Chem. Pharm. Bull. 38(9) 2570—2573 (1990) Vol. 38, No. 9

Juvabione Analogs from *Abies sachalinensis* (Fr. Schm.) Mast. II[1]

Atsushi Numata,* Kenzo Kawai, and Chika Takahashi

Osaka University of Pharmaceutical Sciences, 2-10-65 Kawai, Matsubara 580, Japan. *Received December 27, 1989*

Two new juvabione analogs (4 and 5) were isolated, together with (+)-epijuvabione and (+)-dehydroepijuvabione, from the wood of *Abies sachalinensis* (Fr. Schm.) Mast. (Pinaceae) grown in the southern base of the Taisetsu mountain range of Hokkaido, Japan. The structures of 4 and 5 were respectively established to be methyl 4(*S*)-[1(*S*),5-dimethyl-3-oxohexyl]-3-oxo-1-cyclohexene-1-carboxylate and methyl 4(*R*)-[1(*S*),5-dimethyl-3-oxohexyl]-3-oxo-1-cyclohexene-1-carboxylate on the basis of chemical and spectral evidence. Juvabione-type compounds had been isolated from the wood of this species, whereas this time the epijuvabione-type compounds alone were obtained. This evidence suggests the existence of a tree-to-tree variation in constituents of the *A. sachalinensis* wood.

Keywords *Abies sachalinensis*; juvabione analog; oxoepijuvabione; oxojuvabione; epijuvabione; dehydroepijuvabione; sesquiterpene; Pinaceae

In 1966, Bowers and co-workers isolated (+)-juvabione (**1**) from the wood of North American native balsam fir, *Abies balsamea* (L.) Miller, as the so-called "paper factor" exhibiting juvenile hormone activity on the hemipteran insects of the family Pyrrhocoridae.[2] This evoked wide interest, and the natural occurrence of juvabione-related compounds was examined in the wood of a number of coniferous species.[2-10] Previously,[1] we reported the isolation and identification of (+)-juvabione (**1**) and two analogs from the wood of *Abies sachalinensis* (Fr. Schm.) Mast. (Pinaceae) grown in central Hokkaido, Japan.[11] Our continuing search for juvabione-related compounds led us to examine the constituents from the wood of this species grown in the southern base of the Taisetsu mountain range on Kokkaido, Japan. We report herein the structural identification of (+)-epijuvabione (**2**), (+)-dehydroepijuvabione (**3**) and two new analogs, (+)-oxoepijuvabione (**4**) and (−)-oxojuvabione (**5**), present in this wood.

The acetone extract of the finely cut wood was purified by Sephadex LH-20 and silica gel column chromatographies and high-performance liquid chromatography (HPLC) to afford four oily materials (**2**—**5**). The known compounds **2** and **3** were identified by comparison of the spectral data with published values[4,10,12-14] as (+)-epijuvabione and (+)-4′-dehydroepijuvabione, respectively (Chart 1). Since their proton nuclear magnetic resonance (^{1}H-NMR) spectra had not been discussed in detail, the signals were assigned as shown in Table I.

The molecular formula of compound **4**, $[\alpha]_D^{28}$ +61° (c = 1.05, EtOH), was determined as $C_{16}H_{24}O_4$ on the basis of the high-resolution mass spectrum (HR-MS) [m/z: 280.1671 (M^+)]. This compound showed in the ultraviolet

