Further Studies on the Copper(II) Complexes of Lysine: $[Cu(II)(H_3N \cdot C_5H_{10} \cdot CH \cdot NH_2 \cdot COO)_2][HgI_3]_2$

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Abstract

Spontaneous resolution in the formation of the $[HgI_3]^-$ salts of the copper complex of racemic lysine was previously reported. X-ray and IR studies were used to support this conclusion. Gas chromatographic studies using a chiral phase on the crystals originally studied, and on newly formed crystals using D,L-lysine, do not substantiate the suggestion that spontaneous resolution occurs.

Introduction

Spontaneous resolution has been of interest since its discovery by Pasteur in 1850 [1]. The title complex was first prepared by Taurins in 1950 [2]. He suggested that the same complex was formed with either the racemic or optically active ligand. Blank, Huxtable and O'Brien [3] explained this by proposing spontaneous resolution when the cationic copper(II) complex of D,L-lysine was precipitated with mercury triiodide. They stated that the complexes formed by the optically active and racemic ligand were impossible to distinguish by X-ray powder method [3]. The formula was established as $[Cu(II)(H_3N \cdot C_5H_{10} \cdot CH \cdot NH_2 \cdot COO)_2][HgI_3]_2$ by elemental analysis, IR and UV spectra. They based their conclusion of spontaneous resolution on IR and X-ray data. They proposed that the crystals, formed at temperatures ranging from 20–80 °C from both D,L-lysine and L-lysine, were isomorphic and presumably of the L,L- and D,D-complexes. Spectroscopic and X-ray methods of analyses did not distinguish the complexes prepared from the racemic and optically active lysine; Blank, Huxtable and O'Brien [3] condluded the crystals were identical.

They [3] presented two possible explanations why the crystals were isomorphic, (a) racemization of the L-isomer of the D, L-isomer had occurred or (b) spontaneous resolution had taken place and a mixture of the bis L- and bis D-complexes had formed [4]. Racemization was not considered to be a likely explanation as the conditions of reaction were mild [3]. Furthermore, a check of the optical activity of the complex prepared from L-lysine in 2 M HCl revealed lysine to have retained its optically activity.

We now report further studies by chiral gas chromatography. One sample of the complex reportedly formed from D,L-lysine did not contain any D-lysine. This definitely rules out racemization during the slow crystallization of the copper lysine complex with mercuric triiodide.

Results and Discussion

A representative sample containing many crystals originally studied by Blank, Huxtable and O'Brien [3], which they reported was prepared from D,L-lysine, was analyzed by capillary GC on the optically active phase, *N*-docosanoyl-L-valyl-t-butyl amide. The *N*-TFA isopropyl derivative of lysine separated from this complex using sodium sulfide was chromatographed. This result was compared with those for *N*-TFA-isopropyl ester derivatives of L- and D,L-lysine standards. The results are shown in Fig. 1. This

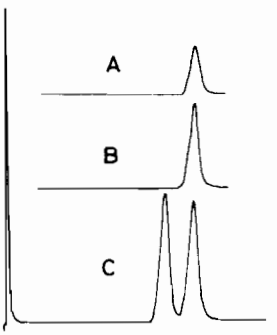

Fig. 1. Gas chromatograms of the N-TFA isopropyl esters of (a) L-lysine standard, (b) O.Brien's lysine sample and (c) D,L-lysine standard.

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study shows that one sample of the complex made and studied by Blank *et al.* reportedly from D,L-lysine, was found to contain 100% of the L-isomer. Further experiments were then carried out. In the first study, three solutions of copper complexes of racemic lysine were precipitated over night, at room temperature, with a solution containing 1.00, 0.50 and 0.25 equivalents of $[HgI_3^-]$. A fourth solution containing 0.1 equivalents of $[HgI_3^-]$ required four days to precipitate the complex. Each complex was analyzed as before using GC analysis. All precipitates revealed that no resolution had occurred. All fractions were racemic. It was expected that there would at least be some preference for the L-enantiomer complex to form.

