ANTITERMITIC ACTIVITY OF ESSENTIAL OILS AND COMPONENTS FROM TAIWANIA (*Taiwania cryptomerioides*)

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Abstract—Antitermitic activity of Taiwania (*Taiwania cryptomerioides* Hayata) against *Coptotermes formosanus* Shiraki was demonstrated in laboratory tests. Blocks of sapwood and heartwood from *T. cryptomerioides* exhibited antitermitic activity. Bioassays revealed that heartwood essential oil exhibited the highest antitermitic activity, followed by sapwood essential oil and then the $n\text{-}C_6H_{14}$ soluble fraction when tested at 10 mg/g. The order of termite mortality of three compounds purified from $n\text{-}C_6H_{14}$ soluble extracts of heartwood was cedrol > α-cadinol > ferruginol. The termite resistance of *T. cryptomerioides* wood can be attributed to the termiticidal activity of cedrol and α-cadinol.

Key Words—*Taiwania cryptomerioides* Hayata, *Coptotermes formosanus* Shiraki, antitermitic activity, essential oils, cedrol, α-cadinol, ferruginol.

INTRODUCTION

Biodegradation is recognized as one of the most significant problems for wood utilization. Among several factors leading to biodegradation, termites are one of the most damaging to wooden structures worldwide. There are several properties that determine wood's natural resistance to termite attack. For example, hardness of wood affects the termite's ability to fragment the wood with its mandibles (Bultman et al., 1979). It is also well known that extractives have a significant effect on the durability of wood (Chang et al., 1999b). Certain extractives from wood

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tissues can provide protection against harmful insects. For nondurable woods, it may be necessary to use inorganic compounds or synthetic pesticides to preserve woods and prolong their application life. To avoid environmental pollution and health problems caused by the use of traditional wood preservatives or synthetic pesticides, there is a trend to search for naturally occurring toxicants in plants. Accordingly, many researchers are focusing on the separation and identification of extractives from some durable woods and examining bioactivities.

Taiwania (*Taiwania cryptomerioides* Hayata) (*Taxodiaceae*) is an endemic tree that grows at elevations of 1800–2600 m in Taiwan's central mountains. The heartwood of *T. cryptomerioides* is yellowish red with distinguished purplish pink streaks. *T. cryptomerioides* timbers are well known in Taiwan for their decay resistance and excellent durability. Recently, we have illustrated the relationships between extractives and wood properties, including the photodiscoloration, antifungal, and antibacterial activity in *T. cryptomerioides* heartwood (Chang et al., 1998, 1999a,b, 2000a,b). To our knowledge, there are no prior studies of the antitermitic activity of chemical constituents from *T. cryptomerioides*. *Coptotermes formosanus* Shiraki is the termite mainly responsible for wood destruction in many countries, including Taiwan, Japan, and parts of the United States. Thus, many researchers have investigated antitermitic compounds of wood species using *C. formosanus* as a test termite (Yaga and Kinjo, 1986; Kinjo et al., 1988; Yaga et al., 1991; Ohtani et al., 1997; Sogabe et al., 2000a,b). In this study, we examined the antitermitic activity of the essential oils and dominant constituents isolated from *T. cryptomerioides* against *C. formosanus*.

METHODS AND MATERIALS

Wood Samples. Test blocks, $2.0 \times 2.0 \times 2.0$ cm, were cut from the sapwood and heartwood of a 27-year-old *T. cryptomerioides* tree collected from the Experimental Forest of National Taiwan University. The blocks were oven-dried ($60 \pm 2°C$, 24 hr) and weighed before treatment. Heartwood and sapwood blocks were extracted in a Soxhlet apparatus with alcohol–toluene (1 : 2) for 24 hr.

Termites. The test termites were from a *C. formosanus* colony from S.-J. Lin's laboratory at the Taiwan Forest Research Institute. The colony has been reared on wood pieces in the dark at 26.5°C and 85% relative humidity for more than two years.

