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Structure−Activity Relationship of Aliphatic Compounds for Nematicidal Activity against Pine Wood Nematode (*Bursaphelenchus xylophilus*)

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Nematicidal activity of aliphatic compounds was tested to determine a structure−activity relationship. There was a significant difference in nematicidal activity among functional groups. In a test with alkanols and 2*E*-alkenols, compounds with C_8−C_{11} chain length showed 100% nematicidal activity against pine wood nematode, *Bursaphelenchus xylophilus*, at 0.5 mg/mL concentration. C_6−C_{10} 2*E*-alkenals exhibited >95% nematicidal activity, but the other compounds with C_{11}−C_{14} chain length showed weak activity. Nematicidal activity of alkyl acetates with C_7−C_{11} chain length was strong. Compounds belonging to hydrocarbons, alkanals, and alkanoic acetates showed weak activity at 0.5 mg/mL concentration. Nematicidal activity of active compounds was determined at lower concentrations. At 0.25 mg/mL concentration, whole compounds except C_8 alkanol, C_8 2*E*-alkenol, and C_7 alkanoic acid showed >80% nematicidal activity. C_9−C_{11} alkanols, C_{10}−C_{11} 2*E*-alkenols, C_8−C_9 2*E*-alkenals, and C_9−C_{10} alkanoic acids showed >80% nematicidal activity at 0.125 mg/mL concentration. Only C_{11} alkanol exhibited strong nematicidal activity at 0.0625 mg/mL concentration, the lowest concentration that was tested.

KEYWORDS: Pine wood nematode; *Bursaphelenchus xylophilus*; aliphatic compounds; structure−activity relationship; nematicidal activity

INTRODUCTION

Pine wood nematode, *Bursaphelenchus xylophilus*, has caused pine wilt disease worldwide including Japan, South Korea, China, Taiwan, and Portugal (*1*). This disease was first reported in Gumjung Mt., Busan city, in 1988 (*2*) and has spread to several areas of the Korean peninsula (*3*). The damaged area was about 6851 ha, and total infected trees were about 81000 in 2008 (*4*). Most damaged trees are red pine tree (*Pinus densiflora*) and black pine tree (*Pinus thunbergii*). Recently, infected *Pinus koraiensis* has been found for the first time in Korea (*5*). *Pinus* species are the predominant tree species in Korean forests and are very susceptible to the pine wood nematode; ecological and economic damages are substantial (*6*).

Control of this disease is primarily by fumigation of disease-infected trees with metham-sodium, aerial application of the synthetic pesticide thiacloprid against *Monochamus alternatus*, the insect vector of this disease, or injection of nematicides such as abamectin and emamectin benzoate (*7*−*9*). Total budget for the control of pine wood nematode was about U.S. $27 million in 2008 (*7*). However, there are environmental and human health concerns with conventional pesticides. To avoid environmental pollution and health problems, there is a need to search for naturally occurring toxicants in plants.

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The volatile chemicals produced by plants include a wide variety of short-chain alcohols, aldehydes, ketones, esters, aromatic phenols, and lactones, as well as mono- and sesquiterpenes (*10*). Short-chain aldehydes and corresponding alcohols are major constituents of volatile organic chemicals produced in response to wounding and insect attack (*11*, *12*). Although antimicrobial and insecticidal activities of short-chain aliphatic compounds have been reported (*13*), little work has been done on their nematicidal activity against pine wood nematode (*14*).

In this study, we investigated the nematicial activity and structure−activity relationship of short-chain aliphatic compounds to find potential alternatives to currently used pine wood nematode control agents or as model compounds for the development of chemically synthesized derivatives with enhanced activity or environmental friendliness.

MATERIALS AND METHODS

Collection of Pine Wood Nematodes. *B. xylophilus* was isolated from chips of infected pine wood collected in the Haman area (March 2004), Gyoungsangnam-do province, Korea, and extracted by Baermann funnel method (*15*). Details of the isolation and culture of pine wood nematode are described by Park et al. (*1*).

Chemicals. Authentic compounds used for bioassays were commercially obtained or synthesized as shown in **Table 1**.

Synthesis. Tetrahydrofuran (THF) and diethyl ether were dried by distillation from sodium benzophenone ketyl. Solutions were dried over

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Table 1. Aliphatic Compounds Tested for Nematicidal Activity

	carbon length (manufacturer, purity)								
	C6	C7	C8	C9	C10	C11	C12	C13	C14
hydrocarbon	Merck 96%	Aldrich 99%	Aldrich 98%	Aldrich 97%	Wako 99%	Wako 99%	Wako 99%	Aldrich 99%	Wako 99%
alkanol	Wako 97%	TCI 98%	TCI 98%	Aldrich 98%	Aldrich 99%	TCI 98%	Aldrich 98%	Aldrich 97%	Aldrich 97%
2*E*-alkenol	Wako 95%	synthetic[a] 99%	synthetic[a] 99%	synthetic[a] 99%	Aldrich 97%	synthetic[b] 98%	synthetic[b] 97%	synthetic[b] 99%	synthetic[b] 95%
alkanal	TCI 98%	Wako 95%	Aldrich 99%	Aldrich 95%	Aldrich 99%	Aldrich 97%	Aldrich 92%	Aldrich 90%	synthetic[c] 92%
2*E*-alkenal	Aldrich 98%	Aldrich 94%	Aldrich 94%	Aldrich 97%	synthetic[b] 98%	synthetic[b] 98%	synthetic[b] 97%	synthetic[b] 99%	synthetic[b] 95%
alkanoic acid	TCI 98%	TCI 96%	TCI 98%	TCI >90%	TCI 98%	TCI 98%	Aldrich 98%	TCI 98%	Aldrich 99%
alkyl acetate	TCI 98%	TCI 98%	Aldrich 99%	synthetic[d] 99%	synthetic[d] 99%	synthetic[d] 99%	Aldrich 97%	TCI 98%	synthetic[d] 99%

[a] Scheme A. [b] Scheme B. [c] Scheme C. [d] Scheme D.

