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Structure–Activity Relationships of Effect of Aryltin Compounds on *Ceratocystis ulmi*

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The effect of aryltin compounds (Ar_4Sn and Ar_3SnCl) on the growth of *Ceratocystis ulmi*, the causative agent of Dutch elm disease, was studied in shake culture. In all cases, the triaryltins were more effective than the tetra-aryltins as inhibitors of *C. ulmi in vitro*. Furthermore, substitution on the phenyl ring at the *meta*- and *para*- positions in the triaryltins did not have a major effect on the biocidal activity for the substituted triaryltins. Quantitative structure–activity relationships (QSARs) gave support to the idea that the species responsible for the inhibition of the fungus is the triaryltin cation. The QSARs further suggest that the interaction of the triaryltin cation with the fungal cell wall of *C. ulmi* is by a non-specific mechanism.

Keywords: Organotin, aryltin, triaryltin, tetra-aryltin, toxicity, Mössbauer, QSAR, quantitative structure–activity, fungus, *Ceratocystis ulmi*, fungicide, Dutch elm disease

INTRODUCTION

The use of organotin compounds of the type R_3SnX as selective biocides has increased in the last several decades because of their favorable environmental and toxicological properties.[1–6] The nature of the organic (R) group is the factor which determines the living species affected by the triorganotin compound.[1–6] For example, the methyl derivatives have a high toxicity towards insects and mammals,[1–6] the tributyltin compounds are toxic to Gram-positive bacteria or fungi[1–6] and the triphenyltin compounds are effective agricultural fungicides.[1–7] The effect of the anionic group (X) is not clear, as reports cite both the importance and insignificance of this group.[1,3,7,8]

Our laboratory has screened several organotin compounds against *Ceratocystis ulmi*, the causative agent of Dutch elm disease (DED), with the intent of developing a more effective fungicide to combat this disease. Previous studies from our laboratory[9–11] have indicated that organotin compounds containing the triphenyltin moiety are an effective inhibitor of *C. ulmi*. Therefore, we have synthesized several substituted tetra-aryltins and triaryltin chlorides. These compounds were screened against the fungus in order to determine whether substitution on the phenyl ring of the aryltins has a major impact on the inhibition of the *C. ulmi*, as well as to gain an insight into the effect of the organotin structure on fungal inhibition.

EXPERIMENTAL

Preparation of the substituted aryltins

The compounds were prepared according to known literature procedures.[12] Tetra-aryltins were synthesized by reaction of the appropriate Grignard reagent in anhydrous diethyl ether or tetrahydrofuran with anhydrous tin(IV) chloride in a 4:1 molar ratio. The triaryltin chlorides were prepared by the reaction of the desired tetra-aryltin with anhydrous tin(IV) chloride in a 3:1 molar ratio. The products were purified by recrystallization from ligroin or petroleum ether (30–60 °C). The elemental analyses and the melting points are given in Table 1.

Elemental analysis

The elemental analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, New York, USA.

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Table 1 Analytical data for substituted phenyltins

Compound	M.p. (°C)	Analysis: found (calcd) (%) C	H	Sn
Tetrasubstituted phenyltins				
(*m*-ClPh)$_4$Sn	186–190	51.26 (51.01)	3.03 (2.85)	20.94 (21.01)
(*m*-CH$_3$OPh)$_4$Sn	87–88	61.33 (61.45)	5.18 (5.17)	21.50 (21.69)
Trisubstituted phenyltin chlorides				
(*m*-ClPh)$_3$SnCl	84–85	44.19 (44.21)	2.76 (2.48)	24.03 (24.27)
(*m*-CH$_3$OPh)$_3$SnCl	99–100	53.03 (53.02)	4.54 (4.46)	24.85 (24.96)
(*p*-t-C$_4$H$_9$Ph)$_3$SnCl	212–216	64.95 (65.05)	7.11 (7.11)	21.63 (21.43)

Preparation of stock organotin solutions

The organotin compounds to be screened against *C. ulmi* were dissolved individually in methanol or acetone to give a final concentration of 10 mg cm^{-3} for the tetrasubstituted aryltin and 2.5 mg cm^{-3} for the trisubstituted aryltin chloride. The solutions were sterilized by filtration through a 0.45 μm Gelman or Whatman Sterile acrodisc. The tin solutions were then stored in sterilized tubes.

