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Insect Antifeedants, Pterocarpans and Pterocarpol, in Heartwood of *Pterocarpus macrocarpus* Kruz.

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The insect antifeedant activities of pterocarpans and a sesquiterpene alcohol from the dichloromethane extract of *Pterocarpus macrocarpus* Kruz. (Leguminosae) were evaluated against the common cutworm, *Spodoptera litura* F. (Noctuidae), and the subterranean termite, *Reticulitermes speratus* (Kolbe)(Rhinotermitidae). Three pterocarpans, (−)-homopterocarpin (1), (−)-pterocarpin (2), and (−)-hydroxyhomopterocarpin (3) and the sesquiterpene alcohol, (+)-pterocarpol (5), were isolated from the dichloromethane extract of the heartwood of *P. macrocarpus* under guidance by a biological assay. Among these natural products, the most active insect antifeedant against both *S. litura* and *R. speratus* was 1. On the other hand, sesquiterpene alcohol 5 showed less insect antifeedant activity than the other pterocarpans against both insect species. While its methylated derivative, (−)-methoxyhomopterocarpin (4), showed high biological activity, 3 showed less insect antifeedant activity in this study. Interestingly, racemic 1 did not show insect antifeedant activity against *S. litura*. However, all of the test pterocarpans and isoflavones showed antifeedant activity against the test termites. Additionally, since these compounds were major constituents of *P. macrocarpus*, these antifeedant phenolics may act as chemical defense factors in this tree. In Thailand, lumber made from this tree is used to make furniture and in building construction due to its resistance to termite attack.

Key words: *Pterocarpus macrocarpus* Kruz.; insect antifeedant; *Spodoptera litura* F.; *Reticulitermes speratus* (Kolbe); pterocarpan

Pterocarpans are a member of the isoflavone family and have two cyclic ether linkage moieties in their chemical structure (Fig. 1). They show various biological activities and contribute to the chemical defense system in plants. Some legumes produce biologically active pterocarpans, *i.e.* pisatin and phaseolin, as phytoalexins to resist infection by phytopathogens and phytophagous insects (Fig. 1).[1)] The insecticidal natural products, rotenoids, are isoflavones produced by a Leguminosae (Fig. 1). These phytophenolics act as a chemical defense system against phytophagous insects and phytopathogens.[2,3)] Flavonoids are widely distributed in many plants and a large amount is accumulated in their tissue. While they show insect antifeedant activity against various phytophagous organisms, this property is not lethal. With regard to their chemical structure, the benzopyranone moiety and loss of a hydroxyl group in the flavonoid appear to be important for their insect antifeedant activity against *S. litura*.[4)]

The common cutworm *Spodoptera litura* F. (Noctuidae) is the most notorious polyphagous insect and the most in famous crop pest in the world. This pest insect seriously damages many crops, and adequate pest control is required for crop production. The subterranean termite, *Reticulitermes speratus* (Kolbe), (Rhinotermitidae) commonly lives in Japanese forests and feeds on rotten wood. Some flavonoids have been shown to have antifeedant activity against the termite, *Coptotermes formosaunus*, the most active compounds being the 3′,4,′5,7-substituted flavonoids, quercetin and taxifolin (Fig. 2).[5)]

In Thailand, the large deciduous tree, *Pterocarpus macrocarpus* Kruz. (Leguminosae) is used to make furniture and in building construction due to its resistance to termite attack. The brownish red color of the heartwood of *Pterocarpus* spp. may be attributed to the presence of aurones.[6)] In this study, the constituents of *P. macrocarpus* were evaluated with regard to their insect antifeedant activity against *S. litura* and *R. speratus*.

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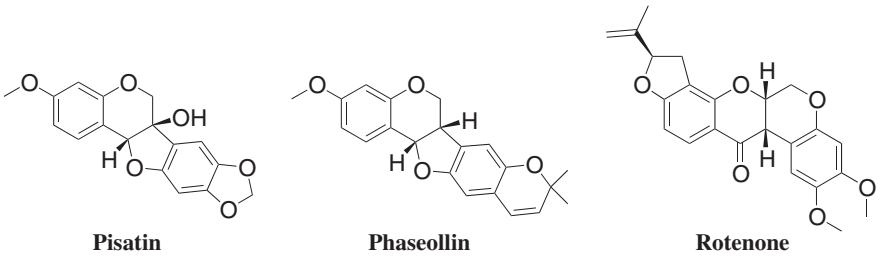

Fig. 1. Leguminosae Pterocarpans, Pisatin and Phaseollin, as Phytoalexins and the Insecticidal Isoflavone, Rotenone.

