

Neurotropic Components from Star Anise (*Illicium verum* Hook. fil.)

Tomonori NAKAMURA, Emi OKUYAMA,* and Mikio YAMAZAKI

Faculty of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263, Japan.

Received May 7, 1996; accepted July 3, 1996

Three new neurotropic sesquiterpenoids, veranisatins A, B and C, were isolated from star anise (*Illicium verum* Hook. fil., Illiciaceae). Veranisatins showed convulsion and lethal toxicity in mice at a dose of 3 mg/kg (*p.o.*), and at lower doses they caused hypothermia. Veranisatin A and the related compound, anisatin, were tested for the other pharmacological activities such as locomotor activity and analgesic effect. Both compounds decreased the locomotion enhanced by methamphetamine at oral doses of 0.1 and 0.03 mg/kg, respectively, and demonstrated the analgesia on acetic acid-induced writhing and tail pressure pain at almost similar doses.

Key words veranisatin; *Illicium verum*; analgesia; convulsion; locomotor activity; hypothermia

Star anise (the fruit of *Illicium verum* Hook. fil., Illiciaceae) is an important spice, and is also used in the treatment of stomach ache *etc.*, as a traditional medicine in China and Japan. During our assay of the neurotropic components of traditional medicines and medicinal plants, the methanol extract of star anise exhibited a significant hypothermic effect ($\Delta T_{\max} - 5.2^\circ\text{C}$, $p < 0.001$, 3 g/kg, *p.o.*) and an analgesic effect on acetic acid-induced writhing (23%, $p < 0.001$, 500 mg/kg, *p.o.*) in mice. As separation of the active components from the extract proceeded, a convulsive effect was also observed. Among the family of Illiciaceae, the fruits of *Illicium anisatum* L. (shikimi in Japanese) are well known as a neurotoxic plant containing the convulsants, anisatin and neoanisatin.¹⁾ However, such components have never before been found in star anise. This paper describes the isolation and structure elucidation of three new neurotropic components, veranisatins A—C, from star anise and pharmacological investigation of veranisatins and anisatin.²⁾

Preliminary experiments resulted in more efficient extraction of the active components from defatted material with ethyl acetate. The hypothermia ($\Delta T_{\max} - 5.6^\circ\text{C}$, $p < 0.001$) of the ethyl acetate extract was induced at a dose of 100 mg/kg, *p.o.*, and the convulsion (4/4) and lethal effect (4/4) at a dose of 500 mg/kg, *p.o.* The extract (100 g) was separated by following biological tests of convulsion and lethal effect, since hypothermia and these effects were observed in the same fractions in the preliminary experiment. Separation by Sephadex LH-20 and silica gel column chromatographies gave the active fractions, 3-A and 3-B. The former fraction was further separated by ODS flash chromatography and then by repeated HPLC using Aquasil eluted with chloroform–methanol–water (100:5:1). Two new convulsive components, veranisatins A (**1**) and B (**2**), were obtained. The latter fraction was separated by Sephadex LH-20 to remove a large amount of 1-(4'-methoxyphenyl)-1,2-propanediol (**4**) which showed no activity, although this is the first isolation of the compound from this plant. HPLC separation of fraction 7-C gave the other convulsive component, veranisatin C (**3**). (Chart 1, Fig. 1)

Veranisatin A (**1**) is colorless prisms, mp 181—182°C, from ethyl acetate. Its molecular formula was determined as $\text{C}_{16}\text{H}_{22}\text{O}_8$ [m/z : 365.1209 ($\text{M} + \text{Na}$)⁺] on the basis

of high resolution fast atom bombardment mass spectrometry (HR-FAB-MS). The IR spectrum showed the presence of hydroxyl (3380 cm^{-1} , br), β -lactone (1830 cm^{-1}) and δ -lactone (1750 cm^{-1}) groups. The ^1H - and ^{13}C -NMR spectra in acetone- d_6 including ^1H - ^1H and ^{13}C - ^1H correlation spectroscopies (COSY) indicated the partial structures of $-\text{C}(15)\text{H}_3-\text{C}(1)\text{H}-\text{C}(2)\text{H}_2-\text{C}(3)\text{H}_2-$, $-\text{C}(8)\text{H}_2-\text{C}(7)\text{H}-\text{O}-$, $-\text{O}-\text{C}(12)\text{H}_2-$, $-\text{C}(14)\text{H}_2-\text{O}-$ and $-\text{C}(10)\text{H}-\text{OH}$. A methoxyl group at δ 3.41 and two other hydroxyl groups at δ 5.52 and 6.05 were also observed in ^1H -NMR, and two ester carbonyls at δ 169.04 and 174.84 and four quaternary carbons at δ 51.97, 64.29, 78.86 and 86.10 in ^{13}C -NMR. The positions of the hydroxyl groups were determined by deuterium-induced shift technique in ^{13}C -NMR.³⁾ The shifts were observed at δ 32.88 (γ -effect), 71.01, 78.86 and 86.10 (0.07, 0.08, 0.12 and 0.12 ppm, respectively) in acetone- d_6 with one additional drop of $\text{CD}_3\text{OD}-\text{CD}_3\text{OH}$ (1:1), indicating that positions of the hydroxyl groups were C-10, C-6, and C-4.

These components were further connected by correlation spectroscopy *via* long-range coupling spectrum (COLOC), as shown in Fig. 2. Connections of C-9 to C-1, C-4, C-8 and C-10 were indicated by the observation of the correlations of $\text{H}_3-15/\text{C}-9$, $4\text{-OH}/\text{C}-9$, $\text{H}_b-8/\text{C}-1$ and $10\text{-OH}/\text{C}-9$. Then, the five-membered ring (A-ring) including a partial structure of $-\text{C}(9)-\text{C}(1)\text{H}-\text{C}(2)\text{H}_2-\text{C}(3)\text{H}_2-$ was estimated by C(3)–C(4) bond formation using the correlation of $4\text{-OH}/\text{C}-3$. The six-membered ring (B-ring) was made up by connection of a partial structure of $-\text{C}(4)-\text{C}(9)-\text{C}(8)\text{H}_2-\text{C}(7)\text{H}-$ and quaternary carbons of C(5) and C(6), because of the observation of the cross peaks, $4\text{-OH}/\text{C}-5$, $\text{H}-7/\text{C}-5$ and C-6, and $\text{H}_a-8/\text{C}-6$. The ester carbonyl of C-11 was connected to C-7 *via* oxygens to constitute δ -lactone ring (C-ring), by the correlation of C-11/H-7 and H-10/C-11. β -Lactone ring composed of C-5, C-13, and C-14 was assigned since the cross peaks among the methylene protons at C-14 and C-4, C-6 and C-13 were observed. Methoxy methylene and hydroxyl substituents at C-6 were suggested by the correlation of $\text{H}_3-16/\text{C}-12$, $\text{H}_b-12/\text{C}-6$ and $6\text{-OH}/\text{C}-6$. From these data, veranisatin A was elucidated to be structure **1** which has the same skeleton as anisatin and neoanisatin. The stereostructure was supported by nuclear Overhauser effect (NOE) experiment. Irradiation of the methyl at δ 1.10

* To whom correspondence should be addressed.

