Structure, Properties, and Function of a Copper(I)–Copper(II) Complex of D-Penicillamine: Pentathallium(I) μ_8-Chloro-dodeca(D-penicillaminato)-octacuprate(I)hexacuprate(II) *n*-Hydrate

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Abstract: The purple complex formed by the reaction of copper(II) and D-penicillamine (H_2Pen = β,β-dimethylcysteine, $HS-C(CH_3)_2CH(COO^-)-NH_3^+$) at physiological pH is shown to be an anionic Cu^I, Cu^{II} cluster complex, $[Cu^{II}_6Cu^I_8(D\text{-}Pen)_{12}Cl]^{5-}$. Crystals were obtained with $Co(NH_3)_6^{3+}$, $Ru(NH_3)_6^{3+}$, and Tl^+ as counterions. The structure of $Tl_5[Cu^{II}_6Cu^I_8(D\text{-}Pen)_{12}Cl]\cdot nH_2O$ was determined by x-ray diffraction. The unit cell is cubic, space group $F432$, with a = 50.847 (5) Å, V = 131461 Å^3, d_m = 1.89 (2), d_x = 1.91 g cm^{-3} for Z = 32 and n = 55. The intensities of only 1469 reflections could be recorded (θ_{max} = 35°). The structure was solved by a combination of Patterson, Fourier, direct phasing, and least-squares methods. The final residual for 1060 reflections with $I > \sigma(I)$ is 0.17. The $[Cu^{II}_6Cu^I_8(D\text{-}Pen)_{12}Cl]^{5-}$ clusters lie on threefold symmetry axes. Each cluster consists of (1) a central Cl^- ion, (2) an icosahedron of 12 thiolate S atoms, (3) a cube of eight Cu^I atoms such that each Cu^I atom is trigonally coordinated by the three S atoms at the corners of an icosahedron face, (4) an octahedron of six Cu^{II} atoms on the exterior of the S_{12}-icosahedron such that each Cu^{II} atom is bonded to the two S atoms on an icosahedron edge, and (5) the remaining atoms of the Pen^{2-} ligands, arranged so that the carboxylate groups project from the surface of the cluster, the side-chain methyl groups shield the Cu^I atoms, and the amino groups complete the cis bis(bidentate) coordination of the Cu^{II} atoms. The clusters are linked in the crystal by hydrogen bonds and by electrostatic interactions with Tl^+ ions. The Tl^+ ions and interstitial water molecules are disordered. The disorder accounts for the low resolution of the x-ray diffraction data. The magnetic susceptibility of the complex is consistent with the result that only 6 of the 14 Cu atoms in each cluster are Cu^{II}. An explanation of the observed ESR spectrum, a single broad resonance without hyperfine structure, is given in terms of dipolar interactions between the Cu^{II} atoms. A reaction scheme for the formation of the purple Cu–penicillamine complex and related thiol complexes is proposed. The effectiveness of D-penicillamine as a Cu scavenger is seen to depend on its ability to act as a reducing agent for Cu^{II} as well as a chelator for Cu^{II} and Cu^I. The structural results thus support an earlier suggestion for the role of D-penicillamine in the chemotherapy for Wilson's disease.

The discovery that D-penicillamine (D-H_2Pen)[1] is effective in promoting the urinary excretion of excess copper from patients with Wilson's disease (hepatolenticular degeneration)[2] has led to a number of studies of the interaction between D-penicillamine and Cu(II) in aqueous solution.[3-6] We recently described the isolation and crystal structure analysis of a Cu^I, Cu^{II} mixed-valence cluster complex, $Tl_5[Cu_{14}(D\text{-}Pen)_{12}Cl]\cdot 55H_2O$.[7] In this paper we report details of the structure of this complex, provide new data concerning its stability, and discuss its relevance to the chemotherapy of Wilson's disease.

At physiological pH, Cu^{II} and H_2Pen react to form an intensely purple complex which is stable for long periods in solution. Solid, purple products isolated by Sugiura and Tanaka,[3] by Wright and Frieden,[4] and by Neagley[6] were reported to have elemental compositions corresponding approximately to $Cu^I_2Cu^{II}(Pen)_2\cdot 2H_2O$, $NaCu^ICu^{II}(Pen)_2\cdot 6H_2O$, and $Cu^I_2Cu^{II}_2(Pen)_3\cdot 4H_2O$, respectively. Wright and Frieden noted the presence of 1.4% of chloride in their solid product, determined that the complex has a molecular weight of 2600 in solution, and assigned to it a formula $[Cu^ICu^{II}(Pen)_2]_n^{n-}$.[4] The persistent presence of chloride in purified purple products was also observed by Musker and Neagley.[5,6]

In the present work, chloride ion was initially present only fortuitously. It was subsequently found, in agreement with the observations of others,[4-6] that chloride is essential for the formation of the purple Cu-Pen complex. Qualitative exploratory experiments (see Preparations, below) led us to the conclusion that the purple complex is anionic. Solutions of the complex were accordingly prepared with a large variety of positive counterions. Crystals were obtained when the counterions were $Co(NH_3)_6^{3+}$, $Ru(NH_3)_6^{3+}$, or Tl^+. Crystallographic considerations made the Tl^+ salt preferable for structure determination.

Experimental Section

Materials. D-Penicillamine (Fluka, puriss.) was used without further purification. All other chemicals and solvents were of reagent grade quality. Sodium acetate buffer was prepared from 0.5 M NaAc and 0.025 M HAc.

Preparations. The intensely colored purple solution containing the Cu^I, Cu^{II} complex of D-penicillamine was prepared as follows. D-Penicillamine (100 mg, 0.67 mmol) was dissolved in 15 mL of a sodium acetate buffer solution (pH 6.2). This solution was added to a solution of $CuCl_2\cdot 2H_2O$ (85 mg, 0.5 mmol) in 2 mL of water. An approximately equal volume of ethanol was added to the resulting purple solution until all the purple complex had precipitated. The precipitate was filtered off, washed with alcohol, and then redissolved in 5 mL of water. We shall refer to the solution prepared in this way as "the purple solution". The absorbance of the purple solution at λ 518 nm corresponded to 1820 mol^{-1} dm^3 cm^{-1} per Cu atom.

