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Synthesis, Characterization, and Comparison of Disinfectant Bioactivity Test of Two Triphenyltin(IV) Compounds

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Abstract: This paper aims to report the synthesis of two new organotin(IV) carboxylate derivatives, triphenyltin(IV) 4-aminobenzoate (**2**) and triphenyltin(IV) 4-nitrobenzoate (**3**) and to examine their antibacterial activity as a disinfectant. These compounds were prepared by reacting triphenyltin(IV) hydroxide (**1**) with 4-aminobenzoic acid and 4-nitrobenzoic acid, respectively. Compound (**2**) was obtained as a yellow solid with a yield of 84.09% and compound (**3**) in the form of a white solid with a yield of 80.70%. These compounds were well characterized using UV-Vis spectrometry, FT-IR spectrometry and NMR spectroscopy. The bioactivity test as a disinfectant was tested against *Salmonella typhosa* and *Staphylococcus aureus*. The activity test was carried out by measuring the optical density (OD) of the tested compounds with concentration variations of $5x10^{-3}$, $1x10^{-3}$, and $5x10^{-4}$ M in methanol and 5% dimethyl sulfoxide (DMSO), commercial Wipol (2.5% pine oil) was used as a positive control with observations monitored at contact times of 0, 5, 10, and 15 minutes. The results showed that of both compounds were active against the two bacteria compared to the positive control with compound **3** found to be more active than compound **2**.

Keywords: antibacterial, disinfectant, *S. typhosa*, *S. aureus*, triphenyltin(IV) compounds

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INTRODUCTION

The interest on organotin(IV) compounds is not only because of their interesting structural features (1-5) but mostly because they have been found to show many biological applications since they have strong effect even at very low concentration (5,6). Biological activities of the compounds are primarily dependent on the organic functional groups bound to Sn (4), and on the anion bearing groups attached to the Sn center as a complementary factor (4).

The current results from the study on organotin(IV) compounds have revealed that some organotin(IV) carboxylates show promising activities in many biological tests. For example, they have been widely used in the biological activities as antifungal (3, 7-9), antioxidant activities (10, 11), antimalarial and antiplasmodial agents (12-15), antiviral agents (16, 17), antitumor and anticancer (18-20), corrosion inhibitor (21-23) and antibacterial (24-30). Moreover, these compounds have been found to exhibit strong activity as disinfectant (31). In this context, it is therefore highly valuable to further explore the potential of organotin(IV) carboxylates as disinfectant agent.

It has also been observed that the strength of the activity of organotin(IV) compounds in many biological test is not only influenced by the number of organic groups attached to the central atom of tin (Sn), but the type of organic groups also plays a role as a determinant in their activity (4, 28). The organic group of phenyl that is bound to the central atom of Sn is known to have a stronger antibacterial activity than the butyl group (7) and the higher the

number of phenyl groups, the higher the antibacterial activity observed (6). In addition, the type of anion ligands bound to Sn atom also has an important role as a secondary determinant of reactivity. Therefore, in this paper, we reported the synthesis of two triphenyltin(IV) compounds with ligands of 4-aminobenzoic acid and 4-nitrobenzoic acid and performed activity tests as disinfectant agent against Gram-positive *S. aureus* and Gram-negative *S. typhosa* bacteria.

EXPERIMENTAL SECTION

Materials

The reagents used were triphenyltin(IV) hydroxide ($[(C_6H_5)_3SnOH]$), 4-aminobenzoic acid $[(C_6H_4(4\text{-}NH_2)COOH]$ (4-HABz), 4-nitrobenzoic acid $[(C_6H_4(4\text{-}NO_2)COOH]$ (4-HNBz), methanol, dimethylsulfoxide ($(CH_3)_2SO$, DMSO), nutrient broth, and nutrient agar. They were obtained from Sigma-Aldrich (Burlington, MA, USA) with Pro Analysis (PA) quality and were used as received. The culture of Gram-positive bacteria *S. aureus* and Gram- negative bacteria *S. typhosa* were obtained from Laboratory of Veterinary Centre, Directorate General of Livestock and Animal Health, Ministry of Agriculture, Lampung, Indonesia. A commercial product Wipol (containing 2.5% pine oil) was used as a positive control.