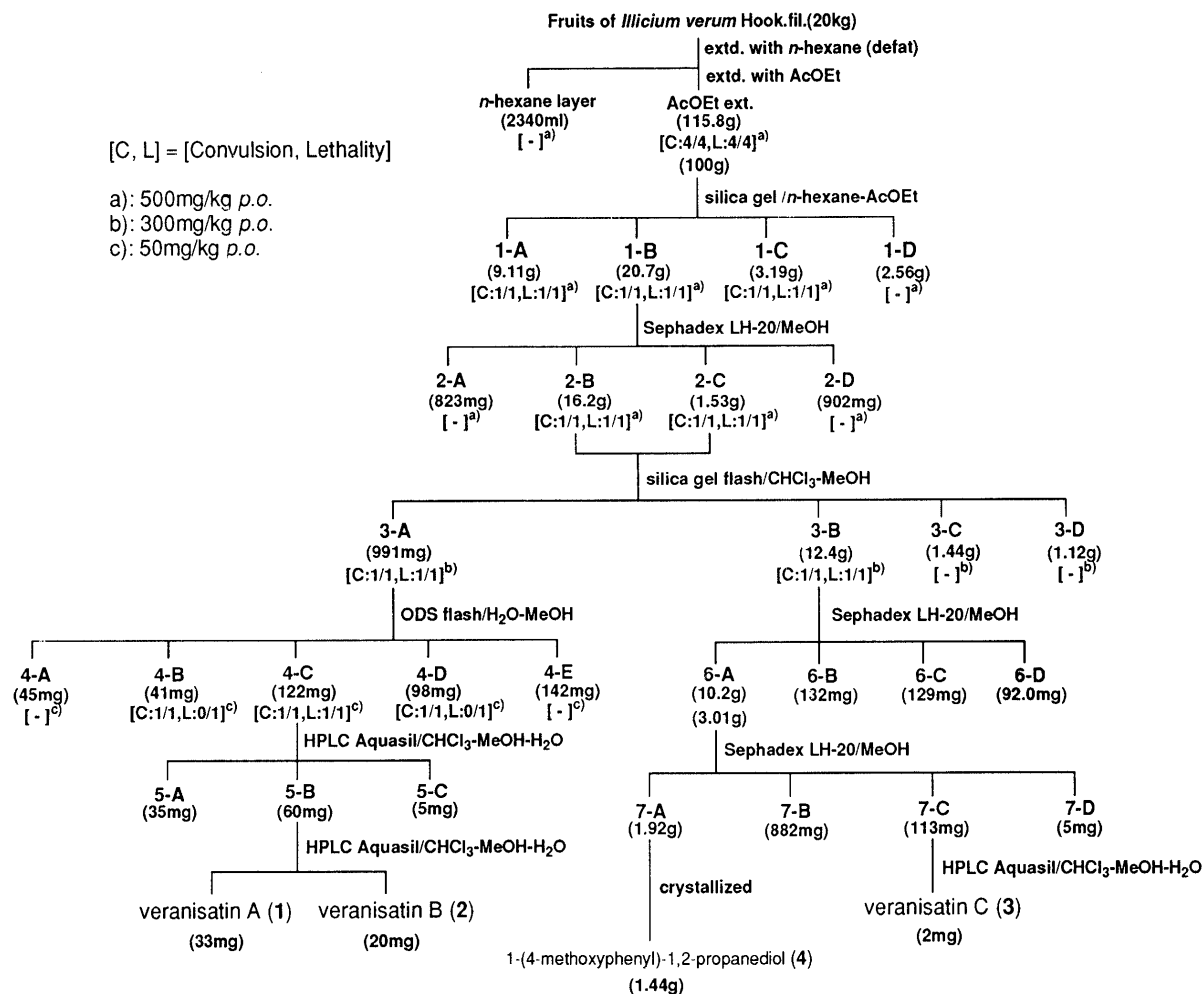


Chart 1. Isolation Procedure

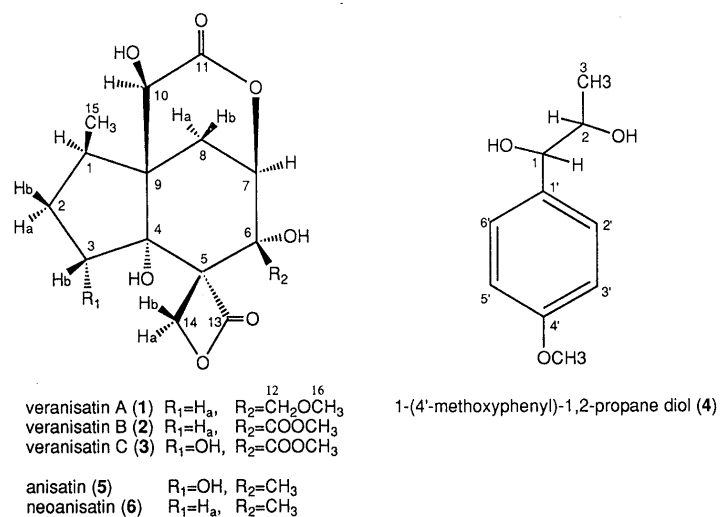


Fig. 1

(H₃-15) and one of methylene signals at δ 3.53 (H_b-12) indicated NOEs of the signals at δ 1.59 (H_a-2) and 4.30 (H-10), and 4.03 (H_a-14), respectively. The absolute stereochemistry was finally provided by direct comparison of the circular dichroism (CD) ($[\theta]_{236} - 1950$ in methanol) with those of anisatin (5) and neoanisatin (6) ($[\theta]_{233} - 2720$ and $[\theta]_{238} - 1940$ in methanol, respectively).⁴⁾

