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Studies on the Constituents of Aconitum Species. III.[1] On the Components of *Aconitum subcuneatum* NAKAI

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A new alkaloid, 14-benzoylneoline, and four known alkaloids, neoline, karakoline, 14-acetyldelcosine, and penduline, were isolated from the roots of *Aconitum subcuneatum* NAKAI. The structures of 14-benzoylneoline and penduline were confirmed by derivation of these compounds from neoline and aconitine, respectively. A radical-type deoxygenation of a bridgehead hydroxyl group at C-13 was used in the transformation of aconitine into penduline through three steps.

Keywords——14-benzoylneoline; neoline; karakoline; 14-acetyldelcosine; penduline; bridgehead hydroxyl deoxygenation; *Aconitum subcuneatum*

The constituents of *Aconitum subcuneatum* NAKAI have been investigated by Suginome and Imato since the isolation of jesaconitine (**2**) was first reported by Majima and Morio.[2] We have also reported the isolation and structure elucidation of deoxyjesaconitine (**4**) as well as several aconitine-type alkaloids from the same plant.[1]

The chloroform extract described in the experimental section was chromatographed over alumina to give five fractions, A, B, C, D, and E. From fraction A, ten alkaloids were isolated: a new alkaloidal compound (**7**) and nine known alkaloids, aconitine (**1**), jesaconitine (**2**), deoxyaconitine (**3**), deoxyjesaconitine (**4**), hypaconitine (**5**), neoline (**6**),[3] karakoline (**8**),[3] 14-acetyldelcosine (**9**),[3] and penduline (**10**).[4] All of these alkaloids are C-19 type diterpene alkaloids.[5] Four compounds among the nine, **6**, **8**, **9**, and **10**, had not previously been reported in this plant. Identification of **6**, **8**, and **9** was carried out by comparison of their melting points and spectral data with those described in the literature.[3] The other fractions, B, C, D, and E, were left for future investigation.

In the present paper, we report the structure elucidation of **7** and the transformation of **1** into **10** through only three steps, including deoxygenation of a bridgehead hydroxyl group at C-13.

Compound **7** showed the following properties; amorphous powder, $[\alpha]_D^{18} = +9.1°$, $C_{31}H_{43}NO_7$. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed a methyl group of an *N*-ethyl moiety, three methoxyl groups, two methines at δ 4.14 (1H, d, J=7.0 Hz, $C_{6\beta}$-H) and 5.18 (1H, t, J=5.0 Hz, $C_{14\beta}$-H), and five aromatic protons. The mass spectrum (MS) showed a molecular ion peak at m/z 541 and a benzoyl cation at m/z 105 as a base peak. The infrared (IR) spectrum suggested the presence of a benzoyl ester group (absorptions at 1720, 1605, and 1270 cm^{-1}). On the basis of these spectral data, the structure of **7** was assigned as 14-benzoylneoline, and this was confirmed by comparison of the carbon-13 NMR (^{13}C-NMR) spectra of **7** and **6**.[6] In the ^{13}C-NMR spectrum of **7**, the benzoyloxy group at C-14 shifted the C-14 resonance downfield (1.0 ppm), and the C-9 and C-13 resonances upfield (2.3 and 0.7 ppm, respectively) as a result of the acylation effect.

Benzoylation of **6** with benzoyl chloride in pyridine did not afford the 14-benzoate but gave the 1-benzoate exclusively. On the other hand, heating of **6** with benzoyl chloride in

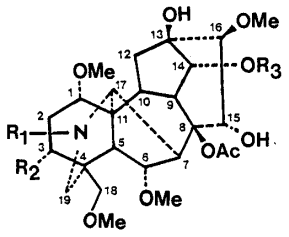
trifluoroacetic acid at 80 °C for 6 h gave three benzoates; the 1-benzoate (27%), 14-benzoate (21%), and 1,14-dibenzoate (12%). The NMR and IR spectra and MS dara of the 14-benzoate were identical with those of the natural compound (**7**).

