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Piscicidal Constituents of *Stellera chamaejasme* L. II[1)]

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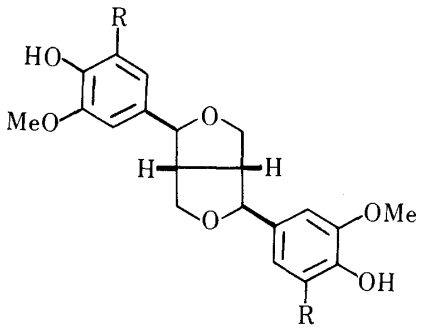
Three lignans have been isolated from the roots of *Stellera chamaejasme* L. (Thymelaeaceae). These compounds were identified as lirioresinol-B, pinoresinol and matairesinol. The former two show piscicidal activity.

Keywords——*Stellera chamaejasme*; Thymelaeaceae; piscicidal activity; lignan; lirioresinol-B; pinoresinol; matairesinol

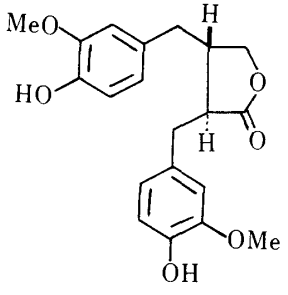
In the course of a search for piscicidal constituents in the plants of Thymelaeaceae, we reported the isolation of four daphnane-type diterpenes showing strong activity from the roots of *Stellera chamaejasme* L., known as "lang du" ("rou doku" in Japanese) in Chinese medicine.[1,2)] We next attempted to isolate other piscicidal substances from the same sourse. In the present paper, we wish to report the isolation and identification of three lignans (lirioresinol-B,[3)] pinoresinol[4)] and matairesinol,[5)] of which the former two show piscicidal activity.

Results and Discussion

The dried roots of *Stellera chamaejasme* L. collected in Sichnan Province (Shisen shō in Japanese) of China were extracted with methanol and the extract was fractionated into hexane-, benzene- and water-soluble fractions. The benzene-soluble fraction was separated by column chromatography (monitored by testing for piscicidal activity on killifish) to afford two active fractions. These fractions were further separated by preparative thin layer chromatography (TLC) followed by high performance liquid chromatography (HPLC) to afford two active lignans (lirioresinol-B and pinoresinol) and one inactive lignan (matairesinol) in 0.00009, 0.0004 and 0.0003% yields from the dried material, respectively. These isolated compounds were identified by comparing their infrared (IR) and nuclear magnetic



I: R=OMe lirioresinol-B
II: R=H pinoresinol



III: matairesinol

Fig. 1

TABLE I. Piscicidal Activity toward Killifish

Concentration (ppm)	Number of test fish surviving after 24 h		
	Lirioresinol-B	Pinoresinol	Matairesinol
Control	5	5	5
10	0	0	5
5	0	4	5
1	0	5	5
0.5	0	5	5
0.1	0	5	5
0.05	5	5	5

resonance (NMR) spectra with those of authentic samples. The piscicidal activities towards killifish are shown in Table I.

Experimental

Optical rotations were measured on a JASCO DIP-181 digital polarimeter using 99.6% CH_3OH as the solvent (cell length, 100 mm). Ultraviolet (UV) spectra were obtained on a JASCO UVIDEC-610 photometer using 99.6% CH_3OH as the solvent. IR spectra were taken in $CHCl_3$ solution on a JASCO IR A-2 spectrophotometer. NMR spectra were taken on a JEOL FX-100 spectrometer using $CDCl_3$ as the solvent; chemical shifts are given in ppm relative to internal tetramethylsilane (TMS). Mass spectra were obtained on a Hitachi M-52 spectrometer operating at an ionization energy of 70 eV.

Isolation——According to the same procedure as reported in the previous paper,[1] the methanol extract (620 g) of the roots (4.6 kg) of the plant was partitioned 3 times between hexane (400 ml) and water (400 ml). The aqueous phase was subsequently extracted 3 times with benzene (400 ml each). The benzene-soluble fraction was repeatedly chromatographed on silica gel (Kieselgel 60, 70—230 mesh (Merck)) with $CHCl_3$–MeOH (100 : 3), with monitoring for piscicidal activity on killifish, to afford two active fractions. The more polar fraction (246 mg) was further separated by the combination of preparative TLC on silica gel (PLK 5F, 1 mm, 20 × 20 cm (Whatman)) with C_6H_6–EtOAc (2 : 1) and preparative HPLC on ODS (Megapak SIL C-18, ϕ10 × 250 mm (JASCO)) with CH_3OH–H_2O (80 : 20) to afford lirioresinol-B (4.5 mg). The other fraction (400 mg) was subjected to preparative TLC on silica gel with $CHCl_3$ and preparative HPLC on ODS with CH_3OH–H_2O (80 : 20) to afford pinoresinol (17.7 mg) and matairesinol (13.4 mg).

Lirioresinol-B——$C_{22}H_{26}O_8$: $[\alpha]_D^{25}$ +3° (c=0.4); m/z 418 (M^+), 387; λ_{max} 240 (ε, 10000), 270 nm (ε, 2600); ν_{max} 3540, 1615, 1510, 1120 cm^{-1}; δ 3.08 (2H, m), 3.8—3.9 (2H, m), 3.88 (12H, s), 4.1—4.4 (2H, m), 4.70 (2H, d, J=4 Hz), 5.48 (2H, br s), 6.56 (4H, s).

Pinoresinol——$C_{20}H_{22}O_6$: $[\alpha]_D^{25}$ +21° (c=1.15); m/z 358 (M^+), 327; λ_{max} 230 (ε, 13200), 280 nm (ε, 5700); ν_{max} 3550, 1610, 1510, 1040 cm^{-1}; δ 3.10 (2H, m), 3.82 (2H, dd, J=8, 4 Hz), 3.90 (6H, s), 4.25 (2H, dd, J=8, 6 Hz), 4.72 (2H, d, J=5 Hz), 5.58 (2H, br s), 6.86 (6H, m).

Matairesinol——$C_{20}H_{22}O_6$: $[\alpha]_D^{25}$ −52° (c=1.22); m/z 358 (M^+); λ_{max} 230 (ε, 16300), 280 nm (ε, 7900); ν_{max} 3550, 1765, 1605, 1510, 1040 cm^{-1}; δ 2.53 (4H, m,), 2.90 (2H, m), 3.80 (6H, s), 3.90 (1H, dd, J=8, 4 Hz), 4.15 (1H, dd, J=8, 6 Hz), 5.56 (2H, br s), 6.3—6.9 (6H, complex).

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