

ANTIBACTERIAL ACTIVITY TEST OF
DIPHENYLTIN(IV) DIBENZOATE AND
TRIPHENYLTIN(IV) BENZOATE
COMPOUNDS AGAINST BACILLUS
SUBSTILIS AND PSEUDOMONAS
AERUGINOSA

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ANTIBACTERIAL ACTIVITY TEST OF DIPHENYLTIN(IV) DIBENZOATE AND TRIPHENYLTIN(IV) BENZOATE COMPOUNDS AGAINST BACILLUS SUBSTILIS AND PSEUDOMONAS AERUGINOSA

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Abstract - The antibacterial activity test of 2 organotin(IV) compounds, diphenyltin(IV) dibenzoate (2) and triphenyltin(IV) benzoate (4) which were prepared by the reaction of diphenyltin(IV) dihydroxide and triphenyltin(IV) hydroxide with benzoic acid has been performed. These compounds were characterized by ¹H and ¹³C NMR, IR, UV-Vis spectroscopies and also based on the microanalytical data. The results of antibacterial activity by diffusion method against *Pseudomonas aeruginosa* and *Bacillus subtilis* showed that both compounds were active at concentration of 200 ppm, which are about 3.89×10^{-4} M for 2 and 4.47×10^{-4} M for 4, while the chloramphenicol, as control positive, at the same concentration 200 ppm (6.1894×10^{-4} M), gave inhibition with halozone was bigger. This result indicated that both compounds have potential to be used as antibacterial substances.

INTRODUCTION

Bacteria resistances towards antibacterial substances are very serious problem in health sector and have attracted much attention in the global perspective (Jawetz *et al.*, 1986). Thus, attempt to find a compound which can be applied as new antibacterial substance is very important and continuously been searched (Jawetz *et al.*, 1986; Lorian, 1980).

Antibacterial are substances which can inhibit the growth or even stop the growth of bacteria by ways of disturbing the metabolism of harmful bacteria. This microorganism may cause the danger due to its ability to infect and cause disease and damage the food material (Lorian, 1980; Pelczar and Chan, 1986).

Organotin(IV) compounds are interesting to be explored not only because their structural features but also they are known to have strong biological activity (Tiekink, 1991; Shahid *et al.*, 2003; Pellerito and Nagy, 2002; Bonire *et al.*, 1998). The factors

affecting their activity are the number and the type of organic ligand attached to the Sn metal (Tiekink, 1991; Pellerito and Nagy, 2002). These compounds and their derivatives have been widely used and known in many biological tests such as antifungal (Bonire *et al.*, 1998; Szorcsik *et al.*, 2002; Hadi *et al.*, 2008; Hadi *et al.*, 2000), anticancer and antitumour (Gielen, 2003; Rehman *et al.*, 2009; Li *et al.*, 2008; Hadi *et al.*, 2010; Hadi *et al.*, 2012), antiviral (Singh *et al.*, 2000), antibacterial (Maiti *et al.*, 1988; Win *et al.*, 2013) and anticorrosion (Kurniasih *et al.*, 2015; Hadi *et al.*, 2015; Singh *et al.*, 2010; Rastogi *et al.*, 2011; Hadi *et al.*, 2016).

In this work, we report the antibacterial activity of diphenyltin(IV) dibenzoate and triphenyltin(IV) benzoate against *B. subtilis* and *P. aeruginosa*.

MATERIALS AND METHODS

Materials

All reagents used were AR grade. Diphenyltin(IV)

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dichloride ($[(C_6H_5)_2SnCl_2]$), triphenyltin(IV) chloride ($[(C_6H_5)_3SnCl]$), benzoic acid were obtained from Sigma, sodium hydroxide (NaOH) and methanol (CH₃OH) were JT Baker products, and the control drug, chloramphenicol were used as received without further purification. Negative gram bacteria *P. aeruginosa* was obtained from Department of Microbiology, University of Indonesia, Jakarta and positive gram bacteria *B. subtilis* was obtained from Biochemistry Laboratory, Department of Chemistry, University of Lampung Indonesia.

