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Termiticidal activity of wood vinegar, its components and their homologues

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Abstract The termiticidal activity of wood vinegar, its components, and their homologues have been studied. Three kinds of wood vinegar made from the mixed chips of *Cryptomeria japonica* and *Pseudotsga menziesii* (wood vinegar A), *Quercus serrata* (wood vinegar B), and *Pinus densiflora* (wood vinegar C) exhibited high termiticidal activities against *Reticulitermes speratus*. Acetic acid, which is the largest content of wood vinegar, exhibited high termiticidal acitivity. The contents of organic fraction of wood vinegars and acetic acid might be responsible for the differences in termiticidal activities among these wood vinegars. The structure and termiticidal activity relations of phenols were studied. Phenol with some substituents revealed higher termiticidal activity than benzene derivatives, which have no hydroxyl group; an ortho substituent of phenol plays an important role in termiticidal activity. It has become apparent that high termiticidal activity cannot be obtained by a phenolic hydroxyl group alone; it can be obtained, however, by some substituents, especially an ortho substituent in addition to a phenolic hydroxyl group. The bulkiness of the substituent at the ortho position participates in termiticidal activity; activity decreases as the size of an ortho substituent increases. It is thought that the interaction at the receptor site of termites is affected by the increased size of the ortho substituent.

Key words Termiticidal activity · Wood vinegar · *Reticulitermes speratus* · Acetic acid · Phenols

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Introduction

The production and consumption of wood vinegar, a condensed liquid of smoke produced by charcoal production, have been increasing recently. Today, around 6.000–7.000 kl of wood vinegar is produced and consumed annually, mainly for agricultural use.[1] We studied the components[2] and the germination- and growth-regulating effects of wood vinegars and found that they promoted plant growth.[3,4] Although several ways to use wood vinegar have been known from olden times,[5] there have been few detailed studies of its chemical characteristics. It is necessary to clarify the characteristics of wood vinegar to establish new uses other than agricultural use. Recently, the use of synthetic termiticides such as organophosphoric termiticide has been restricted owing to environmental pollution. Natural products that cause no or little environmental pollution are recommended as substitutes for synthetic termiticides and are entering widespread use. Wood vinegar has been used as an insect repellent and preservative and is one of the promising substitutes recommended by the related government agency.[6] Although preliminary studies on antitermite measures have been described in papers, magazines, and so on (e.g., a study[7] on antitermite actions using pine wood blocks penetrated with wood vinegar), there are few scientific reports concerning termiticidal activity of wood vinegar. Therefore, we investigated the termiticidal activity of wood vinegar, its components, and their homologues in the course of our study on the characteristics of wood vinegar.

Materials and methods

Wood vinegars

The raw materials of wood vinegars A, B, and C were the mixed chips of *Cryptomeria japonica* and *Pseudotsuga menziesii*, *Quercus serrata*, and *Pinus densiflora*, respectively. Sample A was prepared by a block-type kiln for carbonization of sawdust briquettes prepared by pressurized molding. Samples B and C were prepared using a traditional Japanese black charcoal kiln.

Chemicals

Phenols and other chemicals used for this experiment were special grade chemicals prepared by Wako Pure Chemical Industries.

Identification of components

Identification of each component was done by gas chromatography-mass spectrometry (GC-MS) (HP, 6890-GC/5973-MSD). The GC column was a 30m × 0.25mm glass capillary coated with crosslinked polyethylene glycol (film thickness 0.25μm) (HP-INNOWax). The GC was programmed as follows: started at 35°C, held for 1min, then was increased from 35° to 180°C at 6°C/min, held 1min, increased to 250°C at 10°C/min, and held for 20min. A splitless injection of 1μl was used. The carrier gas was helium (flow rate 1.0ml/min). MS was EI mode (70eV). The peak was confirmed by comparison with a standard in the NIST library data.

Determination of water content

Water content was determined by Karl Fischer volumetric titrators (DL-31, Mettler Toledo). The Karl Fischer reagent used for titration was Hydranal composite 5 (RdH).