anhydrous $MgSO_4$ and concentrated by rotary evaporation under reduced pressure, unless otherwise stated. GC analyses were performed on a HP 6890N gas chromatograph equipped with a FID and a DB-1MS column [25 m × 0.25 mm (i.d.) × 0.25 μm, J&W Scientific]. The purities of all compounds used were checked by GC. NMR spectra were taken on a Varian UI 500 NMR spectrometer using TMS as internal standard (500 MHz for ^1H spectra and 125 MHz for ^{13}C spectra). Mass spectra were taken with an Agilent 6890 gas chromatograph interfaced to a Hewlett-Packard 5973 mass selective detector, using electron impact ionization at 70 eV. DB-5MS [30 m × 0.25 mm (i.d.) × 0.25 μm, J&W Scientific] was used for separating analytes. Infrared (IR) spectra were recorded with a Thermo Nicolet 6700 (Thermo Fisher Scientific Inc.).

Synthesis of 2E-C7:1-OH, 2E-C8:1-OH, and 2E-C9:1-OH (*Scheme A in* ***Figure 1***). To a solution of the corresponding 2*E*-alkenal (1.0 equiv) in diethyl ether was carefully added Red-Al (2.0 equiv, Fluka) at 0 °C. The solution was stirred for 30 min and then slowly warmed to room temperature over 3 h. After the solution had been cooled to 0 °C, 2 N HCl and ice were added. The resulting mixture was diluted with diethyl ether and then washed with H_2O, $NaHCO_3$, and brine and dried. After filtration and concentration, the residue was purified by silica gel chromatography (Wakogel 200).

Synthesis of 2E-C11:1-OH, 2E-C12:1-OH, 2E-C13:1-OH, and 2E-C14:1-OH (*Scheme B in* ***Figure 1***). To a solution of propargyl THP ether (1.0 equiv), which was prepared from propargyl alcohol and dihydropyran, in THF at −78 °C was added a solution of n-BuLi (1.1 equiv, 1.6 M, Aldrich) under nitrogen atmosphere. After 15 min of stirring, HMPA was added to the solution. To this solution was added alkyl bromide (1 equiv). The solution was stirred and left overnight to warm to room temperature. The resulting mixture was diluted with ether, washed with H_2O and brine, and then dried. After the solvent was evaporated, the residue was dissolved in methanol. To the solution was added *p*-toluenesulfonic acid (10% mol). After 1 h of reflux, H_2O was poured into the solution. The reaction mixture was extracted with ether. The organic layer was washed with $NaHCO_3$, H_2O, and brine and dried. After filtration and concentration, the residue was distilled and chromatographed over silica gel to give 2-alkyn-1-ol.

To the solution of 2-alkyn-1-ol (1 equiv) in THF was carefully added Red-Al (1.5 equiv) at 0 °C. The mixture was stirred and left overnight. The mixture was cooled to 0 °C, and ethyl acetate was added to destroy excess Red-Al, followed by the addition of 2 N HCl. After dilution with diethyl ether, the solution was washed with H_2O, $NaHCO_3$, and brine and then dried. The residue was subjected to distillation to afford desired 2*E*-alken-1-ol.

Synthesis of 2E-C10:1-Ald, 2E-C11:1-Ald, 2E-C12:1-Ald, 2E-C13:1-Ald, and 2E-C14:1-Ald (*Scheme B in* ***Figure 1***). 2*E*-Alkenal was obtained by oxidation of the corresponding 2*E*-alken-1-ol following the methods of Matsuo et al. (*16*). Briefly, to the solution of 2*E*-alken-1-ol (1.0 equiv), DBU (1.1 equiv of TCI), and *N-tert*-butylbenzenesulfenamide (10% mol, TCI) in CH_2Cl_2 at 0 °C was added a small portion of *N*-chlorosuccinimide (1.1 equiv of TCI). After 1 h of stirring, 2 N HCl was added to the solution. The resulting solution was extracted with diethyl ether. The organic layer was washed with 2 N HCl, H_2O, $NaHCO_3$, and brine and dried. After filtration and concentration, the residue was purified by silica gel chromatography.

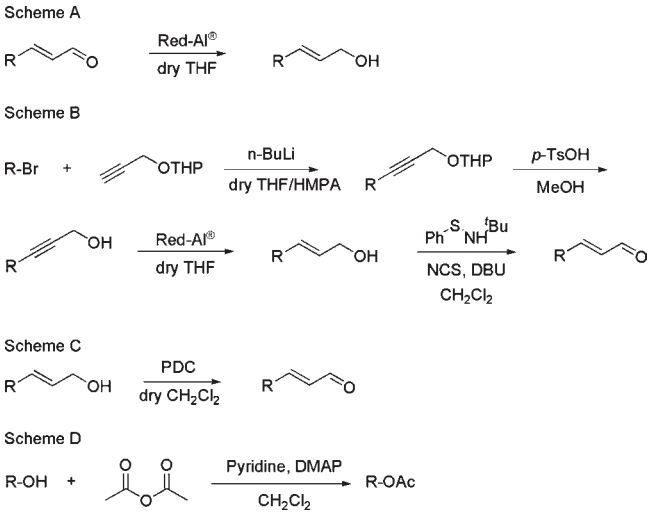

Figure 1. Synthetic schemes of aliphatic compounds.

Synthesis of Tetradecanal (*Scheme C in* ***Figure 1***). Tetradecanal was prepared by oxidation of tetradecanol with PDC (*17*) (scheme C).

Synthesis of Nonyl Acetate (*C9-Ac*)*, Decyl Acetate* (*C10-Ac*)*, Undecyl Acetate* (*C11-Ac*)*, and Tetradecyl Acetate* (*C14-Ac*) (*Scheme D in* ***Figure 1***). The corresponding alcohol was acetylated using acetic anhydride, pyridine, and DMAP. Briefly, the corresponding alcohol (1.0 equiv), acetic anhydride (5.0 equiv), and pyridine (5.0 equiv) were dissolved in CH_2Cl_2 at 0 °C. After a catalytic amount of DMAP had been added to the solution, it was stirred for 1 h at 0 °C and then left overnight at room temperature. The solution was diluted with ether, washed with NH_4Cl, water, and brine, and then dried. After the solvent was removed, the residue was distilled to give the desired alkyl acetate.

