Comparative Study on the Antifungal Activity of Some Di- and Tributyltin(IV) Carboxylate Compounds

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Abstract

A series of di- and tributyltin(IV) carboxylate compounds prepared by reacting the dibutyltin(IV) dichloride and tributyltin(IV) chloride respectively *via* the organotin(IV) oxide with the respective carboxylic acids have been tested for antifungal activity against *Fusarium oxysporum* and *Aspergillus niger* in potatoes dextrose agar. The compounds synthesized were mainly characterized with IR and UV-Vis spectroscopies as well as based on the microanalytical data. The results showed that di- and tributyltin(IV) carboxylates prepared in general exhibit greater fungitoxicity than the organotin chlorides, the intermediate products and the free carboxylic acids. The organotin moiety plays an important role in deciding the antifungal activity of an organotin compound indeed true in our respect that the antifungal activity of tributyltin(IV) carboxylates   is more active than dibutyltin(IV) carboxylates .

Keywords: Antifungal activity test, Minimum inhibition concentration, Organotin(IV) carboxylates

1. Introduction

The organotin(IV) compounds continue to be of interest on account of their interesting structural features (Tiekink, 1991; Shahid et al., 2003; Bhatti et al., 2005) and also because of their wide applications as catalysts, antifouling agents, agricultural biocides, antitumor agents and other biological activities (Blunden and Hill, 1990; Bonire et al., 1998; Pellerito and Nagy, 2002; Gielen, 2003). The organotin(IV) compounds are characterized by the presence of at least one covalent Sn – C bond. These compounds contain tetravalent Sn centers and can be classified as mono-, di-, tri-, and tetraorganotin(IV), depending on the number of alkyl (R) or aryl (Ar) moieties attached to the tin metal. The counter anion is usually chloride, oxide, fluoride, hydroxide, and thiolate (Pellerito and Nagy, 2002). Carboxylate has also been used successfully as counter anion (Bonire et al., 1998; Pellerito and Nagy, 2002; Szorsick et al., 2002; Gielen, 2003).

The organotin(IV) compounds are known to display strong biological activity. Their compounds are normally exhibit high toxicity, even at very low concentration. Their biological activities are fundamentally determined by the number and nature of organic groups bound to the central Sn atom (Pellerito and Nagy, 2002). The nature of the anionic groups seems only as a secondary factor. The current investigations on the coordinating properties of carboxylates toward organotin compounds have led to the isolation of some new organotin(IV) carboxylates and carboxylate derivatives which have shown some interesting biological activities such as antimicrobial (Bonire et al., 1998; Mahmood et al., 2003), anti-tumor (de Vos et al., 1998), and antifungal activity (Ruzika et al., 2002; Mahmood et al., 2003; Hadi et al., 2007; Hadi et al., 2008). Based on the above fact and the opportunity to explore the interesting features of these organotin compounds, in the present work, we report the comparative study on the antifungal activity of di- and tributyltin(IV) carboxylate compounds.

2. Experiment

2.1 Chemicals Required

All reagents were of reagent grades. Dibutyltin(IV) dichlorides ($[(C_4H_9)_2Cl_2]$), tributyltin(IV) chloride ($[(C_4H_9)_3Cl]$), carboxylic acids, dimethylsulfoxide (DMSO), sodium hydroxide   (NaOH), methanol (CH_3OH) were either Sigma or JT Baker products and were used without further purification.

2.2 Experimental Procedure

The preparation of the organotin(IV) carboxylates, for this work and similar compounds with different carboxylate ligands have previously been reported (Hadi et al., 2007; Hadi et al., 2008) and the procedure used was adapted from Szorcsik et al.(2002)   An example procedure in the preparation of dibutyltin(IV) dicarboxylates is as follows:

2.2.1 Preparation of $[(n\text{-}C_4H_9)_2Sn(OH)_2]$ (**2**)

To 3.0383 g (0.01 mol) $[(n\text{-}C_4H_9)_2SnCl_2]$ in 50 mL dry methanol was added with 0.8 g (0.02 mol) NaOH and the reaction mixtures were stirred for about 45 minutes. Compound **2** was precipitated out as white solid, filtered off and dried *in vacuo* till they are ready for IR and for further reaction. The yield in average was 2.3508 g (95 %)