Veranisatin B (2) was obtained as colorless prisms (ethyl acetate), mp 212–213 °C, having the molecular formula

of C₁₆H₂₀O₉ [m/z : 379.1003 (M+Na)⁺] by HR-FAB-MS. The IR spectrum indicated three ester carbonyl groups at 1740, 1760 (δ -lactone) and 1825 (β -lactone) cm⁻¹. The ¹H- and ¹³C-NMR spectra of 2 were similar to those of 1, except for the signals assigned to a substituent at C-6 (Table 1). The ¹³C-NMR indicated the presence of methoxy carbonyl group at δ 53.59 and 170.06, instead of methoxy methylene at δ 60.54 and 76.36 in 1. Deshielding of the methine proton of C-7 at δ 4.88 (1H, dd, $J=3.9$,

2.2) in **2** was caused by the carbonyl substituent at C-6. The CD spectrum ($[\theta]_{237-2370}$) suggested that **2** has the same stereostructure as **1**, as shown in Fig. 1.

Veranisatin C (**3**) was colorless needles (ethanol), mp 228—229.5 °C, which molecular formula of $C_{16}H_{20}O_{10}$ [m/z : 373.1140 ($M+H$)⁺] was determined by HR-FAB-MS. The spectral data of **3** were similar to those of **2**. The ¹H-NMR suggested the presence of one more hydroxyl group at δ 5.09 (1H, d, $J=5.2$; 3-OH) and a methine

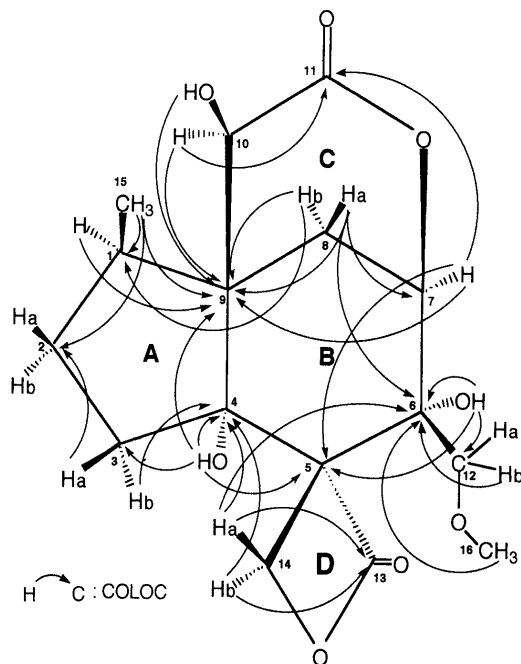


Fig. 2. COLOC Correlations of Veranisatin A (**1**)

Table 1. ¹³C- and ¹H-NMR Data of Veranisatins A—C (**1**—**3**), Anisatin (**5**) and Neoisatin (**6**) in Acetone-*d*₆

Position	1		2		3		5	6
	¹³ C-NMR	¹ H-NMR	¹³ C-NMR	¹ H-NMR	¹³ C-NMR	¹ H-NMR	¹ H-NMR	¹ H-NMR
1	39.50	2.43—2.51 (m)	39.36	2.46—2.50 (m)	37.73	2.55—2.60 (m)	2.50—2.58 (m)	2.42—2.48 (m)
2	31.56	a 1.59 (dddd, 12.4, 11.7, 8.2, 2.5) b 2.07—2.14 (m)	31.19	a 1.65 (dddd, 12.1, 11.7, 8.6, 2.6) b 2.12—2.18 (m)	42.00	a 1.81 (ddd, 12.9, 10.7, 4.4) b 2.07—2.11 (m)	a 1.78 (ddd, 13.2, 10.5, 4.4) b 1.99 (ddd, 13.1, 9.7, 8.6)	a 1.60 (dddd, 12.5, 12.0, 8.3, 2.7) b 2.09—2.16 (m)
3	32.88	a 2.43—2.51 (m) b 1.67 (ddd, 12.4, 9.3, 2.5)	32.87	a 2.54 (ddd, 13.0, 9.0, 6.4) b 1.75 (ddd, 13.0, 9.0, 2.6)	71.06	4.98 (ddd, 9.3, 5.2, 4.4) 5.09 (OH, d, 5.2)	a 4.96 (dt, 9.7, 4.4) b 4.78 (OH, br d, 4.4)	a 2.51 (ddd, 12.4, 12.0, 6.4) b 1.69 (ddd, 12.4, 8.3, 2.7)
4	86.10	5.52 (OH, s)	87.05	5.82 (OH, s)	86.12	5.41 (OH, s)	5.36 (OH, br s)	5.64 (OH, br s)
5	64.29		63.87		61.97			
6	78.86	6.05 (OH, d, 1.5)	80.67	6.85 (OH, br s)	80.10	6.59 (OH, s)	6.02 (OH, br s)	6.18 (OH, br s)
7	79.49	4.26 (dd, 3.9, 2.2)	79.00	4.88 (dd, 3.9, 2.2)	78.44	4.89 (dd, 3.9, 2.0)	4.21 (dd, 3.7, 2.2)	4.20 (dd, 3.6, 2.2)
8	27.80	a 2.07 (dd, 14.7, 3.9) b 2.43 (dd, 14.7, 2.2)	27.51	a 2.18 (dd, 14.9, 3.9) b 2.47 (dd, 14.9, 2.2)	26.69	a 2.24 (dd, 14.9, 3.9) b 2.49 (dd, 14.9, 2.0)	a 2.13 (dd, 14.9, 3.7) b 2.49 (dd, 14.9, 2.2)	a 2.07 (overlapped with solv.) b 2.47 (dd, 14.7, 2.2)
9	51.97		51.48		50.91			
10	71.01	4.30 (d, 3.4) 5.07 (OH, d, 3.4)	71.06	4.36 (d, 3.7) 5.25 (OH, d, 3.7)	70.39	5.39 (OH, d, 3.7) 4.35 (d, 3.7)	5.20 (OH, d, 3.1) 4.29 (d, 3.1)	5.05 (OH, d, 3.2) 4.29 (d, 3.2)
11	174.84		174.50		173.65			
12	76.36	a 4.05 (dd, 11.0, 1.5) b 3.53 (d, 11.0)	170.06		169.45		1.46 (3H, s)	1.49 (3H, s)
13	169.04		168.66		167.52			
14	65.15	a 4.03 (d, 6.1) b 4.30 (d, 6.1)	65.85	a 4.08 (d, 5.9) b 4.36 (d, 5.9)	64.78	a 4.09 (d, 6.0) b 4.41 (d, 6.0)	a 3.99 (d, 6.6) b 4.41 (d, 6.6)	a 4.02 (d, 6.5) b 4.37 (d, 6.5)
15	14.22	1.10 (3H, d, 7.0)	14.11	1.12 (3H, d, 7.1)	13.41	1.06 (3H, d, 7.1)	1.04 (3H, d, 7.1)	1.09 (3H, d, 7.1)
16	60.54	3.41 (3H, s)	53.59	3.82 (3H, s)	53.08	3.81 (3H, s)		