Structure determination of **10** isolated from *A. pendulum* was reported by Limin *et al.* based on the spectral data of **10** and its acetyl derivative,[4] and a partial synthesis was recently reported by Sakai *et al.* through many steps from chasmanine.[7] Compound **10**, mp 167—168 °C, isolated from *A. subcuneatum* was deduced to be penduline from its spectral data. In order to confirm the strucrure, we transformed **1** into **10** through only three steps. The transformation involved deoxygenation of a bridgehead hydroxyl group at C-13 according to the method reported in our previous communication.[8]

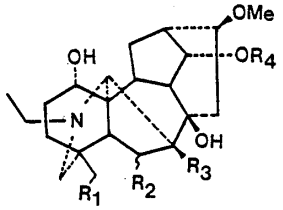
Treatment of **1** with trifluoromethanesulfonic anhydride in pyridine afforded the 13-trifluoromethanesulfonate (**11**), mp 151—152 °C, with simultaneous dehydration in 80% yield. The ^{1}H-NMR spectrum of **11** showed two olefinic protons at δ 6.04 (1H, d, J=9.8 Hz, C_3-H) and 6.29 (1H, dd, J=9.8, 3.7 Hz, C_2-H) as in the case of the trifluoromethanesulfonate of anhydromesaconitine.[8]

Irradiation of **11** with a 2537 Å lamp at room temperature for 3 h gave a deoxygenated compound (**12**), mp 178—179 °C, which could be formed by a radical reaction.[9] The ^{1}H-NMR spectrum of **12** exhibited a proton on a carbon (C-14) carrying a benzoyloxy group at δ 5.06 ppm (t, J=4.8 Hz) in place of that of anhydroaconitine (**13**)[10] at δ 4.90 ppm (d, J=4.4 Hz).

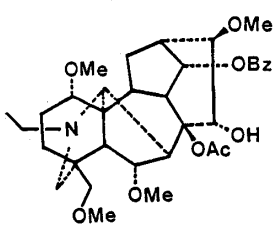
The deoxygenated compound (**12**) was hydrogenated over platinum in ethanol to give **10** in 53% yield. The structure of the compound derived from **1** through these three steps was assigned as 3,13-dideoxyaconitine (penduline[4]) on the basis of the NMR and IR spectra, MS, mp, and mixture melting point test.



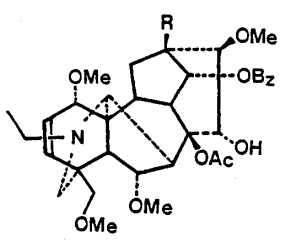
1: R_1=Et, R_2=OH, R_3=Bz
2: R_1=Et, R_2=OH, R_3=An
3: R_1=Et, R_2=H, R_3=Bz
4: R_1=Et, R_2=H, R_3=An
5: R_1=Me, R_2=H, R_3=Bz
Bz=CO–C_6H_5
An=CO–C_6H_4–OMe(*p*)



6: R_1=OMe, R_2=α-OMe, R_3=R_4=H
7: R_1=OMe, R_2=α-OMe, R_3=H, R_4=Bz
8: R_1=R_2=R_3=R_4=H
9: R_1=OMe, R_2=β-OMe, R_3=OH, R_4=Ac



10



11: R=OSO_2CF_3
12: R=H
13: R=OH

Chart 1

TABLE I. ^{13}C-Chemical Shifts and Assignments for Neoline (**6**),[6] 14-Benzoylneoline (**7**), 13-*O*-Trifluoromethanesulfonylanhydroaconitine (**11**), 13-Deoxyanhydroaconitine (**12**), and Penduline (**10**)