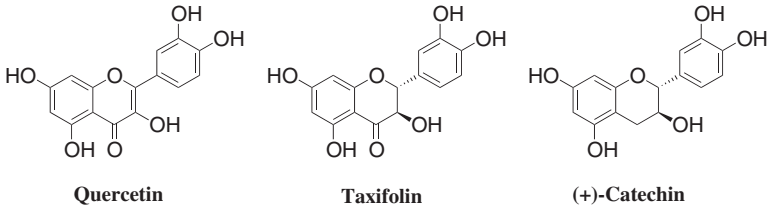

Fig. 2. Antitermite-Active Flavonoids and (+)-Catechin in This Text.

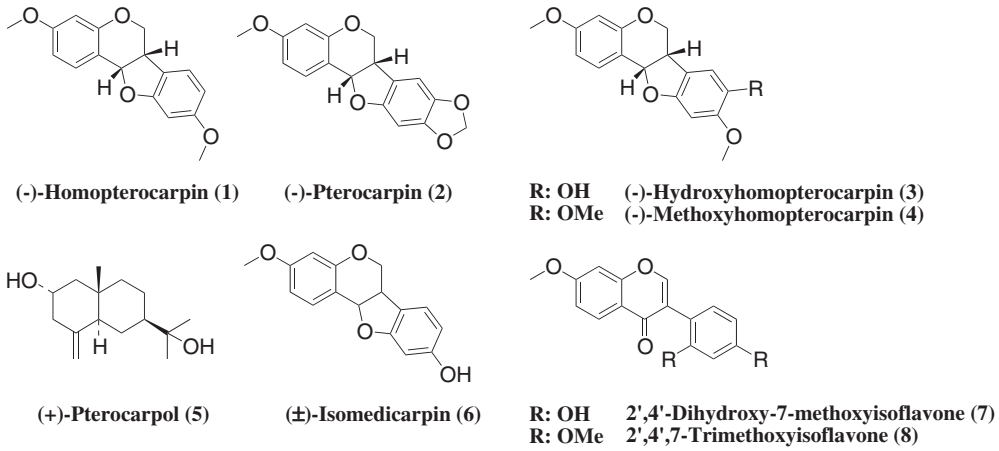

Fig. 3. Pterocarpans and Pterocarpol Used for the Evaluation of Insect Antifeedant Activity.

Material and Methods

General. Optical rotation values were determined at 25 °C with a Horiba SEPA-300 instrument. ^{1}H- and ^{13}C-NMR data were measured with a Jeol 270EX (270 MHz) spectrometer, using TMS as an internal standard. Mass spectra were taken at 70 eV (probe) by a Shimadzu 9100-MK GCMS instrument. These compounds were separated by column chromatography on BW-127ZH and BW-300 silica gel (Fuji Silysia Chemical Ltd., Japan). HPLC was performed with a Shimadzu VP-10 system, and TLC used silica gel plates with a fluorescent indicator (Merck 60 F_{254} silica gel 0.25 mm thick).

Chemicals. (+)-Catechin was purchased from Sigma-Aldrich Co., Ltd. The plant material (*P. macrocarpus*) was purchased from a folk-medicine pharmacy in Bangkok as air-dried heartwood. The plant material was extracted with dichloromethane at Chulalongkorn University (Thailand). (−)-Homopterocarpin (**1**), (−)-pterocarpin (**2**), (−)-hydroxyhomopterocarpin (**3**), and (+)-pterocarpol (**5**) were isolated from the dichloromethane extract of *P. macrocarpus* by silica gel column chromatography (Fig. 3). The extract was separated by silica gel column chromatography with elution by hexane:ethyl acetate (10:3) to afford **1** (15.8% yield from the dichloromethane extract), **3** (6.12% yield from the dichloromethane extract), and **5** (4.84% yield from the dichloromethane extract). Compound **2** was isolated from the fraction that contained **1** by preparative

