STUDIES ON ACONITUM SPECIES. XIII. TWO NEW DITERPENOID ALKALOIDS FROM ACONITUM YESOENSE VAR. MACROYESOENSE (NAKAI) TAMURA. VI[1]

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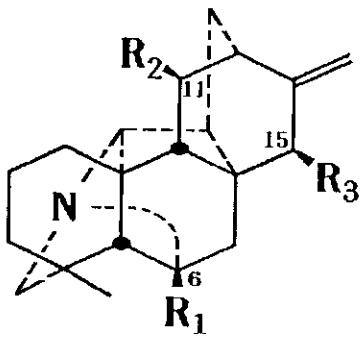
Abstract -- A new C_{20}-diterpenoid alkaloid, yesodine (1), a new C_{19}-diterpenoid alkaloid, yesoensine (2), and two known alkaloids, macrocentridine (3) and subcusine (4), were isolated from Aconitum yesoense var. macroyesoense (Nakai) Tamura. Structures of those new alkaloids were determined on the basis of their spectra and chemical correlation with known alkaloids.

The isolation and structure elucidation of seventeen C_{20}-diterpenoid alkaloids and eleven C_{19}-diterpenoid alkaloids from Aconitum yesoense var. macroyesoense (Nakai) Tamura were reported in our previous papers.[1-3] Our continuing investigations on the constituents of this plant resulted in the isolation of two new diterpenoid alkaloids, yesodine (1) and yesoensine (2), together with two known alkaloids, macrocentridine (3)[4] and subcusine (4).[5]

Alkaloids 3 and 4 have not previously been found in this plant. Alkaloid 3 was determined by comparison of spectral data with those in the literature.[4] Alkaloid 4 was identified by the comparison of melting point and spectral data with those of an authentic sample.[5]

Alkaloid 1 was obtained in an amorphous form, $[\alpha]_D^{22}$ -9.4°, and its molecular formula $C_{25}H_{35}NO_4$ was deduced by the elemental analysis and hrms spectrum indicating as a molecular ion at m/z 413.2598 (calcd 413.2564) and as a fragment ion at m/z 310.1822 ($M^+-COC_4H_9-H_2O$, $C_{20}H_{24}NO_2$, calcd 310.1805). The ^1H-nmr spectrum of 1 showed an angular methyl at δ 1.34 (s), two carbinyl methines at δ 4.00 (d, J=4.6 Hz, C_{11}-H) and 5.59 (s, C_{15}-H), and exomethylene at δ 5.23 and 5.33



1: $R_1=R_2=OH$, $R_3=OCO-\overset{H}{\underset{|}{C}}(\overset{CH_3}{\blacktriangledown})-CH_2CH_3$

5: $R_1=R_2=R_3=OH$

6: $R_1=R_2=R_3=ONB$

7: $R_1=R_3=ONB$, $R_2=OH$

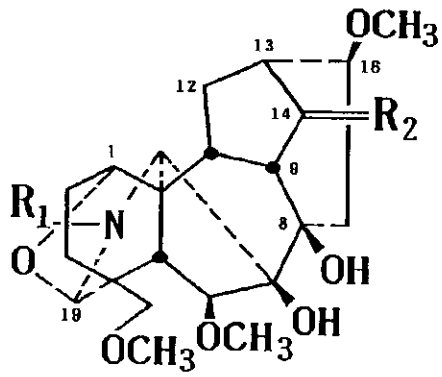
8: $R_1=R_2=ONB$, $R_3=OH$

9: $R_1=ONB$, $R_2=R_3=OH$

10: $R_1=R_2=OH$, $R_3=ONB$

11: $R_1=R_2=ONB$, $R_3=OCO-\overset{H}{\underset{|}{C}}(\overset{CH_3}{\blacktriangledown})-CH_2CH_3$

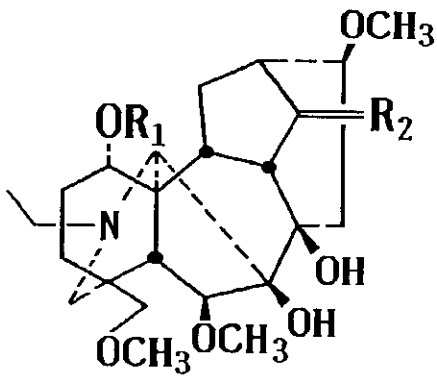
NB=CO-C_6H_4-NO_2(p)



