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Synthesis and Study of Isomers of Bis(4-aminosalicylato)copper(II)

KAREN MOORE and GERALD S. VIGEE*

Department of Chemistry, University of Alabama in Birmingham, Birmingham, Ala. 35294, U.S.A.

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Synthesis of bis(4-aminosalicylato)copper(II) from 4-aminosalicylic acid yields two isomers of the complex that have different physical characteristics, but similar chemical properties. Characterization of the isomers indicates that both are monomeric with coordination to the metal ion occuring through the carbonyl oxygens of the ligand. Both complexes are essentially square planar with normal magnetic moments. Both isomers are thermally stable in the solid state and are insoluble in common solvents. The isomer produced during synthesis is determined by the concentration of reactants.

Introduction

In 1960, Hanic and Michalov reported the synthesis and X-ray analysis of copper(II) salicylate tetrahydrate. This study showed that the compound is monomeric, containing only one copper ion per molecule [1]. Inoue and co-workers subsequently reported the synthesis of two distinct forms of anhydrous copper(II) salicylate [2]. In each case, one form of the complex had a normal magnetic moment, and the other had a subnormal magnetic moment. These results indicate that copper(II) complexes of salicylic acid may be monomeric, dimeric, or polymeric, depending on the reaction conditions.

In 1976, Sorenson reported that copper(II) complexes of salicyclic acid are more active as anti-inflammatory agents than their parent compounds [3]. He interpreted these data to indicate that the active forms of anti-inflammatory drugs may be metal complexes. This observation lead Weser and co-workers to evaluate a group of copper salicylates as enzyme models for superoxide dismutase [4]. The study showed that these copper(II) complexes are very effective superoxide scavengers. Their superoxide dismutase activity is essentially identical to that of the native enzyme cuprein [5]. This work included the first reported synthesis of bis(4-aminosalicylato)copper(II), but no analytical data was given for the complex.

Interest in developing small molecular weight monomeric and dimeric copper(II) complexes as models for copper oxidase enzymes led to this study of the copper(II) complex of 4-aminosalicylic acid. The aim of this research was to synthesize and characterize the complex and then to study its viability as a copper oxidase enzyme model. Synthesis of the complex led unexpectedly to two isomers of the complex. While these isomers have identical molecular weights and similar chemical properties, they have clearly different physical properties.

Experimental

Synthesis

The synthesis of bis(4-aminosalicylato)copper(II) yields two isomers. The isomer obtained is determined by the concentration of reactants used. The two products, denoted CuASA-*Trans* and CuASA-*Cis*, were synthesized as follows.

1. *Trans*-Bis(4-aminosalicylato)copper(II) monohydrate (CuASA-*Trans*)

A 0.1 *M* solution of the sodium salt of 4-aminosalicylic acid was prepared by dissolving 1.751 g of the compound in 100 ml of water, and a 0.1 *M* solution of copper chloride was prepared by dissolving 1.705 g of $CuCl_2 \cdot 2H_2O$ in 100 ml of water. 33.0 ml of the copper chloride solution were added to 100 ml of the 4-amino salicylic acid solution. A bright green precipitate formed immediately. The precipitate was recovered by vacuum filtration and washed three times with deionized water. A test of the wash water with silver nitrate was negative, indicating that all free chloride ions had been removed from the precipitate. The precipitate was dried in a vacuum oven for 12 hours at 60 °C. The

*Author to whom correspondence should be addressed.

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product is a bright green powder. It has a molecular formula of $Cu(NH_2C_6H_3OHCO_2)_2 \cdot H_2O$ and a molecular weight of 385.5 g/m. The complex melts with decomposition at 204–206 °C.

