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

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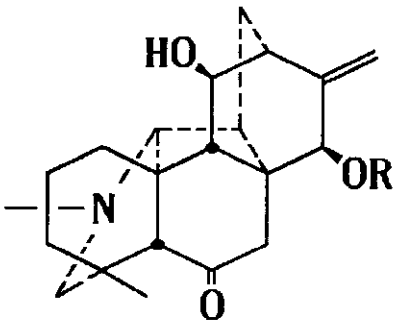
Abstract — A new C_{20}-diterpenoid alkaloid, yesonine (1), a new C_{19}-diterpenoid alkaloid, α-oxobrowniine (2), and five known alkaloids were isolated from Aconitum yesoense var. macroyesoense (Nakai) Tamura. Structures of those new alkaloids were determined on the basis of their spectral and chemical correlation with known alkaloids. Hydrolysis of yesoline (9) and permanganate oxidation of browniine (10) afforded yesonine (1) and α-oxobrowniine (2), respectively.

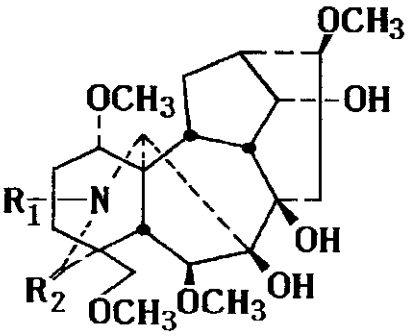
The isolation and structure elucidation of fifteen C_{20}-diterpenoid alkaloids and six C_{19}-diterpenoid alkaloids from Aconitum yesoense var. macroyesoense (Nakai) Tamura were reported in our previous paper.[2,3] Our continued investigations on the constituents of this plant now resulted in the isolation of two new diterpenoid alkaloids, yesonine (1) and α-oxobrowniine (2), together with five known alkaloids, dehydroluciculine[2] (dehydronapelline,[4] 3), flavadine (4),[5] flavamine (5),[5] 18-methoxygadesine (6),[6] nevadensine (7).[7]

Alkaloids 3-7 have not previously been found in this plant. Alkaloid 3 was identified by comparison of the melting point and spectral data with those of the authentic sample.[2] Alkaloids 4 and 5 were determined by comparison of spectral data with those in literature[5] and by chemical correlation as follows. Oxidation of lucidusculine (8)[2] with m-chloroperbenzoic acid gave flavadine (4) and hydrolysis of 4 afforded flavamine (5). The structures of 6 and 7 were determined by comparison of spectral data with those in literature.[6,7]

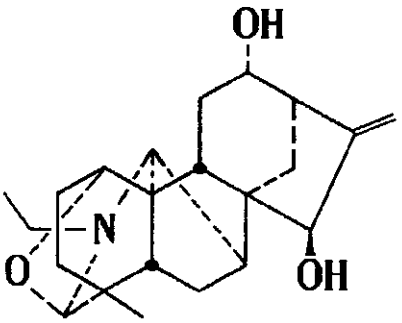
Alkaloid 1 showed the following properties; amorphous, $[\alpha]_D$ +2.4°, $C_{21}H_{29}NO_3$ (M^+



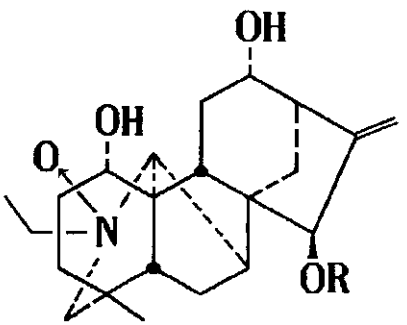
1: R = H
9: R = Veratroyl



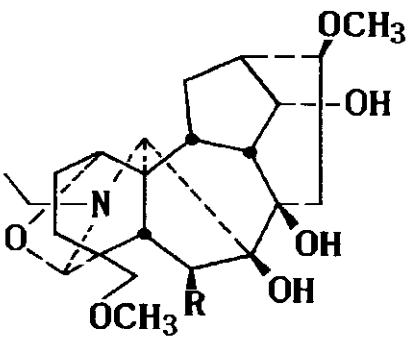
2: R_1 = CHO, R_2 = H_2
10: R_1 = CH_2CH_3, R_2 = H_2
11: R_1 = CH_2CH_3, R_2 = O