2*E*-C7:1-OH: yield, 84.6%; GC-MS, *m*/*z* (%) 114 (M^+, 1.5), 96 ($M^+ - H_2O$, 14.6), 81 (28.6), 71 (13.4), 68 (19.2), 67 (11.3), 57 (100), 41 (40); ^{1}H NMR, δ 0.89 (3H, t, J_1 = 7 Hz, 7-H_3), 1.35 [1.28−1.38] (4H, m, 5-, 6-H_2), 1.79 (1H, br s, −OH), 2.05 (2H, m, 4-H_2), 4.07 (2H, dt, J_1 = 6 Hz, J_2 = 6 Hz, 1-H2), 5.66 [5.72−5.60] (2H, br m, 2-, 3-H, =CH−); ^{13}C NMR, δ 14.0 (CH_3, C7), 22.8 (CH_2, C6), 31.5 (CH_2, C5), 32.0 (CH_2, C4), 63.9 (CH_2, C1), 129.0 (=CH, C2), 133.6 (=CH, C3); IR (cm^{-1}, neat), 3352−3090 (s), 2960−2873 (s), 1672 (w), 1468 (m), 1092 (m), 1011 (s), 970 (s).

2*E*-C8:1-OH: yield, 84.4%; GC-MS, *m*/*z* (%) 128 (M^+, 0.8), 110 ($M^+ - H_2O$, 7.2), 95 (10.4), 81 (25.5), 68 (26.6), 57 (100), 55 (38.1), 43 (31.0); ^{1}H NMR, δ 0.89 (3H, t, J = 7 Hz, 8-H_3), 1.30 [1.20−1.34] (4H, m, 6,7-H_2), 1.38 (2H, quin, J = 7 Hz, 5-H_2), 1.88 (1H, br s, −OH), 2.04 (2H, ddt, J_1 = 7 Hz, J_2 = 6.5, J_3 = 1 Hz, 4-H_2), 4.07 (2H, dd, J_1 = 7 Hz, J_2 = 1 Hz, 1-H_2), 5.66 [5.60−5.72] (2H, br m, 2-, 3-H, =CH−); ^{13}C NMR, δ 14.0 (CH_3, C8), 22.5 (CH_2, C7), 28.9 (CH_2, C5), 31.4 (CH_2, C6), 32.2 (CH_2, C4), 63.7 (CH_2, C1), 128.9 (=CH, C2), 133.5 (=CH, C3); IR (cm^{-1}, neat), 3597−3086 (br s), 2960−2860 (s), 1672 (w), 1468 (m), 1092 (m), 1005 (s), 972 (s).

2*E*-C9:1-OH: yield, 75.7%; GC-MS, *m*/*z* (%) 142 (M^+, 0.4), 124 ($M^+ - H_2O$, 3.3), 109 (2.7), 95 (25.5), 82 (30.8), 70 (17.3), 68 (26.9), 67 (23.6), 57 (100), 41 (45.9); ^{1}H NMR, δ 0.89 (3H, t, J = 7 Hz, 9-H_3), 1.30 [1.20−1.34] (6H, m, 6-, 7-, 8-H_2), 1.38 (2H, m, 5-H_2), 1.80 (1H, br s, −OH), 2.04 (2H, dt, J_1 = 7 Hz, J_1 = 7 Hz, 4-H_2), 4.07 (2H, dd, J_1 = 5.5 Hz, J_2 = 1 Hz, 1-H2), 5.66 [5.72−5.60] (2H, br m, 2,3-H, =CH−); ^{13}C NMR, δ 14.2 (CH_3, C9), 22.8 (CH_2, C8), 29.0 (CH_2, C6), 29.3 (CH_2, C5), 31.9 (CH_2, C7), 32.4 (CH_2, C4), 63.9 (CH_2, C1), 129.0 (=CH, C2), 133.6 (=CH, C3); IR (cm^{-1}, neat), 3580−3084 (s), 2960−2858 (s), 1672 (w), 1468 (m), 1092 (m), 1009 (s), 970 (s).

2*E*-C11:1-OH: yield, 74.1%; bp, 82 °C/5 mmHg; GC-MS, *m*/*z* (%) 170 (M^+, 0.4), 152 (2.4), 137 (0.7), 124 (5.0), 109 (10.3), 95 (25.4), 82 (40.9), 71 (21.5), 69 (29.7), 68 (33.2), 57 (100), 55 (43.8), 43 (43.1), 41 (49.5); ^{1}H NMR, δ 0.88 (3H, t, J = 7 Hz, 1-H_3), 1.27 [1.20−1.35] [10H, m (s-like)], 1.37 (2H, quin, J = 7 Hz), 1.47 (1H, −OH), 2.04 (2H, m, 4-H_2), 4.08 (2H, dd, J_1 = 6 Hz, J_2 = 1 Hz, 1-H_2), 5.66 [5.60−5.72] (2H, m, 2,3-H, =CH−); ^{13}C NMR, δ 14.1 (CH_3), 22.7 (CH_2), 29.1 (CH_2), 29.2 (CH_2), 29.3 (CH_2), 29.5(CH_2), 31.9 (CH_2), 32.2 (CH_2), 63.8 (−CH_2OH), 128.8 (−C=C—CH_2OH), 133.6 (−CH_2—C=C−); IR (cm^{-1}, neat), 3602−3086 (s), 2958−2856 (s), 1674 (w), 1468 (m), 1093 (m), 1011 (m), 970 (s).