2: $R_1=CH_2CH_3$, $R_2=O$

12: $R_1=CH_2CH_3$, $R_2=\alpha$-OH

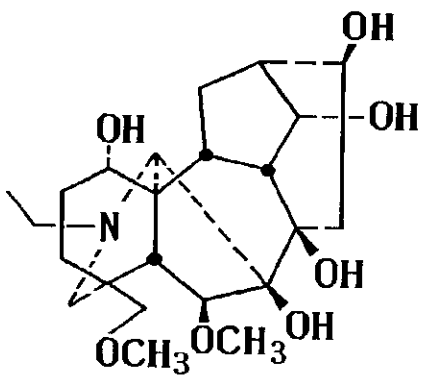
16: $R_1=H$, $R_2=O$



13: $R_1=H$, $R_2=O$

14: $R_1=OCH_3$, $R_2=\alpha$-OH

15: $R_1=OCH_3$, $R_2=O$



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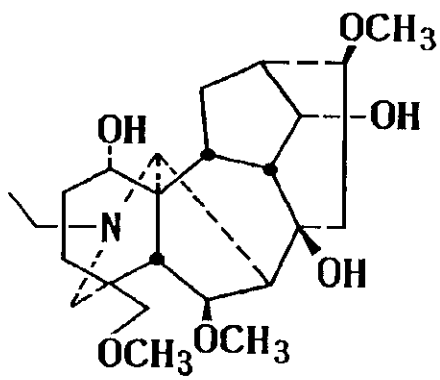

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(each 1H, s). The nmr signals at δ 0.91 (3H, t, J=7.3 Hz) and 1.16 (3H, d, J=6.9 Hz), ir absorptions at 1729 and 1216 cm^{-1}, and two fragment ion peaks at m/z 328 (M^+-COC_4H_9) and 312 (M^+-$OCOC_4H_9$) in the ms spectrum suggested the presence of 2-methylbutyryloxy group. The ^{13}C-nmr spectrum showed a 2-methylbutyryl ester at 175.8 ppm (s, C=O), 41.3 ppm (d), 26.8 ppm (t), 16.6 ppm (q), and 11.6 ppm (q). The molecular formula together with these spectral data suggested that the compound was a C_{20}-diterpenoid alkaloid. The ^{1}H- and ^{13}C-nmr spectra of 1 were very similar to those of pseudokobusine (5),[3] with the exception of the presence of 2-methylbutyryl group, and the ^{1}H-nmr signal at δ 5.59 (1H, s) and ^{13}C-nmr signal at 70.3 ppm (d) suggested the presence of 2-methylbutyryl ester at the C-15. On the basis of the spectral data, the structure was assigned to 15-(2-methylbutyryl)pseudokobusine (1). The structure of yesodine (1) was finally confirmed by the correlation with pseudokobusine (5) as follows. Pseudokobusine (5) was treated with p-nitrobenzoyl chloride to give tri-p-nitrobenzoate (6), 6,15-di-p-nitrobenzoate (7), 6,11-di-p-nitrobenzoate (8), 6-p-nitrobenzoate (9),[3] and 15-p-nitrobenzoate (10) in 3%, 8%, 8%, 37%, and 4% yield, respectively. The ^{1}H-nmr spectrum of 8 showed two carbinyl methines at δ 4.09 (bs, C_{15}-H) and 5.42 (d, J=4.9 Hz, C_{11}-H). The ^{13}C-nmr signals at 72.7 ppm (d, C-11) and 104.9 ppm (s, C-6) suggested the presence of p-nitrobenzoyl esters at the C-6 and C-11. Treatment of 8 with (S)-(+)-2-methylbutyric acid, N,N'-dicyclohexylcarbodiimide, and 4-(dimethylamino)pyridine in dichloromethane at refluxing temperature[6] for 24 h afforded 15-[(S)-2-methylbutyryl]-6,11-di-p-nitrobenzoylpseudokobusine (11) in 29% yield. Hydrolysis of 11 with a mixture of triethylamine, methanol, and water (1:5:1, in volume ratio) gave 15-[(S)-2-methylbutyryl]pseudokobusine in 54% yield. The product showed -9.1° (0.11, $CHCl_3$) as an optical rotation, in excellent agreement with that of isolated yesodine (-9.4°). The ^{1}H- and ^{13}C-nmr chemical shifts for 2-methylbutyryl group of yesodine (1) are also identical to (S)-2-methylbutyryl group of the product. The structure of yesodine (1) was determined as 15-[(S)-2-methylbutyryl]pseudokobusine including the absolute stereochemistry. Alkaloid 2 named yesoensine, amorphous, $[\alpha]_D^{21}$ +39.4°, gave a molecular formula corresponding to $C_{24}H_{35}NO_7$ by the elemental analysis and investigation of ms fragments at m/z 434.2140 (M^+-15, $C_{23}H_{32}NO_7$, calcd 434.2176) and 417.2118 (M^+-CH_3OH, $C_{23}H_{31}NO_6$, calcd 417.2148). The ^{1}H-nmr spectrum of 2 revealed the presence of a methyl of an N-ethyl group at δ 1.10 (t, J=7.2 Hz), three methoxyls at δ 3.31, 3.35, and 3.37 (each, s), a carbinyl methine at δ 3.95 (s, C_6-αH).

