On the Alkaloidal Constituents of *Aconitum sanyoense* NAKAI var. *tonense* NAKAI

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The alkaloidal constituents of the roots and aerial parts of *Aconitum sanyoense* NAKAI var. *tonense* NAKAI collected at Kuzure-sawa (Nagano prefecture) were reinvestigated. From the aerial parts, two new C_{19}-diterpene alkaloids, 10-hydroxyisotalatizidine (5) and 10-hydroxytalatizamine (7), were isolated along with five known diterpene alkaloids and two aporphine alkaloids. Three new alkaloids, *i.e.* 10-hydroxyisotalatizidine (5), 1,15-di-*O*-acetylhypognavine (11), and 1-*O*-acetylhypognavine (12), were obtained from the roots in addition to eight known diterpene alkaloids. The structure of Hanamiyama base (14), found in the previous examination of this plant, was determined to be deacetylhanamisine.

Keywords *Aconitum sanyoense*; C_{19}-diterpene alkaloid; C_{20}-diterpene alkaloid; 10-hydroxyisotalatizidine; 10-hydroxytalatizamine; 1-*O*-acetylhypognavine; 1,15-di-*O*-acetylhypognavine; Hanamiyama base; X-ray analysis; structure elucidation

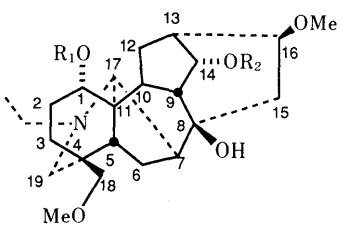
Aconitum sanyoense NAKAI var. *tonense* NAKAI (Zyoshutorikabuto), which is native to Kuzure-sawa (the upper stream of Karasu river) in Nagano prefecture, is classified as a variety of Sanyo-Bushi. Chemical investigation of this plant was conducted by Ochiai *et al.*, in 1956 and three alkaloids, tentatively named Ashio base I, Kajigamori base, and Hanamiyama base were isolated.[1] We reexamined the alkaloidal constituents of this plant collected during the flowering period in August. The whole plants were divided into two parts, *i.e.* roots and aerial parts (stems and leaves), and each was extracted with 10% aqueous ethanol. The crude alkaloids obtained by the usual procedure (see the experimental section) were purified by the combination of Al_2O_3, SiO_2 and preparative thin layer chromatography (p-TLC). The results are shown in Table I. The known alkaloids, isotalatizidine (**1**),[2] condelphine (**2**),[2] talatizamine (**3**),[2] 14-acetyltalatizamine (**4**),[3] sanyonamine (**9**),[4] and hypognavine (**10**),[5] were identified by direct comparison with authentic samples. It became clear that two alkaloids tentatively named Ashio base I and Kajigamori base were talatizamine (**3**) and isotalatizidine (**1**), respectively. Interestingly, two aporphine alkaloids, *N*-methyllaurotetanine (**15**)[6] and isoboldine (**16**),[7] were isolated only from the aerial parts. We describe here the structure elucidation of the new alkaloids.[8]

TABLE I. Alkaloids Isolated from *A. sanyoense* NAKAI var. *tonense*

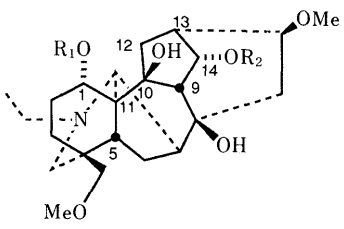
Alkaloid	Root (mg)[a]	Aerial part (mg)[b]
Isotalatizidine (**1**)	401	248
Condelphine (**2**)	281	186
Talatizamine (**3**)	176	130
14-*O*-Acetyltalatizamine (**4**)	361	78
10-Hydroxyisotalatizidine (**5**)[c]	37	63
10-Hydroxytalatizamine (**7**)[c]	—	53
Sanyonamine (**9**)	93	24
Hypognavine (**10**)	20	—
1,15-Di-*O*-acetylhypognavine (**11**)[c]	136	—
1-*O*-Acetylhypognavine (**12**)[c]	321	—
Hanamisine (**13**)	225	—
Hanamiyama base (**14**)	6	—
N-Methyllaurotetanine (**15**)	—	42
Isoboldine (**16**)	—	105

a) From 6.3 g of the crude base. *b*) From 4.0 g of the crude base. *c*) New alkaloids.