Addition of cations such as Cd^{2+}, Zn^{2+}, Pb^{2+}, Hg^{2+}, Al^{3+}, Nd^{3+}, In^{3+}, Th^{4+}, and UO^{2+}_2 to the purple solution resulted in the immediate formation of purple precipitates. In one experiment the solution of D-penicillamine and $CuCl_2\cdot 2H_2O$ was prepared in distilled water instead of NaAc buffer. Neutralization with tetra-*n*-butylammonium hydroxide solution (40%) yielded a purple solution from which a purple, hygroscopic, Me_2SO-soluble solid could be precipitated by adding ethanol and then ether. The formation of solid products from the purple solution with such a variety of positive ions led to the conclusion that the purple solution contains an anionic complex. This conclusion is consistent with the electrophoretic behavior of the complex.[4]

Crystals of $[Co(NH_3)_6]_5[Cu^{II}_6Cu^I_8(D\text{-}Pen)_{12}Cl]_3\cdot xH_2O$. A Pasteur pipet was used to introduce a layer of water (1 mL) gently over the purple solution (2 mL, prepared as above) in a small test tube. Several drops of a dilute aqueous solution of $Co(NH_3)_6Cl_3$ were added to the upper layer. After a few hours the upper layer had diffused into the purple solution and small, dark purple, parallelepiped-shaped crystals had grown on the wall of the test tube. Analytical data for this complex are given in Table I.

Crystals of $[Ru(NH_3)_6]_5[Cu^{II}_6Cu^I_8(D\text{-}Pen)_{12}Cl]_3\cdot yH_2O$. The pro-

Table I. Analytical Data for Copper-Penicillamine Complexes with Different Counterions

Counterion	% Cu	% M	M(exp):Cu[a]	M(calcd):Cu[b]
Tl^+	20.3	22.7	5:14.3	5:14
Cd^{2+}	24.6	7.5	5:29.3	5:28
$Co(NH_3)_6^{3+}$	23.8	2.7	5:40.6	5:42

[a] M(exp):Cu = experimental counterion:Cu ratio. [b] M(calcd):Cu = counterion:Cu ratio calculated on the assumption that the charge on the Cu_{14}(D-Pen)$_{12}Cl$ cluster is −5.

cedure was analogous to that followed for the $[Co(NH_3)_6]^{3+}$ compound.

Crystals of $Tl_5[Cu^{II}_6Cu^{I}_8$(D-Pen)$_{12}Cl]\cdot nH_2O$. A dilute aqueous solution of $TlNO_3$ (1 mL) was added to the purple solution (2 mL) prepared as described above. A layer of ethanol was placed above the aqueous solution. Small purple crystals were deposited on the wall of the test tube within 24 h. Analytical data for this complex are given in Table I.

Precipitation of $Cd_5[Cu^{II}_6Cu^{I}_8$(D-Pen)$_{12}Cl]_2\cdot zH_2O$. When a dilute aqueous solution of $CdSO_4$ was added to the purple solution prepared as described above, a highly insoluble purple Cd^{2+} salt of the complex was precipitated as a powder. It was filtered off and washed with water. Analytical data are given in Table I.

Precipitation of $Na_5[Cu^{II}_6Cu^{I}_8$(D-Pen)$_{12}Cl]\cdot mH_2O$. Addition of ethanol to the purple solution caused the precipitation of a sodium salt, $Na_5[Cu^{II}_6Cu^{I}_8$(D-Pen)$_{12}Cl]\cdot mH_2O$. The composition was inferred from the compositions of the salts which had already been prepared with other counterions (see above).

Stability of $[Cu^{II}_6Cu^{I}_8$(D-Pen)$_{12}Cl]^{5-}$ in Solution. The stability of the sodium salt of the complex dissolved in NaAc buffer, in 0.15 M NaCl solution, and in urine solution was monitored by difference spectrophotometry at 518 nm. The initial concentration of the complex was in each case 3.5×10^{-5} M (based on the formula given above). Solutions were kept in stoppered flasks at ambient temperatures (15–25 °C) under normal conditions of laboratory lighting. The solution in sodium acetate buffer at pH 6.2 showed no decrease in absorbance at 518 nm during a period of 1 year. The solution of the complex in 0.15 M NaCl decomposed with a half-life of approximately 16 days. The solution in urine at pH 6 showed a rapid decrease in absorbance at 518 nm, corresponding to complete decomposition of the complex within 50 h.

Preparation of Cu^{I}(D-HPen)$\cdot H_2O$. A yellow complex characterized as Cu^{I}(D-HPen)$\cdot H_2O$ by Sugiura and Tanaka[3] was prepared from $CuCl_2\cdot 2H_2O$ and H_2Pen as described by these authors. The *absence* of chloride ion from this complex was confirmed by elemental analysis. An identical product was obtained when $CuSO_4\cdot 5H_2O$ was substituted for $CuCl_2\cdot 2H_2O$.

Effect of Halide Ions on Complex Formation. Solid Cu^{I}(D-HPen)$\cdot H_2O$ (see above) was suspended in a solution of $CuCl_2\cdot 2H_2O$ (10^{-3} M) in NaAc buffer (pH 6.2). The solid dissolved slowly and the characteristic color of the purple complex appeared in the solution. No change of color occurred if the solid was stirred in a solution of $CuSO_4\cdot 5H_2O$ in buffer. The addition of NaCl, however, caused the slow formation of the purple complex.

The original preparation of the purple solution was repeated, but $CuCl_2\cdot 2H_2O$ was replaced by (1) $CuBr_2$ and (2) $CuSO_4\cdot 5H_2O$ in the presence of KI (0.05 mol). The maximum absorbances of the resulting solutions were at 523 and 511 nm, respectively.