Table I. ^{13}C-Chemical Shifts and Assignments for Yesoensine (2), 18-Methoxygadesine (12),[7] 14-Dehydrodelcosine (13),[7] and N-Deethylyesoensine (16).

Carbon	2	12	13	16	Carbon	2	12	13	16
1	67.8	68.8[a]	72.1	67.9	13	49.6	36.8	46.8	50.0
2	25.3	25.5	27.3	25.5	14	213.4	75.3	214.9	213.2
3	21.9	21.9	29.6	21.8	15	33.7	33.8	34.8	33.8
4	43.2	43.2	37.5	43.3	16	85.1	81.7	86.5	85.3
5	34.6	38.2	45.3	34.8	17	64.7	64.1	66.4	57.3
6	89.9	90.2	89.7	89.6	18	73.1	73.3	77.0	73.1
7	84.9	85.1	87.3	84.5	19	85.2	85.2[a]	57.3	83.0
8	83.7	76.1	82.9	83.5	N-CH_2	47.4	47.4	50.5	--
9	53.4	49.6	53.1	53.4	CH_3	13.6	13.7	13.6	--
10	46.3	45.3	40.9	46.5	6'	58.9	58.9	56.6	59.0
11	46.8	46.4	49.7	45.8	16'	56.2	56.5	56.0	56.3
12	24.6	27.7	27.5	24.8	18'	59.0	59.1	59.0	59.0

a; The published values for 18-methoxygadesine (12) have been reversed.[8]

The ^{13}C-nmr spectrum exhibited 24 lines corresponding to 24 carbon atoms of the molecule (see Table I). These spectra suggested that compound 2 was a C_{19}-diterpenoid alkaloid. The ir absorption at 1745 cm^{-1} and ^{13}C-nmr signal at 213.4 ppm (s, C-14) suggested the presence of a five-membered carbonyl group. The ir absorption at 1105 cm^{-1}, ^{1}H-nmr signal at δ 3.90 (1H, s, C_{19}-H), and ^{13}C-nmr signals at 67.8 ppm (d, C-1) and 85.2 ppm (d, C-19) suggested the presence of C-1-C-19 inner ether like that in 18-methoxygadesine (12).[7] The spectrum of 2 was very similar to that of 18-methoxygadesine (12) and 14-dehydrodelcosine (13) which were already isolated from this plant.[1,2] The observed molecular ion (m/z 449) of yesoensine (2) was 2 mass units less than that of 18-methoxygadesine (12) and 14-dehydrodelcosine (13). Comparison of the chemical shifts of the carbonyl group in both ^{13}C-nmr spectra of yesoensine (2) and 14-dehydrodelcosine (13) suggested that compound 2 was 14-dehydro derivative of 18-methoxygadesine (12). That the carbonyl group was located at C-14 was confirmed from comparison of the ^{13}C-nmr shifts of C-8, C-9, C-12, C-13, C-14, and C-16 of yesoensine (2) with those of 18-methoxygadesine (12). The deviation of those chemical shifts is similar to those of the pair of browniine (14)[7] and 14-dehydrobrowniine (15)[7] (Table II). On the basis of the spectral data, compound 2 was deduced to be 14-

