Pharmacologically Active Components of Todopon Puok (*Fagraea racemosa*), a Medicinal Plant from Borneo

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The lignans of (+)-pinoresinol, (+)-epipinoresinol, (+)-lariciresinol and (+)-isolariciresinol together with phenols such as syringaldehyde and 7,8-dihydro-7-oxy-coniferyl alcohol were isolated from Todopon Puok (*Fagraea racemosa* JACK *ex* WALL.), a medicinal plant from Borneo, using a bioassay of the relaxation effect on norepinephrine (NE)-induced contraction in rat aortic strips. The plant extract also exhibited analgesic properties in the acetic acid-induced writhing and tail pressure tests in mice, with the activity being concentrated in the lignan fraction. (+)-Pinoresinol showed analgesic effect on writhing symptoms in mice which were dose-dependent, and produced local anesthesia in guinea pigs.

Key words *Fagraea racemosa*; lignan; vasodilation; analgesia; Borneo medicinal plant; (+)-pinoresinol

During our continuing survey of the bioactive components of medicinal plants from Borneo,[1] the extract of Todopon Puok exhibited a relaxation effect on norepinephrine (NE)-induced contractions in rat aortic strips. The root of Todopon Puok has been used as a pain killer by some Kadazans, the major ethnic group of Sabah in Borneo, Malaysia. The botanical name of the plant is *Fagraea racemosa* JACK *ex* WALL. (Loganiaceae), which is also reported to be used for the treatment of fever in Malaysia and India.[2] So far, no chemical and pharmacological studies on this medicinal plant have been carried out. In this paper, we reported the isolation and identification of the active components exhibiting vasodilation and analgesia.

The methanol extract of Todopon Puok caused a relaxing effect on NE-induced contractions in rat aortic strips at 0.2 mg/ml. (Fig. 1) The analgesic effect of the extract was also observed by the acetic acid-induced writhing and tail pressure tests in mice at an oral dose of 3 g/kg as shown in Fig. 2.

The separation of the extract was first carried out by monitoring the relaxation effect as an isolation-guide, as summarized in Chart 1. The extract was partitioned between *n*-butanol and water, and the *n*-butanol layer which showed activity was further separated by column chromatography on Sephadex LH-20 and silica gel. The active fractions, fr. 3-B and -C, yielded compound-1 (292 mg) and -2 (26 mg) and compound-3 (76 mg), respectively.

These three components were found to be related phenols from their spectra. Compound-1 (**1**), mp 119.5—120.5 °C, $[\alpha]_D^{20}$ +72°, had a molecular formula of $C_{20}H_{22}O_6$ by FAB-MS. Its structure was assigned as (+)-pinoresinol by two dimensional (2D)-NMR such as HH-correlation spectroscopy (COSY), CH-COSY and correlation *via* long-range coupling (COLOC) (long-range H/C COSY), and was directly identified with authentic (±)-pinoresinol by TLC (silica gel, *n*-hexane–acetone 1 : 1)

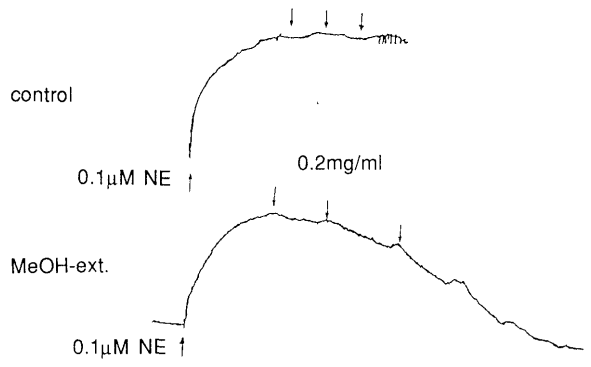

Fig. 1. Relaxation Effect of the Extract of *F. racemosa* (MeOH-ext.) on NE-Induced Contractions in Rat Aortic Strips without Endothelium

The downward arrow indicates 0.2 mg/ml of each application in the case of the MeOH-ext., and vehicle in controls.

