

Synthesis method and analysis of stable silver(I)-theophylline complex, the examination of their antimicrobial agents

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Abstract

The imidazolium salt synthesized in a new clamp type was characterized by experimental $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ analyzes, powder XRD analyses, nuclear magnetic resonance spectra, UV-vis spectra, and mass spectra. Then, density functional theory calculations were performed to obtain molecular configurations, from which theoretical nuclear magnetic resonance and infrared spectra were obtained. The comparison of the calculated spectra with the experimental spectra for each molecule leads to the conclusion that the theoretical results can be considered a good approximation of their molecular configuration. The in vitro biological activities of the salts on the selected bacteria were determined using the liquid dilution method according to the guidelines of the Institute of Clinical and Laboratory Standards. *Bacillus cereus* (ATCC 11778) against bacteria 7,9-bis(3-methoxy-3-oksopropil)-1,3-dimethyl-2,6-diokso-2,3,6,7-tetrahydro-1H-purin-9-ium bromide is low, although the activity of the carbene compound 7,9-bis(3-methoxy-3-oksopropil)-1,3-dimethyl-2,6-diokso-2,3,6,7-tetrahydro-1H-purine-9-ium silver(I) bromide, it was seen that the complex showed higher activity. The in vitro biological activities of the molecules were determined and one of them was found to have significant cytotoxic activity.

Keywords: carbene, silver, cyclophane, the antimicrobial agent.

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1. Introduction

Since their first stable form on an imidazole ring was created, *N*-heterocyclic carbenes (NHCs) have been considered significant ligands [1] because they are the most stable and well-studied family of persistent carbenes. NHCs interact strongly with transition metals in a variety of oxidation states due to their chemical characteristics as a strong δ -donor and a weak π -acceptor [2]. The improved catalytic activity and selectivity of the carbene complexes are the result of these characteristics as well as the steric influence of the substituents on the nitrogen atoms linked to the carbon atoms of the ring [3]. As a result, several in-depth catalytic investigations that were used for organic synthesis have been published [4,5]. Since the synthetic compounds' imidazole rings have many different therapeutic uses, they exhibit biological activity. Additionally, the variety of their structures brought about by extended metal-metal interactions, metal-bridging halides and carbenes, intermolecular hydrogen bonds, and pep interactions may result in interesting structural motifs like supramolecular

architectures and inorganic-organic hybrid materials [6,7]. By presenting two novel pincer-type ligands, 7,9-bis(3-methoxy-3-oxopropyl)-1,3-dimethyl-2,6-diokso-2,3,6,7-tetrahydro-1H-purin-9-ium bromide and 7,9-bis(3-methoxy-3-oxopropyl)-1,3-dimethyl-2,6-diokso-2,3,6,7-tetrahydro-1H-purin-9-ium silver(I) bromide, we add to the study on heterocyclic carbenes. By using X-ray diffraction, compounds' crystal structures were identified. By comparing the NMR and IR spectra of the two ligands in the gas phase with the estimated spectra produced by density functional theory, it was possible to determine the molecular characteristics of the two ligands. We also discussed the synthetic compounds' in vitro biological activity since their imidazole rings can be used for a variety of pharmaceutical purposes.

Xanthines (Theophylline) are several organic compounds that are often present in food. Caffeine and theophylline are two examples. Utilizing safe and sustainable resources to create ligands for organometallic chemistry and catalysis is one of the key objectives of green and sustainable chemistry [8]. In this Special Issue on Sustainable Organometallic Chemistry, we present the first nickel-N-heterocyclic carbene complexes made from Xanthines. The model complex has been described through X-ray crystallography. It also evaluates the steric, electron-donating, and electron-accepting properties. High activity is shown in the Suzuki-Miyaura cross-coupling model. Theophylline is the name of the chemical molecule, also known as 1,3-dimethylxanthine, which has a metastable crystalline phase. Active application of the theophylline molecule is found in medicinal chemistry. It is well known that it bonds to itself in solvents with powerful hydrogen bond donors and inhibits phase changes through nucleation when aggregates are present (Figure 1).

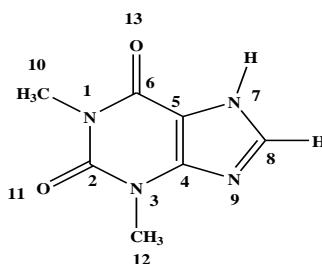


Figure 1. Numerical ranking of theophylline compound

The term "carbenes" refers to a class of molecules that contain carbon atoms that are divalent and/or coordinate and have one lone pair of electrons on each of their X and Y substituents. Decarbonized carbon species known as carbenes are active intermediates that help new carbon-carbon bonds develop (Figure 2).