Analysis:

	%C	%H	%N	%Cu
Found	43.20	3.70	7.12	16.31
Calcd.	43.58	3.63	7.26	16.47

2. *Cis*-bis(4-aminosalicylato)copper(II) monohydrate (CuASA-*Cis*)

133 ml of 0.025 *M* copper chloride solution ($CuCl_2 \cdot 2H_2O$, 0.853 g/200 ml H_2O) were added to 400 ml of 0.025 *M* 4-aminosalicylic acid solution (1.751 g/400 ml H_2O). The solution immediately turned bright green. Fine, dark green crystals precipitated after the solution was stirred for one minute. The product was recovered by vacuum filtration and washed with diionized water three times. A test of the wash water with silver nitrate indicated that it contained no chloride ions. The precipitate was then dried in a vacuum oven at 60 °C for 12 hours. The product is a dark green powder with a molecular formula of $Cu(NH_2C_6H_3OHCO_2)_2 \cdot H_2O$ and a molecular weight of 385.5 g/m. The complex melts with decomposition at 204–205 °C.

Analysis:

	%C	%H	%N	%Cu
Found	43.44	3.69	6.95	16.34
Calcd.	43.58	3.63	7.26	16.47

Chemical analyses were performed by Galbraith Laboratories Inc., Knoxville, Tennessee. The sodium salt of 4-aminosalicylic acid was purchased from Sigma Chemical Company, and the copper chloride dihydrate, AR grade, was purchased from Malinckrodt Chemical Company.

Physical Methods

Infrared Studies

Infrared spectra of these complexes were recorded in KBr disks in the 4000–600 cm^{-1} region, using a Beckman Aculab II Spectrophotometer. Spectra were also measured with a Perkin Elmer Model 283 Spectrophotometer in the 4000–200 cm^{-1} region.

Optical Studies

The visible spectra of CuASA-*Trans* and CuASA-*Cis* were recorded in the 400–800 nm range with a Cary Model 17 Spectrophotometer. The spectrum of each solid complex was determined in a Nujol mull. The solution spectrum of each complex was measured in dimethyl sulfoxide using Coleman 1 cm quartz cells.

Magnetic Susceptibility Studies

The magnetic susceptibility of each isomer was determined by the Gouy method using a system similar to the one developed by Eaton and Eaton [6]. The system was calibrated with $HgCo(SCN)_4$ and $Ni(en)_3S_2O_3$. Diamagnetic corrections were calculated from a table of Pascal's constants [7].

Nuclear Magnetic Resonance Studies

The nuclear magnetic resonance spectra of the ligand, 4-aminosalicylic acid, and both isomers were recorded with a Varian EM 390-90 MHz Spectrometer and with a Nicolet NMC-300/WB Fourier Transform Spectrometer. The studies were done at room temperature in DMSO-d_6.

Electron Spin Resonance Studies

The electron spin resonance spectra of the complexes were measured with a Varian 4502 Spectrometer. The spectra of the solid complexes were determined from undiluted powder samples, and the solution spectra were measured in n-butanol. The studies were done at room temperature.

Oxidase Studies

The ability of CuASA-*Trans* and CuASA-*Cis* to catalyze the oxidation of 4-t-butylcatechol to its o-quinone, 4-t-butyl-1,2-benzoquinone, was determined by spectrophotometrically monitoring the production of the o-quinone at 470 nm. Stock solutions of 1.00×10^{-1} *M* 4-t-butylcatechol and 1.00×10^{-3} *M* CuASA-*Trans* were prepared in DMSO. 3.0 ml of the CuASA-*Trans* solution were added to a 1 cm quartz cell and allowed to equilibrate in the spectrophotometer cavity at 25 °C. A sample of 0.3 ml of 4-t-butyl-1,2-benzoquinone was then followed by monitoring the increasing absorbance at 470 nm. The initial oxidation rate for CuASA-*Cis* was calculated from the average slope of the tangent to the absorbance curve at time zero. The same procedure was used to calculate the initial oxidation rate for CuASA-*Trans*.

Results and Discussions

Elemental analysis of CuASA-*Trans* and CuASA-*Cis* indicates that each compound has a molecular weight of 385.5 grams per mole, and each complex has the same molecular formula, Cu(4-aminosalicylic acid)$_2 \cdot H_2O$. These data indicate that the compounds are geometrical isomers. The nature

TABLE I. Infrared Absorptions of 4-aminosalicylic Acid, CuASA-*Trans* and CuASA-*Cis* in KBr.