IR spectra were recorded on a Bruker VERTEX 70 FT-IR spectrophotometer with KBr discs in the range of 4000-400 cm^{-1} . Elemental analyses (CHNS) were performed on Fison EA 1108 series elemental analyser. ¹H and ¹³C NMR spectra were recorded on a Bruker AV 600 MHz NMR (600 MHz for ¹H and 150 MHz for ¹³C). All experiments were run in DMSO-D₆ at 298K. The number of runs used for ¹H experiments were 32 with reference at DMSO signal at 2.5 ppm, while the ¹³C were 1000-4000 scans with reference DMSO signal at 39.5 ppm. The UV spectra were recorded in the UV region and were measured using a UV-Shimadzu UV-245 Spectrophotometer. Measurements were performed in 1 mL quartz-cells. Solutions were prepared using methanol as the solvent with concentration of 1.0x10⁻⁴M.

Preparation of organotin(IV) benzoate

The organotin(IV) benzoate compounds used in this work were prepared based on the procedure previously reported (Hadi *et al.*, 2008; 2009; 2010; 2012; 2015; 2016). These procedure was obtained from adaption from the work by Szorcik *et al.* (2002). For example the procedure in the preparation of diphenyltin(IV) dibenzoate was as follows:

3.44 g (0.01 mol) $[(C_6H_5)_2SnCl_2]$ in 50 mL methanol was added 0.8 g (0.02 mol) NaOH. The reaction mixtures were stirred for about 45 minutes. Compound $[(C_6H_5)_2Sn(OH)_2]$ (1) was precipitated out as white solid, filtered off and dried *in vacuo* till they are ready for analysis and further reaction. The average yield was 2.33 g (94 %).

0.4605 g (1.5 mmol) compound 1 in 50 mL of methanol was added with 17 mole equivalents of benzoic acid (0.366 g) and was refluxed for 4 hours at 60 - 61°C. After removal of the solvent by rotary evaporator, the produced compounds $[(C_6H_5)_2Sn(OOCC_6H_5)_2]$ were dried *in vacuo* until they are

ready for analysis and further use for antibacterial activity test. The average yields were more than 90 %. The same procedure was also adapted in the preparation of triphenyltin(IV) derivatives, $[(C_6H_5)_3Sn(OOCC_6H_5)]$, where only one mole equivalent of the benzoic acid was added.

Antibacterial Activity Test

Preparation of Media

The media used for the activity test was nutrient agar (NA). 2.8 g of NA was dissolved in 100 mL aquadest, heated and sterilized by autoclave at 121°C, pressure of 1 atm for 15 minutes. 15 mL of steril media was plated on sterilized petri disc. It was carried out in laminar air flow, and left the media to solidify.

Antibacterial activity test by diffusion test

The procedure used for the antibacterial activity with diffusion test was based on the procedure performed by Jawetz *et al.*, (1986)] and as follows: one ose of *P. aeruginosa* and *B. subtilis* was diluted with 2 mL of salin solution (NaCl 0.85%) and was used as bacteria suspension. 1 mL of the suspension was then inoculated on NA, flattened with spreader. 4 paper discs were prepared. The first paper disc contained the positive control (chloramphenicol), the second was negative control containing the solvent used for the test, i.e. DMSO, the third and fourth paper discs containing the organotin(IV) compounds tested. All paper discs were then placed on the surface of media. They were then incubated for 1 day at 37°C and were monitored to see the inhibition zone. The compounds found as the most active, i.e. giving the most effective inhibition was then tested with the dilution method.

Antibacterial activity test with dilution test

Based on the result of diffusion test, the most effective concentration inhibition zones were obtained for both diphenyltin(IV) dibenzoate and triphenyltin(IV) benzoate. These compounds with the most active concentration were then dissolved with aquadest-DMSO and the volumes were then varied for dilution test based on the procedure developed by Lorian (1980). The compound tested with certain volume was then placed to liquid NA media, homogenized with vortex and then pour to petri disc, left them until solidified. The bacteria suspension of *P. aeruginosa* and *B. subtilis*

were then inoculated on the media at temperature of 37°C for 2-3 days. The growth of bacteria was then monitored every day. The volume of the compound tested was varied into 0.5; 1.0; 1.5; 2.0 and 2.5 mL where each of them was mixed with 15 mL of liquid NA media, homogenized with shaker. The most effective compounds tested were a compound which was the compound with the smallest concentration but the inhibition zone was the biggest (Lorian, 1980).