Carbon	6	7	11	12	10	Carbon	6	7	11	12	10
1	72.3	72.0	85.3	81.3	85.2	18	80.3	80.0	75.7	75.5	80.2
2	29.5[a]	29.3[a]	126.1	125.5	29.6	19	57.2	56.9	52.4	52.1	53.2
3	29.9[a]	29.9[a]	137.8	137.8	35.2	N–CH_2	48.2	48.2	48.2	48.5	49.2
4	38.2	38.1	40.4	39.0	38.9	CH_3	13.0	13.0	12.0	12.5	13.4
5	44.9	44.4	45.6	46.8	48.7	1′	—	—	55.6	56.1	56.0
6	83.3	83.3	82.2	84.2	83.6	6′	57.8	57.9	58.5	58.4	57.6
7	52.3	52.9	43.2	44.6	44.4	16′	56.3	56.0	60.8	57.9	57.9
8	74.3	74.8	92.1	92.4	92.1	18′	59.1	59.1	59.3	59.2	59.0
9	48.3	46.0	41.7	44.1	45.1	O=C	—	—	172.3	172.2	172.2
10	40.7	37.4	39.8	40.7	38.6	CH_3	—	—	21.1	21.4	21.4
11	49.6	49.6	50.5	47.9	49.9	O=C	—	166.0	165.1	165.9	166.0
12	29.8[a]	29.5[a]	32.3	27.1	28.8	C_6H_5	—	132.9	134.0	133.1	133.0
13	44.3	43.6	89.3	42.2	44.4		—	130.1	129.7	129.5	130.0
14	75.9	76.9	78.5	76.2	76.2		—	129.5	128.9	128.5	129.5
15	42.7	42.5	78.6	78.2	75.4		—	128.4			128.5
16	82.3	81.9	87.7	88.9	89.2						
17	63.6	63.3	60.8	62.9	61.2	$-OSO_2CF_3$	—	—	118.0	—	—

δ (ppm) downfield from TMS in $CDCl_3$. *a*) Assignments may be interchanged in each column.

Experimental

All melting points are uncorrected. IR spectra were taken with a JASCO IRA-2 spectrometer. Ultraviolet (UV) spectra were measured in EtOH solution with a Shimazu D-300 spectrometer. NMR spectra were measured in $CDCl_3$ solution with a JEOL FX-100 spectrometer using tetramethylsilane (TMS) as an internal standard, and the following abbreviations are used: s=singlet, d=doublet, t=triplet, dd=double doublet, m=multiplet. 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 F_{254} (Merck). Elemental analyses and high-resolution MS were performed by the Analytical Center, Hokkaido University. The rhizoma of *A. subcuneatum* NAKAI were collected at Zenibako-cho, Otaru, in August 1982.

Isolation Procedure——Dried ground rhizoma (17.6 kg) of *A. subcuneatum* NAKAI were extracted with MeOH (153 l) for 4 d to afford the MeOH extract (3.75 kg). A quarter of the extract was added to 5% aqueous HCl (1 l) and partitioned with hexane (1.1 l×3). The aqueous layer was adjusted to pH 9 with 28% aqueous NH_3 and extracted with $CHCl_3$ (1.5 l×5) to afford $CHCl_3$ layer-I. This procedure was repeated 4 times. The hexane layer mentioned above was evaporated to give a residue. The residue was partitioned between 5% aqueous HCl (800 ml) and hexane (500 ml×3). The aqueous layer was adjusted to pH 9 with 28% aqueous NH_3 and extracted with $CHCl_3$ (1 l×4) to afford $CHCl_3$ layer-II. The combined $CHCl_3$ layers (I and II) were dried over anhydrous Na_2SO_4 and evaporated to give the $CHCl_3$ extract (234 g). The extract was chromatographed over alumina (3 kg) to afford the following fractions: elution with $CHCl_3$ (2 l) gave Fr-A (8.9 g), elution with ethyl acetate (2 l) gave Fr-B (91.0 g), elution with $CHCl_3$–MeOH (1 : 1, 2 l) gave Fr-C (55.5 g), elution with MeOH (3 l) gave Fr-D (23.2 g), and further elution with MeOH (3 l) gave Fr-E (3.7 g). Fr-A was chromatographed on silica gel with a mixture of hexane and $CHCl_3$ saturated with 28% aqueous NH_3; the content of hexane was decreased gradually. Purification by repeated column chromatography gave **1** (507 mg), **2** (1030 mg), **3** (89 mg), **4** (8 mg), **5** (86 mg), **6** (312 mg, mp 158—163 °C), **8** (9 mg, mp 184—187 °C), **9** (31 mg, mp 192—193 °C), **10** (7 mg, mp 167—168 °C), and **7**, which was named 14-benzoylneoline (6 mg).