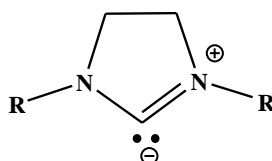


Figure 2. General scheme of carbenes

2. Experiments

Chemical names used in processes under argon gas include methyl-3-bromopropanoate ($C_4H_7BrO_2$), diethyl ether ($C_4H_{10}O$), deuterated chloroform ($CDCl_3$), deuterium oxide (D_2O), ethanol (C_2H_5OH), sodium carbonate (Na_2CO_3), silver(I)oxide (Ag_2O), ethyl acetate ($C_4H_8O_2$), and Thermo Scientific Nicolet N10 brand Pye Unicam, Shimadzu UV 1600 spectrophotometer, Thermogravimetric analysis TGA 400, A vacuum oven, and X-Ray diffraction (XRD) analysis are examples of instruments used in chemical processes. Elemental analysis is performed using a Sundry SDCHN636 Carbon Hydrogen and Nitrogen

Analyzer, an ATI Unicam 1000 FT-IR spectrometer, an Electrothermal-9200 melting point equipment, and a Multi-Analyzer-MRC Lab. pH, Conductivity, Do, Temp, and More.

2.1. Synthesis Methods of Carbene (1)

In this study, 1,3-dimethyl-1H-purine-2,6(3H,7H)-dione and 1-bromo-5-methoxypentan-3-one interacted to obtain the main skeleton. After the structure of this obtained compound was elucidated, it interacted with this compound to obtain the carbene compound. The conductivity value of the carbene compound was characterized by spectroscopic methods such as $^1\text{H-NMR}$ - $^{13}\text{C-NMR}$ analysis, FT-IR spectrum [9], TGA analysis, melting point, and XRD analysis (Figure 3).

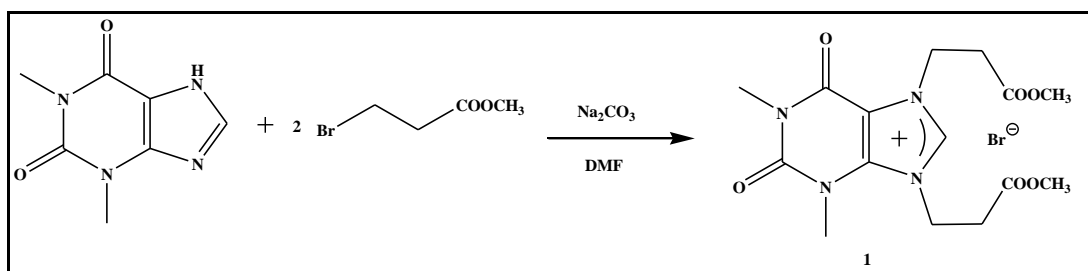


Figure 3. Synthesis of carbene

Under argon gas, methyl-3-bromopropanoate (0.365 g, 2.2 mmol), 1,3-dimethyl-1H-purine-2,6(3H,7H)-dione (0.180 g, 1 mmol) and Na_2CO_3 (0.11 g, 1 mmol) was taken and DMF (20 mL) was dissolved at room temperature. After the mixture was backwashed at 153 °C for 14 hours, the resulting brown mixture was extracted three times with dichloromethane and water. The mixture remaining in the water phase was followed by ethyl acetate-hexane (1:5) column chromatography to obtain the brown compound. Yield: 26% (0.112 g). Melting point: 322-324 °C. Molecular formula: $\text{C}_{15}\text{H}_{23}\text{BrN}_4\text{O}_6$. LC-Mass: found: 353.1174 $[\text{M}]^+$, calc: 353.1600 $[\text{M}]^+$ g/mol. UV-Vis (λ max, (log ϵ) nm ($\sim\text{M}^{-1}\text{cm}^{-1}$) in Acetonitrile: $\pi \rightarrow \pi^*$ transition at 300 (4.95) 382 (5.09), 429 (4.30) and 445 (4.00) nm. %Elemental analysis; calc.: C: 41.39, H: 5.33, N: 12.87, found: C: 41.33, H: 5.18, N: 12.13. FTIR spectrum in benzene - C=C-group at 3120 and 2781, in CH_3 and CH_2 , at 978 and 927 cm^{-1} , the stretching -C=C- at 1563 and 1440 cm^{-1} , and the sharp peak of the carbonyl -C=O stretch in acetate structure at 1705 and 1660 cm^{-1} . $^1\text{H-NMR}$ (300 MHz, DO): δ 8.85 (s, 1H, carbene), 3.34 (s, $J = 9.8$ Hz, 4H), 3.16 (m, $J = 6.8$ Hz, 4H), 2.83 (m, $J = 7.1$ Hz, 2H), 2.64 (m, $J = 9.3$ Hz, 6H). $^{13}\text{C-NMR}$ (75.5 MHz, DO): δ 152.5 (N-C-N, carbene), 34.8 (CH_2), 34.8 (CH_2), 55.5 (OCH_3), 28.5 (CH_2), 30.7 (CH_2), 28.5 (CH_3), 30.7 (CH_3), 176.3 (C=O), 155.9 (C=O), 147.6 (C=O), 107.3 C=C) ppm.

