(*N*-Salicylidene-L-glutamato)copper(II) Complexes Containing Imidazole and Pyridine Derivatives

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Synthesis, properties, and antimicrobial activity of copper(II) complexes of the composition Cu(sal-L-glu)X, where $(\text{sal-L-glu})^{2-}$ represents Schiff base derived from salicylaldehyde and L-glutamic acid and X = 1-methylimidazole, 2-methylimidazole, 4-methylimidazole, 2-ethylimidazole, 2-methylpyridine, 3-methylpyridine, and 4-methylpyridine, are reported. On the basis of infrared and electronic spectral data the stereochemical arrangement of individual components is discussed. The complexes prepared are supposed to adopt square pyramidal geometry, the basal plane sites are occupied by the donor atoms of tridentate Schiff base and by *N*-donor atom of the corresponding *N*-molecular ligand. The apical position is occupied by carboxylic oxygen of adjacent complex unit. The complexes studied exhibit significant activity against bacteria *Staphylococcus aureus* and are considered as potential SOD mimetics.

In the recent years the special interest has been devoted to Cu(II) complexes containing tridentate Schiff bases as anionic ligands. This type of complexes has been studied predominantly thanks to their observed antimicrobial [1—3] and antiradical as well as radioprotective activities [4]. As it was stated elsewhere [5—7] the square pyramidal arrangement of ligands in these complexes is also close to the coordination sphere of Cu(II) in active centre of superoxide dismutase metalloenzyme Cu, Zn—SOD [8, 9], the most efficient antiradical enzyme catalyzing the dismutation of the superoxide radicals in living systems [10, 11]. From the group of Schiff bases containing amino acids the special emphasis has been given to amino acids containing two carboxylic groups, in the first place to glutamic acid [12]. As it has been confirmed on the basis of infrared spectral data the one terminal carboxylic group of glutamic acid in Schiff base was found to be uncoordinated even after complexation [13] and this fact is believed to influence the chemical behaviour, first of all the solubility, of this type of complexes.

As it was recently published [12] the group of copper(II) complexes containing tridentate Schiff bases (TSB) derived from salicylaldehyde and glutamic acid (sal-L-glu), and water or diazoles (pyrazole, 3,5-dimethylpyrazole, and imidazole) as additional ligand was found to be SOD mimetics. From this point of view we found it interesting to study the (*N*-salicylidene-L-glutamato)copper(II) complexes, containing 1-methylimidazole (1-Meim), 2-methylimidazole (2-Meim), 4-methylimidazole (4-Meim), 2-ethylimidazole (2-Etim), 2-methylpyridine (2-Mepy), 3-methylpyridine (3-Mepy), 4-methylpyridine (4-Mepy) as molecular ligand.

EXPERIMENTAL

The used parent aqua complex (*N*-salicylidene-L-glutamato)copper(II) monohydrate was synthesized according to [13].

Copper(II) acetate (34.0 g; 0.17 mol) was dissolved in 700 cm^3 of water and the formed solution was heated to 60—65 °C. Under continuous stirring salicylaldehyde (21.5 g; 0.17 mol) and then after *ca.* 10 min L-glutamic acid (25.0 g; 0.17 mol) were added and the system was heated to 60 °C. The formed transparent dark green solution was then filtered at elevated temperature and allowed to cool (to room temperature). After 24 h the deposited crystalline product was washed out with water and air dried.

For $CuC_{12}H_{17}O_8N$ (M_r = 366.82) w_i(calc.): 39.26 % C, 3.82 % N, 4.67 % H; w_i(found): 39.60 % C, 3.89 % N, 4.87 % H.

(*N*-Salicylidene-L-glutamato)copper(II) complexes containing imidazole and pyridine derivatives were prepared in the mole ratio of components 1 : 2 in ethanol as described below.

Cu(sal-L-glu)(1-Meim)

$[Cu(sal\text{-}L\text{-}glu)(H_2O)_2]H_2O$ (2.0 g; 0.0054 mol) was dissolved in 40 cm^3 of ethanol and to this solution the mixture of 1-methylimidazole (0.9 g; 0.011 mol) in 20 cm^3 of ethanol was added. After 24 h the deposited product was isolated, washed out with ethanol and air dried.

