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Complex Formation between 5-Aminoorotic Acid and Copper(II) Ions in Dimethylsulfoxide Solution

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Abstract—Complex formation between 5-aminoorotic acid and copper(II) ions in DMSO solution has been studied by means of electron absorption, NMR, and IR spectroscopy. The complex composition and its formation constant have been determined by means of spectrophotometry. NMR and IR spectroscopy data have revealed the donor sites of 5-aminoorotic acid involved in the coordination with copper(II) ions. The complex structure has been suggested.

Keywords: complex formation, copper(II), 5-aminoorotic acid

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Orotic acid and its derivatives are essential substrates of biosynthesis of pyrimidine bases, building blocks of nucleic acids. The synthesis is known to occur in the presence of metal ions. The coordination chemistry of orotic acid complexes with transition metals has been well studied [1–5]; it has been shown that orotic acid forms the 1 : 1 complexes with Co(II) and Ni(II) ions and the 2 : 1 complexes with Fe(III), Cu(II), and Cd(II) ions [4]. Complexes of orotic acid with the mentioned ions as well as its supramolecular complex with Mn(II) ions [5] are formed via the coordination at the donor sites: the N^1 atom of uracil ring and oxygen atom of the carboxylate group. Structure of Mn(II) complex with orotic acid has been confirmed by X-ray diffraction (XRD) analysis.

The data on formation of the complexes with 5-substituted derivatives of orotic acid have been scarce so far [6–9]. The introduction of a substituent in the position 5 of the uracil ring has been shown to significantly modify the ligand complex forming properties. We have earlier studied Cu(II) ions complex formation with 5-hydroxyorotic acid [7]. The formation of the metal ions complexes with 5-aminoorotic acid (AOA) may involve the heterocyclic nitrogen atoms, the two exocyclic carbonyl oxygen atoms, the amino moiety, and the carboxy group. The 1 : 3 complexes of AOA with lanthanides have been prepared [8, 9]; the complexes structure has been confirmed by Raman, IR, ^{1}H NMR, and ^{13}C NMR spectral data as well as by elemental analysis to show that both oxygen atoms of the carboxy groups are involved in the coordination with the metal ions. The structure of three complexes of Pt(II) with 5-aminoorotic acid have been elucidated by XRD analysis [10].

This work aimed at investigating the complex formation between AOA and ions of Cu(II), an important biogenic metal, by means of electron absorption, IR, and ^{13}C NMR spectroscopy as well as elemental analysis techniques. DMSO was chosen as the solvent in view of the good solubility of the ligand.

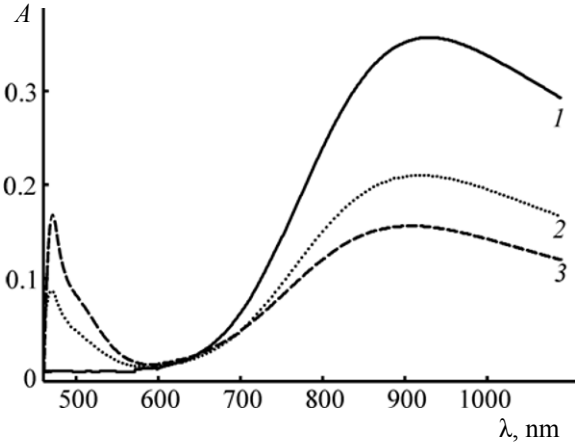


Fig. 1. Absorption spectra of solutions of copper(II) chloride (5×10^{-3} mol/L) (*1*) in the absence and (*2, 3*) in the presence of 5-aminoorotic acid. (*2*) $c_{Cu}/c_{AOA} = 1 : 2$ and (*3*) and 1 : 8.