A) C_{19}-Diterpene Alkaloid Type. 10-Hydroxyisotalatizidine (5) The new alkaloid (**5**) was obtained as an amorphous powder, whose high-resolution mass spectrum (MS) showed M^+ corresponding to the formula $C_{23}H_{37}NO_6$. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed the characteristic signals of a C_{19}-diterpene alkaloid due to N-CH_2CH_3, OMe×2, and 14-H (δ 4.67 1H, t, J=5 Hz). The mass spectral fragmentation pattern of **5** [M^+ (15%), M^+-OH (100%)] strongly indicated the presence of a hydroxy group at the C-1 position.[9] Treatment of **5** with acetic anhydride in pyridine at room temperature afforded the diacetate (**6**) (mp 156—158.5 °C). In the ^{1}H-NMR spectrum of **6**, two characteristic signals were observed at δ 5.29 (1H, t, J=5 Hz) and δ 5.44 (1H, dd, J=10, 7 Hz), which appeared at δ 4.67 (1H, t, J=5 Hz) and δ 4.07 (1H, br s), respectively, in the spectrum of **5**. This indicates that two secondary hydroxy groups exist at the C-1 and C-14 positions in **5**.[2,10] The carbon-13 NMR



1 : R_1=R_2=H isotalatizidine
2 : R_1=H, R_2=Ac condelphine
3 : R_1=Me, R_2=H talatizamine
4 : R_1=Me, R_2=Ac 14-*O*-acethyltalatizamine



5 : R_1=R_2=H 10-hydroxyisotalatizidine
6 : R_1=R_2=Ac
7 : R_1=Me, R_2=H 10-hydroxytalatizamine
8 : R_1=Me, R_2=Ac

Fig. 1

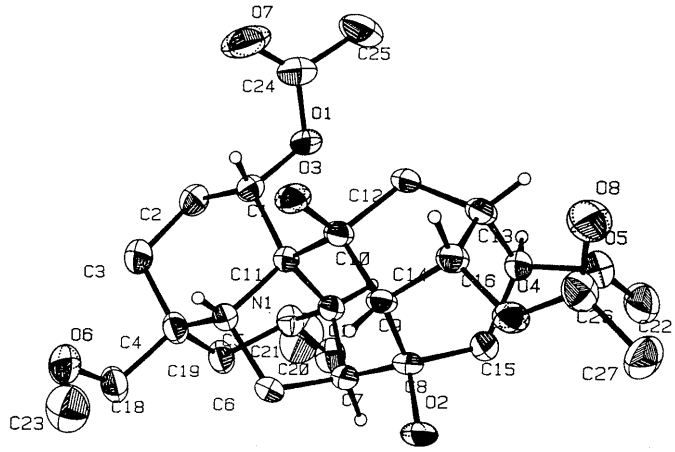


Fig. 2. ORTEP Drawing of **6**

(^{13}C-NMR) spectrum of **5** resembled that of isotalatizidine (**1**) except for a few changes (Table II). The appearance of the signal at δ 82.3 in the spectrum of **5**, as well as other data described above, afforded evidence for the presence of an additional tertiary hydroxy group in **5** compared with isotalatizidine (**1**). The signals due to C-9, C-11, and C-12 of **5** were observed at lower field by 9.5, 4.8, and 12.4 ppm, respectively, than the corresponding signals of **1**. These phenomena can be interpreted in terms of the introduction of a hydroxy group at the C-10 position in **1**.[11] The structure of **5** proposed on the basis of spectroscopic analysis was confirmed by an X-ray analysis of the diacetate derivative (**6**). The ORTEP drawing of the structure of 1,14-di-*O*-acetyl-10-hydroxyisotalatizidine (**6**) is shown in Fig. 2.

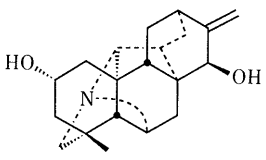
10-Hydroxytalatizamine (7) The second new alkaloid **7** was isolated as an amorphous powder. The high resolution MS of **7** showed M^+ corresponding to the formula $C_{24}H_{39}NO_6$. This is 14 a.m.u. (Me) higher than the corresponding peak in **5**. From the fragmentation pattern in the MS [M^+ (1%), M^+-OMe (100%)] the presence of a methoxyl group at the C-1 position was suggested. On acetylation with acetic anhydride in pyridine, **7** gave a monoacetate (**8**). The major difference in the ^{1}H-NMR spectrum between the acetate (**8**) and the original alcohol (**7**) was the downfield shift (Δ 0.61 ppm) of the signal at δ 4.72 (1H, t, J=5.1 Hz) in **7**. This indicated the presence of a hydroxy group at C-14 in **7**. By comparison of the ^{13}C-NMR spectra of **7** and talatizamine (**3**), the presence of a tertiary hydroxy group at C-10 was confirmed. Similar phenomena were found in **5**, *i.e.*, appearance of a signal at δ 81.1 and the downfield shift of C-9 (Δ 9.0 ppm), C-11 (Δ 5.2 ppm), and C-12 (Δ 8.9 ppm). Per-*O*-methylation of **5** and **7** with sodium hydride (NaH)–methyl iodide (MeI) in dimethylformamide (DMF), respectively, gave the same hexamethyl ether compound. Therefore, the structure of the new alkaloid **7** was concluded to be 10-hydroxytalatizamine.

