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# Nematicidal Activity of *N*-Substituted and *N,N*-Disubstituted Alkylamines against the Pine Wood Nematode, *Bursaphelenchus xylophilus*

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In the random screening of nematicidal compounds against the pine wood nematode, *Bursaphelenchus xylophilus*, using the immersion test, a series of *N*-alkyl- and *N,N*-dialkyl oleylamines, dialkylamines, and their related compounds were tested. Among these tested, didecylamine was identified as the most potentially-active nematicidal compound ($LC_{50}$; 4.19 $\mu$M, 1.24 ppm). Amongst a total of 23 alkylamines, six *N*- and *N,N*-dialkyl oleylamines, including oleylamine, and three dialkylamines showed a higher activity than octadecylamine ($LC_{50}$; 8.61 $\mu$M, 2.08 ppm) on a concentration basis. A total of 9 compounds tested indicated higher activity than octadecylamine on a molar basis.

In the previous paper, several *n*-alkylmonoamines such as tetradecylamine ($LC_{50}$; 9.80 $\mu$M), hexadecylamine ($LC_{50}$; 9.06 $\mu$M) and octadecylamine ($LC_{50}$; 8.61 $\mu$M) were demonstrated to show a remarkable nematicidal activity against the pine wood nematode, *Bursaphelenchus xylophilus*.[1],[*1] On the other hand, several *N*-substituted and *N,N*-disubstituted alkyl amines are known to exhibit unique activities such as nematicide against saprophytic nematode, *Panagrellus redivivus*, and a root-knot nematode, *Meloidoglyne incognita*.[2] Furthermore these amines and several amides are known as powerful acaricides against a psoroptic scabies mite of live stock,[3∼5] as nematicides against the medium stomach worm of cattle, *Ostertagia ostertagi*,[6] and as antimicrobial agents against some of the mastitic bacteria of cattle.[7,8] All of the amines hitherto tested, however, have been restricted to $C_{10}$-$C_{18}$ amines with *N*- or *N,N*-dimethyl or ethyl residue, and no assay has been conducted on amines possessing oleyl residue and symmetrically substituted di-alkylamines.

The pine wood nematode infested in the pine tree from the previous season is known to aggregate around the pupal case of the symbiotic carrier of the longhorn beetle, *Monochamus alternatus*, and to invade the tracheae of the carrier beetle in the following spring to disperse. One of the active compounds for this aggregation of the nematodes has been identified as oleic acid emitted from these longhorn beetles.[9]

On the other hand, oleylamine, *N*-methyloleylamine and *N,N*-dimethyloleylamine have been identified as potent nematicides against this nematode during an aggregation attractant study.[10] Therefore we systematically studied the nematicidal activity of *N,N*- and *N*-substituted oleylamines and symmetrically disubstituted alkylamines, and found that some compounds were potent nematicides against the pine wood nematode. Some relationship between the nematicidal activi-

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[*1] Used to be known as *Bursaphelenchus lignicolus*.

ty and chemical structure are also discussed.

## MATERIALS AND METHODS

*Nematode and bioassay method.* The same nematode, *Bursaphelenchus xylophilus* and the same method of bioassay were used as previously reported.[1] The bioassay data were processed and $LC_{50}$ values were determined. All test compounds were solubilized using Tween 80 and EtOH, as already reported.[1]

*Test compounds.* All of the following compounds were commercially available, and used without further purification: di-*n*-propylamine, di-*n*-butylamine, di-*n*-hexylamine, dioctylamine, didecylamine, oleyl alcohol, oleic acid, oleamide and oleylamine. The other amines and amides appearing in the text were all prepared by synthesis.

*Syntheses.* All bps and mps were uncorrected. The IR spectra were obtained in film or KBr-pellet with a JASCO model IRA-1 infrared spectrometer. The PMR spectra were measured with a JEOL MH-100 spectrometer in $CDCl_3$ with TMS as an internal standard. All amines were prepared by the following general procedure: An appropriate acyl chloride was condensed to an amine or ammonia to give the corresponding amide, which was reduced without further purification to the corresponding amine by $LiAlH_4$. All of the synthesized amines were purified by distillation or recrystallization from benzene–ethanol or acetone–pentane. In Table I, the physical data of the synthesized amines and amides appearing in the text are summarized. As a typical example of the preparation of amines, the following experimental detail for N-methyloleylamine is given.