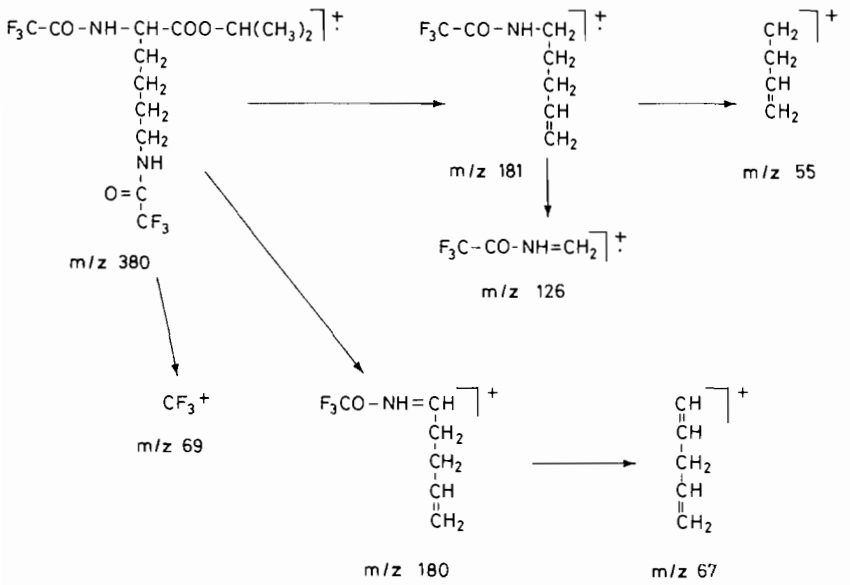
This lead us to believe that we were not carrying out the crystallization in the same manner in the U.S.A. as had been done in London. For this reason we carried out a second study. Crystalline racemic lysine was sent to O'Brien's laboratory where he prepared the complex from the racemic lysine supplied to him by us and from the same batch of lysine that had been used for our GC studies. O'Brien sent two batches (a first crop and a second crop) of crystals to be analyzed by GC. These samples were treated in the same manner as before. The results showed the material to be racemic.

We conclude that the structures of the salts formed from D,L-lysine and L-lysine are so similar that conventional spectroscopic techniques cannot distinguish between the racemic and optically pure complex. This has been confirmed by IR and X-ray (powder) measurements on D, L and L complexes prepared in the present study. It is possible that the salt formed from racemic lysine has a pronounced tendency to form microcrystalline conglomerates of bis-D-lysine complex and the bis-L-lysine complex. Apparently one sample studied by X-rays as reported by Blank, Huxtable and O'Brien [3] was not representative of all the crystals present. They may have, by chance, chosen a crystal which consisted of only the bis-L-lysine complex. Another possibility is that the lysine used initially to prepare the complex [3], which was thought to be racemic, was the optically active L-lysine.

Mass spectral analysis was also performed on the *N*-TFA isopropyl esters (Table I). Scheme 1 shows possible structures for the ions observed in the mass

TABLE I. Mass Spectral Analysis of (a) L-Lysine Standard, (b) O'Brien's Sample and (c) D,L-Lysine Standard

(a) Mass	180	67	126	69	181	55	68	294	70	139
Intensity	10000	2745	1683	1617	1192	1013	980	702	620	588
(b) Mass	180	67	126	69	181	55	68	294	70	152
Intensity	10000	2733	1759	1610	1103	1013	904	815	636	626
(c) Mass	180	67	126	69	181	294	55	68	70	168
Intensity	10000	2489	1620	1527	1139	1021	970	911	624	624



Scheme 1.

spectral analyses of the lysine derivatives. The base peak of 180 and the next four intense peaks were the same for all three samples.

Although X-ray studies have been used in the past as a method of studying crystalline structure it may not be suitable for determining if spontaneous resolution has occurred simply by a consideration of the powder picture of these compounds. This may be because the heavy $[HgI_3]^-$ ion dominates the diffraction pattern.

