Synthesis and comparative study on the antibacterial activity organotin(IV) 3-hydroxybenzoate compounds

By Sutopo Hadi

WORD COUNT 3479
TIME SUBMITTED 28-JUN-2021 02:35PM
PAPER ID 73862858

Conference Paper

Sutopo Hadi*, Sri Lestari, Tati Suhartati, Hardoko Insan Qudus, Mita Rilyanti, Dian Herasari and Yandri Yandri

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https://doi.org/10.1515/pac-2020-1103

Abstract: The synthesis and comparative study on the antibacterial activity of three organotin(IV) compounds, namely dibutyltin(IV) bis-(3-hydroxybenzoate), [$Bu_2Sn(3\text{-}HBz)_2$] (**7**), diphenyltin(IV) bis-(3-hydroxybenzoate), [$Ph_2Sn(3\text{-}HBz)_2$] (**8**), and triphenyltin(IV) 3-hydroxybenzoate, [$Ph_3Sn(3\text{-}HBz)$] (**9**) which were prepared by the reaction of dibutyltin(IV) oxide, [Bu_2SnO] (**4**), diphenyltin(IV) dihydroxide, [$Ph_2Sn(OH)_2$] (**5**), and triphenyltin(IV) hydroxide, [Ph_3SnOH] (**6**) with 3-hydroxybenzoic acid (3-HBz) has successfully been performed. The characterization of these compounds were done using ^1H and ^{13}C NMR, IR, UV spectroscopies and their compositions were determined based on microanalytical data. Antibacterial activity of these compounds was demonstrated at concentrations of 1.89×10^{-4}, 1.81×10^{-4}, and 1.72×10^{-4} M, respectively by dilution method against *Pseudomonas aeruginosa*. Similarly, the compounds were active at concentration of 1.87×10^{-4}, 1.79×10^{-4}, and 1.71×10^{-4} M, respectively, against *Bacillus subtilis*. These activities are comparable to that of streptomycin at a concentration of 1.70×10^{-4} M as a positive control, but the halozone of compounds **7**, **8**, and **9** were slightly lower than that of streptomycin's halozone. The results obtained suggest that the compounds synthesized have potential as antibacterial agents.

Keywords: Antibacterial activity; *B. subtilis*; chemistry and its applications; organotin(IV) 3-hydroxybenzoate; *P. Aeruginosa*; VCCA-2020.

Introduction

Organotin(IV) compounds have been extensively studied not only because their interesting structural features but also they have been known to show strong many biological activities [1–4]. Their activities mainly are affected by the functional organic ligand attached to the Sn metal center as well the numbers of the ligand present [2], as a result many derivatives have been synthesized and investigated for their biological activity, such as antifungal [1, 5], anticancer and antitumor [6–9], antiviral [10], antibacterial [11, 12], antimalarial [13, 14], and inhibitor of corrosion [15–18].

The resistance of bacteria to antibacterial drugs has become a very serious problem in various sectors, thus special attention is needed to overcome the problem [19, 20]. For this reason, attempts to find new antibacterial drug have been extensively conducted [20, 21].

Article note: A collection of invited papers based on presentations at the Virtual Conference on Chemistry and its Applications (VCCA-2020) held on-line, 1–31 August 2020.

***Corresponding author:** Sutopo Hadi, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Lampung, Bandar Lampung 35145, Indonesia, e-mail: sutopo.hadi@fmipa.unila.ac.id
Sri Lestari, Tati Suhartati, Hardoko Insan Qudus, Mita Rilyanti, Dian Herasari and Yandri Yandri, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Lampung, Bandar Lampung 35145, Indonesia

A compound considered as antibacterial if it has the ability to inhibit the growth of bacteria. As a result of antibacterial action, the ability of microorganism to infect and cause disease, and damage foodstuffs is prevented [20, 21].

Based on the potential application of organotin(IV) carboxylate compound as describe above, in this work, we report the synthesis and comparative study on the antibacterial activity of three derivative organotin(IV) compounds of dibutyltin(IV), diphenyltin(IV), and triphenyltin(IV) with 3-hydroxybenzoate as ligand against two bacteria, Gram-positive *Bacillus subtilis* and Gram-negative *Pseudomonas aeruginosa*.