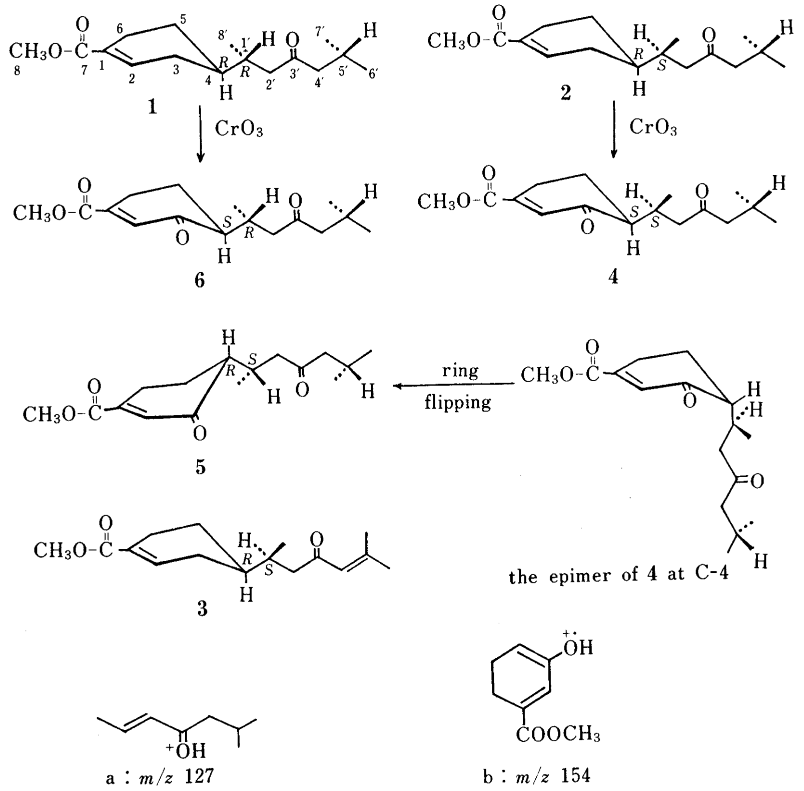

Chart 1

Table I. ^{1}H-NMR Chemical Shifts and Coupling Constants of **2**, **3**, **4** and **5** in $CDCl_3$

Position	**2**		**3**		**4**		**5**	
	δ ppm	$J_{\mathrm{H-H}}$ Hz	δ ppm	$J_{\mathrm{H-H}}$ Hz	δ ppm	$J_{\mathrm{H-H}}$ Hz	δ ppm	$J_{\mathrm{H-H}}$ Hz
2	6.96 m		6.97 m		6.69 dd	2.8 (6α) 1.3 (6β)	6.69	2.5 (6β) 1.3 (6α)
3α	2.18 m		2.18 m					
3β	1.94 m		1.94 m					
4	1.45 ttd	12.5 (3β, 5β) 5.0 (3α, 5α) 2.9 (1′)	1.47 ttd	12.5 (3β, 5β) 5.0 (3α, 5α) 2.5 (1′)	2.31 ddd	11.0 (5β) 8.5 (1′) 4.8 (5α)	2.25 td	11.0 (5α, 1′) 5.0 (5β)
5α	1.78 dtd	12.5 (5β) 5.0 (4, 6α) 2.5 (6β)	1.78 dtd	12.5 (5β) 5.0 (4, 6α) 2.3 (6β)	2.10 dtd	13.5 (5β) 4.8 (4, 6α) 3.6 (6β)	1.89 dddd	14.0 (5β) 11.0 (4) 9.6 (6β) 5.0 (6α)
5β	1.23 qd	12.5 (4, 5α, 6α) 5.2 (6β)	1.25 qd	12.5 (4, 5α, 6α) 5.2 (6β)	1.84 dtd	13.5 (5α) 11.0 (4, 6α) 4.6 (6β)	2.04 dq	14.0 (5α) 5.0 (4, 6α, 6β)
6α	2.14 m		2.14 m		2.48 dddd	19.5 (6β) 11.0 (5β) 4.8 (5α) 2.8 (2)	2.74 dtd	20.0 (6β) 5.0 (5α, 5β) 1.3 (2)
6β	2.47 br d	17.0 (6α)	2.45 br d	16.9 (6α)	2.76 dddd	19.5 (6α) 4.6 (5β) 3.6 (5α) 1.3 (2)	2.49 dddd	20.0 (6α) 9.6 (5α) 5.0 (5β) 2.5 (2)
8	3.72 s		3.72 s		3.83 s		3.83 s	
1′	2.02 m		2.03 m		2.60 m		2.73 m	
2′A	2.19 dd	15.9 (2′B) 7.0 (1′)	2.21 dd	15.4 (2′B) 8.9 (1′)	2.31 dd	16.1 (2′B) 7.6 (1′)	2.35 dd	16.0 (2′B) 7.6 (1′)
2′B	2.44 dd	15.9 (2′A) 4.5 (1′)	2.48 dd	15.4 (2′A) 4.7 (1′)	2.53 dd	16.1 (2′A) 5.0 (1′)	2.42 dd	16.0 (2′A) 5.2 (1′)
4′	2.27 d	6.6 (5′)	6.07 septet	1.1 (6′, 7′)	2.28 d	6.6 (5′)	2.27 d	6.5 (5′)
5′	2.13 nonuplet	6.6 (4′, 6′, 7′)			2.11 nonuplet	6.6 (4′, 6′, 7′)	2.12 nonuplet	6.6 (4′, 6′, 7′)
6′	0.92 d	6.6 (5′)	2.15 d	1.1 (4′)	0.90 d	6.6 (5′)	0.90 d	6.5 (5′)
7′	0.93 d	6.6 (5′)	1.89 d	1.1 (4′)	0.91 d	6.6 (5′)	0.90 d	6.5 (5′)
8′	0.88 d	6.6 (1′)	0.89 d	6.6 (1′)	0.95 d	6.6 (1′)	0.90 d	6.5 (1′)