B) C_{20} Diterpene Alkaloid Type. 1,15-Di-*O*-acetylhypognavine (11) The new alkaloid (**11**), $[\alpha]_D^{30}$ +83.0° (c=0.44, $CHCl_3$), was obtained as an amorphous solid and its formula, $C_{31}H_{35}NO_7$, was confirmed by the elemental analysis of the picrate derivative (mp 233—239 °C). In the ^{1}H-NMR spectrum, in addition to some characteristic

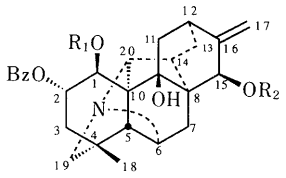
TABLE II. ^{13}C-NMR Data for **1**, **5**, **3** and **7** in $CDCl_3$

Carbon	**1**[14,15]	**5**	**3**[16]	**7**
1	72.2	69.2	86.3	78.5
2	29.6	30.8	25.8	25.7
3	28.5	26.6	32.8	32.5
4	37.2	36.9	38.7	38.5
5	41.5	40.5	45.9	42.0
6	24.8	25.0	24.8	25.4
7	45.1	44.7	46.0	45.2
8	74.2	73.4	72.9	72.1
9	46.6	56.1	47.0	56.0
10	43.9	82.3	46.0	81.1
11	48.5	53.3	48.8	54.0
12	26.6	39.1	27.7	37.6
13	39.9	37.5	37.7	37.7
14	75.7	74.3	75.6	74.1
15	42.3	43.4	38.5	39.5
16	81.9	81.3	82.3	81.7
17	64.0	64.8	62.9	63.9
18	78.9	78.9	79.5	79.4
19	56.5	56.6	53.3	52.9
N–CH_2	48.4	48.5	49.5	49.5
CH_3	13.0	13.0	13.6	13.7
1′OMe	—	—	56.3	56.0
16′OMe	56.3	56.3	56.5	56.4
18′OMe	59.4	59.4	59.5	59.5

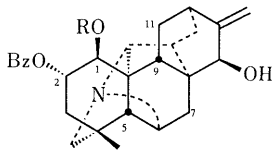
Chemical shifts in δ downfield from TMS.



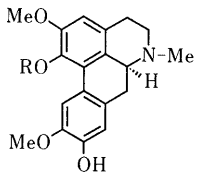
9 sanyoamine



10 : R_1=R_2=H hypognavine
11 : R_1=R_2=Ac
12 : R_1=Ac, R_2=H



13 : R=Ac hanamisine
14 : R=H Hanamiyama base



15 : R=Me *N*-methyllaurotetanine
16 : R=H isoboldine

Fig. 3

signals of a C_{20}-diterpene alkaloid having a kobusine-type skeleton [18-H_3 (δ 1.09, 3H, s), 17-H_2 (δ 4.98 and 5.10, each 1H, s), 15-H (δ 5.56, 1H, s), 6-H (δ 3.41, 1H, br s), 20-H (δ 3.21, 1H, s), and 19-H (δ 2.93, 2.58, each 1H, d, J=12.4 Hz)], two singlets at δ 2.13 (3H) and 2.14 (3H), and five aromatic protons were observed. In addition to these data, the mass spectral fission pattern, *i.e.* *m/z* 474 (M^+-OAc, 30%) and *m/z* 414 ($M^+-OAc-HOAc$, 100%), and the infrared (IR) absorption at 1735 and 1720 cm^{-1}, showed the presence of two acetoxyls and one benzoyloxy group in the molecule. From these data, the new alkaloid (**11**) was deduced to be a diacetyl derivative of hypognavine (**10**). The signals due to 1-H (δ 4.33) and 15-H (δ 4.04) in hypognavine (**10**) were shifted downfield at δ 5.46 (1H, d, J=2.2 Hz) and δ5.56 (1H, s), respectively, in **11**. The ^{13}C-NMR spectrum of **11** assigned by using

^1H–^{13}C correlation spectroscopy (COSY) and long-range coupling (COLOC) spectra[12] closely resembled that of **10** except for a few changes (Table III). That can be explained by the introduction of acetyl groups at the C-1 and C-15 hydroxyl groups. The conclusion obtained from the above spectroscopic analysis was confirmed by chemical derivatization. Thus, on acetylation with acetic anhydride in pyridine, hypognavine (**10**) afforded the diacetate, which was identical with natural **11**.