Instrumentantion

Elemental analysis was carried out on an EA Fission 1108 series elemental analyzer, the UV spectra were recorded in the UV region and measured using a UV-Shimadzu UV-245 Spectrophotometer. Measurements were carried in 1 mL quartz cells. The solution was prepared using methanol solvent with a concentration of 1.0×10^{-5} M. The IR spectra were recorded on a Bruker VERTEX 70 FT-IR spectrophotometer with a KBr disc in the range of 4000-400 cm^{-1}. ^1H and ^{13}C NMR spectra were recorded on a Bruker AV 600 MHz NMR (600 MHz for ^1H and 150 MHz for ^{13}C). All experiments were run in DMSO-d_6 at 298 K.

Preparation of Triphenyltin(IV) Compounds

Two target compounds of triphenyltin(IV) 4-aminobenzoate (**2**) and triphenyltin(IV) 4-nitrobenzoate (**3**) were prepared by the reaction between the starting compound triphenyltin(IV) hydroxide (**1**) with 4-HABz and 4-HNBz using the published method (14,15, 20, 22, 24-28). The following procedure was performed:

Triphenyltin(IV) 4-aminobenzoate, $[(C_6H_5)_3Sn(4\text{-}OCOC_6H_4NH_2)]$ ($Ph_3Sn4\text{-}ABz$) (2)

1.1010 g of compound **1** in 20 mL of methanol was reacted with 0.4114 g 4-HABz in 10 mL of methanol (mole ratio was 1:1) and they were refluxed for 4 hours at 60-61 °C. The water formed in the synthesis process was separated by a Dean and Stark apparatus. The remaining methanol solvent was evaporated by putting the synthesized solution into a vial and covered with aluminium foil that had been perforated and stored in a desiccator until it was obtained. The same procedure was applied in the preparation of compound, $[Ph_3Sn(4\text{-}NBz)]$ (**3**). The compounds synthesized obtained were as follows:

$[Ph_3Sn(4\text{-}HABz)]$ (**2**): yellow solid; UV $\lambda_{max.}$ (MeOH) nm (log ε): 234 and 278; IR $\nu_{max.}$ (KBr) cm^{-1}: 3473.09 (NH), 3049.00 (C-H Phen), 1602.08 (C=O), 1528.02 (CO_2 asym), 1551.8; 730.8 (phen), 1177.08 (Sn-O-C), 782.07 (Sn-O); ^{1}H-NMR (in DMSO-D_6, 600 MHz) δ (ppm): H_2= H_6 7.440 (6H, d, Ar-H); H_3 & H_5 7.460 (6H, d, Ar-H); H in benzoate: $H_{9,\ 13}$= 7.843-7.838 (6H, d); $H_{10,12}$=7.750-7.744 (6H, d); ^{13}C-NMR (in DMSO-D_6, 150 MHz): δ (ppm): C(phen): C_2 & C_6= 131.6, C_3 & C_5= 129.1 , C_4= 126.9; C_7= 163.8 (C7 C=O); C(NBz) C_8= 135.9; C_9 & C_{13} = 130.2; C_{10} & C_{12} = 129.1; C_{11}= 129.5; microelemental analysis: found (calculated): C 61.52 (61.70), H 4.29 (4.32), N 2.80 (2.88).

$Ph_3Sn(4\text{-}HNBz)]$ (**3**): white solid; UV $\lambda_{max.}$ (MeOH) nm (log ε): 234 and 290; IR $\nu_{max.}$ (KBr) cm^{-1}: 3047.20 (C-H Phen), 1600.23 (C=O), 1520.08 (CO_2 asym), 1551.8; 730.8 (phen), 1334.50 (N-O),1170.04 (Sn-O-C), 723.53 (Sn-O); ^{1}H-NMR (in DMSO-D_6, 600 MHz) δ (ppm): H_2= H_6 7.508 (6H, d, Ar-H); H_3 & H_5 7.481 (6H, d, Ar-H); H in benzoate: $H_{9,\ 13}$= 7.867-7.862 (6H, d); $H_{10,12}$=7.774-7.769 (6H, d); ^{13}C-NMR (in DMSO-D_6, 150 MHz): δ (ppm): C(phen): C_2 & C_6= 131.8, C_3 & C_5= 129.5, C_4= 127.3; C_7= 164.5 (C7 C=O); C(NBz) C_8= 136.9; C_9 & C_{13} = 130.5; C_{10} & C_{12} = 129.3; C_{11}= 129.8; microelemental analysis: found (calculated): C 58.23 (58.14), H 3.84 (3.68), N 2.75 (2.71).