Bioassay for termiticidal activities

The test termites were *Reticulitermes speratus* (Kolbe) workers that had been cultured in a chamber maintained at room temperature on a pine log as feed. The termiticidal activities against termites were examined for each test using a multidish (Nunc made), with six dishes (35mm diameter, 20mm depth) in two lines (i.e., three dishes in each line). Filter paper (33mm diameter) with a certain amount (0.1–0.001ml) of a test sample was placed in each of three dishes on one side; and a piece of filter paper with 0.4ml of distilled water was placed in each of three dishes on the other side to prevent drying. Six to ten termites were then placed in each dish with a test sample, and the multidish was covered. The multidishes were then kept in a chamber maintained at 25°C (light 10h, dark 14h) for 7 days, and the survivors were counted each day. The same amount of distilled water as that of wood vinegar or chemical was placed in a multidish as a control. Each determination was made with three replicates of three dishes, for a total of nine dishes.

Deep dishes (90mm diameter, 75mm depth) were used for the bioassay of termiticidal activities of phenol to study the influence of substituted groups of phenol on termiticidal acitivities. A piece of filter paper (88mm diameter) with phenol (0.10 or 0.01mg) dissolved in 1ml of methanol was put in the bottom of a deep dish, and the methanol was volatilized; 0.3ml of distilled water was then added. The dish treated with 1ml of methanol and 0.3ml of distilled water, as noted above, was used as a control. Twenty worker termites were put in the dishes, and the tops of the dishes were covered. The dishes were then allowed to stand in a chamber maintained at 25°C for 10 days. The survivors were counted each day, and the percentages (based on the control numbers) were calculated. Each determination was made with three replicates of 20 termites each. The standard deviations for the survival rates in Tables 1 and 3 (see below) were 9 at most.

Results and discussion

Bioassay of wood vinegars for termiticidal activities

All three wood vinegar samples used in this experiment exhibited high termiticidal activities at a dosage of 0.1ml, although the activity of wood vinegar C was slightly weaker than that of A or B (Table 1). The survival rates for the three samples were 0% or nearly 0% at 0.1ml 1 day after the tests were started. The difference in the activities among the three samples appeared obvious at the diluted dosage (at 0.01ml). At a dosage of 0.01ml, wood vinegar A exhibited a survival rate of 0% at 3 days after the test was started, whereas wood vinegar B and C exhibited survival rates of

Table 1. Termiticidal activities of wood vinegars

Sample	Dosage (ml)	Survival rate (%) over 1–7 days						
		1	2	3	4	5	6	7
Wood vinegar A	0.10	0						
	0.01	27	12	0				
Wood vinegar B	0.10	0						
	0.01	94	85	78	75	70	67	65
Wood vinegar C	0.10	5	3	0				
	0.01	100	100	95	93	91	69	67
Control		100	100	100	100	100	100	100

The raw materials of wood vinegars A, B, and C were the mixed chips of *Cryptomeria japonica* and *Pseudotsuga menziesii*, *Quercus serrata*, and *Pinus densiflora*, respectively
Results are the average of nine replicates
Distilled water was used as a control

Table 2. Contents of main acids and phenols of wood vinegars

Wood vinegar	Water content (%)	Acids					Phenols								
		Acetic	*n*-Propionic	Butanoic	*n*-Valeric	Total	(1)	(3)	(4)	(6)	(7)	(8)	(12)	(13)	Total
A	84.22	45.19	4.65	3.38	0.25	53.47	3.52	–	0.12	7.32	3.60	0.92	0.71	0.05	16.24
B	84.68	48.90	2.75	1.85	0.15	53.65	1.82	–	–	2.12	1.20	0.48	0.57	6.58	12.77
C	88.13	27.64	1.85	1.83	0.27	31.89	1.66	0.92	0.86	4.73	3.49	1.44	1.66	8.87	23.63

The raw materials of wood vinegars A, B, and C were the mixed chips of *Cryptomeria japonica* and *Pseudotsuga menziesii*, *Quercus serrata*, and *Pinus densiflora*, respectively
Results are the percentages of each compound in the organic fraction of wood vinegar

65% and 67%, respectively, after 7 days. The contents of the organic fraction of wood vinegar and acetic acid, which were the highest contents in the wood vinegars, seems to be the reason the activity of sample C was lower than that of A or B. As shown in Table 2, wood vinegars A and B contained about 84% water and about 15% organic fraction, whereas C contained about 12% organic fraction. Furthermore, the acetic acid content of vinegars A and B were about 45% and 49%, respectively, whereas that of vinegar C was only 28%. It can be presumed that acetic acid contributes to termiticidal activity of wood vinegar, as it was one of the most termiticidally active compounds, as shown in Table 3. *n*-Propionic acid, which was present in higher quantity in vinegars A and B than in vinegar C and had high termiticidal activity at 0.01 ml, would also be responsible for a small part of the termiticidal activity.

