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Thermal Rearrangement of the Copper(II)–L-Cystine Complex

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Polycrystalline Cu(II), Ni(II), Zn(II), Pb(II), and Co(II) chelates of L-cystine have been prepared and analyzed. The compounds may be characterized from their infrared and Raman spectra, in particular by their S–S stretching vibrations. A Cu(II) compound precipitates at temperatures <40 °C with ν(S–S) at 497 cm^{-1}, similar to ν(S–S) in solid, uncomplexed L-cystine, but in the temperature range 50–65 °C (pH 9.45) the complex forms with ν(S–S) at 510 cm^{-1}. The apparent activation energy of reaction is 17 ± 4 kcal mol^{-1}. The behaviour is interpreted in terms of ligand conformations adopted in the formation of crystals.

Introduction

The structures and reactions of metal complexes of cysteine and related amino acid thiols (*e.g.* Cu(II), Mn(II), Fe(II), Co(II), *etc.*) have been studied extensively [1–3]. By comparison, complexes of the corresponding disulphides have received considerably less attention, perhaps because many of them are insoluble and possibly polymeric. An exception has been two recent studies of the crystal and molecular structures of soluble bis[copper(II)-D-penicillamine disulphide] hydrates [4, 5].

One of the earliest procedures to isolate cystine from protein hydrolysates involved the preparation of a copper salt [6–9]. Rây and coworkers referred briefly to a pale green copper(II)–cystine compound, empirical formula $Cu(C_6H_{10}O_4N_2S_2)\cdot 2H_2O$, and they later described a method to prepare blue $Cu(C_6H_{10}O_4N_2S_2)\cdot H_2O$ by neutralization of a boiling solution equimolar in copper(II) ion and cystine [10]. Several structural investigations of the blue polycrystalline salt were prompted by its unusual fibrous morphology. From electron micrographic and conductometric analyses Kahler, Lloyd and Eden [11] assumed that the copper(II)–cystine complex was monomeric in solution and formed a polymeric chain structure upon crystallization. Similarly, Otto, Ferenc and Tamas [12] concluded from a powder X-ray diffraction study that the fibrous, needle-like crystals resulted from the molecular packing of a polymeric chelate in a special stereo arrangement. Hawkins and Perrin [13] computed stability constants based on the assumption that a non-weighted, statistical distribution of polynuclear copper(II)–cystine species could exist in equilibrium at 20 °C. Their model discounted, on steric grounds, the possibility of a monomer in solution with both ends of the dibasic cystine molecule attached to the same copper(II) ion. They postulated that the major species to be precipitated from solution was dimeric and this molecularity has yet to be confirmed experimentally.

The present study has sought to characterize some metal complexes of cystine by infrared and Raman spectroscopy, and to investigate the nature of the two Cu(II) complexes of L-cystine which form at low and high temperatures of preparation [14].

Experimental

Preparation of Compounds

Stock solutions of the amino acids were prepared freshly in 0.2 *M* $HClO_4$ and the aqueous solutions containing the metal ions were analyzed by standard volumetric or gravimetric procedures. Aliquots of a solution, made to contain equivalent amounts of metal ion and amino acid, were neutralized with 0.1 *M* NaOH to pH 7–8 at the various temperatures. An Orion model 701 pH meter with Automatic Temperature Compensator probe, model 07-01-10, was used to determine the pH. The highly insoluble precipitates were collected in a crucible with a fritted disc (4–5.5μ ASTM F) and washed successively with distilled water, absolute ethanol and ether.

Investigations of Temperature and pH Effects

Sealed bulbs containing 5.0 ml of the amino acid solutions (4×10^{-2} *M* approx.) were broken, after temperature equilibrium had been attained, into 150 ml portions of deaerated borax buffer (0.025*M*; with added 1 *M* NaOH or $HClO_4$). The mixtures were stirred continuously with a Teflon coated, magnetic bar. After 15 seconds, another sealed bulb was

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TABLE 1. Elemental Analyses of Metal Complexes with Cystine.

