, H-4'), 8.02 (2H, br d, *J* = 6.9 Hz, H-2', 6'); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): 83.2 (C-1), 35.7 (C-2), 71.1 (C-3), 43.4 (C-4), 46.2 (C-5), 82.3 (C-6), 44.3 (C-7), 91.5 (C-8), 43.7 (C-9), 40.9 (C-10), 50.0 (C-11), 34.7 (C-12), 74.0 (C-13), 78.9 (C-14, 15), 90.0 (C-16), 63.2 (C-17), 76.2 (C-18), 49.5 (C-19), 42.4 (*N*-CH_3), 56.3 (1-OCH_3), 58.1 (6-OCH_3), 61.2 (16-OCH_3), 59.1 (18-OCH_3), 129.8 (C-1'), 129.7 (C-2', 6'), 128.6 (C-3', 5'), 133.2 (C-4'), 166.0 (C=O), 175.1 (O<u>C</u>Oacyl), 130.2, 130.1, 128.4, 128.1 (olefinic carbons of acyl group), 14.0, 14.1 (terminal CH_3 of acyl group), 22.5, 24.1, 27.1, 27.2, 28.9, 29.0, 29.5, 30.0, 31.5, 31.9, 33.9 [$(CH_2)_n$]; (+)-FAB-MS, *m/z* 856 (acyl = stearoyl), 854 (acyl = oleoyl), 852 (acyl = linoleoyl), 828 (acyl = palmitoyl) [M+H]$^+$, 572 [M-RC≡O]$^+$, 105 [C_6H_5CO]$^+$.

Lipohypaconitine (5): Colorless oil; $[\alpha]_D^{17}$ + 19.6° (*c* = 0.048, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 0.86~0.91 [m, $(CH_2)_n$<u>CH_3</u>], 1.26 [br s, $(CH_2)_n$], 2.34 (3H, s, *N*-CH_3), 3.15, 3.27, 3.28, 3.74 (3H each, s, 4×OCH_3), 3.08, 3.32 (1H each, d, *J* = 8.4 Hz, H-18), 3.97 (1H, br d, *J* = 6.9 Hz, H-6β), 4.42~4.44 (2H, m, H-15, 15-OH), 4.36 (1H, t, *J* = 4.8 Hz, H-14β), 5.32~5.38 (m, olefinic H), 7.44 (2H, br t, *J* = 7.5 Hz, H-3', 5'), 7.56 (1H, tt, *J* = 1.2, 7.5 Hz, H-4'), 8.03 (2H, dt, *J* = 1.5, 6.9 Hz H-2', 6'); (+)-FAB-MS, *m/z* 840 (acyl = stearoyl), 838 (acyl = oleoyl), 836 (acyl = linoleoyl), 812 (acyl = palmitoyl) [M+H]$^+$, 556 [M-RC≡O]$^+$.

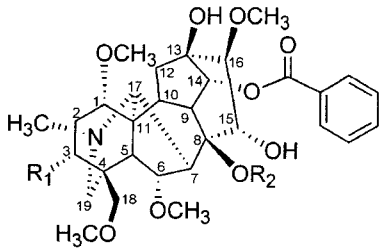
15α-Hydroxyneoline (6): White powder, mp 206~207°C; $[\alpha]_D^{23}$ + 19.3° (*c* = 0.27, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.11 (3H, t, *J* = 7 Hz, *N*-CH_2<u>CH_3</u>), 2.16 (1H, br d, *J* = 6.3 Hz, H-5), 3.16 (1H, br d, *J* = 7.8 Hz, H-16α), 3.19, 3.66 (1H each, d, *J* = 8.1 Hz, H-18), 3.33, 3.34, 3.44 (3H each, s, 3×OCH_3), 3.67 (overlap, H-1), 4.13 (1H, br d, *J* = 5.1 Hz, H-6β), 4.14 (1H, br d, *J* = 6.6 Hz, H-14β), 4.41 (1H, d, *J* = 7 Hz, H-15β); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): Table I; EI-MS, *m/z* (rel. int., %): 453 [M]$^+$ (20), 438 [M-CH_3]$^+$ (24.6), 436 [M-OH]$^+$ (100), 420 [M-OH-O]$^+$ (10), 366 (14.7).

