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 syringalddehyde and 7,8-dihydro-7-oxo-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 (I), mp 119.5—120.5°C, [α]20D +72°, had a molecular formula of C20H22O6 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](image1)

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

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![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](image2)

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and $^1$H-NMR. The purity of I 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 (±)-epipinoresinol. The structural determination of compound-2 (2), mp 139—140 °C, $[\alpha]_D^{19} + 79^\circ$ was based on analysis of the spectra including 2D-NMR and nuclear Overhauser effect (NOE) difference spectrum (NOEDS) and a comparison with I. 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, $[\alpha]_D^{19} + 32^\circ$, seemed to be a tetrahydrofurane-type lignan because $^1$H-NMR methylene signals at $\delta$ 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 $\delta$ 4.86 (1H, d, $J = 5.4$ Hz) in 2. The spectra of 3 were identical with published data for (±)-lariciresinol.\(^5\)

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 syringicaldehyde. 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 $\delta$ 6.14 (1H, s) and $\delta$ 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 $\delta$ 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 $\delta$ 7.54 signal were observed by irradiation of a methoxy methyl signal at $\delta$ 3.96 (3H, s) of a methylene at $\delta$ 3.19 (2H, t, $J = 5.3$ Hz) which constituted a hydroxymethyl side-chain together with the methylene at $\delta$ 4.02 (2H, dt, $J = 6.3, 5.3$ Hz) and a hydroxy group at $\delta$ 3.76 (1H, brt, $J = 6.3$ Hz). From these data, the structure of 5 was proposed as 7,8-dihydro-7-oxo-coniferol alcohol and this is its first isolation from a plant to our knowledge. Compound-6 (6), mp 156.5—157.5 °C, $[\alpha]_D^{19} + 98^\circ$, 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 (cyclocariciresinol).\(^4\)

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. Furanoifuran-type lignans, 1 and 2, exhibited activity,
while dihydrofuran- and aryltetrahydro-naphthalene-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. 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
Experimental

Melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. IR spectra were recorded on a Hitachi 260-10 spectrophotometer, UV on a Hitachi U-3400 spectrophotometer, 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. 1H- and 13C-NMR spectra were recorded on JEOL-JNM-GSX 400 and JEOL-JMN-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 (388 mg). Fraction 1-C was separated by silica gel flash chromatography, and active fr. 2-A (180 mg) 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 (3:1:1) 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)). Compound-1 (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.113 mp 119—120°C), HR-FAB-MS (NBA + KI) *m/z* 397 1048 [M+K]+ (err. -0.5 mmu for C29H32K2O8), 381 1415 [M]+, (err. -0.1 mmu for C29H32O8). ORD (c=0.100 mg/ml, MeOH) [α]20 (nm): +72° (589) ([lit.8] [α]D 77.5° (CHCl3)), +104° (500), +180° (400), +1100° (300), +1400° (287, peak), +960° (265, trough). The NMR in CDCl3 was identical with published data.39 HPLC using a chiral column (opti-pak X, 9 × 330 mm (Waters), n-hexane-ethyl alcohol (1:1), 9.5 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 [H-NMR data].39

compound-2 (2): White powder, mp 139—140°C (lit.98 mp 138—139°C). EI-MS *m/z* (%): 358 (M+), 25, 327 (24, 284 (4), 256 (11), 205 (11), 163 (19), 151 (100), 137 (60). ORD (c=0.100 mg/ml, MeOH) [α]20
(nm): +79° (589) (lit.100 $\text{[X]}_{p}+110^\circ$ (MeOH)), +120° (500), +200°
(400), +640° (300), +640° (300), +640° (236), +240° (233, trough). UV 
$\lambda_{\text{max}}$ (nm) (log ε): 232 (4.11), 282 (3.73), 288 (3.64). \(\text{H}-\text{NMR (CDCl}_3\): D: \(\delta \)), 2.88 – 2.93 (m, 1H), 3.11 (H-NMR (CDCl3): D: \(\delta \)), 50.12 (8.1, 3.3), 3.32 – 3.37 (1H, m, H-8), 3.84 (1H, dd, J = 8.6, 7.6 Hz, H-9q), 3.85 (1H, dd, J = 9.6, 6.3 Hz, H-9q), 3.90 (3H, s, H-10), 3.93 (2H, s, H-10), 4.12 (1H, dd, J = 9.6, 1.0 Hz, H-9a), 4.43 (1H, dd, J = 7.0 Hz, H-7), 4.86 (1H, d, J = 5.4 Hz, H-7), 5.60 (1H, s, H-4 or 4'), 5.62 (1H, s, H-4 or 4'), 5.79 (1H, d, J = 8.1, 1.7 Hz, H-6), 6.34 (1H, d, J = 8.1, 1.7 Hz, H-6), 6.39 (1H, d, J = 8.1, 5.2 Hz, H-6), 7.05 (1H, d, J = 8.1, 4.0 Hz, H-6). 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 blind reflex in guinea pigs was used. The test solution administered to the skin was instilled in saline. 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 stimulus caused blinking at least twice on the three occasions.

Local Anesthesia (Infiltration Anesthesia): The trystch reflex in guinea pigs was used. Samples were instilled into the skin on the back of guinea pigs after removal of hair. The papilla 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 stimulus did not cause a reaction in at least four of the six trials. The experiment was continued until all trystch responses were observed.

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

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