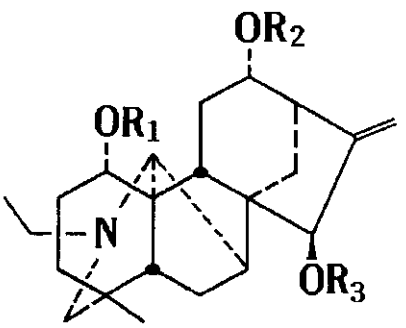
3



4: R = $COCH_3$
5: R = H



6: R = OCH_3
7: R = H



8: R_1 = R_2 = H, R_3 = $COCH_3$
12: R_1 = $COCH_3$, R_2 = R_3 = H
13: R_1 = R_2 = R_3 = $COCH_3$
14: R_1 = R_3 = $COCH_3$, R_2 = H
15: R_1 = H, R_2 = R_3 = $COCH_3$
16: R_1 = R_2 = R_3 = H

Table I. Alkaloids Isolated from Crude Base.

Alkaloids	Yield (mg)[a]	Yield (mg)[b]
Yesonine (1)	--	14
α-Oxobrowniine (2)	6	19
Dehydroluciculine (3)	--	10
Flavadine (4)	--	14
Flavamine (5)	--	7
18-Methoxygadesine (6)	--	17
Nevadensine (7)	--	18

In a and b columns, alkaloids were obtained from their crude base by isolation procedures-1 and -2[2], respectively.

343.2152, calcd 343.2147). The ^{1}H-nmr spectrum of 1 revealed the presence of an N-methyl group at δ 2.46 (s). The remaining carbon number of the molecule led the compound to C_{20}-diterpenoid alkaloid with an exomethylene moiety, of which protons were observed at δ 5.15 and 5.25 (each 1H, s, $C=CH_2$) in the ^{1}H-nmr and at 149.1 ppm (s, C-16) and 113.9 ppm (t, C-17) in the ^{13}C-nmr spectra. The ^{1}H-nmr spectrum of 1 showed an angular methyl group at δ 1.47 (s) and two carbinyl methines at δ 3.95 (s, C_{15}-H) and 4.08 (d, J=5.0 Hz, C_{11}-H). The ir absorption at 1720 cm^{-1} and ^{13}C-nmr signal at 191.2 ppm (s, C-6) suggested the presence of a ketone moiety. These spectra were very similar to those of yesoline (9)[3], with the exception of the absence of veratroyl group, and the structure corresponded to N-methyl-N,6-seco-6-dehydropseudokobusine (1).[3] N-Methyl-N,6-seco-6-dehydropseudokobusine (1) derived from pseudokobusine was identical with the natural compound (1) in terms of the ir and nmr spectra, and the tlc behaviors, and the structure of yesonine (1) was established.

Alkaloid 2 showed the following properties; amorphous, $[\alpha]_D$ +70.7°. Alkaloid 2 gave a molecular formula corresponding to $C_{24}H_{37}NO_8$ (M^+, m/z 467) by the investigation of ms fragment at 437.2395 ($C_{23}H_{35}NO_7$, M^+-CH_2O, calcd 437.2413). The ^{1}H-nmr spectrum of 2 revealed the presence of four methoxyl groups at δ 3.18, 3.35, 3.38, and 3.40 (each 3H, s), and two carbinyl methines at δ 3.73 (s, C_6-αH) and 4.04 (t, J=4.6 Hz, C_{14}-βH). The ir absorption at 1650 cm^{-1} and ^{13}C-nmr signal at 166.5 ppm (d) suggested the presence of an N-formyl group. The

upfielded chemical shift value of N-formyl proton (δ 7.44) could be explained by steric factor. These spectra suggested that the compound was a C_{19}-diterpenoid alkaloid. The ^{1}H- and ^{13}C-nmr spectra of 2 were similar to those of browniine (10),[8] with the exception of the presence of an N-formyl group and the absence of an N-ethyl group. On the basis of the spectral data, compound 2 was assigned to be α-oxobrowniine.

Treatment of browniine (10) with $KMnO_4$ in anhyd. acetone gave α-oxobrowniine (2) and oxobrowniine (11)[9] in 19% and 20% 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.