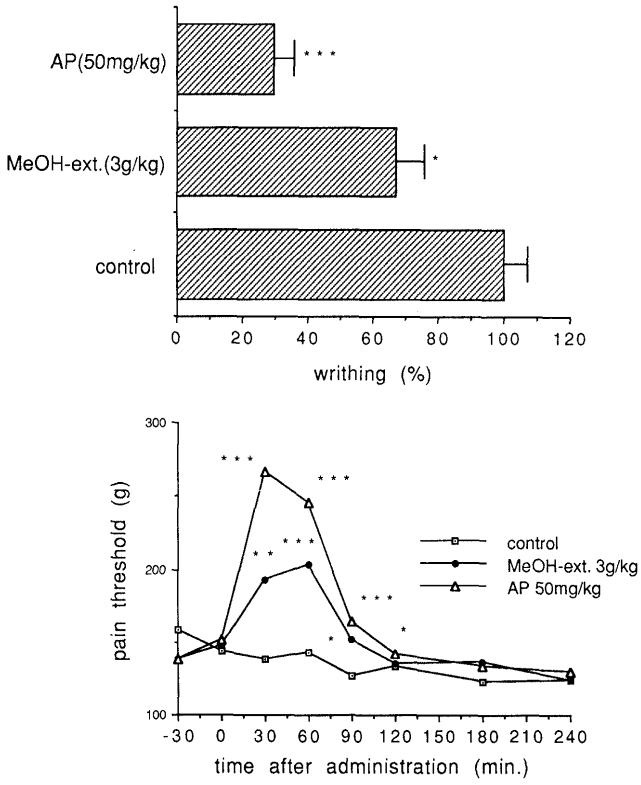

Fig. 2. Analgesic Effect of the Extract of *F. racemosa* (MeOH-ext.) on Acetic Acid-Induced Writhing (Above) and on the Pressure Pain Threshold (Below) in Mice

AP, aminopyrine. $*p<0.05$, $**p<0.01$, $***p<0.001$. $n=6$ (above) and 8 (below).

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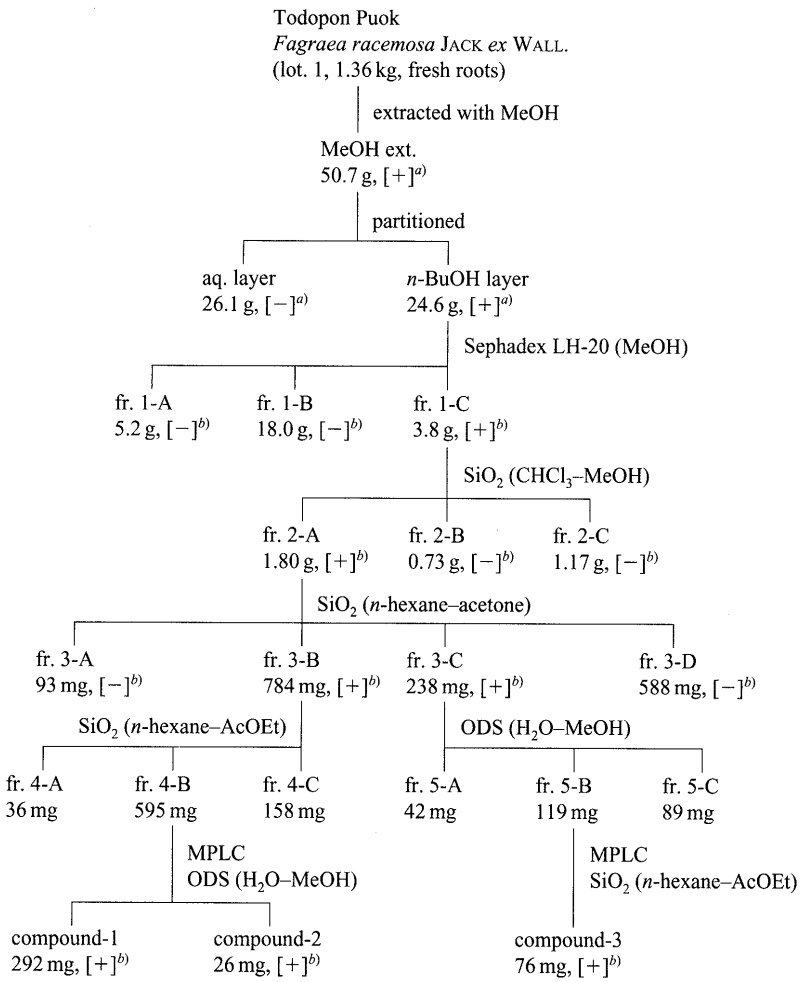

Chart 1. Separation Procedure for Todopon Puok Using a Bioassay-Guide of the Inhibitory Activity on NE-Induced Contractions in Rat Aortic Strips without Endothelium

[+], inhibitory effect on NE-induced contractions in rat aortic strips without endothelium; [−], no effect; *a*) 0.2 mg/ml; *b*) 0.1 mg/ml.

and ^{1}H-NMR. The purity of **1** was checked by HPLC using a chiral column. It presented a single HPLC peak using a chiral column, with a retention time identical with one of the two peaks of (±)-pinoresinol. The structural determination of compound-2 (**2**), mp 139—140 °C, $[\alpha]_D^{20}$ +79° was based on analysis of the spectra including 2D-NMR and nuclear Overhauser effect (NOE) difference spectrum (NOEDS) and a comparison with **1**. It was identified as (+)-epipinoresinol by comparing it directly with authentic (±)-epipinoresinol using ^{1}H-NMR, TLC (silica gel, *n*-hexane–acetone, 1 : 1), and its purity was confirmed by HPLC using a chiral column. Compound-3 (**3**), mp 166.5—168 °C, $[\alpha]_D^{20}$ +32°, seemed to be a tetrahydrofuran-type lignan because ^{1}H-NMR methylene signals of δ 2.55 (1H, dd, J=13.6, 10.9 Hz) and 2.92 (1H, dd, J=13.6, 5.3 Hz) were observed in place of the methine at δ 4.86 (1H, d, J=5.4 Hz) in **2**. The spectra of **3** were identical with published data for (+)-lariciresinol.[3]

