

## Analgesic Components of Saposhnikovia Root (*Saposhnikovia divaricata*)

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**By activity-oriented separation using the writhing method in mice, the analgesic components of Saposhnikovia root (*Saposhnikovia divaricata* Schischkin; Umbelliferae) were identified to be chromones, coumarins, polyacetylenes and 1-acylglycerols.**

**Two new components, divaricatol and (3′S)-hydroxydeltoidin, were also isolated. The most potent analgesia was observed in chromones such as divaricatol, ledebouriellol and hamaudol, which inhibited writhing inhibition at an oral dose of 1 mg/kg in mice. Acylglycerols also showed inhibition significantly at a dose of 5 mg/kg. In some pharmacological tests using *sec-O*-glucosylhamaudol, the compound showed analgesia by the tail pressure and the Randall & Selitto methods, and its writhing inhibition was not reversed by naloxone.**

**Key words** *Saposhnikovia divaricata*; analgesia; divaricatol; (3′S)-hydroxydeltoidin; chromone; 1-acylglycerol

In view of their scientific understanding and potential usage, we have continuously investigated traditional medicines to clarify the active components and their pharmacological properties.<sup>1–4</sup> Saposhnikovia root (*Saposhnikovia divaricata* SCHISCHKIN, syn. *Ledebouriella seseloides* WOLFF; Umbelliferae) is an important Chinese and Japanese traditional medicine, called Fang-feng and Bofu, respectively.<sup>5,6</sup> The herb is listed as a high-grade drug in the old Chinese Materia Medica, *Shen Nung Pen Tsao Ching*, and is applied for headaches, vertigo, generalized aching and arthralgia due to “wind, cold and dampness” in the traditional medical system.<sup>7</sup> It has been used in many prescriptions, including those for the treatment of analgesic symptoms.

Pharmacological experiments using the extract reported its suppression of adjuvant arthritis, inhibitory effects on the CNS and peptic ulcers, and febrifugal analgesic, anti-convulsant and anti-inflammatory activities, etc.<sup>8–11</sup> Chemical study on this herb had been done, and many components such as chromones, coumarins and polyacetylenes were isolated.<sup>12–14</sup> However, it has not been clearly identified which components contribute to the pharmacological efficacy, such as analgesia, of this traditional medicine. In Japan, Glehnia root, named Hama-Bofu (*Glehnia littoralis* FR. SCHMIDT ex MIQUEL; Umbelliferae), is used for the same purpose as Bofu (*Saposhnikovia* root), and in prescriptions the two medicines are often confused. With respect to Glehnia root, we recently identified the active components which are responsible for the analgesia and/or a prolongation effect on pentobarbital-induced hypnosis caused by the inhibition of liver-metabolizing enzymes.<sup>2</sup> Some of the active components in Glehnia root, such as furanocoumarins and polyacetylenes, are the same as the constituents in Bofu. By applying activity-oriented separation to the extract of Saposhnikovia root, the analgesic components of this herbal medicine can be clarified in this paper, and the activity-mechanisms of the components are also discussed.

### Results and Discussion

The methanol extract of Saposhnikovia root showed an analgesic effect at a dose of 2 g/kg in mice by the acetic acid-induced writhing method. The extract also caused a prolongation effect on pentobarbital-induced hypnosis in mice at a

dose of 3 g/kg. The separation of the extract was mainly carried out by following the writhing inhibition in mice with oral administration of samples as an isolation guide (Chart 1). Partition using ethyl acetate, *n*-butanol and water concentrated the activity to the ethyl acetate fraction, which was further separated by Sephadex LH-20 chromatography. The obtained analgesic fraction, fr. 1-C, also showed the prolongation effect on pentobarbital-induced sleeping time in mice. The following silica gel chromatography, however, gave two active fractions, fr. 2-A and fr. 2-B. The latter fraction showed only analgesia at a dose of 150 mg/kg and no prolongation effect at a dose of 200 mg/kg, while the former exhibited both effects at doses of 300 mg/kg and 400 mg/kg, respectively. Fraction 2-B was further separated by repeated chromatography using silica gel, ODS and Sephadex LH-20 guided by the analgesic effect. Compounds **1** and **2** were finally obtained as the major components, as shown in Chart 1-1, together with minor components of compounds **3–10**, and **12** in Chart 1-2. The other active fraction, fr. 2-A, was separated by silica gel chromatography to give two types of active fractions, fr. 2-Ab and fr. 2-Ad. Compared with the results of Glehnia root, these fractions were found to be polyacetylenes- and furanocoumarins-containing fractions, respectively.<sup>2</sup> Fraction 2-Ab showed analgesia at a dose of 150 mg/kg without the sleeping-prolongation effect at 200 mg/kg. From this fraction, compound **11** was obtained as a major component. Fraction 2-Ad, showing both analgesic and sleeping-prolongation effects at doses of 80 and 50 mg/kg, respectively, was analyzed by ODS-HPLC (acetonitrile-water) with photodiode array detection (Fig. 1). The following components in fr. 2-Ad were detected by comparison with each retention time and the UV spectrum of the authentic samples: psoralen, xanthotoxin, bergapten, imperatorin, deltoin and isoimperatorin.

The structures of the isolated compounds were estimated by spectra, including 2D-NMR, and compounds **1**, **2**, **6** and **7** were identified by comparison with the reported data to be *sec-O*-glucosylhamaudol, cimifugin, ledebouriellol and hamaudol, respectively.<sup>12,13,15</sup> Compound **3** was identified with isofraxidin, which has been isolated from *Fraxinus japonica*, and compound **5** with fraxidin reported to be produced by hydrolysis of fraxin methyl ether.<sup>16</sup> Compounds **4**