4-aminosalicylic acid (cm^{-1})	CuASA-I (cm^{-1})	CuASA-II (cm^{-1})	Assignment
–	3280	3280(s)	N–H asym stretch
3240	3235	3235	N–H sym stretch
–	3140	3140	C–H stretch
1640	1585	1600	COO^- asym stretch
1495	1490	1490	C=C stretch
1355	1380	1380	COO^- sym stretch
1155	1155	1155	N–H twist
685	685	685	C–C bend
–	405	405	Cu–O asym stretch
–	285	285	Cu–O sym stretch

of their structural relationship must be determined by analytical methods.

Thermochromism is well known in some copper complexes and has been shown to arise from temperature-dependent distortions about the copper ion site, as in the case of isomers of bis(N,N-diethylethylenediamine)copper(II) complexes [8]. Temperature studies of CuASA-*Trans* and CuASA-*Cis*, however, have shown both complexes to be thermally stable in the solid state. Heating the complexes to 200 °C effected no observable change in the color or structure of either compound. Attempts to convert one isomer to the other by dehydration were also unsuccessful. These studies confirm the stability of each isomer, indicating that the difference in their structures is more critical than subtle geometrical coordination effects.

In preparing the two isomers of bis(4-aminosalicylato)copper(II), the product obtained is controlled by the concentration of reactants. When the concentration of reactants, $CuCl_2 \cdot 2H_2O$ and 4-aminosalicylic acid, is above 0.075 *M*, the combination of equimolar or greater ratios of ligand to copper will produce the bright green product, CuASA-*Trans*. When the concentration of reactants is 0.075 *M*, the product is controlled by the mole ratio of ligand to copper. The ratio of one mole of ligand to one mole of copper ions produces the dark green isomer, CuASA-*Cis*, while higher ligand ratios produce a mixture of the two complexes. At reactant concentrations below 0.075 *M*, the dark green product, CuASA-*Cis*, is produced regardless of the mole ratio of ligand to copper. After initial formation of the dark green product, the addition of ligand will cause the product to be converted to the light green isomer over a period of twelve hours.

The isomer formed during the reaction is also influenced by the reaction temperature. The room temperature reaction of 0.1 *M* solutions combined in the ligand-to-copper mole ratio of one produces the light green isomer. The same reaction run at 40 °C initially yields the light green isomer which slowly converts to the dark green product. When the same reaction is run at 60 °C, the dark green product forms immediately. If these reaction mixtures are cooled to room temperature after formation of the product, the dark green isomer rearranges to the light one, yielding the same product as the reaction run at room temperature.

In view of these temperature and concentration studies, additional analytical data are necessary to elucidate the structure of each isomer. Initially, the nature of the ligand coordination must be resolved because both nitrogen and oxygen donor atoms are available for bonding to the metal ions.

Diagnostic infrared absorptions of CuASA-*Trans* and CuASA-*Cis* are compared in Table I. The infrared spectra of CuASA-*Trans* and CuASA-*Cis* are very similar in the 1600 cm^{-1} region. Both complexes show a shift in the carboxylate ion stretching frequencies. $\nu_a(CO_2^-)$, which occurs at 1640 cm^{-1} in the ligand, is shifted to 1585 cm^{-1} in CuASA-*Trans* and to 1600 cm^{-1} in CuASA-*Cis*. $\nu_s(CO_2^-)$ appears at 1355 cm^{-1} in the ligand, but occurs at 1380 cm^{-1} in both CuASA-*Trans* and CuASA-*Cis*. Hence, the energy difference between the asymmetric and symmetric carboxylate stretching frequencies is smaller for each complex than it is for the ligand. This decrease is characteristic of complexes in which the carboxylate ion is coordinated to a metal ion in a bidentate structure [10].

Isolation of the NH_2 bending absorption in the 1600 cm^{-1} region is difficult, but the NH_2 twisting frequency is seen clearly at 1155 cm^{-1} in the ligand and in both complexes. This amine absorption is known to be very sensitive to interaction with metal ions, and the similarity of its frequency in the ligand to that of the complexes indicates that the amine nitrogens are not involved in coordination

TABLE II. Optical Spectra of CuASA-*Trans* and CuASA-*Cis*.