dehydro-18-methoxygadesine. Treatment of 14-dehydrodelcosine (13) with potassium permanganate in acetone-H_2O (5:1 v/v)[9] gave yesoensine (2) and N-deethylyesoensine (16) in 52% and 18% yield, respectively. The ir and nmr spectra, and tlc behavior of the former one (2) were identical with those of the natural compound, and the structure was established.

EXPERIMENTAL

All melting points are uncorrected. Optical rotations were measured with a JASCO DIP-4 polarimeter. Ir spectra in KBr disks or $CHCl_3$ solution were taken with a JASCO FT/7000 and JASCO IRA-2 spectrophotometers. Nmr spectra were measured in $CDCl_3$ solution with a JEOL FX-100 and GX-270 spectrometers using TMS as an internal standard. Ms and hrms were measured with JEOL JMS-D300, JMS-DX303, Shimadzu LKB-9000B, and Hitachi M-2000 mass spectrometers.

Table II. ^{13}C-Nmr Data of Compounds 14, and 15.

Carbon	14	15
8	76.3	85.5
9	49.6	53.8
12	27.5	25.3
13	36.4	49.5
14	75.3	216.3
16	81.7	85.5

Isolation procedure -- In the previous paper,[1-3] we already reported the extraction and isolation of several alkaloids from the rhizoma of the title plant. Flash column chromatography (silica gel, $CHCl_3$ saturated with 28% ammonia) of the remaining crude alkaloid gave several fractions. Flash column chromatography (silica gel, 5% methanol-ether saturated with 28% ammonia) of a less polar fraction gave two new alkaloids 1 (13 mg) and 2 (11 mg) and a known alkaloid 4 (3 mg). Column chromatography (silica gel, $CHCl_3$ saturated with 28% ammonia) of a polar fraction gave a known alkaloid 3 (5 mg).

Yesodine (1) -- Amorphous powder (from ether). $[\alpha]_D^{22}$ -9.4° (c=0.34, $CHCl_3$). Hrms (m/z): 413.2598 (M^+, $C_{25}H_{35}NO_4$, calcd 413.2564), 310.1822 (M^+-COC_4H_9-H_2O, $C_{20}H_{24}NO_2$, calcd 310.1805). Ir (ν, cm^{-1}): 3548, 1729, 1216. Ms (m/z): 413 (M^+), 328, 312 (base peak). Anal. Calcd for $C_{25}H_{35}NO_4 \cdot 1/3(C_2H_5)_2O \cdot 2/3H_2O$: C, 70.24; H, 8.88; N, 3.11. Found. C, 70.03; H, 8.63; N, 2.99. ^{1}H-Nmr (δ): 0.91 (3H, t, J=7.3 Hz), 1.16 (3H, d, J=6.9 Hz), 1.34 (3H, s), 4.00 (1H, d, J=4.6 Hz), 5.23 and 5.33 (each 1H, s), 5.59 (1H, s). ^{13}C-Nmr (ppm): 175.8 (s, C=O), 144.2 (s, C-16), 118.7 (t, C-17), 100.0 (s, C-6), 72.3 (d, C-20), 70.3 (d, C-15), 67.3 (d, C-11), 60.0 (d), 58.4 (t, C-19), 55.4 (d), 49.9 (s, C-10), 44.8 (s, C-8), 41.3

(d), 41.1 (d), 40.4 (d), 39.5 (t), 37.7 (s, C-4), 35.3 (t), 30.0 (q, C-18), 28.0 (t), 27.1 (t), 26.8 (t), 19.1 (t), 16.6 (q), 11.6 (q).

p-Nitrobenzoylation of pseudokobusine (5) -- A mixture of pseudokobusine (5, 50 mg), pyridine (2 ml), and p-nitrobenzoyl chloride (85 mg) was stirred for over night at room temperature. Usual work-up and purification by column chromatography (silica gel, $CHCl_3$ saturated with 28% ammonia) and preparative tlc (silica gel, $CHCl_3$ saturated with 28% ammonia) afforded tri-p-nitrobenzoate (6, 3 mg), 6,15-di-p-nitrobenzoate (7, 7 mg), 6,11-di-p-nitrobenzoate (8, 7 mg), 6-p-nitrobenzoate (9, 27 mg),[3] and 15-p-nitrobenzoate (10, 3 mg) in 3%, 8%, 8%, 37%, and 4% yield, respectively.