Reaction of D-Penicillamine with $[Cu$(Gly-H$_{-1}$Gly-L-H$_{-1}$His)$]^-$. Diglycyl-L-histidine (40 mg) and $CuCl_2\cdot 2H_2O$ (21 mg) were dissolved in sodium acetate buffer (pH 6.2, 15 mL). The resulting solution was red-violet (λ_{max} 525 nm), as expected.[8] D-Penicillamine (25 mg) in water (2 mL) was added. The color immediately became much more intense (λ_{max} 518 nm). The absorbance at 518 nm indicated that at least 50% of the total amount of copper was present as the purple penicillamine complex.

Physical Measurements. UV–visible spectra were recorded on a Beckman Acta V spectrophotometer. Metal analyses were made with a Varian Techtron AA6 atomic absorption spectrophotometer. *X*-Band ESR measurements were carried out at 120K using a Varian E12 ESR spectrometer.

Selection of Derivative for Structure Analysis. Crystal Data. Crystal data for the three crystalline derivatives are shown in Table II. The

Table II. Crystal Data for Derivatives of Cu-Pen Complex

Counterion	$Co(NH_3)_6^{3+}$	$Ru(NH_3)_6^{3+}$	Tl^+
Diffraction data	Films (Weissenberg and precession)		Counter
Crystal system	Monoclinic		Cubic
Space group	$P2_1$		$F432$
a	39.5 Å		50.847 (5) Å
b	22.8 Å		
c	35.4 Å		
β	117°		
Unit cell volume (V)	28 406 Å^3		131 461 Å
Multiplicity of general atomic positions (n)	2		96
Volume occupied by asymmetric unit (V/n)	14 203 Å^3		1 369 Å

$Co(NH_3)_6^{3+}$ and $Ru(NH_3)_6^{3+}$ derivatives are isomorphous. The space group of the Tl^+ derivative is uniquely determined as $F432$ from the systematic absences (reflections absent for odd values of $h + k$, $k + l$, and $l + h$), from the fact that the complex must be noncentrosymmetric because it contains a chiral ligand, and from the Laue symmetry ($m3m$; space group $F23$ has Laue symmetry $m3$).

The Tl^+ derivative was selected for structure determination in the expectation that the disadvantages inherent in its very high symmetry would be compensated by the smaller size of the asymmetric unit in comparison with those of the two hexaammine derivatives (Table II). The density of the crystals is 1.89 (±2) g cm^{-3} (determined by flotation in $CHCl_3/CHBr_3$ immediately after drying the crystals on filter paper). If the formula unit has threefold symmetry, as was shown subsequently to be the case, the formula weight of the compound is 4714 (±50). This corresponds to a composition $Tl_5[Cu_{14}$(D-Pen)$_{12}Cl]\cdot nH_2O$ with $n = 55$ (±3). There are 32 formula units in the unit cell.

Diffraction Measurements. The crystals were sealed in capillary tubes containing some of the mother liquor. Crystals which were not protected in this way lost their diffracting power within minutes.

Preliminary crystal data were obtained with a Nonius-Weissenberg camera and a Supper precession camera (100 mm crystal–film distance). Counter data for the Tl^+ derivative were recorded on a Nonius CAD-4/F diffractometer. Accurate cell dimensions for this compound were obtained by least-squares refinement of 2θ values for 16 automatically centered strong reflections ($\theta > 10°$). All data were collected using Ni-filtered Cu Kα radiation [λ(Cu Kα_1) = 1.5405, λ(Cu Kα_2) = 1.5443 Å]. The take-off angle at the tube was 2.8°. The vertical detector aperture was 4 mm. The horizontal aperture was (1.50 + 0.14 tan θ) mm. Standard Nonius accessories were used for low-temperature measurements. The intensity data for the Tl^+ derivative were recorded with a crystal of dimensions 0.011 × 0.014 × 0.014 cm parallel to a, b, and c, respectively. The crystal had the shape of a square prism with well developed (1 0 0), ($\bar{1}$ 0 0), (0 1 0), (0 $\bar{1}$ 0), (0 0 1), and (0 0 $\bar{1}$) faces and a large (2 2 1) face exposed on one corner. The diffractometer ϕ axis was approximately parallel to $-a$.

The intensities of 1469 independent reflections were measured up to a limit of $\sin\theta/\lambda = 0.372$ Å^{-1} ($\theta = 35°$). No reflections could be found at higher θ values. A profile analysis of a suitable reflection was made in a series of θ scans. This led to the selection of the $\omega - (2/3)2\theta$ scan mode of the instrument for data collection. The ω-scan angle was (1.50 + 0.14 tan θ)°. The scan speeds were determined by a required precision of $\sigma(I) < 0.03I$ with a maximum scan time of 100 s per reflection. Each reflection was scanned in 96 steps. The peak (P) was assumed to consist of the 64 central steps, leaving 16 steps at each end of the scan to measure the background (B_1 and B_2). The intensity (I) was calculated as $I = s[P - 2(B_1 + B_2)]$, where s is a factor to account for differences in scan speed. The standard deviation in I was defined by the equation $\sigma(I) = [s(P + 4(B_1 + B_2) + c^2I^2)]^{1/2}$, where c is an optional constant which equals zero for weights derived from counting statistics alone. Three intensity control reflections were monitored after every 10 000 s of x-ray exposure time. No decomposition was detected. The orientation of the crystal was automatically checked after every 100 reflections. The data were corrected for absorption by the crystal ($\mu = 134$ cm^{-1}, based on the calculated com-

Table III. Fractional Positional Parameters and Thermal Parameters ($Å^{-2}$)[a,b] for Atoms in $Tl_5[Cu^{II}_6Cu^{I}_8(D\text{-Pen})_{12}Cl]\cdot nH_2O$