For $CuC_{16}H_{17}O_5N_3$ (M_r = 394.93) w_i(calc.): 48.73 % C, 10.63 % N, 4.30 % H; w_i(found): 48.74 % C, 10.62 % N, 4.42 % H.

Cu(sal-L-glu)(2-Meim)

$[Cu(sal\text{-}L\text{-}glu)(H_2O)_2]H_2O$ (2.0 g; 0.0054 mol) was dissolved in 40 cm^3 of ethanol and afterwards mixed with 2-methylimidazole (0.9 g; 0.011 mol) in 20 cm^3 of ethanol. The formed dark green coloured product, crystallizing in two hours, was washed out with ethanol and air dried.

For $CuC_{16}H_{17}O_5N_3$ (M_r = 394.93) w_i(calc.): 48.73 % C, 10.63 % N, 4.30 % H; w_i(found): 48.65 % C, 11.08 % N, 4.51 % H.

Cu(sal-L-glu)(4-Meim)

$[Cu(sal\text{-}L\text{-}glu)(H_2O)_2]H_2O$ (2.0 g; 0.0054 mol) in 40 cm^3 of ethanol was mixed with 4-methylimidazole (0.9 g; 0.011 mol) in 20 cm^3 of ethanol. In two hours deposited bluish green product was isolated and after washing out with ethanol air dried.

For $CuC_{16}H_{17}O_5N_3$ (M_r = 394.93) w_i(calc.): 48.73 % C, 10.63 % N, 4.3 % H; w_i(found): 48.91 % C, 10.71 % N, 4.55 % H.

Cu(sal-L-glu)(2-Etim)

$[Cu(sal\text{-}L\text{-}glu)(H_2O)_2]H_2O$ (2.0 g; 0.0054 mol) was dissolved in 40 cm^3 of ethanol. To the formed solution 2-ethylimidazole (1.05 g; 0.011 mol) in 20 cm^3 of ethanol was added. In two hours deposited dark green crystalline product was isolated, washed out with ethanol and air dried.

For $CuC_{17}H_{19}O_5N_3$ (M_r = 409.82) w_i(calc.): 49.88 % C, 10.24 % N, 4.64 % H; w_i(found): 50.11 % C, 10.30 % N, 4.76 % H.

Cu(sal-L-glu)(2-Mepy) (*a*) and Cu(sal-L-glu)(3-Mepy) (*b*)

$[Cu(sal\text{-}L\text{-}glu)(H_2O)_2]H_2O$ (4.6 g; 0.00315 mol) was dissolved in 80 cm^3 of ethanol and after filtration to the formed solution 2-methylpyridine (3-methylpyridine) (1.5 cm^3; 0.0063 mol) in 5 cm^3 of ethanol was added. The colour of solution immediately turned dark green and greyish green (*a*) and dark green (*b*) product crystallized. The crystals were isolated after two days, washed out with ethanol and air dried.

For $CuC_{18}H_{18}O_5N_2$ (M_r = 405.88) w_i(calc.): 53.26 % C, 6.90 % N, 4.47 % H; w_i(found): 53.17 % C, 6.91 % N, 4.57 % H (*a*), 53.55 % C, 6.99 % N, 4.54 % H (*b*).

Cu(sal-L-glu)(4-Mepy)

$[Cu(sal\text{-}L\text{-}glu)(H_2O)_2]H_2O$ (4.6 g; 0.00315 mol) together with 4-methylpyridine (1.5 cm^3; 0.0063 mol) were dissolved in 80 cm^3 of ethanol. Under continuous stirring the system was heated to 60—65 °C and afterwards filtered. After 48 h the separated bluish green crystals were washed out with ethanol and dried in air.