Figures in parenthesis indicate a proton coupling with that in question.

Table II. ^{13}C-NMR Chemical Shifts[a)] (δ ppm) of **1**, **2**, **4** and **5**

Position	**1**	**2**	**4**	**5**
1	130.19 (q)	130.21 (q)	147.72 (q)	147.61 (q)
2	139.27 (t)	139.23 (t)	133.61 (t)	133.31 (t)
3	29.73 (s)	28.52 (s)	201.14 (q)	201.22 (q)
4	37.74 (t)	37.69 (t)	50.95 (t)	50.54 (t)
5	24.76 (s)	26.13 (s)	24.76 (s)	23.32 (s)
6	24.55 (s)	24.95 (s)	25.01 (s)	24.14 (s)
7	167.74 (q)	167.79 (q)	166.94 (q)	166.94 (q)
8	51.45 (p)	51.48 (p)	52.59 (p)	52.59 (p)
1′	32.65 (t)	32.88 (t)	28.38 (t)	27.01 (t)
2′	47.77 (s)	47.93 (s)	47.38 (s)	47.80 (s)
3′	210.43 (q)	210.51 (q)	210.44 (q)	209.99 (q)
4′	52.46 (s)	52.43 (s)	52.02 (s)	52.15 (s)
5′	24.55 (t)	24.55 (t)	24.59 (t)	24.14 (t)
6′	22.62 (p)	22.62 (p)	22.61 (p)	22.57 (p)
7′	22.56 (p)	22.56 (p)	22.54 (p)	22.57 (p)
8′	16.53 (p)	16.42 (p)	16.66 (p)	16.98 (p)

a) The data were taken at 75.4 MHz for comparison with those of the other compounds. Letters, p, s, t and q, in parenthesis indicate respectively primary secondary, tertiary and quaternary carbons, assigned by distortionless enchancement by polarization transfer.