1-*O*-Acetylhypognavine (12) The new alkaloid (**12**) was obtained as colorless prisms, mp 127—128 °C and its molecular formula ($C_{29}H_{33}NO_6$) corresponded to the monoacetate of hypognavine (**10**). The mass spectral fragmentation pattern, [*m/z* 491 (M^+, 0.9%), 432 (M^+ − OAc, 100%)], as well as the appearance of a signal at δ 2.13 (3H, s) in the ^1H-NMR spectrum showed the presence of an acetoxyl group in **12**. On hydrolysis with aqueous 2.5% K_2CO_3 in MeOH at room temperature, **12** gave hypognavine (**10**) in 64% yield. In the ^1H-NMR spectrum, a signal observed at δ 5.44 (1H, d, J=2.0 Hz) in **12** was shifted upfield at δ 4.33 in the hydrolysis product. Furthermore, on oxidation with pyridinium dichromate (PDC) in dichloromethane (CH_2Cl_2), **12** afforded an α,β-unsaturated ketone, mp 253—255°C, that was characterized by the appearance of a signal at δ 5.95 (1H, d, J=1.4 Hz, one of 17-H), deshielded by the anisotoropy of the C-15 carbonyl group. Therefore, the structure of **12** was determined to be 1-*O*-acetylhypognavine.

Hanamiyama Base (14) Hanamiyama base, mp 243—244.5 °C, $C_{27}H_{31}NO_4$, was first isolated from *Aconitum sanyoense* NAKAI by Ochiai *et al.*,[1] but its structure was not determined. The intense peak at 1705 cm^{-1} in the IR spectrum of **14** showed the presence of an ester group. In the ^1H-NMR spectrum, in addition to some characteristic signals of a C_{20}-diterpene alkaloid having a kobusine-type skeleton [18-H_3 (δ 1.00, 3H, s), 17-H_2 (δ 5.21 and 5.01, each 1H, d like J=1.6 Hz), 15-H (δ 4.32), 6-H (δ 3.47), 20-H (δ 3.45), 19-H_2 (δ 3.21 and 2.68, each 1H, d, J=12.1 Hz)], five aromatic protons were observed. The ^1H–^1H COSY spectrum indicated that the two protons at 5.88 (1H, m) and δ 4.68 (1H, br s) were located on vicinal carbons each having an oxygen function. The mass spectral fragmentation pattern [(M^+, 21%), $(M-OH)^+$, 100%] indicated the presence of a hydroxy group at the C-1 position.[9] These data indicated the presence of a hydroxy group at C-1 and a benzoyloxy group at C-2, as in hypognavine (**10**). In the ^{13}C-NMR spectrum of **14**, a signal (δ 80.4) due to a quaternary carbon having a hydroxy group observed in **10** was displaced by a higher field signal at δ 43.4. The signals due to C-5 and C-7 (β position to C-9) were observed at lower field by 6.1 and 4.3 ppm, respectively, while the signal due to C-11 (α position to C-9) was observed at higher field by 11.9 ppm, as compared with the corresponding signals of hypognavine. From these data, **14** was deduced to be 9-deoxyhypognative. Finally, the structure of **14** was proved by partial hydrolysis of the acetyl group in hanamisine (**13**), whose structure has already been established by X-ray diffraction analysis.[13]

TABLE III. ^{13}C-NMR Data[a] for **10**, **11**, **12**, **13** and **14**

Carbon	**10**[b]	**11**[c]	**12**[c]	**13**[c]	**14**[b]
1	67.9	69.5	70.2[d]	69.8	66.7
2	74.1	70.2	70.3[d]	69.9	75.3
3	33.4	33.4	33.5	33.7	33.9
4	36.0	35.6	35.7	36.4	36.9
5	51.1	51.3	51.5	56.9	57.2
6	64.6	64.3	64.7	65.1	65.6
7	29.4	28.3	28.9	32.7	33.7
8	44.8	44.4	44.7	44.7	45.2
9	80.4	79.1	79.8	42.4	43.4
10	55.2	54.4	54.4	52.1	54.0
11	39.5	39.7	39.3	26.8	27.6
12	35.5	34.9	34.8	33.4	34.8
13	33.4	32.5	33.1	33.2	34.1
14	42.9	42.4	42.2	43.9	44.8
15	72.2[d]	73.1	72.6[e]	71.4	71.2
16	155.5	149.4	154.5	156.1	157.7
17	109.3	111.7	109.9	108.8	108.0
18	29.4	29.1	29.2	29.0	29.1
19	63.9	63.1	63.5	63.5	64.3
20	72.0[d]	71.8	72.2[e]	73.8	74.1
CO	165.5	164.9	165.0	165.1	165.9
	130.4	129.5	129.7	129.8	130.9
	129.6	129.4	129.6	129.6	129.8
	128.8	128.7	128.7	128.6	129.0
	133.2	133.3	133.3	133.2	133.4
1CO	—	169.6	169.6	169.8	—
CH_3	—	21.12	21.2	20.9	—
15CO	—	170.3	—	—	—
CH_3	—	21.07	—	—	—