A previous report[7] noted that pine wood blocks penetrated by wood vinegar showed no antitermite activity; and even if wood vinegar had activity, it was weak. It is presumed that antitermite activity of wood vinegar might be weaker than that of synthetic termiticides. Its activity depends on its quality, however, as can be seem in the differences of activity among wood vinegars (Table 1).

Table 3. Termiticidal activities of components of wood vinegars

Dosage (ml)	Survival rate (%) at 1–7 days						
	1	2	3	4	5	6	7
Acetic acid							
0.010	0						
0.001	14	0					
Formic acid							
0.010	0						
0.001	0						
n-Propionic acid							
0.010	0						
0.001	100	83	76	73	70	68	66
n-Lactic acid							
0.010	0						
0.001	0						
n-Valeric acid							
0.010	0						
0.001	0						
Methanol							
0.010	100	100	100	100	100	85	80
0.001	100	100	100	100	100	100	100
Control							
0	100	100	100	100	100	100	100

Figures are the average of nine replicates
Distilled water was used as a control

Termiticidal activities of main components of wood vinegar

Usually acid acounts for more than 50% of wood vinegar. Acetic acid is the largest component of the acid portion and is the largest of all the components of wood vinegar. Acetic acid killed perfectly at a dosage of 0.01 ml 1 day after the test was started and at 2 days at a dosage of 0.001 ml, as shown in Table 3. High termiticidal activities were also observed with other acids, such as *n*-lactic acid, *n*-valeric acid, and propionic acid (0% survival rate at dosages of 0.01 and 0.001 ml, except for propionic acid at 0.001 ml). Therefore it was suggested that the acids in wood vinegar greatly contribute to termiticidal activity, and the activity is mostly influenced by acetic acid because of its high content in wood vinegar. Formic acid was not found in the wood vinegar in this study, although it is sometimes found in wood vinegars as a minor component and shows strong termiticidal acitivity. *n*-Lactic acid, which was not found in wood vinegars A, B, and C, showed strong termiticidal activity at dosages of 0.01 and 0.001 ml.

Structure and termiticidal activity relation of phenols

Figure 1 shows the termiticidal activities of a series of phenols. Among these phenols, compounds (1), (3), (4), (6), (7), (8), (9), (12), and (13) were found in the wood vinegars used in this report. In addition to these phenols, others were tested to investigate their influence on the termiticidal activity of their substituents. In the wood vinegar used in this study, 2,6-dimethoxyphenols, which are found in pyrolysis products of wood,[2,8] were found only in wood vinegar C as 4-propenyl-2,6-dimethoxy phenol, for which the production mechanism is not yet clear; it might be a secondary reaction product under pyrolysis. Further detailed survey in minor products is needed to find 2,6-dimethoxyphenols. However, even if they are contained in wood vinegar, their contents would be small and they might make a relatively small contribution to termiticidal activity. Therefore, the 2,6-dimethoxyphenols for termiticidal activity were not bioassayed for this report, although it is the next subject to be studied.

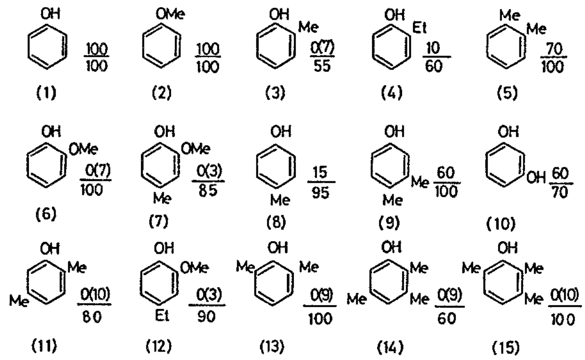

Fig. 1. Termiticidal activities of phenols. The *denominator* shows the percentage of live termites at a dosage of 0.01 mg after 10 days; the *numerator* shows it at a dosage of 0.1 mg. The accompanying figures in *parentheses* show the day by which all termites were killed