2.2. Synthesis Methods of Metal Carbene Complex (2)

The carbene compound (0.432 g, 1 mmol) and Ag_2O (0.081 g; 0.36 mmol), and DMF (20 mL) were added to stir at room temperature for 8 hours. The solution was filtered and all solvent was removed. The white compound was obtained after crystallizing the crude product from a mixture of CH_2Cl_2 - Et_2O (1:2) (Figure 4). LC-Mass: 460.0510 $[\text{M}]^+$ and 538.9695 $[\text{M-Br}]$ g/mol. UV-Vis (λ max, (log ϵ) nm ($\sim\text{M}^{-1}\text{cm}^{-1}$) in acetonitrile: peak at 276 (4.72) nm and $\pi \rightarrow \pi^*$ transition, 475 (4.99) 513 (5.06) and 592 It peaks at (4.86) nm and transitions to LCMT ($n \rightarrow \pi^*$). %Elemental analysis; calc.: C: 33.29, H: 3.91, N: 10.35. Found: C: 32.95, H: 3.28, N: 11.14. $^1\text{H NMR}$ (300 MHz, D_2O): 4.26 (m, $J = 9.1$ Hz, 4H), 3.27 (t, $J = 7.9$ -8.0 Hz, 4H), 3.11 (t, $J = 8.7$ -8.6 Hz, 6H), 2.53 (s, $J = 9.3$ Hz, 6H). $^{13}\text{C NMR}$ (75.5 MHz, D_2O): 194.8 (N-

C-N, carbene), 179.0 (C=O), 158.8 (C=O), 152.8 (C=O), 146.5 (C=C), 103.6 (C=C), 50.6 (OCH₃), 44.6 (CH₂), 43.3 (CH₂), 36.0 (CH₂), 35.3 (CH₂), 31.0 (CH₃), 28.2 (CH₃).

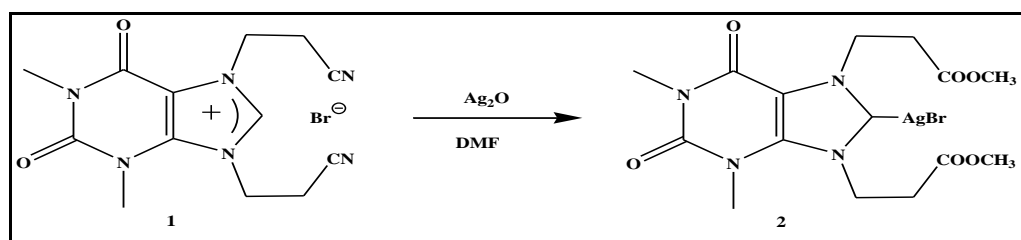


Figure 4. Synthesis of metal carbene complex

2.3. Antimicrobial Activity Measurements

In a sterile 5 mL tube of sterilized LB broth solution, a cultivated microbe is inserted and cultured before being stirred at 37 °C overnight. The following stage was transferring a 3 ml sterile solution of these silver(I)-NHC complexes to a 10 ml sterile tube in order to conduct serial dilution studies referred to as "A, B, C, D, and E." The standard medication Amphillicine was used to investigate the antifungal activity of the carbene (**1**) and silver(I)-NHC complex (**2**). The evidence collected indicates that NHC carbenes are less effective than silver(I) complex (**2**). (Table 1) (Figure 5).

Samples	<i>Escherichia coli</i>	<i>Listeria monocytogenes</i>	<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>	<i>Candida albicans</i>
Compound 1	84.52	89.53	89.67	81.49	89.65
Compound 2	0.02	0.03	0.03	0.01	0.02
Drug	-	-	-	-	-

Table 1. MICs values against the microorganism's resistance of compounds **1** and **2**