proton at δ 4.98 (1H, ddd, $J=9.3, 5.2, 4.4$; H-3) in **3** instead of one of the methylenes in **2**. The position of the hydroxyl group was assigned at C-3 by two dimensional (2D)-NMRs including the field gradient heteronuclear multiple bond connectivity (FG-HMBC) shown in Fig. 3. The stereochemistry was presented by NOEs of H-3/10-OH, H_b-14 and H_a-14 (negative NOE), H_a-8/H-7 and H-10 and H₃-15/H-10. Irradiation of methyl signal at C-16 fortunately indicated small NOEs of H-7 and H_a-14, suggesting the same configuration of the substituent at C-6 as **2**. (Fig. 4) The CD spectrum ($[\theta]_{235-3730}$ (methanol)) confirmed that **3** has the same stereostructure as **2**.

The plain structure of **4** was determined to be 1-(4'-methoxyphenyl)-1,2-propanediol by the aid of ¹H- and ¹³C-NMR including ¹H-¹H-COSY and distortionless enhancement by polarization transfer (DEPT) method. Compound **4** was further separated to two isomers, **4A** (mp 118—119 °C) and **4B** (mp 67.5—68.0 °C), by ODS-MPLC (medium pressured liquid chromatography). As the optical rotations in both **4A** and **4B** were almost 0°, these compounds seemed to be mixtures of the racemic compounds. Their structures were established as 1*R*/2*S*- and 1*S*/2*R*-isomers and 1*R*/2*R*- and 1*S*/2*S*-isomers at C-1 and C-2 positions, respectively, since the melting points were similar to the published data (mp 111.5—116.5 °C, mp 62.8—63.2 °C, respectively).⁵⁾ To confirm the stereochemistry, we tried to lead **4B** from *trans*-anethole by oxidation. The *trans*-anethole was obtained by purification of commercial anethole which had the *trans*-isomer as a major component. Oxidation of *trans*-anethole with osmium tetroxide gave **4C**, a mixture of 1*R*/2*R*- and

1*S*/2*S*-diols. The melting point (66.5–67.0 °C) and the spectral data (^1H - and ^{13}C -NMR, IR, UV) of **4C** were identical with those of **4B**. Therefore, **4B** was confirmed to be 1-(4'-methoxyphenyl)-(1*R*,2*R* and 1*S*,2*S*)-propane-1,2-diol, while **4A** is a mixture of 1*R*,2*S*- and 1*S*,2*R*-isomers.

Veranisatins A–C (**1**–**3**) showed convulsive effects and lethal toxicity at an oral dose of 3 mg/kg in mice, while anisatin caused the same effects at a dose of 1 mg/kg, *p.o.* (Table 2) Administration of 1 mg/kg of **1**–**3** did not cause convulsion, but did elicit strong hypothermia in mice with

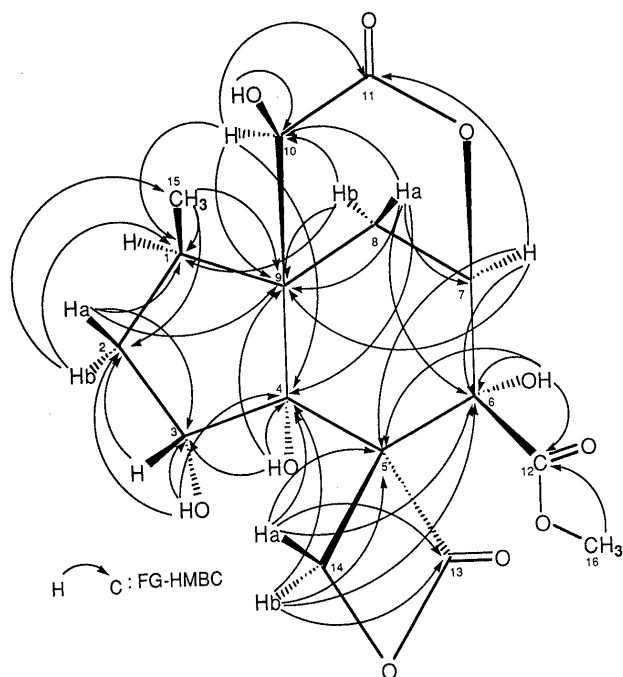


Fig. 3. FG-HMBC Correlations of Veranisatin C (**3**)

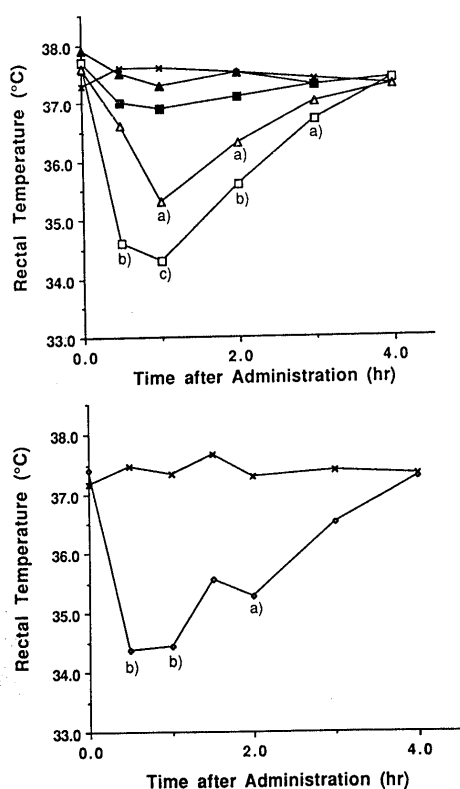


Fig. 5. Effects of Veranisatins A–C (**1**–**3**) and Anisatin (**5**) on Body Temperature in Mice

a) $p < 0.05$, b) $p < 0.01$, c) $p < 0.001$. $n = 3$ – 4 .