crystallization, using a hexane-ethyl acetate solvent system. Separation was monitored by measuring the melting point and by an HPLC analysis. The analytical conditions for HPLC were an Imtakt cadenza column (CD-C18, 100 mm × 4.6 i.d.), eluent of 50% acetonitrile in water, flow rate of 0.8 ml/min, detection at UV 280 nm. These natural products were identified by comparing their spectral data with those in the literature.[7,8] Compound **4** was obtained by methylating **3** with methyl iodide and K_2CO_3 (95.7% yield). 2′,4′-Dibenzyloxy-7-methoxyisoflavone and (±)-isomedicarpin (**6**) were prepared by known synthetic methods.[9] 2′,4′-Dibenzyloxy-7-methoxyisoflavone was hydrolyzed with HCl–AcOH (1:2) at 60 °C to obtain 2′,4′-dihydroxy-7-methoxyisoflavone (**7**, 29.6% yield). Compounds **6** and **7** were methylated with methyl iodide in acetone with K_2CO_3 to obtain racemic homopterocarpin [(±)-**1**, 54.4% yield] and 2′,4′,7-trimethoxyisoflavone (**8**, 48.6% yield). These synthetic analogues have had their chemical structures confirmed by comparison with literature data on the basis of their EI-MS, and ^{1}H- and ^{13}C-NMR spectra.[10]

Insects. Common cutworms (*Spodoptera litura* F.) were purchased from Sumika Technoservice Co., Ltd. (Takarazuka, Japan). The insects were reared on an artificial diet (Insecta LF, Nihon Nosan Kogyo Co., Japan) in a controlled environment at 26.5 °C and 60% humidity. Subterranean termites, *Reticulitermes speratus* (Kolbe), were collected from a pine forest near the Enju coast (Wakayama Pref. Japan, N35°53′15″, E135°07′51″) in 2004, brought to our university, and fed on pieces of pine wood in a controlled environment at 26.5 °C until bing used in this test.

Antifeedant bioassays. The antifeedant test against common cutworms used leaf disks of 2 cm in diameter that had been prepared with a cork borer from the leaves of fresh sweet potato (*Ipomoea batata* cv. *narutokintoki*) cultivated on the farm at Kinki University (Nara Pref., Japan). Two disks were treated with test compounds in an acetone solution and two other disks were treated only with acetone as a control. The four disks were set in alternating positions in the same petri dish on moistened filter paper at the bottom. After the acetone had completely evaporated, 15 larvae (3rd instar) were released into the dish. The dishes were then kept in an insect-rearing room at 26.5 °C in the dark for 5–6 h.[11] Images of the partially consumed leaf disks were digitized. Data were analyzed on a PC by using NIH Image (http://rsb.info.nih.gov/nih-image/). The data for an intact disk were measured and compared to those of a treated disk for each experiment. To measure the activity of a test compound, we used the antifeedant index AFI = % of treated disks consumed/(% of treated disks consumed + % of control disks consumed) × 100. The AFI value was converted to the feeding inhibition rate (%) = (50-AFI) × 2. The potency of a test compound was evaluated in terms of the ED_{50} value for the rate of feeding inhibition calculated from the area of the leaf disk consumed. A straight line was fitted to the points obtained by the bioassay, and the ED_{50} value was calculated as the dose corresponding to the midpoint between complete inhibition and no effect. The leaf disks were replaced by paper disks of 6 mm in diameter for the termites made from filter paper Toyo No. 1). Treated paper disks were placed in a petri dish of 5 cm in diameter on moistened vermiculite *ca.* 5 mm thick. Four paper disks were placed in alternating positions in the same Petri dish. Twenty workers from the same colony were released into the dish and fed on the disks for 2 weeks in darkness at 27 °C. To keep the paper from drying, the disks were occasionally sprayed with water. Partially consumed paper disks were pasted on black paper and their images digitized. Data were analyzed on a PC using NIH Image after black and white inversion. For each experiment, the data for an intact disk were measured and compared to those of a treated disk. To measure the activity of a test compound, we used the antifeedant index AFI = % of treated disks consumed/(% of treated disks consumed + % of control disks consumed) × 100. The AFI value was converted to the feeding inhibition rate FI (%) = (50-AFI) × 2. An FI value under 40% when treated at 1 mg/disk (*ca.* 1.2 μmol/cm^2) is indicated as inactive in this study.

Results and Discussion

The antifeedant effects of the test compounds greatly differed between the phytophagous worms and termites. In this study, the subterranean termites, *R. speratus*, were highly sensitive to all of the test compounds, except for sesquiterpene alcohol **5** and the phytophenol, (+)-catechin. (+)-Catechin was used as a negative control in the termite feeding test, and did not significantly inhibit termite feeding at 100 μg/disk (Table 1).