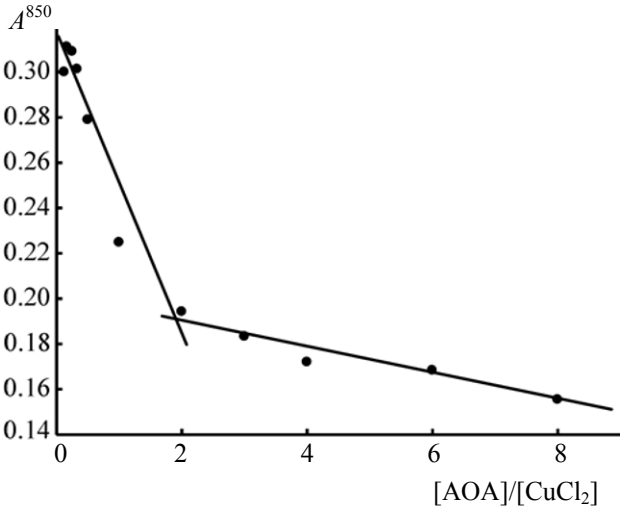


Fig. 2. Solution absorbance at 850 nm as a function of 5-aminoorotic acid concentration. $CuCl_2$ concentration 10^{-4} mol/L.

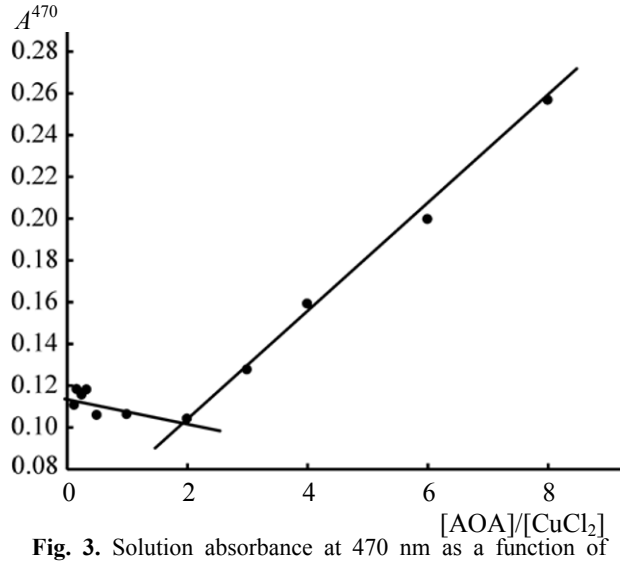


Fig. 3. Solution absorbance at 470 nm as a function of 5-aminoorotic acid concentration. $CuCl_2$ concentration 10^{-4} mol/L.

Electron absorption spectrum of copper(II) chloride solution in DMSO contained an absorption band with a maximum at 850 nm (Fig. 1). The addition of AOA to that solution resulted in the appearance of a new absorption band at 470 nm, characteristic of square-planar complexes of tetracoordinate copper(II) ion [11]. The intensity of the band at 850 nm decreased and the band at 470 nm became stronger with the increasing ligand-to-metal concentrations ratio in the studied solution. The composition of the formed complex was determined by the molar ratios method at the constant concentration of copper(II) ions. The plot of the absorbance at 850 nm as a function of the solution composition (Fig. 2) evidenced the formation of the 2 : 1 complex.

Figure 3 shows the similar plot for absorbance at 470 nm. The presence of two bands (470 and 850 nm) in the electron absorption spectra pointed at the formation of the two copper(II) complexes: the octahedral one with the hexacoordinate Cu(II) ion and the square-planar one with the tetracoordinate Cu(II) ion.

In order to determine what donor sites of the ligand were involved in coordination with Cu(II) ions, we analyzed the ^{13}C NMR spectra of the solution of AOA in DMSO-d_6 in the presence of $CuCl_2$ at the 2 : 1 molar ratio (Table 1). The change in the chemical shift was the most prominent for the C^5 and C^7 atoms of the ligand, evidencing the Cu(II) coordination with the nitrogen atom of the primary amino group and with the carboxy groups.

We further simulated the structures of the two possible complexes of AOA with copper(II) ions (Fig. 4) at the CSGT-PCM(DMSO)-B97-2/pcS-3//B3LYP/6-31+G(d) (m6-31G* for Cu) level (Table 2).

In the case of complex **I** (formed exclusively via the coordination with the carboxy groups, Fig. 4), the only change of NMR signals associated with the complex formation should have been the downfield shift of the C^7 signal; that was not in agreement with the experimental observations.

The formation of the complex **II** (via the amino and the carboxy groups, Fig. 4) should have resulted in the strong shift of the signals of carbon atoms at the double bond and a smaller shift of the signal of the carbonyl carbon; the C^5 signal should have been shifted upfield, and the C^6 one should have been moved downfield. That coincided with the experimental data, provided that inversion of the C^5 and C^6 signals positions occurred.