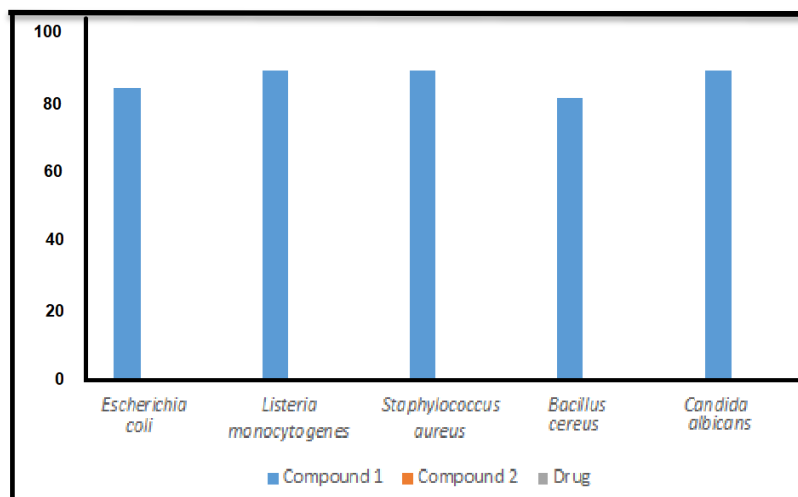


Figure 5. Antimicrobial activities of compounds **1** and **2**

3. Results and discussion

An organic substance or some metal complexes can be identified using the NMR and IR spectra. By comparing computed spectra with comparable actual spectra, they may also be used to confirm the estimated molecular structure, yielding structural parameters, and a

comprehensive description of the substances under investigation.

While the IR spectra were captured in the solid KBr pellet, the NMR measurements were made in a diluted D₂O solution. Because of this, NMR and IR spectra may show various chemical structures, such as a molecule surrounded by D₂O molecules in the former and intermolecular interactions, particularly on -CH and -OH bonds, in the latter [10]. Due to the molecules' single crystal form being produced for X-ray examination, the theoretical calculations for the crystalline phase were disregarded. With a 2% maximum error margin, the gas phase calculations were used, taking into account variations with other phases, particularly with regard to the lengths of the -CH and -OH bonds.

Using the spectra of compounds 1 and 2, the actual and theoretical chemical shifts of ¹H NMR and ¹³C NMR were determined. Because D₂O was used as the solvent for the specimens that exchanged -OH protons with deuterium, there were no proton signals for -OH in any of the experimental ¹H NMR spectra. The computed shifts of 1.73 ppm of -OH in compound 1 and 0.5 ppm in compounds 1 and 2, respectively, are in the normal chemical shift range of R-OH groups, even if their thorough investigation is left to the IR spectra in the next section.

Compounds 1 and 2's experimental infrared spectra in the 450–4000 cm⁻¹ range are provided in spectra along with their calculated IR spectra. For compounds 1 and 2, a full description of the IR spectra that includes each molecule's in-plane stretching, scissoring and rocking as well as out-of-plane wagging and twisting vibrations is offered.

Six different strains of bacteria and yeast were used in the antibacterial testing of the four compounds that had been produced. The inhibitory effects of the different doses of LA and LB on *Candida albicans* ATCC 10231 and *Bacillus cereus* ATCC 11778 (Gram-positive), as well as those of the antibiotic controls, are shown in Figure 5.

4. Conclusion

In this study, for the synthesis of metal carbene complexes, two distinct synthesis techniques have been used.

In the first approach, carbene compounds were created using theophylline as the starting material, and their structures were then discovered and examined using various spectroscopic techniques.

In the second technique, metal-bound carbene compounds were created as a result of the structure-identified carbenes reacting with Ag₂O. These complexes' structures were examined using several spectroscopic techniques, and their chemical compositions were determined. According to the information in the literature, analyses and spectroscopic techniques were used, and it was found that the results were consistent with the literature. To create symmetric *N*-heterocyclic carbenes (NHC) [(3a-c)], carbenes were created by adding 2-bromopropionitrile (2a), ethyl-3-bromopropanoate (2b), and 2-bromoethanamine (2c).

In the third procedure, silver(I)-NHC complexes [(4a-4c)] were made using unique type carbenes that had been synthesized.

The antimicrobial activities of carbene and silver carbene complex, whose structures were elucidated, were measured and published for the first time in the literature. Ag(I)-NHC complex showed better antimicrobial activity than carbene against bacteria and fungi and was determined to have antimicrobial agent properties.

1,3-bis(2-hydroxyethyl) imidazolium bromide, a novel imidazole salt or *N*-Heterocyclic carbene ligand, was created, and it was initially validated by LC-MS and elemental analysis techniques. Their theoretically derived chemical structures were then verified by contrasting computed ¹H, ¹³C NMR, and IR spectra with those of experimentally collected data. Further supporting the estimated structures were the XRD findings on a related imidazole salt [11]. Utilizing spectrophotometric techniques, the synthesized compounds'

antibacterial and cytotoxic effects on a few bacteria were quantified. It was shown that the silver(I)-NHC complexes inhibited *Bacillus cereus* ATCC 11778 more effectively than the chosen antibiotic.

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