Complex	Preparation Temperature	Colour	Yield, %	Analyses, weight %					
					C	H	N	S	Metal
Cu(II)–cystine									
$Cu(C_6H_{10}O_4N_2S_2)\cdot\frac{1}{2}H_2O$	Room	Pale blue–violet	95.2 ± 0.1	Found	23.3	3.6	9.0	20.5	19.9
				Calc.	23.2	3.6	9.0	20.6	20.4
Cu(II)–cystine									
$Cu(C_6H_{10}O_4N_2S_2)\cdot H_2O$	Boiling	Pale blue (green if impure)	94.0 ± 0.5	Found	22.9	3.8	8.7	20.0	19.8
				Calc.	22.5	3.8	8.8	20.0	19.9
Ni(II)–cystine									
$Ni(C_6H_{10}O_4N_2S_2)\cdot H_2O$	Boiling	Very pale green	97.7 ± 0.8	Found	23.1	3.9	9.0	19.8	18.0
				Calc.	22.9	3.8	8.9	20.4	18.6
Zn(II)–cystine									
$Zn(C_6H_{10}O_4N_2S_2)\cdot H_2O$	Room	Cream	96	Found	22.8	3.6	8.7	19.5	19.9
				Calc.	22.4	3.8	8.7	19.9	20.3
Pb(II)–cystine									
$Pb(C_6H_{10}O_4N_2S_2)$	Room	White	95	Found	15.9	2.3	6.2	14.3	46.6
				Calc.	16.2	2.3	6.3	14.4	46.5
Co(II)–cystine									
$Co(C_6H_{10}O_4N_2S_2)\cdot H_2O$	Room	Pale pink	91	Found	24.3	3.9	9.3	21.2	18.0
				Calc.	22.9	3.8	8.9	20.3	18.7

broken containing an equivalent amount of the metal ion in solution. This procedure was found necessary to avoid inconsistent mixing of the solutions. After 1800 seconds, ~100 ml cold, distilled water was poured into the mixtures and they were immediately filtered. The precipitates were washed, as previously described, and dried over P_2O_5 in a vacuum desiccator.

Spectral Measurements of Compounds

Infrared spectra were measured with Perkin–Elmer spectrometers; a model 337 was used for routine Nujol mulls and a model 521 instrument for KBr discs, of the compounds. The spectra were calibrated with polystyrene film and atmospheric carbon dioxide. The peak positions quoted in the Tables are considered to be accurate to at least ±5 cm^{-1}. Quantitative ir data were obtained in KBr discs as follows: 20 mg amounts of samples were carefully ground with 100 mg KBr (ir grade) in an agate mortar and 20 mg taken of these mixtures, mixed with 200 mg portions of previously ground, dried KBr and compressed (10 tons/½"; 5 min) in a stainless steel die with oil pump evacuation. Peak absorbances (A_p) were corrected for blank KBr backgrounds, $A_p = \log_{10}(T_{KBr,\nu}/T_\nu)$, and linear Beer–Lambert calibration curves for a low and high temperature Cu(II) complex were obtained for the experimental concentration ranges.

The Raman spectra were recorded with a Jarrell Ash 25-300 spectrometer using the Ar^+ laser blue line (488.0 nm excitation), Coherent Radiation model 52G laser. Samples were sealed in standard capillary tubes and cooled to approximately −40 °C by nitrogen gas flow from boiling liquid nitrogen. The calibration lines were taken with a neon emission lamp.

Analyses and Chemicals

Elemental analyses of the complexes for C, H, N and S were made by the Schwarkopf Microanalytical Labs., N.Y.. The amino acid stock solutions for the compounds listed were prepared from L-cystine, mol. wt. 240.3 (Aldrich Chem. Co. and Fisher Scientific brands). The preparation and purification of *meso*-cystine and separation of cystine from cystine dihydrochloride were by the procedures outlined in Greenstein and Winitz [1]. Cystine may be extracted from the copper complexes, as a precipitate of cystine dihydrochloride, by treatment of 0.5 g complex with 50 ml acetone containing 2 ml conc. HCl.