RESULTS AND DISCUSSION

The syntheses of diphenyltin(IV) dibenzoate (2) and triphenyltin(IV) benzoate (4) were performed by reacting diphenyltin(IV) dihydroxide (1) and triphenyltin(IV) hydroxide (3) with benzoic acid using the similar procedure we have used previously (Hadi *et al.*, 2008; 2009; 2010; 2012; 2015; 2016).

The reactions that occurred in the preparation of 2 and 4 were shown in Figure 1 and 2.

The important vibrations information of IR spectra from compounds 2 and 4 as well as the

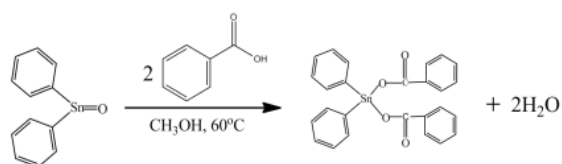


Fig. 1 The preparation of diphenyltin(IV) dibenzoate

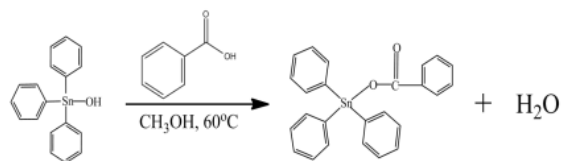


Fig. 2 The preparation of triphenyltin(IV) benzoate

intermediate compounds are tabulated in Table 1.

From Table 1, compound 1 has characteristic stretch for Sn-O bond at 693.56 cm⁻¹. When 1 was converted to 2, the new stretch at 1176.89 cm⁻¹ appeared, this peak is characteristic that Sn-O-C has been formed which indicated that bond formation between Sn and carboxyl group from the acid has been formed. The other characteristic vibration is at 1624.04 cm⁻¹, specific for C=O stretch, indicated that carbonyl is present in the compounds. The similar data are also observed in the case of compound 4 which indicated that the intended compound synthesized has been obtained.

The data of microanalysis for the compounds synthesized in this work are shown in Table 2. The result showed that in general the values obtained are in a good agreement with the calculated theoretical values, and they were less than 1%.

Table 2. Microanalytical data of the compounds synthesized

Compounds	Elemental Analysis found (Calculated)	
	C	H
[(C ₆ H ₅) ₂ Sn(C ₆ H ₅ COO) ₂] (2)	60.4 (60.6)	3.7 (3.6)
[(C ₆ H ₅) ₃ Sn(C ₆ H ₅ COO)] (4)	63.5 (63.7)	4.3 (4.1)

Table 3. The λ_{max} of the UV spectra of the organotin(IV) compounds

Compound	λ _{max} (nm)	
	π→π*	n→π*
Benzoic acid	210.00	280.00
[(C ₆ H ₅) ₂ Sn(OH) ₂] (1)	207.00	263.00
[(C ₆ H ₅) ₂ Sn(C ₆ H ₅ COO) ₂] (2)	235.00	309.00
[(C ₆ H ₅) ₃ Sn(OH)] (3)	204.00	293.00
[(C ₆ H ₅) ₃ Sn(C ₆ H ₅ COO)] (4)	234.00	309.00

Table 1. Some selected and important IR band of the compounds synthesized

Compound	1	2	3	4	References (cm ⁻¹)
Sn-O	693.56	593.36	691.28	598.45	800-600
Sn-O-C	-	1176.89	-	1158.10	1250-1000
Sn-ph	1077.18	1072.90	1076.83	1074.67	1100-1000
OH	3445.83	3447.12	3437.37	3438.79	3100-3500
C=O	-	1624.04	-	1624.77	1760-1600
CO ₂ sym	-	1406.87	-	1532.91	1500-1400
C=C	1426.76	1521.16	1479.29	1551.81	1650-1400
Aromatic C-H	3011.16	3067.38	3061.29	3069.22	3100-3000

