APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. **14**, 34–39 (2000)

Studies in Aryltin Chemistry: Part 13. Spectroscopic and Fungicidal Studies of some *m*- and *o*-substituted Triaryltin Acetates, Oxides and Hydroxides

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The fungicidal activity of a series of aryltin compounds, Ar_3SnOAc (Ar = *m*-Tol (*m*−$CH_3C_6H_4$), 3,5-Xyl [3,5-$(CH_3)_2C_6H_3$], *o*-Tol (*o*−$CH_3C_6H_4$) or Mes [2,4,6-$(CH_3)_3C_6H_2$]) and $(Ar_3Sn)_2O$ [Ar = *m*-Tol, *m*-Anis (*m*-$CH_3OC_6H_4$), or *o*-Tol] as well as $(Mes)_3SnOH$, for which IR and NMR (^{119}Sn) data are reported, has been assessed by radial growth assays on *Aspergillus niger, Botrytis cinera, Mucor hiemalis, Fusarium solani* and *Penicillium chrysogenum*, and the results are compared with those for the corresponding triphenyl- and tris (*p*-tolyl)-tin compounds. In general, sterically hindered systems (Ar = Mes) which are unlikely to achieve a trigonal-bipyramidal five-coordinate geometry at tin with oxygen atoms in the axial positions, are ineffective as fungicides. However the *o*-tolyltin compounds, particularly the acetate, show some fungicidal activity. A larger size (*m*-Anis) or number (3,5-Xyl) of *meta* groups decreases fungicidal activity (to zero against *P. chrysogenum*) in comparison with $(m\text{-Tol})_3SnX$. Indeed, where test substances are inactive as fungicides, they promote the growth rate of *P.chrysogenum* by up to 60%. The steric effects implied by these data suggest that dimensions of active sites in the F_0 unit of the ATPase enzyme may differ significantly for each fungus studied. A model for the active site is proposed, based on the need of the Ar_3Sn^+ unit first to be able to reach the active site and then to occupy it with the required five-coordinate geometry so as to inhibit the activity of the ATPase enzyme.

Keywords: fungicidal activity; triaryltin; active site,

Received 19 October 1998; accepted 28 May 1999

INTRODUCTION

Recently we reported[1] on the fungicidal activities of some tris(*p*-substituted phenyl) tin acetates, oxides and hydroxides and compared them with the data for the unsubstituted biocides already in use, 'Brestan' (Ph_3SnOAc) and 'Du-Ter' (Ph_3SnOH). In most cases there was little change or even a slight decrease in fungicidal activity when the *para* substituent (Z) was F, Cl, CH_3 or C_2H_5, but with *p*-Z = CH_3O the triaryltin compounds were completely inactive. Generally R_3Snx biocides are thought to act by inhibiting the mitochondrial function in eukaryotic cells, with 'R_3Sn' the active species involved. The most significant process is usually assumed to be the inhibition of oxidative phosphorylation or ATP hydrolysis by the R_3Sn moiety disallowing the rapid transmembrane proton flow through the F_0 unit of ATPase that is necessary for the multisite processes catalysed by this enzyme.[2] The F_0 unit consists of subunits *a*, *b* and *c*, and inhibitors such as R_3SnCl (R = Bu, Ph) bind to at least one of the *c* subunits. In all ATP synthase complexes there is a free carboxyl group from Asp or Glu residues in subunit *c*, that appears to be involved in transmembrane proton conduction by hydrogen bonding to amino-acids in subunit *a*. Thus it was suggested that triorganotins freeze the structure of F_0 so that the rapid proton transmem-

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Contract/grant sponsor: Fonds FCAR (Programmes ACSAIR, 'Actions spontanées') of the government du Québec.