Deuteration of the exchangeable proton in L-cystine, or the complexes, was achieved by dissolution in ~1 *M* DCl and reprecipitation at various temperatures with ~1 *M* NaOD (Aldrich Chem. Co. solutions), to a Universal paper endpoint. The deuterated compounds were washed with D_2O (99.8 atom%), followed by dried acetone.

Results

Elemental Analyses

These are recorded in Table I. Traces of water or impurities in the cystine might explain the consistently low yields of the complexes, since samples of the

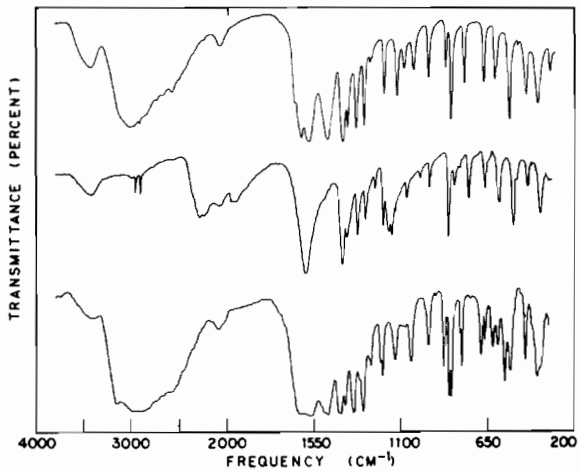

Figure 1. Infrared spectra of L-cystine (upper), N-deuterated L-cystine (middle), and *meso*-cystine (lower) in KBr discs.

low temperature Cu(II)–cystine complex could be dissolved in dilute acid and reprecipitated with 98.2 ± 0.1% recovery by weight.

Infrared Spectra

The infrared spectra of the free ligands used in this study are contained in Figure 1 and the spectra of some complexes are illustrated in Figure 2. It may be noted that the ir spectrum of L-cystine (Figure 1) has a water peak similar to that found in the Cu(II)–cystine complex (A, Figure 2). Ir spectra revealed that the water (occluded solvent or water of crystallisation) could be removed by heating the low temperature Cu(II) complex to constant weight at 120 °C, and that no other solid phase change resulted.

A strong band at 857 cm^{-1} is present in the spectrum of the light blue–violet precipitate of Cu(II)–L-cystine formed at temperatures 0–40 °C. It gradually diminishes and is replaced by the spectrum due to another complex as the temperature of preparation is increased in the range 40–100 °C. The 857 cm^{-1} band, which is of equal intensity but shifted 12 cm^{-1} from a similar band in the L-cystine spectrum, might be associated with the ligand chain C–C–N stretching vibrations [15]. In the spectrum of *meso*-cystine, the band is split at 847 cm^{-1} and 836 cm^{-1} (Figure 1). The high temperature complex could be dissolved in dilute acid and reprecipitated, by neutralization, to produce the low temperature Cu(II)–L-cystine complex. Spectra of Cu(II) complexes formed with *meso*-cystine at room temperature and 100 °C were found to be practically indistinguishable and to have an overall profile like the spectrum of the high temperature Cu(II)–L-cystine complex. Interestingly, ir measurements indicated that only one form of deuterated Cu(II)–L-cystine could be made at high or low temperatures. Correspondingly, only one form