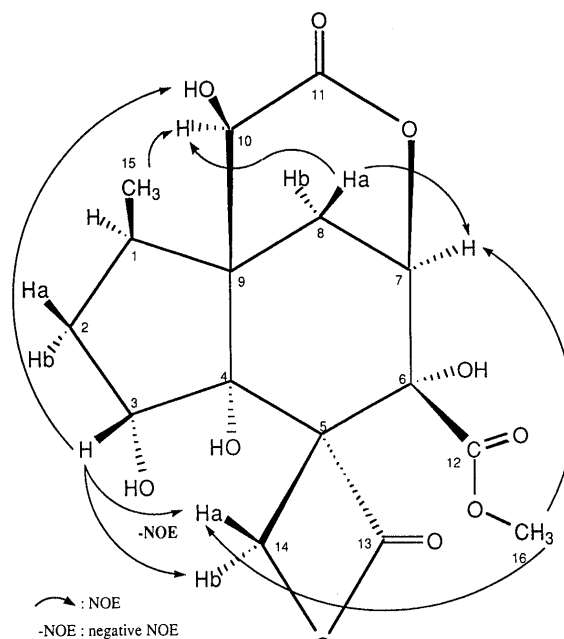
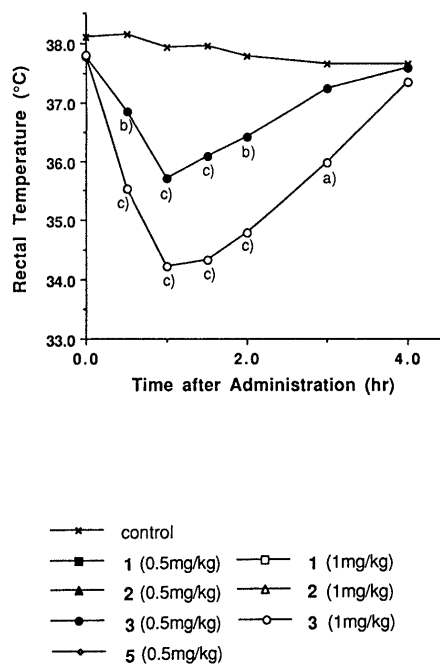


Fig. 4. NOEs of Veranisatin C (**3**)

Table 2. Toxicity of Veranisatins A–C (**1**–**3**) and Anisatin (**5**)

	Dose	Convulsion	Lethality
Veranisatin A	1 mg/kg <i>p.o.</i>	0/3	0/3
	3 mg/kg <i>p.o.</i>	3/3	3/3
Veranisatin B	1 mg/kg <i>p.o.</i>	0/3	0/3
	3 mg/kg <i>p.o.</i>	3/3	3/3
Veranisatin C	1 mg/kg <i>p.o.</i>	0/4	0/4
	3 mg/kg <i>p.o.</i>	4/4	4/4
Anisatin ⁶⁾	1 mg/kg <i>p.o.</i>	3/3	3/3

The number of affected mice/treated mice is shown.



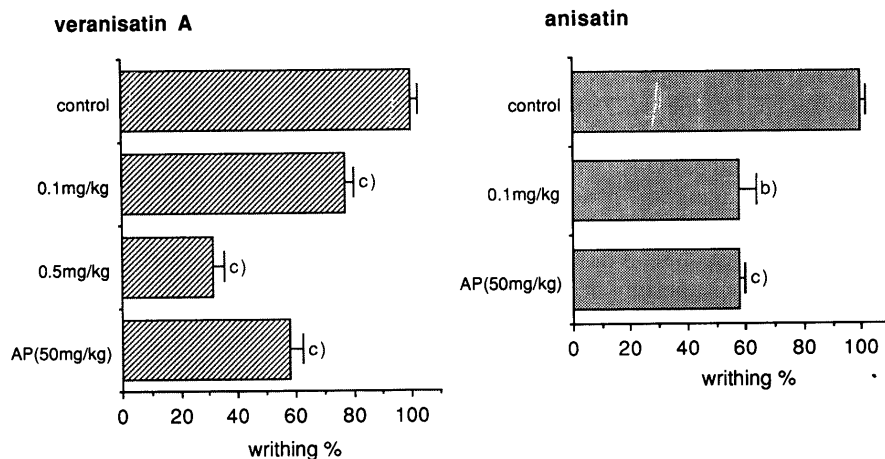


Fig. 6. Analgesic Effects of Veranisatin A (1) and Anisatin (5) on Acetic Acid-Induced Writhing in Mice

Each bar represents the mean $\pm$ S.E. The number of squirms in each control (26.3 ± 0.68 and 26.3 ± 0.44 for left and right panels, respectively) was taken as 100%. AP: aminopyrine. a) $p < 0.05$, b) $p < 0.01$, c) $p < 0.001$. $n = 6$.