 CCC 0268–2605/2000/010034–06 $17.50

brane flow needed for multisite processes is no longer allowed.[3]

Both chemical[1] and structural[4] evidence indicates that the tin atom in the biocidally ineffective $(p\text{-}CH_3OC_6H_4)_3Sn$ moiety is very reluctant to become five-coordinate with oxygen atoms in the axial positions. Therefore we proposed that the biocidal activity of triaryltins is dependent on their ability to attain a *trans*-C_3SnO_2 trigonal-bipyramidal geometry, the structural motif most often adopted by triorganotin derivatives with oxygen donor ligands,[5] and that the active site occupied by the Ar_3Sn species consists of a pendant carboxylate group from one *c* subunit and a carbonyl group, perhaps from a neighbouring *a* subunit.[1] These two groups can then act as transaxial ligands to the Ar_3Sn moiety which is then strongly bound to a *c* subunit in the F_0 component of the enzyme ATPase. This then blocks the rapid proton transport required for multisite ATP synthesis or hydrolysis, which are then disallowed.

The effects of *meta* or *ortho* substituents on the fungicidal activities of triaryltins have received only cursory attention. Very early, the activities of $(p\text{-Tol})_3SnOAc$ and $(m\text{-Tol})_3SnOAc$ were found to be very similar,[6] while later $(o\text{-Tol})_3SnOAc$ was also found to be active,[7] albeit with MIC values ten times those for its *para* analogue. More recently, results from screening various Ar_3SnCl compounds against *C ulmi* indicated that compounds with *meta* substituents ($m\text{-Z} = CH_3$, Cl) were slightly more effective than their *para* analogues, although still less effective than the archetype, triphenyltin chloride.[8] In this report, we concentrate on the effects of methyl and methoxy substituents in the *meta* or *ortho* positions on the fungicidal activities of some Ar_3SnOAc and $(Ar_3Sn)_2O$ systems, again chosen for direct comparison with their phenyltin analogues.

EXPERIMENTAL

All experimental details, including the materials used and the precursors required, $(m\text{-}CH_3C_6H_4)_3SnX$ (X = Br, I) and Ar_3SnCl (Ar = $m\text{-}CH_3OC_6H_4$ and $3,5\text{-}(CH_3)_2C_6H_3]_3$), as well as microanalyses and IR (Nujol mull) and NMR (^{13}C, ^{119}Sn; $CDCl_3$) procedures, were as described earlier.[1,9]

Syntheses

Trimesityltin acetate (mesityl = 2,4,6-trimethylphenyl) has already been reported[4] trimesityltin hydroxide was prepared from the bromide by the literature method.[10] Standard syntheses[1,11] were used to prepare the compounds listed below. All products were recrystallised from ethanol. Spectroscopic data for all compounds used for bioassays in this work are given in Table 1.

Tris (*m*-tolyl)tin acetate
M.p. 100–102 °C (lit.[6] 91–95 °C).

Tris (o-tolyl)tin acetate
M.p. 98–100 °C (lit.[11] 95 °C).

Tris (3,5−dimethylphenyl)tin acetate
M.p. 98–100 °C. Analysis: found: C, 63.60; H, 6.52; calcd for $C_{26}H_{30}O_2Sn$: C, 63.32; H, 6.13%.

Tris (*m*-tolyl)tin oxide
M.p. 71–78 °C (*dec*). Analysis: found: *C*, 62.97; *H*, 5.35; *calcd for* $C_{42}H_{42}O_2Sn$: *C*, 63.04; *H*, 6.29%.

Tris (o−tolyl)tin oxide
M.p. 214–217 °C. Analysis: found: C, 63.01; H, 5.32; calcd for $C_{42}H_{42}OSn_2$: C, 63.04; H, 6.29%.

Tris (*m*-methoxyphenyl)tin oxide
M.p. 65–66 °C. Analysis: found: *C*, 57.25; *H*, 5.12; *calcd for* $C_{42}H_{42}O_7Sn_2$: C, 56.29; H, 4.72%.