The next separation involved a bioassay guide of analgesia carried out on the extract of *F. racemosa*. The analgesic activity was also concentrated in the lignan fraction. From the active fraction, compounds-4 to -6 together with compounds-1, -2 and -3 were isolated by repeated flash chromatography and/or medium pressure liquid chromatography (MPLC) as described in detail in the experimental section. Compound-4 (**4**) was directly identified by comparison with authentic syringaldehyde. Compound-5 (**5**), mp 96.5—97.5 °C, $C_{10}H_{12}O_4$ was a phenolic compound having a conjugated ketone, as suggested by absorption bands of 3520 and 1670 cm^{-1} in the IR and the signals at δ 6.14 (1H, s) and δ 199.14 in the ^{1}H- and ^{13}C-NMR spectra, respectively. The ^{1}H-NMR indicated 1, 2 and 4-substituted aromatic signals at δ 6.96 (1H, d, J=8.0 Hz), 7.55 (1H, d, J=8.0, 1.9 Hz) and 7.54 (1H, d, J=1.9 Hz). The NOEs at the δ 7.54 signal were observed by irradiation of a methoxy methyl signal at δ 3.96 (3H, s) and of a methylene at δ 3.19 (2H, t, J=5.3 Hz) which constituted a hydroxyethyl side-chain together with the methylene at δ 4.02 (2H, dt, J=6.3, 5.3 Hz) and a hydroxy group at δ 2.76 (1H, br t, J=6.3 Hz). From these data, the structure of **5** was proposed as 7,8-dihydro-7-oxy-coniferyl alcohol and this is its first isolation from a plant to our knowledge. Compound-6 (**6**), mp 156.5—157.5 °C, $[\alpha]_D^{20}$ +98°, has similar spectra and the same molecular weight (360) as **3**. The structure of **6**, which was proposed by analyzing COSY spectra, was confirmed by direct comparison with published data for (+)-isolariciresinol (cyclolariciresinol).[4]

Figure 4 presents the relaxation effects of compounds-1, -2 and -3 together with compounds-4 to -6 on NE-induced contractions in rat aortic strips without endothelium. Furanofuran-type lignans, **1** and **2**, exhibited activity,