The λ_{\max} of all compounds were obtained by UV spectroscopy analyses. The data obtained are shown in Table 4. From these data, it is clear that there was some important shifting change in the λ_{\max} for each compound. In the spectra, all compounds produced 2 peaks due to transition of $\pi \rightarrow \pi^*$ and $n \rightarrow \pi$. For example in compounds 1, the λ_{\max} observed were 207 and 263 nm. In 2 and 4, there were large shift in $\pi \rightarrow \pi^*$ the transition, due to the bound of benzoate to Sn atom. The bathochromic shift from the starting material to the synthesized compounds were due to the substitution of oxygen in 1 and 3 which was replaced by oxygen atom in benzoate (Hadi *et al.*, 2008; 2009; 2010; 2012; 2015; 2016). The large shifts observed in 2 and 4 due to the increase of conjugate bond in these compounds causing the energy difference between HOMO and LUMO orbitals were decreased making the λ_{\max} absorbs were increased (Sudjadi, 1985).

The ¹H and ¹³C NMR data of the organotin(IV) compounds prepared are tabulated in Table 4. The characteristic chemical shift in the spectra of the compounds prepared were characterized carefully and compared to some previous results (Hadi *et al.*, 2012; Kurniasih *et al.*, 2015; Hadi *et al.*, 2015; Nath *et al.*, 1997). Based on the data of ¹H NMR spectra for compound 2 and 4, the chemical shifts of the vinyl protons attached to tin metal appeared in the range of 7.5- 7.6 ppm, as expected and the protons in benzoate ring appeared at 7.7-7.9 ppm. The ¹³C NMR of the compounds synthesized are very similar to the results obtained by others (Hadi *et al.*, 2012; Kurniasih *et al.*, 2015; Hadi *et al.*, 2015; Nath *et al.*, 1997). The analyses are as follows carbon in the carboxyl group appeared in the region of 165-166 ppm, as expected. The δ of carbons in the phenyl ligand in both compounds of 128-130 ppm and the carbons in the benzoate are in δ range of 130-135

ppm (Hadi *et al.*, 2012; Kurniasih *et al.*, 2015; Hadi *et al.*, 2015; Nath *et al.*, 1997; Hadi *et al.*, 2003 and Hadi and Appleton, 2010).

The results of antibacterial activity test with diffusion method for the compounds synthesized are shown in Table 5-8. The halozone was observed in all concentrations of compounds 2 and 4, but no halozone was observed for the starting material and the ligand used. This result indicated that the synthesized compounds tested have antibacterial activity mean they disturb the metabolism in the bacteria.

Based on the data on Table 5-8, both compounds have inhibition activity toward the two bacteria used and they were effective at the same concentration, i.e. 200 ppm. Similar observation was also recorded for the positive control chloramphenicol which also gave inhibition at 200 ppm. This method is used in order to find the most effective concentration that inhibit the growth of bacteria, therefore the ratio comparison was used to find the effectivity of the two compounds tested by dividing the wide of halozone with the amount of active compound in the media. After careful observation, the concentration 200 ppm had the biggest ratio. Although the higher concentration of the compound tested gave wider halozone, but ratio was smaller than the ratio for 200 ppm. Although the both compounds tested have the same concentration in this test, but their comparison activity can be seen from ratio of the test at higher concentration (Table 5-8). Compound 2 was observed to have a better activity than 4.

The most active concentrations for both compounds were then subjected for dilution test. The results of the test were shown in Table 9 and 10.

The result of dilution test is shown in Table 9-10. The compound 2 and 4 in concentration 3.89×10^{-4}

Table 4. ¹H and ¹³C spectra of the organotin(IV) compounds

Compound	H in phenyl (ppm)	H in benzoate (ppm)	C in phenyl and benzoate (ppm)
[(C ₆ H ₅) ₂ Sn(C ₆ H ₅ COO) ₂] (2)	H2 & H5 7.52 (d, 4H); H3 & H5 7.56 (t, 4H); H4 7.52 (t, 2H)	7.71-7.91 (m)	C1-6 (phenyl): 129.1 - 128.5; C7 165.7; C8 131.4; C9 130.2; C10 134.0; C11 133.8; C12 130.0; C13 128.4
[(C ₆ H ₅) ₃ Sn(C ₆ H ₅ COO)] (4)	H5 & H6 7.5 (d, 6H); H3 & H5 7.49 (t, 6); H4 7.47	7.74-7.95 (d)	C1-6 (phenyl): 129.2-128.7; C7: 165.4; C8: 131.3; C9: 130.3; C10:134.0; C11: 134.0; C12: 130.0; C13: 128.2