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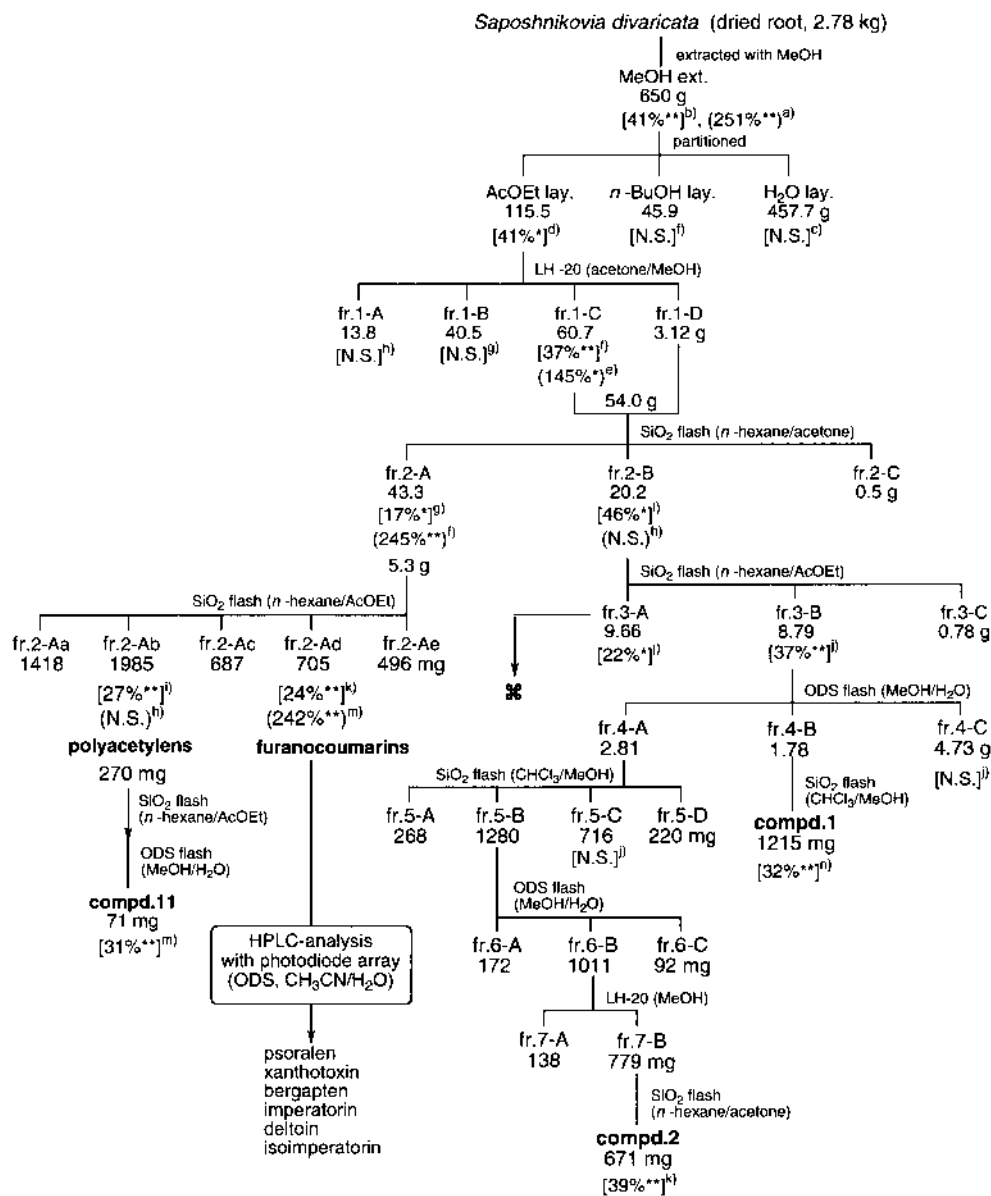


Chart 1-1. Activity-Oriented Separation Procedure of *Saposhnikovia* Root [Writhing Inhibition: % vs. Control], (Sleeping Time: % vs. Control)

a) 3 g/kg, b) 2 g/kg, c) 1.5 g/kg, d) 600 mg/kg, e) 500 mg/kg, f) 400 mg/kg, g) 300 mg/kg, h) 200 mg/kg, i) 150 mg/kg, j) 100 mg/kg, k) 80 mg/kg, l) 60 mg/kg, m) 50 mg/kg. \*:  $p < 0.05$ , \*\*:  $p < 0.01$ .

and **11** were directly identified with the authentic scopoletin and panaxynol, respectively.

The NMR of **9** and **10** suggested the compounds to be glycerol monoacylates. The Mass spectra gave the molecular ions of  $m/z$  354 and 356, and characteristic fragment ions of  $m/z$  262 and 264, respectively. The <sup>1</sup>H- and <sup>13</sup>C-NMR of compound **9** were very similar to those of glycerol monolinoleate.<sup>17</sup> The stereochemistry of the (2*R*)-glycerol moiety was considered by comparison of its optical activity,  $[\alpha]_{589} -3^\circ$ , to the reported data of *R*(-)-glycerol monolinoleate,  $[\alpha]_{\text{D}} -5.2^\circ$ .<sup>18</sup> Compound **10** was estimated to be glycerol monooleate by comparing the <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of oleic acid together with those of **9**. Compounds **3**, **5**, **9** and **10** were isolated from this plant for the first time.

Compounds **8** and **12** were new compounds, and the structures were estimated as follows. Compound **8** is a white crystalline powder, mp 168–171 °C,  $[\alpha]_{589} -30^\circ$ . The molecular formula, C<sub>17</sub>H<sub>18</sub>O<sub>7</sub> (MW 334), was obtained by the HR-FAB-

MS. The <sup>1</sup>H- and <sup>13</sup>C-NMR of **8** were similar to those of **6** (ledebouriellol), except there was no observation of the signals of an angeloyl group. The signals at  $\delta$  2.01 (3H, s) in the <sup>1</sup>H-NMR and  $\delta$  21.0 and 170.3 in the <sup>13</sup>C-NMR were assigned to an acetyl group in **8**, instead of an angeloyl group. The compound, therefore, was elucidated as a 3'-*O*-acetyl derivative and named divaricatol. The stereochemistry at C-3' position was determined to be *S*-configuration because of very similar ORD and CD to those of 3'-*O*-acetylhamaudol and **6**, respectively.<sup>12,15</sup>

Compound **12** is a white powder,  $[\alpha]_{589} -45^\circ$ , the HR-FAB-MS spectrum of which gave the molecular formula, C<sub>19</sub>H<sub>20</sub>O<sub>6</sub>. The <sup>1</sup>H- and <sup>13</sup>C-NMR suggested the compound was a coumarin derivative having a hydroxy and an angeloyl group in the C-ring. The positions at C'-3 and C'-2, respectively, were determined by the PFG-HMBC cross peaks observed between the methyl groups at  $\delta$  1.66 and 1.67 and  $\delta$  98.2 (C-2'), and between the hydroxyl group at  $\delta$  5.05 and  $\delta$