*N-Methyloleylamine.* To the methylamine (3.1 g, 0.10 mol) in dry benzene (20 ml), a benzene solution (15 ml) of oleyl chloride (15 g, 0.05 mol) was added, keeping the temperature below 5°C. After addition, the mixture was stirred for 1 hr at room temperature. To the mixture, water was then added and the mixture was acidified. The product was extracted from the mixture with benzene three times and the combined extracts were washed with 2 N-HCl and dried over $MgSO_4$. After evaporation of the solvent, the crude amide was obtained as a deep yellow slushy solid (14.0 g, 94%). IR $\nu_{max}^{film}$ cm$^{-1}$; 3280, 3080, 1635, 1540 and 710.

The amide (7.6 g, 0.026 mol) suspended in ether (20 ml) was added dropwise to an ether suspension (25 ml) of $LiAlH_4$ (1.2 g, 0.03 mol) and the mixture was refluxed for 6 hr. After the careful addition of 2 N-NaOH to the reaction mixture, the product was extracted with ether for

TABLE I. PHYSICAL DATA FOR AMIDES AND AMINES PREPARED FOR THE TEST

| Compounds | Yield (%) | bp (°C/mmHg) | Lit. bp (°C/mmHg) |
|---|---|---|---|
| *N*-Methyloleamide | 94.0 | Slushy solid | |
| *N,N*-Dimethyloleamide | *ca.* 100 | Viscous oil | |
| *N*-Ethyloleamide | 70.0 | Viscous oil | |
| *N,N*-Diethyloleamide | 81.5 | Viscous oil | |
| *N*-Propyloleamide | 97.4 | Viscous oil | |
| *N,N*-Dipropyloleamide | 99.5 | Viscous oil | |
| *N*-Butyloleamide | 97.2 | Viscous oil | |
| *N,N*-Dibutyloleamide | *ca.* 100 | Viscous oil | |
| *N*-Methyloleylamine | 31.4 | 175 ~ 180/6 | 151 ~ 155/4[11] |
| *N,N*-Dimethyloleylamine | 36.9 | 178 ~ 179/7 | |
| *N*-Ethyloleylamine | 58.3 | 165 ~ 167/3 | |
| *N,N*-Diethyloleylamine | 47.8 | 178/3 | |
| *N*-Propyloleylamine | 58.2 | 168 ~ 173/1 | |
| *N,N*-Dipropyloleylamine | 49.5 | 169 ~ 189/1 | |
| *N*-Butyloleylamine | 53.2 | 175 ~ 178/1 | |
| *N,N*-Dibutyloleylamine | 26.4 | 186 ~ 188/1 | |
| Elaidylamine | | Slushy solid | |
| Linolenylamine | | Viscous oil | |
| Eicosanylamine | | 60 ~ 64 (mp) | 57.8 (mp)[12] |
| Diundecylamine | | 62 ~ 64 (mp) | 51.5 ~ 52.5 (mp)[13] |
| *N*-Ethyloctadecylamine | | 36 ~ 38 (mp) | |
| *N*-Butylhexadecylamine | | Slushy solid | 31 ~ 32 (mp)[14] |
| *N*-Hexyltetradecylamine | | Slushy solid | |
| *N*-Octyldodecylamine | | Viscous oil | |

12 hr using a continuous extraction apparatus. The ether layer was dried over $MgSO_4$ and distilled fractionally. Yield; 2.3 g (31.4%), bp 175~180°C/6 mmHg (lit.,[11] 151~155°C/4 mmHg). IR $\nu_{max}^{film}$ $cm^{-1}$; 3300, 1640 and 705. PMR $\delta$; 0.87 (3H, br. t), 1.30 (25H, br. s), 1.8~2.2 (4H, m), 2.41 (3H, s), 2.4~2.7 (2H, m) and 5.32 (2H, m).

## RESULTS AND DISCUSSION

Oleylamine and its related compounds were collected and their nematicidal activities were compared (Table II). Oleic acid and oleyl alcohol were completely inactive and oleylamine was active at $10^{-5}$ M. Elaidylamine gave the same level of activity as oleylamine, but linoleylamine showed a weaker activity than oleylamine and elaidylamine. Therefore, not only an optimum carbon-chain length, as indicated in the previous paper,[1] but also an optimum number of carbon-carbon double bonds seemed to be present in the molecule to enhance the activity, although the geometry of double bond did not seem to be important. For the series of *N*-alkyl-substituted oleylamines, the effect of the alkyl chain length on nematicidal activity was investigated (Table II). In the present assay results, all compounds from the methyl to butyl residue indicated almost the same level of activity and were active at $10^{-5}$ M. A more detailed comparison of activity based on $LC_{50}$-values is described later.