For $CuC_{18}H_{18}O_5N_2$ (M_r = 405.88) w_i(calc.): 53.26 % C, 6.90 % N, 4.47 % H; w_i(found): 53.15 % C, 6.93 % N, 4.50 % H.

The elemental analysis was carried out using an analyzer EA 1108 (Carlo Erba Instruments, Italy) based on chromatographic principle at temperature 1800 °C and in the atmosphere of helium.

To verify the retaining of L-form of glutamic acid in complexes studied the specific optical rotation using solution in DMSO at temperature 20 °C and at the wavelength 546 nm was measured.

Electronic absorption spectra measurements were performed with spectrophotometer Specord M40 (Zeiss Jena) in the $\tilde{\nu}$ range 12000—30000 cm^{-1} using nujol mull technique. Infrared spectra measurements were carried out with the aid of single beam FTIR spectrophotometer Impact 400 D Nicolet and KBr discs were used. Magnetic susceptibility data at room temperature were obtained on apparatus based on the Gouy principle.

For antimicrobial activity evaluation the microorganisms *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* were used. As cultivation media the nutrient broth and nutrient agar for bacteria and Sabouraud medium and Sabouraud agar for fungi were used.

DISCUSSION

The presented group of complexes, prepared by the reaction of parent aqua complex, $[Cu(sal\text{-}L\text{-}glu)(H_2O)_2]H_2O$ [13], with corresponding molecular ligand X, where X = 1-methylimidazole, 2-methylimidazole, 4-methylimidazole, 2-ethylimidazole, 2-methylpyridine, 3-methylpyridine, 4-methylpyridine, was of the Cu(sal-L-glu)X composition.

According to findings reported in [5] the used parent aqua complex exhibits square pyramidal arrangement of ligands around the Cu(II) atom with approximately trans-planar chromophore [Cu, N, O_3]. As it was stated using the X-ray diffraction method, the basal plane sites are occupied by the donor atoms of tridentate [*N*-salicylidene-L-glutamato]$^{2-}$ anionic ligand and by the O donor atom of water molecule. The further water molecule is localized at apical position (Fig. 1). To confirm the supposed formation of complexes studied as well as the presence and coordination of individual groups the results of infrared spectral data were used (Table 1). The presence of uncoordinated carboxylic group in L-glutamic acid man-

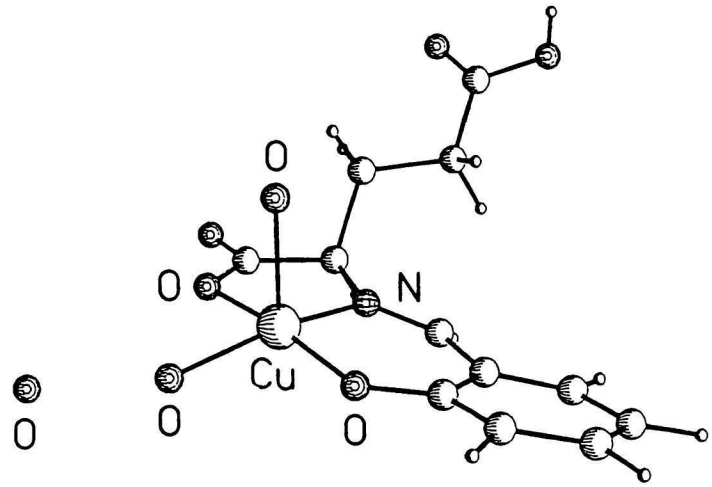

Fig. 1. Molecular structure of diaqua-(*N*-salicylidene-L-glutamato)copper(II) monohydrate [5]; for clarity the hydrogen atoms of H_2O molecules are omitted.