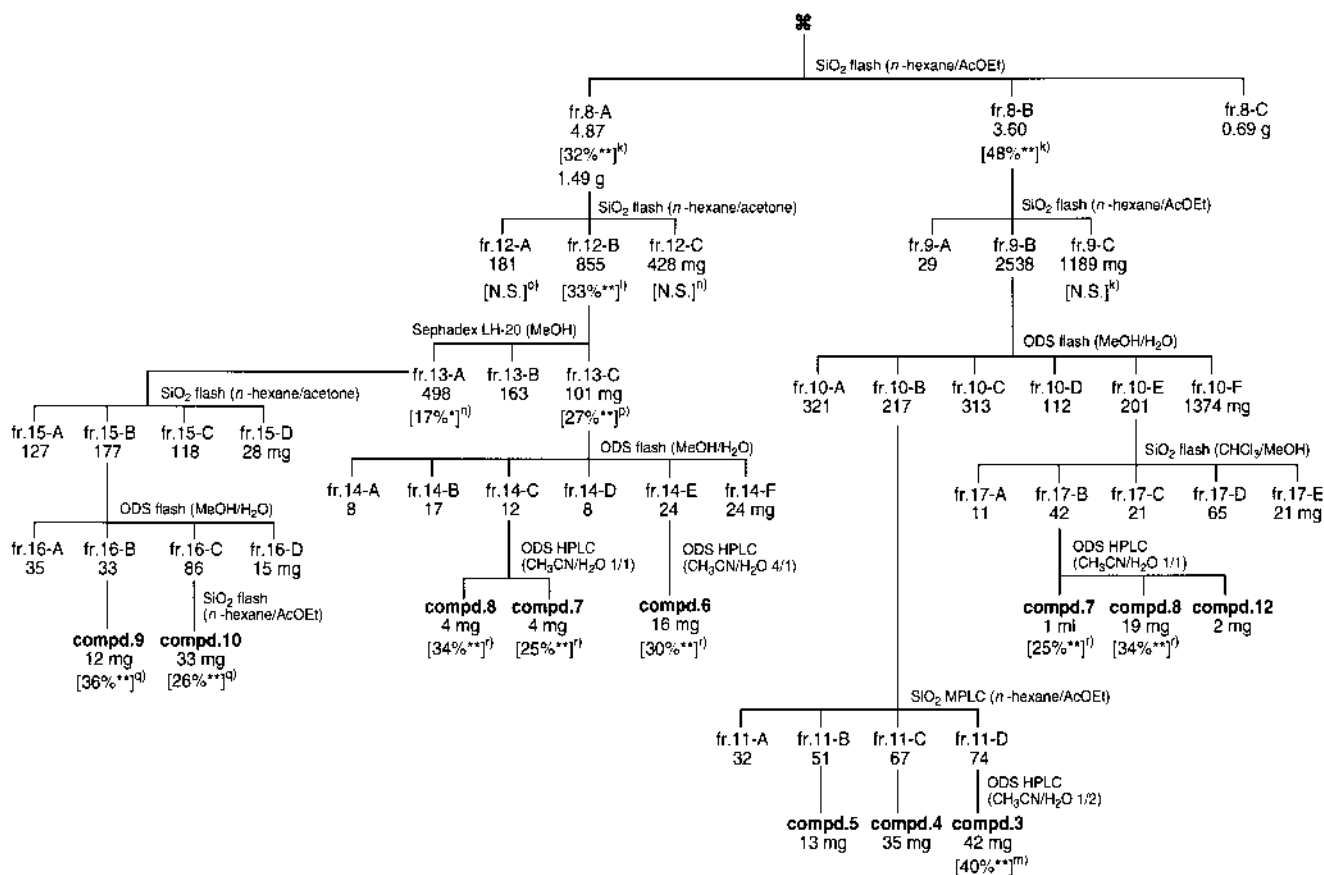


Chart 1-2. Activity-Oriented Separation Procedure of Saposhnikovia Root [Writhing Inhibition: % vs. Control]

k) 80 mg/kg, l) 60 mg/kg, m) 50 mg/kg, n) 40 mg/kg, o) 30 mg/kg, p) 20 mg/kg, q) 5 mg/kg, r) 1 mg/kg. \*:  $p < 0.05$ , \*\*:  $p < 0.01$ .

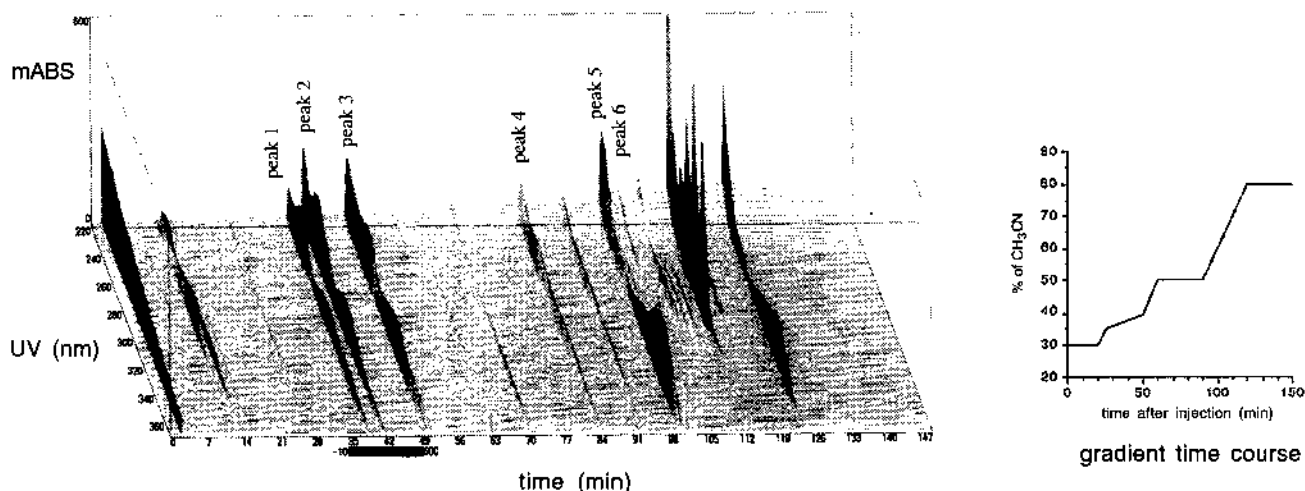


Fig. 1. HPLC Profile of Fr. 2-Ad by Photodiode Array Detection

Retention time, min: peak 1 (psoralen), 38.8; peak 2 (xanthotoxin), 41.8; peak 3 (bergapten), 50.3; peak 4 (imperatorin), 84.6; peak 5 (deltoin), 100.6; peak 6 (isoimperatorin), 101.8.

72.4 (C-3') and  $\delta$  98.2 (C-2'). The 2D-NMRs did not conclude a five- or six-membered ring for the C-ring, since no typical correlation such as a cross peak between  $\delta$  4.73 and  $\delta$  164.4 (2'-H/C-7 in the case of the former) or  $\delta$  167.2 (3'-H/carbonyl C in the latter case) was clearly observed in HMBC. The chemical shifts of the methyl groups, however, were similar to those of deltoin rather than the six-membered derivative, L1-2 from *Libanotis laticalycina*, which resulted

in the structure shown in Chart 2.<sup>19)</sup> *Trans* substitution at the 2' and 3'-position in the C-ring of **12** was estimated by the NOEs between  $\delta$  5.51 (3'-H) and  $\delta$  1.66 and 1.67 (4'-dimethyl) in the <sup>1</sup>H-NMR. The absolute stereochemistry was obtained by direct comparison of the CD with deltoin, which showed the similarity in both compounds (see experimental part). Therefore, compound **12** was determined as (3'*S*)-hydroxydeltoin.