Disinfectant Bioactivity Test

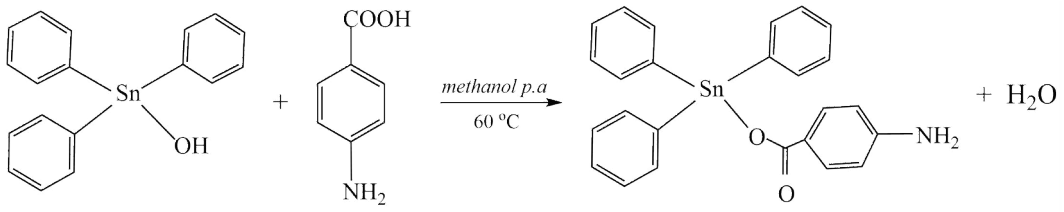
The disinfectant bioactivity test was carried using procedure similar to the previous work (31) and as follows: Bacterial inoculum was made by taking one dose of rejuvenated *S. aureus* and *S. typhosa*, each bacteria was placed into 2 different Erlenmeyer flasks containing 100 mL of sterile Nutrient Broth media, and then they were shaker at room temperature for 24 hours. The optical density was measured at a wavelength of 600 nm using a UV-Visible Spectrophotometer. The tested solutions were prepared with varying concentrations of $5x10^{-3}$, $1x10^{-3}$, and $5x10^{-4}$ M and 5 mL of each compounds tested were placed into three different test tubes. Each tube was added with 500 µL of *S. aureus* and *S. typhosa* inoculums and then vortexed. At contact times of 0, 5, 10, and 15 minutes, the optical density of this mixture was measured using a UV-Visible Spectrophotometer instrument. Then, the same treatment was also carried out with a solution of methanol added with 5% dimethyl sulfoxide as a negative control, and a positive control solution of Wipol (2.5% pine oil).

RESULTS AND DISCUSSION

The synthesis of organotin(IV) compound

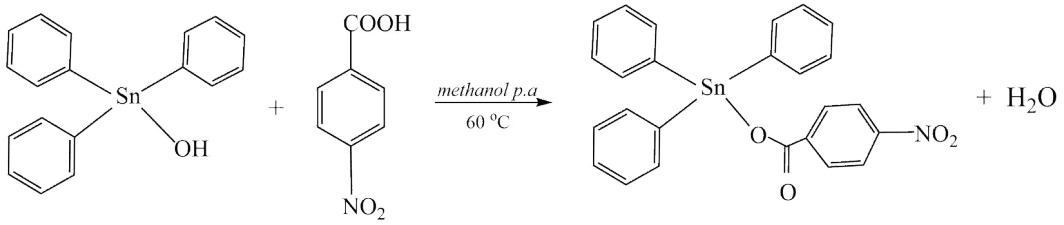
Two organotin(IV) compounds, [Ph_3Sn(4-HABz)] (**2**) and Ph_3Sn(4-HNBz)] compounds were obtained as yellow and white solid, respectively have been successfully synthesized from the reaction of compound **1** with 4-HABz and 4-HNBz based on the procedures described in the literature (14,15, 20, 22, 24-28). The schematic reaction for the synthesis of compounds **2** and **3** are shown in Figure 1, the products of the synthesis for compounds **2** and **3** were 84.09% and 80.70%, respectively. The elemental microanalyses of the compounds synthesized are in accordance with the calculated data.