General Procedures. Merck (Germany) Kieselgel 60F$_{254}$ sheets were used for analytical thin layer chromatography (TLC). High performance liquid chromatography (HPLC) was performed with a Jasco model PU980 pump equipped with a Jasco RI-930 RI detector and Hibar Lichrosorb Si-60 column (25×1 cm ID). FTIR spectra were recorded on a Bio-Rad model FTS-40 spectrophotometer, and the MS was obtained using a Finnigan MAT-95s Mass spectrometer. The ^{13}C and ^{1}H NMR spectra were recorded on a Bruker Avance-500 MHz FT-NMR.

Extraction and Isolation. T. cryptomerioides heartwood chips were prepared from the freshly cut tree. The essential oils from sapwood and heartwood of this tree were obtained by water distillation. The air-dried heartwood chips (5.7 kg) were exhaustively extracted with methanol (MeOH). The extracts were condensed to 286.4 g by rotary evaporation, followed by extraction with *n*-hexane (*n*-C_6H_{14}), chloroform ($CHCl_3$), ethyl acetate (EtOAc), and methanol (MeOH) successively. After removing solvents from the combined extracts, the *n*-C_6H_{14}, $CHCl_3$, EtOAc, and MeOH soluble fractions and MeOH insoluble fraction were obtained. The *n*-hexane soluble fraction (5 g) of methanol extracts was fractionated initially by gradient elution with EtOAc/*n*-C_6H_{14} on a silica gel column (800 g). Fractions were tracked by TLC, and compounds with similar R_f values were pooled to give 41 subfractions (H1–H41). α-Cadinol (**1**) (27.6 min retention time) was isolated and purified from H16 to H22 by semipreparative HPLC [Si-60 column, EtOAc– *n*-C_6H_{14} (30 : 70) mobile phase, 1.0 ml/min flow rate]. Ferruginol (**2**) (16.2 min retention time) was collected from H2–H8 with the same HPLC system. The mobile phase was changed to EtOAc–*n*-C_6H_{14} (10 : 90). Cedrol (**3**) (20.0 min retention time) was isolated from H10 with the same HPLC system. The mobile phase was changed to EtOAc–*n*-C_6H_{14} (20 : 80), 1.0 ml/min flow rate. Structures of compounds isolated from *T. cryptomerioides* were identified using FTIR, MS, and NMR spectrometry. Their spectral data are consistent with those reported in the literature (Chang et al., 1998, 2000a,b).

*α-Cadinol (**1**)*. Colorless needle crystal; mp 74–75°C; EI-MS for $C_{15}H_{26}O$ found 222; IR ν_{max}: 3372, 3046, 1660 cm^{-1}; ^{1}H NMR (in CDCl$_3$): δ (ppm) 0.74 (d, $J = 7.0$, H-12), 0.89 (d, $J = 7.0$, H-13), 1.08 (s, H-14), 1.64 (s, H-15), 2.13 (m, H-11), 5.47 (s, H-4); ^{13}C NMR: δ (ppm) 15.13 (C-12), 20.77 (C-14), 21.51 (C-13), 22.00 (C-1), 22.68 (C-7), 23.81 (C-15), 26.00 (C-11), 30.95 (C-2), 39.89 (C-5), 42.22 (C-8), 46.73 (C-6), 50.04 (C-10), 72.47 (C-9), 122.35 (C-4), 134.94 (C-3).