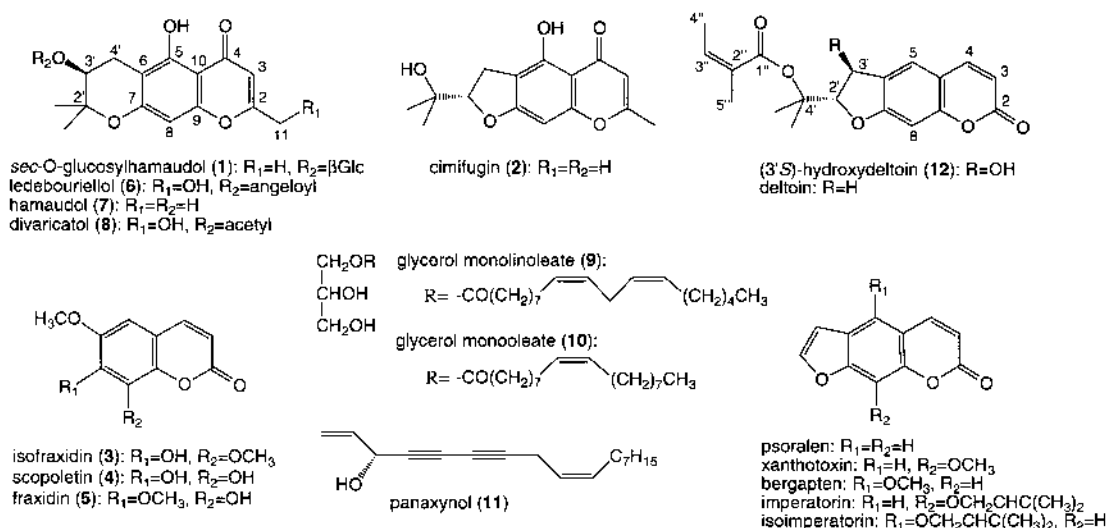


Chart 2. Structures of the Components of Saposhnikovia Root

Figure 2 summarizes the analgesic effects of the components by the acetic acid-induced writhing method in mice. The chromones such as **1** (*sec-O*-glucosylhamaudol) and **2** (cimifugin) indicated analgesia significantly by oral administration of 40 and 80 mg/kg, respectively. Compound **7**, the aglycone part of **1**, increased the potency to exhibit a significant effect at doses of 1, 5 and 10 mg/kg, although it did not show clear dose dependency. Both **6** and **8**, having a dihydropyran-type C-ring, showed a potent analgesic effect at doses of 1 and 5 mg/kg, in spite of the modification with acyl-substituents at the C-3' position and a hydroxy group at C-11. Although the other compounds having a dihydrofuran-type C-ring, except for cimifugin and deltoin, were not isolated by this activity-oriented isolation, some of them have been reported as major components in Saposhnikovia root.<sup>12,13</sup> Therefore, the compounds, such as *prim-O*-glucosylcimifugin, 4'-*O*-glucosyl-5-*O*-methylvissaminol and 5-*O*-methylvissaminol, which were kindly offered by Dr. T. Deyama, were tested at a dose of 10 mg/kg. None of these showed significant effects at this dose. For the potency of analgesic effect of chromones, therefore, dihydropyran-type C-ring without glycosylation may have an important role.

The writhing inhibition of coumarins, such as scopoletin (37% inhibition,  $p < 0.01$ , 50 mg/kg), has been reported in Glehnia root.<sup>2</sup> Compound **3** (isofraxidin) also showed the activity to a similar extent (40% inhibition,  $p < 0.01$ , 50 mg/kg).

It has been reported in a patent that a mixture of 1-acylglycerols from *Aconitum chinense* inhibited carrageenan-induced edema by peritoneal injection of 100 mg/kg.<sup>20</sup> In our writhing test, both 1-acylglycerols, **9** and **10**, inhibited the writhing at a lower dose, 5 mg/kg orally.

Our previous paper on Glehnia root has already summarized the writhing inhibitions at doses of 50–100 mg/kg of **11** (panaxynol) and furanocoumarins such as psoralen, xanthotoxin, bergapten, imperatorin and isoimperatorin, which were also detected in Saposhnikovia root by HPLC with a photodiode array detector. Prolongation effects on pentobarbital-induced hypnosis in mice were observed in the case of furanocoumarins, but not in panaxynol. The effect seems to be a characteristic property of furanocoumarins that causes the inhibition of metabolizing enzymes in mouse liver rather

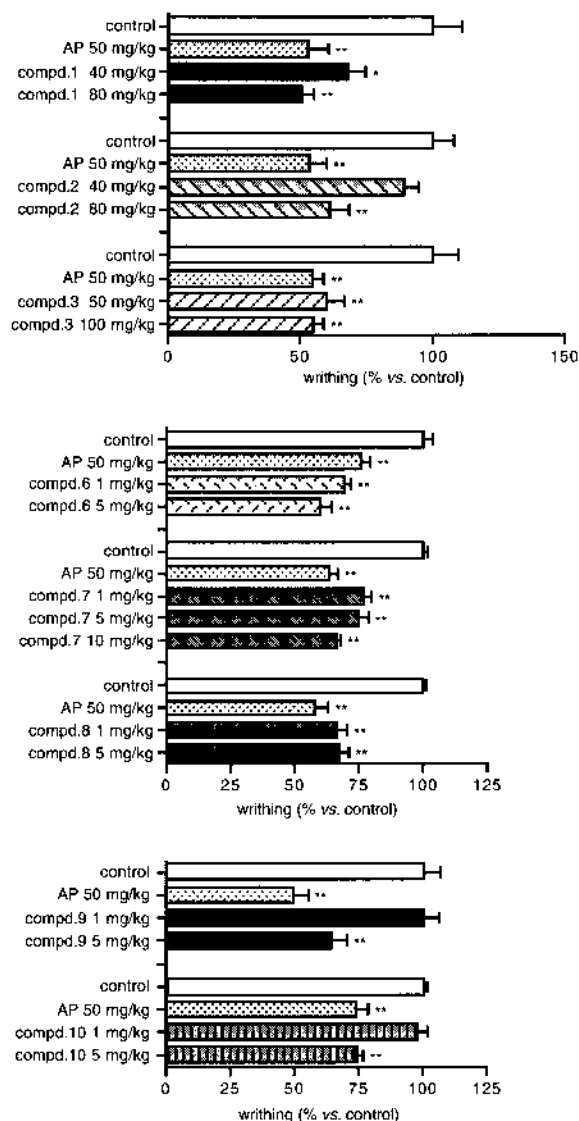


Fig. 2. Analgesic Effects of the Components from Saposhnikovia Root on Acetic Acid-Induced Writhing in Mice

AP: aminopyrine. \*:  $p < 0.05$ , \*\*:  $p < 0.01$ ,  $n = 5-6$ . Each bar represents the mean  $\pm$  S.E.M. The number of squirms in each control was taken as 100% (control squirms from up to down,  $29.5 \pm 3.2$ ,  $24.8 \pm 2.0$ ,  $22.9 \pm 2.2$ ,  $27.6 \pm 1.0$ ,  $27.4 \pm 0.6$ ,  $28.0 \pm 0.4$ ,  $26.8 \pm 1.9$  and  $29.1 \pm 0.4$ , respectively).

than CNS depression.<sup>2,25a)</sup> We also tested a chromone, **1**, on aminopyrine *N*-demethylase activity in liver microsomes of mice, but did not observe the inhibition until 0.5 mM. These activity differences of the components reflected the observed activities in the isolation process, in which some fractions showed both activities of analgesia and sleeping time-prolongation and some others had only the analgesic effect.

In our preliminary experiment, *Saposhnikovia* root increased the pain threshold in the tail pressure method by oral administration of the extract of 3 g/kg. The result suggested that the analgesia of this herbal drug include the CNS effect. Compound **1**, a major component, was applied to additional pharmacological tests. The analgesic effect of **1** by the tail pressure method is shown in Fig. 3. The compound increased the pain threshold significantly at an oral dose of 80 mg/kg in mice. The modified Randall & Selitto method in Fig. 4 also described that **1** raised the pain threshold of both the inflamed and non-inflamed hind paws in mice when it was administered at a dose of 80 mg/kg.<sup>21)</sup> Aminopyrine 50 mg/kg was used as a positive control in both methods. Compound **1**, however, did not show a significant hypothermic effect on lipopolysaccharide-induced pyrexia at doses of 80 and 160 mg/kg, which is not shown here. These experiments suggested that the compound affects the central nervous system.

Figure 5 shows the naloxone counteraction of **1** together with the effect of morphine as a positive control. The writhing inhibition of **1** at a dose of 80 mg/kg in mice was reversed by subcutaneous (*s.c.*) injection of naloxone (1 mg/

kg). From this fact, it seems that the analgesic pathway of the compound is related to an opioid receptor, although the activity was much less than that of morphine.

