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6

Antibacterial Activity of Diphenyltin(IV) and Triphenyltin(IV) 3-Chlorobenzoate Against *Pseudomonas aeruginosa* and *Bacillus subtilis*

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ABSTRACT

In this paper, we reported the syntheses and antibacterial activity test of 2 organotin(IV) compounds, diphenyltin (IV) di-3-chlorobenzoate (2) and triphenyltin (IV) 3-chlorobenzoate (4). These two compounds were prepared by the reaction of diphenyltin (IV) dihydroxide and triphenyltin (IV) hydroxide with 3-chlorobenzoic acid. 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 the triphenyltin(IV) 3-chlorobenzoate was active at concentration of 3.956×10^{-4} M (200 ppm), while the chloramphenicol gave inhibition of 6.1894×10^{-4} M (200 ppm), although the halozone was bigger. This result indicated that compound 4 is potentially to be used as antibacterial substance, although the search of other derivative of organotin (IV) with other ligands is still needed to get much higher and much better activity.

Keywords: antibacteria, *B. subtilis*, organotin(IV) 3-chlorobenzoate, *P. aeruginosa*

INTRODUCTION

Infectious disease is one of the main problems in the world health, as almost every country faces this problem¹. The main cause of infectious diseases is due to bacteria, of them are negative gram bacteria *P. aeruginosa* and positive gram bacteria *B. subtilis*¹. *P. aeruginosa* causes many diseases such as infections of wounds, burns, bluish green pus, urinary channel, respiratory causing the

pneumonia with necrosis, light external otitis on swimmers and eyes. *B. subtilis* can cause damage on canned food which can cause gastroenteritis to the consumers².

One of the ways that can be carried out to inhibit the growth of bacteria in every organism is by applying antibacterial substances³. Based on the use of antibacterial substances, they can be categorized as disinfectant, antiseptic, sterilizer,

sanitizer⁴. The inhibition of bacterial growth by antibacterial substances can be done by cell disruption mechanism which is by inhibiting its formation or changing it after being formed, alteration of cytoplasm membran permeability that cause the nutrient is out from inside the cell, inhibition of enzyme activity and preventing the synthesis of nucleid acid and protein¹.

Organotin (IV) compounds are well known to have wide applications and one of the organometallic compounds widely applied and used due to their significants in many biological activities⁵⁻²¹. These compounds and their derivatives have been known as antifungal⁹⁻¹¹, anticancer and antitumour¹²⁻¹⁵, antiviral¹⁶, antibacterial¹⁷⁻¹⁸ and anticorrosion²⁰⁻²⁴. The biological activity of these compounds because of the number and basic character of organic ligand attached to metal center Sn¹⁹. The anions bound to Sn metal are the secondary factor in determining the organotin (IV) compounds.

In this paper, we report the antibacterial activity of organotin (IV) derivatives of diphenyltin (IV) and triphenyltin (IV) with 3-chlorobenzoic acid againts negative gram bacteria *P. aeruginosa* and positive gram bacteria *B. subtilis*

EXPERIMENTAL

All reagents used were AR grade. Diphenyltin(IV) dichloride [(C₆H₅)₂SnCl₂], triphenyltin(IV) chloride [(C₆H₅)₃SnCl], 3-chlorobenzoic 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.

The UV spectra were obtained using a UV-Shimadzu UV-245 Spectrophotometer. The measurements were conducted in the UV region and were measured in 1 mL quartz-cells. The solvent used to dissolve sample was methanol. The concentration used in the measurements were

1.0x10⁻⁴ M. A Bruker AV 600 MHz NMR (600 MHz for ¹H and 150 MHz for ¹³C) were used to obtain ¹H and ¹³C NMR spectra. All experiments were run in DMSO-D₆ at 298K. The number of scans used for ¹H experiments were 32 with reference at DMSO signal at 2.5 ppm, while the ¹³C were 1000-4000 scans with the reference of DMSO signal at 39.5 ppm. A Bruker VERTEX 70 FT-IR spectrophotometer were used to record the IR Spectra, KBr discs were used in all measurements. The range of 4000-400cm⁻¹ was applied. Elemental analyses (CHNS) were performed on Fision EA 1108 series elemental analyser.