2.2.2 Preparation of $[(n\text{-}C_4H_9)_2Sn(OOCR)_2]$

To 0.37338 g (1.5 mmol) compound **2** in 50 mL dry methanol was added with 2 mole equivalents of carboxylic acids and was refluxed for 4 hours at 60 – 70°C. The product compounds $[(n\text{-}C_4H_9)_2Sn(OOCR)_2]$ were obtained after removal of the solvent by rotary evaporator and dry *in vacuo* until they are ready for IR spectroscopy and used further for antifungal test The average yield was more than ~ 90 %.

The similar procedure was used to prepare tributyltin(IV) carboxylates, $[(n\text{-}C_4H_9)_3Sn(OOCR)]$, where in this reaction the carboxylic acids added was 1 mole equivalent.

2.3 The Antifungal Activity Test

The procedure of the antifungal activity test undertaken based on the procedure reported previously (Hadi et al., 2007; Hadi et al., 2008) and as follows:

1.   The *F. oxysporum* and *A. niger* isolates are available in the botany laboratory, the University of Lampung.

2.   The fungi isolates were taken from their pure culture and transferred to PDA (*Potatoes Dextrose Agar*) media to get their optimum growth.

3.   The activity test of organotin(IV) compounds was performed with (1) the *disk diffusion test method* and (2) *dilution method.*

2.3.1 Disk diffusion test method

This method was done as follows: transferred the pure culture of the fungi which has grown optimum by cutting the colony of the fungi at the edge of their culture growth in 1 x 1 cm size. This colony agar blocks is put at the middle of PDA. It is incubated at 25 °C for 1-2 day to ensure its growth. When the growth of the fungi was seen clearly, three *filter disk papers,* which have been kept in the solution containing the organotin(IV) compound tested in DMSO was placed in surround of the inoculums aseptically. The media test was then incubated further at 25°C for 7 consecutive days and was daily checked. The observation was monitored by looking at the inhibition zone. If the inhibition zone was formed (the area where the fungi was unable to grow), then it indicated the compound tested showed the antifungal activity. If there is no any inhibition zone then the organotin used is inactive or the concentration used may be less than required.

2.3.2 Dilution test method

This method was done as follows: the organotin compound being used is dissolved in DMSO using the concentration which was the most effective from the disk diffusion test method. The compound is then mixed with 25 mL of agar media with the volume variation of 0.5; 1; 1.5; 2; and 2.5 mL. The inoculums block of the fungus is placed in the middle of the PDA media at 25°C for 10 consecutive days and was daily checked. The minimum inhibition concentration is then calculated based on the smallest concentration of the compound used which maximally inhibits the growth of the fungi.

Based on the results of the inhibition zone obtained from disk diffusion test and the smallest concentration used in dilution test, tributyltin(IV) carboxylates are in general more active three times than those of any analogous compound of dibutyltin(IV) carboxylates.

3. Results and Discussion

The preparations of dibutyltin(IV) dicarboxylates, $[(n\text{-}C_4H_9)_2Sn(OOCR)_2]$ (**3-5**) and tributyltin(IV) carboxylates, $[(n\text{-}C_4H_9)_3Sn(OOCR)]$ (**6-8**), were successfully done from their chlorides $[(n\text{-}C_4H_9)_2SnCl_2]$ (**1a**) and $[(n\text{-}C_4H_9)_3SnCl]$ (**1b**), respectively. To maximize the product obtained, the reactions in all cases were done *via* $[(n\text{-}C_4H_9)_2SnO]$ (**2a**) and $[\{(n\text{-}C_4H_9)_3Sn\}_2O]$ (**2b**) respectively similar to those previously reported (Hadi et al., 2007; Hadi et al., 2008). The antifungal activities of compounds **3** and **4** have previously been reported (Hadi et al., 2008), and included here to compare their activity values to the respective tributyltin(IV) compounds. The reaction occurred in each step for dibutyltin(IV) dicarb*o*xylates, for example, is shown in Scheme 1. The microanalytical data of all compounds prepared are very good and   all values obtained are close to the theoretical values as shown in Table 1.