In conclusion, the analgesic property of *Saposhnikovia* root is a mixture of several types of compounds, such as chromones, coumarins, furanocoumarins, polyacetylenes, and monoacylglycerides, although the contents and potencies of such are variable. The analgesia may involve both anti-inflammatory and CNS effects. The inhibitory effect of furanocoumarins on liver metabolizing enzymes also contributes to prolongation of the activity. The content level of each component, therefore, may alter the analgesia of this herb qualitatively and quantitatively, especially in decoctions together with other herbs.

#### Experimental

Melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. UV spectra were recorded on a Hitachi U-3400 spectrometer, and ORD on a JASCO J-20 polarimeter, CD on a JASCO J-720WI spectropolarimeter. EI-MS and HR-FAB-MS were taken with JEOL JMS-AM20 and HX-110A spectrometers, respectively. <sup>1</sup>H- and <sup>13</sup>C-NMR were measured with a JEOL JNM GSX400A and 500A spectrometers with tetramethylsilane or a deuterated solvent as an internal standard. Column chromatographies were performed on Sephadex LH-20, Wakogel C-200, Nacalai Silica Gel 60 and Chromatorex ODS (100–200 mesh). Pre-packed columns for HPLC, Senshu Pak. ODS-5251-S and Pegasil ODS, were also used for purification.

**Material** *Saposhnikovia* radix (the chopped and dried root of *Saposhnikovia divaricata* SCHISCHKIN; specimen No. LNP19804-02) was purchased

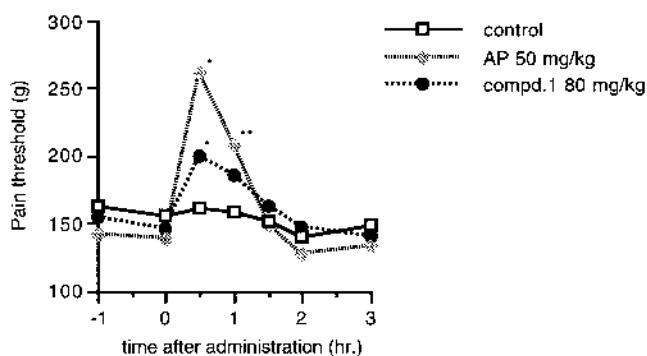


Fig. 3. Analgesic Effect of Compound **1** by the Tail Pressure Method in Mice

AP: aminopyrine. \*:  $p < 0.05$ , \*\*:  $p < 0.01$ ,  $n = 6$ .

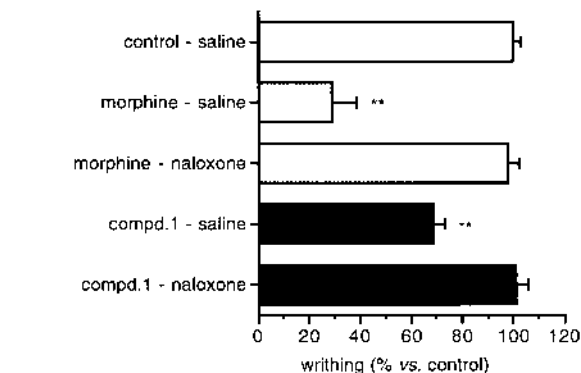
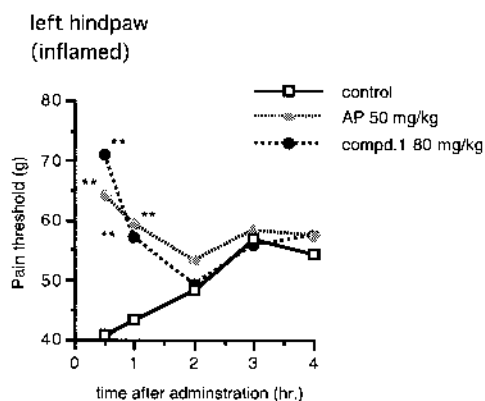


Fig. 5. Effect of Compound **1** with Naloxone on the Writhing Inhibition in Mice

AP: aminopyrine. \*\*:  $p < 0.01$ ,  $n = 6$ . Each bar represents the mean  $\pm$  S.E.M. The number of control squirms (control-saline,  $31.6 \pm 0.9$ ) was taken as 100%, and each significance was shown versus control-saline.

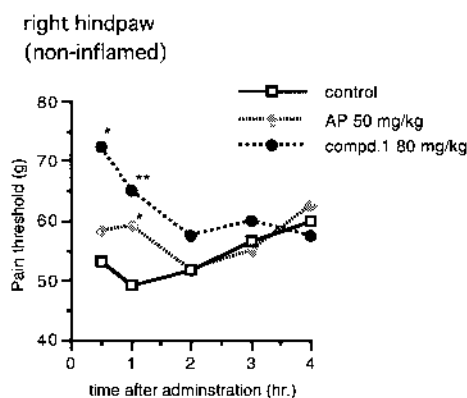


Fig. 4. Analgesic Effect of Compound **1** by the Randall & Selitto Method in Mice

AP: aminopyrine. \*:  $p < 0.05$ , \*\*:  $p < 0.01$ ,  $n = 6$ .

in April, 1995, from Uchida Wakanyaku, a commercial outlet of traditional medicines in Japan, which carried out their own identification of the plant (lot. No. US042323).

**Isolation** Saposhnikovia root 2.78 kg was extracted four times with methanol at room temperature. After evaporation of the solvent, 650 g of the methanol extract was obtained. Oral administration of the extract induced analgesia by both the acetic acid-induced writhing and the tail pressure methods in mice at doses of 2 and 3 g/kg, respectively. The extract at a dose of 3 g/kg in mice also prolonged the sleeping time in mice, which was induced by pentobarbital sodium. The isolation was carried out by following the activity-guide of writhing inhibition together with sleeping time-prolongation in mice.

The extract was partitioned with ethyl acetate, *n*-butanol and water, and the activity was only found in the ethyl acetate fraction. Separation of the active fraction (115.5 g) by Sephadex LH-20 with a solvent of acetone-methanol 1:1 concentrated the analgesic effect into fr. 1-C (60.7 g). The sleeping prolongation was also observed in the fraction at a dose of 500 mg/kg. As the TLC spots in fr. 1-D were overlapped with those of fr. 1-C, both fractions were combined to be separated by silica gel chromatography. Two active fractions by the writhing method, fr. 2-A (43.3 g) and fr. 2-B (20.2 g), were obtained by eluting with *n*-hexane/acetone 5:1 and 3:1—acetone, respectively. The sleeping prolongation, however, was only indicated in the former fraction and not in the latter.

Fraction 2-B, having analgesia at a dose of 150 mg/kg, was separated again by silica gel chromatography with *n*-hexane/ethyl acetate, and one of two active fractions, fr. 3-B (8.79 g), was applied on ODS flash column chromatography. As fr. 4-C (4.73 g) eluted with acetone showed no effect, fr. 4-A (2.81 g) and fr. 4-B (1.78 g) of the methanol/water 2:3 and 3:2 eluate, respectively, were further separated. Fraction 4-B was purified by silica gel with CHCl<sub>3</sub>/methanol 10:1 to give a major compound, **1** (1215 mg). Fraction 4-A was separated by silica gel using CHCl<sub>3</sub>/methanol eluent, which removed non-active fr. 5-C (716 mg) with 5:1 as an eluent. Compound **2** (671 mg) was purified from fr. 5-B (1280 mg) of the eluate 20:1 by repeated chromatography on ODS (methanol/water 1:2—2:3), Sephadex LH-20 (methanol) and silica gel (*n*-hexane/water 1:1—2:3).

