SYNTHESIS, SPECTROSCOPIC AND ANTIMICROBIAL STUDIES OF HEVY METALS COMPLEXES OF TEMPO-AMINE

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ABSTRACT: Complexes of TEMPO-amine (2,2,6,6-tetramethyl-4-amino-piperidine-N-oxide) were obtained by reaction with salts of heavy metals like Ni(NO₃)₂·6H₂O, VOSO₄·5H₂O, Na₂WO₄·2H₂O and Na₂MoO₄·2H₂O in aqueous solutions at room temperature. The complexes were characterized by elemental chemical analysis and FT-IR. The probable structures of these compounds were suggested. The antibacterial properties of these compounds were determined by comparing the growth curves of Escherichia coli in pure nutrition medium and one containing complexes of TEMPO-amine. The bacteria growth was studied by determination of the optical density (OD) at wavelength of λ 420 nm. According to their antibacterial activities, the complexes of TEMPO-amine can be arranged in the following order: Ni²⁺ > WO₂²⁻ > VO²⁺ > MoO²⁺.

Key words: Tempo-amine; complexes; E. coli

Introduction

Chemical substances have various effects on microorganisms, depending basically on substance nature, its concentration, contact duration, etc. Many chemical substances change medium composition, thus stimulating, suppressing or terminating microorganisms’ growth. On the other hand, a number of chemical substances kill microbial cells at some higher or lower rate.

Metal-free N,N-dimethylbiguanidium acetate and novel complexes with Mn(II), Ni(II), Cu(II) and Zn(II) were screened for their antimicrobial properties against Gram-positive (Bacillus subtilis, Listeria monocytogenes, Staphylococcus aureus), Gram-negative (Escherichia coli, Klebsiella pneumoniae, Psedomonas aeruginosa) bacteria and fungal (Candida albicans) strains. The tested compounds exhibited a good antimicrobial activity with Mn(II) and Zn(II) complexes [1]. New tetradentate donor Schiff bases and their mononuclear Co(II), Ni(II), Cu(II) and Pd(II) complexes were synthesized. All the ligands and complexes were screened for their in vitro antibacterial activity against two Gram-positive bacteria (Bacillus subtilis, Staphylococcus aureus), Gram-negative (Klebsiella pneumoniae, Escherichia coli, Psedomonas aeruginosa) bacteria and fungal (Candida albicans) strains. The tested compounds exhibited a good antimicrobial activity with Mn(II) and Zn(II) complexes [1]. New tetradentate donor Schiff bases and their mononuclear Co(II), Ni(II), Cu(II) and Pd(II) complexes were synthesized. All the ligands and complexes were screened for their in vitro antibacterial activity against two Gram-positive bacteria (Bacillus subtilis, Staphylococcus aureus) and two Gram-negative bacteria (Escherichia coli, Klebsiella pneumoniae). All the metal complexes have shown moderate to good antibacterial activity against Gram-positive and Gram-negative bacteria [2]. Eight new copper (II)-thiophene-2,5-dicarboxylate have been synthesized and characterized by elemental analyses, thermal analyses and IR spectra. Antimicrobial activities of the complexes were tested according to the minimal inhibitory concentration method (MIC). All of the complexes showed lower antimicrobial activity against the studied wild-type microorganisms [3]. Copper and cobalt complexes with 4-chloro-3-nitrobenzoate and the nitrogen ligands 1,3-diaminopropane or o-phenylenediamine were prepared and characterized. Copper complex shows high antibacterial activity as indicated by its ability to inhibit the growth of Staphylococcus aureus and Enterococcus faecalis [4]. The chemistry of macrocycles has aroused increasing interest in recent years due to their use as models for protein metal binding sites in metalloproteins, as therapeutic agents in chelate therapy for the treatment, in catalysis, and in the treatment of cancer. A new tetra-aza macrocyclic ligand and its complexes with Ni(II), Co(II) and Cu(II) were synthesized and characterized by the spectral and analytical techniques. All the synthesized metal complexes were screened for their in vitro antimicrobial activity against selected species of pathogenic bacteria and fungi [5]. The antibacterial activity of morin, sodium salt of morin-5-sulfonic acid and new complexes of La(III), Gd(III) and...
Lu(III) with morin were tested against three bacterial strains: *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and compared with the activity penicillin. All of the complexes possess inhibitory action against the tested strains [6]. 3-acetyl and 3-decanoyltetramic acids with various substituents at the 5-position and their copper complexes, which possess a tricarbonylmethane structure, were prepared and tested for antimicrobial activity. 3-decanoyltetramic acid derivatives and their copper complexes, which possess a tricarbonylmethane structure, were prepared and tested for antimicrobial activity. 3-decanoyltetramic acid derivatives and their copper complexes, which possess a tricarbonylmethane structure, were prepared and tested for antimicrobial activity. 3-decanoyltetramic acid derivatives and their copper complexes, which possess a tricarbonylmethane structure, were prepared and tested for antimicrobial activity. 3-decanoyltetramic acid derivatives and their copper complexes, which possess a tricarbonylmethane structure, were prepared and tested for antimicrobial activity.