*Ferruginol (**2**)*. Yellow oil; EI-MS for $C_{20}H_{30}O$ found 286; IR ν_{max}: 3370, 1612, 1501, 1440 cm^{-1}; ^{1}H NMR (in CDCl$_3$): δ (ppm) 0.89 (3H, s, H-18), 0.93 (3H, s, H-19), 1.15 (3H, s, H-20),1.22 (3H, d, $J = 7.0$ Hz, H-16), 1.29 (3H, d, $J = 7.0$ Hz, H-17), 2.77 (1H, ddd, $J = 17.0, 10.5, 7.0$ Hz, H-7a), 2.81(1H, ddd, $J = 2.0, 6.5, 17.0$ Hz, H-7b), 3.11 (sept, $J = 7.0$ Hz, H-15), 6.62 (1H, s, H-11), 6.81 (1H, s, H-14); ^{13}C NMR: δ (ppm) 19.12 (C-6), 19.18 (C-2), 21.46 (C-19), 22.50 (C-16), 22.67 (C-17), 24.60 (C-20), 26.47 (C-15), 29.60 (C-7), 33.17 (C-18), 33.23 (C-4), 37.27 (C-10), 38.65 (C-1), 41.57 (C-3), 50.23 (C-5), 110.86 (C-11), 126.47 (C-14), 126.27 (C-8), 131.69 (C-13), 148.16 (C-9), 150.95 (C-12).

*Cedrol (**3**)*. Colorless needle crystal; mp 87°C; EI-MS for $C_{15}H_{26}O$ found 222; IR ν_{max}: 3342, 1373 cm^{-1}; ^{1}H NMR (in CDCl$_3$): δ (ppm) 0.81 (d, $J = 7.2$, H-15), 0.97 (s, H-13), 1.23 (s, H-14), 1.29 (s, H-12); ^{13}C NMR (in CDCl$_3$): δ (ppm) 15.54 (C-15), 25.34 (C-13), 27.61 (C-14), 28.89 (C-12), 30.16 (C-11), 31.58 (C-9), 35.33 (C-10), 36.99 (C-3), 41.45 (C-7), 41.96 (C-4), 43.38 (C-2), 54.08 (C-1), 56.51 (C-5), 61.03 (C-6), 75.10 (C-8).

Termiticidal Tests with Wood Blocks. Sapwood and heartwood (extracted and unextracted) blocks were tested using the no-choice test methods described by the American Wood-Preservers' Association (AWPA, 1997) against *C. formosanus. Pinus taiwanensis* sapwood is well recognized in Taiwan for its poor durability (Chang and Wang, 1995). It was selected as the control in this assay. Oven-dried wood blocks as well as 45 workers and 5 soldiers of *C. formosanus* were placed into the test container (75 mm diameter × 65 mm high). Three replicates of each treatment were tested in a growth chamber maintained at 26.5°C and 80% relative humidity for four weeks. Termite mortality (percent) and weight loss (percent) of the blocks were recorded periodically up to four weeks, using the following equations:

$$\text{Termite mortality } (\%) = \frac{\text{Number of dead termites}}{\text{Total number of test termites}} \times 100$$

$$\text{Weight loss } (\%) = \frac{W_1 - W_2}{W_1} \times 100$$

where W_1 is the oven-dried weight before termiticidal test (grams) and W_2 is the oven-dried weight after termiticidal test (grams).

We used Duncan's multiple-range test to examine the difference of the results of termiticidal test. Results with $P < 0.05$ were considered statistically significant.