Table 1. Infrared Spectral Data of Cu(II) Complexes Studied ($\tilde{\nu}_i/cm^{-1}$)

Complex	$\tilde{\nu}_{OH}$		$\tilde{\nu}_{CH}$	$\tilde{\nu}_{CO}$			$\tilde{\nu}_{CO}$	γ	
	assoc.	arom.	aliph.	COO^- lone	COO^- asym.	COO^- sym.	phenol.	out-of-plane	
$[Cu(sal\text{-}L\text{-}glu)(H_2O)_2]H_2O$	3419			1690	1633	1446	1193	766	
				1598			1298		
Cu(sal-L-glu)(1-Meim)		3136	2968	1719	1644	1450	1189	780	740
			2900		1610				
Cu(sal-L-glu)(2-Meim)		3140	2957	1725	1637	1448	1193	761	743
			2935		1599				
Cu(sal-L-glu)(4-Meim)		3144	2993	1705	1638	1446	1209	750	742
		3101	2917		1601				
Cu(sal-L-glu)(2-Etim)		3131	2980	1721	1633	1439	1189	760	744
			2935		1614				
Cu(sal-L-glu)(2-Mepy)		3031	2936	1727	1643	1449	1193	762	774
			2921		1600				
Cu(sal-L-glu)(3-Mepy)		3068	2955	1745	1637	1451	1197	759	803
			2939		1566				
Cu(sal-L-glu)(4-Mepy)		3092	2934	1737	1644	1450	1201	760	498
			2916		1603				

Table 2. Electronic Spectral Data of Cu(II) Complexes Studied ($\tilde{\nu}_i/cm^{-1}$)

Complex	$\tilde{\nu}_{1\ max}$	$\tilde{\nu}_{2\ max}$
$[Cu(sal\text{-}L\text{-}glu)(H_2O)_2]H_2O$	15440	26440
Cu(sal-L-glu)(1-Meim)	16040	27280
Cu(sal-L-glu)(2-Meim)	17240	26480
Cu(sal-L-glu)(4-Meim)	16520	26480
Cu(sal-L-glu)(2-Etim)	17400	27000
Cu(sal-L-glu)(2-Mepy)	17960	27280
Cu(sal-L-glu)(3-Mepy)	16920	25960
Cu(sal-L-glu)(4-Mepy)	16280	27800

ifests the band observed at about 1700 cm^{-1} [13] and for the associated water molecule in parent aqua complex used the band at 3419 cm^{-1} is characteristic. Comparing with the parent aqua complex in complexes containing *N*-donor molecular ligands the stronger bands in the range 2900—3100 cm^{-1} were found. This fact together with observed positions of out-of-plane vibrations of aromatic rings confirms the coordination of *N*-donor ligands [14]. In agreement with found positions of absorption band maxima in electronic spectra (Table 2), the original square pyramidal arrangement of ligands in coordination sphere of Cu(II) atom is believed to be retained (Fig. 1). Thus it seems reasonable to conclude that during the reactions the used *N*-donor ligand substituted "in plane" linked H_2O molecule, while the chelate type of TSB bonding was maintained. The solved crystal structures of two *N*-salicylidene-L-glutamato copper(II) complexes

Table 3. Antimicrobial Activity of Cu(II) Complexes Studied (μg cm^{-3})

Complex	*Staphylococcus aureus*	*Escherichia coli*	*Candida albicans*
[Cu(sal-L-glu)$(H_2O)_2$]H_2O	195	3125	2500
Cu(sal-L-glu)(1-Meim)	156	1250	1250
Cu(sal-L-glu)(2-Meim)	39.1	1250	1250
Cu(sal-L-glu)(4-Meim)	312.5	1250	1250
Cu(sal-L-glu)(2-Etim)	312.5	1250	1250
Cu(sal-L-glu)(2-Mepy)	156	1250	1250
Cu(sal-L-glu)(3-Mepy)	78.1	1250	1250
Cu(sal-L-glu)(4-Mepy)	78.1	1250	1250
1-Methylimidazole	625	1250	1250
2-Methylimidazole	625	1250	1250
2-Ethylimidazole	625	1250	1250
DMSO	5000	2500	2500

DMSO = dimethyl sulfoxide.

containing pyridine [6] or 4-methylpyridine [7] as *N*-donor molecular ligand supported this assumption. The found values of effective magnetic moment (μ_{eff}) at room temperature for two representative substances of this group [Cu(sal-L-glu)$(H_2O)_2$]H_2O (1.80 μ_B) and [Cu(sal-L-glu)(4-Mepy)] (1.87 μ_B) are typical of one unpaired electron in mononuclear copper(II) compounds without any significant interaction between paramagnetic Cu(II) centres.