Experimental

Materials

All reagents used were of AR grade. Dibutyltin(IV) dichloride, ($[Bu_2SnCl_2]$), (**1**), diphenyltin(IV) dichloride ($[Ph_2SnCl_2]$) (**2**), triphenyltin(IV) chloride ($[Ph_3SnCl]$) (**3**), 3-hydroxybenzoic acid, (3-HBz), and the control drug, streptomycin were obtained from Sigma–Aldrich, sodium hydroxide (NaOH) and methanol (CH_3OH) were JT Baker products and they were used as received without further purification. Gram-negative bacteria *P. aeruginosa* was obtained from Department of Microbiology, University of Indonesia, Jakarta and Gram-positive bacteria *B. subtilis* ITBCCB148 was obtained from Biochemistry Laboratory, Department of Chemistry, University of Lampung Indonesia.

Instrumentations

Elemental analyses (CHNS) were conducted on Fision EA 1108 series elemental analyzer. IR spectra were recorded on a Bruker VERTEX 70 FT-IR spectrophotometer with KBr discs 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. The number of runs used for ^1H experiments were 32 with reference at DMSO signal at 2.5 ppm, while the ^{13}C were 1000–4000 scans with the 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 1.0×10^{-4} M.

Preparation of organotin(IV) 3-hydroxybenzoate

The organotin(IV) 3-hydroxybenzoate compounds used in this work were prepared based on the procedures previously reported [8, 9, 12, 14, 16–18], adapted from the work available in the literature [3]. For example the procedure in the preparation of $[Ph_3Sn(3\text{-}HBz)]$ (**9**) was as follows:

A mass of 3.855 g (0.01 mol) $[Ph_3SnCl]$ (**3**) in 50 mL methanol was added 0.4 g (0.01 mol) NaOH. The reaction mixtures were stirred for about 60 min. Compound $[Ph_3SnOH]$ (**6**) was precipitated out as white solid, filtered off, washed with double distilled water then with methanol three times and dried *in vacuo* till they are ready for analysis and further reaction. The yield was 3.42 g (93 %).

A mass of 0.5505 g (1.5 mmol) compound **6** in 30 mL of methanol was added with 1 mol equivalent of 3-HBz (0.207 g) and was refluxed for 4 h at 60–61 °C. After removal of the solvent by rotary evaporator, the compound $[[Ph_3Sn(3\text{-}HBz)]$ (**9**) which was obtained was dried *in vacuo* until they are ready for analysis and further use for antibacterial activity test. The yield was 0.672 g (92 %). The same procedure was also used in the synthesis of $[Bu_2Sn(3\text{-}HBz)_2]$ (**7**) from compound **4** and $Ph_2Sn(3\text{-}HBz)_2]$ (**8**) from compound **5** where 2 mol equivalents of 3-HBz acid were added. The compounds synthesized obtained were as follows:

[$Bu_2Sn(3\text{-}HBz)_2$] (**7**): white-yellowish solid; UV $\lambda_{max.}$ (MeOH) nm (log ε): 294; IR $\nu_{max.}$ (KBr) cm^{-1}: 2925.63 (Bu), 1584.07 (C=O), 1506.87 (CO_2 asym), 1243.4 (Sn–O–C), 778.7 (Sn–Bu), 435.7 (Sn–O); ^{1}H NMR (in DMSO-d_6, 600 MHz) δ (ppm): Hα: 1.6 (t), Hβ:1.4 (m); Hγ: 1.29 (t); Hδ: 0.93 (t), H in benzoate = 7.34–783 (m); ^{13}C NMR (in DMSO-d_6, 150 MHz): δ (ppm): Cα: 26.2, Cβ: 25.4, Cγ: 21.8, Cδ: 14.1, C1: 165.0; C2: 139.3, C3: 132.2, C4: 138.4, C5: 125.1, C6: 128.6, C7: 129.7; microelemental analysis: found (calculated): C 51.76 (52.07), H 5.48 (5.52).

[$Ph_2Sn(3\text{-}HBz)_2$] (**8**): white solid; UV $\lambda_{max.}$ (MeOH) nm (log ε): 2[illegible] and 297; IR $\nu_{max.}$ (KBr) cm^{-1}: 1597.2 (C=O), 1390.1 (CO_2 sym), 1479.3; 725.7 (phen), 1289.2 (Sn–O–C), 598.4 (Sn–O); ^{1}H NMR (in DMSO-d_6, 600 MHz) δ (ppm): H2 & H6 7.52 (d, 4H); H3 & H5 7.56 (t, 4H); H4 7.42 (t, 2H), H in benzoate: 7.70–7.90 (m); ^{13}C NMR (in DMSO-d_6, 150 MHz): δ (ppm): C(phen) C1: 129.3, C2 & 6: 129.1, C3 & 5: 128.9, C4: 128.1, C7 165.7, C8 131.4, C9 130.2, C10 134.0, C11 133.8, C12 130.0, C13 128.4; microelemental analysis: found (calculated): C 56.65 (57.04), H 3.61 (3.66).