a) Chemical shifts in δ downfield from tetramethylsilane. b) In pyridine-d_5. c) In $CDCl_3$. d, e) The assignments with the same symbol may be interchanged in any column.

Experimental

All melting points were determined on a Yamato MP-21 apparatus and are uncorrected. The instruments used in this study were as follows; UV spectra, Hitachi U3400 spectrophotometer; IR spectra, Hitachi 260 spectrophotometer; MS, Hitachi RMU-6E and RMU-7M spectrometers; ^1H- and ^{13}C-NMR spectra, JEOL JNM-MH 100 (100 MHz), JEOL JNM GX270 (270 MHz), JEOL GSX500 and JEOL JNM A500 (500 MHz) instruments (compounds were dissolved in $CDCl_3$ unless otherwise noted, with tetramethylsilane as an internal standard, and chemical shifts are recorded in δ values). Thin-layer chromatography (TLC) was performed on Merck precoated Silicagel 60 F_{254} plates. Column chromatography was carried out on Merck Silica gel 60 (230—400 mesh for flash chromatography) and Merck Al_2O_3 90 (activity II—III). Organic solutions were dried over anhydrous Na_2SO_4. Abbreviations used are: singlet (s), doublet (d), triplet (t), multiplet (m), shoulder (sh).

Extraction and Separation of the Alkaloidal Fraction The plant material was cellected at Kuzure-sawa, the upper stream of Karasu river, Nagano prefecture, in August. It was identified by Professor J. Haginiwa, Faculty of Pharmaceutical Sciences, Chiba University, and a voucher specimen is stored in the authors' laboratory. The dried powdered roots (1.4 kg) of the plant were extracted with 10% aqueous EtOH 3 times. The combined extract obtained by concentration of the solution under reduced pressure was dissolved in 1 N HCl solution and washed with ethyl acetate (AcOEt). The aqueous layer was basified with solid Na_2CO_3 at 0 °C and extracted with $CHCl_3$. The organic layer was washed with brine, dried and evaporated to give the crude base (6.3 g, 0.45% from the dry roots). The same treatment of the dried powdered aerial parts (2.1 kg) of the plant gave the crude base (4.0 g, 0.19% from the dried leaves and stems). The crude base was roughly separated by column chromatography with a 30-fold amount of Al_2O_3, and then purified with SiO_2, Al_2O_3 and/or p-TLC as described below.

Isolation of (+)-*N*-Methyllaurotetanine (15) The 5% MeOH/AcOEt–MeOH eluate from the Al_2O_3 column chromatography of the crude base from the aerial part was purified successively by SiO_2 column chromatography (5—10% EtOH/$CHCl_3$) and p-TLC (Al_2O_3, benzene : AcOEt = 1 : 1) to give 42 mg of **15**. $[\alpha]_D^{19}$ +78.2° (c=0.10, EtOH), HBr salt; colorless needles, mp 224—226 °C (lit.[6] mp 223—224 °C). The IR and ^1H-NMR spectra of **15** were identical with authentic charts provided by Prof. H. Furukawa.

Isolation of (+)-Isoboldine (16) The 5% MeOH/AcOEt–MeOH eluate from the Al_2O_3 column chromatography of the crude base from

the aerial part was purified by SiO_2 column chromatography, and then 5—20% EtOH/$CHCl_3$ fraction was crystallized from MeOH to give 105 mg of **16** as colorless prisms. mp 175°C (lit.[7] mp 180°C). $[\alpha]_D^{16}$ +49.8° (c=0.55, $CHCl_3$). The IR and ^{1}H-NMR spectra of **16** was identical with authentic charts provided by Prof. H. Furukawa.