(UV) spectrum an absorption band at 238 nm and in the infrared (IR) spectrum bands at 1720, 1705, 1675 and 1620 cm^{-1}, characteristic of an unsaturated ester, a ketone, an unsaturated ketone and a double bond, respectively. The ^{1}H-NMR spectrum of **4** exhibited signals corresponding to three *sec*-methyl groups at δ 0.90, 0.91 and 0.95, one methyl ester at δ 3.83 and one olefinic proton at δ 6.69 (Table I). These spectral properties suggest that **4** is a juvabione analog. Comparison of the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra of **4** and **1** or **2** indicated that one methylene group of the latter compound was replaced by an unsaturated ketone function (δ 201.14) in **4** (Table II). The ^{13}C-NMR chemical shifts of C-2′ to C-8′ in **4** were similar to those in **1** and **2**. In addition, the two fragments at m/z 127 and 154 in the electron ionization mass spectrum (EI-MS) were assigned to ions a and b (Chart 1), respectively, thus indicating the presence of the same side-chain moiety as that of **1** or **2**. In the ^{1}H-NMR spectrum of **4**, the signals of the C-6 methylene protons appeared at δ 2.48 and 2.76, whereas the C-3 methylene protons were not observed. In addition, the C-4 methyne proton exhibited a greatest chemical shift difference (*ca.* 0.86 ppm) relative to **1** and **2**, and the splitting pattern and coupling constants showed the side-chain moiety at C-4 to be affixed in an equatorial manner. Hence, it was assumed that **4** is a 3-oxo derivative of either **1** or **2**. The absolute configuration of this compound was determined by comparison of **4** with the allylic oxidation product of **2**. (+)-Epijuvabione (**2**) was treated with chromium trioxide[15)] to afford only one product having the 3-keto group, as deduced from its

^{1}H-NMR spectrum. This compound was identical with **4** in all respects examined, including the optical rotatory dispersion (ORD) and circular dichroism (CD) spectra. Based on this evidence, **4** was assigned as methyl 4(*S*)-[1(*S*),5-dimethyl-3-oxohexyl]-3-oxo-1-cyclohexene-1-carboxylate and may be described as (+)-oxoepijuvabione.

The general features of the UV, IR and NMR spectra of compound **5**, $C_{16}H_{24}O_4$ [HR-MS *m/z*: 280.1665 (M^+)], $[\alpha]_D^{28}$ −38° (*c*=1.57, EtOH), closely resembled those of **4** except that the C-5 and C-1′ signals exhibited a greater chemical shift difference (*ca.* 1.4 ppm) than observed for **4** in the ^{13}C-NMR spectrum (Table II). This evidence showed **5** to be an epimer of **4** at either C-4 or C-1′. Since the epimer of **4** at C-1′ is a juvabione-type compound, it was derived from **1** by allylic oxidation for comparison with **5**. The UV, IR and NMR spectral properties of the synthetic material (**6**) were identical with those of **5**. However, the ORD and CD curves of **6** were opposite to those of **5**. Based on this evidence, **5** was established to be the enantiomer of (+)-oxojuvabione (**6**), namely, methyl 4(*R*)-[1(*S*),5-dimethyl-3-oxohexyl]-3-oxo-1-cyclohexene-1-carboxylate [(−)-oxojuvabione] (Chart 1). This compound (**5**) corresponds to the C-4 epimer of **4** having the side chain in a more thermodynamically stable equatorial orientation (Chart 1). Therefore, **5** may be described as an epijuvabione-type compound rather than a juvabione-type one.

As mentioned above, (+)-juvabione (**1**) and the other juvabione-type compounds were previously isolated from the *A. sachalinensis* wood grown in central Hokkaido,[1)] whereas this time **1** was not detected, but (+)-epijuvabione (**2**) was instead isolated together with the other epijuvabione-type compounds from the wood of this species grown in the southern base of the Taisetsu mountain range. This evidence suggests that there is a tree-to-tree variation in constituents of the *A. sachalinensis* wood.

Experimental

CD curves were recorded on a JASCO J-500A spectropolarimeter. Other spectral measurements were carried out with the instruments described in the previous paper.[16)] HPLC was carried out on a Waters ALC-200 instrument equipped with a differential refractometer (R-401) and a Shim-pack PREP-SIL column (25 cm × 20 mm i.d.).