Bioassys

Biological activity was assayed by radial growth studies using *Aspergillus niger, Botrytis cinera, Mucor hiemalis, Fusarium solani* and *Penicillium chrysogenum* as test fungi provided by the Department of Plant Science, MacDonald College.[1] Each fungus was cultured for 7–10 days on potato dextrose agar (PDA). By aseptic procedures, ethanol (95%) solutions (6.0 ml) containing the test compound were added to molten PDA (50 °C) to give solutions with concentrations of 1.0, 2.0, 4.0, 6.0 and 8.0 mg l^{-1} of the fungicide as well as a control with zero fungicide, which were then poured into 9-cm Petri dishes — three for each fungus and concentration. A 10-mm disc of the fungus was placed on the centre of each plate, which was then incubated at 25 ± 1 °C for 7–10 days. Colony diameters (along two 90 ° axes) were measured every 24 h for two days, and then every 48 h. Average growth rates (mm d^{-1}), after the

Table 1 Spectroscopic (IR and NMR) data

		Ar_3SnOAc			
	Ar =	*m*-Tol	3,5-Xyl	*o*-Tol	Mes
IR data					
$\nu_{as}(CO)_2$	(cm^{-1})	1534	1536	1665	1674
$\nu_s(CO)_2$	(cm^{-1})	1411	1433	1292	1280
$\Delta\nu(CO)_2$	(cm^{-1})	123	103	373	394
NMR data[a]					
$\delta(^{119}Sn)$	(ppm)	− 110.1	− 106.9	− 102.4	-129.9_5
$^1J(^{119}Sn–^{13}C)$	(Hz)	642.0	n.a.[b]	631.4	627.6
$^2J(^{119}Sn–^{13}C)$	(Hz)	48.0[c] 46.8	47.2	44.8[c] 47.6	48.4
$^3J(^{119}Sn–^{13}C)$	(Hz)	64.0[c] 66.4	67.2	52.3[c] 62.9	55.2
		$(Ar_3Sn)_2O$			
	Ar =	*m*-Tol	*o*-Tol	*m*-Anis	Mes[d]
IR data					
ν_{as}(Sn-O-Sn)	(cm^{-1})	n.a.[b]	839	761	—
NMR data[a]					
$\delta(^{119}Sn)$	(ppm)	− 81.27	− 77.20	− 83.93	− 101.78
$^2J(^{119}Sn–^{119}Sn)$	(Hz)	417.0	576.1	429.0	—
$^1J(^{119}Sn–^{13}C)$	(Hz)	620.3	617.1	—	614.7

[a] NMR: Ext. ref. Sn $(CH_3)_4$. Complete ^{13}C NMR data are available from the author.
[b] n.a., not measured or not observed.
[c] On substituent side of phenyl ring.
[d] Data are for $(Mes)_3SnOH$.

initial slow phase, were then used to provide relative radial growth rates (errors $\leq$5%). Probit analysis[12] of the radial growth inhibition data gave ED_{50} values, i.e the concentrations necessary for 50% reduction in fungal growth compared with the control for each fungus–compound combination. These are listed in Table 2 for the compounds studied here together with previously determined ED_{50} values for the phenyl and *p*-tolyl analogues.[1] (Complete radial growth data for all the inhibitor–fungus combinations used here, are available from the author.)

RESULTS

Synthesis and characterisation

For Ar = *m*- or *o*-Tol, both Ar_3SnOAc and $(Ar_3Sn)_2O$ compounds were made but only one compound of each type was synthesised for Ar = 3,5-Xyl (acetate) and Ar = *m*-Anis (oxide). Infrared data (Table 1) show that both *meta*-substituted acetates are polymeric in the solid state[13] but $(o\text{-Tol})_3SnOAc$ is monomeric like trimesityltin acetate.[4] The oxides were obtained by recrystallising from ethanol the product of the reaction of Ar_3SnX (X = Cl or Br) with 10% KOH (aq). With Ar = *m*-Tol, the oxide readily absorbed water from moist air to form Ar_3SnOH, as monitored by the growth of ν (OH) at 3580 cm^{-1}, while in the ^{119}Sn NMR spectrum ($CDCl_3$) of this oxide, the minor peak observed at 81.61 ppm was assigned to the Ar_3SnOH species. In contrast, tris (*o*-tolyl) tin oxide could not be converted to the hydroxide even after refluxing the oxide with ethanol–water (1:1) for several hours. This fact, together with the monomeric nature of $(o\text{-Tol})_3SnOAc$, implies that the tin atom in the tris (*o*-tolyl) tin unit is somewhat reluctant to become five-coordinate with oxygen atoms in the axial positions. Infrared and NMR data for $(Ar_3Sn)_2O$ (Ar = *m*-Tol or *m*-Anis) are consistent with both of these compounds having a bent