Preparation of organotin (IV) chlorobenzoate

The organotin(IV) chlorobenzoate compounds used in this work were prepared based on the procedure previously reported^{9, 20, 21}. These procedure was obtained from adaption from the work by Szorcsik *et al.*²⁴. For example the procedure in the preparation of dibutyltin(IV) dihydroxybenzoate was as follows:

To 3.44 g (0.01 mol) [(C₆H₅)₂SnCl₂] in 50 mL methanol was added 0.8 g (0.02 mol) NaOH. 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 analysis and further reaction. The average yield was 2.33 g (94 %).

To 0.4605 g (1.5 mmol) compound 2 in 50 mL of methanol was added with 2 mole equivalents of 3-chlorobenzoic acid (0.313 g) and was refluxed for 4 hours at 60 – 61°C. After removal of the solvent by rotary evaporator, the product compounds [(C₆H₅)₂Sn(OOCC₆H₄Cl)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 %.

A similar procedure was also adapted in the preparation of triphenyltin(IV) derivatives, [(C₆H₅)₃Sn(OOCC₆H₄Cl)], where only one mole equivalent of the 3- chlorobenzoic acid was used.

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 placed on sterilized petri disc. It was carried out in laminar air flow, and left the media to solidify.

Antibacterial activity test by diffusion test

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 dilution Test

Based on the result of diffusion test, the most effective concentration inhibition zones were obtained for both diphenyltin (IV) di-3-chlorobenzoate and triphenyltin (IV) chlorobenzoate. These compounds with the most active concentration were then dissolved with aquadest- DMSO and the

volumes were then varied. 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 suspensions 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 most effective compounds tested were a compound which was the compound with the smallest concentration but the inhibition zone was the biggest²⁶.

RESULTS AND DISCUSSION

The syntheses of diphenyltin(IV) di-3-chlorobenzoate (2) and triphenyltin(IV) 3-chlorobenzoate (4) were performed by reacting diphenyltin (IV) dihydroxide (1) and triphenyltin(IV) hydroxide(3) with 3-chlorobenzoic acid using the a similar procedure reported previously^{8, 14,15,21,22}.

Some important vibration of IR spectra for the synthesized compound are presented 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 1155.86 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.

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

| Compound | 1 | 2 | 3 | 4 | References |
|----------|---------|---------|---------|---------|------------|
| Sn-O | 693.56 | 652.68 | - | - | 800-600 |
| Sn-O-C | - | 1155.86 | - | 1158.10 | 1250-1000 |
| Sn-ph | 1077.85 | 1072.90 | 1076.83 | 1072.90 | 1100-1000 |
| OH | 3445.83 | 3447.12 | 3615.51 | 3438.79 | 3100-3500 |
| C=O | - | 1697.63 | - | 1629.72 | 1760-1600 |
| C=C | 1470.87 | 1591.60 | 1479.29 | 1551.81 | 1650-1566 |

Table2: Microanalytical data of the compounds synthesized

| Compounds | Elemental Analysis found (Calculated) | |
|---|---------------------------------------|-------------|
| | C | H |
| $[(C_6H_5)_2Sn(m-C_6H_4(Cl)COO)_2]$ (2) | 52.35 (53.42) | 3.05 (3.08) |
| $[(C_6H_5)_3Sn(m-C_6H_4(Cl)COO)]$ (4) | 58.00 (59.40) | 3.69(3.76) |

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

| Compound | λ_{\max} (nm) $\pi \rightarrow \pi^*$ | $n \rightarrow \pi^*$ |
|---|--|-----------------------|
| $[(C_6H_5)_2Sn(OH)_2]$ (1) | 207.00 | 263.00 |
| $[(C_6H_5)_2Sn(m-C_6H_4(Cl)COO)_2]$ (2) | 235.00 | 272.00 |
| $[(C_6H_5)_3Sn(OH)]$ (3) | 204.00 | 293.00 |
| $[(C_6H_5)_3Sn(m-C_6H_4(Cl)COO)]$ (4) | 236.00 | 285.00 |

The other characteristic vibration is at 1697.63 cm^{-1} , specific for C=O stretch, indicated that carbonyl is present in the compounds.