Atom	10^4x	10^4y	10^4z	10^3U_{iso}	Atom	10^4x	10^4y	10^4z	10^3U_{iso}	Occupancy
Cu (1)	1082 (8)	3918 (8)	1082 (8)	52 (8)	Tl (1)	0	3157 (22)	0	44	0.16 (3)
Cu (2)	1725 (9)	3275 (9)	1725 (9)	63 (8)	Tl (2)	0	3318 (9)	0	44	0.37 (3)
Cu (3)	1221 (3)	3048 (3)	1362 (3)	67 (6)	Tl (3)	0	4020 (6)	0	44	0.261 (2)
Cu (4)	880 (3)	3548 (3)	1559 (3)	55 (6)	Tl (4)	1046 (9)	2156 (9)	149 (8)	44	0.094 (7)
Cu (5)	1094 (3)	3067 (3)	2098 (3)	81 (6)	Cl	1413 (12)	3587 (12)	1413 (12)	23 (11)	
Cu (6)	886 (3)	4304 (3)	1710 (3)	77 (6)						

Atom	10^3x	10^3y	10^3z	10^2U_{iso}	Atom	10^3x	10^3y	10^3z	10^2U_{iso}
S (A)	79.4 (5)	397.2 (5)	142.2 (5)	4 (1)	S (B)	139.7 (6)	299.2 (6)	178.0 (6)	6 (1)
C (1A)	43 (2)	436 (2)	133 (2)	3 (3)	C (1B)	124 (2)	253 (2)	200 (2)	1 (3)
C (2A)	7 (2)	447 (2)	135 (2)	3	C (2B)	131 (2)	221 (2)	201 (2)	2 (3)
C (3A)	45 (3)	397 (3)	130 (3)	12 (5)	C (3B)	150 (2)	265 (2)	189 (2)	3
C (4A)	24 (2)	390 (2)	151 (2)	7 (4)	C (4B)	160 (2)	254 (2)	163 (2)	3
C (5A)	40 (2)	395 (3)	105 (3)	7 (4)	C (5B)	165 (2)	267 (2)	214 (2)	3
N (A)	52 (2)	447 (2)	155 (2)	5 (3)	N (B)	110 (1)	268 (1)	220 (1)	1 (2)
O (1A)	4 (3)	441 (2)	114 (2)	18 (5)	O (1B)	131 (2)	204 (2)	186 (2)	12 (3)
O (2A)	0 (2)	444 (1)	159 (2)	12 (3)	O (2B)	125 (1)	220 (2)	226 (2)	10 (3)
S (C)	100.7 (5)	348.8 (5)	197.8 (5)	3 (1)	S (D)	79.9 (5)	320.8 (5)	130.1 (5)	4 (1)
C (1C)	78 (3)	348 (3)	255 (3)	11 (5)	C (1D)	51 (2)	275 (2)	109 (2)	3 (3)
C (2C)	58 (3)	336 (3)	273 (4)	13 (6)	C (2D)	21 (2)	258 (2)	120 (2)	3 (3)
C (3C)	74 (2)	360 (2)	222 (2)	3	C (3D)	58 (3)	288 (3)	137 (3)	11 (5)
C (4C)	75 (2)	391 (2)	224 (2)	2 (3)	C (4D)	64 (1)	277 (1)	158 (2)	1 (2)
C (5C)	49 (2)	344 (2)	219 (2)	3	C (5D)	27 (2)	313 (2)	144 (2)	6 (3)
N (C)	91 (1)	315 (1)	244 (1)	5 (3)	N (D)	46 (1)	295 (1)	95 (1)	2 (2)
O (1C)	62 (1)	366 (1)	280 (1)	7 (3)	O (1D)	27 (3)	238 (3)	130 (3)	23 (6)
O (2C)	52 (3)	321 (3)	280 (3)	18 (7)	O (2D)	6 (2)	273 (1)	106 (1)	8 (3)

[a] For explanation of labels see text and Figure 3. Estimated standard deviations are given in parentheses. [b] The temperature factors are $\exp[-8\pi^2U(\sin\theta/\lambda)^2]$. U values for which no standard deviations are shown were kept fixed in the final stages of refinement.

position and the observed density (see above), and standard values of the mass absorption coefficients[9]). Lorentz and polarization corrections were applied in the usual way.

Solution of the Structure. The structure was solved by a combination of Patterson, Fourier, and direct methods and full-matrix least-squares refinements.[10] Scattering factor curves for Tl^+, Cu, S, O, N, C, and Cl^- were taken from the International Tables for X-Ray Crystallography.[11] The Tl^+, Cu, S, and Cl^- atoms were treated as anomalous scatterers. Only data in the range $0° < \theta < 30°$ were used for the Patterson and direct methods calculations. The complete data up to $\theta = 35°$ were included in Fourier and least-squares refinement calculations. A Patterson search[12] program was written to take advantage of the high symmetry of the space group $F432$. The asymmetric unit of the unit cell (in real space) was scanned at intervals of 0.5 Å in x, y, and z. For each grid point, the vectors to all symmetry-related positions were generated. The Patterson function was then sampled at the points corresponding to all these vectors. The original grid point in the real cell was accepted as a possible heavy-atom position if the Patterson function had maxima above a subjectively fixed threshold at all the relevant points or at points not more than 0.76 Å from them. The procedure led to the identification of two heavy atom positions, one on a threefold axis (A or B in Figure 1) and another on a fourfold axis (C or D in Figure 1). The alternatives in each case corresponded to different choices of origin.

The ambiguity was resolved by a single application of the direct methods program MULTAN.[10] Some solutions with very high combined figures of merit could be rejected since all the calculated phase angles were 360°. An E map was calculated for the solution which had the highest figure of merit and which included phase angles other than 360°. The two highest peaks in the E map were at 0, 0.33, 0 and 0.11, 0.39, 0.11 in agreement with combination BD from the solution of the Patterson function. Phases were calculated for a trial structure consisting of a Tl^+ ion at 0, 0.33, 0 and a Cu atom at 0.11, 0.39, 0.11. The rest of the structure was found in a series of Fourier and difference Fourier maps. Atoms were tentatively placed at the positions of all major Fourier peaks in each map. Least-squares refinement of temperature factors eliminated those peaks which did not correspond to atoms. This process led to the identification of the positions of all the atoms in the complex anion as well as four Tl^+ ions.