14-Benzoylneoline (7)——Amorphous powder, $[\alpha]_D^{18}=+9.1°$ ($c=0.11$, MeOH). UV λ_{max}^{EtOH} nm (log ε): 229 (3.62). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580, 1720, 1605, 1505, 1270. ^{1}H-NMR δ: 1.14 (3H, t, $J=7.0$ Hz), 3.26 (3H, s), 3.35 (6H, s), 4.14 (1H, d, $J=7.0$ Hz), 5.18 (1H, t, $J=5.0$ Hz), 7.32—7.59 (2H, m), 7.80—8.03 (3H, m). MS *m/z*: 541 (M^+), 105 (base peak). High-resolution MS: Calcd for $C_{31}H_{47}NO_7$ 541.303, Found 541.302.

Benzoylation of 6——i) Benzoyl chloride (26.6 mg) was added to a solution of **6** (47 mg) in pyridine (4 ml) and the mixture was stirred at −18 °C for 2 h. The resulting mixture was poured into water, made alkaline with $NaHCO_3$, and extracted with $CHCl_3$. The $CHCl_3$ solution was worked up in the usual manner to afford a residue. The residue was purified by column chromatography on silica gel to afford the 1-benzoate (39 mg) in 67% yield.

ii) Compound **6** (40 mg) was added to a mixture of benzoyl chloride (21.7 mg) and trifluoroacetic acid (0.2 ml), and the whole was stirred at 80 °C for 6 h. The reaction mixture was poured into water and made alkaline with 10% aqueous NH_3, and the resulting mixture was extracted with $CHCl_3$. The $CHCl_3$ solution was worked up in the usual manner to afford a residue. The residue was purified by TLC on silica gel to afford the 1-benzoate (14 mg), 14-benzoate (11 mg), and 1,14-dibenzoate (9 mg).

1-Benzoate: Amorphous powder. *Anal.* Calcd for $C_{31}H_{43}NO_7$: C, 68.74; H, 8.00; N, 2.59. Found: C, 68.48; H, 8.21; N, 2.30. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540, 1705, 1605, 1275. ^{1}H-NMR δ: 1.21 (3H, t, J=7.0 Hz), 3.29 (3H, s), 3.34 (3H, s), 3.36 (3H, s), 4.08 (1H, t, J=5.0 Hz, $C_{14\beta}$-H), 4.27 (1H, d, J=7.0 Hz, $C_{6\beta}$-H), 5.12 (1H, dd, J=12.0, 7.0 Hz, $C_{1\beta}$-H), 7.32—7.68 (2H, m), 7.92—8.14 (3H, m). MS m/z: 541 (M^+), 420 (M^+ − benzoyloxy, base peak), 105.

14-Benzoate: Amorphous powder. *Anal.* Calcd for $C_{31}H_{43}NO_7 \cdot 1/2H_2O$: C, 67.61; H, 8.05; N, 2.54. Found: C, 67.68; H, 8.05; N, 2.50. The IR, ^{1}H- and ^{13}C-NMR spectra and MS were identical with those of natural 14-benzoylneoline.