Extraction and Separation A 20 cm diameter butt log was obtained from an *A. sachalinensis* tree grown on the southern base of Mt. Upepesanke located at Shikaoi-cho, Kato-gun, in Hokkaido, Japan.[17)] The debarked whole wood (2.73 kg) was finely cut and extracted three times with acetone (20 l each) at room temperature overnight and then once with hot acetone (20 l) for 3 h. The combined extracts were evaporated at 40 °C *in vacuo*. The oily residue (79 g) was passed through a Sephadex LH-20 column using acetone–CH_2Cl_2 (1 : 1) as the eluent. The third fraction (5.02 g) was repeatedly chromatographed on a silica gel column with an MeOH–CH_2Cl_2 gradient as the eluent. The fraction eluted with CH_2Cl_2 gave **2** (620 mg). The oily fraction (778 mg) eluted with 0.5% MeOH in CH_2Cl_2 was chromatographed on a silica gel column with an AcOEt–benzene gradient as the eluent. The fraction (377 mg) eluted with 2.5% AcOEt in benzene was subjected to HPLC with 5% AcOEt in benzene to afford **3** (44.2 mg), **4** (22.5 mg) and **5** (15.7 mg).

(+)-Epijuvabione (2) A colorless oil, $[\alpha]_D^{28}$ +89° (*c*=2.02, EtOH). HR-MS *m/z*: 266.1873 (M^+) (Calcd for $C_{16}H_{26}O_3$: 266.1875). The UV, IR, NMR, ORD and CD data of this compound were in accord with the published values.[4,12−14)]

(+)-Dehydroepijuvabione (3) A colorless oil, $[\alpha]_D^{28}$ +41.5° (*c*=1.59, EtOH). HR-MS *m/z*: 264.1714 (M^+) (Calcd for $C_{16}H_{24}O_3$: 264.1719). ORD (*c*=1.59, EtOH, 28 °C): $[\phi]_{500}$ +176.1, $[\phi]_{430}$ +292.4, $[\phi]_{350}$ +658.6, $[\phi]_{314}$ +525.0, $[\phi]_{274}$ +1296.0. CD (*c*=1.00, EtOH, 23 °C): $[\theta]_{388}$ 0, $[\theta]_{325}$ +898.2, $[\theta]_{278}$ +396.3, $[\theta]_{261}$ +2707.8, $[\theta]_{241}$ +8981.9, $[\theta]_{238}$ +6340.1. The UV, IR, and NMR data of this compound were identical with the published values.[4,10,12,14)]

(+)-Oxoepijuvabione (4) A colorless oil, $[\alpha]_D^{28}$ +61° (*c*=1.05, EtOH). UV λ_{max}^{EtOH} nm (log ε): 238 (3.96). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720 ($COOCH_3$), 1705 (>C=O), 1675 (α, β-unsaturated ketone), 1620 (C=C). EI-MS *m/z* (%) (HR-MS: Calcd; Found): 280 (M^+, 52) (280.1672 for $C_{16}H_{24}O_4$; 280.1671), 223 (26) (223.0969 for $C_{12}H_{15}O_4$; 223.0964), 195 (24) (195.1019 for $C_{11}H_{15}O_3$; 195.0990), 181 (100) (181.0863 for $C_{10}H_{13}O_3$; 181.0887), 154 (ion b, 89) (154.0628 for $C_8H_{10}O_3$; 154.0644), 153 (11) (153.0551 for $C_8H_9O_3$; 153.0589), 127 (ion a, 12) (127.1122 for $C_8H_{15}O$; 127.1125), 121 (16) (121.0288 for $C_7H_5O_2$; 121.0281), 85 (22) (85.0652 for C_5H_9O; 85.0660). ORD (*c*=1.15, EtOH, 28°C): $[\phi]_{450}$ +453.6, $[\phi]_{410}$ +837.9, $[\phi]_{366}$ +186.8, $[\phi]_{305}$ +3090.0, $[\phi]_{286}$ +2316.1. CD (*c*=1.15, EtOH, 25 °C): $[\theta]_{430}$ 0, $[\theta]_{390}$ +377.6, $[\theta]_{360}$ 0, $[\theta]_{340}$ −304.5, $[\theta]_{319}$ 0, $[\theta]_{276}$ +1949.0, $[\theta]_{268}$ +2192.6, $[\theta]_{253}$ +5725. 1, $[\theta]_{241}$ +4301.3.