Another active fraction, 3-A (9.66 g), was separated by silica gel with the eluent of *n*-hexane/ethyl acetate 2:1—ethyl acetate and gave active fractions, fr. 8-A (4.87 g) and fr. 8-B (3.60 g). After removal of the non-active part from the latter (the acetone and methanol eluate on silica gel), fr. 9-B (2538 mg) which showed fluorescent spots on TLC under UV<sub>360</sub> was applied on ODS column. From fr. 10-B (217 mg) eluted with methanol/water 2:3, **3—5** were obtained in yields of 42, 35 and 13 mg, respectively. Fraction 10-E (201 mg) of the 2:1 eluate gave **7** (1 mg), **8** (19 mg) and **12** (2 mg) by silica gel with CHCl<sub>3</sub>/methanol 100:1 and then ODS-HPLC with acetonitrile/water 1:1.

Silica gel separation of a part of fr. 8-A, 1489 mg, concentrated the activity to fr. 12-B (855 mg) by eluting with *n*-hexane/acetone 5:1. Fraction 12-B was chromatographed on Sephadex LH-20 with methanol to obtain active fr. 13-A (498 mg) and fr. 13-C (101 mg), which showed analgesia at doses of 40 and 20 mg/kg, respectively. Fraction 13-C yielded **6** (16 mg) together with **7** (4 mg) and **8** (4 mg) by ODS flash chromatography using methanol/water 4:1 and HPLC with acetonitrile/water 4:1 for **6** and 1:1 for **7** and **8**. Compounds **9** and **10** were obtained from fr. 13-A in yields of 12 and 33 mg, respectively, by separation of silica gel with *n*-hexane/acetone 3:1, ODS with methanol/water 4:1—9:1 and then silica gel with *n*-hexane/ethyl acetate 1:1.

Fraction 2-A indicated polyacetylenes and furanocoumarins on TLC, which were separated as fr. 2-Ab and fr. 2-Ad by silica gel chromatography with *n*-hexane/ethyl acetate 30:1—10:1 and 5:1—3:1, respectively. Fraction 2-Ab showed analgesia at a dose of 150 mg/kg, but no sleeping prolongation effect, while fr. 2-Ad exhibited both activities. A major component in fr. 2-Ab, **11**, was isolated by silica gel chromatography with *n*-hexane/ethyl acetate 80:1—50:1 and ODS with methanol/water 3:1. Fraction 2-Ad was analyzed by HPLC with a photodiode array detector by comparing the retention time and UV spectrum of each peak with the authentic samples. Shimadzu LC Workstation CLASS-LC10 with SPD-M10AVP Diode Array Detector; column, Deverosil ODS-UG-5 (φ10×250 mm) with the eluent of acetonitrile—water in CTO-10A Column Oven at 40 °C; flow rate, 2 ml/min; samples, ca. 10 μl injection of a 10 mg/ml sample solution or 0.1 mg/ml authentic samples.

Compound **1** (*sec*-*O*-glucosylhamaudol), yellow needles, mp 236—237 °C (lit.<sup>12</sup>) mp 229—230 °C.  $[\alpha]_D^{26}$  -48° (*c*=0.27, methanol) (lit.<sup>12</sup>)  $[\alpha]_D$  -48.5° in methanol. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ: 1.27 (3H, s, 2'-CH<sub>3</sub>), 1.32 (3H, s, 2'-CH<sub>3</sub>), 2.34 (3H, s, 2'-CH<sub>3</sub>), 2.59 (1H, dd, *J*=17.1, 6.6 Hz, 4'-Ha),

2.87 (1H, dd, *J*=17.1, 5.4 Hz, 4'-Hb), 2.92 (1H, ddd, *J*=8.3, 7.5, 5.3 Hz, 2''-H), 3.02 (1H, td, *J*=9.2, 5.1 Hz, 4''-H), 3.13 (1H, ddd, *J*=9.3, 6.1, 1.9 Hz, 5''-H), 3.15 (1H, td, *J*=8.8, 4.9 Hz, 3''-H), 3.41 (1H, ddd, *J*=11.7, 5.9, 5.6 Hz, 6''-Ha), 3.67 (1H, ddd, *J*=11.7, 6.3, 1.9 Hz, 6''-Hb), 3.96 (1H, dd, *J*=6.6, 5.4 Hz, 3'-H), 4.32 (1H, d, *J*=7.5 Hz, 1''-H), 4.37 (1H, dd, *J*=6.3, 5.6 Hz, 6''-OH), 4.84 (1H, d, *J*=5.3 Hz, 2''-H), 4.86 (1H, d, *J*=5.1 Hz, 4''-OH), 4.87 (1H, d, *J*=4.9 Hz, 3''-OH), 6.17 (1H, s, 3-H), 6.36 (1H, s, 8-H), 13.17 (1H, s, 5-OH). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>) δ: 20.0 (C-11), 21.5 (C-4'), 21.7 (2'-CH<sub>3</sub>), 25.3 (2'-CH<sub>3</sub>), 61.4 (C-6''), 70.3 (C-4''), 72.7 (C-3'), 73.4 (C-2''), 76.90 (C-3'' or 5''), 76.96 (C-5'' or 3''), 94.3 (C-8), 100.6 (C-1''), 103.4 (C-6 or 10), 103.5 (C-10 or 6), 107.7 (C-3), 155.5 (C-9), 158.7 (C-5 or 7), 158.8 (C-7 or 5), 167.9 (C-2), 181.9 (C-4). The UV, IR and <sup>1</sup>H-NMR were identical with the data published, although the sugar moiety was not assigned in the <sup>1</sup>H-NMR.<sup>12,15</sup>

Compound **2** (cimifugin), colorless needles, mp 112—113 °C (lit.<sup>22</sup>) mp 107—109 °C,  $[\alpha]_D^{23}$  +87° (*c*=0.61, ethanol) (lit.<sup>15</sup>)  $[\alpha]_{589}$  +88.5° in ethanol. <sup>1</sup>H-NMR (acetone-*d*<sub>6</sub>) δ: 1.24 (3H, s, 4'-CH<sub>3</sub>), 1.28 (3H, s, 4'-CH<sub>3</sub>), 3.27 (1H, dd, *J*=16.1 Hz, 9.3, 3'-Ha), 3.32 (1H, dd, *J*=16.1, 7.8 Hz, 3'-Hb), 3.77 (1H, s, 5-OCH<sub>3</sub>), 3.88 (3H, s, 5-OCH<sub>3</sub>), 4.45 (2H, br d, *J*=4.8 Hz, 11-H<sub>2</sub>), 4.74 (1H, br, 11-OH), 4.78 (1H, dd, *J*=9.3, 7.8 Hz, 2''-H), 6.12 (1H, t-like, *J*=1.0 Hz, 3-H), 6.50 (1H, s, 8-H). The MS, UV, IR and <sup>13</sup>C-NMR were identical with the published data.<sup>12</sup>