Table 2 Radial growth inhibition data; ED_{50}[a] values[b]

	Ar_3SnOAc					
	Ar =	Ph	*p*-Tol	*m*-Tol	3,5-Xyl	*o*-Tol	Mes
A. niger		2.0 (0.1)	8 (8)	2[c]	22[c];	9.6 (4.7)	NE[d]
B. cinera		5.2 (2.6)	5.0 (1.4)	6[c]	13; (12);	16; (11)	NE
M. hiemalis		1.9 (0.6)	7.6 (4.4)	3.6 (0.9)	4[c]	4.2 (1.4)	NE
F. solani		0.7 (0.2)	3.8 (1.5)	3.4 (2.3)	7.5 (6.3)	11[c]	NE
P. chrysogenum		5.0 (1.0)	12 (4)	9.1 (8.4)	NE	9.6 (2.7)	NE
	$(Ar_3Sn)_2O$					
	Ar =	Ph	*p*-Tol	*m*-Tol	*m*-Anis	*o*-Tol	Mes[e]
A. niger		0.5 (0.1)	4.3 (1.6)	1.2 (0.5)	6.6 (1.4)	NE	NE
B. cinera		5 (4)	6[c]	5.1 (4.0)	6.7 (4.8)	19[c]	NE
M. hiemalis		1.5 (0.4)	7 (7)	3.0 (2.1)	3.3 (2.9)	7[c]	13[c]
F. solani		1.1 (0.2)	3.4 (0.7)	3.8 (3.6)	5.3 (3.6)	NE	NE
P. chrysogenum		3.7 (0.9)	5.5 (2.2)	8.8 (7.6)	NE	NE	NE

[a] Concentration (mg L^{-1}) required to decrease radial growth to 50% of that of the control.
[b] Errors given in parentheses.
[c] Error greater than ED_{50} value.
[d] NE, No effect.
[e] Data are for $(Mes)_3SnOH$.

Sn-O-Sn skeleton[14,15] but the higher ν_{as} (SnOSn) and $^2J(^{119}Sn–^{119}Sn)$ values for tris (*o*-tolyl) tin oxide are consistent with its linear (Sn-O-Sn) skeleton and an Sn-O bond slightly shorter than that found in the bent structures.[16]

Bioassays

As shown by the ED_{50} values (Table 2) both $(m\text{-Tol})_3SnOAc$ and $[(m\text{-Tol})_3Sn]_2O$ have similar fungicidal activity which is consistent with our earlier results.[1] They are generally more effective than their *p*-tolyl analogues but are still less effective than the parent phenyl compounds.[8] Two *m*-CH_3 groups, as in $(3,5\text{-Xyl})_3SnOAc$, cause an overall decrease in activity — except with *M. hiemalis* — and complete loss of activity versus *P. chrysogenum*. In contrast to its para analogue, which is completely ineffective against all the test fungi,[1] $[(m\text{-Anis})_3Sn]_2O$ is relatively active except against *P. chrysogenum*, for which it is ineffective at the test substance concentrations used here.

The *ortho*-tolyl compounds present a less uniform aspect of biological activity. Thus $(o\text{-Tol})_3SnOAc$ is only slightly less effective overall than $(m\text{-Tol})_3SnOAc$ or $(p\text{-Tol})_3SnOAc$ for the fungi tested here, but for the oxide the results unexpectedly depend on the fungus being tested. Both trimesityltin compounds are ineffective fungicides but they do not promote fungal growth,