The characterization of the products synthesized was confirmed mainly by analyzing them with FT-IR spectroscopy in the frequency range of $4000 - 250$ cm^{-1} and the results are presented in Table 2. The characteristic band of the starting materials (**1a,b**) is the appearance of strong stretching band of Sn - Cl bond at $390 - 310$ cm^{-1}, and in the spectrum of **1a**, this bond appeared at frequency of 334.2 cm^{-1}. The other characteristic bands of this compound appear as stretching band from butyl ligands at 1069 cm^{-1}, and bending vibration of C-H aliphatic stretch of the butyl at frequency of $2956 - 2865$ cm^{-1}.

When compound **1a** is converted to compound **2a**, the main stretching band of Sn – Cl disappeared and a new strong band at frequency of 417.4 cm^{-1} appeared as one of the main stretching band. This band is characteristic for Sn – O bond in compound $[(n\text{-}C_4H_9)_2SnO]$ (**2a**). The stretching band due to the butyls and their bending vibrations are still appeared as expected although the frequencies have little bit moved. The formation of dibutyltin (IV) dicarboxylate compounds, $[(n\text{-}C_4H_9)_2Sn(RCOO^-)_2]$, (**3 - 5**) is confirmed by the strong asymmetric stretching bands of the carboxylates which occurred at *ca.* 1400 cm^{-1} and the symmetric stretch at *ca.* 1600 cm^{-1}, confirming the success of the substitution reaction (Hadi et al., 2008).

The UV-vis spectroscopy analyses have also been taken for all the compounds used. The $\lambda_{max.}$ of all the compounds is summarized in Table 3. The data obtained from Table 3 are clear that there was a shifting change in the $\lambda_{max.}$ for each compound in any steps of the reaction. For example, the compound **1a** has $\lambda_{max.}$ of 210.7 nm, while compound **2a** has $\lambda_{max.}$ of 202.9 nm. This information gave an indication that there was a shift to a shorter λ maximum when the conversion of compound **1a** to **2a** taken place. The wave length shift to a shorter $\lambda_{max.}$ could be occurred due to either the solvent used or the effect of auxochrome. However in this study, this can not be due to the effect of solvent as the solvent used for all measurements is the same, which is methanol. This change must be due to the auxochrome effect. In case of compound **1a** and **2a** can be closely looked at that in compound **2a** there is oxide group which has electron drawing effect bigger than that of chloride group in **1a**, as a result in **2a**, the electron transition is hard to occur, thus the λ_{max} measured is getting shorter (Sudjadi, 1985). The similar observations are also observed for other changes as can be seen from the Table 3. In compound **3**, for example, the electron drawing effect of o-$C_6H_4(OH)COOH$ is less than chloride in **1a**, so the electron transition in its molecule will be easier (the energy required is less), thus producing longer $\lambda_{max.}$, 307.8 nm.

The antifungal activity of salicylic acid and its derivative has long been known (Coates et al., 1957). However, compare to the organotin compounds used in the present study, the free salicylic acid and other carboxylic acids presented weaker antifungal activity against the tested fungi (see Table 4). Table 4 clearly illustrated that the Minimum Inhibition Concentration (MIC) values of the organotin(IV) salicylates (compounds **3** and **6**), in general, are smaller than other organotin(IV) carboxylate compounds, the derivatives of acetylsalycilate (**4, 7**) and benzoate (**6, 8**) . These data agreed to those previously reported (Hadi et al., 2008). This indicates that the presence of the metal ions plays an important role in the increased antifungal activity when the acids are coordinated. In this respect, our results are consistent with a well-known fact that many biologically active compounds become more active upon complexation than in their uncomplexed forms (Gershon, 1974). The fact that the organotin(IV) carboxylates are more active against the tested fungi than their parent organotin(IV) compounds (the organotin(IV) chloride), the intermediate products, and the carboxylic acids suggests that the carboxylate groups play a role in the fungi toxicities of these compounds. According to Crowe (1989), the actual biological activity of diorganotin compounds of the type RR'SnXY (R and R' = alkyl or aryl; X and Y= anions) is determined solely by the RR'Sn^{2+} moiety.