1,14-Dibenzoate: Amorphous powder. *Anal.* Calcd for $C_{38}H_{47}NO_8$: C, 70.68; H, 7.34; N, 2.17. Found: C, 70.69; H, 7.61; N, 1.84. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580, 1720 (sh), 1710, 1605, 1585, 1275. ^{1}H-NMR δ: 1.21 (3H, t, J=7.0 Hz), 3.10 (3H, s), 3.33 (3H, s), 3.36 (3H, s), 4.21 (1H, d, J=7.0 Hz, $C_{6\beta}$-H), 4.98—5.27 (2H, m, $C_{1\beta}$ and $C_{14\beta}$-H), 7.37—7.64 (4H, m), 7.78—8.16 (6H, m). MS m/z: 645 (M^+), 524 (M^+ − benzoyloxy, base peak), 105.

13-*O*-Trifluoromethanesulfonylanhydroaconitine (11)——Trifluoromethanesulfonic anhydride (0.24 ml) was added to a solution of **1** (290 mg) in pyridine (1.5 ml) at 0 °C and the mixture was stirred at room temperature for 18 h. The resulting mixture was poured into water to afford a precipitate. The precipitate was crystallized from aqueous MeOH to give needles (280 mg, 82%), mp 151—152 °C. *Anal.* Calcd for $C_{35}H_{44}F_3NO_{12}S$: C, 55.33; H, 5.84; N, 1.84; S, 4.22. Found: C, 55.23; H, 5.76; N, 1.78; S, 4.33. IR ν_{max}^{KBr} cm^{-1}: 3500, 1735, 1715. ^{1}H-NMR δ: 1.26 (3H, t, J=7.0 Hz), 1.46 (3H, s), 3.25 (3H, s), 3.32 (3H, s), 3.47 (3H, s), 3.77 (3H, s), 4.17 (1H, d, J=6.0 Hz, $C_{6\beta}$-H), 5.29 (1H, d, J=7.0 Hz, $C_{14\beta}$-H), 6.04 (1H, d, J=9.8 Hz, C_3-H), 6.29 (1H, dd, J=9.8, 3.7 Hz, C_2-H), 7.35—8.15 (5H, m).

13-Deoxyanhydroaconitine (12)——A solution of **11** in a mixture of hexamethylphosphoric triamide–water (95 : 5) was irradiated with a 2537 Å lamp under N_2 gas for 3 h, then 500 ml of water was added. The mixture was made alkaline with 10% aqueous NH_3 and extracted with ether. The ether solution was worked up as usual to afford a residue. The residue was purified by TLC followed by crystallization from MeOH to give needles (23 mg, 29%), mp 178—179 °C. *Anal.* Calcd for $C_{34}H_{45}NO_9$: C, 66.76; H, 7.41; N, 2.29. Found: C, 66.66; H, 7.42; N, 2.13. IR ν_{max}^{KBr} cm^{-1}: 3450, 1720, 1710. ^{1}H-NMR δ: 1.15 (3H, t, J=7.0 Hz), 1.47 (3H, s), 3.20 (3H, s), 3.33 (3H, s), 3.55 (3H, s), 4.12 (1H, d, J=6.0 Hz, $C_{6\beta}$-H), 5.06 (1H, t, J=4.8 Hz, $C_{14\beta}$-H), 5.78 (1H, d, J=10.0 Hz, C_3-H), 6.08 (1H, dd, J=10.0, 3.2 Hz, C_2-H), 7.40—8.15 (5H, m). MS m/z: 611 (M^+).

Hydrogenation of 12——Platinum oxide (10 mg) was added to a solution of **12** (23 mg) in EtOH (1.5 ml) and the mixture was stirred at room temperature for 1.5 h, then filtered, and the filtrate was evaporated to give a residue. The residue was purified by TLC followed by crystallization from acetone–hexane to afford needles (12.1 mg, 53%), mp 165—166 °C, underpressed by admixture with the natural product (**10**). *Anal.* Calcd for $C_{34}H_{49}NO_9$: C, 66.54; H, 7.72; N, 2.28. Found: C, 66.34; H, 7.74; N, 2.11. The IR, ^{1}H- and ^{13}C-NMR spectra and MS were identical with those of the natural product (**10**).

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