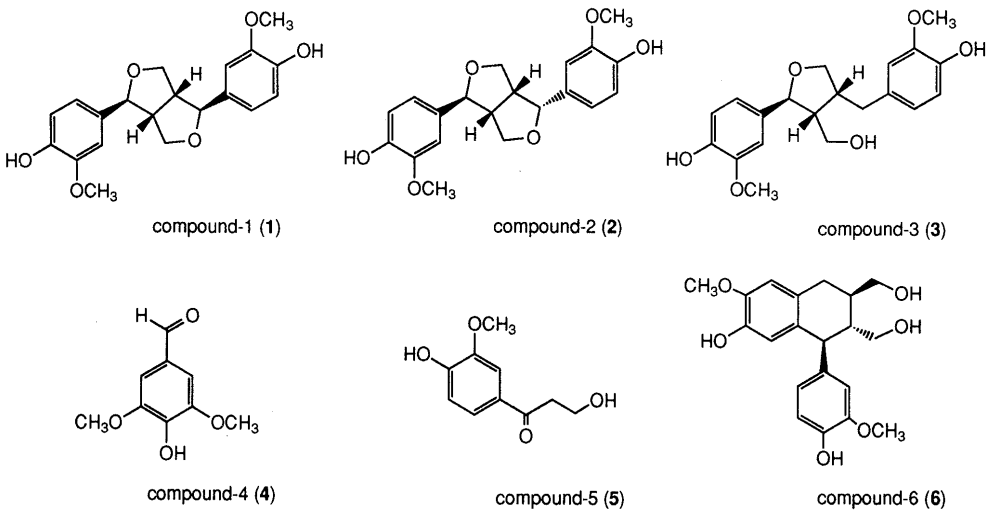

Fig. 3. Structures of Compounds-1—-6

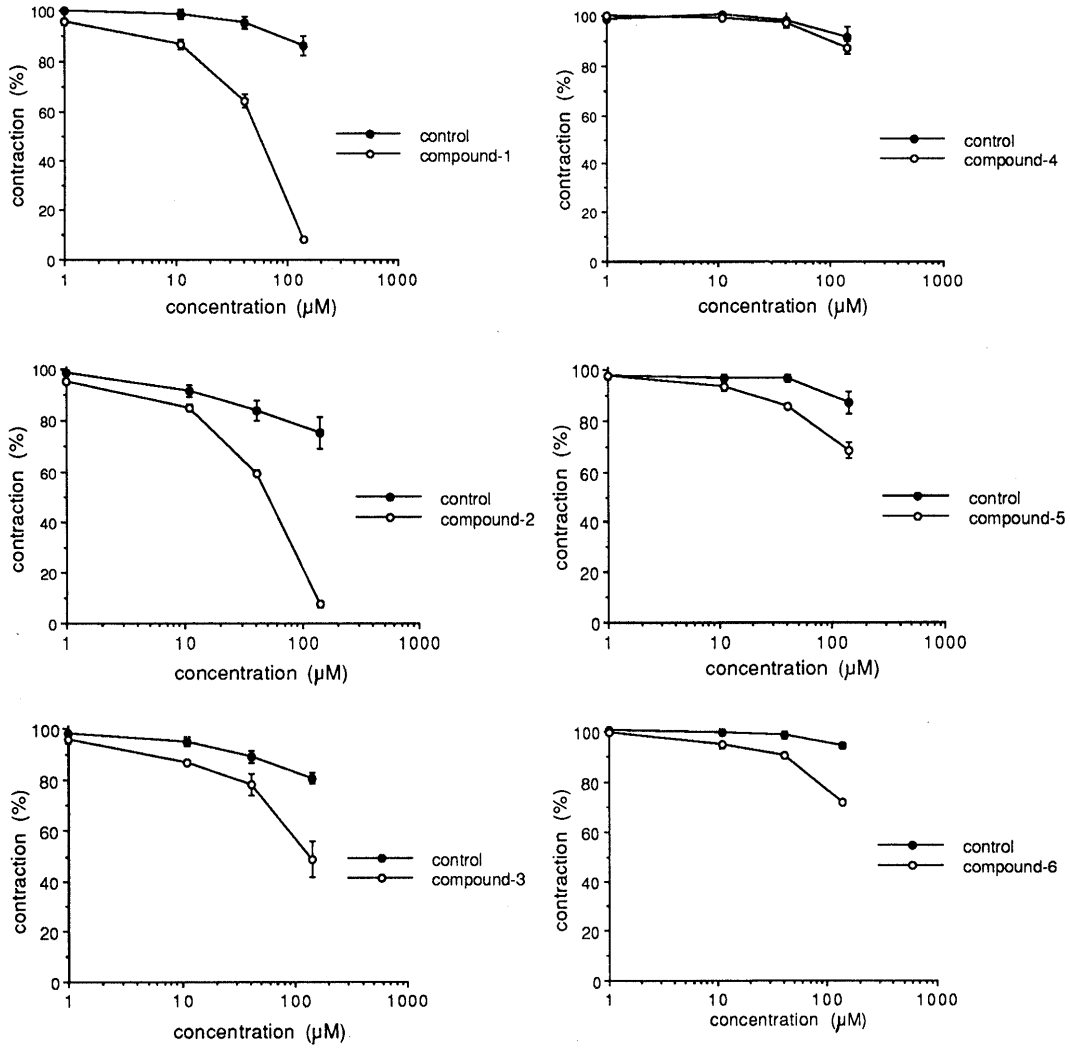

Fig. 4. Relaxation Effects of Compounds-1—-6 on NE-Induced Contractions in Rat Aortic Strips without Endothelium

Data are plotted as means ± S.E.M. for five experiments.

while dihydrofuran- and aryltetrahydronaphthalene-types, such as **3** and **6**, were less potent. The relaxation effect was not observed to any significant extent in the simple phenols, **4** and **5**. Some lignans including pinoresinol are reported to have Ca^{2+} antagonist activity and inhibit cyclic AMP phosphodiesterase.[5] Therefore, the inhibitory effects of **1** and **2** on NE-induced vasoconstriction were probably involved in the related mechanisms for smooth muscle.

Since analgesic activity was concentrated in the lignan fraction, as mentioned in the isolation procedure, compound-1, a major lignan was tested for activity. The

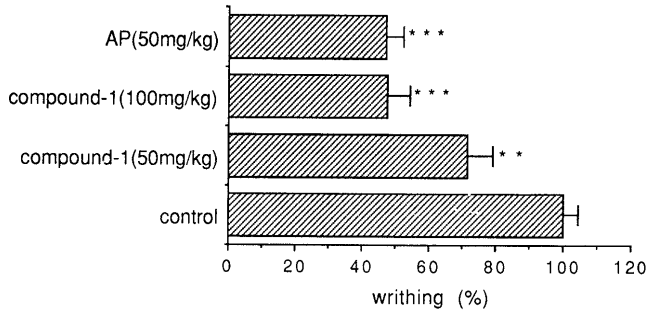

Fig. 5. Analgesic Effect of Compound-1 on Acetic Acid-Induced Writhing in Mice

AP, aminopyrine. $**p<0.01$, $***p<0.001$. Data are expressed as means ± S.E.M. for 6—8 mice.

