Journal of Organometallic Chemistry 725 (2013) 46–53

Contents lists available at SciVerse ScienceDirect

Journal of Organometallic Chemistry

journal homepage: www.elsevier.com/locate/jorganchem

Silver(I) *N*-heterocyclic carbene complexes: Synthesis, characterization and antibacterial activity

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ARTICLE INFO

Article history:
Received 14 September 2012
Received in revised form
17 October 2012
Accepted 21 October 2012

Keywords:
Antibiotic
Silver complexes
Carbene ligands

ABSTRACT

Five new silver complexes having bidentate *N*-heterocyclic carbene ligands were synthesized and characterized by elemental analysis, IR, NMR and mass spectroscopic methods. Four of the ligands used are neutral, having an alcohol group on alkyl substituent of one of the two nitrogen atoms of the heterocycle [NHC–OH], the fifth, having a ligand alkoxide, is mono-anionic [NHC–O]. A study on the rate of hydrolysis of complexes synthesized, showed that they are stable to hydrolysis even after 24 h. Probably, the pincer effect of both [NHC–OH] and [NHC–O] ligands stabilizes these compounds. All the synthesized complexes do not show cytotoxicity, but they have a significant antibacterial activity. They were tested on *Escherichia coli* and *Bacillus subtilis* and data obtained demonstrate that the synthesized Ag-complexes are very promising candidates to be used as antimicrobial compounds.

1. Introduction

Silver has been since antiquity used as antimicrobial agent, it was earlier employed in the purification of drinking water, wine and vinegar, and also Hippocrates remarked its therapeutic properties [1]. Still today silver salts are used particularly in the treatment of chronic ulcers, extensive burns and to prevent conjunctivitis into newborn's eyes as well as other bacterial infections [2].

The activity of silver against Gram-positive and Gram-negative bacteria, fungi and yeast is due to Ag cations, which can interact with the cell membrane, interfere with the electron transport system of the cell and interact with thiol groups of the vital enzymes of bacteria [3].

The efficacy of a silver-drug as antibacterial agent is, of course, linked to its bioavailability which must be slow and continues for an appropriate time in the affected area [4]. In fact, silver nitrate and silver sulfadiazine, commonly used topical antibacterials, have very quick bactericidal action, but rapidly lose their effectiveness by exposing the wound-site to a possible re-infection.

Thus, the search for a silver-compound that slowly releases silver cations into the wound, maintaining a constant source of antibacterial agents to prevent infection, is a topic of great interest in the medical community.

The slow release of Ag cation at the wound-site is closely related to the choice of ancillary ligands to silver, which can play an important role stabilizing the complexes, thus retaining the antibacterial effect over a longer period of time.

N-heterocyclic carbenes (NHC), due to their excellent σ-donating properties and, as theoretical and structural studies suggest, π-backbonding ability [5,6], are widely used as ancillary ligands to stabilize both main group and transition metals [7–9].

In recent years many *N*-heterocyclic silver compounds have been synthesized, mainly for use in transmetalation of *N*-heterocyclic carbenes from silver to other metals, making it the most important synthetic method to give other metal NHC compounds [10].

Silver-*N*-heterocyclic carbene complexes can slowly release silver ions into the wound, enabling better prevention of infection and promoting healing [11,12].

Pyridine linked NHC-[1-benzyl-3-tert-buthylimidazol-2-ylidene]-, *N*,*N*′-methylate-NHC, with varying substituents on the 4

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http://dx.doi.org/10.1016/j.jorganchem.2012.10.040

and 5 position of the imidazole ring, -silver complexes have been synthesized and tested on clinically important microorganisms [10,13–15].

NHC having a pendant amine-alkoxide, and cyclopentadienyl-group have been recently synthesized. The concomitant presence of a strong electron-donating ylidene carbon with coordinating group generates ligand [NHC–X] able to produce complexes with noticeable stability and having steric rigidity [16,17].

In this paper, we report the preparation and characterization of some iodide silver complexes stabilized by *N*-heterocyclic carbene ligands having an alcohol group on alkyl substituent of one of the two nitrogen atoms of the heterocycle (NHC–OH). Moreover, we also detail on the synthesis of a silver complex having an *N*-heterocyclic carbene ligand bringing an alkoxy group on one of two nitrogen of the heterocycle (NHC–O). The latter ligand is bidentate–monoanionic, while the former are bidentate–neutral, however in all cases they can act as a chelating agent, and therefore significantly stabilize the resulting complex.

In Scheme 1 are reported the ligands utilized, which produced the corresponding complexes **1**, **2**, **3**, **4** and **1**′.

The antibacterial activity was evaluated for all the reported compounds, using a standard assay against a Gram negative (*Escherichia coli*) and a Gram positive (*Bacillus subtilis*) strain.

2. Results and discussion

2.1. Chemistry

Main Imidazolium-*N*-methyl-*N*′-cyclopentan-2-ol-iodide, imidazolium-*N*-methyl-*N*′-cyclohexane-2-ol-iodide, imidazolium-*N*-methyl-*N*′-ethane-2-ol-iodide were prepared following the strategy proposed by Arnold and co-workers [16], and applying the procedures previously reported by us [17].