Compound **3** (isofraxidin), white powder, mp 150—152 °C (lit.<sup>23</sup>) mp 148.5—150 °C. <sup>1</sup>H-NMR (acetone-*d*<sub>6</sub>) δ: 3.89 (3H, s, 6-OCH<sub>3</sub>), 3.95 (3H, s, 8-OCH<sub>3</sub>), 6.20 (1H, d, *J*=9.5 Hz, 3-H), 6.99 (1H, s, 5-H), 7.85 (1H, d, *J*=9.5 Hz, 4-H), 8.60 (1H, br s, 7-OH). <sup>13</sup>C-NMR (acetone-*d*<sub>6</sub>) δ: 56.8 (6-OCH<sub>3</sub>), 61.3 (8-OCH<sub>3</sub>), 105.1 (C-5), 111.7 (C-10), 113.5 (C-3), 135.8 (C-8), 144.4 (C-9), 144.8 (C-7), 145.1 (C-4), 146.4 (C-6), 160.8 (C-2). The UV and IR were identical with the data published by Takemoto *et al.*<sup>23</sup>

Compound **4** was directly identified with the authentic scopoletin.<sup>2</sup>

Compound **5** (fraxidin), white powder, mp 199—201 °C (lit.<sup>16</sup>) mp 198—200 °C. <sup>1</sup>H-NMR (acetone-*d*<sub>6</sub>) δ: 3.89 (6H, s, 6,7-OCH<sub>3</sub>), 6.29 (1H, d, *J*=9.5 Hz, 3-H), 6.80 (1H, s, 5-H), 7.85 (1H, d, *J*=9.5 Hz, 4-H), 8.58 (1H, br s, 8-OH). The UV, IR and <sup>13</sup>C-NMR (acetone-*d*<sub>6</sub>) were identical with the data published.<sup>16</sup>

Compound **6** (ledebouriellol), white needles, mp 95—99 °C (lit.<sup>12</sup>) mp 97—99 °C, HR-FAB-MS (NBA/PEG 200+400): 375.1440 (M+1)<sup>+</sup>, (err. -0.4 mmu for C<sub>20</sub>H<sub>23</sub>O<sub>7</sub>). The <sup>1</sup>H- and <sup>13</sup>C-NMR and CD were identical with the published data.<sup>12</sup>

Compound **7** (hamaudol), white crystalline powder, mp 202—203 °C (lit.<sup>12</sup>) mp 202—202.5 °C,  $[\alpha]_{589}^{24}$  -32° (*c*=0.13, ethanol) (lit.<sup>15</sup>)  $[\alpha]_{589}$  -19.6° in ethanol. CD (*c*=0.031 mg/ml, methanol)  $\Delta\epsilon^{22}$  (nm): -0.15 (332, trough), -0.02 (306, max.), -0.30 (282, trough), 0 (270), +1.74 (257, max), 0 (241), -1.13 (230, trough). The <sup>1</sup>H-NMR and UV were identical with the published data.<sup>12,24</sup>

Hydrolysis of compound **1** (*sec*-*O*-glucosylhamaudol) As the CD spectrum of **7** was not identical with the published one,<sup>12</sup> the enzymatic hydrolysis of **1** was carried out. To a solution of **1** (50 mg) in DMSO (3 ml) and water (9 ml) was added β-glucosidase (30 mg). After stirring at 37 °C for 4 h, the solution was extracted with ethyl acetate. The extract was chromatographed by silica gel with CHCl<sub>3</sub> as an eluent to give the aglycone (19 mg), which was identical with **7**.

Compound **8** (divaricatol), white crystalline powder, mp 168—171 °C. HR-FAB-MS (NBA/PEG 200+400): 335.1116 (M+1)<sup>+</sup>, (err. -1.4 mmu for C<sub>17</sub>H<sub>19</sub>O<sub>7</sub>). EI-MS *m/z* (%): 334 (M<sup>+</sup>, 2), 274 (14), 259 (100), 221 (9). <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 1.34 (3H, s, 2'-CH<sub>3</sub>), 1.37 (3H, s, 2'-CH<sub>3</sub>), 2.01 (3H, s, CH<sub>3</sub>COO), 2.37 (1H, br s, 11-OH), 2.78 (1H, dd, *J*=17.7, 4.8 Hz, 4'-Ha), 2.99 (1H, dd, *J*=17.7, 5.3 Hz, 4'-Hb), 4.55 (2H, br s, 11-H<sub>2</sub>), 5.11 (1H, t-like, *J*=5.0 Hz, 3'-H), 6.31 (1H, br s, 3-H), 6.34 (1H, s, 8-H), 12.90 (1H, s, 5-OH). <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ: 21.0 (CH<sub>3</sub>COO), 22.6 (C-4'), 23.3 (2'-CH<sub>3</sub>), 24.7 (2'-CH<sub>3</sub>), 61.4 (C-11), 69.7 (C-3'), 76.7 (C-2'), 94.9 (C-8), 102.7 (C-6), 104.9 (C-10), 106.4 (C-3), 155.9 (C-9), 159.0 (C-5 or 7), 159.6 (C-7 or 5), 167.9 (C-2), 170.3 (CH<sub>3</sub>COO), 182.5 (C-4). UV  $\lambda_{max}$  (ethanol) nm (log  $\epsilon$ ): 210 (4.39), 230 (4.19), 251 (4.21), 257 (4.20), 295 (3.92), 318 (sh., 3.65). ORD (*c*=0.13, ethanol)  $[\alpha]_{24}$  (nm): -30° (589), -35° (550), -40° (500), -45° (450), -54° (400). CD (*c*=0.041 mg/ml, methanol)  $\Delta\epsilon^{15}$  (nm): +1.02 (294, max.), +0.66 (276, trough), +3.65 (258, max.), 0 (236), -3.49 (229, trough).

Compound **9** (glycerol monolinoleate), colorless oil. EI-MS *m/z* (%): 354 (M+, 8), 336 (3), 280 (7), 262 (100), 234 (9); % shown in the case that *m/z* 262 was taken as a base peak. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 0.89 (3H, t-like, *J*=6.9 Hz, 18'-H<sub>3</sub>), ca. 1.25 (m, methylene), 1.61—1.65 (2H, m, 3'-H<sub>2</sub>), 2.05 (4H, m, 8', 14'-H<sub>2</sub>), 2.35 (2H, t-like, *J*=7.5 Hz, 2'-H<sub>2</sub>), 2.77 (2H, t-like, *J*=6.4 Hz, 11'-H<sub>2</sub>), 3.57—3.63 (1H, m, 3-Ha), 3.68—3.71 (1H, m, 3-Hb),

3.91—3.94 (1H, m, 2-H), 4.15 (1H, dd,  $J=11.7$ , 6.1 Hz, 1-Ha), 4.21 (1H, dd, 11.7, 4.6, 1-Hb), 5.32—5.38 (4H, m, 9', 10', 12', 13'-H).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : 14.1 (C-18'), 22.6 (C-17'), 24.9 (C-3'), 25.6 (C-11'), 27.17 (C-8' or 14'), 27.19 (C-14' or 8'), 29.07 ( $\times 2$ ), 29.13, 29.3, 29.6, 31.5 (C-16'), 34.1 (C-2'), 63.3 (C-3), 65.2 (C-1), 70.3 (C-2), 127.9 (C-10' or 12'), 128.1 (C-12' or 10'), 130.0 (C-9' or 13'), 130.2 (C-13' or 9'), 174.3 (C-1'). ORD ( $c=0.36$ , methanol)  $[\alpha]_D^{22}$  (nm):  $+3^\circ$  (589),  $ca.$  0 (535),  $-5^\circ$  (400).