The ^{13}C-nmr spectrum of a series of luciculine type alkaloids led to the confirmation of whole assignment of ^{13}C-chemical shifts for flavadine (4), flavamine (5), lucidusculine (8), 1-acetylluciculine (12),[10] triacetylluciculine (13),[10] 1,15-diacetylluciculine (14),[10] 12-acetyllucidusculine (15),[3,4] and luciculine (16)[10] (Table II). Carbon resonances at C-3, C-5, C-11, C-13, and C-14 for flavadine (4), flavamine (5), lucidusculine (8), 1-acetylluciculine (12), and luciculine (16) should be revised on the basis of acetylation effects, as follows. Introduction of an acetyl group at C-1 hydroxy group shifted the C-1 resonance downfield (4.6-5.0 ppm), the C-2 and C-10 resonances upfield (4.2-4.4 and 2.5-2.9 ppm, respectively), and the C-3, C-5, and C-9 resonances downfield (1.6-1.8, 3.1-3.2, and 0.3-1.5 ppm, respectively) as the result of acylation effect. The acetylation of hydroxy group at C-12 likewise shifted the C-12 resonance downfield (1.1-1.3 ppm), the C-11 and C-13 resonances upfield (2.7-3.2 and 2.1-2.4 ppm, respectively) as the result of β effect, and the C-9 and C-14 resonances downfield (0.6-1.1 and 0.5-0.7 ppm, respectively) as the result of γ effect. The acetylation of hydroxy group at C-15 shifted the C-15 resonance downfield (0.1 ppm), the C-8 and C-16 resonances upfield (0.4-1.1 and 5.7-7.3 ppm, respectively) as the result of β effect, and the C-9, C-13, and C-14 resonance downfield (0.6-1.1, 0.4-0.6, and 0.5-0.7 ppm, respectively) as the result of γ effect.

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

Table II. ^{13}C-Chemical Shifts and Assignments for Flavadine (4),[5] Flavamine (5),[5] Lucidusculine (8),[10] 1-Acetylluciculine (12),[10] Triacetylluciculine (13), 1,15-Diacetylluciculine (14), 12-Acetyllucidusculine (15), and Luciculine (16).[10]

Carbon	4[a,c]	4	5[a,c]	5	8[c]	12[c]	13	14	15	16[b,c]	16
1	68.0	66.6	68.1	66.7	69.9	74.5	74.1	74.2	69.6	70.5	69.5
2	29.9	30.2	30.1	30.3	31.6	27.0	26.8	26.8	31.7	31.9	31.2
3	35.2	34.6	35.3	34.6	36.5	37.9	37.7	37.8	36.8	38.4	36.1
4	36.4	35.4	36.2	35.3	34.0	34.4	34.3	34.3	34.0	34.7	34.2
5	48.4	47.3	48.5	46.9	48.8	50.1[d]	50.1	50.2	48.4	49.8	48.0
6	23.5	23.0	22.9	22.5	23.7	23.2	23.3	23.4	23.6	23.6	23.2
7	47.2	45.8	47.3	46.1	43.7	44.7	44.3	44.3	43.6	45.0	43.9
8	49.6	48.3	49.8	49.0	49.6	50.0[d]	48.9	49.2	49.4	50.3	50.0
9	40.7	39.3	39.1	38.0	37.7	36.9	37.7	38.1	37.2	38.2	36.6
10	55.2	54.1	55.5	54.3	52.5	50.5[d]	50.1	50.0	52.6	53.5	52.9
11	31.1	29.4	31.3	29.5	30.5	30.1	25.4	29.8	25.9	32.4	30.7
12	76.4	75.3	76.7	75.6	75.5	75.7	77.1	75.0	77.5	76.2	75.8
13	47.2	46.3	48.5	46.2	47.7	47.9[d]	45.0	48.3	44.7	49.4	47.1
14	29.9	29.2	29.6	28.7	29.1	28.6	29.1	29.0	29.3	29.4	28.6
15	77.8	76.7	77.6	76.9	77.5	77.5	76.9	77.3	77.1	77.8	77.4
16	154.3	151.9	158.9	158.1	153.1	159.2	151.6	153.2	151.7	160.8	158.9
17	110.8	110.8	109.3	109.5	109.5	108.6	111.2	109.6	111.1	107.4	108.6
18	26.3	26.4	26.5	26.4	26.4	26.0	25.8	25.9	26.3	26.4	26.3
19	74.8	74.3	75.3	74.3	57.9	57.4	57.1	57.2	57.9	57.7	58.1
20	81.1	80.3	81.5	80.4	65.7	65.2	65.1	65.0	65.5	66.2	65.4
N-CH_2	68.0	67.1	67.9	67.0	50.8	51.0[d]	50.6	50.6	50.9	51.6	51.4
CH_3	7.9	7.6	7.8	7.7	13.4	13.4	13.4	13.4	13.4	13.3	12.7
C=O	172.3	170.7	-	-	170.6	171.2	170.1	170.7	170.5	-	-
							170.7	170.9	170.7		
CH_3	21.3	21.6	-	-	21.6	22.1	21.2	21.6	21.3	-	-
							21.6	22.0	21.6		
							21.8				

a; Spectrum was taken in CD_3OD.