[$Ph_3Sn(3\text{-}HBz)$] (**9**): white solid; UV $\lambda_{max.}$ (MeOH) nm (log ε): 234 and 293; IR $\nu_{max.}$ (KBr) cm^{-1}: 3437.3 (OH), 1624.7 (C=O), 1632[illegible] (CO_2 asym), 1551.8; 730.8 (phen), 1290.1 (Sn–O–C), 726.4 (Sn–O); ^{1}H NMR (in DMSO-d_6, 600 MHz) δ (ppm): $H_2 = H_6$ 7.59 (6H); H_3 & H_5 7.46 (t, 6H); H_4: 7.33 (t, 3H), H in benzoate: H_9 = 7.83 (s); H_{11} = 7.60 (d); H_{12} = 7.60 (d); H_{13} = 7.60 (d); ^{13}C NMR (in DMSO-d_6, 150 MHz): δ (ppm): C(phen) C_2 & C_6 = 131.7, C_3 & C_5 = 129.2, C_4 = 126.9; C_7 = 165.3; C_8 = 137.2; C_9 = 132.9; C_{10} = 129.5; C_{11} = 128.4; C_{12} = 128.2; C_{13} = 130.0; microelemental analysis: found (calculated): C 60.79 (61.60), H 4.02 (4.11).

Antibacterial activity test

Antibacterial activity test by diffusion test

Nutrient agar (NA) was used as the media for the antibacterial activity test. In 100 mL aquadest was dissolved 2.8 g of NA, heated and sterilized by autoclave at 121 °C, pressure of 1 atm for 15 min Fifteen milliliters of sterile media was placed on sterilized petri disc. The preparation of the media was conducted at laminar air flow, and left the media to solidify.

The diffusion test method was performed based on the procedure available in the literature [22, 23] and as follows: one ose of *B. subtilis* and *P. aeruginosa* was diluted with 2 mL of saline solution (NaCl 0.85 %) and was used as bacteria suspension. One milliliter of the suspension was then inoculated on NA, flattened with spreader. Four paper discs were prepared. The first paper disc was for the positive control (streptomycin), the second was for negative control containing the DMSO, solvent used for the test, 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 one day at 37 °C and were monitored to see the inhibition zone. Each experiment was repeated at least three times. The compounds giving the most effective inhibition were then chosen for the dilution method.

Antibacterial activity test with dilution test

The most effective concentration inhibition zones obtained for all organotin(IV) 3-hydroxybenzoate compounds tested with diffusion test, then were tested with dilution test. They were dissolved with aquadest-DMSO and the volumes were varied for dilution test based on the procedure described by other [22, 23].The compounds tested with certain volume were 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 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 [22, 23].

Results and discussion

The syntheses of $[Bu_2Sn(3\text{-}HBz)_2]$ (**7**), $[Ph_2Sn(3\text{-}HBz)_2]$ (**8**), and $[Ph_3Sn(3\text{-}HBz)]$ (**9**) were conducted by reacting the compounds of $[Bu_2SnO]$ (**4**), $[Ph_2Sn(OH)_2]$ (**5**), and $[Ph_3SnOH]$ (**6**) with 3-HBz acid by the use of the procedures previously reported [5, 8, 9, 12, 14, 16–18]. The elemental compositions of all synthesized compounds, as revealed by the results of microanalysis for each compound, are very reliable and in accordance with the calculated data. An example on the preparation of compound **9** was shown in Fig. 1.

Several spectroscopic techniques were used to justify the successful synthesis of the targeted compounds. The FT-IR of compound **3** has characteristic stretch for Sn–Cl bond at 448.2 cm^{-1}, upon conversion of **3**–**6**, the new stretch at 726.4 cm^{-1} appeared, this peak is a characteristic for Sn–O bond which indicated that this bond has been formed, while Sn–Cl disappeared and also the presence of wide peak at 3437.3 cm^{-1} is an indication the presence of hydroxyl group in the tin atom. After the reaction of **6** and 3-hydrobenzoic acid to form compound **9**, the new vibration of Sn–O–C appeared at 1290.1 cm^{-1}, which indicated that oxygen from carboxyl group is bound to Sn atom, and the presence of vibration at 1624.7 cm^{-1}, which is specific for C=O stretch indicated that carbonyl in the carboxyl is now present in the compound. The other vibrations are still present in the region close to the starting material [1, 8, 9, 12, 14, 16–18, 24].