The fungus

The preparation of the fungus for the toxicity studies has been previously described.[9–11]

Fungicidal activity

The biocidal activity of the organotin compounds was tested by incorporating the appropriate amounts of toxicant into 100 cm^3 of potato dextrose broth (PDB). The amounts of toxicants added represented a range of concentrations to be studied. 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. Stock suspension (0.1 cm^3) of cells was added to the amended PDB and the resulting suspension was then shaken in an incubator–shaker (7 days; 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 until a constant weight was obtained. 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 added. The concentration at which 50% of the fungus was inhibited was taken as the IC_{50} value.

Mössbauer Spectroscopy

The Mössbauer spectra were measured at 80K on a Mössbauer spectrometer model MS-900 (Ranger Scientific Co., Burelson, TX) in the acceleration mode with a moving source geometry using a liquid nitrogen cryostat (CYRO Industries of America, Inc., Salem, NH). The samples were mounted in teflon holders. The source was 15 mCi $Ca^{119m}SnO_3$, and the velocity was calibrated at ambient temperature using a composition of $BaSnO_3$ and Sn foil (splitting = 2.52 mm^{-1}). The resultant spectra were analyzed by a least-square fit to Lorenzian shaped lines.

RESULTS AND DISCUSSION

Mössbauer spectroscopy for the elucidation of organotin molecular structure is well established. The Mössbauer data for these compounds are listed in Table 2. Structural information has been determined based on quadrupole splitting and

Table 2 Mössbauer spectral parameters[a] for substituted phenyltins

Compound	QS	IS	ρ
Tetrasubstituted phenyltins			
(*p*-FPh)$_4$Sn	1.22 ± 0.02		
(*m*-CH$_3$Ph)$_4$Sn	1.22 ± 0.02		
(*m*-ClPh)$_4$Sn	1.19 ± 0.02		
(*p*-CH$_3$OPh)$_4$Sn	1.24 ± 0.02		
(*m*-CH$_3$OPh)$_4$Sn	1.23 ± 0.02		
(*m*-CH$_3$SPh)$_4$Sn	1.24 ± 0.02		
Trisubstituted phenyltin chlorides			
(*m*-ClPh)$_3$SnCl	2.41 ± 0.07	1.28 ± 0.02	1.88
(*m*-CH$_3$OPh)$_3$SnCl	2.43 ± 0.07	1.28 ± 0.02	1.89
(*m*-CH$_3$Ph)$_3$SnCl	2.76 ± 0.07	1.34 ± 0.02	2.05
(*p*-t-C$_4$H$_9$Ph)$_3$SnCl	2.31 ± 0.07	1.26 ± 0.02	1.83
(*p*-FPh)$_3$SnCl	2.35 ± 0.07	1.25 ± 0.02	1.88
(*p*-CH$_3$OPh)$_3$SnCl	2.69 ± 0.07	1.29 ± 0.02	2.08

[a] All values in mm s^{-1} relative to $BaSnO_3$ at 80 K.

Table 3 Inhibitory concentrations[a] (IC_{50}, log mM) of tetra-substituted phenyltins against *C. ulmi* in potato dextrose broth at 22 °C

Compound	IC_{50} (mg dm^{-3})	Log IC_{50} (mM)
Ph_4Sn	7.6	−1.75
(*m*-ClPh)$_4$Sn	15.7	−1.54
(*m*-$CH_3OPh)_4Sn$	19.4	−1.45
(*m*-$CH_3Ph)_4Sn$	11.1	−1.63
(*p*-ClPh)$_4$Sn	21.9	−1.40
(*p*-$CH_3SPh)_4Sn$	15.4	−1.60
(*p*-$CH_3Ph)_4Sn$	11.5	−1.62
(*p*-t-$C_4H_9Ph)_4Sn$	10.1	−1.81

[a] The concentration at which 50% of the fungus is inhibited.

isomer shift values as well as the ρ value, the ratio of the quadrupole splitting to the isomer shift. The observation of only a single peak in the Mössbauer spectra for the tetra-aryltin compounds indicates that the stereochemistry of these compounds is tetrahedral. The observed isomer shifts range from 1.19 mm s^{-1} to 1.24 mm s^{-1}, which is in agreement with the known values for tetra-aryltin compounds[13] cited in the literature.