b; Spectrum was taken in pyridine-d_5.

c; Previous assignments[5,10] of C-3, C-5, C-11, C-13, and C-14 in 4, 5, 8, 12, and 16 were revised.

d; Those assignments were revised by private communication from Prof. Shin-ichiro Sakai, Chiba, Japan, April, 11th, 1987.

$CDCl_3$ solution with a JOEL FX-100 and GX-270 spectrometers using TMS as an internal standard. Ms and hrms were measured with JOEL JMS-D300, JMS-DX303, and Shimadzu LKB-9000B mass spectrometers.

Isolation procedure---In the previous paper[2,3], we already reported the extraction and isolation of several alkaloids from the rhizoma of the title plant. Column chromatography of the remaining crude alkaloid gave two new alkaloids 1 and 2 and five known alkaloids 3-7 (See Table I).

Yesonine (1) -- Amorphous. $[\alpha]_D$ = +2.4° (c=0.17, EtOH). Hrms (m/z): calcd for $C_{21}H_{29}NO_3$ 343.2147, found 343.2152. Ir (ν, cm^{-1}): 3400, 1720, 910. Ms (m/z): 343 (M^+, base peak). ^{1}H-Nmr (δ): 1.47 (3H, s), 2.46 (3H, s), 3.95 (1H, s), 4.08 (1H, d, J=5.0 Hz), 5.15 (1H, s), 5.26 (1H, s). ^{13}C-Nmr (ppm): 191.2 (s, C-6), 149.1 (s, C-16), 113.9 (t, C-17), 77.6 (d), 69.1 (d, C-15), 67.5 (d, C-11), 61.9 (t), 60.4 (d), 55.1 (d), 45.3 (s x 2 and t), 41.3 (q, N-CH_3), 41.2 (d), 40.8 (d), 39.3 (t), 37.9 (s, C-4), 31.2 (t), 31.1 (t), 30.6 (q, C-18), 18.7 (t).

α-Oxobrowniine (2) -- Amorphous, $[\alpha]_D$ = +70.7° (c=0.15, EtOH). Hrms (m/z): calcd for $C_{23}H_{35}NO_7$ (M^+-CH_2O) 437.2413, found 437.2395. Ir (ν, cm^{-1}): 3450, 1650, 1094. Ms (m/z): 467 (M^+), 437 (M^+-CH_2O), 422, 406. ^{1}H-Nmr (δ): 3.18 (3H, s), 3.35 (3H, s), 3.38 (3H, s), 3.40 (3H, s), 3.73 (1H, bs), 4.04 (1H, t, J=4.6 Hz), 7.44 (1H, s). ^{13}C-Nmr (ppm): 166.5 (d, N-CHO), 91.3 (d, C-6), 86.7 (s, C-7), 83.1 (d, C-1), 81.6 (d, C-16), 76.1 (s, C-8), 75.4 (d, C-14), 74.6 (t, C-18), 65.1 (d, C-17), 59.2 (q, C-18'), 58.1 (q, C-6'), 56.5 (q, C-16'), 56.1 (q, C-1'), 49.6 (s, C-11), 47.8 (s, C-4), 47.0 (d), 45.1 (d), 45.0 (d), 36.9 (d), 33.0 (t), 27.4 (t), 26.3 (t), 23.8 (t).

Oxidation of browniine (10) -- A mixture of browniine (10, 20 mg), potassium permanganate (10 mg), and anhyd. acetone (10 ml) was stirred for 1 h at room temperature. The excess permanganate was decomposed with sodium sulfite. The aqueous solution was worked up in usual manner to afford a residue. The residue was purified by flash column chromatography (silica gel, hexane : $CHCl_3$ saturared with 28% ammonia = 1 : 4) to give α-oxobrowniine (2, 4 mg) and oxobrowniine (11, 4 mg) in 19% and 20% yield, respectively. The ir and nmr spectra and tlc behavior of 2 were identical with those of the natural compound.