Consequently the group X and Y would only influence the delivery of the active RR'Sn^{2+} ion to the cell. The higher activities of the orgnotin(IV) carboxylates relative to their parent organotin(IV) compounds (the organotin(IV) chlorides), the intermediate products, and the carboxylic acids appear to be an additive (not a synergistic) effect of the metal ions and the carboxylate groups, with the possibility of a common mode of action. In this regard, the hypothesis that relates the toxicity and nontoxicity of metal complexes to the penetration and nonpenetration of the fungus by the toxicant is of interest (Crowe, 1989). Therefore the free carboxylic acids show some explanation for our results. The groups like acetylsalicylate and $(n\text{-}C_4H_9)Sn^{2+}$ considerably change the molecular parameter that influence the orientation of the molecule on biological receptor and facilitate penetration into the cell membranes (Bonire et al., 1998).

The observation that the tributyltin(IV) carboxylate compounds are more active than their dibutyltin(IV) is in line with the notion that the number of carbon atoms in the organotin moiety affects its activity (Crowe, 1989). In tributyltin(IV) derivatives optimal activity has been associated with 12 carbon atoms and this may be the case in the present work, where the dibutyltin(IV) derivatives contain only 8 carbon atom (Chohan and Rauf, 1996). These data are also in agreement with our previous result where in general, the derivative of triphenyltin(IV) carboxylate which contain 18 carbon atoms has smallest MIC values in the series (Hadi et al., 2008). As an example, the triphenyltin(IV) salycilate, $[(n\text{-}C_6H_5)_3Sn(o\text{-}C_6H_4(OH)COO^-)]$ has MIC value of 0.61 mM.

4. Conclusion

The results presented in the present study clearly have again indicated that the organotin(IV) carboxylates synthesized and described in this work showed strong antifungal activities. The MIC values obtained for tributyltin(IV) carboxylate compound are smaller than respective dibutyltin(IV) carboxylates compound. However, the MIC values of tributyltin(IV) compound are higher compared to their respective triphenyltin(IV) carboxylate. This observation does agree to the fact that the number of carbon atoms in the organotin moiety affects its activity against fungi tested.

Acknowledgments

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$[(C_4H_9)_2SnCl_2]$ $\xrightarrow[\substack{\text{refluxed} \\ \text{60 mins}}]{\text{NaOH in MeOH}}$ $[(C_4H_9)_2SnO]$ $\xrightarrow[\substack{\text{refluxed} \\ \text{4 h in MeOH}}]{\text{Carb. Acid desired}}$ $[(C_4H_9)_2Sn(OOCR)_2]$

1a   **2a**   **3 - 5**
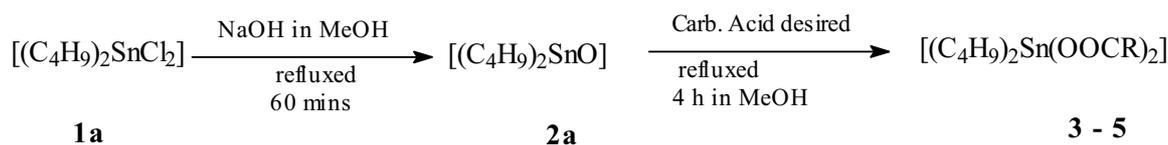
Scheme 1. The preparative route of $[(C_4H_9)_2Sn(OOCR)_2]$