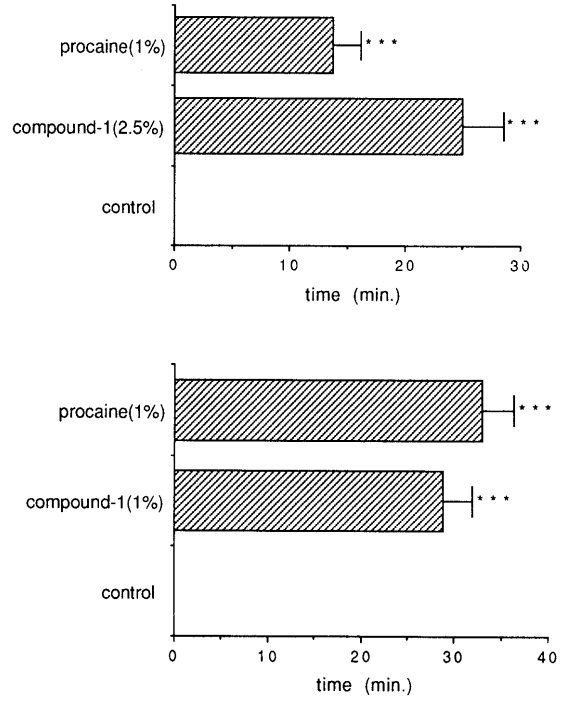

Fig. 6. Local Anesthesia of Compound-1 in the Blink Test (Above) and Twitch Response Test (Below) in Guinea Pigs

$***p<0.001$. Each bar is presented as a mean ± S.E.M. ($n=4$).

compound exhibited a dose-dependent inhibition of the acetic acid-induced writhing in mice at oral doses of 50 (28%, $p<0.01$) and 100 mg/kg (53 %, $p<0.001$), as shown in Fig. 5.

Considering the uses of Todopon Puok, it was also expected to have some local anesthesia. Figure 6 indicates the results of **1** in surface and infiltration anesthesia in guinea pigs using the blink and twitch response tests, respectively. The solutions containing 2.5% and 1% of **1**, respectively, exhibited significant effects in these anesthesia models.

Dihydrofuran- and aryltetrahydronaphthalene-types lignan glycosides from *Stauntonia chinensis* have been reported to show analgesic effects.[6] Furanofuran-type lignans, such as syringaresinol di-*O*-glucoside, were investigated for stress reducing activity.[7] Considering on these data, pinoresinol and related lignans seem to contribute significantly to the pharmacological effects of Todopon Puok.

Experimental

Melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. IR spectra were recorded on a Hitachi 260-10 spectrometer, UV on a Hitachi U-3400 spectrometer, HR-FAB-MS on a JEOL HX-110, and EI-MS on a JEOL AUTO MS-20. Optical rotations were measured with a JASCO J-20 polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL-JNM-GSX 400 and JEOL-JNM-GSX-500 spectrometers with tetramethylsilane or solvent as an internal standard. Column chromatography was performed on Sephadex LH-20, Wakogel C-200, Nakalai Silica gel 60 and Chromatorex ODS (100—200 mesh). Pre-packed columns (Kusano CPO-HS-221-20 and CPS-HS-221-05) were used for MPLC.

Plant Material and Isolation The fresh roots of Todopon Puok were collected in Sabah, Borneo, Malaysia in May, 1991 (lot 1) and May, 1992 (lot 2). The plant was identified as *Fagraea racemosa* JACK *ex* WALL. by Mr. Julius Kulip, Forest Research Center (FRC), Forestry Department, Sabah, Malaysia, and a specimen is kept in the herbarium of FRC.

Fresh roots (1.36 kg, lot 1) were extracted with methanol at room temperature, and the methanol extract (50.7 g) was separated using a bioassay-guide of the relaxation effect on NE-induced contractions in rat aortic strips without endothelium. The extract was partitioned between *n*-butanol and water, and the former produced a relaxation effect. The *n*-butanol fraction (24.6 g) was chromatographed on Sephadex LH-20 with methanol to give the active fraction, fr. 1-C (3.8 g). Fraction 1-C was separated by silica gel flash chromatography, and active fr. 2-A (1.80 g) was obtained from the chloroform–methanol (20 : 1) eluate. Fraction 2-A was flash chromatographed on silica gel, and the eluate of *n*-hexane–acetone (3 : 1 and 1 : 1) gave active fractions, fr. 3-B (784 mg) and -C (238 mg), respectively. Further separation of fr. 3-B by flash chromatography (silica gel, *n*-hexane–ethyl acetate (1 : 1)) and then by MPLC (ODS, methanol–water (1 : 1)) yielded compound-1 (292 mg) and -2 (26 mg) as active components. Fraction 3-C was also separated by flash chromatography (ODS, methanol–water (1 : 1)) and MPLC (silica gel, *n*-hexane–ethyl acetate (1 : 2)) to give compound-3 (76 mg).