Hokbusine A (7): White powder, mp 140~144°C; $[\alpha]_D^{23}$ + 8.2° (*c* = 9.3, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 2.34 (3H, s, *N*-CH_3), 2.56 (1H, br t, *J* = 6.0 Hz, H-9), 2.82 (1H, br s, H-7), 3.10 (3H, s, 8-OCH_3), 3.23 (overlap, H-16), 3.25 (6H, s, 1,6-OCH_3), 3.28 (3H, s, 18-OCH_3), 3.55, 3.61 (1H each, d, *J* = 8.7 Hz, H-18), 3.69 (3H, s, 16-OCH_3), 3.75 (1H, dd, *J* = 5.1, 9.9 Hz, H-3), 4.01 (1H, d, *J* = 6.6 Hz, H-6β), 4.52 (1H, d, *J* = 6 Hz, H-15β), 4.81 (1H, d, *J* = 5.1 Hz, H-14β), 7.41 (2H, br t, *J* = 7.5 Hz, H-3', 5'), 7.52 (1H, br t, *J* = 6.9 Hz, H-4'), 8.00 (2H, br d, *J* = 6.9 Hz, H-2', 6'); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): Table I; EI-MS, *m/z* (rel. int., %) 603 [M]$^+$ (2.5), 572 [M-OCH_3]$^+$ (97.5), 554 [M-(OCH_3+H_2O)]$^+$ (14.), 105 [C_6H_5CO]$^+$ (100)

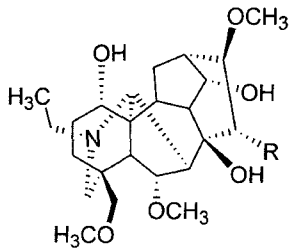
Napelline (8): White powder, mp 114~116°C; $[\alpha]_D^{23}$ + 19.3° (*c* = 0.27, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 0.76 (3H, s, 18-CH_3), 1.05 (3H, t, *J* = 7.2 Hz, *N*-CH_2<u>CH_3</u>), 3.36 (1H, br s, H-20), 3.54 (1H, dd, *J* = 6.6, 9.3 Hz, H-12β), 3.92 (1H, dd, *J* = 6.0, 8.0 Hz, H-1β), 4.17 (1H, br s, H-15), 5.13 (1H, dd, *J* = 1.5, 1.8 Hz, C=C<u>H_2</u>), 5.15 (1H, dd, *J* = 1.2, 2.4 Hz, C=C<u>H_2</u>); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): Table I; EI-MS, *m/z* (rel. int., %) 359 [M]$^+$ (100), 342 [M-OH]$^+$ (35.0), 331 (14.4), 314 (20), 300 (38.5).

RESULTS AND DISCUSSION

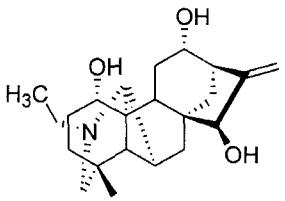
Repeated chromatographic separation followed by recrystallization gave 8 alkaloidal compounds. Compound **1** was obtained as colorless needles and showed positive reaction to Dragendorff's reagent. In the IR spectrum, it showed the presence of hydroxyl groups at 3532, 3395 cm^{-1} and no absorption in the carbonyl region. Its EI-MS showed a molecular ion peak at *m/z* 437 with other significant fragment ion peaks at *m/z* 420 [M-OH]$^+$, and 388 [M-OH-OCH_3]$^+$, which indicated an α-hydroxyl group was attached to C-1 in C_{19}-norditerpenoid alkaloid (Takayama



2 R_1 = OH, R_2 = Ac
3 R_1 = H, R_2 = Ac
4 R_1 = OH, R_2 = fatty acyl group
5 R1 = H, R2 = fatty acyl group
7 R_1 = OH, R_2 = CH_3