The $\lambda_{max.}$ of all compounds were obtained by UV spectroscopy analyses. From the data obtained for each compound, it is clear that there was some important shifting change in the λ_{max} for each compound. For example in compounds **8**, the λ_{max} observed were 220 and 258 nm. In **9**, there were large shift in both $\pi \rightarrow \pi^*$ and $n\text{-}\pi^*$ transitions, due to the replacement of OH group by 3-hydroxybenzoate. The 3-hydroxybenzoate ligand is a strong chromophore group due to the present –C=O– and –C=C– bonds. The large shifts observed in **9** were due to the increase of conjugate bond in these compounds causing the energy difference between HOMO and LUMO orbitals were decreased making the $\lambda_{max.}$ absorbs were increased [5, 8, 9, 12, 14, 16–18, 25]. These observations were also found for compounds **7** and **8**.

The ^1H and ^{13}C NMR data of the organotin(IV) compounds synthesized were carefully analyzed to prove the successful of synthesis. The characteristic chemical shift in the spectra of the compounds prepared were characterized carefully and compared to the data available in the literatures [5, 8, 9, 12, 14, 16–18, 24]. Based on the data of ^1H NMR spectrum for compound **9**, the chemical shifts of phenyl protons attached to tin metal appeared as expected in the range from 7.33 for H_4 to 7.59 ppm for H_2 and H_6, while the protons in benzoate ring appeared at 7.60–7.83 ppm. The ^{13}C NMR values of the compounds synthesized were close to the values reported by others [5, 8, 9, 12, 14, 16–18, 24]. The analyses are as follows the carbon in the carboxyl group as expected appeared in the region of 165–166 ppm. The δ of carbons in the phenyl ligand in compounds **8** and **9** are at 126.9–131.7 ppm and the carbons in the benzoate are in δ range of 128.2–1329 ppm [5, 8, 9, 12, 14, 16–18, 24].

The results of antibacterial activity test by diffusion method and then followed with dilution test for the compounds synthesized are shown in Table 1. The halozone was observed in all concentrations from compounds **7**–**9**, while for the starting materials (**1**–**3**) and intermediate products (**4**–**6**) the halozone observed were very small or no halozone were observed. This result indicated that the synthesized compounds tested have antibacterial activity and have ability to disturb the metabolism in the bacteria.

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 [21–23].

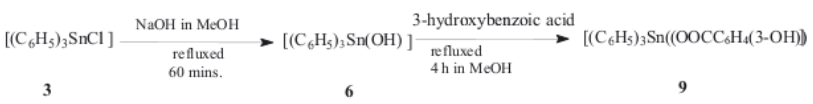

Fig. 1: The preparation of triphenyltin(IV) 3-hydroxybenzoate, $[Ph_3Sn(3\text{-}HBz)]$ (**9**).

Table 1: MIC values of all compounds tested compared with streptomycin.

Compound	Minimum inhibitory concentration (MIC) (x 10^{-4} M)	
	B. subtilis	***P. aeruginosa***
Streptomycin	1.70	1.71
$[Bu_2Sn(3\text{-}HBz)_2]$ (**7**)	1.87	1.89
$[Ph_2Sn(3\text{-}HBz)_2]$ (**8**)	1.79	1.81
$[Ph_3Sn(3\text{-}HBz)]$ (**9**)	1.71	1.72

In this biological activity test, the compounds **8** and **9** where their molecules are more electropositive than **7**, were able to 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 die due to the disruption of the tested compounds [21–23].

Conclusions

The preparation of three organotin(IV) compounds, dibutyltin(IV)-, diphenyltin(IV)-, and triphenyltin(IV) with 3-Hbz acid have successfully been carried out and successfully tested as antibacterial. Based on the results obtained, all compounds synthesized are potentially to be used as antibacterial agents. The data also indicated that the triphenyltin(IV) has stronger antibacterial activities. The result also showed that this finding directly proportional with to the number of carbon atom present in each compound. The data also reveal that it correlates with 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. Further investigations with other bacteria both Gram-positive and Gram-negative are now still on going in our laboratory. It will also be interesting to test the activity of these compounds as antifungal.

Acknowledgment: Special thanks must go to Directorate of Intellectual Property Right (**DPKI**), Directorate General of Strengthening Research and Development for giving SH support to present the paper in the international conference, International Conference on the Coordination and Organometallic Chemistry of Germanium, Tin and Lead (ICCOC-GTL) September 1-6, 2019 Saitama, Japan.
Research funding: The authors are grateful to Directorate of Research and Community Services, The Ministry of Research, Technology and Higher Education, Indonesia that provided fund for this project to be undertaken through Penelitian Disertasi Doktor (Doctoral Research Grant Scheme) 2019 and 2020 with contract number 858/UN26.21/PN/2019 and 179/SP2H/ADM/LT/DRPM/2020, respectively.

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