2*E*-C12:1-OH: yield, 91.0%; bp, 104 °C/4 mmHg; GC-MS, *m*/*z* (%) 184 (M^+, 0.5), 166 (3.5), 138 (5.9), 123 (6.0), 109 (15.4), 95 (31.8), 82 (58.0), 71 (24.0), 57 (100), 55 (45.0), 43 (44.1); ^{1}H NMR, δ 0.88 (3H, t, J = 7 Hz), 1.27 [1.00−1.34] (12H, s-like), 1.37 (2H, m), 1.91 (1H, br s, −OH), 2.03 (2H, dt, J_1 = 7 Hz, J_2 = 7 Hz, 4-H_2), 4.06 (2H, dd, J_1 = 6 Hz, J_2 = 1 Hz), 5.65 [5.71−5.59] (2H, br m); ^{13}C NMR, δ 14.30 (CH_3), 22.89 (CH_2), 29.39 (CH_2), 29.42 (CH_2), 29.55 (CH_2), 29.73 (CH_2), 29.80 (CH_2), 32.12 (CH_2), 32.44 (CH_2), 63.72 (CH_2), 128.91 (=CH, C2), 133.43 (=CH, C3); IR (cm^{-1}, neat), 3599−3111 (br s), 2931−2862 (s), 1672 (w), 1468 (m), 1093 (m), 1012 (m), 929 (s).

2*E*-C13:1-OH: yield, 75.6%; bp, 108 °C/3 mmHg; GC-MS, *m*/*z* (%) 180 ($M^+ - H_2O$, 4.7), 152 (4.0), 123 (7.3), 95 (25.3), 82 (54.7), 71 (29.3), 68 (38.6), 57 (100), 43 (48.8); ^{1}H NMR, δ 0.88 (3H, t, J = 7 Hz), 1.26 [1.00−1.34] (14H, s-like), 1.36 (2H, m), 1.51 (1H, br s, −OH), 2.03 (2H, dt, J = 7 Hz, 7, 4-H_2), 4.06 (2H, dd, J_1 = 6 Hz, J_2 = 1 Hz), 5.65 [5.71−5.59] (2H, br m); ^{13}C NMR, δ 14.30 (CH_3), 22.89 (CH_2), 29.17 (CH_2), 29.22 (CH_2), 29.36 (CH_2), 29.53 (CH_2), 29.64 (CH_2), 29.65 (CH_2), 31.93 (CH_2), 32.24 (CH_2), 63.83 (CH_2), 128.85 (=CH, C2), 133.58 (=CH, C3); IR (cm^{-1}, neat), 3540−3111 (br s), 2931−2856 (s), 1674 (w), 1468 (m), 1093 (m), 1011 (m), 970 (m).

2*E*-C14:1-OH: yield, 79.4%; bp, 116−118 °C/3 mmHg; GC-MS, *m*/*z* (%) 194 ($M^+ - H_2O$, 2.8), 166 (3.4), 151 (176.4), 124 (6.7), 111 (7.3), 109 (16.8), 96 (37.8), 82 (66.4), 71 (25.4), 68 (39.6), 57 (100), 43 (43.8); ^{1}H NMR, δ 0.88 (3H, t, J_1 = 7 Hz, 1-H3), 1.26 [1.20−1.42] [18H, m (s-like)], 2.03 (3H, m, 4-H_2, −OH), 4.06 (2H, d-like), 5.66 [5.590−5.71] (2H, m); ^{13}C NMR, δ 14.1 (CH_3, C-14), 22.7 (CH_2), 29.22 (CH_2), 29.26 (CH_2), 29.40 (CH_2), 29.57 (CH_2), 29.67 (CH_2), 29.69 (CH_2), 29.73 (CH_2), 32.0 (CH_2), 32.3 (CH_2), 63.7 (−CH_2OH), 128.9 (−C=C—CH_2OH), 133.4 (−CH_2—C=C−); IR (cm^{-1}, neat), 3614−3120 (br s), 2943−2866 (s), 1674 (w), 1468 (m), 1095 (m), 1011 (m), 972 (m).

2*E*-C10:1-al: yield, 70.4%; GC-MS, *m*/*z* (%) 154 (M^+, 0.04), 136 (1.8), 121 (5.3), 110 (11.4), 98 (19.7), 83 (50.2), 70 (86.3), 57(58.7), 55 (100); ^{1}H NMR, δ 0.89 (3H, t, J = 7.8 Hz), 1.20 −1.38 (8H, m), 1.51 (2H, pentuplet, J = 7.5 Hz), 2.34 (2H, ddt, J_1 = 7.5 Hz, J_2 = 7.0 Hz, J_3 = 1.5 Hz), 6.12 (1H, ddt, J_1 = 15.5 Hz, J_2 = 7.0 Hz, J_3 = 1.5 Hz), 6.86 (1H, dt, J_1 = 15.5 Hz, J_2 = 7.0 Hz), 9.15 (1H, d, J = 7 Hz); ^{13}C NMR, δ 194.1 (=CHO), 159.0 (=CH), 133.0 (=CH), 32.7 (CH_2), 31.7 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 27.9 (CH_2), 22.6 (CH_2), 14.1 (CH_3); IR (cm^{-1}, neat), 2930−2858 (s), 1697 (s), 1634 (w), 1468 (m), 1146 (m), 1105 (m), 976 (s).

2*E*-C11:1-al: yield, 88.5%; GC-MS, *m*/*z* (%) 150 ($M^+ - H_2O$), 135 (4.1), 124 (11.0), 121 (19.5), 111 (15.2), 97 (25.8), 83 (70.4), 70 (100), 69 (51.3), 57 (70.9), 55 (66.4), 41 (63.0); ^{1}H NMR, δ 0.89 (3H, t, J = 7 Hz), 1.20 −1.38 [1.28] (10H, s-like), 1.50 (2H, pentuplet, J = 7 Hz), 2.34 (2H, ddt, J_1 = 7 Hz, J_2 = 7 Hz, J_3 = 1.5 Hz), 6.13 (1H, ddt, J_1 = 15.5 Hz, J_2 = 7 Hz, J_3 = 1.5 Hz), 6.86 (1H, dt, J_1 = 15.5 Hz, J_2 = 7 Hz), 9.15 (1H, d, J = 7 Hz); ^{13}C NMR, δ 14.09 (CH_3), 22.67 (CH_2), 27.87 (CH_2), 29.18 (CH_2), 29.21 (CH_2), 29.34 (CH_2), 31.85 (CH_2), 32.78 (CH_2), 134.02 (=CH), 159.25 (=CH), 194.32 (−CHO); IR (cm^{-1}, neat), 2931−2858 (s), 1697 (s), 1639 (w), 1468 (m), 1146 (m), 1105 (m), 976 (s).