The donor sites of the ligand involved in the complex formation were elucidated by means of IR spectroscopy as well. IR spectrum of the complex con-

Table 1. Parameters of ^{13}C NMR spectra (δ_C, ppm) of 5-aminoorotic acid and its complex with Cu(II)

Compound	C^2	C^4	C^5	C^6	C^7
Ligand	147.77	161.40	129.29	109.44	164.55
Complex	145.72	160.01	123.05	106.95	158.44
$\Delta\delta$	–2.05	–1.39	–6.24	–2.49	–6.11

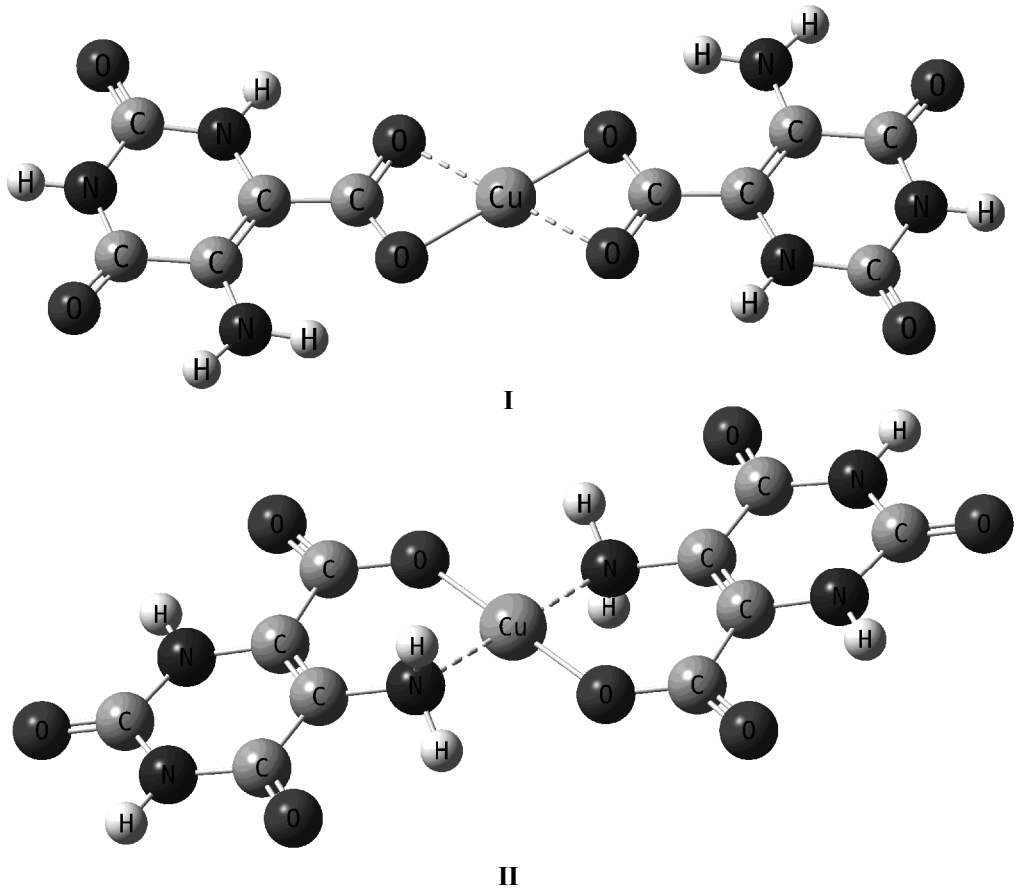


Fig. 4. Optimized geometry of the Cu(II) complexes with 5-aminoorotic acid.

tained weak bands at 560 (Cu–O) and 498 (Cu–N) cm^{-1} (Table 3). The appearance of absorption bands at 1687 and 1373 cm^{-1} pointed at the monodentate coordination of copper(II) ion with the COO^- group. The simulated IR spectra [B3LYP/6-31+G(d), Table 3] evidenced the formation of the complex via the amino and the carboxy groups as well.