Oxobrowniine (11) -- Amorphous. Hrms (m/z): calcd for $C_{25}H_{39}NO_8$ 481.2676, found 481.2683. Ir (ν, cm^{-1}): 3450, 1717, 1096. Ms (m/z): 481 (M^+), 466 (M^+-15, base peak). ^{1}H-Nmr (δ): 1.12 (3H, t, J=7.3 Hz), 3.23 (3H, s), 3.32 (3H, s), 3.39 (3H, s), 3.43 (3H, s).

Dehydroluciculine (3) -- mp 98.5-101.5°C (from ether-hexane). Ir (δ, cm^{-1}): 3350, 1640, 1110, 890. Ms (m/z): 357 (M^+, base peak), 301 ($M^+-C_3H_4O$). ^{1}H-Nmr (δ): 0.82 (3H, s), 1.02 (3H, t, J=7.1 Hz), 3.66 (1H, t, J=8.2 Hz), 3.69 (1H, s), 4.06 (1H, d, J=5.3 Hz), 4.22 (1H, bs), 5.17 (2H, bs).

Flavadine (4) -- mp 200-202°C (from acetone-methanol). Hrms (m/z): calcd for $C_{24}H_{35}NO_4$ (M^+-O) 401.2566, found 401.2537. Ir (ν, cm^{-1}): 3408, 1742, 1655, 1236, 953, 901. Ms (m/z): 417 (M^+), 401 (M^+-O), 323 (base peak). ^{1}H-Nmr (δ): 0.85 (3H, s), 1.44 (3H, t, J=6.9 Hz), 2.13 (3H, s), 4.93 (1H, s), 5.18 (1H, s), 5.53 (1H, s). The ^{13}C-nmr spectrum was shown in Table II.

Oxidation of Luciduscuiine (8) -- A mixture of luciduscuiine (8, 21 mg), m-chloroperbenzoic acid (24 mg), and $CHCl_3$ (1 ml) was stirred for 1 h at room temperature. The excess peracid was decomposed with 10% sodium sulfite. The aqueous solution was extracted with $CHCl_3$ (15 ml x 3). The $CHCl_3$ solution was worked up in usual manner to afford a residue. The residue was purified by flash column chromatography (silica gel, 1% methanol-$CHCl_3$ saturated with 28% ammonia) to give flavadine (4, 12 mg) in 56% yield. The mp, ir and nmr spectra, and tlc behavior of 4 were identical with those of the natural compound.

Flavamine (5) -- Amorphous. Hrms (m/z): calcd for $C_{22}H_{31}NO_3$ (M^+-H_2O) 357.2304, found 357.2318. Ir (ν, cm^{-1}): 3426, 1655, 955, 905. Ms (m/z): 359 (M^+-O, base peak), 357 (M^+-H_2O). ^{1}H-Nmr (δ): 0.85 (3H, s), 1.45 (3H, t, J=6.9 Hz), 4.20 (1H, s), 5.17 (1H, s), 5.23 (1H, s). The ^{13}C-nmr spectrum was shown in Table II.

Hydrolysis of flavadine (4) -- flavadine (4, 11.5 mg) in a 5% KOH-methanol (2 ml) was stirred for 2 h at room temperature. Usual work-up aforded flavamine (5, 4.2 mg) in 41% yield. The mp, ir and nmr spectra, and tlc behavior of 5 were identical with those of the natural compound.

18-Methoxygadesine (6) -- mp 137-140°C (from acetone-hexane). Hrms (m/z): calcd for $C_{24}H_{37}NO_7$ 451.2570, found 451.2577. Ir (ν, cm^{-1}): 3450, 1102. Ms (m/z): 451 (M^+), 436 (M^+-15, base peak), 395 ($M^+-C_3H_4O$). ^{1}H-Nmr (δ): 1.08 (3H, t, J=7.3 Hz), 3.31 (3H, s), 3.37 (3H, s), 3.39 (3H, s), 3.84 (1H, s), 3.92 (1H, s).

Nevadensine (7) -- Amorphous. Hrms (m/z): calcd for $C_{23}H_{35}NO_6$ 421.2464, found 421.2442. Ir (ν, cm^{-1}): 3442, 1098. Ms (m/z): 421 (M^+), 406 (M^+-15), 365 ($M^+-C_3H_4O$). ^{1}H-Nmr (δ): 1.10 (3H, t, J=7.3 Hz), 3.32 (3H, s), 3.37 (3H, s), 3.96 (1H, s).

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