2*E*-C12:1-al: yield, 91.9%; GC-MS, *m*/*z* (%) 182 (M^+, 0.5), 164 (1.6), 138 (9.4), 121 (20.0), 111 (17.9), 97 (40.6), 83 (78.0), 70 (100), 69 (47.3), 55 (66.3), 43 (54.4); ^{1}H NMR, δ 0.88 (3H, t, J = 7 Hz), 1.20 −1.38 [1.25] (12H, m), 1.51 (2H, pentuplet, J = 7 Hz), 2.33 (2H, ddt, J_1 = 7.5 Hz, J_2 = 7 Hz, J_3 = 1.5 Hz), 6.11 (1H, ddt, J_1 = 15.5 Hz, J_2 = 8 Hz, J_3 = 1.5 Hz), 6.86 (1H, dt, J_1 = 15.5 Hz, J_2 = 7.5 Hz), 9.47 (1H, d, J = 8 Hz); ^{13}C NMR, δ 14.11 (CH_3), 22.64 (CH_2), 28.03 (CH_2), 29.32 (CH_2), 29.45 (CH_2), 29.54 (CH_2), 29.65 (CH_2), 32.04 (CH_2), 32.99 (CH_2), 133.00 (=CH), 159.02 (=CH), 194.07 (−CHO); IR (cm^{-1}, neat), 2937−2858 (s), 1699 (s), 1670 (w), 1468 (m), 1147 (m), 1105 (m), 978 (s).

2*E*-C13:1-al: yield, 90.2%; GC-MS, *m*/*z* (%) 196 (M^+, 0.8), 181 (0.4), 165 (1.9), 152 (6.0), 135 (9.6), 121 (15.3), 111 (22.1), 97 (41.0), 83 (77.7), 70 (100), 69 (43.7), 55 (66.0), 41 (59.2); ^{1}H NMR, δ 0.86 (3H, t, J = 7 Hz), 1.25 [1.20−1.38] (16H, m), 1.49 (2H, pentuplet, J = 7 Hz), 2.34 (2H, q-like, J = 7.5 Hz), 6.09 (1H, ddt, J_1 = 15.5 Hz, J_2 = 7.5 Hz, J_3 = 1.5 Hz), 6.84 (1H, dt, J_1 = 15.5 Hz, J_2 = 7.5 Hz), 9.48 (1H, d, J = 7.5 Hz); ^{13}C NMR, δ 14.11 (CH_3), 22.69 (CH_2), 27.87 (CH_2), 29.17 (CH_2), 29.32 (CH_2), 29.37 (CH_2), 29.53 (CH_2), 29.59 (CH_2), 31.91 (CH_2), 32.76 (CH_2), 132.98 (=CH), 159.14 (=CH), 194.22 (−CHO); IR (cm^{-1}, neat), 2929−2856 (s), 1697 (s), 1639 (w), 1467 (m), 1143 (m), 1106 (m), 977 (m).

2*E*-C14:1-al: yield, 88.4%; GC-MS, *m*/*z* (%) 210 (M^+, 0.7), 192 (1.1), 166 (5.0), 135 (11.9), 121 (18.9), 111 (24.2), 97 (41.9), 83 (79.4), 70 (100), 69 (42.6), 55 (64.5), 41 (59.0); ^{1}H NMR, δ 0.86 (3H, t, J = 7 Hz), 1.25 [1.20−1.38] (16H, m), 1.49 (2H, pentuplet, J = 7 Hz), 2.34 (2H, q-like, J = 7.5 Hz), 6.09 (1H, ddt, J_1 = 15.5 Hz, J_2 = 7.5 Hz, J_3 = 1.5 Hz), 6.84 (1H, dt, J_1 = 15.5 Hz, J_2 = 7.5 Hz), 9.48 (1H, d, J = 7.5 Hz); ^{13}C NMR, δ 14.13 (CH_3), 22.72 (CH_2), 27.90 (CH_2), 29.19 (CH_2), 29.38 (CH_2), 29.40 (CH_2), 29.55 (CH_2), 29.65 (CH_2), 29.66 (CH_2), 31.95 (CH_2), 32.77 (CH_2), 133.00 (=CH), 159.04 (=CH), 194.09 (−CHO); IR (cm^{-1}, neat), 2931−2856 (s), 1697 (s), 1641 (w), 1468 (m), 976 (m).

Tetradecanal: yield, 42.0%; IR (cm^{-1}, neat), 2918−2850 (s), 1707 (s), 1471 (s).

C9-Ac: yield, 82.5%; bp, 104 °C/22 mmHg; GC-MS, 171 ($M^+ - CH_3$, 0.2), 127 (1.4), 126 (13.0), 116 (16.9), 97 (38.9), 83 (37.6), 70 (54.1), 69 (46.1), 56 (56.6), 55 (46.7), 43 (100); IR (cm^{-1}, neat), 2933−2860 (s), 1747 (s), 1470 (m), 1365 (m), 1240 (s), 1041 (s).

C10-Ac: yield, 81.3%; bp, 87−90 °C/3 mmHg; GC-MS, *m*/*z* (%) 185 ($M^+ - CH_3$, 0.3), 141 (1.5), 140 (12.7), 112 (30.9), 97 (41.2), 83 (54.3), 70 (62.4), 69 (51.3), 56 (47.9), 55 (51.8), 43 (100); IR (cm^{-1}, neat), 2933−2858 (s), 1747 (s), 1470 (m), 1365 (m), 1240 (s), 1047 (s).