(a)



1 4-aminobenzoic acid **2**

(b)



1 4-nitrobenzoic acid **3**

Figure 1: The preparation of **(a)** compound **2**; **(b)** compound **3**.

Characterization of Organotin(IV) Compounds

The success of the synthesis the targeted compounds was analyzed using some spectroscopy techniques. The result of IR characterization was proven by the appearance and disappearance of certain characteristic peaks. The appearance of the characteristic absorption in the two target compounds **2** and **3** is the presence of peak in the regions of 782.07 cm^{-1} and 723.53 cm^{-1} which are characteristics for the vibration of the Sn-O bond, and it is supported with the peaks from Sn-O-C bond in 1177.08 cm^{-1} and 1170. 04 cm^{-1} which indicated that the central atom of tin (Sn) has been bonded to the ligands of 4-HABz and 4-HNBz via oxygen (O) atom (3, 14).

The UV spectroscopic analysis produced the maximum wavelength (λ_{max}) of the compound measured. The data indicated there are several important shifts for each compound. The two compounds give two main characteristic bands of π→π* and n→π* transitions. For example, in compound **1**, there is π→π* transition at 234 nm, after conversion **1** to **2** there are changes in λ_{max} for the n→π* transition to 234 and 278 nm. The presence of a bathochromic shift is an indication that the substitution of ligand has occurred at the central atom because the 4-HABz ligand is a chromophore molecule, with the presence of a –C=O group and a -C=C- bond which causes a shift in wavelength towards a longer one and the effect of auxochrome with the presence of the $–NH_2$ group. Compound **3** also undergoes a change in max for the n→π* transition to 234 and 290 nm originating from the free electrons of O atoms such as $–NO_2$ and –COOH groups from 4-HNBz ligand.

The NMR data of the compounds synthesized were carefully analyzed to ascertain the successful in the synthesis of compounds **2** and **3**. The typical chemical shifts for compounds **2** and **3** prepared were characterized carefully and compared to the data available in the literature (14,15, 20, 22, 24-28). Based on the data of ^1H NMR spectrum for compound **2**, the chemical shifts of phenyl protons attached to tin metal appeared as expected in the range of 7.440 for H_2 and H_6 to 7.460 ppm for H_3 and H_5, while the protons in benzoate ring appeared at 7.744-7.843 ppm. The ^{13}C NMR values of the compounds synthesized were close to the values

reported by others (14,15, 20, 22, 24-28). The analyses are as follows the carbon in the carboxyl group as expected appeared in the region of 164 ppm. The δ of carbons in the phenyl ligand in compounds **2** and **3** are at 126.9-131.8 ppm and the carbons in the benzoate are in δ range of 129.1-136.9 ppm (14,15, 20, 22, 24-28).

The disinfectant bioactivity tests for compounds **2** and **3** at variation concentrations of $5x10^{-3}$, $1x10^{-3}$, and $5x10^{-4}$ M, solvent as negative control, and positive control with contact times of 0, 5, 10 and 15 minutes were carried. This is performed to find out the optical density of the disinfectant solutions that show the ability to inhibit the bacterial growth (Tables 1-3). The result clearly showed that compounds **2** and **3** have strong antibacterial activity against Gram-positive *S. aureus* and Gram-negative *S. typhosa* bacteria.

The compounds **2** and **3** have strong activity as a disinfectant, which characterized by the decrease in the absorbance value with variations from the maximum concentration to the minimum concentration where the longer the contact time to the disinfectant agent, the more disinfectant agent was absorbed by bacteria, and because of this process causing the destruction of bacteria and inhibits the growth of these bacteria, so that the absorbance value will decrease (31).

Compounds **2** and **3** were more capable in inhibiting *S. aureus* than *S. typhosa*, this was indicated by a greater decrease in the absorbance value when these compounds were tested against *S. aureus* bacteria. The difference in the decrease of absorbance values is because the two bacteria have different sensitivities and it was fount that *S. aureus* has a higher sensitivity than *S. typhosa*. This is due to fact that the differences in the structure of the cell walls of the two bacteria, causing differences in the decrease of optical density to the compounds tested in the intracellular bacteria. Gram positive bacteria *S. aureus* has a greater sensitivity level than Gram negative bacteria *S. typhosa* because the cell wall of Gram negative bacteria *S. typhosa* is composed of an outer membrane, an inner membrane and peptidoglycan with a more complex structure than that of Gram-positive bacteria (32-34).