Tri-p-nitrobenzoylpseudokobusine (6) -- mp: 254-256°C (from acetone-hexane). Hrms (m/z): 776.2299 (M^+, $C_{41}H_{36}N_4O_{12}$, calcd 776.2329). Ir (ν, cm^{-1}): 1720, 1600, 1520, 1340, 1270. Ms (m/z): 776 (M^+), 626, 459, 292, 167, 150 (base peak). ^{1}H-Nmr (δ): 0.97 (3H, s), 5.24 and 5.48 (each 1H, s), 5.52 (1H, d, J=4.9 Hz), 5.83 (1H, bs), 7.99, 8.05, 8.06, 8.08, 8.13, and 8.24 (each 2H, d, J=8.9 Hz).

6,15-Di-p-nitrobenzoylpseudokobusine (7) -- mp: 253-254°C (from acetone-hexane). Hrms (m/z): 627.2202 (M^+, $C_{34}H_{33}N_3O_9$, calcd 627.2216). Ir (ν, cm^{-1}): 3400, 1715, 1705, 1600, 1520, 1350, 1295, 1270, 895. Ms (m/z): 627 (M^+), 477 (base peak), 310, 150. ^{1}H-Nmr (δ): 0.96 (3H, s), 4.17 (1H, d, J=4.6 Hz), 5.30 and 5.45 (each 1H, s), 5.77 (1H, bs), 8.13, 8.20, 8.24, and 8.32 (each 2H, d, J=8.9 Hz).

6,11-Di-p-nitrobenzoylpseudokobusine (8) -- mp: 255-258°C (from acetone-hexane). Hrms (m/z): 627.2208 (M^+, $C_{34}H_{33}N_3O_9$, calcd 627.2216). Ir (ν, cm^{-1}): 3400, 1720, 1600, 1530, 1350, 1280. Ms (m/z): 627 (M^+), 477 (base peak), 310, 150. ^{1}H-Nmr (δ): 0.98 (3H, s), 4.09 (1H, bs), 5.13 and 5.29 (each 1H, s), 5.42 (1H, d, J=4.9 Hz), 8.15, 8.20, 8.29, and 8.30 (each 2H, d, J=8.9 Hz).

15-p-Nitrobenzoylpseudokobusine (10) -- Amorphous. Hrms (m/z): 478.2113 (M^+, $C_{27}H_{30}N_2O_6$, calcd 478.2104). Ir (ν, cm^{-1}): 3400, 1720, 1600, 1520, 1350, 1270. Ms (m/z): 478 (M^+, base peak), 312, 150. ^{1}H-Nmr (δ): 1.33 (3H, s), 4.12 (1H, d, J=4.6 Hz), 5.33 and 5.43 (each 1H, s), 6.01 (1H, bs), 8.18 and 8.30 (each 2H, d, J=8.9 Hz).

15-[(S)-2-Methylbutyryl]-6,11-di-p-nitrobenzoylpseudokobusibe (11) -- A solution of 6,11-di-p-nitrobenzoylpseudokobusine (8, 58.6 mg), S-(+)-2-methylbutyric acid (94.9 mg), N,N'-dicyclohexylcarbodiimide (191.6 mg), and 4-(dimethylamino)pyridine 22.7 mg) in dichloromethane (10 ml) was heated at reflux[6] for 24 h. The white precipitate was collected, and filtrate was treated with 1.5% sulfuric acid (10 ml). The aqueous layer was extracted with dichloromethane (15 ml x 3). The combined organic layers were worked up in usual manner to afforded a residue. The residue was purified by column chromatography (silica gel, hexane : $CHCl_3$ saturated with 28% ammonia= 2:3 v/v) to give 11 (19 mg) in 29% yield. mp: >300°C (from acetone-hexane). Ir (ν, cm^{-1}): 1715, 1595, 1520, 1340, 1275. SI-ms (m/z): 712 (M^++1). ^{1}H-Nmr (δ): 0.87 (3H, t, J=7.4 Hz), 0.96 (3H, s), 1.12 (3H, d, J=6.9 Hz), 5.13 and 5.19 (each 1H, s), 5.43 (1H, d, J=5.0 Hz), 5.64 (1H, s), 8.18 and 8.21 (each 2H, d, J=8.9 Hz), 8.28 (4H, d, J=8.9 Hz).