1 R_1 = H
6 R_1 = OH



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et al., 1982). In its ^{1}H-NMR spectrum, the characteristic signals of aconitine-type norditerpenoid alkaloids were observed. The methyl signal of *N*-$CH_2\underline{CH_3}$ and three methoxyl signals were observed at δ 1.12 (3H, t, *J* = 6.8 Hz) and 3.31 (OCH_3) and 3.32 (2×OCH_3), respectively. The appearance of H-18 methylene signals at δ 3.25 and 3.61 (1H each, d, *J* = 8.1 Hz) indicated C-3 did not bear a hydroxyl group (Hanuman and Katz, 1994a). A triplet signal at δ 4.17 was assigned to H-14 methine proton, indicative of the absence of a hydroxyl group at C-13. A sequence of connectivities through an oxygen-bearing methine proton at δ 4.16 (br d, *J* = 6.6 Hz, H-6) and two methine protons at δ 1.97 (br s, H-7) and at δ 2.15 (br d, *J* = 6.6 Hz, H-5), an oxygen-bearing methine proton at δ 3.34 and methylene protons at δ 2.03 (1H, dd, *J* = 6, 15.9 Hz) and δ 2.34 (1H, dd, *J* = 9.3, 15.9 Hz, H-15), respectively, was observed in the ^{1}H-^{1}H COSY spectrum, and their signals could be assigned to the H-6, H-7, H-5, H-16 and H-15, respectively. The ^{13}C-NMR spectrum of **1** gave 24 carbon signals. The DEPT spectrum revealed four CH_3 carbons at δ 56.3, 57.8, 59.2, and 12.9, seven CH_2 carbons at δ 29.2, 29.4, 29.8, 42.7, 48.4, 57.0, and 80.2, ten CH carbons at δ 40.2, 44.1, 44.9, 48.3, 52.2, 63.9, 72.2, 75.9, 81.7, and 83.0, and three quaternary carbons at δ 38.1, 49.5, and 74.1. On the basis of the above evidences, the structure of **1** was assigned as neoline. The spectral and physicochemical data of **1** were in agreement with the previous reported data of neoline (Pelletier *et al.*, 1976; Kitagawa *et al.*, 1982; 1984).

Compound **2** was obtained as colorless needles. It showed hydroxyl (3507 cm^{-1}), aromatic (1638 cm^{-1}), ester carbonyl (1713 cm^{-1}), and acetoxyl (1277 cm^{-1}) bands in its IR spectrum. The ^{1}H-NMR spectrum showed that it contained a *N*-methyl group at δ 2.32, an acetoxyl group at δ 1.35, and four methoxyl groups at δ 3.14, 3.26, 3.27, and 3.71. The spectrum also exhibited three one-proton doublets at δ 3.30 (*J* = 4.8 Hz), 4.01 (*J* = 6.3 Hz), and 4.85 (*J* = 5.1 Hz) attributed to H-16α, H-6β, and H-14β, respectively, as well as an one-proton doublet of doublets signal at δ 4.44 (*J* = 3.0, 5.4 Hz) attributed to H-15β. Aromatic protons with A_2B_2X pattern at δ 7.44 (2H, tt, *J* = 1.2, 7.2 Hz), 7.56 (1H, tt, *J* = 1.2, 7.2 Hz), and 8.01 (2H, dt, *J* = 1.2, 7.2 Hz) indicated the presence of benzoyl group. Being affected by aromatic ring to upfield, an acetate group was attached to C-8 and a benzoate group was attached to C-14. These signals are characteristics of aconitine-type norditerpenoid alkaloids (Hanuman and Katz, 1994a; Kitagawa *et al.*, 1984). The H-18 methylene signals at δ 3.53 and 3.62 (1H each, d, *J* = 9.1 Hz) suggested a hydroxyl group was attached to C-3, which was supported by ^{13}C-NMR spectrum. C-4 and C-18 signals appeared at δ 43.4 and 76.2 due to the β- and γ-gauche effects of the C-3 hydroxyl group. Thus the structure of **2** was suggested to be mesaconitine. EI-MS spectrum showed a molecular ion peak at *m/z* 631 with other significant fragment ions at *m/z* 600 $[M\text{-}OCH_3]^+$, 540 $[M\text{-}OCH_3\text{-}CH_3COOH]^+$, and 105 $[C_6H_5CO]^+$. The structure of **2** was determined as mesaconitine on the basis of spectral data, and confirmed with direct comparison with an authentic sample (Kitagawa *et al.*, 1982; 1984).

Compound **3** was obtained as white powder. The ^{1}H-NMR spectrum of **3** was similar to that of **2** except that H-18 methylene protons appeared at δ 3.11 and 3.62 (1H each, d, *J* = 8.4 Hz), which indicated C-3 did not bear a hydroxyl group. Consistent with this, a comparison of the ^{13}C-NMR spectra of **2** and **3** revealed only significant differences involving ring A, and carbon atoms C-10, 18 and C-19. Its EI-MS spectrum showed a fragment ion peak at *m/z* 584 due to the loss of a methoxyl group attached to C-1 from the molecular ion even though the molecular ion peak did not appear. Thus the structure of **3** was determined as hypaconitine on the basis of the above spectral data. This result was further confirmed by direct comparison with an authentic sample (Kitagawa *et al.*, 1982; 1984).