The effect of the alkyl chain on the *N*,*N*-dialkyl-substituted oleylamines was also compared (Table II). *N*,*N*-Dimethyloleylamine and *N*,*N*-diethyloleylamine showed the same activity and were active at $10^{-5}$ M. On the other hand, the longer alkyl chain homologues, *N*,*N*-dipropyl- and *N*,*N*-dibutyloleylamine, were less active. Consequently, an optimum carbon number for the substituted alkyl residue was noticed in this series.

Several secondary and tertially alkyl amides are known as potent acaricides against *Psoroptes cuniculi* and *P. ovis*.[3] Therefore, a total of eight *N*- and *N*,*N*-disubstituted oleamides, which were prepared as the intermediates for the syntheses of the substituted oleylamines, were assayed (Table III). *N*,*N*-Dimethyloleamide showed the highest activity ($10^{-5}$ M, 47.5%) amongst these amides. *N*-Methyloleamide was active at $10^{-3}$ M and the other six amides, except for *N*,*N*-dibutyloleamide were only active at $10^{-2}$ M. *N*,*N*-Dibutyloleamide was completely inactive.

On the symmetrical dialkylamines, the effect of the alkyl chain was examined (Table IV). Di-*n*-propylamine, di-*n*-butylamine and di-*n*-hexylamine were all active at $10^{-3}$ M. Didecylamine indicated the highest activity ($10^{-6}$ M; 9.3%). Both of the higher and lower homologues, dioctylamine ($10^{-5}$ M; 12%) and

TABLE II. NEMATICIDAL ACTIVITY OF OLEYLAMINES AND RELATED COMPOUNDS

| | Concentration (M) | | | | |
|---|---|---|---|---|---|
| | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ |
| Oleyl alcohol | − | − | | | |
| Oleic acid | − | − | | | |
| Oleamide | + | − | − | | |
| Oleylamine | + | + | + | 98.9% | − |
| Elaidylamine | + | + | + | 96.5% | − |
| Linolenylamine | + | + | − | − | − |
| *N*-Methyloleylamine | + | + | + | 98.1% | − |
| *N*-Ethyloleylamine | + | + | + | 95.7% | − |
| *N*-Propyloleylamine | + | + | + | + | − |
| *N*-Butyloleylamine | + | + | + | + | − |
| *N*,*N*-Dimethyloleylamine | + | + | + | 99.4% | − |
| *N*,*N*-Diethyloleylamine | + | + | + | 99.5% | − |
| *N*,*N*-Dipropyloleylamine | + | + | − | − | |
| *N*,*N*-Dibutyloleylamine | + | + | − | − | |

TABLE III. NEMATICIDAL ACTIVITY OF SUBSTITUTED OLEAMIDES

| | Concentration (M) | | | | |
|---|---|---|---|---|---|
| | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ |
| Oleamide | + | – | – | | |
| *N*-Methyloleamide | + | + | – | – | |
| *N*,*N*-Dimethyloleamide | + | + | + | 47.5% | – |
| *N*-Ethyloleamide | + | – | – | | |
| *N*,*N*-Diethyloleamide | + | – | – | | |
| *N*-Propyloleamide | + | – | – | | |
| *N*,*N*-Dipropyloleamide | + | – | – | | |
| *N*-Butyloleamide | + | – | – | | |
| *N*,*N*-Dibutyloleamide | – | – | – | | |

TABLE IV. NEMATICIDAL ACTIVITY OF DIALKYLAMINES

| | Concentration (M) | | | | |
|---|---|---|---|---|---|
| | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ |
| Di-*n*-propylamine | + | 46.9% | – | – | |
| Di-*n*-butylamine | + | 19.1% | – | – | |
| Di-*n*-hexylamine | + | + | – | – | |
| Dioctylamine | + | + | + | 12.0% | – |
| Didecylamine | + | + | + | + | 9.3% |
| Diundecylamine | + | + | + | – | – |
| *N*-Octyldodecylamine | + | + | + | 96.1% | – |
| *N*-Hexyltetradecylamine | + | + | + | 98.6% | – |
| *N*-Butylhexadecylamine | + | + | + | 59.9% | – |
| *N*-Ethyloctadecylamine | + | + | + | 37.8% | – |
| Eicosanylamine | + | + | + | – | – |

diundecylamine ($10^{-4}$ M; 100%), gave a lower activity than didecylamine. As a result, the optimum carbon number for inducing nematicidal activity was also confirmed for these types of compounds.