Preparation of 4 from 2 A solution of **2** (10 mg) in benzene (2 ml) was added with stirring at below 20 °C to a solution of CrO_3 (22 mg), acetic anhydride (10 ml) and glacial acetic acid (5 ml) in benzene (5 ml), according to the procedure of Nakayama *et al.*[15)] The reaction mixture was allowed to stand at 20 °C overnight, diluted with water, neutralized with aqueous sodium hydroxide solution, and extracted with benzene. The organic layer was dried and evaporated to dryness. The oily residue (8.6 mg) was purified by HPLC with 5% AcOEt in benzene as the eluent to afford the oxidation product (**4**, 4 mg) as a colorless oil besides **2** (4 mg). This product was identical with the natural product (**4**) in all respects examined, including ORD and CD spectra.

(−)-Oxojuvabione (5) A colorless oil, $[\alpha]_D^{28}$ −38° (*c*=1.57, EtOH). UV λ_{max}^{EtOH} nm (log ε): 238 (3.90). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720 ($COOCH_3$), 1705 (>C=O), 1675 (α,β-unsaturated ketone), 1620 (C=C). EI-MS *m/z* (%) (HR-MS: Calcd; Found): 280 (M^+, 49) (280.1672 for $C_{16}H_{24}O_4$; 280.1665), 223 (29) (223.0969 for $C_{12}H_{15}O_4$; 223.0969), 195 (28) (195.1019 for $C_{11}H_{15}O_3$; 195.1019), 181 (96) (181.0863 for $C_{10}H_{13}O_3$; 181.0862), 154 (ion b, 100) (154.0628 for $C_8H_{10}O_3$; 154.0628), 153 (9) (153.0551 for $C_8H_9O_3$; 153.0557), 127 (ion a, 18) (127.1122 for $C_8H_{15}O$; 127.1135), 121 (16) (121.0288 for $C_7H_5O_2$; 121.0249), 85 (21) (85.0652 for C_5H_9O; 85.0652). ORD (*c*=1.57, EtOH, 28 °C): $[\phi]_{450}$ −207.0, $[\phi]_{415}$ −321.2, $[\phi]_{380}$ 0, $[\phi]_{365}$ +128.5, $[\phi]_{358}$ 0, $[\phi]_{294}$ −1784.8, $[\phi]_{288}$ −1641.8. CD (*c*=1.05, EtOH, 25 °C): $[\theta]_{425}$ 0, $[\theta]_{392}$ −272. 8, $[\theta]_{368}$ 0, $[\theta]_{342}$ +389.8, $[\theta]_{302}$ 0, $[\theta]_{265}$ −1315.6, $[\theta]_{261}$ −1071.9, $[\theta]_{250}$ −3410.8, $[\theta]_{240}$ −5870.2.

Preparation of (+)-Oxojuvabione (6) from 1 A solution of **1** (10 mg) in benzene (2 ml) was treated with a solution of CrO_3 (22 mg), acetic anhydride (10 ml) and glacial acetic acid (5 ml) in benzene (5 ml), according to the procedure mentioned above, to afford, in addition to **1** (3.4 mg), (+)-oxojuvabione (**6**) (2.4 mg) as a colorless oil, $[\alpha]_D^{28}$ +38.3° (*c*=2.4, EtOH). Its UV, IR and NMR spectra were in accord with those of **5**, but the ORD and CD curves were opposite to those of **5**. ORD (*c*=2.4, EtOH, 23°C): $[\phi]_{450}$ +241.8, $[\phi]_{415}$ +452.9, $[\phi]_{374}$ 0, $[\phi]_{365}$ −149.4, $[\phi]_{358}$ 0, $[\phi]_{294}$ +2497.0, $[\phi]_{270}$ +2101.3. CD (*c*=1.20, EtOH, 25 °C): $[\theta]_{425}$ 0, $[\theta]_{392}$ +350.2, $[\theta]_{367}$ 0, $[\theta]_{342}$ −583.7, $[\theta]_{306}$ 0, $[\theta]_{267}$ +2101.3, $[\theta]_{258}$ +2941.8, $[\theta]_{250}$ +5720.0.