Based on the test results, compound **3** has activity as a better disinfectant because it is characterized by a greater decrease in absorbance value than compound **2**, this is because compound **3** has the effect of electron withdrawing anion (NO_2) which causes the central atom of Sn to become more positive, so it is easier to penetrate the peptidoglycan layer on the bacterial cell wall which is electronegative, causing inhibition of bacterial cell growth.

The results of the bioactivity test of organotin disinfectant with a comparison of solvent as negative control and positive control against *S. aureus* and *S.typhosa* bacteria showed that both organotin compounds **2** and **3** had activity as effective disinfectants, compared to solvents and positive control characterized by a greater decrease in absorbance. large compared to the decrease in absorbance of the solvent and positive control. The MIC value (Minimum Inhibitory Concentration) of the two organotin compounds tested against *S. aureus* and *S. typhosa* was $5x10^{-4}$ M. This MIC value is stronger than fractions obtained from the stem roots extracts of *Archidendron jiringa* (35) or other synthetic products reported by others (36). The most effective contact time to inhibit the growth of the test bacteria was 15 minutes. This proves that the length of contact time has an effect on the magnitude of the inhibition of growth and reproduction of bacteria.

Table 1: The OD values of compounds **2** against *S. aureus* and *S. typhosa.*

Type of bacteria	Results											
	5 x 10^{-3} M				**1 x 10^{-3} M**				**5 x 10^{-4} M**			
	0′	5′	10′	15′	0′	5′	10′	15′	0′	5′	10′	15′
S. aureus **($A_{initial}$ = 0.655)**	0.585	0.480	0.420	0.375	0.301	0.210	0.218	0.122	0.256	0.226	0.185	0.110
S.typhosa **($A_{initial}$ = 0.661)**	1.112	0.452	0.330	0.133	0.300	0.252	0.260	0.110	0.235	0.193	0.195	0.102

Table 2: The OD values of compound **3** against *S. aureus* and *S. typhosa.*

Type of bacteria	Results											
	5 x 10^{-3} M				**1 x 10^{-3} M**				**5 x 10^{-4} M**			
	0′	5′	10′	15′	0′	5′	10′	15′	0′	5′	10′	15′
S. aureus **($A_{initial}$ = 0.655)**	0.200	0.160	0.113	0.075	0.135	0.127	0.085	0.070	0.127	0.082	0.065	0.025
S.typhosa **($A_{initial}$ = 0.661)**	0.172	0.157	0.110	0.080	0.113	0.089	0.077	0.045	0.089	0.073	0.055	0.032

Table 3: The OD values of solvent and positive control against *S. aureus* and *S. typhosa.*

Compound	Results							
	S. aureus ($A_{initial}$ = 0.655)				*S. typhosa* ($A_{initial}$ = 0.661)			
	0′	5′	10′	15′	0′	5′	10′	15′
P	0.522	0.494	0.492	0.490	0.598	0.486	0.480	0.484
KP	0.651	0.596	0.572	0.570	0.638	0.624	0.599	0.594

Note:
P = solvent as negative control
KP = positive control

CONCLUSIONS

The synthesis of two organotin(IV) compounds, triphenyltin(IV) 4-aminobenzoate (**2**) and triphenyltin(IV) 4-nitrobenzoate (**3**) has been successfully carried out. The synthesized compounds, the ligands and the starting compounds **1** have been tested for their antibacterial activity. Based on optical density data, compound **3** showed the best antibacterial activity at a minimum inhibitory concentration value of $5x10^{-4}$ M and the most effective contact time to inhibit the growth of the test bacteria was 15 minutes. Organotin compounds **2** and **3** have activity as effective disinfectants, compared to the ligands, starting compound and positive control characterized by a greater decrease in absorbance compared to other substances during the test.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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