Because the triaryltin chloride molecule is asymmetric, Mössbauer data are expected to contain both quadrupole splitting and isomer shift values. A review of the literature indicates that both the quadrupole splitting and isomer shift parameters obtained are within the range of those found for other triorganotin chlorides.[13] Observed ρ values of greater than 2.1 further indicate that the coordination of the tin atom is greater than four.[14,15] The above results confirm that the triaryltin chlorides have the expected tetrahedral structure.

The results of the bioassay in a shake culture medium for the tetra-aryltin and triaryltin compounds are summarized in Tables 3 and 4, respectively. A comparison of the log IC_{50} values for the tetra-aryltin compounds with those for the triaryltin compounds clearly indicates that compounds which contain three aryl groups are far superior to those containing four in their inhibition of *C. ulmi*. This is consistent with known antifungicidal activity of organotin compounds.[2,4-6] The biological activity of tetraorganotin compounds arises from their dealkyl- or dearyl-ation to the corresponding triorganotin compounds.[16,17] The data indicate that all substituted tetra-aryltin compounds tested against *C. ulmi*, with the exception of tetrakis-(*p*-t-butyltin), are less effective in the inhibition of the fungus when compared with the parent compound, tetraphenyltin. It is possible that tetrakis-(*p*-t-butyltin) is easier to dearylate. This may be attributed to a steric effect due to the bulky t-butyl group.

Results of the screening of the triaryltins, in general, indicate that compounds with *meta* substituents on the phenyl ring have a slightly higher inhibition of the *C. ulmi* than those compounds with para substituents. However, in both cases, the fungal inhibition of these compounds is lower than that of the parent compound, triphenyltin chloride.

Previously, our laboratory[18] has studied the interaction between a series of triphenyltin compounds and *C. ulmi* using Mössbauer spectroscopy. It was concluded that it was the triphenyltin cation (Ph_3Sn^+) that binds to the cell wall and is the species which is responsible for the inhibition of the *C. ulmi*. We speculate that a similar species, Ar_3Sn^+, would be responsible for the inhibition of *C. ulmi* in the present study.

A quantitative structure–activity relationship (QSAR) is a regression equation that relates some measurable biological activity to properties associated with the structure of the molecule. A commonly used descriptor for QSARs studies is the partition coefficient.[19] Other descriptors include various electronic and topological parameters. These types of correlations have proved useful for predicting the toxicities of organotin compounds. In previous studies, high correlations were obtained between the total surface areas (TSAs) and the biotoxicities for several series of organotin compounds.[20–22] In order to determine whether such relationships exist for the present study, QSARs between the triaryltin chlorides and the inhibition of *C. ulmi* were obtained by plotting log $1/C$ ($C = IC_{50}$ in mM) against various

Table 4 Inhibitory concentrations[a] (IC_{50}, log mM) of trisubstituted phenyltin chlorides against *C. ulmi* in potato dextrose broth at 22 °C

Compound	IC_{50} (mg dm^{-3})	Log IC_{50} (mM)
Ph_3SnCl	2.10	−2.26
(*m*-ClPh)$_3$SnCl	2.10	−2.37
(*m*-$CH_3OPh)_3SnCl$	1.60	−2.47
(*m*-$CH_3Ph)_3SnCl$	2.30	−2.27
(*p*-ClPh)$_3$SnCl	1.45	−2.53
(*p*-$CH_3SPh)_3SnCl$	2.50	−2.32
(*p*-$CH_3Ph)_3SnCl$	2.60	−2.22
(*p*-FPh)$_3$SnCl	1.85	−2.48
(*p*-t-$C_4H_9Ph)_3SnCl$	4.60	−2.08

[a] The concentration at which 50% of the fungus is inhibited.

electronic, partition and topological descriptors. In contrast to earlier work, a poor correlation ($r=0.55$) was obtained with respect to the TSAs in the present study. Similarly, a poor correlation was also obtained when the partition coefficient, π, was used. These results indicate that neither topological nor partitioning effects are important in determining the activity of the triaryltin chlorides used in this study.

If the earlier hypothesis, that the triaryltin cation is responsible for the inhibition, is correct, then ring substituents would be expected to affect the stability and therefore the reactivity of the cation. This effect can be studied quantitatively by carrying out a correlation between the σ substituent parameters and the biotoxicity of the triaryltin chlorides. A plot of log $1/C$ versus σ resulted in a parabolic curve (Fig. 1) with the following equation ($r=0.93$, $n=9$):

$$\log 1/C = 2.36 + 0.92\sigma - 1.98\sigma^2$$

However, when the electronic effects were dissected into polar and resonance components, no further improvement in the correlation was observed. This indicated that the inhibition was equally sensitive to inductive and resonance effects.