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Results and Discussion

Preparation of complexes

By the solution of sodium molybdate in water, Mo(VI) anions are obtained according to the equation:

$$\text{Na}_2\text{MoO}_4 \leftrightarrow 2\text{Na}^+ + \text{MoO}_4^{2-}$$

By the acid hydrolysis of the molybdenyl ions, polymer anions are formed according to the equations:

$$7 \text{MoO}_4^{2-} + 8\text{H}^+ \leftrightarrow \text{Mo}_7\text{O}_{24}^{6-} + 4 \text{H}_2\text{O}$$

$$8 \text{MoO}_4^{2-} + 12\text{H}^+ \leftrightarrow \text{Mo}_8\text{O}_{26}^{4-} + 6 \text{H}_2\text{O}$$

In acidic solutions, the polymer anions depolymerize to give molybdenyl cation according to the reactions:

$$\text{Mo}_7\text{O}_{24}^{6-} + 20 \text{H}^+ \leftrightarrow 7\text{MoO}_2^{2+} + 10 \text{H}_2\text{O}$$

$$\text{Mo}_8\text{O}_{26}^{4-} + 20 \text{H}^+ \leftrightarrow 8\text{MoO}_2^{2+} + 10 \text{H}_2\text{O}$$

The tungstenyl cations (WO$_2^{2+}$) are prepared by similar reactions.

The dissolution of vanadyl sulphate or nickel nitrate in water directly gives cations according to the reactions:

$$\text{VOSO}_4 \leftrightarrow \text{VO}^{2+} + \text{SO}_4^{2-}$$

$$\text{Ni(NO}_3)_2 \leftrightarrow \text{Ni}^{2+} + 2 \text{NO}_3^-$$

The aim of the present work is to study the antibacterial activity of complexes of Temponamine with heavy metals on the growth of *Escherichia coli* bacteria.

Experimental

1. Preparation of molybdenum complex

To 20 ml bidistilled water, 0.24 g (1.45x10$^{-3}$ M) TEMPO-amine were added and the solution was acidified with diluted nitric acid to pH 2.0 – 2.2. To 20 ml bidistilled water, 0.23 g (6.9x10$^{-3}$ M) sodium molybdate were added and the solution was acidified with diluted nitric acid to pH 2.0 – 2.2. The two solutions were mixed at room temperature under vigorous agitation. The precipitation obtained was washed with distilled water until neutral reaction. The complex obtained was dried under vacuum at 40°C until constant weight. The complex of sodium tungstenate with TEMPO-amine was obtained by similar procedure.

2. Preparation of vanadium complex

To 20 ml bidistilled water, 0.24 g (1.45x10$^{-3}$ M) TEMPO-amine and 1.83 g (7.25x10$^{-3}$ M) vanadyl sulphate were added. The solution obtained was stirred intensely at room temperature for 12 h and then kept in refrigerator for 24 h. The precipitate obtained was washed with distilled water until neutral reaction. The complex obtained was dried in vacuum at 40°C until constant weight. The complex of nickel nitrate with TEMPO-amine was prepared by the same technique.

Fig. 1. FT-IR spectrum of TEMPO-amine (1) and TEMPO-amine+MoO$_2^{2+}$ (2)
Fig. 1 shows the FT-IR spectra of TEMPO-amine before and after interaction with molybdenyl cations. Bands at 430 and 800 cm$^{-1}$ were observed which can be attributed to the Mo-N and Mo-O-Mo co-ordination bonds, respectively. In the complex spectrum, intense absorption bands were observed at 909 and 961 cm$^{-1}$, characteristic for molybdenyl cation. [10,11]. The bands at 1465 and 3283 cm$^{-1}$ are characteristic for symmetric and deformation vibration of the N-H group. Based on the elemental analysis and the spectral characteristics, the following structure of the complex can be suggested (scheme 1).

![Scheme 1. Suggested structure of the complex](image)

The antimicrobial activities of the complexes obtained and characterized were studied. The activity was determined by comparing the growth curves for cultures in pure nutrient and one containing some of the complexes studied. The optical density was measured at wavelength of 420 nm. All the experiments were carried out on a rotating shaker at 37°C for 12 h, at concentration of antibacterial compounds of 120 mg/L.

The curves of biomass growth in standard (pure) nutrient and in one containing complexes of molybdenum and tungsten with TEMPO-amine are presented in Fig. 3.

![Fig. 3. Curves without and with complexes](image)

As can be seen for the standard nutrient, the initial phase (lag-phase) duration was about 2 h. The exponential phase was about 8 h and then cell growth stopped. For this reason, all the cultivations were of the same duration of 12 h. Both the molybdenum and tungsten complexes exerted inhibiting effect up to the sixth hour followed by a slow transition to the exponential phase. This effect was slightly better pronounced with the tungsten complex.
The curves of biomass growth in absence and presence of vanadium and nickel complexes are shown in Fig. 4. In presence of vanadium, the initial phase was prolonged to the 5th hour and the exponential one – to the 9th hour. The strongest antibacterial effect was observed with the nickel complex. The initial phase was about 7 hours long, followed by growth of biomass until 9th hour and inhibition after that. With the nickel complex, the inhibition was about 50% compared to the reference.

![Fig. 4. Curves without and with complexes](image)

It is well known that the antibacterial substances affect the microbes by different mechanisms. For instance: a) Inhibition of cell metabolism; b) Inhibition of bacterial cell wall synthesis; c) Some antibacterial agents interact with the plasma membrane of bacterial cells to affect membrane permeability.

Most probably, the complexes cause changes in cell membrane permeability by covering the cell surface. Thus, they have bacteriostatic rather than bactericide effect. By their extent of antibacterial activity, the complexes of TEMPO-amine can be arranged in the following order: Ni^{2+}> WO_2^{2-}> VO_2^{2+}> MoO_2^{2+}.

**Conclusion**

The proper conditions for formation of complexes between TEMPO-amine and salts of heavy metals were determined. On the basis of spectral methods, structures of the antibacterial compounds were suggested. Bacteriostatic effect was observed with the complexes of TEMPO-amine with salts of heavy metals.

**References**