Isolation of 10-Hydroxyisotalatizidine (5) From the roots: The 50% MeOH/AcOEt–MeOH eluate from the first Al_2O_3 column chromatography was successively subjected to Al_2O_3 column chromatography (benzene : AcOEt : EtOH = 70 : 20 : 5) and SiO_2 column chromatography (5% MeOH/$CHCl_3$) to give 37 mg of **5**. From the aerial parts: The 10—50% MeOH/AcOEt eluent from the first Al_2O_3 column chromatography was purified by SiO_2 column chromatography (5% MeOH/$CHCl_3$) and then by p-TLC (Al_2O_3, benzene : AcOE : EtOH = 7 : 2 : 1) to give 63 mg of **5**. Colorless amorphous powder. $[\alpha]_D^{14}$ +7.0° (c=0.28, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3650, 2940, 1090. ^{1}H-NMR (500 MHz), δ: 4.67 (1H, t, J=5.0 Hz, 14-H), 4.07 (1H, br s, $W_{1/2}$=6.0 Hz, 1-H), 3.34, 3.33 (each 3H, s, OCH_3 × 2), 1.12 (3H, t, J=7.0 Hz, $NCH_2C\underline{H}_3$). ^{13}C-NMR (Table II). MS m/z (%): 423 (M^+, 15), 406 (M^+ − OH, 100). High-resolution MS Calcd for $C_{23}H_{37}NO_6$: 423.2618. Found: 423.2636.

Acetylation of 5 A mixture of **5** (33 mg), acetic anhydride (0.3 ml), and dry pyridine (0.5 ml) was stirred at room temperature for 28 h. After removal of the solvent, 5% aqueous $NaHCO_3$ was added to the residue, and the solution was extracted with $CHCl_3$. The organic layer was washed with brine, dried and evaporated. The residue was crystallized from ether to give 10 mg of **6** as colorless prisms. The mother liquid was purified by Al_2O_3 column chromatography to give 6 mg of **6**. Colorless prisms. mp 156—158.5°C, $[\alpha]_D^{12}$ −1.4° (c=0.13, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1740, 1720, 1260, 1250. ^{1}H-NMR (100 MHz), δ: 5.44 (1H, dd, J=10, 7 Hz, 1-H), 5.29 (1H, t, J=5 Hz, 14-H), 3.29, 3.21 (each 3H, s, OCH_3 × 2), 2.04, 2.00 (each 3H, s, $COCH_3$ × 2), 1.08 (3H, t, J=7 Hz, $NCH_2C\underline{H}_3$): MS m/z (%): 448 (M^+ − OAc, 100). *Anal.* Calcd for $C_{27}H_{41}NO_8$: C, 63.88; H, 8.14; N, 2.76. Found: C, 64.01; H, 8.07; N, 2.71.

Crystal Structure of 6 Colorless prismatic crystals from ether were mounted on a Rigaku AFC-5 diffractometer and exposed to graphite-monochromated MoK_α radiation. The unit cell parameters are a=13.694 (4) Å, b=14.160 (3) Å, and c=13.143 (2) Å in space group $P2_12_12_1$ (z=4). Of the 6257 reflections measured with a $\omega \leqq 30° \leqq \omega$–$2\theta$ scan, 4433 were independently observed at the level of $F > 3\sigma$ (F). The structure was solved by the direct method using the program MULTAN and the atomic parameters were refined by the block-diagonal least-squares method. The final R factor was 0.066. Atomic co-ordinates, bond lengths and angles, and thermal parameters have been deposited at the Analysis Center of Chiba University.

Isolation of 10-Hydroxytalatizamine (7) The 10% MeOH/AcOEt eluate from the first Al_2O_3 column chromatography of the aerial parts was separated successively by SiO_2 column chromatography (50% EtOH/$CHCl_3$) and Al_2O_3 column chromatography (benzene : AcOEt : EtOH = 7.2 : 2 : 0.5) to give 53 mg of **7**; amorphous powder, $[\alpha]_D^{20}$ +6.4° (c=0.33, $CHCl_3$). IR$_{max}^{CHCl_3}$ cm^{-1}: 3600—3200, 2920, 1085. ^{1}H-NMR (270 MHz), δ: 4.72 (1H, t, J=5.1 Hz, 14-H), 3.76 (1H, dd, J=10.2, 6.6 Hz, 1-H), 3.35, 3.30, 3.27 (each 3H, s, OCH_3 × 3), 1.07 (3H, t, J=7.1 Hz, $NCH_2C\underline{H}_3$). ^{13}C-NMR (Table II). MS m/z (%): 437 (M^+, 1), 406 (M^+ − OMe, 100). High-resolution MS Calcd for $C_{24}H_{39}NO_6$: 437.2775. Found: 437.2799.