C11-Ac: yield, 63.8%; bp, 120 °C/22 mmHg; GC-MS, *m*/*z* (%) 199 ($M^+ - CH_3$, 0.2), 155 (1.3), 154 (9.6), 126 (21.5), 111 (22.6), 97 (47.4), 83 (53.3), 70 (53.0), 69 (56.7), 56 (45.8), 55 (51.6), 43 (100); IR (cm^{-1}, neat), 2933−2858 (s), 1745 (s), 1470 (m), 1365 (m), 1244 (s), 1043 (s).

C14-Ac: yield, 72.1%; bp, 130−132 °C/1 mmHg; GC-MS, *m*/*z* (%) 256 (M^+, 0.1), 241 (0.4), 196 (10.5), 168 (16.8), 140 (6.3), 125 (18.7), 111 (41.7), 97 (75.2), 83 (87.0), 69 (69.8), 55 (60.5), 43 (100); IR (cm^{-1}, neat), 2935−2858 (s), 1747 (s), 1468 (m), 1365 (m), 1246 (s), 1043 (s).

Nematicidal Activity. Solutions of aliphatic compounds were prepared by serial dilution with distilled water containing Triton X-100 (5 mg/mL). Test solutions were introduced into wells of 96-well plates (Falcon, USA). In each well, the concentration of nematodes was about 50−150 nematodes (mixtures of juvenile and adult nematodes, male/female/juvenile ≈ 1:1:2) per 100 μL of water. Controls received distilled water−Triton X-100 solutions. Treated and control nematodes were held under

Table 2. Nematicidal Activity[a] of Aliphatic Compounds against Pine Wood Nematode, *Bursaphelenchus xylophilus*

	mortality of each compound group (mean ± SEM, $N = 12$)						
carbon length	hydrocarbon	alkanol	2*E*-alkenol	alkanal	2*E*-alkenal	alkyl acetate	alkanoic acid
C6	4.4 ± 1.3 a[b] (c)[c]	12.6 ± 2.4 c (c)	13.0 ± 3.2 c (c)	4.2 ± 0.6 b (c)	95.6 ± 1.9 a (a)	1.5 ± 0.3 bc (c)	31.3 ± 6.8 b (b)
C7	2.6 ± 0.5 a (d)	29.8 ± 6.8 b (c)	50.9 ± 6.3 b (b)	6.0 ± 1.3 ab (d)	100 a (a)	7.4 ± 0.9 a (d)	100 a (a)
C8	3.8 ± 0.6 a (c)	100 a (a)	97.4 ± 0.8 a (a)	13.6 ± 2.1 a (b)	100 a (a)	4.9 ± 0.9 ab (c)	100 a (a)
C9	2.3 ± 0.5 a (c)	100 a (a)	100 a (a)	14.2 ± 2.4 a (b)	100 a (a)	3.1 ± 0.6 bc (c)	100 a (a)
C10	1.6 ± 0.4 a (c)	100 a (a)	100 a (a)	7.9 ± 1.7 ab (b)	100 a (a)	1.6 ± 0.3 bc (c)	100 a (a)
C11	1.6 ± 0.4 a (c)	100 a (a)	100 a (a)	7.4 ± 1.4 ab (c)	24.8 ± 4.8 b (b)	2.3 ± 0.7 bc (c)	98.5 ± 0.6 a (a)
C12	1.6 ± 0.4 a (b)	3.1 ± 0.7 c (ab)	4.7 ± 0.7 c (ab)	6.6 ± 1.2 ab (a)	5.1 ± 0.8 c (ab)	1.7 ± 0.3 bc (b)	1.9 ± 0.3 c (b)
C13	3.3 ± 1.6 a (ab)	4.4 ± 0.7 c (ab)	3.5 ± 1.0 c (ab)	7.6 ± 0.8 ab (a)	4.9 ± 0.6 c (ab)	1.4 ± 0.3 c (b)	4.4 ± 0.7 c (ab)
C14	1.7 ± 0.3 a (a)	4.0 ± 0.7 c (a)	2.6 ± 0.3 c (a)	3.5 ± 0.7 b (a)	3.3 ± 0.9 c (a)	0.9 ± 0.2 c (a)	3.8 ± 0.7 c (a)

[a] 0.5 mg/mL concentration. [b] Means within a column followed by the same letters are not significantly different ($P = 0.05$, Scheffe's test). [c] Means within a row followed by the same letters are not significantly different ($P = 0.05$, Scheffe's test).

the same conditions as used for colony maintenance. Mortality of nematodes was recorded after 48 h under a microscope. Nematodes were defined as dead if their bodies were straight and they did not move, even after transfer to clean water. Three trials on different days were used to test the nematicidal activity (four replicates in each trial, and total replicates were 12 times).

Statistical Analysis. Nematode mortality was transformed to arcsine square root values for analysis of variance (ANOVA). Treatment means were compared and separated by Scheffe's test (*18*).

RESULTS

Nematicidal activity of aliphatic compounds is shown in **Table 2**. There was a significant difference in nematicidal activity among functional groups. In a test with alkanols and 2*E*-alkenols, compounds with C_8-C_{11} chain length showed 100% nematicidal activity at 0.5 mg/mL concentration. C_6-C_{10} 2-alkenals exhibited >95% nematicidal activity, but the other 2-alkenals with $C_{11}-C_{14}$ chain length showed weak activity. Alkanoic acids with C_7-C_{11} chain length showed 100% nematicidal activity at 0.5 mg/mL concentration. Compounds belonging to hydrocarbons, alkanals, and alkyl acetate showed weak nematicidal activity at 0.5 mg/mL concentration. The nematicidal activity of compounds that showed strong nematicidal activity at 0.5 mg/mL concentration was tested at a lower concentration (**Table 3**). At 0.25 mg/mL concentration, whole compounds except C_8 alkanol, C_8 2*E*-alkenol, and C_7 alkanoic acid showed >80% nematicidal activity. C_9-C_{11} alkanols, $C_{10}-C_{11}$ 2*E*-alkenols, C_8-C_9 2*E*-alkenals, and C_9-C_{10} alkanoic acids showed >80% nematicidal activity at 0.125 mg/mL concentration. Only C_{11} alkanol exhibited strong nematicidal activity at 0.0625 mg/mL concentration. Mortality of control was <2%.