Refinement of the Structure. The model, consisting of the complex anion and four Tl^+ ions, was refined by full-matrix least squares with isotropic thermal parameters. The function minimized was $\Sigma w(|F_o| - k|F_c|)^2$, with $w = 1$. Two other weighting schemes were tried. The first was an empirical weighting scheme based on a polynomial fitted to a curve of $(\Delta F)^2$ vs. $|F_o|$ after each refinement cycle. The second was a statistical weighting scheme with $w = \sigma^{-2}(F)$, where $\sigma(F)$ was derived in the usual way from $\sigma(I)$, and $\sigma(I)$ was calculated from the expression given under "Diffraction Measurements", using a value of 0.03 for the optional constant c. Both weighting schemes led to very large ΔF values for reflections with low weights, failed to produce more acceptable values of the light-atom–light-atom bond lengths, and caused the disappearance of some light atoms for which there was good evidence from Fourier maps and chemical necessity. A damping factor of 0.3 was applied to the calculated parameter shifts for the atoms Cu(1), Cu(2), and Cl, which lie on a threefold axis. The refinement was terminated when all the parameter changes were smaller than the estimated standard deviations. Certain difficulties, which were no doubt associated with the disorder in the structure (see below), were experienced during the refinement. They were overcome by "brute force" methods which might not have been acceptable if we had been refining a smaller structure or using a data set with a greater ratio of observations to refinement variables. Firstly, the temperature factors of the Tl^+ ions refined to unrealistically high values ($B > 10$ $Å^{-2}$). These temperature factors were reset to a value of 3.5 $Å^{-2}$ at an early stage of the refinement, and were then kept fixed while the occupancies of the Tl^+ positions were refined. Secondly, the temperature factors of six carbon atoms tended to become slightly negative. These were reset to a value of 2.0 $Å^{-2}$ and were not varied in the final refinement cycles. Thirdly, the least-squares calculations were begun with the 1094 reflections with $I > \sigma(I)$. At the stage when the residual R, defined as $(\Sigma||F_o| - |F_c||)/(\Sigma|F_o|)$, was 0.24, an analysis of R vs. $\sin\theta/\lambda$ showed that the agreement between F_o and F_c was least satisfactory at low angles. The 34 reflections for which $\sin\theta/\lambda < 0.09$ $Å^{-1}$ were excluded from subsequent refinement cycles. The fate of these 34 reflections, and the evidence that they were affected by disorder, are presented in the following section. A total of 131 positional parameters, 37 temperature factors, and 4 occupancies were refined. The final residual R for the 1060 reflections used in the refinement is 0.17.[13] The atomic positional and vibrational parameters are shown in Table III.

Investigation of the Disorder in the Structure. The resolution of the diffraction data for all three of the D-penicillamine complexes crystallized by us was limited to about 1.36 Å ($\sin\theta/\lambda < 0.37$ $Å^{-1}$). The diffraction measurements for the Tl^+ derivative were repeated at 105K

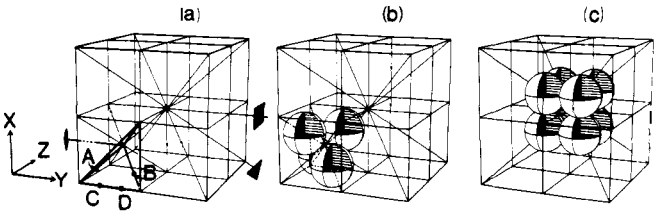


Figure 1. The unit cell showing (a) the symmetry axes and initial heavy-atom positions mentioned in the text, (b) the tetrahedral arrangement of four complexes (represented as spheres with 8 Å radii) in each octant of the cell, and (c) the cubic arrangement of eight complexes at the intersection of three fourfold axes.

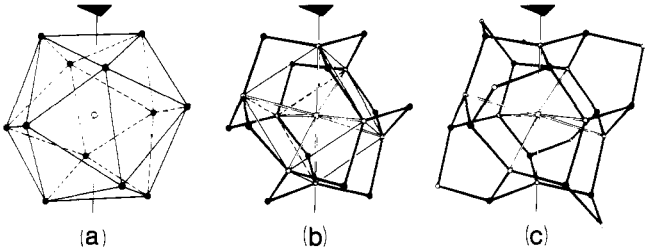


Figure 2. The arrangement of S, Cl, and Cu atoms in $[Cu^{II}_6Cu^{I}_8(D\text{-Pen})_{12}Cl]^{5-}$. (a) Icosahedron of 12 thiolate S atoms centered around a Cl^- ion. (b) Cube of eight Cu^I atoms oriented so that each Cu^I atom lies at the center of a face of the S_{12} icosahedron. (c) Six Cu^{II} atoms lying outside the S_{12} icosahedron and forming bridges between pairs of S atoms.

without any improvement in resolution, indicating that the effect was due to disorder rather than thermal motion.

Before we discuss the nature of the disorder, it is necessary to establish that the stoichiometry of the compound has been correctly determined. There is convincing evidence that the complex anion has a charge of −5 and that this charge is balanced exclusively by 5 Tl^+ ions. The analytical data for the Cd^{2+}, $Co(NH_3)_6^{3+}$, and Tl^+ derivatives (Table I) all indicate a counter-charge of +5 per complex anion in the solid compounds. Identical crystals of the Tl^+ derivative are obtained whether the neutralization step in the preparation is carried out with tetra(*n*-butyl)ammonium hydroxide or with sodium acetate buffer. It follows that no positive ions other than Tl^+ are required for the crystallization of this derivative, and that there are 5 Tl^+ per $[Cu_{14}(D\text{-Pen})_{12}Cl]^{5-}$ complex. The structure analysis, however, accounts for only 0.875 Tl^+ instead of 5 Tl^+ per formula unit; and none of the H_2O molecules could be found in the electron density maps. The disorder in the crystals evidently affects that part of the structure which contains the Tl^+ ions and H_2O molecules.