Acetylation of 7 A mixture of **7** (28 mg), acetic anhydride (0.3 ml) and dry pyridine (0.5 ml) was stirred at room temperature for 44 h. The residue obtained by the usual work-up was chromatographed on Al_2O_3 using 2% benzene/AcOEt as an eluent to give 22 mg (72%) of **8** as an amorphous powder. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740. ^{1}H-NMR (270 MHz), δ: 5.33 (1H, t, J=5.1 Hz, 14-H), 3.74 (1H, dd, J=10.0, 6.8 Hz, 1-H), 3.30, 3.29, 3.22 (each 3H, s, OCH_3 × 3), 2.06 (3H, s, $COCH_3$), 1.08 (3H, t, J=7.0 Hz, $NCH_2C\underline{H}_3$). MS m/z (%): 479 (M^+, 1), 448 (M^+ − OMe, 100).

***O*-Methylation of 5** To a stirred solution of **5** (27 mg, 0.064 mmol) in dry DMF (1 ml), sodium hydride (49 mg, 50% suspension in oil, 0.255 mmol) was added, followed by methyl iodide (100 μl, 0.403 mmol) at 0°C under an argon atmosphere. After 12 h the reaction mixture was poured into chilled water and extracted with ether three times. The organic layer was washed with water, dried and evaporated. The residue was purified by Al_2O_3 column chromatography using 30% benzene : hexane as an eluent to afford 14 mg (46%) of the hexamethyl ether compound as a colorless amorphous powder. $[\alpha]_D^{23}$ −12.1° (c=0.13, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1090. ^{1}H-NMR (270 MHz), δ: 3.86 (1H, t, J=4.8 Hz, 14-H), 3.73 (1H, dd, J=9.9, 6.6 Hz, 1-H), 3.39, 3.37, 3.35, 3.29, 3.28, 3.15 (each 3H, s, OCH_3 × 6), 1.06 (3H, t, J=7.1 Hz, $NCH_2C\underline{H}_3$). MS m/z (%): 479 (M^+, 1), 448 (M^+ − OMe, 100).

***O*-Methylation of 7** Compound **7** (18 mg, 0.042 mmol) was methylated by the same procedure described above, by using NaH (42 mg) and methyl iodide (100 μl) in dry DMF (1 ml). The hexamethyl ether (14 mg, 74%) obtained after purification was identical with the derivative from **5** ($[\alpha]_D$, behavior on TLC, IR and ^{1}H-NMR spectra).

Isolation of 1,15-Di-*O*-acetylhypognavine (11) The 5—7% AcOEt/benzene eluate of the first Al_2O_3 column chromatography of the roots was purified by Al_2O_3 column chromatography using 20% AcOE/hexane as an eluent followed by SiO_2 column chromatography (2% MeOH/$CHCl_3$) to give 136 mg of **11** as a colorless amorphous powder. [picrate mp 233—239°C (dec. from 20% H_2O/EtOH)]. $[\alpha]_D^{30}$ +83.0° (c=0.44, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3575, 1735, 1720 (sh), 1272, 1232. ^{1}H-NMR (270 MHz), δ: 8.00—7.44 (5H, arom H), 5.56 (1H, s, 15-H), 5.46 (1H, d, J=2.0 Hz, 1-H), 5.24 (1H, m, 2-H), 5.10, 4.98 (each 1H, s, 17-H_2), 3.41 (1H, br s, 6-H), 3.21 (1H, s, 20-H), 2.93, 2.58 (each 1H, d, J=12.4 Hz, 19-H_2), 2.37 (1H, s, 5-H), 2.13 (6H, s, $COCH_3$ × 2), 1.09 (3H, s, 18-H_3). ^{13}C-NMR (Table III). MS m/z (%): 474 (M^+ − OAc, 30), 473 (M^+ − HOAc, 23), 414 (M^+ − OAc − HOAc, 100). *Anal.* Calcd for $C_{31}H_{35}NO_7 \cdot C_6H_3N_3O_7$: C, 58.26; H, 5.02; N, 7.35. Found: C, 58.23; H, 5.00; N, 7.29.

Preparation of 11 from Hypognavine (10) A mixture of **10** (30 mg) and acetic anhydride (0.1 ml) in dry pyridine (1.5 ml) was stirred at room temperature under argon for 24 h and then at 70°C for 1 h. After removal of the solvent under reduced pressure, chilled 5% aqueous $NaHCO_3$ was added to the residue and the whole was extracted with $CHCl_3$. The organic phase was washed with brine, dried and then evaporated. The residue was subjected to Al_2O_3 column chromatography (20% AcOEt/hexane) to give 37 mg (99%) of **11** as an amorphous powder, which was identical with natural **11** by comparison of their chromatographic behavior, IR, MS and ^{1}H-NMR spectra.