Table 1. The microanalytical data of the organotin(IV) compounds synthesized

No.	Compound	Elemental analyses found (calculated)	
		C	**H**
3	$[(n\text{-}C_4H_9)_2Sn(o\text{-}C_6H_4(OH)COO^-)_2]$	55.98 (57.04)	3.26 (3.32)
4	$[(n\text{-}C_4H_9)_2Sn(o\text{-}C_6H_4(O_2CCH_3)COO^-)_2]$	56.23 (57.05)	3.76 (3.8)
5	$[(n\text{-}C_4H_9)_2Sn(C_6H_5COO^-)_2]$	54.39 (55.58)	5.94 (5.89)
6	$[(n\text{-}C_4H_9)_3Sn(o\text{-}C_6H_4(OH)COO^-)]$	55.04 (55.47)	7.90 (7.79)
7	$[(n\text{-}C_4H_9)_3Sn(o\text{-}C_6H_4(O_2CCH_3)COO^-)]$	53.0 (53.4)	7.37 (7.49)
8	$[(n\text{-}C_4H_9)_3Sn(C_6H_5COO^-)_2]$	61.47 (61.61)	7.18 (7.25)

Table 2.The characteristic and important IR bands of the organotin(IV) compounds (cm^{-1}) synthesized

Compound	**3**	**4**	**5**	**6**	**7**	**8**	References
Sn-O	434.5	435.7	491.8	464.2	444.1	456.2	600-400
Sn-O-C	1029.9	1028.1	1027.3	1030.8	1023.8	1029.4	1050-900
Sn-Bu	674.8	678.3	709.4	698.5	678.9	684.2	740-660
CO_2 asym	1419.6	1418.2	1452.1	1483.9	1458.8	1462.3	1500-1400
CO_2 sym	1558.7	1560.7	1589.3	1658.3	1648.7	1638.6	1660-1550
C-H aliphatic	2955 – 2862	2955 – 2866	2956-2865	2924-2856	2957-2855	2944-2855	2960 – 2850

Table 3. The $\lambda_{maximum}$ of the UV spectra of the organotin(IV) compounds

Compound	$\lambda_{maximum}$ (nm)
$[(n\text{-}C_4H_9)_2SnCl]$ (1a)	210.7
$[(n\text{-}C_4H_9)_2SnO]$ (2a)	202.9
$[(n\text{-}C_4H_9)_2Sn(o\text{-}C_6H_4(OH)COO^-)_2]$ (3)	307.8
$[(n\text{-}C_4H_9)_2Sn(o\text{-}C_6H_4(O_2CCH_3)COO^-)_2]$ (4)	305.7
$[(n\text{-}C_4H_9)_2Sn(C_6H_5COO^-)_2]$ (5)	287.7
$[(n\text{-}C_4H_9)_3Sn(o\text{-}C_6H_4(OH)COO^-)]$ (6)	308.9
$[(n\text{-}C_4H_9)_3Sn(o\text{-}C_6H_4(O_2CCH_3)COO^-)]$ (7)	302.3
$[(n\text{-}C_4H_9)_3Sn(C_6H_5COO^-)_2]$ (8)	282.4

Table 4. The Minimum Inhibition Concentration (MIC) of starting materials and organotin(IV) carboxylates

Compounds	*F. oxysporum* (mM)	*A. Niger* (mM)
Salicylic acid	18.7	18.5
Acetylsalicylic acid	13.7	13.9
$[(n\text{-}C_4H_9)_2SnCl_2]$ (1a)	16.5	16.0
$[(n\text{-}C_4H_9)_3SnCl]$ (1b)	12.2	11.8
$[(n\text{-}C_4H_9)_2SnO]$ (2a)	19.0	18.5
$[(n\text{-}C_4H_9)_3O]$ (2b)	9.4	9.2
$[(n\text{-}C_4H_9)_2Sn(o\text{-}C_6H_4(OH)COO^-)_2]$	6.4	6.6
$[(n\text{-}C_4H_9)_2Sn(o\text{-}C_6H_4(O_2CCH_3)COO^-)_2]$	6.1	6.2
$[(n\text{-}C_4H_9)_2Sn(C_6H_5COO^-)_2]$	7.3	7.1
$[(n\text{-}C_4H_9)_3Sn(o\text{-}C_6H_4(OH)COO^-)]$	2.61	2.62
$[(n\text{-}C_4H_9)_3Sn(o\text{-}C_6H_4(O_2CCH_3)COO^-)]$	2.54	2.56
$[(n\text{-}C_4H_9)_3Sn(C_6H_5COO^-)_2]$	2.98	3.14