Compound **10** (glycerol monooleate), white waxy solid. EI-MS  $m/z$  (%): 356 ( $\text{M}^+$ , 3), 325 (5), 299 (11), 264 (64), 239 (24), 221 (6), 98 (100).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : 0.87 (3H, t-like,  $J=7.0$  Hz, 18'-H<sub>3</sub>),  $ca.$  1.25 (m, methylene), 1.58—1.66 (2H, m, 3'-H<sub>2</sub>), 1.98—2.03 (4H, m, 8' and 11'-H<sub>2</sub>), 2.34 (2H, t-like,  $J=7.5$  Hz, 2'-H<sub>2</sub>), 3.59 (1H, dd,  $J=11.5$ , 5.9 Hz, 3-Ha), 3.69 (1H, dd,  $J=11.5$ , 3.9 Hz, 3-Hb), 3.90—3.95 (1H, m, 2-H), 4.14 (1H, dd,  $J=11.7$ , 6.1 Hz, 1-Ha), 4.19 (1H, dd,  $J=11.7$ , 4.8 Hz, 1-Hb), 5.30—5.38 (2H, m, 9' and 10'-H<sub>2</sub>).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : 14.1 (C-18'), 22.7 (C-17'), 24.9 (C-3'), 27.1 (C-8' or 11'), 27.2 (C-11' or 8'), 29.07, 29.12, 29.30 ( $\times 2$ ), 29.50, 29.66 ( $\times 2$ ), 29.74, 31.9 (C-16'), 34.1 (C-2'), 63.3 (C-3), 65.1 (C-1), 70.3 (C-2), 129.7 (C-9' or 10'), 130.0 (C-10' or 9'), 174.3 (C-1'). ORD ( $c=0.42$ , methanol)  $[\alpha]_D^{26}$  (nm):  $ca.$  0 (589—400).

Compound **11** (panaxynol), colorless oil. The  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR and the ORD were identical with the authentic sample.<sup>2)</sup>

Compound **12** ((3'S)-hydroxydeltolin), white amorphous solid. HR-FAB-MS (NBA/PEG 200+400): 345.1338 ( $\text{M}+1$ )<sup>+</sup>, (err. 0.0 mmu for  $\text{C}_{19}\text{H}_{21}\text{O}_6$ ). EI-MS  $m/z$  (%): 344 ( $\text{M}^+$ , 1), 244 (6), 229 (100), 189 (6), 83 (41).  $^1\text{H-NMR}$  (acetone- $d_6$ )  $\delta$ : 1.54 (3H, quintet,  $J=1.5$  Hz, 5''-H<sub>3</sub>), 1.66 (3H, s, 4'-CH<sub>3</sub>), 1.67 (3H, s, 4'-CH<sub>3</sub>), 1.82 (3H, dq,  $J=7.3$ , 1.5 Hz, 4''-H<sub>3</sub>), 4.73 (1H, d,  $J=4.0$  Hz, 2'-H), 5.05 (1H, d,  $J=7.0$  Hz, 3'-OH), 5.51 (1H, br dd,  $J=6.1$ , 4.0 Hz, 3'-H), 5.94 (1H, qq,  $J=7.3$ , 1.5 Hz, 3'-H), 6.20 (1H, d,  $J=9.7$  Hz, 3-H), 6.77 (1H, s, 8-H), 7.66 (1H, s, 5-H), 7.92 (1H, d,  $J=9.7$  Hz, 4-H).  $^{13}\text{C-NMR}$  (acetone- $d_6$ )  $\delta$ : 15.6 (C-4''), 20.6 (C-5''), 21.9 (4'-CH<sub>3</sub>), 22.3 (4'-CH<sub>3</sub>), 72.4 (C-3'), 82.3 (C-4'), 98.2 (C-2'), 98.4 (C-8), 113.1 (C-3), 114.0 (C-10), 126.1 (C-5), 128.7 (C-6), 129.8 (C-2''), 137.6 (C-3''), 144.9 (C-4), 157.7 (C-9), 160.9 (C-2), 164.4 (C-7), 167.2 (C-1''). UV  $\lambda_{\text{max}}$  (ethanol) nm (log  $\epsilon$ ): 221 (4.26), 247 (3.55), 259 (3.47), 301 (3.81), 329 (4.12), 340 (4.00). ORD ( $c=0.078$ ,  $\text{CHCl}_3$ )  $[\alpha]_D^{26}$  (nm):  $-45^\circ$  (589),  $-50^\circ$  (550),  $-66^\circ$  (500),  $-103^\circ$  (450). CD ( $c=0.16$  mg/ml, ethanol)  $\Delta\epsilon^{22}$  (nm):  $-3.76$  (328, trough),  $-1.86$  (299, sh.),  $-0.12$  (266, max.),  $-0.52$  (259, trough),  $-0.33$  (254, max.),  $-0.65$  (248, trough), 0 (243). Deltolin, CD ( $c=0.092$  mg/ml, ethanol)  $\Delta\epsilon^{23}$  (nm):  $-3.72$  (332, trough),  $-1.39$  (299, sh.),  $-0.05$  (267, max.),  $-1.94$  (258, trough),  $-1.63$  (254, max.),  $-1.98$  (249, trough).

**Pharmacological Assay** Male ddy strain mice weighing 24—36 g were used. The animals (4 weeks old) bred at Japan SLC, Hamamatsu, Japan, were housed for about a week in a controlled 12-h light—dark environment at 22—25 °C, water and food being provided *ad libitum*. The following materials were used for pharmacological assays: aminopyrine (Wako Pure Chemical Industries), pentobarbital sodium (Tanabe), carrageenin (Nacalai Tesque), naloxone hydrochloride (Sigma), morphine hydrochloride (Takeda), Tween 80 (Wako Pure Chemical Industries), and saline (Otsuka).

Assay for analgesia by the acetic acid-induced writhing and tail pressure methods, the effect on pentobarbital-induced hypnosis, anti-pyretic effect and aminopyrine *N*-demethylase activity was carried out as previously described.<sup>2,25)</sup> Samples were dissolved or suspended in water with 5% Tween 80. Aminopyrine 50 mg/kg was used as a positive control, if without notice.

Analgesic effect on inflamed and non-inflamed hindpaws in mice was measured by the modified method of Randall & Setillo.<sup>21)</sup> Mice having a pain threshold range of 40—75 g in the preliminary experiment, 30 min prior to the test, were used. Sample (0.1 ml/10 g mouse) was administered orally 90 min after the injection of 0.1% carrageenin (0.02 ml) to the left hindpaw in mice. Then, the pain thresholds of both hindpaws in each mouse were measured after 30 min, 1 h, 2 h, 3 h and 4 h.

Naloxone counteraction in mice was tested in the writhing method.<sup>26,27)</sup> Naloxone (1 mg/kg) or saline was injected subcutaneously to mice 15 min before sample administration. The sample administration was followed after 25 min by intraperitoneal injection of 0.7% acetic acid. After 5 min, the number of squirms was counted in each mouse for the next 15 min. Morphine (5 mg/kg) was used as a positive control.

Statistics: After the data were analyzed for outlier by Smirnov-Groubbs,

statistical significance was evaluated by Student's *t*-test or 1-way ANOVA followed by the Dunnett test.

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