The ordered part of the unit cell consists of 32 complex anions. They can be described roughly as spheres with 8 Å radii, packed in the unit cell as shown in Figure 1(b) and (c). The disordered part can then be described as the volume outside the 32 spheres. The average electron density in this volume is 0.49 e $Å^{-3}$. Fourier transformation of the disordered part of the unit cell should give the contribution of the disordered atoms to the structure amplitudes. To carry out this transformation, the contents of the unit cell were described by a grid of 28 × 28 × 28 points. Every grid point outside the 32 spheres representing the complex anions was given a value 0.49 e $Å^{-3}$. All other grid points were given a value of zero. The Fourier transformation then produced contributions to the structure amplitudes on an absolute scale. The three-dimensional array of points was transformed using a fast Fourier transform program. Structure factors were calculated down to a resolution of 3.7 Å. At this resolution the calculated contribution of the disordered atoms to the structure factors became negligible.

The structure factor contributions from the disordered part of the unit cell were large for only a limited number of low-angle reflections. These reflections all had particularly large ΔF values when their structure factors were based on the ordered atoms of the refined model alone (e.g., (0,0,2), (0,0,4), (0,0,6), (0,4,4), (1,1,1) and (1,1,3)). The inclusion of the contributions of the disordered atoms produced a dramatic improvement in the agreement between the observed and calculated structure factors. At the end of the refinement of the ordered part of the structure, the 34 reflections which had not been used in the final refinement cycles (see "Refinement", above) had a residual $R = 0.52$. When the structure factors were corrected for the contributions of the disordered atoms, the R dropped to 0.31.[13]

Description of the Structure. The $[Cu_{14}(D\text{-Pen})_{12}Cl]^{5-}$ Clusters. The compound crystallizes in a cubic space group which is unique among amino acid complexes. The unit cell contains 32 symmetry-related complex anions. Each of these complex anions is a $[Cu_{14}(D\text{-Pen})_{12}Cl]^{5-}$ cluster. The structure of such a cluster is best explained in three stages which are shown in Figure 2.

We first observe that the 12 sulfur atoms of the penicillaminate ligands are arranged in an approximately regular icosahedron with the single chloride ion at the center (Figure 2(a)). The sulfur icosahedron has 20 triangular faces. A crystallographic threefold rotation axis passes through two of the faces. The approximate centers of eight of the icosahedron faces, including the two through which the threefold axis passes, are occupied by Cu atoms. No two Cu atoms are in adjacent sulfur triangles (Figure 2(b)). In addition to the trigonal-planar coordination by three sulfur atoms, each of the Cu atoms has the central Cl^- ion as a weakly bound fourth ligand at a mean distance of 2.8 Å. The coordination geometry of these eight Cu atoms identifies them as Cu^I. The remaining six Cu atoms of the cluster are bonded to pairs of sulfur atoms which define six of the icosahedron edges (Figure 2(c)). Each of these Cu atoms is coordinated not only by two sulfur atoms but also by the amino nitrogen atoms of the two penicillaminate ligands to which the sulfur atoms belong (Figure 3). The square-planar coordination (cis bisbidentate chelation) suffices to identify the six additional Cu atoms as Cu^{II}.

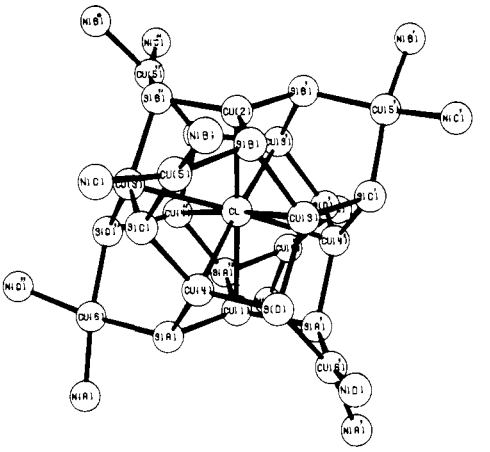


Figure 3. The $Cu^{II}_6Cu^{I}_8S_{12}N_{12}Cl$ core of the complex ion. The atomic labelling is explained in the text and in Table IV.

The method which we have used to describe the Cu–S cluster has been applied to related compounds.[14] The symmetry properties of the icosahedron, and the positions which the Cu^I and Cu^{II} atoms occupy in relation to the sulfur icosahedron, lead to some interesting consequences. The eight Cu^I atoms lie at the corners of a cube (see Figure 2(b)). The average Cu^I–Cu^I distance along a cube edge is 3.30 (2) Å [range 3.24–3.37 (2) Å]. The average Cu^I–Cu^I–Cu^I angle is 90 (2)° [range 86.1 (5) to 93.8 (7)°]. The six Cu^{II} atoms are at the corners of an octahedron with an average Cu^{II}–Cu^{II} separation of 6.71 Å (range 6.88–6.76 Å). The icosahedron, and hence the cube and octahedron, deviate slightly from ideal regular geometry. The reason is that those icosahedron edges which are bridged by Cu^{II} atoms are significantly shorter than the rest [S–S(Cu-bridged) = 3.36–3.40 (4) Å, S–S (other) = 3.84–4.05 (4) Å].

A complete anionic cluster complex is shown in Figure 4. In chemical terms, each pencillaminate ligand chelates one Cu^{II} atom through the amino and thiol groups, and forms bonds from the thiol group to two Cu^I atoms. The configurations of the C–S and S–Cu bonds at the ligand sulfur atoms are approximately tetrahedral. The carboxylate groups of the ligands project from the surface of the cluster and are not involved in the coordination of the Cu atoms. The orientations of the penicillaminate side-chain methyl groups are such that each Cu^I atom is covered by three methyl groups from three different ligands (Figure 5). As a result, the Cu^I atoms are buried

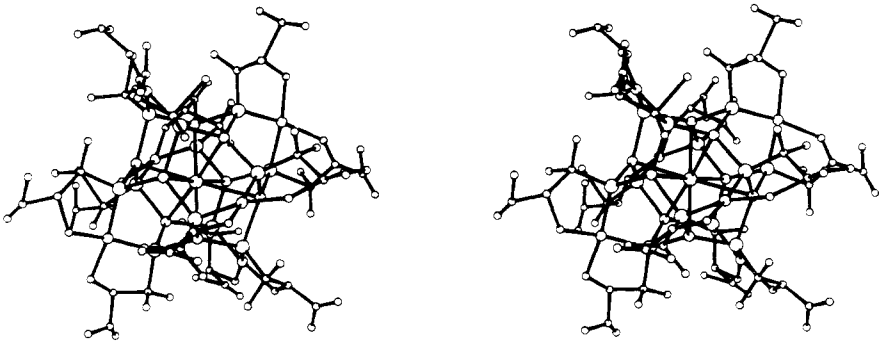


Figure 4. ORTEP stereodrawing of the $[Cu^{II}_6Cu^{I}_8(D\text{-Pen})_{12}Cl]^{5-}$ cluster.