Table 2 presents the data of microanalysis for the organotin (IV) compounds synthesized. The values obtained generally are very good and compared to the calculated theoretical values were less than 1%.

The λ_{\max} of all compounds were obtained by UV spectroscopy analyses. The data obtained are shown in Table 3. Although the shift was not very high, but 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 3 and 4, there were large shift in the $\pi \rightarrow \pi^*$ transition, due

to the bound of chlorobenzoate 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 chlorobenzoate^{9,14,115,20,21}.

The data of 1H and ^{13}C chemical shifts of the organotin (IV) compounds prepared are tabulated in Table 4. The characteristic chemical shift in the spectra of the compounds prepared (Fig. 1) obtained were characterized carefully and compared to some previous results^{9,14,115,20,21}. Based on the 1H NMR obtained for compound 2 and 4, the chemical shifts of phenyl protons attached to tin metal as expected appeared in the range of 7.4 – 7.6 ppm, while the protons in the benzoate ring were at 7.7-7.9 ppm. The chemical shifts of carbon for both compounds also close to the reported values^{9,14,115,20,21}. The carbon in the carboxyl group as predicted appeared in the region of 165-166 ppm, while carbon of the phenyl ligand appeared in δ of 128-130 ppm. The carbons in the chlorobenzoate as expected appeared in δ range of 130 – 135 ppm close to the reported values of similar compounds^{9,14,115,20,21}.

The results of antibacterial by diffusion method are shown Table 5. The increase of halozone diameter was observed with the increase of concentration of all compounds tested. The triphenyltin (IV) 3-chlorobenzoate was more active in inhibiting *B. subtilis* than *P. Aeruginosa*, as the

Table 4: 1H and ^{13}C spectra of the organotin(IV) compounds

| Compound | H in phenyl (ppm) | H in benzoate (ppm) | C in phenyl and benzoate (ppm) |
|-------------------------------------|--|---------------------|--|
| $[(C_6H_5)_2Sn(m-C_6H_4(Cl)COO)_2]$ | H2 & H6 7.538 (d,4H); H3 & H5 7.554 (t, 4H); H4 7.570 (t,2H) | 7.703-7.991 (m) | C1-6 (phenyl): 129,3 – 128,8; C7 166,9; C8 131,6; C9 130,3; C10 134,2; C11 1340 ; C12 130,0; C13 128,4 |
| $[(C_6H_5)_3Sn(m-C_6H_4(Cl)COO)]$ | H2&H6 7.46 (d,6H); 3&H5 7.43 (t 6); H4 7.41 | 7.84-7.85 (d) | C1-6 (phenyl): 129.3 – 128.8 128.8; C7: 165; C8: 131.6; C9: 130.3; C10: 134.2; C11: 134.0; C12: 130.0; C13: 128.4 |

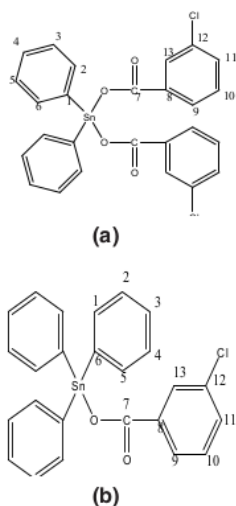


Fig. 1: Structure of (a) diphenyltin(IV) di-3-chlorobenzoate, (b) triphenyltin(IV) 3-chlorobenzoate.

inhibition zone observed was bigger in *B. subtilis*, although it was still smaller than the inhibition zone of the positive control used, chloramphenicol. The differences in inhibition zone of both bacteria is because *B. subtilis* has higher sensitivity compared to *P. Aeruginosa* perhaps due to the differences of cell wall structure of the bacteria²⁵⁻²⁷.