Hydrolysis of 11 -- A solution of 11 (12 mg) dissolved in a mixture of triethylamine, methanol, and water (1, 5, and 1 ml, respectively) was stirred at 60°C for 6 d. After evaporation of the solution, the residue was purified by flash column chromatography (silica gel, $CHCl_3$ saturated with 28% ammonia) to give 1 (3.8 mg, 54% yield) that exhibited $[\alpha]_D$ -9.1° (c=0.11, $CHCl_3$) as the optical rotation, in excellent agreement with that of yesodine (-9.4°) isolated from the plant.

Yesoensine (2) -- Amorphous powder (from hexane), $[\alpha]_D^{21}$+39.4° (c=1.67, $CHCl_3$). Hrms (m/z): 434.2140 (M^+-15, $C_{23}H_{32}NO_7$, calcd 434.2176), 417.2118 (M^+-CH_3OH, $C_{23}H_{31}NO_6$, calcd 417.2148). Ir (ν, cm^{-1}): 3500, 1745, 1105. SI-ms (m/z): 450 (M^++1), 434, 418. Anal. Calcd for $C_{24}H_{35}NO_7 \cdot 1/3C_6H_{14}$: C, 65.29; H, 8.36; N, 2.93. Found. C, 65.32; H, 8.37; N, 2.95. ^{1}H-Nmr (δ): 1.10 (3H, t, J=7.2 Hz), 3.31, 3.35, and 3.37 (each 3H, s), 3.90 (1H, s), 3.95 (1H, s).

Oxidation[9] of 14-dehydrodelcosine (13) -- A solution of potassium permanganate (32 mg) in 32 ml of water-acetone (1:5 v/v) was added to a solution of 14-dehydrodelcosine (13, 31.9 mg) in acetone (18 ml). The mixture was stirred for 1 h at room temperature, and then an additional solution of potassium permanganate (32 mg) in 32 ml of water-acetone (1:5 v/v) was added, and the mixture was warmed by immersing in a water bath heated to 80-90°C for 20 min. After a removal of

acetone under reduced pressure, water (30 ml) was added, and excess potassium permanganate was decomposed by addition of about 20 mg of sodium sulfite. The mixture was cooled in ice bath and made basic with 28% ammonia, followed by extraction with $CHCl_3$ (5 x 30 ml). The $CHCl_3$ solution was dried over anhydrous sodium sulfate and was concentrated to give 30.6 mg of a residue. The residue was purified by flash column chromatography (silica gel, 1% methanol-ether saturared with 28% ammonia, 1% and 5% methanol-$CHCl_3$ saturated with 28% ammonia) to give yesoensine (2, 16.5 mg) and N-deethylyesoensine (16, 5.5 mg) in 52% and 18% yield, respectively. The ir and nmr spectra and tlc behavior of 2 were identical with those of the natural compound.

N-Deethylyesoensine (16) -- mp: 277-278°C (from $CHCl_3$-ether). Hrms (m/z): 406.1822 (M^+-15, $C_{21}H_{28}NO_7$, calcd 406.1863), 390.1935 (M^+-OCH_3, $C_{21}H_{28}NO_6$, calcd 390.1915). Ir (ν, cm^{-1}): 3500, 1750, 1110. API-ms (m/z): 422 (M^++1). ^{1}H-Nmr (δ): 3.31, 3.34, and 3.39 (3H, s), 3.89 (1H, d, J=4.9 Hz), 3.97 (1H, s), 4.06 (1H, s).

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