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# Fungicidal Activity of Some Organotin Compounds against Ceratocystis ulmi

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**Three series of organotin compounds were screened *in vitro* against *Ceratocystis ulmi*, the causative agent of Dutch elm disease. The series that were most active against this deadly fungus were those that contained the triphenyltin moiety. In addition, the fungicidal activity of the triphenyltin compounds were found to be independent of the anionic group attached to the tin atom. However, there was a marked increase in the activity of the triphenyltins when a biologically active group which did not dissociate upon dissolution was incorporated into the overall molecule.**

**Keywords: *Ceratocystis ulmi*; dialkyltin; Dutch elm disease; fungicide; organotin; triphenyltin**

## INTRODUCTION

Organotin compounds, especially triphenyltins [$(C_6H_5)_3SnX$], have been used as broad-spectrum fungicides against various agricultural crops.[1–3] Earlier *in vitro* studies from this laboratory have indicated that this class of organotin compounds is an effective inhibitor of *Ceratocystis ulmi*, the fungus responsible for Dutch elm disease (DED).[4–7] DED has destroyed millions of elm trees since its first identification by a Dutch investigator, Marie Schwarz, in the early 1920s.[8] The development of a more effective fungicide than those currently being used would help reduce the immense financial and aesthetic losses resulting from the destruction of the elms. In our continuing efforts to develop more effective fungicides in the inhibition of *C. ulmi*, our laboratories have synthesized several series of organotin compounds and screened them *in vitro* against this fungus. The first of the three series is the dialkyltin *N*-arylidene-$\alpha$-amino acid complexes; the second series contains the triphenyltin adducts of *N*-alkylsalicylideneimines; the third series comprises triphenyltins containing sulfur ligands covalently bonded to the tin atom. The results of these screening studies are reported herein.

## EXPERIMENTAL

### Synthesis of the compounds

All starting materials were obtained commercially and used without further purification. The organotin derivatives were synthesized by previously published methods.[9–12]

### Preparation of stock organotin solutions and fungicidal activity

The activity of the organotin compounds was tested by incorporating the appropriate amounts of organotin compound into 100 $cm^3$ of potato dextrose broth (PDB). The amount of organotin compound added represented the ranges of concentrations to be studied. Stock solutions of organotins were made up in methanol or acetone (1000 mg $dm^{-3}$). The appropriate solvent, methanol or acetone, was added to the control and test flasks so that the total volume (methanol or acetone and organotin solution) was 400 µl. A stock suspension (1.0 $cm^3$) of cells (concentration = $10^6$ cells $cm^{-3}$) of *C. ulmi*, strain 32437, obtained from the American Type Culture Collection, Rockville, Maryland, was added to the amended PDB, and the resulting suspension was

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then shaken in an incubator–shaker (7 d; 22 °C) in total darkness. The contents of the flasks were then filtered and rinsed thoroughly with distilled water. The fungal growth was dried and weighed. Three replicates were used for each concentration tested.

The $IC_{50}$ values were obtained by plotting the percentage growth of the fungus versus the concentration of organotin compound (parts per million) added. The concentration at which 50% of the fungus is inhibited is taken as the $IC_{50}$ value.

## RESULTS AND DISCUSSION

The first series to be screened was the dialkyltin *N*-arylidene-$\alpha$-amino acid complexes. As is evident from the results presented in Table 1, this series did not prove to be an effective inhibitor of the fungus, since the average $IC_{50}$ value is 10.5 mg $dm^{-3}$ as compared with 3.4 mg $dm^{-3}$ for the triphenyltin adducts of *N*-alkylsalicylideneimines (Table 2) and 0.9 mg $dm^{-3}$ for the triphenyltins containing sulfur ligands (Table 3). This observation is in agreement with the known biological properties of organotin compounds, in that diphenyltins are not effective inhibitors of fungi.[1,2]