Using the most active compound, didecylamine, as the lead compound, the position of the secondary amino group was modified by shifting each by two carbons from the center to the terminal of the molecule. As a result, four dialkylamines with different chain lengths in the molecule such as $C_8$-NH-$C_{12}$, $C_6$-NH-$C_{14}$, $C_4$-NH-$C_{16}$ and $C_2$-NH-$C_{18}$ were prepared and assayed (Table IV). The assay result indicates that the nematicidal activity decreased gradually along with the position of the secondary amino group in a molecule as it was shifted from the center to the terminal. The order of activity between *N*-octyldodecylamine ($10^{-5}$ M; 96.1%) and *N*-hexyltetradecylamine ($10^{-5}$ M; 98.6%) in the present assay result was reversed, but a more precise comparison, using the $LC_{50}$-value which is described later, indicates that *N*-octyldodecylamine was more active.

For the active and interesting compounds, the $LC_{50}$-value was determined (Table V). Within the *N*-alkyl substituted oleylamines, *N*-propyloleylamine was the most active compound, and its $LC_{50}$ was 4.24 $\mu$M and 1.31 ppm. In the case of the *N*,*N*-disubstituted oleylamines, *N*,*N*-dimethyloleylamine was the most active (6.13 $\mu$M and 1.81 ppm). On a concentration basis, six compounds within a total of nine *N*- and *N*,*N*-disubstituted oleylamines, including oleylamine, indicated $LC_{50}$-

TABLE V. $LC_{50}$ VALUES AND THEIR FIDUCIAL LIMITS

| Compound | $LC_{50}$ ($\mu$M) | $LC_{50}$ (ppm) | Fiducial limits ($\mu$M) |
|---|---|---|---|
| Oleylamine | 6.88 | 1.84 | 6.68 ~ 7.08 |
| *N*-Methyloleylamine | 5.20 | 1.46 | 4.86 ~ 5.57 |
| *N*-Ethyloleylamine | 5.08 | 1.50 | 4.73 ~ 5.45 |
| *N*-Propyloleylamine | 4.24 | 1.31 | 3.98 ~ 4.53 |
| *N*-Butyloleylamine | 4.30 | 1.39 | 4.16 ~ 4.56 |
| *N,N*-Dimethyloleylamine | 6.13 | 1.81 | 5.82 ~ 6.46 |
| *N,N*-Diethyloleylamine | 9.86 | 3.18 | 9.33 ~ 10.4 |
| Dioctylamine | 20.4 | 4.92 | 18.6 ~ 22.4 |
| Didecylamine | 4.19 | 1.24 | 4.02 ~ 4.37 |
| *N*-Octyldodecylamine | 5.40 | 1.60 | 5.09 ~ 5.72 |
| *N*-Hexyltetradecylamine | 5.73 | 1.70 | 5.56 ~ 6.09 |
| *N*-Butylhexadecylamine | 8.84 | 2.62 | 8.28 ~ 9.44 |
| *N*-Ethyloctadecylamine | 10.2 | 3.02 | 9.56 ~ 10.9 |

values less than 2 ppm, and gave a higher activity than octadecylamine (8.61 $\mu$M and 2.08 ppm), which had been identified as the most potent nematicidal alkylamine in the previous paper.[1] All of the *N,N*-disubstituted oleylamines were less active than the corresponding *N*-alkyloleylamines.

In the case of the symmetrically substituted dialkylamines, didecylamine was the most active ($LC_{50}$; 4.19 $\mu$M and 1.24 ppm). Its activity was the highest of all the compounds tested. As already mentioned, the biological activity decreased gradually along with the shift of the secondary amino group from the center to the terminal of the molecule. On a concentration basis, *N*-octyldodecylamine and *N*-hexyltetradecylamine showed their $LC_{50}$-values at 1.60 ppm and 1.70 ppm, respectively, which is still more active than oleylamine (1.84 ppm). Again, the three dialkylamines showed a higher activity than octadecylamine (8.61 $\mu$M and 2.08 ppm), not only on a molar basis but also on a concentration basis.

From above results, it was found that *N*-propyloleylamine ($LC_{50}$; 4.24 $\mu$M and 1.31 ppm) was the most active of the series of *N*-substituted oleylamines. In the case of the *N,N*-disubstituted oleylamines, the longer the carbon chain, the lower became the activity, and *N,N*-dimethyloleylamine ($LC_{50}$; 6.13 $\mu$M and 1.81 ppm) was the most active. In the case of the symmetrically substituted dialkylamines, didecylamine ($LC_{50}$: 4.19 $\mu$M and 1.24 ppm) was the most active amongst all the compounds tested, although the activity of dinonylamine was not then known. Therefore, in all cases the optimum carbon number for inducing nematicidal activity was confirmed. In the case of the unsymmetrically substituted dialkylamines the position of the secondary amino group in the molecule was important for enhancement of the activity.

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