2.1.1. Synthesis of N-methyl, N′-[(2-hydoxy-2-phenyl)ethyl] imidazolium iodide

The *N*-methyl, *N*′-[(2-hydroxy-2-phenyl)ethyl]-imidazolium iodide was synthesized by reacting phenylethylenoxide with imidazole, followed addition of iodomethane in acetonitrile (see Scheme 2). After distillation of the solvent, the product was purified by crystallization in acetone, obtaining the imidazolium salt as a white solid in high yield.

Elemental analysis (C, H, N) is in agreement with the proposed formulation. ^{1}H COSY and NOESY experiments allowed to assign all proton resonances of the ^{1}H NMR spectrum, whereas resonances of ^{13}C NMR spectrum were attributed using support of DEPT and HMQC experiments (see Fig. 1). All signal attributions are reported in the experimental part. The singlet at 8.23 ppm is assigned to imidazolium proton on carbon 2, whereas the resonances of hydrogen atoms of methines of the backbone fall at 6.84 and 6.82 ppm, respectively. At 6.60–6.40 ppm are the signals of protons of aromatic ring. The doublet at 5.10 was attributed to –OH and the multiplet at 4.11 to –CH–O, while the protons of methylene $-CH_2-N$, give rise to two signals at 3.57 and 3.36 ppm, because they are not equivalent, probably because the free rotation around the C–C bond is prevented by the coordination of oxygen to the carbocation. The protons of the methyl bonded to nitrogen atom give a singlet at 3.01 ppm.

Signal at 136.9 is assigned to C_2 of imidazolium and that at 123.0 ppm to two carbons of imidazolium backbone. Aromatic carbons resonate at 141.2, 128.3, 127.8, 125.9. The resonances of methine carbons bonded to oxygen and to nitrogen atoms are at 70.6 and 55.6 ppm, respectively, whereas the chemical shift of methyl carbon is at 35.8 ppm.

2.1.2. Synthesis of silver complex bearing [NHC–O] ligand (**1**′)

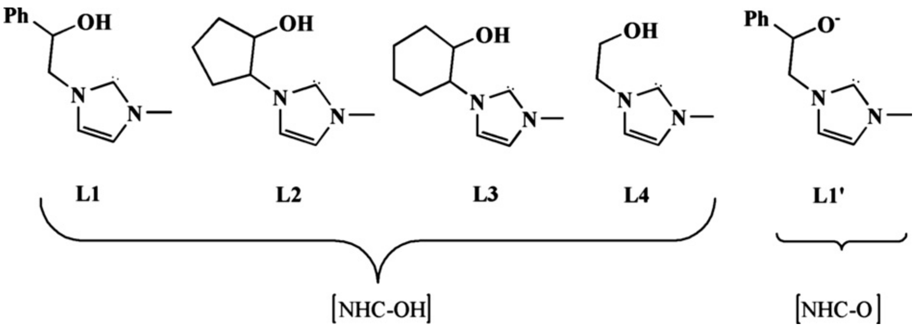
Reaction of *N*-methyl, *N*′-[(2-hydroxy-2-phenyl)ethyl] imidazolium iodide with an excess of strong base (*i.e.* potassium-hexamethyldisilazide), used both for the deprotonation of the alcohol group and for the formation of carbene, produced potassium alkoxide carbene, to which was added silver acetate CH_3COOAg to give, after purification (see Section Experimental part), [NHC–O]Ag ({*N*-methyl,*N*′-[(2-phenyl)-2-ethoxy]imidazole-2-ylidine}-silver(I)) as a white microcrystalline solid. Elemental analysis, mass spectroscopy analysis, NMR and FT-IR measurement are according with formulation reported in Scheme 3.

In fact, MS spectrum of complex showed the presence of the signals at 309 and 311 *m*/*z* assignable to the molecular ion $C_{12}H_{13}AgN_2O$. The presence of the signals M and M + 2 are due to the two isotopes of silver (^{107}Ag and ^{109}Ag) of abundance nearly equal. The elemental analysis data of [NHC–O]Ag found are in good agreement with those calculated (see Section Experimental part) and the IR spectrum does not show absorbance attributable to –OH. ^{1}H and ^{13}C NMR spectra show the signals of phenyl ring, of methine of the backbone, of *N*-methyl group, and of methine and methylene bonded to heteroatoms, O and N, respectively. In the ^{13}C NMR it is possible to observe only one sharp resonance attributable to carbene at 180.9 ppm. This fact, evidently due to the non coupling ^{13}C–^{107,109}Ag, is understandable given the lability of the carbene–silver bond and therefore explainable considering the fluxional behavior of **1**′ [18]. In Fig. 2 are reported ^{1}H and ^{13}C NMR and in the experimental part are detailed all the attribution of ^{1}H and ^{13}C NMR signals.

2.1.3. Synthesis of silver complexes bearing [NHC–OH] ligands (**1**–**4**)

The *in situ* deprotonation of imidazolium salt with basic silver precursors (*i.e.* silver oxide) was used to give *N*-methyl-*N*′alkylhydroxy-imidazole-2-ylidine [14,18].

Reaction of *N*-methyl, *N*′-[(2-hydroxy-2-phenyl)ethyl] imidazolium iodide (1.0 mmol) with the stoichiometric amount of silver



Scheme 1. Structures of substituted carbene ligands: *N*-methyl-*N*′-alkylhydroxy [NHC–OH] and *N*-methyl-*N*′-(2-alkoxyde-2-phenyl-ethyl) [NHC–O].