In order to gain a better understanding of the electronic effects in this series of compounds, a correlation was carried out between log $1/C$ and tin-119 chemical shifts. Tin-119 chemical shifts might be expected to be a better descriptor of the electronic effects in the present system since σ constants are derived from the ionization of benzoic acids,[23] while tin-119 chemical shift values reflect the electronic density at the tin nucleus.

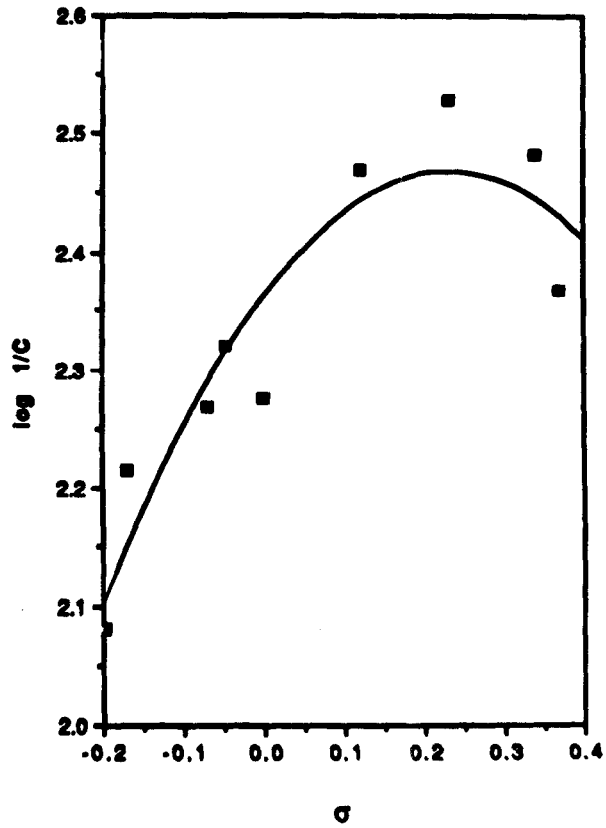

Figure 1 A plot of the logarithm of the reciprocal of the triaryltin inhibitory concentrations (IC_{50}) log $1/C$ versus hammett sigma values (σ).

Using tin-119 chemical shift data from the literature,[12] a plot of log $1/C$ versus tin-119 substituent chemical shift ($\delta_{subs}-\delta_H$) was obtained. This is shown in Fig. 2. The same type of parabolic relationship ($r=0.84$) is obtained as in the

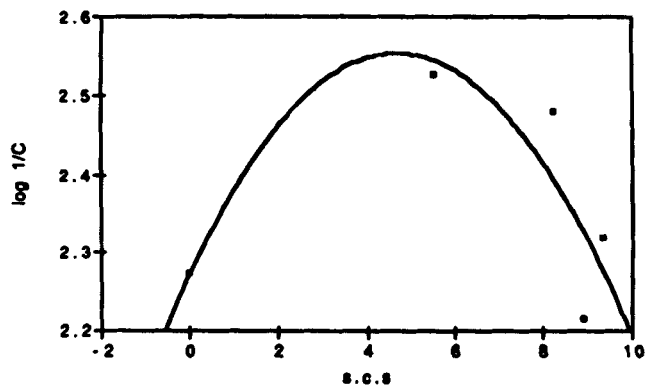

Figure 2 A plot of the logarithm of the reciprocal of the triaryltin inhibitory concentrations (IC_{50}) log $1/C$ versus tin-119 substituent chemical shift values (s.c.s.).

earlier plot of log $1/C$ values and σ constants (Fig. 1).