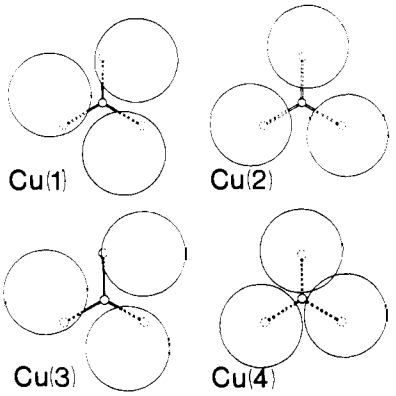
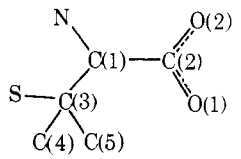


Figure 5. Projections along the four crystallographically independent Cu^{I}-Cl bonds, showing the shielding of each Cu^{I} atom by the $-CH_3$ groups of three D-Pen ligands. The $-CH_3$ groups are drawn as spheres with van der Waals radii of 2.1 Å. The three S atoms coordinated to each Cu^{I} are also included.

inside the cluster and are sterically protected against chemical attack.

Molecular Dimensions. The six crystallographically independent Cu atoms are labeled Cu(1), Cu(2), Cu^{I} atoms on threefold axis through cluster; Cu(3), Cu(4), Cu^{I} atoms in general positions; Cu(5), Cu(6), Cu^{II} atoms in general positions. The numbering of the atoms in each ligand molecule is as follows



where O(2) is the oxygen atom closest to N and C(5) is the methyl group closest to C(2). Each ligand is given a letter code. The ligands, and the Cu atoms to which their S atoms are bonded, are *A*, Cu(1), Cu(4), and Cu(5); *B*, Cu(2), Cu(3), and Cu(5); *C*, Cu(3), Cu(4), and Cu(5); and *D*, Cu(3), Cu(4), and Cu(6). Operations of the threefold symmetry axis are indicated by superscripts in the atomic labels.

The lengths of the metal–ligand bonds (i.e., the bonds shown in Figure 3) are listed in Table IV. The low precision is a consequence of the low resolution of the x-ray data. A list of the relevant bond angles is available as supplementary material.[13] The individual bond lengths in the penicillamine ligands are not listed. The values lie in the ranges 1.95 ± 0.05 Å for S-C, 1.65 ± 0.35 Å for C-N and C-C, and 1.25 ± 0.35 Å for C-O (with esd's 0.1, 0.2, and 0.2 Å, respectively).

Packing of the Clusters in the Unit Cell. The unit cell can be divided into symmetry-related octants. Each octant contains four of the anionic clusters in a tetrahedral arrangement (Figure 1(b)). The center of the tetrahedron is at the intersection of four threefold and three twofold axes (Figure 1(a)). Each cluster is also a member of a cubic group of eight clusters which are related by the operation of three intersecting fourfold axes (Figure 1(c)).

Table IV. Copper–Ligand Bond Lengths[a] (Å) in $Tl_5[Cu^{II}_6Cu^{I}_8(D\text{-Pen})_{12}]\cdot nH_2O$

Bond	Length	Bond	Length
Cu^{I} Atoms		Cu^{II} Atoms	
Cu(1)-Cl	2.90 (4)	Cu(5)-S(B)	2.27 (3)
Cu(1)-S(A)	2.28 (4)	Cu(5)-S(C)	2.27 (3)
Cu(2)-Cl	2.75 (5)	Cu(5)-N(B)	2.04 (6)
Cu(2)-S(B)	2.22 (4)	Cu(5)-N(C)	2.01 (7)
Cu(3)-Cl	2.92 (3)	Cu(6)-S(A)	2.26 (3)
Cu(3)-S(B)	2.32 (3)	Cu(6)-S(D″)	2.31 (3)
Cu(3)-S(C′)	2.34 (3)	Cu(6)-N(A)	2.12 (7)
Cu(3)-S(D)	2.32 (3)	Cu(6)-N(D″)	2.15 (7)
Cu(4)-Cl	2.82 (6)		
Cu(4)-S(A)	2.31 (3)		
Cu(4)-S(C)	2.24 (3)		
Cu(4)-S(D)	2.21 (3)		

[a] Estimated standard deviations in parentheses. Superscripts in this table and in Table V are related to those shown in Table III by the following symmetry operations: ′ = $\frac{1}{2} - y, \frac{1}{2} - z, x$; ″ = $z, \frac{1}{2} - x, \frac{1}{2} - y$; ‴ = z, y, z.

Table V. Distances (Å) from Partially Occupied Tl^+ Positions to Oxygen Atoms[a]

Atoms	Distance
Tl(1)-O(2A′)	3.15 (9)
Tl(2)-O(2A′)	2.91 (7)
Tl(3)-O(1A′)	3.1 (1)
Tl(4)-O(1C′)	2.81 (8)
Tl(4)-O(1D‴)[b]	1.8 (1)
Tl(4)-O(2D‴)	2.94 (8)

[a] Estimated standard deviations in parentheses. The significance of superscripts is indicated in the footnote of Table IV. [b] See comment in text concerning this distance.