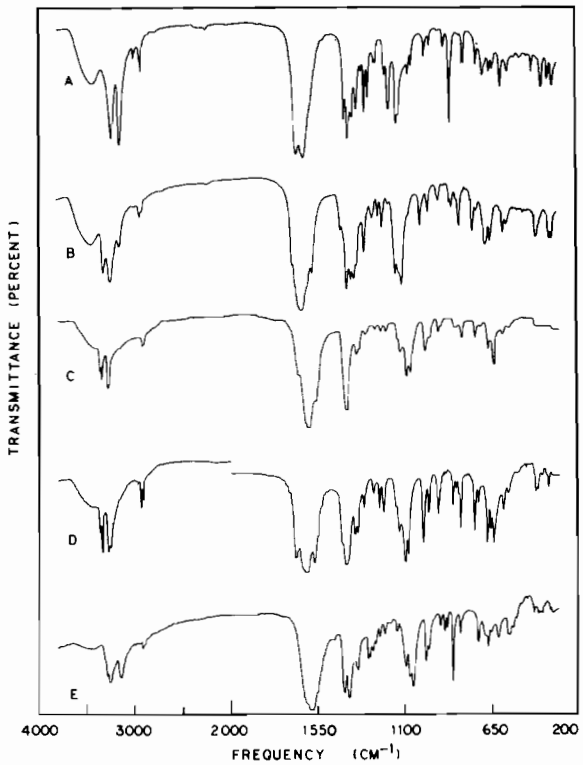

Figure 2. Infrared spectra of Cu(II)–L-cystine (0 °C prepn.), A; Cu(II)–L-cystine (100 °C prepn.) B; Ni(II)–L-cystine C; Zn(II)–L-cystine D; and Pb(II)–L-cystine E; in KBr discs.

each of L-cystine complexes could be isolated with Ni(II), Zn(II), Pb(II) or Co(II), although the Ni(II) complex prepared at room temperatures was gelatinous, in comparison with its high temperature form, and gave a poorly resolved spectrum.

To investigate whether racemization of the ligand ensued in preparations of the Cu(II) complex at 100 °C, cystine was recovered from complexes prepared with *meso*- and (D + L)-cystine. Analyses by ir spectroscopy showed that high yields of *meso*- and (D + L)-cystine could be recovered from their respective high temperature complexes and no evidence for extensive disulphide bond cleavage was detected for the *meso*-cystine complex.

The strong peak in the spectrum of L-cystine at 1485 cm^{-1}, assignable to $\nu(NH_3^+)$ symmetrical vibrations, is absent from the spectra of the complexes. Antisymmetric and symmetric $\nu(C{=}O)$ and $\nu(NH_2)$ stretching vibrations, in the region 1300–2000 cm^{-1} of the spectrum of the Cu(II)–cystine complex formed at 100 °C, are consistent with the assignments for square planar coordination of the amino acid groups in Cu(II) bis-glycinates [16], Cu(II) bis-methionine [17], or Cu(II) bis-valine [18], Table II. The C=O and C–O stretching bands show virtually no shift upon deuteration of the compounds.

TABLE II. Infrared Frequencies (cm^{-1}) for Some Non-deuterated and N-deuterated Amino Acid Complexes of Cu(II) and Ni(II).

Cu(II)–L-cystine (Prep. at 100 °C)		Ni(II)–L-cystine (Prep. at 25 °C)		$Cu(II)(glycine)_2$ (Ref. 16)		Assignments
H	D	H	D	H	D	
3310 medium	2640 strong	3360 medium	2520 medium	3360	2465	Antisymm. and symm.
3240 strong	2400 medium	3340 strong	2500 strong	3260	2350	NH_2 and ND_2 stretch
3140 weak	2380 medium	3275 strong	2420 strong			
	2350 medium					
	2320 medium					
1568	1170	1557	1150	1606	1190	NH_2 and ND_2 scissoring
1620	–	1593	–	1595	–	(C=O) antisymm. stretch
1363	–	1398	–	1389	–	(C–O) symm. stretch

TABLE III. Raman Spectra of Solid Ligands and Complexes with L-Cystine in the 450–800 cm^{-1} Region.