Isolation of 1-*O*-Acetylhypognavine (12) The 30—50% AcOEt/benzene eluate from the first Al_2O_3 column chromatography (roots) was crystallized from acetone and the crude crystals were further purified by Al_2O_3 column chromatography (80% AcOEt/benzene) to yield 321 mg of **12**. mp 127—128°C (dec.) (from acetone). $[\alpha]_D^{30}$ +116.7° (c=0.21, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3450, 2950, 1730, 1272, 1239. ^{1}H-NMR (270 MHz), δ: 8.00—7.44 (5H, arom H), 5.44 (1H, d, J=2.0 Hz, 1-H), 5.26 (1H, m, 2-H), 5.05, 5.02 (each 1H, s, 17-H_2), 4.07 (1H, s, 15-H), 3.47 (1H, br s, 6-H), 3.18 (1H, s, 20-H), 2.72 (1H, s, 5-H), 2.13 (3H, s, $COCH_3$), 1.10 (3H, s, 18-H_3). ^{13}C-NMR (Table III). MS m/z (%): 491 (M^+, 1), 432 (M^+ − OAc, 100). *Anal.* Calcd for $C_{29}H_{33}NO_6 \cdot C_3H_6O$: C, 69.92; H, 7.15; N, 2.55. Found: C, 69.83; H, 7.14; N, 2.50.

Alkaline Hydrolysis of 12 A mixture of **12** (25 mg) and K_2CO_3 (15 mg) in 2.5% aqueous MeOH (2 ml) was stirred at room temperature for 1 h. The residue obtained by the usual work-up was crystallized from acetone to afford 13 mg (64%) of **10** as colorless prisms. mp 239—239.5°C. The chromatographic behavior on TLC and the IR spectrum were identical with those of natural hypognavine (**10**).

PDC Oxidation of 12 PDC (50 mg, 0.133 mmol) was added to a stirred solution of **12** (49 mg, 0.10 mmol) in dry CH_2Cl_2 (1 ml) at 0°C and the mixture was stirred at room temperature for 5 h. The reaction mixture was diluted with CH_2Cl_2 and filtered. The filtrate was washed with brine, dried and evaporated to give a residue, which was purified by Al_2O_3 column chromatography (40% hexane/AcOEt) to afford 28 mg (64%) of the α,β-unsaturated ketone as colorless prisms. mp 253—255°C (from MeOH). IR ν_{max}^{KBr} cm^{-1}: 1740, 1730, 1695. ^{1}H-NMR (500 MHz), δ: 5.95 (1H, d, J=1.4 Hz, 17-H), 5.50 (1H, d, J=1.5 Hz, 1-H), 5.31 (1H, m, 2-H), 5.14 (1H, d, J=1.4 Hz, 17-H), 3.45 (1H, br s, 6-H), 3.30 (1H, s, 20-H), 2.14 (3H, s, $COCH_3$), 1.10 (3H, s, 18-H_3). MS m/z (%): 489 (M^+, 8), 472 (10), 430 (96), 105 (100). High-resolution MS Calcd for $C_{29}H_{31}NO_6$: 489.2149. Found: 489.2107.

Isolation of Hanamiyama Base (14) The 10% MeOH/AcOEt eluate from the first Al_2O_3 column chromatography (roots) was subjected to SiO_2 column chromatography, and from the 40% MeOH–$CHCl_3$ fraction colorless plates of Hanamiyama base (**14**) (6 mg) were obtained: mp 243—244.5°C (from acetone/MeOH) (lit.[1] mp 243—245°C) $[\alpha]_D^{16}$ +130.0° (c=0.11, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3505, 1705, 1270. ^{1}H-NMR (500 MHz, pyridine-d_5). δ: 5.88 (1H, m, 2-H), 5.21 and 5.01 (each 1H, d like, J=1.6 Hz, 17-H_2), 4.68 (1H, s, 1-H), 4.32 (1H, s, 15-H), 3.47 (1H, br s, 6-H), 3.45 (1H, s, 20-H), 3.21, 2.68 (each 1H, d, J=12.1 Hz, 19-H_2), 1.00 (3H, s, 18-H_3). ^{13}C-NMR (Table III). MS m/z (%): 433 (M^+, 21), 416 (100), 311 (62). High-resolution MS Calcd for $C_{27}H_{31}NO_4$: 433.2251. Found: 433.2253.

Preparation of 14 from Hanamisine (13) Aqueous K_2CO_3 solution (25 mg of K_2CO_3 in 0.08 ml of water) was added to a stirred solution of

hanamisine (**13**) (40 mg) in MeOH (4 ml), and the mixture was stirred at room temperature for 1.5 h. The residue obtained by the usual work-up was subjected to Al_2O_3 column chromatography to give 16 mg (44%) of **14** as colorless prisms, mp 241—243 °C (from acetone/MeOH). This product was identical with natural **14** by TLC comparison, mixed melting point determination, and comparisons of their IR and MS spectra.

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