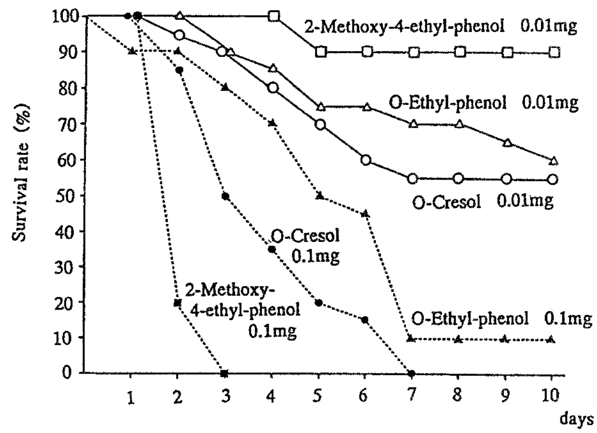

Fig. 2. Time course of termiticidal activities of phenols

The denominator of the fraction on the right side of each structure in Fig. 1 shows the percentage of live termites at a dosage of 0.01 mg 10 days after the bioassay was started, and the numerator is the percentage at a dosage of 0.1 mg. The figures in parentheses show the day by which all termites were killed.

At a dosage of 0.1 mg, compounds (3), (6), (7), (11), (12), (13), (14), and (15) killed all termites within 10 days. The strongest termiticidal activity was observed in for (7) and (12), showing that all termites were killed in the shortest time (3 days) among the test samples at a dosage of 0.1 mg.

Because phenol (1) did not exhibit any activity under our conditions, it can be presumed that termiticidal activity cannot be obtained by a phenolic hydroxyl group alone. Oviposition attractants and stimulants of phenols were studied for the tree-hole mosquito *Aedes triseriatus* (Say) by Bentley et al.[9] They found that the simple presence of a benzene ring, even when hydroxylated or methylated like phenol or toluene, is insufficient for an attractant or stimulant to induce increased oviposition. In our experiment of the termiticidal activity of phenols, phenol (1) was inactive, which was the same result as oviposition activity for the tree-hole mosquito. Compound (3), which has a methyl group at the ortho position of the hydroxyl group, has termiticidal activity. Therefore, phenols that have some substituents in addition to a hydroxyl group, display termiticidal activity, though it is unknown why the activity of phenol (3) is higher than that of (1). The termiticidal activity of (3) was higher than that of *o*-xylene (5), in which the hydroxyl group was substitued by a methyl group. The phenols that have a substituent at the ortho position showed more activity than phenols that have no substituent at the ortho position; for example, the activities of (8), (9), and (10) were lower than that of phenols with ortho substituents. This fact also showed that phenols with some substituents revealed higher termiticidal activity than benzene derivatives, which have no hydroxyl group; and ortho substituents play an important role in termiticidal activity. Therefore, it is possible that phenols with a relatively small ortho substituent fit the receptor site of a termite and so display termiticidal activity.

Phenols with substituents at the para position of the hydroxyl group exhibited higher termiticidal activity than the original phenols; for example, (7) and (12) showed higher activity than (6), and compound (8) showed higher activity than (1). This is the same result as the oviposition attractant activity for the tree-hole mosquito.[7] The size of the para substituent of phenols used in this report was almost independent of the degree of activity; there was only a small difference between the activities of (7) and (12).

Concerning the size of ortho substitutents of phenols and the termiticidal activity relationship, the activities of (4) and (6) were lower than that of (3), of which the substituent at the ortho position was smaller than those of (4) and (6). The activity of (14) was higher than that of (15), whose bulkiness around the hydroxyl group was greater than that of (14). These results supported the supposition that the bulkiness of the substituent at the ortho position participates in the termiticidal activity and that as the size of an ortho substituent increases the activity decreases. It is thought that an interaction at the receptor site of termites is affected by the increased size of the ortho substituents. The increasing bulkiness of phenols with bulky substituents decreases volatility and is responsible for the decreasing activity of phenols. Compounds (13) and (15), which have 2,6-dimethyl groups, showed less activity than (3), which has only two substituents. This may be influenced by the interaction of the phenolic OH being blocked by the two neighboring methyl groups. The termiticidal activities of 2,6-dimethyoxyphenols, which have a bulkier methoxy group than a methyl group, are presumed to be weaker than 2,6-dimethylphenols.

Figure 2 shows the time course of the termiticidal activity of phenols. The activities of *o*-ethyl phenol and *o*-cresol at a dosage of 0.01 mg were mild, and the differences in the activities between 0.10 mg and 0.01 mg were relatively small, whereas the activity of 2-methoxy-4-ethyl phenol at a dosage of 0.10 mg was extremely high but extremely low at 0.01 mg. The activities changed over the wide range between 0.10 and 0.01 mg.

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