Experimental

Copper(II) Mercuric Triiodide Lysine Complex [2]

Reagent A was prepared by adding 6.65 g KI and 9.09 g HgI_2 to 100 ml of water. After continuous stirring, a yellow cloudy solution of $KHgI_3$ resulted.

A one equivalent lysine copper chloride solution was prepared by dissolving 2.0 g D,L-lysine·HCl in 100 ml water. To the solution was added 2.45 g of $CuCO_3(OH)_2$ and the mixture heated to boiling. This gave a deep blue solution with some green precipitate. The solution was allowed to cool before filtering. Water was added to the solution making a 200 ml of a deep blue clear solution of $Cu(lysine)_2$-Cl_2.

Three 50 ml aliquots of the copper lysine solution were heated almost to boiling in small bottles. A second set of solutions of one-tenth concentration were prepared by diluting the remaining 50 ml to 500 ml. Three 50 ml aliquots of this dilute solution were also heated almost to boiling. Reagent A was added to each aliquot to prepare samples of 1.00, 0.50 and 0.25 equivalents in Reagent A.

The samples were allowed to cool at room temperature overnight. The three samples of greatest copper lysine concentration precipitated purple crystals overnight. The dilute copper lysine solution containing only one equivalent of $[HgI_3]^-$, took four days to precipitate. The other two solutions showed no precipitation even after being placed in a refrigerator for one month.

Derivatization for GC analysis [5]

5 mg of the copper(II) lysine mercuric iodide complexes obtained from L-lysine and from D,L-lysine (originally prepared by Blank *et al.* [3]) were placed in separate vials and 5 ml of 6 N HCl was added to each. This yielded a yellow–orange solution and an orange precipitate. Sodium sulfate was added in excess, and a grey–brown precipitate of CuS resulted. The mixes were filtered yielding clear colorless solutions. Each solution was divided and placed into three glass tubes. The liquid was blown to dryness by forced air with the tubes placed in an oil bath at 120 °C. 2 ml of 4 N hydrogen chloride in isopropyl alcohol was added to each tube; these tubes were sealed and placed in an oil bath at 120 °C for 1.5 h, to effect esterification. The excess alcohol was removed by evaporation under forced air. The resultant esters were further derivatized by adding 2 ml of 30% trifluoroacetic anhydride in methylene chloride. The solvent, CH_2Cl_2, was evaporated at room temperature overnight. A D,L-lysine standard was prepared for comparison by the same derivatization precedure but no sodium sulfide was added. A sample of racemic lysine used for GC study was sent to O'Brien. This lysine was converted into the complex and two samples were sent back to be studied by GC. They were treated in the same manner as the original sample described above.

GC Analysis

GC analysis of the *N*-TFA-i-propyl esters of the lysines was performed using a Hewlett-Packard 5880 series gas chromatograph. A 46 meter stainless steel capillary column loaded with *N*-docosanoyl-L-valyl-t-butyl amide was used as the chiral phase. At 145 °C and 175 kg cm^{-2} pressure of the nitrogen carrier gas, D-lysine derivative showed a retention time of 60 min while L-lysine eluted at 70 min (Fig. 1). The derivatized samples were dissolved in methylene chloride for injection.

Mass Spectral Analysis

Low resolution mass spectra were obtained on the *N*-TFA-isopropyl ester derivatives of the lysines separated from the two complexes (L- and D,L-complexes) and D,L-lysine using an LKB 2091 magnetic sector mass spectrometer (separator temperature 225 °C, trap current 27 μA, ionization voltage 70 eV). The samples were injected through a fused silica capillary OV 101 GC column with helium as the carrier gas. The initial oven temperature was 80 °C and was immediately increased to 180 °C after the solvent, CH_2Cl_2 peak was observed. The lysine derivatives were detected in this achiral column under these conditions within 10 min.

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