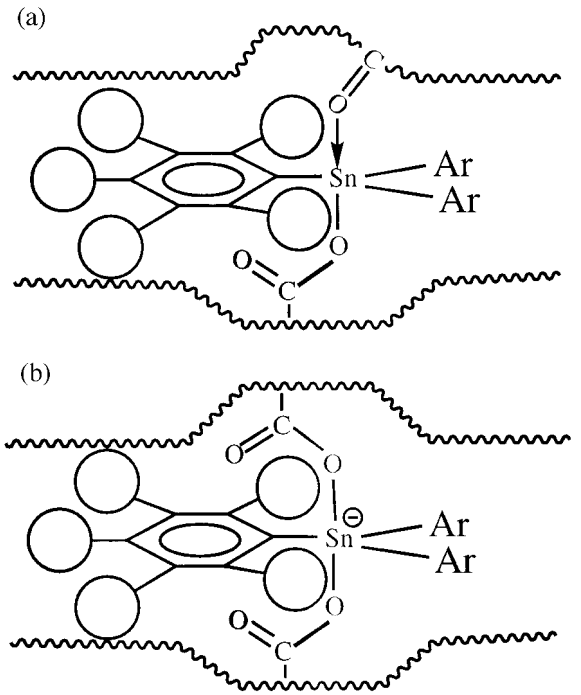

Figure 1 Proposed architectures for strong binding sites having two trans covalent bonds, in the F_0 part of the F-ATPase enzyme. These must be occupied for inhibition of proton transfer across the mitochondrial membrane to occur: (a) active site has a carboxylate group (from *c*) and a carbonyl group (from *a*) to hold a planar Ar_3Sn^+ ion; (b) active site has two carboxylate groups from *c* subunits to hold a planar Ar_3Sn^+ ion.

although $(Mes)_3SnOH$ appears to show some residual activity against *M. hiemalis*.

However, ED_{50} values alone do not give the full picture of biological activity for all the triaryltins examined in this work. Thus while $(Mes)_3SnX$ (X = OAc, OH) and $[(o\text{-Tol})_3Sn]_2O$ have no measurable fungicidal effect over the concentration range used here, they also have little effect on fungal growth rate. This contrasts with the equally ineffective $(p-CH_3OC_6H_4)_3Sn$ moiety, which appears to promote the fungal growth rate by about 10% for most of the fungi considered here.[1] Much more surprising is the behaviour of the two organotins which are ineffective only against *P. chrysogenum*: they even more strongly promote the growth of this fungus, by up to 80% at low gel concentrations (1–2 mg L^{-1}). However the increase in growth rate becomes less at higher fungicide concentrations, but is still about 20% at 8 mg L^{-1}.

4 DISCUSSION

Previously we have suggested that the readiness of the Ar_3Sn moiety to attain a *trans*-C_3SnO_2 trigonal-bipyramidal geometry is one of the necessary conditions for the fungicidal activity of triaryltins.[1] Thus the activity of monomeric $(o\text{-Tol})_3SnOAc$ at first glance is unexpected since methyl groups in the *ortho* positions will provide steric crowding around tin and thus will inhibit the approach of *trans* ligands to form five-coordinate species. However, although $(o\text{-Tol})_3SnNCS$ is four-coordinate and monomeric, the existence of the adduct $(o\text{-Tol})_3SnNCS{\cdot}HMPA$[17] shows that if the two ligands are sterically undemanding, a *trans* five-coordinate geometry is allowed. This would imply that both donor groups, one of which is a carboxylate ion, in the active site must be relatively unhindered for the $(o\text{-Tol})_3Sn$ moiety to act as a fungicide. While one CH_3 in a *meta* position does not seriously affect fungicidal activity, two *m*-CH_3 groups or a larger methoxy radical in the *meta* position clearly cause a decrease in activity against some of the test fungi. This selectivity could indicate that steric effects away from the centre of the active site may also play a rôle in the biocidal activity of triaryltins.