A parabolic (convex) Hammett plot is obtained when there is a change in the rate-determining step in multistep processes.[23] Inhibition by triaryltin chlorides consists of two basic processes: formation of the aryltin cation and its subsequent reaction with the cell wall. The curvature observed in Fig. 1 is attributed to a change in the rate-determining step from the formation of the aryltin cation to its reaction with the cell wall. When electron-donating substituents are present, the slow step is the interaction of the aryltin cation with the cell wall, and when electron-withdrawing substituents are present, the slow step is the formation of the cation. Examples of such changes in rate-determining steps are well known in carbocation chemistry.[23]

The QSARs presented in this study support the proposed mechanism in which the reactive intermediate is a triaryltin cation. However, it does not provide any direct evidence as to how the triaryltin cation interacts with the cell wall of the *C. ulmi*. In general, drugs act by either a structurally specific or non-specific mechanism.[24] In the former case they usually react with a specific receptor, whereas in the latter case they do not become attached to any specific receptor. Examples of non-specific drugs are antiseptics, disinfectants and preservatives.[24] In these cases, the activities have been found not to be very sensitive to changes in the structure of the compounds. Therefore, the data in Table 4 would suggest a non-specific mode of action for the triaryltin chlorides used in this study, since only small variations in activity are observed.

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REFERENCES

1. S. Nicklin and M. W. Robson, *Appl. Organomet. Chem.* **2**, 487 (1988).
2. A. J. Crowe, *Appl. Organomet. Chem.* **1**, 143 (1987).
3. A. K. Saxena, *Appl. Organomet. Chem.* **1**, 39 (1987).
4. S. J. Blunden and A. Chapman, in *Organometallic Compounds in the Environment*, edited by P. J. Craig, p. 111. New York (1986).
5. A. G. Davies and P. J. Smith, in *Advances in Inorganic Chemistry and Radiochemistry*, edited by H. J. Emeléus and A. G. Sharpe, Vol. 23, p. 1. Academic Press, New York (1980).
6. A. G. Davis and P. J. Smith, in *Comprehensive Organometallic Chemistry*, edited by G. Wilkinson, F. G. A. Stone and E. W. Abel, Vol. 2, p. 519. Pergamon Press, New York (1982).
7. V. G. Kumar Das, A. J. Kuthubutheen, S. Balabaskaran and S. W. Ng, *Main Group Metal Chem.* **12**, 389 (1989).
8. C. J. Evans in *Chemistry of Tin*, edited by P. G. Harrison, p. 421. Chapman and Hall, New York (1989).
9. G. Eng, S. P. Coddington, L. L. Stockton and A. D. W. Acholonu, *Pestic. Sci.* **26**, 117 (1989).
10. G. Eng and A. D. W. Acholonu, *Appl. Organomet. Chem.* **5**, 131 (1991).
11. G. Eng, L. E. Khoo, L. L. Stockton and S. P. Coddington, in *Chemistry and Technology of Silicon and Tin*, edited by V. G. Kumar Das, S. W. Ng and M. Gielen, p. 334. Oxford University Press, New York (1992).
12. I. Wharf, *Inorg. Chim. Acta* **159**, 41 (1989).
13. J. J. Zuckerman, in *Advances in Organometallic Chemistry*, edited by F. G. A. Stone and R. West, p. 21. Academic Press, New York (1970).
14. R. H. Herber, H. A. Stöckler and W. T. Reichle, *J. Chem. Phys.* **42**, 2447 (1965).
15. W. T. Reichle, *Inorg. Chem.* **5**, 87 (1966).
16. J. E. Cremer, *Biochem. J.* **68**, 685 (1958).
17. E. C. Kimmel, R. H. Fish and J. E. Casida, *J. Agric. Food Chem.* **25**, 1 (1977).
18. L. May, G. Eng, S. P. Coddington and L. L. Stockton, *Hyperfine Interact.* **42**, 909 (1988).
19. Y. C. Martin, in *Quantitative Drug Design*, p. 62. Marcel Dekker, New York (1978).
20. G. Eng, E. J. Tierney, J. M. Bellama and F. E. Brinckman, *Appl. Organomet. Chem.* **2**, 171 (1988).
21. G. Eng, E. J. Tierney, G. J. Olson, F. E. Brinckman and J. M. Bellama, *Appl. Organomet. Chem.* **5**, 37 (1991).
22. E. Luedke, E. Lucero and G. Eng, *Main Group Metal Chem.* **14**, 37 (1991).
23. C. D. Johnson, in *The Hammett Equation*, pp. 1, 55. Cambridge University Press, Cambridge (1973).
24. M. S. Tute, in *Quantitative Structure–Activity Relationships of Drugs*, edited by J. G. Topliss, p. 25. Academic Press, New York (1983).