Table 2 contains the screening results for the 13 triphenyltin adducts of N-alkylsalicylideneimines. As is evident from the data, this class of compounds is an effective inhibitor of *C. ulmi*. The $IC_{50}$ values for this series ranged from 1.9 to 5.6 mg $dm^{-3}$. Variation of the R groups on the Schiff base portion of the molecule did not result in an observable trend. This is most likely due to the fact that the R group is too far removed from the tin atom to have any major influence on the effect of the triphenyltin moiety. The results also suggest that the anionic group X attached to the tin atom does not play a significant role in the activity of the adducts, since the average $IC_{50}$ values for X=chlorine and X=thiocyanate are 3.2 and 3.7 mg $dm^{-3}$, respectively. This observation supports earlier results that the fungicidal activity of $Ph_3SnX$ compounds against *C. ulmi* was found to be independent of the anionic X group.[4,5] The generalization that the fungicidal activity of triphenyltin compounds is independent of the anionic X group attached to the tin atom has also been reported in previous studies.[3,13] Earlier screening results with other triorganotins suggested that the species responsible for the inhibition of the *C. ulmi* is the $Ph_3Sn^+$ cation or its hydrated species, which is produced through a series of dissociation steps.[4,6,7] The data from this study would support this idea.

The last series of organotin compounds screened against *C. ulmi* were five triphenyltins

**Table 1** Inhibitory concentrations ($IC_{50}$ values) of dialkyltin *N*-arylidene-$\alpha$-amino acid complexes against *C. ulmi* in potato dextrose broth at 22 °C

| $(OArCR'{=}NCHR''CO_2)SnR_2$ | | | | |
|---|---|---|---|---|
| Ar | R | R′ | R″ | $IC_{50}$ (mg $dm^{-3}$) |
| $C_6H_4$ | $CH_3$ | H | $CH_3$ | 8.5 |
| $C_6H_4$ | $CH_3$ | H | $C_6H_5$ | 9.9 |
| $C_6H_4$ | $CH_3$ | H | $CH_2CONH_2$ | 4.3 |
| $C_6H_4$ | $CH_3$ | H | $CH_2OH$ | 7.1 |
| $C_6H_4$ | $CH_3$ | H | H | 15.5 |
| $C_6H_4$ | n-$C_4H_9$ | H | $CH_3$ | 5.9 |
| $C_6H_4$ | n-$C_4H_9$ | H | $C_6H_5$ | 3.1 |
| $C_6H_4$ | n-$C_4H_9$ | H | $CH_2CONH_2$ | 14.7 |
| $C_6H_4$ | n-$C_4H_9$ | H | $CH_2OH$ | 11.8 |
| $C_6H_4$ | n-$C_4H_9$ | H | $CH(CH_3)_2$ | 13.9 |
| $C_6H_4$ | n-$C_4H_9$ | H | H | 11.9 |
| $C_6H_4$ | n-$C_4H_9$ | $CH_3$ | H | 18.1 |
| $C_{10}H_6$ | $CH_3$ | H | H | 9.9 |
| $C_{10}H_6$ | n-$C_4H_9$ | H | H | 12.3 |
| Average for the series | | | | 10.5 |

**Table 2** Inhibitory concentration ($IC_{50}$) values for triphenyltin *N*-alkylsalicylideneimine adducts against *C. ulmi* in potato dextrose broth at 22 °C

| $(C_6H_5)_3SnX$·*o*-$HOC_6H_4CH{=}NR$ | | |
|---|---|---|
| R | X | $IC_{50}$ (mg $dm^{-3}$) |
| c-$C_6H_{11}$ | NCS | 5.5 |
| $C_6H_5CH_2$ | Cl | 2.0 |
| $C_6H_5CH_2$ | NCS | 2.3 |
| $(CH_3)_3C$ | NCS | 2.4 |
| $(CH_3)_3C$ | Cl | 2.8 |
| $CH_3$ | Cl | 5.6 |
| $HOCH_2CH_2$ | Cl | 3.4 |
| $HOCH_2CH_2$ | NCS | 3.6 |
| c-$C_6H_{11}$ | Cl | 1.9 |
| n-$C_6H_{13}$ | Cl | 3.9 |
| $CH_3$ | NCS | 4.7 |
| $CH_2COOCH_2CH_3$ | Cl | 2.8 |
| $CH(CH_3)COOCH_3$ | Cl | 3.2 |
| Average for the series | | 3.4 |
| Average for X=Cl | | 3.2 |
| Average for X=NCS | | 3.7 |