Table 2. Calculated chemical shifts (ppm) of carbon atoms of 5-aminoorotic acid and its complex with Cu(II)

Compound	C^2	C^4	C^5	C^6	C^7
Ligand	143.91	159.24	130.63	105.93	162.25
Complex **I**	143.64	158.42	133.35	104.35	177.93
$\Delta\delta_{calc}$[a]	–0.27	–0.82	2.72	–1.58	15.68
Complex **II**	145.63	160.97	113.41	138.39	159.64
$\Delta\delta_{calc}$[a]	1.72	1.73	–17.22	32.46	–2.61
$\Delta\delta_{exp}$	–2.05	–1.39	–22.34[b]	13.61[b]	–6.11

[a] $\Delta\delta_{calc} = \delta^{calc}_{complex} - \delta^{calc}_{ligand}$. [b] According to the data in Table 1 assumung inversion of the C^5 and C^6 signals.

In summary, the following scheme of the complex formation between 5-aminoorotic acid and copper(II) ion in DMSO solution was elucidated from the experimental data (Scheme 1).

Equilibrium constant of the complex formation calculated from the spectrophotometry data recorded for the mixtures with varied ligand-to-metal ratio was 47.5±4.1.

EXPERIMENTAL

The studied solutions were prepared from weighed amounts of $CuCl_2 \cdot 2H_2O$ (chemically pure grade) and 5-aminoorotic acid (Aldrich, 99%).

Electron absorption spectra of the solutions were recorded in a quartz cell (l = 5 cm) at 400–900 nm using a Specord M40 spectrophotometer and referenced to the spectrum of pure DMSO recorded under the same conditions. IR spectra of the mineral oil suspensions were recorded at 400–4000 cm^{-1} using an IR Prestige-21 spectrophotometer (Shimadzu Corp.).

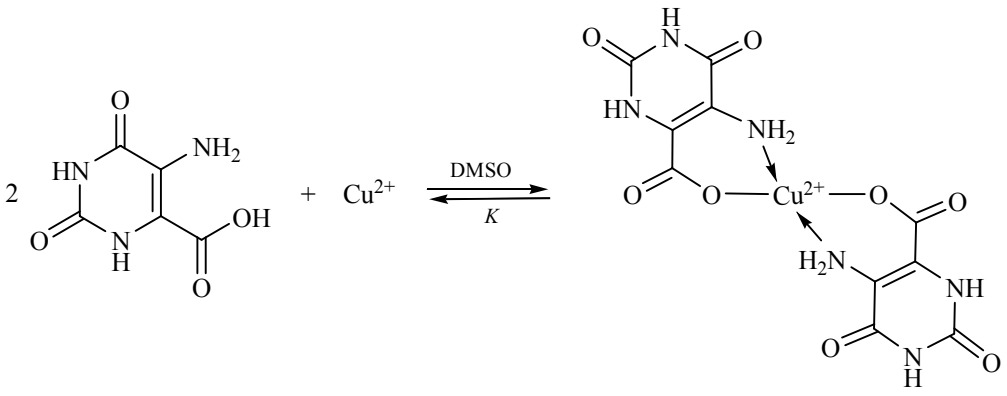
Table 3. Assignment of the absorption bands in the experimental and simulated (B3LYP/6-31+G*, m6-31G*) IR spectra of 5-aminoorotic acid and its complex with Cu(II)

5-Aminoorotic acid			Complex		
assignment	ν, cm^{-1}	ν_{calc}, cm^{-1}	assignment	ν, cm^{-1}	ν_{calc}, cm^{-1}
H–N–H	3458 s		N^3–H	3514 s	3499
O–H	3333 s		N^1–H		3446
H–N–H, N–H	3196 s		NH_2 (as.)		3380
			NH_2	3186 s	3334
C^2=O	1700 s	1762	C^2=O	1740 s	1770
C=O C^4=O	1677 s	1725 1716	C^4=O COO^- (as.)	1687 s	1707 1694
C=C	1606 s	1644	C=C, δ(H–N–H)	1633 s	1670
δ(H–N–H)	1567 m	1571	C=C, δ(H–N–H)	1581 m	1624
			C^6–N^1	1509 m	1485
			δ(N^1–H)	1420 m	1350
δ(N–H)	1438 m 1406 m	1397, 1387, 1366	COO^-	1373 m	1312
γ(uracil)	1312 m	1290	C–NH_2 δ(H–N–H)	1220 m	1227
δ(O–H)	1237 m	1150	δ(H–N–H)	1160 w	1124
			ρ(C–NH_2)	590 w	590
			Cu–O	560 w	
			δ(H_2N–Cu–NH_2)	498 w	478

The absorption bands were assigned accounting for the reference data [3, 8]. ^{13}C NMR spectra (25°C, DMSO-d_6 as solvent, and Me_4Si as internal reference) were recorded using a Bruker Avance 500 spectrometer.