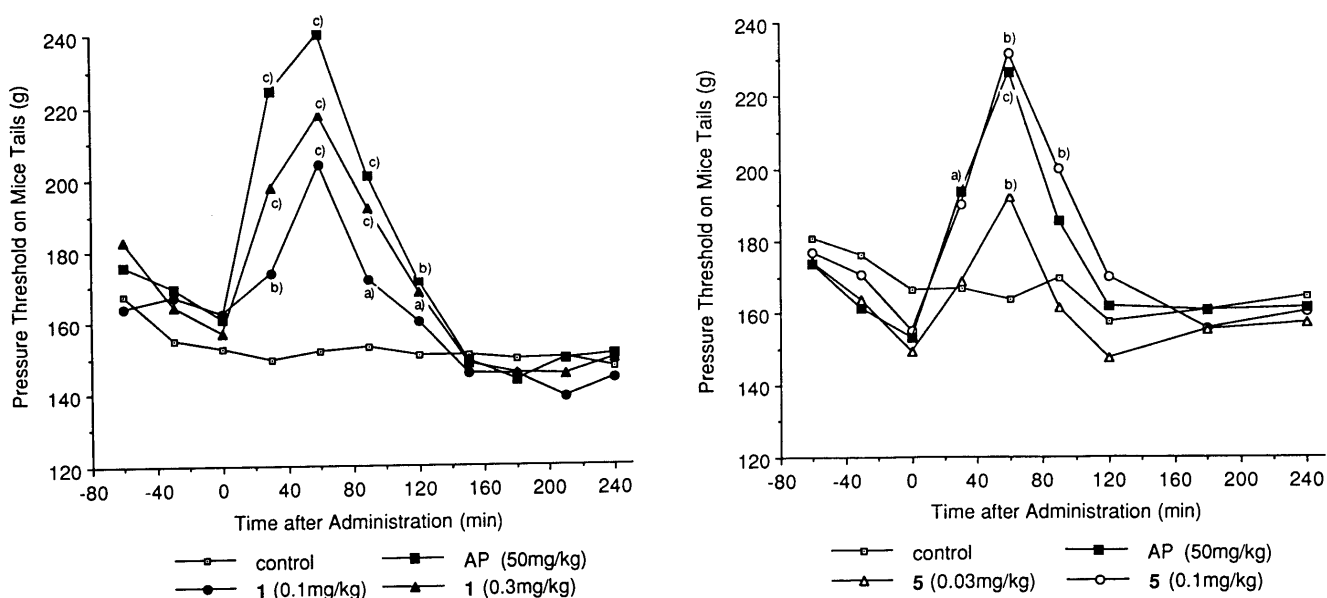


Fig. 7. Analgesic Effects of Veranisatin A (1) and Anisatin (5) on Pressure Pain Threshold in Mice

AP: aminopyrine. a) $p < 0.05$, b) $p < 0.01$, c) $p < 0.001$. $n = 7-8$.

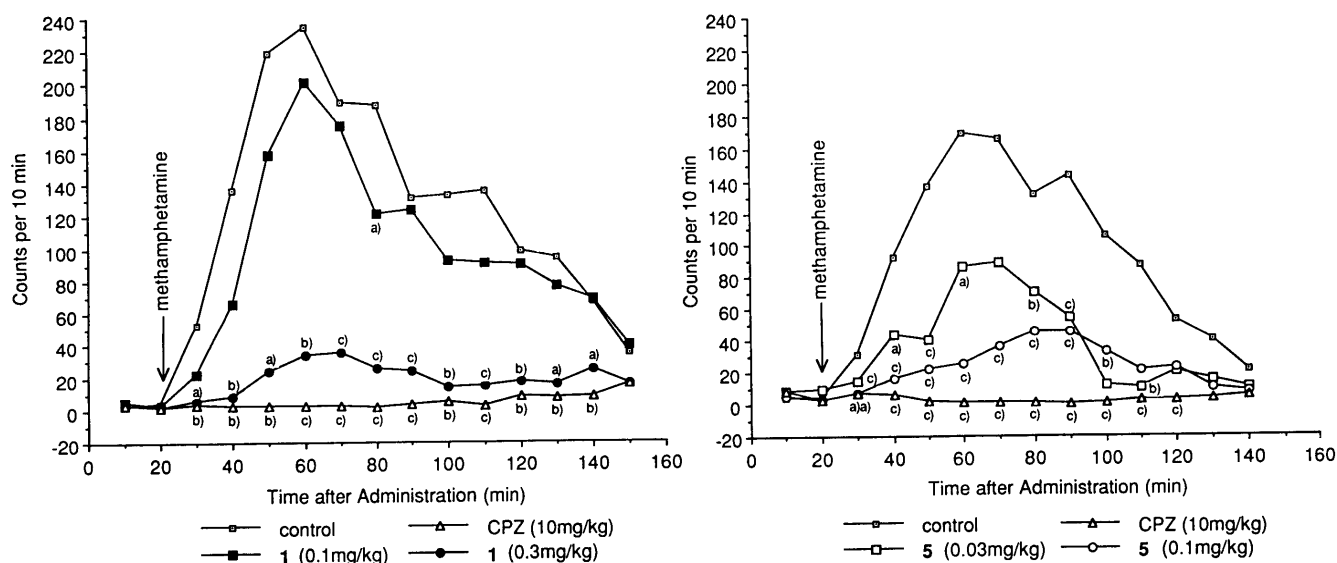


Fig. 8. Effects of Veranisatin A (1) and Anisatin (5) on Methamphetamine-Induced Locomotor Activity in Mice

CPZ: chlorpromazine (10 mg/kg, *p.o.*). a) $p < 0.05$, b) $p < 0.01$, c) $p < 0.001$. $n = 10$.

their $\Delta T_{\max}$ of -4.2°C , -2.4°C and -3.6°C , respectively. (Fig. 5) Anisatin also decreased body temperature at a dose of 0.5 mg/kg, *p.o.*

The other pharmacological activities of veranisinatin A (1) and anisatin (5) were tested at a dose that causing convulsions. In veranisinatin A (1), the analgesic effect on acetic acid-induced writhing was observed dose-dependently at doses of 0.1 and 0.5 mg/kg (*p.o.*) without hypothermia, and increase in the pressure pain threshold at doses of 0.1 and 0.3 mg/kg, as shown in Figs. 6 and 7, respectively. On the other hand, anisatin exhibited analgesia at doses lower than 0.1 mg/kg. Figure 8 presents the sedative effects of veranisinatin A and anisatin. Both compounds decreased the locomotor activity enhanced by methamphetamine dose-dependently when administered at 0.1 and 0.3 mg/kg, and 0.03 and 0.1 mg/kg, respectively. From these data, the pharmacological potent of veranisinatins seemed to be slightly weaker than anisatin. The toxicity of the plants seemed to depend on the content of these components. The yield of anisatin (0.021% from dried fruits) in *I. anisatum* was much higher than those of veranisinatins A—C (0.00016, 0.00010, 0.000015%, respectively, from dried fruits) in *I. verum*. The difference in the content of these components distinguishes between *I. verum* and *I. anisatum* as to whether they are medicinal or toxic. The pharmacological effects of veranisinatins, such as analgesia and sedation, may be related to clinical usage of star anise.

It was shown earlier that anisatin acts as a picrotoxin-like, non-competitive γ -aminobutylic acid (GABA) antagonist.⁷⁾ The mechanisms of convulsive effects of anisatin and veranisinatins are probably similar, but their analgesic and sedative effects remain subjects for which the mechanisms are yet to be revealed.