Table 3. Nematicidal Activity of Aliphatic Compounds against Pine Wood Nematode, *Bursaphelenchus xylophilus*

	mortality of each compound group (mean ± SEM, $N = 12$)		
functional group	0.25 mg/mL	0.125 mg/mL	0.0625 mg/mL
alkanol			
C8	17.0 ± 3.0 d[a]	–[b]	–
C9	100 a	81.0 ± 3.5 ab	4.7 ± 1.0 d
C10	100 a	100 a	15.6 ± 3.9 d
C11	100 a	99.4 ± 0.3 a	98.3 ± 1.1 a
2*E*-alkenol			
C8	31.5 ± 2.0 d	–	–
C9	100 a	45.5 ± 4.7c	–
C10	100 a	100 a	43.3 ± 4.9 bc
C11	100 a	100 a	45.1 ± 3.6 b
2*E*-alkenal			
C6	83.1 ± 3.7 b	22.0 ± 3.2 d	–
C7	97.8 ± 1.0 a	76.3 ± 4.9 b	18.0 ± 3.3 d
C8	99.0 ± 0.6 a	90.6 ± 2.3 ab	22.6 ± 3.3 cd
C9	100 a	99.8 ± 0.1 a	47.4 ± 2.2 b
C10	94.8 ± 2.0 ab	37.0 ± 3.3 cd	–
alkanoic acid			
C7	53.7 ± 5.6 c	–	–
C8	98.9 ± 0.9 a	50.1 ± 4.3 c	–
C9	100 a	100 a	40.1 ± 4.3 bc
C10	100 a	100 a	59.8 ± 4.8 b
C11	96.0 ± 1.2 ab	51.3 ± 2.7 c	–

[a] Means within a column followed by the same letters are not significantly different ($P = 0.05$, Scheffe's test). [b] Not tested.

DISCUSSION

Many phytochemicals belonging to isothiocyanates, alkaloids, terpenoids, and phenolics have been reported to show nematicidal activity (*19*). Nematicidal activity of plant essential oils and their components against pine wood nematode have also been reported (*1*, *14*). In this study, we investigated the nematicidal activity of aliphatic compounds with C_6-C_{14} chain length against pine wood nematode to identify a structure−activity relationship. The nematicidal activity of test compounds varied according to functional groups. Aliphatic alcohols, aldehydes, and acids were more effective than hydrocarbons and acetates. This result agreed with previous reports (*14*, *20*, *21*) that aldehydes and alcohols were more effective than other hydrocarbons and ketones. There was a significant difference in nematicial activity between alkanals and 2*E*-alkenals. Kim et al. (*14*) reported that 2-decenal, an α,β-unsaturated aldehyde, showed strong nematicidal activity compared to alkanals with C_8-C_{12} chain length. In this study, the nematicidal activity of 2*E*-alkenals was stronger than that of the corresponding alkanals. This and earlier studies suggest that the enhancement of nematicidal activity of aldehydes is attributed to the existence of a double bond at the α,β-position of the carbonyl group.

Legal et al. (*22*) tested the relationship between structures and toxicity of oxygenated aliphatic compounds to two fruit flies, *Drosophila melanogaster* and *Drosophila sechellia*. Among linear carboxylic acids with C_4-C_{10} chain lengths, octanoic acid was the most toxic to *D. melanogaster* followed by heptanoic, hexanoic, nonanoic, and pentanoic acid. The authors claimed that octanoic acid was capable of penetrating insect cuticles, leading to high toxicity to *D. melanogaster* flies. Grodnitzky and Coats (*23*) studied the QSAR of monoterpenoids against house flies, *Musca domestica*. There was an optimum shape and size requirement that monoterpenoids must possess to fit into a site of toxic action. In our study, there was a significant difference in nematicidal activity against pine wood nematode according to chain length. In alkanols and 2*E*-alkenols, the nematicidal activity of compounds with C_9-C_{11} chain length was stronger than that of compounds

of other chain lengths. Compounds with C_8-C_9 and C_9-C_{10} chain lengths showed strong nematicidal activity in 2*E*-alkenals and alkanoic acids group, respectively. Compounds with $C_{12}-C_{14}$ chain length showed weak nematicidal activity against pine wood nematode. Weak nematicidal activity was also observed in compounds with C_6-C_7 chain length except 2*E*-alkenals and alkanoic acids. Our results indicated that proper chain length is necessary for nematicidal activity against pine wood nematode.

Mougabure Cueto et al. (*24*) investigated toxic effect of aliphatic alcohols against head lice, *Pediculus humanus capitis*. Toxicity to head lice systematically increased with the increase in carbon atoms in the aliphatic alcohol moiety. Our result suggested that the toxicity of aliphatic alcohol was very closely related to hydrophobicity. In this study, nematicidal activities of alkanols and 2*E*-alkenols with C_8-C_{11} chain length were stronger than those of compounds with C_6-C_7 chain length, and these results agreed with those of Mougabure Cueto et al. (*24*). However, $C_{12}-C_{14}$ alkanols and 2*E*-alkenols showed weak activity, which might be attributed to the size of these chemicals as already described.

Pine wood nematode bodies treated with the muscle activity blockers levamisole hydrochloride and morantal tatrate usually exhibited semicircular and coiling shapes, respectively (*25*). In our study, the dead body of *B. xylophilus* treated with aliphatic compounds was usually straight and without movement. These results suggest that the nematicide modes of action between the aliphatic compounds tested in this study and commercial nematicides might be different. However, the exact mode of action of aliphatic compounds against pine wood nematode is not well understood. SAR study of aliphatic compounds could be used in the future to develop new effective nematicides, as well as contribute to the understanding of their mechanisms of action.