Dried roots (1.14 kg, lot 2) were extracted with methanol to give a methanol extract (100 g). The analgesic effect in the writhing test was used as a bioassay-guide for separating the extract. The extract was partitioned between *n*-butanol and water, and the *n*-butanol phase (51.2 g, wet) which exhibited analgesia (24% inhibition, $p<0.05$, 2 g/kg, *p.o.*) was chromatographed on Sephadex LH-20 with methanol. Fraction C, the lignan-containing fraction only inhibited writhing (49%, $p<0.01$, 1 g/kg, *p.o.*). This fraction (1.4 g) was separated by silica gel column chromatography with gradient elution using *n*-hexane–acetone (5 : 1—0 : 1) to give fr. 2-A (94 mg), fr. 2-B (43 mg), -C (286 mg), -D (222 mg) and -E (772 mg). Fraction 2-B to fr. 2-D were further separated independently, since the TLC pattern of fr. 2-A was similar to that of the non-active fraction and fr. 2-E did not show any effect at a dose of 500 mg/kg. Fraction 2-B was purified by MPLC (silica gel, *n*-hexane–ethyl acetate (2 : 1)) to yield compound-4 (13 mg). Fraction 2-C was separated by ODS flash chromatography with methanol–water (1 : 1), and then fr. 3-B (162 mg) and fr. 3-C (91 mg) were subjected to repeated MPLC (silica gel, *n*-hexane–ethyl acetate (1 : 1 or 1:2); ODS, water–methanol (1 : 1)). Compounds-3 (25 mg) and -5 (5 mg) were obtained from the former fraction, and compounds-1 (36 mg) and -2 (6 mg) from the latter. Compound-6 (8 mg) was isolated from fr. 2-D by silica gel and ODS flash chromatography (chloroform–methanol (20 : 1) and methanol–water (2 : 1), respectively) and MPLC (silica gel, chloroform–methanol (20 : 1)).

Compound-1 (**1**): White powder, mp 119.5—120.5 °C (lit.[8] mp 119—120 °C), HR-FAB-MS (NBA+KI) *m/z*: 397.1048 $(M+K)^+$ (err. −0.5 mmu for $C_{20}H_{22}KO_6$), 358.1415 $(M)^+$, (err. −0.1 mmu for $C_{20}H_{22}O_6$). ORD ($c=0.100$ mg/ml, MeOH) $[\alpha]^{20}$ (nm): +72° (589) ((lit.[8] $[\alpha]_D$ +77.5° ($CHCl_3$)), +104° (500), +180° (400), +1100° (300), +1400° (287, peak), +960° (265, trough). The NMR in $CDCl_3$ was identical with published data.[9] HPLC using a chiral column (opti-pak XC 3.9 × 300 mm (Waters), *n*-hexane–ethanol (1 : 1), 0.5 ml/min, UV 280 nm) t_R: compound-1, 30.5 min; (±)-pinoresinol, 16 min and 30.5 min. The diacetate was also identified by comparison with published ^{1}H-NMR data.[9a]

Compound-2 (**2**): White powder, mp 139—140 °C (lit.[10] mp 138—139 °C). EI-MS *m/z* (%): 358 (M^+, 25), 327 (2), 284 (4), 256 (11), 205 (11), 163 (19), 151 (100), 137 (60). ORD ($c=0.100$ mg/ml, MeOH) $[\alpha]^{20}$

(nm): +79° (589) (lit.[10]) $[\alpha]_D$ +110° (MeOH)), +120° (500), +200° (400), +640° (300), +3100° (236, peak), +2400° (233, trough). UV λ_{max}^{EtOH} nm (log ε): 232 (4.11), 282 (3.73), 288 (sh, 3.64). ^{1}H-NMR ($CDCl_3$) δ: 2.88—2.93 (1H, m, H-8), 3.31 (1H, dd, J=8.6, 8.3 Hz, H-9′ax), 3.32—3.37 (1H, m, H-8′), 3.84 (1H, dd, J=8.6, 7.6 Hz, H-9eq), 3.85 (1H, dd, J=9.6, 6.3 Hz, H-9eq), 3.90 (3H, s, H_3-10), 3.92 (3H, s, H_3-10′), 4.12 (1H, dd, J=9.6, 1.0 Hz, H-9ax), 4.43 (1H, d, J=7.0 Hz, H-7), 4.86 (1H, d, J=5.4 Hz, H-7′), 5.60 (1H, s, HO-4 or 4′), 5.62 (1H, s, HO-4′ or 4), 6.79 (1H, ddd, J=8.1, 1.7, 0.7 Hz, H-6′), 6.84 (1H, dd, J=8.1, 1.9 Hz, H-6), 6.89 (2H, d, J=8.1 Hz, H-5, 5′), 6.91 (1H, d, J=1.9 Hz, H-2), 6.95 (1H, d, J=1.7 Hz, H-2′). ^{13}C-NMR ($CDCl_3$) δ: 50.12 (C-8′), 54.46 (C-8), 55.92 (C-10 or 10′), 55.98 (C-10′ or 10), 69.68 (C-9′), 70.97 (C-9), 82.08 (C-7′), 87.72 (C-7), 108.32 (C-2′), 108.50 (C-2), 114.20 (C-5, 5′), 118.38 (C-6′), 119.16 (C-6), 130.31 (C-1′), 133.02 (C-1), 144.57 (C-4′), 145.30 (C-4), 146.40 (C-3′), 146.70 (C-3). HPLC using a chiral column (opti-pak XC 3.9×300 mm (Waters), n-hexane–ethanol (1:1), 0.5 ml/min, UV 280 nm) t_R: FR-B 13.5 min; (±)-epipinoresinol 6.5 and 13.5 min.