**Table 3** Inhibitory concentration ($IC_{50}$) values for triphenyltin sulfides against *C. ulmi* in potato dextrose broth at 22 °C

| $(C_6H_5)_3SnSR$ | |
|---|---|
| R | $IC_{50}$ (mg $dm^{-3}$) |
| 2-($C_5H_4NO$) | 0.45 |
| 4-$C_6H_4NH_2$ | 1.35 |
| $C(S)N(CH_2CH_2CH_3)_2$ | 0.80 |
| $CH_2CH_2OH$ | 0.50 |
| $C(N{=}NC_6H_5)NHNHC_6H_5$ | 1.20 |
| Average for the series | 0.86 |

that contained a sulfur ligand covalently bonded to the tin atom. Ligands containing one or two sulfur atoms were selected for this study since many sulfur-bearing compounds possess biological properties. For example, dithiocarbamates are known to possess fungicidal properties.[14–16] In addition, the sulfur-containing group is not expected to dissociate upon dissolution. The results are presented in Table 3. The average $IC_{50}$ for this group of compounds is 0.9 mg $dm^{-3}$, indicating that there is a marked improvement over the activity for the triphenyltin adducts of *N*-alkylsalicylideneimines ($IC_{50}$=3.4 mg $dm^{-3}$). The significant improvement in the activity for this series may be attributed to the addition of a biologically active group. Thus, incorporating a biologically active ligand on the triphenyltin molecule may enhance the overall activity of the compound through a synergistic effect. An alternative explanation might be that the non-dissociating S ligand may assist in carrying the biocidal tin center through the cell wall of the fungus.

## CONCLUSIONS

This study has shown that organotins containing the triphenyltin moiety are better inhibitors of *C. ulmi* than dialkyltin compounds. It is further evident that incorporating a biologically active ligand can enhance the overall fungicidal activity of the organotin compound. This observation is significant in that it can aid in the design of new compounds for the treatment of Dutch elm disease.

## REFERENCES


1. A. G. Davis and P. J. Smith, in *Comprehensive Organometallic Chemistry*, Wilkinson, G., Stone, F. G. A. and Abel, E. W. (eds.), Vol. 2, Pergamon Press, New York, 1982, p. 519.
2. S. J. Blunden and A. Chapman, in *Organometallic Compounds in the Environment*, Craig, P. J. (ed.), John Wiley and Sons, New York, 1986, p. 111.
3. A. K. Saxena, *Appl. Organomet. Chem.* **1**, 39 (1989).
4. G. Eng, S. P. Coddington, L. L. Stockton and A. D. W. Acholonu, *Pestic. Sci.* **26**, 117 (1989).
5. G. Eng and A. D. W. Acholonu, *Appl. Organomet. Chem.* **5**, 131 (1991).
6. G. Eng, L. E. Khoo, L. L. Stockton and S. P. Coddington, in *Chemistry and Technology of Silicon and Tin*, Kumar Das, V. G., Ng, S. W. and Gielen, M. (eds), Oxford University Press, New York, 1992, p. 334.
7. G. Eng, Y. Z. Zhang, D. Whalen, R. Ramsammy, L. E. Khoo and M. DeRosa, *Appl. Organomet. Chem.* **8**, 445, (1994).
8. F. W. Holmes, in *Dutch Elm Disease Research*, Sticklen, M. B. and Sherald, J. L. (eds), Springer, New York, 1993, p. 9.
9. L. E. Khoo and F. E. Smith, *Polyhedron* **4**, 447 (1985).
10. S. Chandra, B. D. James, B. J. Macauley and R. J. Magee, *J. Chem. Tech. Biotechnol.* **39**, 65 (1987).
11. B. D. James, S. Chandra, R. J. Magee and J. D. Cashion, *J. Organomet. Chem.* **436**, 155 (1992).
12. F. E. Smith, R. C. Hynes, T. T. Ang, L. E. Khoo and G. Eng, *Can. J. Chem.* **70**, 1114 (1992).
13. R. C. Poller, *The Chemistry of Organotin Compounds*, Academic Press, New York, 1970, p. 276.
14. G. D. Thorn and R. A. Ludwig, *The Dithiocarbamates and Related Compounds*, Elsevier, New York, 1991.
15. H. L. Klopping and G. J. M. van der Kerk, *Recl. Trav. Chim. Pays-Bas* **70**, 917 (1951).
16. G. J. M. van der Kerk and H. L. Klopping, *Recl. Trav. Chim. Pays-Bas* **71**, 117 (1952).