Compound **4** was obtained as colorless oil and showed positive reaction to Dragendorff's reagent. The ^{1}H- and ^{13}C-NMR spectra of **4** were close similar to those of **2**. The NMR spectra showed that **4** lacked an acetoxyl group but had a long-chain fatty acid ester group [δ_H 0.86 ~0.91; δ_C 14.0 and 14.1 for terminal CH_3, δ_H 1.26; δ_C 22.5~33.9 for long-chain CH_2, δ_H 5.32~5.38; δ_C 128.1, 128.4,

130.1, and 130.2 for double bonds; δ_C 175.1 for acyl C=O. This result suggested that the fatty acid acyl group was substituted for an acetoxyl group at C-8 (Hanuman and Kaiz, 1994b). Positive FAB-MS spectrum showed a series of four protonated molecular ion peaks $[M + H]^+$ at *m/z* 856, 854, 852, and 828, as well as the fragment ion peak at *m/z* 572 due to loss of acylium ions suggesting mesaconitine acylated with a mixture of fatty acids such as stearic acid, oleic acid, linoleic acid, and palmitic acid (Kawata *et al.*, 1999; Sun *et al.*, 1999). Alkaline hydrolysis of **4** yielded a mixture of long-chain fatty acids displaying molecular ion peaks for the methyl esters of stearic, oleic, linoleic, and palmitic acids in the GC-MS. On the basis of the above evidence, the structure of **4** was elucidated as lipomesaconitine (Kitagawa *et al.*, 1982; 1984).

Compound **5** was also obtained as colorless oil. The ^{1}H-NMR spectrum of **5** were close similar to that of **4** except that H-18 methylene signals at δ 3.52 and 3.62 (1H each, d, J = 9.0 Hz) in **4** were shifted to δ 3.08 and 3.62 (1H each d, J = 8.4 Hz) in **5**, indicating the absence of hydroxy group at C-3. Positive FAB-MS spectrum of **5** was also supported this result. It showed a series of four protonated molecular ion peaks $[M + H]^+$ for stearoyl (*m/z* 840), oleoyl (*m/z* 838), linoleoyl (*m/z* 836), and palmitoyl (*m/z* 812) esters of hypaconitine. On the basis of the above spectral data, the structure of **5** was assigned as lipohypaconitine and confirmed by comparison with the previous published data (Kitagawa *et al.*, 1982; 1984).

Compound **6** was obtained as amorphous powder. It showed positive reaction to Dragendorff's reagent and bluish green color to 20% aqueous H_2SO_4. The molecular ion peak $[M]^+$ at *m/z* 453 and a significant fragment ion peak $[M-OH]^+$ at *m/z* 436 in EI-MS spectrum indicated that a hydroxyl group was attached to C-1α in norditerpenoid alkaloid. The ^{1}H-NMR spectrum of **6** resembled the reported spectrum of neoline (**1**), except for the absence of C-15 methylene resonances at δ 2.03 (1H, dd, J = 6.0, 15.9 Hz) and 2.34 (1H, dd, J = 9.3, 15.9 Hz) and the presence of two oxygenated methine protons at δ 3.16 (1H, br d, J = 7.8 Hz, H-16α) and 4.41 (1H, d, J = 6.6 Hz, H-15β) which is characteristic for C-15 and C-16 dioxygenated pattern. The appearance of the downfield-shifted methoxyl signal at δ 50.1 in the ^{13}C-NMR spectrum suggested the introduction of additional hydroxyl group at C-15. On the basis of the above data, the structure of **6** was assigned to 15α-hydroxyneoline (= senbusine C) and the spectral data of **6** were in good agreement with the previous reported data (Takayama *et al.*, 1982b; Konno *et al.*, 1982).