Table 5. The size of halozone of diphenyltin(IV) dibenzoate against *B. subtilis*

Concentration (ppm) (X)	Halozone (cm)(Y)	Effectivity (Y/X)
200	0.8.7	0.004
250	0.8.7	0.0032
300	1.2	0.004
400	1.0.7	0.0025
500	1.0	0.002

Table 6. The size of halozone of diphenyltin(IV) dibenzoate against *P. aeruginosa*.

Concentration (ppm) (X)	Halozone (cm)(Y)	Effectivity (Y/X)
200	0.8.7	0.004
250	0.9.7	0.0036
300	0.6	0.002
400	0.6.7	0.0015
500	1.2	0.0024

Table 7. The size of halozone of triphenyltin(IV) benzoate against *B. subtilis*

Concentration (ppm) (X)	Halozone (cm)(Y)	Effectivity (Y/X)
200	0.6.7	0.003
250	0.5.7	0.002
300	0.8	0.0026
400	0.7.7	0.0017
500	0.8	0.0016

Table 8. The size of halozone of triphenyltin(IV) benzoate against *P. aeruginosa*.

Concentration (ppm) (X)	Halozone (cm)(Y)	Effectivity (Y/X)
200	0.4.7	0.002
250	0.2.7	0.0008
300	0.4	0.0013
400	0.4.7	0.001
500	0.6	0.0012

Table 9. The result of dilution test against *B. subtilis*

Compound	Volume (mL)				
	0.5	1	1.5	2	2.5
2	++++	+++	+++	+	+
4	++++	++++	+++	++	+

Note of Bacterial growth:

++++ = very high; +++ = high; ++ = medium; + = little; - = no growth

Table 10. The result of dilution test against *P. aeruginosa*.

Compound	Volume (mL)				
	0.5	1	1.5	2	2.5
2	++++	++++	+++	+	+
4	++++	++++	+++	++	+

Note of Bacterial growth:

++++ = very high ; +++ = high ; ++ = medium ; + = little ; - = no growth

M (200 ppm) and 4.6×10^{-4} with volume used 2.5 mL per media was able to inhibit maximally the growth of both bacteria, while at other volumes, the bacteria were still able to grow.

The microorganism inhibition mechanism by antibacterial substances may be caused by some factors, namely (1) the disturbance in the compound composition of cell wall; (2) the increase of cell membrane permeability which cause the loss of component cell structure; (3) the inactivation of enzyme; and (4) destruction or damaging the

function of genetic materials (Lorian, 1980; Jawetz *et al.*, 1986; Pelczar and Chan, 1986).

In this biological activity test, the compound 4 where its compound is more electropositive than 3, disturb the electronegative bacteria cell wall, thus the interaction cause the disruption of bacteria growth. This is because the bacteria wall cell is composed by macromolecule of peptidoglycan which was composed by tetrapeptideglycan that functioned to feed the cell and to give strength, protect the cell and carry out intracellular material

exchange with their environment. When the cell wall is disrupted, it will cause the cell inside is not protected as a result the bacteria will be death due the disruption (Lorian, 1980; Pe;czr and Chan, 1986).

CONCLUSION

We have successfully prepared the derivatives of organotin(IV) compound, diphenyltin(IV) dibenzoate and triphenyltin(IV) benzoate. Based on the data reported [2], both compounds synthesized are potentially to be used as antibacterial agents. The triphenyltin(IV) derivative has been shown to be more active than diphenyltin(IV) derivative. This finding was in line with other data relating to the number of carbon atom present in the compound and might also relate to the ability of phenyl ligand to draw electron from the metal center as a result the metal became more positive and reacted actively with electronegative cell of bacteria, thus the growth of bacteria was disrupted. However, we aim to have a better antibacterial substance which has much smaller inhibition concentration.

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