Compound-3 (**3**): White powder, mp 166.5—168 °C (lit.[3a] 167—169 °C). EI-MS m/z (%): 360 (M^+, 45). ORD (c=0.100 mg/ml, MeOH) $[\alpha]^{20}$ (nm): +34° (589) (lit.[3a] $[\alpha]_D$ +19.5° (MeOH)), +55° (500), +78° (400), +106° (350). UV λ_{max}^{EtOH} nm (log ε): 231 (4.11), 282 (3.76), 287 (sh, 3.71). ^{1}H-NMR ($CDCl_3$) δ: 1.37 (1H, dd, J=5.1, 4.6 Hz, HO-9), 2.39—2.44 (1H, m, H-8), 2.55 (1H, dd, J=13.6, 10.9 Hz, H-7′a), 2.70—2.77 (1H, m, H-8′), 2.92 (1H, dd, J=13.6, 5.3 Hz, H-7′b), 3.75 (1H, dd, J=8.5, 6.2 Hz, H-9′), 3.78 (1H, ddd, J=10.7, 6.3, 5.1 Hz, H-9a), 3.88 (3H, s, H_3-10′), 3.90 (3H, s, H_3-10), 3.92 (1H, ddd, J=10.7, 6.8, 4.6 Hz, H-9b), 4.06 (1H, dd, J=8.5, 6.6 Hz, H-9′), 4.79 (1H, d, J=6.8 Hz, H-7), 5.49 (1H, s, HO-4′), 5.57 (1H, HO-4), 6.69 (1H, d, J=2.0 Hz, H-2′), 6.70 (1H, dd, J=8.3, 2.0 Hz, H-6′), 6.80 (1H, dd, J=8.3, 2.0 Hz, H-6), 6.84 (1H, d, J=8.3 Hz, H-5′), 6.87 (1H, d, J=2.0 Hz, H-2), 6.88 (1H, d, J=8.3 Hz, H-5). ^{13}C-NMR ($CDCl_3$) δ: 33.26 (C-7′), 42.37 (C-8′), 52.55 (C-8), 55.88 (C-10 or 10′), 55.89 (C-10′ or 10), 60.84 (C-9), 72.85 (C-9′), 82.78 (C-7), 108.29 (C-2), 111.19 (C-2′), 114.16 (C-5), 114.40 (C-5′), 118.71 (C-6), 121.16 (C-6′), 132.25 (C-1′), 134.74 (C-1), 143.95 (C-4′), 144.99 (C-4), 146.51 (C-3′), 146.62 (C-3). The assignment of C-1 and C-1′ in the literature[3b] may be the opposite one.

Compound-4 (**4**): Yellowish white powder. The data were identical with those of available syringaldehyde.

Compound-5 (**5**): White powder, mp 96.5—97.5 °C. HR-FAB-MS (NBA) m/z: 197.0808 $(M+H)^+$ (err. -0.6 mmu for $C_{10}H_{13}O_4$). EI-MS m/z (%): 196 (M^+, 95), 153 (99), 137 (71), 123 (96), 108 (100), 77 (96). IR $\nu_{max}^{CDCl_3}$ cm^{-1}: 3600, 3520, 2960, 1660, 1600, 1455, 1350. UV λ_{max}^{EtOH} nm: 231, 277, 305; $\lambda_{max}^{EtOH+NaOH}$ nm: 250, 347. ^{1}H-NMR ($CDCl_3$) δ: 2.75 (1H, br t, J=6.3 Hz, HO-9), 3.19 (2H, t, J=5.3 Hz, H-8), 3.96 (3H, s, OCH_3), 4.02 (2H, dt, J=6.3, 5.3 Hz, H-9), 6.14 (1H, s, HO-4), 6.96 (1H, d, J=8.0 Hz, H-5), 7.54 (1H, d, J=1.9 Hz, H-2), 7.55 (1H, dd, J=8.0, 1.9 Hz, H-6). ^{13}C-NMR ($CDCl_3$) δ: 39.70 (C-8), 56.08 (OCH_3), 58.18 (C-9), 109.57 (C-2), 113.86 (C-5), 123.67 (C-6), 129.63 (C-1), 146.63 (C-3), 150.70 (C-4), 199.14 (C-7).