Complexes	nm(solid)	nm(DMSO solution)	(M^{-1} cm^{-1})
CuASA-*Trans*	580	765	97
	420	400	325
CuASA-*Cis*	630	765	95
	420	400	295

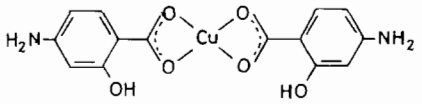

Fig. 1. *Cis* isomer of Bis(4-aminosalicylato)copper(II).

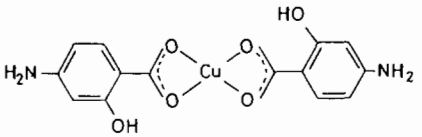

Fig. 2. *Trans* isomer of Bis(4-aminosalicylato)copper(II).

to the copper ions [11]. Each complex shows a broad band at 1040 cm^{-1}, which is characteristic of the C–O stretch in hydrogen-bonded alcohols. This absorption and the O–H stretching absorption above 3400 cm^{-1} are evidence that the phenolic oxygens are not coordinated to the copper ions.

The true nature of the metal to ligand interaction in CuASA-*Trans* and CuASA-*Cis* should be clearly demonstrated in the 500–200 cm^{-1} region where metal–nitrogen and metal–oxygen stretching bands occur. There has been much controversy concerning the assignment of Cu–N and Cu–O bands [12–14]. Based on normal coordinate analysis of bis(glycinato)copper(II) complexes, Nakamoto and Kincaid have assigned the Cu–N stretching bands in the 480–450 cm^{-1} region [13]. Comparison of the spectra of CuASA-*Trans* and CuASA-*Cis* with that of the ligand shows no new bands in this region. However, new bands appear in both complexes at 405 cm^{-1} and 285 cm^{-1}. These bands may be assigned to the asymmetric and symmetric Cu–O stretch, respectively, according to Nakamoto's calculations. These absorptions substantiate the conclusion that the copper ions are coordinated through the oxygens of the carboxylate group and that the nitrogen atoms are not involved in coordination. It is noted that the Cu–O stretching frequencies are identical for the two complexes, indicating that ν(C–O) is insensitive to structural differences between CuASA-*Trans* and CuASA-*Cis*.

Analysis of the infrared and molecular weight data for these complexes leads to the conclusion that CuASA-*Cis* and CuASA-*Trans* are the *cis* and *trans* isomers of bis(4-mainosalicylato)Cu(II) as shown below in Figures 1 and 2.

While *cis* and *trans* isomers of a few copper amino acid complexes have been prepared and characterized [12–14], such isomerism is unusual in copper(II) complexes. *Cis–trans* isomerism has not been previously reported in copper complexes of salicylic acid.

From symmetry considerations, the *cis* complex is expected to exhibit more infrared-active absorptions than the *trans* isomer. Comparison of the spectra of CuASA-*Trans* and CuASA-*Cis* from this aspect indicates that CuASA-*Cis* may be the *cis* isomer, but the infrared data are not conclusive.

A comparison of the d–d transitions of CuASA-*Trans* and CuASA-*Cis* in the visible are shown in Table II.

Each solid complex has a broad, weak absorption in the 600 nm region with a shoulder at 420 nm. These absorptions occur in the range expected for square planar complexes, and the low intensity of the absorptions is characteristic of complexes with essentially planar coordination sites. The energy of the d–d transitions of a complex generally increases as the complex approaches square planar from either octahedral or tetrahedral [12]. Hence, it appears that CuASA-*Trans* is the more planar of the two complexes because it absorbs at the shorter wave length. The solution spectra of these complexes show a broad absorption peak at 765 nm. This shift in absorption maxima to a longer wave length in solution indicates that solvent molecules are coordinating axially, giving the complexes a structure in solution that approaches octahedral. The similarity of the absorption curves of the complexes in solution implies that one of the complexes rearranges so that the structure of the two complexes in DMSO is identical.

NMR studies of these complexes showed the general signal broadening characteristic of Cu(II) complexes. While the ligand absorptions shifted little upon coordination to the metal ion, the amine and hydroxyl peaks were so broadened that no information could be gained about the structure of the complexes.

TABLE III. ESR Data for CuASA-*Trans* and CuASA-*Cis* in n-Butanol and as a Powder.