Termiticidal Test with Essential Oils and Compounds. The termiticidal test followed the method of Kang et al. (1990). Each compound (**1, 2** and **3** as shown in Figure 1) and the essential oils (sapwood and heartwood) were dissolved in acetone, to make various concentrations of solutions, which were then applied to filter paper (8.6 cm diameter, Whatman No. 3) and air-dried at room temperature. Control filter paper was treated with solvent only. Fifty active termites (45 workers and 5 soldiers) were put onto the filter paper impregnated with the test materials in the Petri dish (9 cm diam. × 1.5 cm high). The test dishes were placed in a growth chamber maintained at 26.5°C and 80% realtive humidity for 14 days. A few drops of water were periodically put onto the bottom edge of the dishes. Three replicates were made for each sample, and termite mortality was determined daily. Paired Student's *t* tests were used to evaluate the difference of the results of termiticidal test. Results with $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Antitermitic Activity of Woods. Unextracted sapwood of *Pinus taiwanensis* (PSN), used as a control, showed no antitermitic activity (Table 1). In contrast, both the sapwood and heartwood of *T. cryptomerioides* displayed antitermitic activity. Termite mortalities for THN (unextracted *T. cryptomerioides* heartwood), THE (alcohol–toluene-extracted *T. cryptomerioides* heartwood), TSN (unextracted
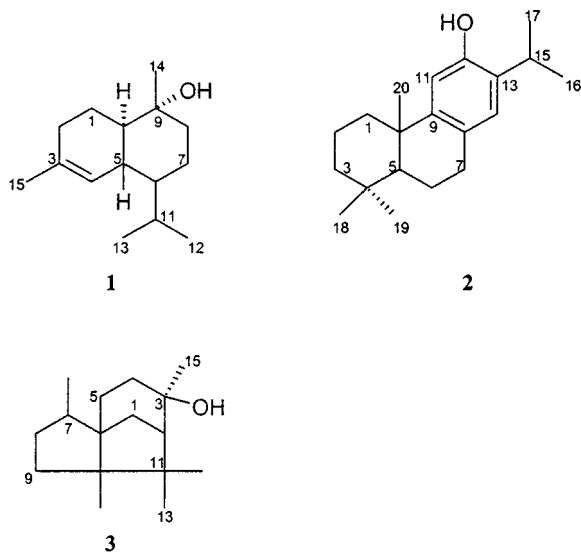
FIG. 1. Structures of α-cadinol (**1**), ferruginol (**2**), and cerdol (**3**) isolated from *T. cryptomerioides* heartwood.

T. cryptomerioides sapwood) and TSE (alcohol–toluene-extracted *T. cryptomerioides* sapwood) after four weeks were 57%, 50%, 48% and 43%, respectively. Duncan's multiple range test showed significant differences in termite mortality between extracted and unextracted wood ($P < 0.05$). Weight losses of heartwood and sapwood blocks extracted by alcohol–toluene (8.2% and 7.8%) were larger than those of unextracted blocks (1.1% and 1.3%) after four weeks. These differences were significant at $P < 0.05$. We conclude that the presence of

TABLE 1. TERMITE MORTALITY AND WEIGHT LOSS OF WOOD BLOCKS ATTACKED BY *Coptotermes formosanus**

Samples**	Termite mortality (%)	Weight loss (%)
TSE	43 ± 1.2c	7.8 ± 0.2b
TSN	48 ± 2.0b	1.3 ± 0.1c
THE	50 ± 1.6b	8.2 ± 0.1b
THN	57 ± 2.3a	1.1 ± 0.1c
PSN	9 ± 2.1d	29.1 ± 0.7a

*Means ($N = 3$) using 50 termites per replicate. Numbers followed by the different letter (a, b, c, d) are significantly different at the level of $P < 0.05$ according to Duncan's multiple range test.
**TSE: alcohol–toluene-extracted *T. cryptomerioides* sapwood; TSN: unextracted *T. cryptomerioides* sapwood; THE: alcohol–toluene-extracted *T. cryptomerioides* heartwood; THN: unextracted *T. cryptomerioides* heartwood; PSN: unextracted pine sapwood.

T. cryptomerioides wood extractives decreases the weight loss of wood in the presence of termites.

Antitermitic Activity of Essential Oils and Fractions. In our previous studies, the essential oils and n-C_6H_{14}-soluble fraction of MeOH extracts of *T. cryptomerioides* were demonstrated to have antifungal and antibacterial activity (Chang et al., 1998, 1999b, 2000a,b). Therefore, the antitermitic activities of essential oils (heartwood and sapwood) and the n-C_6H_{14}-soluble fraction of MeOH extracts from *T. cryptomerioides* were determined in this study.