Ligands	cm^{-1}	Relative Intensity	Complex with L-cystine	Temperature Preparation, °C	cm^{-1}	Relative Intensity
L-cystine	497	100	Cu(II)	25	497	strong
	539	–				
	616	4	Cu(II)	100	510 (broad)	medium
	678	9				
	784	10	Ni(II)	100	512 (broad)	100
					670	34
					708	23
L-cystine (N-deuterated)	497	100	Zn(II)	25	514 (broad)	100
	516	–				
	593	–			580	–
	669	8			670	29
	755	–			713	25
	794	–			732	–
			Co(II)	25	510 (broad)	100
					667	30
					708	20
Meso-cystine	479	29	Pb(II)	25	484	90
	507 (broad)	100			508	100
	661	18			549	19
					604	13
					661	66
					677	20

Raman Spectra

Raman spectra of the ligands and the complexes with L-cystine are summarized in Table III for the region of the S–S and C–S stretching vibrations. The strong, characteristic bands of the S–S stretch dominate the Raman spectra and these were the only bands that could be observed for the solid copper(II) complexes. On the other hand, the Ni(II), Zn(II) and Pb(II) compounds gave detailed, well-defined spectra. The S–S stretching wave numbers (at 497 cm^{-1}) coincide for L-cystine, deuterated L-cystine and the low temperature form of the Cu(II)–L-cystine complex. The spectra for the L-cystine complexes with Cu(II) (high temperature form), Ni(II), Zn(II) and Co(II) are similar and contain a relatively weak, broad S–S stretch at 510–514 cm^{-1}. The spectrum for *meso*-cystine and that for the Pb(II)–cystine complex each contain a fairly strong peak, but of different intensities, at about 480 cm^{-1}, which may be due to

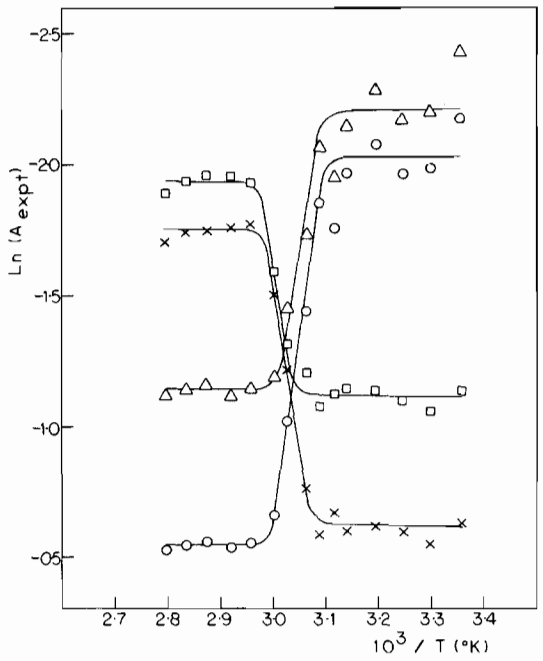

Figure 3. The variation of experimental absorbances, at 788 cm^{-1} (×), 806 cm^{-1} (○), 895 cm^{-1} (□), and 914 cm^{-1} (△) of mixed Cu(II)–L-cystine complexes, as a function of temperature; buffer pH 9.45.

splitting of the S–S bands as a result of site symmetry or correlation effects.

Copper(II) Chelates of L-Cystine

A study was made of the experimental parameters that affect the interconversion of the low and high temperature Cu(II)–L-cystine complexes. Preliminary qualitative analyses of mixed precipitates, by ir spectroscopy, had revealed that the yield of high temperature complex was strongly dependent on the temperature at neutralization and that a final pH in the range 7–10 was required.

Figure 3 illustrates the interconversion of the two species with temperature. The ir peaks at 788 cm^{-1} and 895 cm^{-1} for the low temperature and at 806 cm^{-1} and 914 cm^{-1} for the high temperature forms were chosen since they occur in mutually accessible spectral regions. The experimental absorbance values, A_{expt}, were appropriately corrected for background due to the KBr disc. Assuming that the precipitate is either low temperature form, high temperature form, or a mixture of these two complexes,

$$A_{expt} = x(\epsilon_\lambda^L - \epsilon_\lambda^H) + \epsilon_\lambda^H$$

where x is the mole fraction of the low temperature form, ϵ_λ^L the extinction at wavelength λ for the pure low temperature complex, and ϵ_λ^H the extinction at wavelength λ for the pure high temperature complex, for the standard disc conditions. The values of $\ln(A_{expt})$ may be directly proportional to the extent of molecular conversion (*vide infra*) and an estimate of an apparent energy of activation may be made from a plot of $\ln(A_{expt})$ against 1/T, where T is the temperature of preparation. A least squares analysis of the spectral data, for the temperature range 50–65 °C, gave an average value of $E_A = 17 \pm 4$ kcal mol^{-1}.