Acknowledgement The authors are very grateful to Dr. G. Murata, Department of Botany, Faculty of Science, Kyoto University, and Drs. J. Samejima and K. Takahashi, Hokkaido Research Center, Forestry and Forest Products Research Institute, for the plant identification, and to Director H. Nagai of Shimizu Local Forestry Office and Chief H. Takahashi of the Enterprise Section in the office for providing the wood of *A. sachalinensis*. Thanks are also due to Mrs. M. Yoneda and Miss M. Danjo, this university, for the NMR and MS measurements, respectively.

References and Notes

1) Part I: A. Numata, K. Hokimoto, T. Takemura, S. Matsunaga, and R. Morita, *Chem. Pharm. Bull.*, **31**, 436 (1983).
2) W. S. Bowers, H. M. Fales, M. J. Thompson, and E. C. Uebel, *Science*, **154**, 1020 (1966).
3) J. F. Manville, *Can. J. Chem.*, **54**, 2365 (1976).
4) *a*) V. Cerny, L. Dolejs, L. Labler, F. Sorm, and K. Slama, *Tetrahedron Lett.*, **12**, 1053 (1967); *b*) *Idem, Coll. Czech. Chem. Commun.*, **32**, 3926 (1967); *c*) J. F. Manville, L. Greguss, K. Slama, and E. von Rudloff, *ibid.*, **42**, 3658 (1977).
5) J. F. Manville, *Can. J. Chem.*, **53**, 1579 (1975).
6) J. F. Manville and C. D. Kriz, *Can. J. Chem.*, **55**, 2547 (1977).
7) J. F. Manville, K. Bock, and E. von Rudloff, *Phytochemistry*, **16**, 1967 (1977).
8) *a*) T. Sakai and Y. Hirose, *Chem. Lett.*, **1973**, 491; *b*) T. Sakai and

Y. Hirose, *ibid.*, **1973**, 825.

9) *a*) I. H. Rogers and J. F. Manville, *Can. J. Chem.*, **50**, 2380 (1972); *b*) I. H. Rogers, J. F. Manville, and T. Sahoda, *ibid.*, **52**, 1192 (1974).

10) P. Bhan, B. S. Pande, R. Soman, N. P. Damodaran, and S. Dev, *Tetrahedron*, **40**, 2961 (1984).

11) Unfortunately we did not know precisely the location in central Hokkaido where the tree had grown.

12) J. F. Manville and K. Bock, *J. Org. Magn. Reson.*, **9**, 596 (1977).

13) B. A. Pawson, H.-C. Cheung, S. Gurbaxani, and G. Sancy, *J. Am. Chem. Soc.*, **92**, 336 (1970).

14) As pointed out by Manville,[5)] juvabione and dehydrojuvabione described by Cerny *et al.*[4a,b)] are, in fact, epijuvabione and dehydroepijuvabione, respectively, and juvabione and epijuvabione described by Pawson *et al.*[13)] are reversed.

15) M. Nakayama, S. Shinke, Y. Matsushita, S. Ohira, and S. Hayashi, *Bull. Chem. Soc. Jpn.*, **52**, 184 (1979).

16) A. Numata, P. Yang, C. Takahashi, R. Fujiki, M. Nabae, and E. Fujita, *Chem. Pharm. Bull.*, **37**, 648 (1989).

17) A voucher specimen (Numata No. 2) has been deposited at the Herbarium of the Department of Botany, Faculty of Science, Kyoto University, Kyoto, Japan.