Experimental

The melting points (uncorrected) were determined on a Yanagimoto micro melting point apparatus. The optical rotations were measured with a JASCO J-20 polarimeter. CD was measured with a JASCO J-500 and a JASCO J-720WI spectrometer. IR spectra were recorded on a Hitachi 260-10 spectrometer and a JASCO FT/IR-230 spectrometer, while UV spectra on a Hitachi U-3200 spectrometer. The MS spectra were taken with a JEOL JMS-HX 110A spectrometer. ^1H - and ^{13}C -NMR including 2D-NMR were recorded on JEOL JNM GSX-400, GSX-500 and GSX-A500, and FG-HMBC on GSX-A500 with a FG (pulse field gradient) control unit spectrometer (with tetramethyl silane and a solvent as an internal standard). Columns of Kusano ODS-CIG-22 and Senshu Aquasil SN-342N and SS-852N were used for MPLC and HPLC, respectively.

Materials Star anise (*Illicium verum* Hook. fil.) was purchased from a commercial outlet of traditional medicines in Japan, and was kindly confirmed by Mr. S. Terabayashi, Tsumura Co., Ltd. Shikimi (*Illicium anisatum* L.) was collected in Chiba University Medicinal Plant Gardens in Chiba and Tateyama, Chiba Prefecture, Japan, in October, 1990.

Isolation of Veranisinatins A, B and C (1, 2, 3) Star anise (20 kg, dried fruit) was crushed by grinder, defatted with *n*-hexane (18 l $\times$ 3), and then extracted with ethyl acetate (18 l $\times$ 3) at room temperature to obtain the ethyl acetate extract (115.8 g), which caused severe convulsion and lethality in mice (4/4) at dose of 500 mg/kg (*p.o.*). The extract (100.0 g) was chromatographed by silica gel eluted with *n*-hexane-ethyl acetate (10:1—1:1) to get the fractions, 1-A (9.11 g), 1-B (20.7 g), 1-C (3.19 g) and 1-D (2.56 g) and to remove a large amount of the polar residue. The active fraction 1-B (the eluate of 5:1) was separated by Sephadex LH-20 column chromatography using methanol to give the fractions 2-A (823 mg), 2-B (16.2 g), 2-C (1.53 g) and 2-D (902 mg). The active 2-B and 2-C were combined and then separated by silica gel flash chromatography

using chloroform-methanol (1:0—5:1) as an eluent. Active fractions, 3-A (991 mg) and 3-B (12.4 g), were obtained together with 3-C (1.44 g) and 3-D (1.12 g). ODS flash chromatography of 3-A using water-methanol (2:1—0:1) yielded fractions 4-A (45 mg), 4-B (41 mg), 4-C (122 mg), 4-D (98 mg) and 4-E (142 mg). Although 4-B, 4-C and 4-D showed the activity, the most active fraction, 4-C was separated by repeated HPLC (Aquasil) eluted with chloroform-methanol-water (100:5:0.1), which was followed by crystallization to give 1 and 2 (33 and 20 mg, respectively). The other active fraction 3-B was separated repeatedly by Sephadex LH-20 column chromatography with methanol to remove 7-A (1.92 g) which gave 4 (1.44 g) by crystallization. Repeated HPLC of 7-C (113 mg) eluted with chloroform-methanol-water (100:5:0.1) yielded 3 (2 mg).

Veranisinatin A (1): Colorless prisms from ethyl acetate, mp 181—182 $^{\circ}\text{C}$, $[\alpha]_{\text{D}}^{25} -15^{\circ}$ ($c=1.0$, methanol). HR-FAB-MS m/z : 365.1209 $[(\text{M}+\text{Na})^+]$; err. -0.3 mmu for $\text{C}_{16}\text{H}_{22}\text{NaO}_8$. IR: $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3380 (br), 1830, 1750. CD ($c=0.20$ mg/ml, methanol) $[\theta]^{17}$ (nm): -1950 (236) (negative maximum).

Deuterium-Induced Shift Technique: ^{13}C -NMR (acetone- d_6 with additional one drop of CD_3OD and CD_3OH) δ : 13.75 (C-15), 27.35 (C-8), 30.71 (C-2), 32.33, 32.40 (C-3), 39.04 (C-1), 51.52 (C-9), 60.08 (C-16), 63.83 (C-5), 64.70 (C-14), 70.44, 70.56 (C-10), 75.90 (C-12), 78.31, 78.39 (C-6), 79.02 (C-7), 85.50, 85.63 (C-4), 168.64 (C-13), 174.43 (C-11).

Veranisinatin B (2): Colorless prisms from ethyl acetate, mp 212—213 $^{\circ}\text{C}$, $[\alpha]_{\text{D}}^{25} -15^{\circ}$ ($c=1.0$, methanol). HR-FAB-MS m/z : 379.1003 $[(\text{M}+\text{Na})^+]$ err. -0.2 mmu $\text{C}_{16}\text{H}_{20}\text{NaO}_9$. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3480 (br), 1825, 1760, 1740. CD (0.2 mg/ml, methanol) $[\theta]^{17}$ (nm): -2370 (237) (negative maximum).

Veranisinatin C (3): Colorless needles from ethanol, mp 228—229.5 $^{\circ}\text{C}$. HR-FAB-MS m/z : 373.1140 $[(\text{M}+\text{H})^+]$; err. -0.5 mmu for $\text{C}_{16}\text{H}_{21}\text{O}_{10}$. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3450 (br), 1825 (br), 1760, 1743. CD (0.50 mg/ml, methanol) $[\theta]^{23}$ (nm): -3730 (235) (negative maximum).

Separation of 1-(4'-Methoxyphenyl)-1,2-propanediol (4) Compound 4 was further separated into two mixtures of diastereomers, 4A and 4B, by ODS-MPLC with methanol-water (2:3) as an eluent.

1-(4'-Methoxyphenyl)-(1R,2S and 1S,2R)-propanediol (4A): Colorless plates from benzene, mp 118—119 $^{\circ}\text{C}$, $[\alpha]_{\text{D}}^{19} 0^{\circ}$ ($c=0.89$, MeOH). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3250 (br), 1610, 1250. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 224 (11800), 274 (1550), 280 (1330). ^1H -NMR (acetone- d_6) δ : 1.03 (3H, d, 6.3; H-3), 3.49 (1H, brs; 2-OH), 3.77 (3H, s; OCH₃), 3.84 (1H, dd, 6.3, 5.0; H-2), 4.09 (1H, brs; 1-OH), 4.50—4.51 (1H, m; H-1), 6.86—6.88 (2H, m; H-3', H-5'), 7.26—7.29 (2H, m; H-2', H-6'); the signals at δ 3.49 and δ 4.09 were eliminated by D_2O addition. ^{13}C -NMR (acetone- d_6) δ : 18.08 (C-3), 55.43 (OCH₃), 72.01 (C-2), 77.88 (C-1), 113.98 (C-3', C-5'), 128.74 (C-2', C-6'), 135.56 (C-1'), 159.72 (C-4').