LITERATURE CITED

(1) Park, I. K.; Park, J. Y.; Kim, K. H.; Choi, K. S.; Choi, I. H.; Kim, C. S.; Shin, S. C. Nematicidal activity of plant essential oils and components from garlic (*Allium sativum*) and cinnamon (*Cinnamomum verum*) oils against the pine wood nematode (*Bursaphelenchus xylophilus*). *Nematology* **2005**, *7*, 767–774.

(2) Yi, C. K.; Byun, B. H.; Park, J. D.; Yang, S. I.; Chang, K. H. First finding of the pine wood nematode, *Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle and its insect vector in Korea. *Res. Rep. For. Res. Inst.* **1989**, *38*, 141–149.

(3) Chung, Y. J. Occurrence and spread of pine wilt disease in Korea. *Kor. Tree Prot.* **2002**, *7*, 1–7.

(4) Korea Forest Research Institute. *Annual Report of Monitoring for Forest Insect Pests and Diseases in Korea*; Korea Forest Research Institute: Seoul, Republic of Korea, 2008; 158 pp.

(5) Korea Forest Research Institute. *Proceedings of International Symposiums on Pine Wilt Diseases*; Korea Forest Research Institute: Seoul, Republic of Korea, **2008**; 94 pp.

(6) Korea Forest Service. *Statistical Yearbook of Forestry*; Korea Forest Service: Daejeon, Republic of Korea, 2009; 495 pp.

(7) Korea Forest Service. *Guideline for the Control of Forest Diseases and Insect Pests*; Korea Forest Service: Daejeon, Republic of Korea, 2008; 115 pp.

(8) Kishi, Y. *The Pine Wood Nematode and the Japanese Pine Sawyer*; Thomas: Tokyo, Japan, 1995; 302 pp.

(9) Lee, S. M.; Chung, Y. J.; Moon, Y. S.; Lee, S. G.; Lee, D. W.; Choo, H. Y.; Lee, C. K. Insecticidal activity and fumigation conditions of several insecticides against Japanese pine sawyer (*Monochamus alternatus*) larvae. *J. Kor. For. Soc.* **2003**, *92*, 191–198.

(10) Bernays, E. A.; Chapman, R. F. *Host-Plant Selection by Phytophagous Insects*; Chapman and Hall: New York, 1994; 312 pp.

(11) Noordermeer, M. A.; Veldink, G. A.; Vliegenthart, J. F. G. F. Fatty acid hydroperoxide lyase: a plant cytochrome P450 enzyme involved in wound healing and pest resistance. *ChemBioChem* **2001**, *2*, 495–504.

(12) Matsui, K. Green leaf volatiles: hydroperoxide lyase pathway of oxylipin metabolism. *Curr. Opin. Plant Biol.* **2006**, *9*, 274–280.

(13) Santino, A.; Poltronieri, P.; Mita, G. Advances on plant products with potential to control toxigenic fungi: a review. *Food Addit. Contam.* **2005**, *22*, 389–395.

(14) Kim, J.; Seo, S. M.; Lee, S. G.; Shin, S. C.; Park, I. K. Nematicidal activity of plant essential oils and components from coriander (*Coriandrum sativum*), oriental sweetgum (*Liquidambar orientalis*), and valerian (*Valeriana wallichii*) essential oils against pine wood nematode (*Bursaphelenchus xylophilus*). *J. Agric. Food Chem.* **2008**, *56*, 7316–7320.

(15) Chawla, M. L.; Prasad, S. K. Techniques in nematology. II. Comparative efficiency of sampling tools and nematode extraction methods. *Ind. J. Nematol.* **1975**, *4*, 115–123.

(16) Matsuo, J.; Iida, D.; Yamanaka, H.; Mukaiyama, T. *N-tert*-Butylbenzenesulfenamide-catalyzed oxidation of alcohols to the corresponding carbonyl compounds with *N*-chlorosuccinimide. *Tetrahedron* **2003**, *59*, 6739–6750.

(17) Corey, E. T.; Schmidt, G. Useful procedures for the oxidation of alcohols involving pyridinium dichromate in approtic media. *Tetrahedron Lett.* **1979**, *20*, 399–402.

(18) SAS Institute. *SAS/STAT User's Guide*, release 9.0; SAS Institute: Cary, NC, 2002.

(19) Chitwood, D. J. Phytochemical based strategies for nematode control. *Annu. Rev. Phytopathol.* **2002**, *40*, 221–249.

(20) Choi, I. H.; Kim, J.; Shin, S. C.; Park, I. K. Nematicidal activity of monoterpenoids against the pine wood nematode (*Bursaphelenchus xylophilus*). *Russ. J. Nematol.* **2007**, *15*, 35–40.

(21) Park, I. K.; Kim, J.; Lee, S. G.; Shin, S. C. Nematicidal activity of plant essential oils and components from ajowan (*Trachyspermum ammi*), allspice (*Pimenta dioica*) and litsea (*Litsea cubeba*) essential oils against pine wood nematode (*Bursaphelenchus xylophilus*). *J. Nematol.* **2007**, *39*, 275–279.

(22) Legal, L.; Moulin, B.; Jallon, J. M. The relation between structure and toxicity of oxygenated aliphatic compounds homologous to the insecticide octanoic acid and the chemotaxis of two species of *Drosophila*. *Pestic. Biochem. Phys.* **1999**, *65*, 90–101.

(23) Grodnitzky, J. A.; Coats, J. R. QSAR evaluation of monoterpenoids' insecticidal activity. *J. Agric. Food Chem.* **2002**, *50*, 4576−4580.

(24) Mougabure Cueto, G.; Gonzalenz Audino, P.; Vassena, C. V.; Picollo, M. I.; Zerba, E. N. Toxic effect of aliphatic alcohols against susceptible and permethrin-resistant *Pediculus humanus capitis* (Anoplura: Pediculidae). *J. Med. Entomol.* **2002**, *39*, 457–460.

(25) Kong, J. O.; Lee, S. M.; Moon, Y. S.; Lee, S. G.; Ahn, Y. J. Nematicidal activity of plant essential oils against *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae). *J. Asia−Pac. Entomol.* **2006**, *9*, 173–178.

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