At 54.5 °C, the fraction of low temperature complex in mixtures decreased from 87 to 46%, approximately, as the pH of the buffer was changed from 7.6 to 10.0. Degradation of the cystine became evident at pH values >10, *cf.* [19], or, at ~9, if the copper ion were added to the cystine/buffer system after 5–30 minutes of incubation.

Discussion

The ir and Raman spectra of particular metal chelates of cystine have similarities that enable the compounds to be placed into common categories. For spectra of metal complexes with large organic chelating ligands, not only have band assignments to be made on an empirical basis in most cases, but the conditions under which the solid, polycrystalline KBr discs permit spectra to be made can also introduce difficulties. Herlinger, Wenhold and Long [20] have catalogued the copper(II)–ligand stretching frequencies for some amino acid complexes, the structures of which are known from X-ray determinations, and have suggested vibrational criteria to distinguish between cis and trans configurations. The form of the spectrum in the region of metal–ligand stretches, 600–300 cm^{-1}, of the low temperature Cu(II)–L-cystine complex is remarkably analogous to those for Cu(II) bis-L-isoleucine and Cu(II) bis-D,L-phenylalanine complexes, for which *cis* configurations have been assigned. However, additional experimental evidence would be required to establish unequivocally the geometrical isomerism of this Cu(II)–L-cystine complex. The complications involved in the differentiation of geometrical isomers by infrared spectroscopy have been discussed by Hawkins [21]. Intramolecular interactions, on the basis of which criteria for isomer selection are made, are often of magnitude similar to intermolecular crystal effects. Schugar [22] has cautioned that splitting of metal/ligand modes may cause difficulties of interpretation. In addition, hydrogen bonding or other interactions can also cause shifts in the frequencies of some modes, and peak assignments become correspondingly more difficult to make on an empirical basis. Nevertheless, the assignments of the C–S and S–S stretching vibrations are more equivocal in view of the molecular conformation studies available for cystine and its derivatives by Raman spectroscopy [23–25].

The marked similarities between certain infrared and Raman stretching frequencies, in particular the

S–S stretches, of solid L-cystine and the low temperature Cu(II)–L-cystine complex may be taken as evidence that the ligand chain dispositions in their crystals are similar. In aqueous solution (room temperatures), the S–S stretching frequency of L-cystine occurs at about 508 cm^{-1} and the reason for the lower value in its crystalline phase, at 497 cm^{-1}, is not fully understood [26]. Factors such as the nature of the metal–ligand bonding, the hydroxyl ion concentrations in the solutions and hydrogen bonding in the solid have an undetermined role in structural traits. The inability to prepare two forms of N-deuterated Cu(II)–cystine may reflect the considerable importance of hydrogen interactions. The rearrangement of the Cu(II) complex, alone of the metal complexes investigated to exhibit two isomeric forms, seemingly involves specific and subtle factors that influence crystal formation. The L-cystine ligand may adopt different conformations in the crystal structures of the two copper complexes because of an irreversible change in rotamer populations as the solid phase is formed. The apparent activation energy for such a rearrangement, E_A ~17 kcal mol^{-1}, is consistent in magnitude with the estimated barrier values for freedom of rotation about the bond between divalent sulphur atoms, *e.g.* 13.2 kcal mol^{-1} for diethyl disulphide [27]. However, the experimental activation energy is also comparable with that estimated for the hydrolysis of the disulphide bond in cystine [28] and it is not known if metal ion chelation or conformational effects influence the rates of hydrolysis. Fission and recombination of the S–S bond, as well as rotation, is therefore an alternative, but less likely mode for the molecular rearrangement, especially in view of the failure of the high temperature complex of *meso*-cystine to undergo extensive racemization.

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