Compound **7** was obtained as amorphous powder. Its ^{1}H-NMR spectrum showed characteristic signals for the aconitine skeleton with a *N*-CH_3 at δ 2.34, five methoxyl groups at δ 3.10, 3.25 (2×OCH_3), 3.28 and 3.69, and five aromatic protons at δ 7.41 (2H, br t, J = 7.5 Hz),7.52 (1H, br t, J = 6.9 Hz) and 8.00 (2H, br d, J = 6.9 Hz) for benzoyl group. It also showed three doublet signals due to H-6 at δ 4.01 (1H, d, J = 6.6 Hz), H-14 at δ 4.81 (1H, d, J = 5.1 Hz), and H-15 at δ 4.52 (1H, d, J = 6.0 Hz). ^{1}H and ^{13}C signals in the ^{1}H- and ^{13}C-NMR spectra of **7** were similar to those of **2**, except for the absence of acetoxyl resonances (δ_H 1.35; δ_C 21.4, 172.4) and the presence of the highly upfield shifted methoxyl signal (δ_H 3.10; δ_C 49.6) due to the anisotropy effect. In alkaloids bearing an aromatic substitution on C-14 a shielding effect is observed on the substituent groups on C-8 in the ^{1}H- and ^{13}C-NMR spectra of these compounds (Desai and Pelletier, 1989; Hang *et al.*, 1988). Consequently, the position of a methoxyl group of **7** was clarified as the C-8 position and thus the structure of **7** was determined as hokbusine A (Hikino *et al.*, 1983a; Hang *et al.*, 1988).

Compound **8** was obtained as amorphous powder and showed positive reaction to Dragendorff's reagent. The ^{1}H-NMR spectrum showed some characteristic signals for C_{20}-diterpene alkaloids having napelline-type skeleton at δ 1.05 (3H, t, J = 7.2 Hz, *N*-$CH_2\underline{CH_3}$), 0.76 (3H, s, 18-CH_3), 3.36 (1H, br s, H-20), and at 5.13 (1H, dd, J = 1.5, 1.8 Hz) and δ 5.15 (1H, dd, J = 1.2, 2.4 Hz, exomethylene H). In addition to these signals, there are three carbinol protons at δ 3.54 (1H, dd, J = 6.6, 9.3 Hz, H-12), 3.92 (1H, dd, J = 6.0, 8.0 Hz, H-1), and 4.17 (1H, br s, H-15). The exo cyclic methylene protons exhibit long-range coupling to a broad singlet carbinol proton at δ 4.17 in the ^{1}H-^{1}H COSY spectrum. This evidence indicates that the carbinol proton could be assigned to H-15α, suggesting the presence of 15β-hydroxyl functional group. The EI-MS spectrum showed a molecular ion peak $[M]^+$ at *m/z* 359 and a significant fragment ion peak $[M-OH]^+$ at *m/z* 342, indicative of the presence of a hydroxy group at C-1α. In its ^{13}C-NMR spectrum, 22 carbon signals were observed including carbon signals of ethylamino group at δ 51.6 and 13.3, exomethylene carbon signals at δ 108.5 and 159.1, and three oxygenated methine carbon signals at δ 70.5, 76.2, and 77.8. These are characteristics of napelline-type C_{20}-diterpenoid alkaloids having 1α,12α,15β-trihydroxy-16,17-exocyclic double bond functionalities. From the above evidence, the structure of **8** was assigned as napelline (= luciculine). This result was further confirmed by comparison with the previous reported data (Takayama *et al.*, 1982a; Wada *et al.*, 1989).

In a previous study with *A. jaluense* collected from Mt. Bultasan in the north part (Hwanghae province), a group of norditerpenoid alkaloids (deoxyaconitine, hypaconitine, mesaconitine, neoline, aconitine, acetyltalatizamine, talatizamine, and benzoylmesaconine), together with three uncharacterized ones, were isolated (Kim *et al.*, 1990). In our present study, with plant material collected from Mt. Changbaek in the south part (Kangwon province), we

have only obtained three of the above compounds, neoline (**1**), mesaconitine (**2**), hypaconitine (**3**) and other known alkaloids, lipomesaconitine (**4**), lipohypaconitine (**5**), 15α-hydroxyneoline (**6**), hokbusine A (**7**), and napelline (**8**) were also isolated. The differences in the alkaloidal contents indicated that the two collections represent two different chemotypes. Therefore, the reason for this variation could be explained by the fact that climatic and soil conditions as well as the date of harvesting influence the alkaloid content and composition (Hikino *et al.*, 1983a, b; Chen *et al.*, 1982). This suggestion could be supported by the fact that the constituents of the processed tuber from *A. carmichaeli* were very different from those of the other previous reports (Shim *et al.*, 2003). Five alkaloids such as lipomesaconitine, lipohypaconitine, 15α-hydroxyneoline, hokbusine A, and napelline were isolated for the first time from this plant.

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