1-(4'-Methoxyphenyl)-(1R,2R and 1S,2S)-propanediol (4B): Colorless prisms from benzene, mp 67.5—68.0 $^{\circ}\text{C}$, $[\alpha]_{\text{D}}^{19} 0^{\circ}$ ($c=0.73$, MeOH). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3250 (br), 1610, 1245. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 224 (11700), 274 (1570), 280 (1330). ^1H -NMR (acetone- d_6) δ : 0.93 (3H, d, 6.3; H-3), 3.69—3.72 (1H, m; H-2), 3.78 (3H, s; OCH₃), 3.79 (1H, brd, 6.3, 5.0; 2-OH), 4.25—4.27 (2H, m; H-1, 1-OH), 6.86—6.88 (2H, m; H-3', H-5'), 7.26—7.29 (2H, m; H-2', H-6'), δ 3.79, δ 4.25—4.27 disappeared with D_2O addition. ^{13}C -NMR (CDCl_3) δ : 19.24 (C-3), 55.45 (OCH₃), 72.69 (C-2), 78.44 (C-1), 114.15 (C-3', C-5'), 129.02 (C-2', C-6'), 135.51 (C-1'), 160.02 (C-4').

Preparation of 4C *trans*-Anethole (561 mg) was isolated from commercial anethole (1.14 g) by silica gel column chromatography eluted by methanol. Osmium tetroxide (230 mg) was added to a solution of *trans*-anethole (103 mg) in pyridine (3.5 ml), and the mixture was stirred for 85 min at room temperature. After stirring for 60 min with a solution of NaHSO_4 (310 mg) in 8.7 ml of pyridine-water (2:3), the mixture was extracted with ethyl acetate. The organic layer was washed with water and saturated NaCl solution, dried with Na_2SO_4 , and concentrated to leave an oily residue. Crystallization from benzene afforded 4C (61 mg) as colorless prisms, mp 66.5—67.0 $^{\circ}\text{C}$. The physicochemical data of 4C was identified as that of 4B.

Pharmacological Assay Male ddY mice were purchased from Nippon SLC Co., Ltd. Mice (5 w, 25—28 g) were used in the experiments after having been kept in an animal room for around one week under standard conditions (temperature of $22 \pm 1^{\circ}\text{C}$, humidity of $60 \pm 10\%$, circadian cycle of 12 h light and 12 h darkness), standard diet and water *ad libitum*. In each experiment, samples were suspended with 5%-arabic gum, and the vehicle without samples was used for control.

Hypothermic Effect: Rectal temperature in mice was measured up to 4 h after oral administration of samples with a thermister (Takara

Instrumentals Co., Ltd., Japan).

Analgesic Effect by the Acetic Acid-Induced Writhing Method: A slight modification of Whittle's method was used.⁸⁾ Samples were orally administered 40 min before intraperitoneal injection of 0.7% acetic acid (0.1 ml/10 g body weight). The number of squirms was counted in each mouse for 15 min from 5 min after the injection. Aminopyrine (50 mg/kg, *p.o.*) was used as a positive control.

Analgesic Effect by the Tail Pressure Method: A gradient pressure was given at the base of the mouse tail using a BASILE Analgesy-Meter (Ugo Basile, Italy).^{8b)} Mice tested twice before the experiment to have a pain threshold of 100–200 g were used. The pain threshold was measured every 30 min after sample-administration. Aminopyrine (50 mg/kg, *p.o.*) was used as a positive control.

Effect on the Locomotor Activities Enhanced by Methamphetamine: The locomotor activities were counted every 10 min using as Ambulometer AMB-10 (O'hara & Co., Ltd., Japan). The samples were orally administered 20 min before subcutaneous injection of methamphetamine hydrochloride (2 mg/kg). Chlorpromazine hydrochloride (10 mg/kg, *p.o.*) was used as a positive control.

Statistics: Statistical significance was evaluated by the Student's *t*-test.

Acknowledgements We thank Professor K. Yamada, Nagoya University, for his kind gift of authentic anisatin and neoanisatin; Dr. F. Ikegami, of this Faculty, for his helpful suggestion on collecting *I. anisatum*; Ms. Y. Watabe and Ms. C. Fukai for their good technical assistance; and the staff of the Analytical Center of Chiba University

for measurement of NMR, MS.

References and Notes

- 1) Lane J. F., Koch W. T., Leeds N. S., Gorin G., *J. Amer. Chem. Soc.*, **74**, 3211–3215 (1952); Takada S., Nakamura S., Yamada K., Hirata Y., *Tetrahedron Lett.*, **1966**, 4739–4744.
- 2) Okuyama E., Nakamura T., Yamazaki M., *Chem. Pharm. Bull.*, **41**, 1670–1671 (1993).
- 3) Gagnaire D., Vincendon M., *J. Chem. Soc., Chem. Commun.*, **1977**, 509–510; Sato H., Otake N., Koyama M., Ogino H., Kodama Y., Nishizawa N., Tsuruoka T., Inoue S., *Tetrahedron Lett.*, **24**, 495–498 (1983).
- 4) Anisatin was used for measurement of spectra and animal experiment after our isolation from the fruits of *I. anisatum* L. and identification with the authentic samples.
- 5) "Dictionary of Organic Compounds," 5th ed., Chapman & Hall, London, 1982, p. 3712; Alexandre St. P., *Helv. Chim. Acta*, **22**, 382–391 (1939).
- 6) The LD₅₀ of **5** and **6** was reported as 1.03 and 1.62 mg/kg, *i.p.*, respectively, in the following paper; Kouno I., Kawano N., Yang C.-S., *J. Chem. Soc., Perkin Trans. I*, **1988**, 1537–1539.
- 7) Matsumoto K., Fukuda H., *Neuroscience Lett.*, **32**, 175–179 (1982); *idem*, *Brain Res.*, **270**, 103–108 (1983); Kudo Y., Oka J., Yamada K., *Neuroscience Lett.*, **25**, 83–88 (1981).
- 8) a) Whittle B. A., *Br. J. Pharmacol. Chemother.*, **22**, 246–253 (1964); b) Okuyama E., Umeyama K., Yamazaki M., Kinoshita Y., Yamamoto Y., *Planta Med.*, **61**, 101–198 (1995).