Based on data presented in Table 6, the compound 4 at concentration of 3.956×10^{-4} M, was the most effective concentration that inhibit the growth of the both bacteria. The diffusion method has been applied in order to find the smallest concentration that is able to inhibit the growth of bacteria, thus the ratio comparison has been used to determine the effectivity of the two compounds tested, by observing the wide of clear zone per the number of active compound in the media. The result showed that the concentration of 6 at 3.956×10^{-4} M (200 ppm) has the biggest ratio, even when it is compared with the increased of the concentration of the compound tested.

Table 5: The result of diffusion test againts *P. aeruginosa* and *B. subtilis*

| Compound (ppm) | Inhibition Zone (cm) | | | | | | | | | |
|---|----------------------|-----|--------------|-----|--------------|-----|--------------|-----|--------------|-----|
| | 200 (ppm) | | 250 (ppm) | | 300 (ppm) | | 400 (ppm) | | 500 (ppm) | |
| | P.a | B.s | P.a | B.s | P.a | B.s | P.a | B.s | P.a | B.s |
| $[(C_6H_4(Cl)COO)]$ | - | - | - | - | - | - | - | - | - | - |
| $[(C_6H_5)_2Sn(O)]$ (1) | - | 0.8 | - | 0.9 | - | 0.9 | - | 1.0 | - | 1.0 |
| $[(C_6H_5)_2Sn(m-C_6H_4(Cl)COO)_2]$ (2) | - | - | - | - | 0.8 | - | 0.8 | - | - | - |
| $[(C_6H_5)_3Sn(OH)]$ (3) | - | 0.8 | - | 0.9 | - | 1.0 | - | 1.1 | - | 1.1 |
| $[(C_6H_5)_3Sn(m-C_6H_4(Cl)COO)]$ (4) | 0.8 | 1.0 | 0.7 | 1.1 | 0.8 | 1.2 | 0.9 | 1.3 | 0.9 | 1.4 |
| Chloramphenicol | 1.5 | 2.5 | 1.5 | 2.5 | 1.5 | 2.5 | 1.5 | 2.5 | 1.5 | 2.5 |

P.a. = *Pseudomonas aeruginosa*, B.s. = *Bacillus subtilis*,
Positive control = Chloramphenicol

Table 6: The inhibition percentage of compound tested against *P. aeruginosa* and *B. subtilis*

| Compound | Inhibition | | | | | | | | | |
|---|--------------|-------|--------------|-------|--------------|-------|--------------|-------|--------------|-------|
| | 200 (ppm) | | 250 (ppm) | | 300 (ppm) | | 400 (ppm) | | 500 (ppm) | |
| | P.a | B.s | P.a | B.s | P.a | B.s | P.a | B.s | P.a | B.s |
| $[(C_6H_5)_2Sn(m-C_6H_4(Cl)COO)_2]$ (2) | - | - | - | - | 0.003 | - | 0.003 | - | - | - |
| $[(C_6H_5)_3Sn(m-C_6H_4(Cl)COO)]$ (4) | 0.004 | 0.005 | 0.003 | 0.004 | 0.003 | 0.004 | 0.002 | 0.003 | 0.002 | 0.003 |

P.a. = *Pseudomonas aeruginosa*, B.s. = *Bacillus subtilis*,
Positive control = Chloramphenicol

Table 7: The result of dilution test of compound 4 against *P. aeruginosa* and *B. subtilis*

| Bacteria | Volume of 4 (mL) | | | | |
|---|------------------|---------|-------|------|-----|
| | 0.5 | 1 | 1.5 | 2 | 2.5 |
| <i>P. aeruginosa</i> / <i>B. subtilis</i> | +++++++ | +++++++ | +++++ | ++++ | — |

Note of Bacterial growth:

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

The result of dilution test is shown in Table 7. The compound 4 in concentration 3.956×10^{-4} M (200 ppm) 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, such as the disturbance in the compound composition of cell wall, the increase of cell membrane permeability which cause the loss of component cell structure, the inactivation of enzyme and destruction or damaging the function of genetic materials²⁵.

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.

CONCLUSION

The derivatives of organotin(IV) compound, diphenyltin(IV) di-3-chlorobenzoate and

triphenyltin(IV) 3-chlorobenzoate were prepared. Based on the result obtained, they have shown to be potentially used as antibacterial substances. 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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