Possible architectures of the site needed for Ar_3SnX to block the rapid proton transport required for multisite ATP synthesis or hydrolysis, and which can account for the substituent effects noted in this work and earlier,[1] are shown in Fig. 1. In one case (a) the Ar_3Sn^+ unit occupying the site is held in a *trans*-C_3SnO_2 arrangement by covalent bonds from a pendant carboxylate ion from one *c* chain and a carbonyl group from an amide linkage in a neighbouring *a* chain, while in the other (b) both *trans* ligands are carboxylates from adjacent *c* chains. In addition, although the central cavity of the active site is well able to accommodate the C_3Sn^+ centre of the biocidal agent (the planar Ar_3Sn^+ ion), the environs of the cavity are more restricted. In this picture, with the expected propeller geometry of phenyl rings in planar Ar_3Sn species, replacing *para*-hydrogen by various substituents, unless they are very large, should have little steric effect on the ability of the triaryltin to reach and then occupy the active site. This would then leave the fungicidal action of the organotin relatively unaffected as is indeed the case.[1]

In contrast, both *ortho* and *meta* substituents will increase the thickness of the planar Ar_3Sn^+ ion which will then be less able to fit into the active site. The consequent decrease in activity appears when

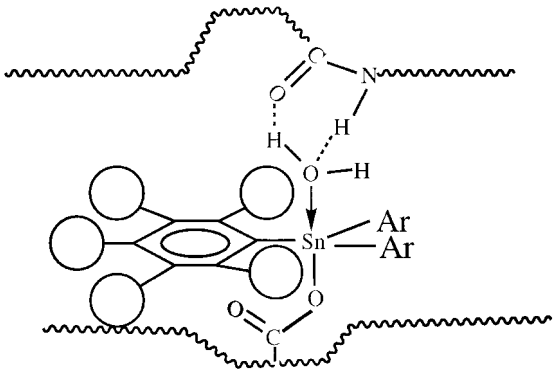

Figure 2 One possible weaker binding site in the F_0 part of the FATPase enzyme, having one covalent bond from a carboxylate group in *c* and hydrogen bonding interaction involving an amide group in *a*, to hold a non-planar $Ar_3Sn(H_2O)^+$ ion.

there are two *m*-CH_3 groups or one CH_3O- in the *meta* position, and is also fungus dependent. In the extreme case, *P. chrysogenum*, a much narrower channel around the centre of the active site will prevent the $(m\text{-Anis})_3Sn^+$ or $(3,5\text{-Xyl})_3Sn^+$ ions from occupying the active site, rendering both of these organotins ineffective as fungicides and leaving them to be metabolised by the fungus.

This picture (Fig. 1) does not account for the perceptible, albeit weak, biocidal activity of $(Mes)_3SnX$ (X = OAc or OH) towards *M. hiemalis*, since steric hindrance will prevent a planar trimesityltin moiety from achieving a *trans*-C_3SnO_2 trigonal-bipyramidal geometry. However, in this fungus, the F_0 unit may be sufficiently flexible to expand and thus allow weaker binding sites to come into use. One such possibility is shown in Fig. 2 where the $[(Mes)_3SnOH_2]^+$ species is now held in place by a covalent bond to the pendant carboxylate group and hydrogen bonding from the amide group to a water molecule relatively weakly bound to the sterically hindered tin atom. This environment around tin is analogous that in $[Ph_3SnO_2CCCl_3(CH_3OH)]$[18] where the Sn atom lies out of the trigonal plane, in the direction towards the carboxylate O atom, and shows a tendency towards a tetrahedral distortion.

Acknowledgements The support of the Fonds FCAR (Programmes ACSAIR, 'Actions spontanées') of the Gouvernement du Québec is most gratefully acknowledged, as is the assistance of the Department of Chemistry, McGill University, where much of this work was carried out. Special thanks are due to the Medical Laboratory Technology Department of Dawson College for enabling radial-growth assays to be carried out using their facilities, and in particular to Wendy Somerville and students Rina Pierpaoli and Lisa Artuso who performed the assays, as well as to Professor Tim Paulitz of the Department of Plant Science, MacDonald College, for providing the test fungi used here. The radial growth data were analysed by Dr Graeme Welch of the Department of Chemistry, Dawson College. The administration of Dawson College is thanked for supporting this research with release time (IW).

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