The antimicrobial activity of investigated copper(II) compounds was evaluated using bacteria *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. The results are presented in terms of MIC (minimal inhibition concentration), representing the minimum mass concentration of substance (μg cm^{-3}) inactivating the microorganism reproducibility. The found values are listed in Table 3. As it is evident the studied substances exhibit the highest efficacy against bacteria *Staphylococcus aureus* and comparing with the observed activity of used uncoordinated ligands the whole group was more active.

The predicted SOD-like activity of these complexes was recently also proved. The two representatives of this group Cu(sal-L-glu)(2-Meim) and Cu(sal-L-glu)(4-Meim) were found to be SOD mimetics with values of IC_{50} 1.57×10^{-7} mol dm^{-3} and 3.93×10^{-7} mol dm^{-3}, respectively [15].

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REFERENCES

1. Blahová, M., Sokolík, J., Sedláčková, S., Burianová, E., and Mlynarčík, D., *Cesk. Farm. 42*, 137 (1993).
2. Valent, A., Kohútová, M., Krätsmár-Šmogrovič, J., Mlynarčík, D., Blahová, M., and Sokolík, J., in *Contribution to Development of Coordination Chemistry.* (Sirota, A. and Ondrejovič, G., Editors.) P. 437. STU Press, Bratislava, 1993.
3. Sokolík, J., Blahová, M., Čukanová, G., Kohútová, M., Mišíková, E., and Mlynarčík, D., *Cesk. Slov. Farm. 47*, 186 (1998).
4. Valentová, J., Žemlička, M., Vaníčková, M., and Labuda J., in *Progress in Coordination and Organometallic Chemistry*, Vol. *3*. (Ondrejovič, G. and Sirota, A., Editors.) P. 251. STU Press, Bratislava, 1997.
5. Krätsmár-Šmogrovič, J., Pavelčík, F., Soldánová, J., Sivý, J., Seressová, V., and Žemlička, M., *Z. Naturforsch. 46b*, 1323 (1991).
6. Krätsmár-Šmogrovič, J., Soldánová, J., Pavelčík, F., and Sokolík, J., *Proc. 10th Conf. Coord. Chem., Smolenice, 1985*, p. 209.
7. Kožíšek, J., Kožíšková, Z., Valko, M., Pelikán, P., and Krätsmár-Šmogrovič, J., *Proc. 13th Conf. Coord. Chem., Smolenice, 1991*, p. 125.
8. McCord, J. M. and Fridovich, I., *J. Biol. Chem. 244*, 6049 (1969).
9. Richardson, D. C. in *Superoxide and Superoxide Dismutases.* (Michelson, A. M., McCord, J. M., and Fridovich, I., Editors.) P. 217. Academic Press, London, 1977.
10. Sawyer, D. T. and Valentine, J. S., *Acc. Chem. Res. 14*, 393 (1981).
11. Bergendi, Ľ., Krätsmár-Šmogrovič, J., Ďuračková, Z., and Žitňanová, I., *Free Radical Res. Commun. 12—13*, 195 (1998).
12. Andrezálová, L., Ďuračková, Z., Valent, A., and Devínsky, F., *Pharmazie 53*, 5 (1998).
13. Nakao, Y., Sakurai, K., and Nakahara, A., *Bull. Chem. Soc. Jpn. 40*, 1536 (1967).
14. Horák, M. and Papoušek, D., *Infračervená spektra a struktura molekul.* (Infrared Spectra and Molecular Structure.) Academia, Prague, 1976.
15. Kohútová, M., Valent, A., and Ďuračková, Z., in *Coordination Chemistry at the Turn of Century*, Vol. *4*. (Ondrejovič, G. and Sirota, A., Editors.) P. 365. STU Press, Bratislava, 1999.