The insect antifeedant activities of **1**, **2**, **3** and **5** from

Table 1. Insect Antifeedant Activities of the Test Flavonoids against *S. litura* Larvae and *R. speratus*

Compound	*S. litura*	*R. speratus*
	ED_{50} (95% CI) (μmol/cm^2)	FI (%) (SD) 50 μg/disk
1	0.14 (0.098–0.1839)	89.5 (0.80)
2	0.37 (0.322–0.419)	86.2 (2.36)
3	0.91 (0.798–1.053)	80.9 (3.43)
4	0.10 (0.058–0.158)	85.8 (0.32)
5	1.05 (0.892–1.255)	16.4 (7.21)
6	inactive	93.1 (0.24)
7	inactive	79.9 (1.36)
8	inactive	94.2 (0.12)
1 (racemic)	inactive	86.4 (2.19)
(+)-Catechin[a]	NT	38.4[a]

[a]Used as negative control for the termite antifeedant test at 100 μg/disk
Inactive, FI < 40% (1 mg/disk, *ca.* 1.2 μmol/cm^2)
NT, Not Tested

P. macrocarpus were between 0.1 and 1.04 μmol/cm^2 based on the ED_{50} values against *S. litura*. However, natural pterocarpan **1** corresponding to 2′,4′,7-trisubstituted isoflavones **7** and **8** did not show insect antifeedant activity. 2′,4′,7-Trisubstituted isoflavones **7** and **8** have been predicted to be biosynthetic precursors of their respective pterocarpans (**1**–**3**).[10] The results with the corresponding isoflavones (**7** and **8**) did not show biological activity, suggesting that the presence of a hydrofuran moiety played an important role. We have previously reported that various simple dihydrobenzofurans showed insect antifeedant activity against *S. litura* larvae.[2]

Interestingly, racemic **1** showed no biological activity, even though **1** showed strong insect antifeedant activity against *S. litura* larvae. A similar change in the biological activity of flavonoids attributed to an optical isomer or racemic form has been reported, in that (−)-medicarpin showed insect antifeedant activity, but racemic medicarpin did not show such activity against larvae of the beetle, *Costelytra zealandica*.[1] In addition, (−)-phaseolin and (−)-rotenone have shown potent insect antifeedant activity against *C. zealandica*, this activity not being restricted to a particular class of isoflavonoid (Fig. 1). Moreover, Lane *et al.* have reported that (+)-pisatin, which differs from pterocarpin only with regard to the presence of one hydroxyl group, showed the same biological activity (Fig. 1). The structural similarity between pisatin and **2** suggests that it is reasonable to assume that **2** would show antifeedant activity against *S. litura* larvae in this study. The results of the test for insect antifeedant activity against *S. litura* larvae demonstrate that **1** and **6** were likely to be inactivated by racemization (Table 1). In a previous QSAR study of benzopyranones, including flavones, a high-melting-point property and the introduction of a hydroxyl group tended to decrease the insect antifeedant activity as evaluated by a leaf disk bioassay.[12] In this case, the insect antifeedant activity of optically active pterocarpans tended to decrease with the introduction of a hydroxyl group as compared between **3** and **1** and **4**.

Among these pterocarpans, **1** had the greatest antifeedant activity against termites. Compound **1** was also the most effective insect antifeedant against *S. litura* larvae (Table 1). In this study, the termite antifeedant activities of all of the test compounds were independent of the presence of a hydroxyl group and optical activity against termite feeding inhibition. Ohmura has shown that such flavonoids as quercetin and taxifolin that had antifeedant activity against the subterranean termite, *C. formosanus*, had some hydroxyl groups (Fig. 2).[5] Moreover, this termite species was sensitive to biochanin A (5,7-dihydroxy-4′-methoxyisoflavone) by the choice test. In this present study, some isoflavones showed as much antitermite activity as the test pterocarpans (Table 1). This result suggests that, in the pterocarpan structure, antifeedant activity against termites is independent of the B–C cyclic ether linkage in isoflavones. Pterocarpans **1**–**4** showed a slight correlation between their biological activity against the common cutworm and that against the termite.

Insect antifeedant **1** moderately inhibited the activities of both α-amylase and 5α-reductase (data not shown). Some insect antifeedants inhibit α-amylase[13] and other enzymes that participate in metamorphosis and physiological functions. However, the mode of action of these pterocarpans for insect antifeedant activity was not demonstrated in this study.

Finally, the fact that lumber made from *P. macrocarpus* is used to make furniture and in construction in Thailand is reasonable considering its resistanse to termite attack. Pterocarpans **1**–**3** found in *P. macrocarpus* may be useful as a natural termite repellent and could be used as lead compounds for the development of termite repellents and other agents for the protection of wood products.

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