After 14 days, the order of termite mortality from essentials oils and the n-C_6H_{14}-soluble fraction at 10 mg/g was: heartwood essential oil (56%) > sapwood essential oil (32%) > n-C_6H_{14}-soluble fraction (22%) (Figure 2). All samples demonstrated significant termiticidal activity compared to controls (termite mortality = 4%). According to our previous data, the cadinanes (T-cadinol, T-muurolol, α-cadinol) were the main constituents in heartwood essential oils (66.7%), followed by sapwood essential oils (37.1%), and then n-C_6H_{14} extracts (27.4%) (Chang et al., 2000a). These results suggest that antitermitic activity of *T. cryptomerioides* may be related to the cadinane constituents of essential oils or the n-C_6H_{14} soluble fraction.

Although the antitermitic activity of the n-C_6H_{14}-soluble fraction at 10 mg/g was lower than heartwood and sapwood essential oils, termite mortality increased to 72% when the dosage increased to 50 mg/g.

Antitermitic Activity of Isolated Compounds. The n-C_6H_{14} soluble fraction from *T. cryptomerioides* had excellent antitermitic activity at 50 mg/g. HPLC was used to isolate and purify α-cadinol, ferruginol and cedrol for further bioassay. Figure 3 shows the antitermitic activity of the three dominant compounds. The order of antitermitic activity against *C. formosanus* was cedrol, followed by α-cadinol, and then ferruginol. Termite mortalities (at 5 mg/g after 14 days) were
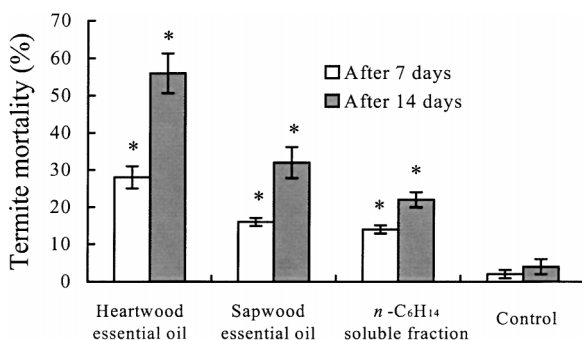

FIG. 2. Antitermitic activity of essential oils and the n-C_6H_{14} soluble fraction from *T. cryptomerioides* (bars = SD). $^*P < 0.05$; statistically different from the data of control.
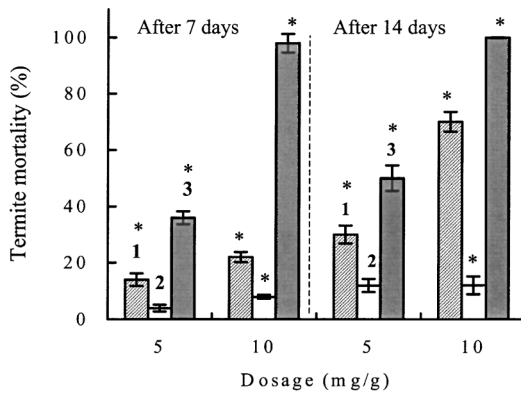
FIG. 3. Antitermitic activity of α-cadinol (**1**), ferruginol (**2**), and cedrol (**3**), (bars = SD). $^*P < 0.05$; statistically different from the data of control.

50%, 30%, and 12%, respectively. The antitermitic activities of 10 mg/g α-cadinol and cedrol increased after 14 days, whereas no dosage-dependent-effects occurred with ferruginol. The decreasing order of termite mortality at 10 mg/g dosage for 14 days was cedrol (100%) > α-cadinol (70%) > ferruginol (12%). Overall, cedrol possessed the strongest antitermitic activity.

During the test periods all the termites were killed within eight days at a dosage of 10 mg/g. This suggests that the resistance of *T. cryptomerioides* wood to termite attack may be attributed to the action of both cedrol and α-cadinol. Yaga and Kinjo (1986) previously showed that cedrol from *Sciadopitys verticillata* S. et Z was termiticidal. They also found that α-cadinol was the main termiticidal constituent of *Chamaecyparis obtusa* Endl (Kinjo et al., 1988).

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