Study of the complex formation between 5-aminoorotic acid and copper(II) ions. The composition of the formed complex was determined from the spectrophotometry data using the molar ratios approach. To do so, a series of mixtures with the varied ligand-to-metal ratio was prepared from the fresh solutions of AOA (5×10^{-2} mol/L) and $CuCl_2$ (5×10^{-2} mol/L) in DMSO. The mixtures were purged with argon during 5 min and transferred to a sealed

Scheme 1.



quartz cell to prevent the possible oxidation of AOA. The mixtures absorbance at 470 and 850 nm was recorded.

^{13}C NMR spectra were obtained as follows. The solutions of $CuCl_2 \cdot 2H_2O$ (34.1 mg in 0.4 mL of DMSO-d_6) and AOA (68.5 mg in 0.4 mL of DMSO-d_6) were purged with argon during 15 min and transferred to the ampule pre-purged with argon.

The complex formation constant was calculated from the spectrophotometry data [12] for each of the studied mixtures:

$$K = \frac{[CuL_2]/[H^+]^2}{[Cu^{2+}]/[HL]^2}. \quad (1)$$

Molar absorptivity of the complex was calculated assuming that all Cu(II) ions were bound in the complex at the excess of the ligand (as confirmed by the plateau in the plots of absorption as a function of the mixture composition):

$$\varepsilon(CuL_2) = \frac{A}{c_{Cu^{2+}} l}. \quad (2)$$

Equilibrium concentration of the complex was determined as follows:

$$[CuL_2] = \frac{\Delta A}{\varepsilon(CuL)_2 l}, \quad (3)$$

with ΔA being the absorption change upon the complex formation.

Equilibrium concentrations of AOA and copper(II) ions were found as follows.

$$[HL] = c_L - 2[CuL_2], \quad (4)$$

$$[Cu^{2+}] = c_{Cu^{2+}} - [CuL_2]. \quad (5)$$

The values of the complex formation constant calculated at different ligand-to-metal ratios were averaged.

The density functional theory simulations were performed applying the B3LYP three-parameter hybrid exchange functional [13, 14]. The standard 6-31+G(d) biexponential basis set was used for C, H, N, and O atoms; the m6-31G* basis set was used for Cu atoms, known to yield the simulation results coinciding with the experimental data [15]. All the geometry parameters of the molecules and the complexes were optimized without any symmetry constraints. The optimized structures nature was determined via analysis of the Hessian matrix eigenvalues [16]. The vibration frequencies were obtained using the correction factor of 0.975 [17]. Isotropic ^{13}C chemical shifts were calculated in the redundant intrinsic coordinates using the Schlegel optimization algorithm [18]. Isotropic shielding constants $\sigma(^{13}C)_{comp}$ were calculated using the B97-2 hybrid exchange potential [19] and the pcS-3 (C, O, N, and H) and m6-31G* (Cu) basis sets in combination with the continuous set of gauge transformations (CSGT) method [15, 16, 20–22]. The solvent effect was accounted for in the frame of the polarization continuum model PCM. The chemical shifts $\delta(^{13}C)$ were calculated from the isotropic shielding constants as follows: $\delta(^{13}C) = 171.97 - 0.9303\sigma(^{13}C)_{comp}$; the equation parameters were obtained via linear correlation analysis of the $\sigma(^{13}C)_{comp}$ and $\delta(^{13}C)$ data for the series of model compounds (tetramethylsilane, acetone, acetonitrile, nitromethane, pyridine, butan-2-one, *tert*-butyl methyl ether, diglyme, ethyl acetate, and triethylamine), the correlation coefficient being $r^2 = 0.9996$. Experimental values of chemical shifts of the model compounds in DMSO were extracted from [23]. Reducing systematic error of $\delta(^{13}C)$ values determination using the described linear regression method has been demonstrated earlier in [24]. The quantum-chemical simulations were performed using the GAUSSIAN'09 software package [25].

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