Compound-6 (**6**): White powder, mp 156.5—157.5 °C. (lit.[4a] 155—157 °C). ORD (c=0.200 mg/ml, MeOH) $[\alpha]^{20}$ (nm): +98° (589) (lit.[4b] $[\alpha]_D$ +44.3° (MeOH)), +163° (500), +230° (400), +100° (299, trough), +5450° (281, peak). EI-MS m/z (%): 360 (M^+, 100). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3530, 1705, 1590. UV λ_{max}^{EtOH} nm (log ε): 230 (sh, 4.06), 284 (3.76). ^{1}H-NMR (acetone-d_6) δ: 1.81 (1H, tdd, J=10.4, 4.6, 2.8 Hz, H-8′), 1.94—2.02 (1H, m, H-8), 2.73 (1H, dd, J=15.6, 4.9 Hz, H-7a), 2.79 (1H, dd, J=15.6, 10.7 Hz, H-7b), 3.40 (1H, dd, J=10.7, 4.2 Hz, H-9′a), 3.68—3.75 (3H, m, H-9b, H_2-9′), 3.78 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 3.82 (1H, br d, J=10.7 Hz, H-7′), 6.19 (1H, s, H-5), 6.62 (1H, dd, J=7.9, 2.1 Hz, H-6′), 6.66 (1H, s, H-2), 6.75 (1H, d, J=2.1 Hz, H-2′), 6.77 (1H, d, J=7.9 Hz, H-5′), 7.12 (1H, br s, OH), 7.40 (1H, br s, OH). ^{13}C-NMR (acetone-d_6) δ: 33.76 (C-7), 40.43 (C-8), 48.12 (C-7′), 48.33 (C-8′), 56.18 (OCH_3), 56.27 (OCH_3), 62.09 (C-9′), 65.91 (C-9), 111.99 (C-2), 113.57 (C-2′), 115.54 (C-5′), 116.94 (C-5), 122.88 (C-6′), 128.51 (C-1), 134.00 (C-1′), 138.50 (C-6), 145.28* (C-4), 145.82* (C-4′), 146.47* (C-3), 148.25* (C-3′); * the signals are interchangeable.

Pharmacological Assay Male rats (Wistar, 8—10 weeks, 280—350 g) and male guinea pigs (Hartley, 7—8 weeks, 250—330 g) bred at Takasugi Experimental Animal Corporation (Saitama, Japan) and male mice (Std: ddY, 5 weeks, 24—33 g) at Japan SLC, Inc. (Hamamatsu, Japan) were used. The animals were housed for about one week under a 12-h light/dark cycle at 20—25 °C and allowed free access to food and water.

Effect on Contracted Aorta: Isolated thoracic aorta was maintained at 37 °C in Krebs–Henseleit solution (NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 11.1 mM) aerated with 95% O_2 and 5% CO_2-mixture. The aorta was cleaned of fat and connective tissues and cut into helical strips. The luminal surface of the preparation was gently rubbed to remove endothelium. The strips were mounted in 5 ml organ baths and allowed to stabilize for 60 min with a tension of 1 g. The test samples suspended in water with 0—0.2% dimethylsulfoxide were applied after 10^{-7} M NE-induced contractions were produced.

Analgesic Effect in the Acetic Acid-Induced Writhing Test: Samples were orally administered 40 min prior to intraperitoneal injection of 0.7% acetic acid (0.1 ml/10 g mouse). After 5 min, the number of squirms was counted for each mouse for the next 15 min. Aminopyrine (50 mg/kg, *p.o.*) was used as a positive control.

Analgesic Effect in the Tail Pressure Test: The gradient pressure was applied at the base of the mouse tail using BASILE Analgesy-Meter (Ugo Basile, Italy). Prior to the experiment, mice were tested twice, and those having a pain-reaction pressure range of 100—250 g were used. The reaction threshold in each animal was measured and noted at 30, 60, 90, 120 and 180 min after sample administration. Samples were suspended in saline with 5% gum arabic. Aminopyrine (50 mg/kg, *p.o.*) was used as a positive control.

Local Anesthesia (Surface Anesthesia): The blink reflex in guinea pigs was used. The test solution adjusted to pH 8—9 and a positive control (1% procaine) was administered to the conjunctival sac. The cornea was stimulated three times at intervals of 5 min with a porcine hair. The experiment was continued until blinking was observed at all three times, and it was judged to be no activity when the stimulant caused blinking at least twice on the three occasions.

Local Anesthesia (Infiltration Anesthesia): The twitch response in guinea pigs was used. Samples were intracutaneously injected into the skin on the back of guinea pigs after removal of hair. The papula caused by injection was stimulated six times at intervals of 5 min with a needle. The anesthetic activity was judged to be positive if the stimulant did not cause a response in at least four of the six trials. The experiment was continued until all twitch responses were observed.

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

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