Complex	g		g	
	solid	solution	solid	solution
CuASA-I	2.25	2.28	1.97	1.94
CuASA-II	2.28	2.27	1.93	1.94

TABLE IV. Comparison of Oxidase Activity of CuASA-*Trans* and CuASA-*Cis* in DMSO.

Complex	Initial Oxidation Rate $R \times 10^6$ (M min^{-1})
CuASA-*Trans*	2.2
CuASA-*Cis*	2.2
Fsal(Lys)	7.2
Fsal(Glu)	0.45

The magnetic susceptibility of each of the solid complexes was measured by the Gouy method [6], and the magnetic moment of each complex was calculated to be 1.90 B.M. and 1.84 B.M. for CuASA-*Trans* and CuASA-*Cis* respectively.

The magnetic moments are similar to the magnetic moment of copper salicylate tetrahydrate, which Inoue measured at 1.92 B.M. [2]. X-ray crystallographic studies of copper(II) salicylate tetrahydrate indicate that the complex is monomeric, containing only one copper ion *per* molecule, and that the copper ion is coordinated to the carbonyl oxygens in a *trans* configuration [1]. The similarity of the magnetic moments of the copper salicylates prepared in this work confirms that they are both monomeric complexes in which the copper ions exist in essentially square planar environments.

The solid esr spectra were recorded on powdered samples, and the solution spectra were measured in n-butanol. The spectrum of CuASA-*Trans* contains a broad, asymmetric signal centered near 3300 G. No hyperfine coupling is observed. The spectra of the solid complexes differ slightly in that the absorption of CuASA-*Cis* is slightly more asymmetric than that of CuASA-*Trans*. The solution spectra of both isomers are essentially identical to the solid spectrum of CuASA-*Trans*. Based on esr and optical data, it can be concluded that CuASA-*Cis* rearranges in DMSO solution to form CuASA-*Trans*. Hence, CuASA-*Trans* is the more thermodynamically stable isomer. Although the *cis* isomer is less stable than the *trans* isomer, its structure is probably stabilized to some extent through hydrogen bonding of the hydroxyl groups which are located on the same side of the benzene ring.

The $g_{\parallel}$ and $g_{\perp}$ were calculated from the esr spectra and are listed in Table III. These values fall in the range reported for monomeric Cu(II) complexes that are essentially planar [13, 14].

Unfortunately, the esr absorptions for the complexes prepared in this work are so broad that no specific information can be gained about the ligand–to–copper bonding in the complexes.

CuASA-*Trans* and CuASA-*Cis* are insoluble in water, hence the determination of their ability to catalyze the oxidation of catechols in aqueous systems was not possible. However, oxidation studies were conducted in DMSO solution to evaluate the potential of these complexes as oxidase enzyme models. The ability of CuASA-*Trans* and CuASA-*Cis* to oxidize 4-t-butylcatechol to 4-t-butyl-1,2-benzoquinone was determined spectrophotometrically. The production of 4-t-butyl-1,2-benzoquinone was observed at 470 nm where its molar absorptivity has a value of 1.78×10^4 M^{-1} cm^{-1} [15]. Initial oxidation rates were calculated for both complexes and are compared to two enzyme model complexes which were previously studied [16]. The results of these studies are given in Table IV.

Bis(4-aminosalicylato)copper(II) isomers show measurable ability to catalyze the oxidation of 4-t-butylcatechol, having an initial oxidation rate of 2.2×10^{-6} M min^{-1}. The identical oxidation rate of the two isomers is further confirmation of the rearrangement of CuASA-*Cis* in solution.

Conclusions

During the synthesis of copper complexes as models for copper oxidase enzymes, two geometrical isomers, CuASA-*Cis* and CuASA-*Trans* were prepared. The isomer prepared depends upon the concentration of the reactants. The isomers retain their structure over a wide temperature range in the solid but when dissolved in DMSO, the *cis* complex rearranges to the *trans* isomer. Two 4-aminosalicylate ligands bond as bidentate ligands through the carboxylate oxygens to form square planar mononuclear complexes typical of Copper II complexes. The *meta*-stable *cis* structure is probably stabilized through hydrogen bonding of the hydroxy groups on the same side of the benzene ring.

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