The crystal structure is stabilized by hydrogen bonds between carboxyl groups and amino groups of penicillamine ligands in adjacent clusters. The eight clusters in the cubic group centered on the intersection of three fourfold axes at $\frac{1}{2}, \frac{1}{2}, \frac{1}{2}$ (Figure 1(c)) are linked by 24 hydrogen bonds. Four of these are shown in Figure 6(a). In addition there are two symmetry-related hydrogen bonds between pairs of clusters in each octant (Figure 6(b)).

The disordered Tl^+ ions and water molecules which fill the spaces between the anionic complex clusters presumably also contribute to the stability of the structure. The Tl^+ sites for which partial occupancies have been established are surrounded by carboxylate groups. The environment of Tl(2) is illustrated in Figure 6(a), and a list of $Tl^+ \cdots O$ distances is given in Table V. One of these distances, Tl(4)···O(1D‴), is clearly too short (1.8 Å). However, the Tl(4) site has an occupancy of only 0.094, and O(1D) has a very large temperature factor (B = 18 (5) $Å^{-2}$). It is likely that the carboxylate oxygen is displaced whenever the Tl(4) site is occupied.

Discussion

Comparison with Related Structures. The $[Cu^{II}_6Cu^{I}_8(D\text{-Pen})_{12}Cl]^{5-}$ cluster belongs to a growing family of structures.

The same type of $[Cu^I_8S_{12}]$ core, consisting of a cube of copper(I) atoms inside an icosahedron of sulfur atoms (but without a central halide ion), has been found in three copper(I) complexes with 1,1- and 1,2-dithiolate ligands.[15,16] A $[Cu^{II}_6Cu^I_8(SR)_{12}Cl]^{7+}$ cluster, identical with that in the present structure except for the omission of the 12 carboxylate groups, is formed when D-penicillamine is replaced by β,β-dimethylcysteamine (HSR = $HSC(CH_3)_2CH_2NH_2$).[17] A recent structure analysis of a cadmium–thioglycolate complex has revealed the existence of an analogous $[Cd_8(SR)_{12}I]^{3+}$ cluster (HSR = $HSCH_2CH_2OH$).[18]

Apart from their general shape, the $[Cu^I_8S_{12}]$ clusters in the D-penicillamine, β,β-dimethylcysteamine, and dithiolate complexes have a structural feature in common: six of the S_{12}-icosahedron edges in each cluster have additional S–X–S bridges between the sulfur atoms. In the D-penicillamine and β,β-dimethylcysteamine complexes the S–X–S bridges are provided by the S_2N_2-chelated Cu^{II} atoms. In the dithiolate complexes X represents one or two carbon atoms of the dithiolate ligands. It has been suggested that S–X–S bridges are essential for the stabilization of $[Cu^I_8S_{12}]$ clusters.[14] The lengths of the bridged icosahedron edges depend on the bridging groups (mean S···S = 3.39 (2) Å in the present complex, 3.04 (1)–3.92 (1) Å in the three dithiolate complexes[16]), whereas the lengths of the unbridged edges lie in a relatively small range (mean S···S = 3.94 (1) Å in the present complex, 3.81 (1)–3.88 (1) Å in the dithiolate complexes[16]). The Cu^I–S bonds in all the $[Cu^I_8S_{12}]$ clusters under discussion have approximately equal lengths [mean Cu^I–S = 2.25 (6) Å (present complex), 2.28 Å (β,β-dimethylcysteamine complex[17]), 2.247 (3), 2.25 (4), 2.26 (1) Å (dithiolate complexes[16])]. However, the Cu^I_8 cubes are considerably larger in the D-penicillamine and β,β-dimethylcysteamine complexes (mean $Cu^I\cdots Cu^I$ = 3.30 (2) and 3.3 Å, respectively) than in the dithiolate complexes (mean $Cu^I\cdots Cu^I$ = 2.84 (2), 2.83 (1), and 2.79 (1) Å[16]). The $Cu^I\cdots Cu^I$ distances in the D-penicillamine and β,β-dimethylcysteamine complexes are so large that metal–metal bonding interactions, such as those which have been postulated for the dithiolate complexes,[19] are clearly absent. We suggest that the expansion of the Cu^I_8 cubes is caused by charge transfer to the Cu^{II} atoms coordinated at the thiolate groups, and that this enables the clusters to gain additional stability by the inclusion of an atom such as Cl^-.

Magnetic Properties. Magnetic susceptibility measurements on purple Cu-Pen complexes by a number of antecedent authors have consistently yielded values lower than those expected for normal Cu^{II} complexes. Wilson and Martin[20] reported a ratio Cu^{II}/Cu_{total} = 0.44 for solutions prepared from $CuCl_2$ and H_2Pen. Wright and Frieden[4] stated that the magnetic susceptibility of the Na salt of a Cu-Pen complex was equivalent to 0.48 spin per Cu, which we interpret to mean that 0.48 of the Cu atoms behaved as Cu^{II}. Neagley and Musker[5] found μ_{eff} = 1.22 μ_B for a solid complex; this value corresponds to a ratio Cu^{II}/Cu_{total} = 0.5–0.3, if reasonable assumptions are made about the method of calculation and μ_{eff} for Cu^{II} is taken as 1.75–2.20 μ_B. In each of these experiments the results were consistent with the formation of $[Cu^{II}_6Cu^I_8Pen_{12}Cl]^{5-}$, since the 6 Cu^{II} atoms in this complex represent $^6/_{14}$ = 0.43 of the total Cu.

Compositions of Previously Reported Cu-Pen Complexes. The purple solid complexes isolated by several groups of workers from the reaction of Cu(II) with D-H_2Pen appear to have been salts of $[Cu^{II}_6Cu^I_8Pen_{12}Cl]^{5-}$ with various proportions of Na^+ and Cu^{2+} as counterions. (In the light of our own difficulties in producing pure solid derivatives and maintaining them in constant states of hydration it is no criticism of others to suggest that the reported analytical results have to be interpreted rather flexibly.) For example, we now know that the complex described by Sugiura and Tanaka[3] as

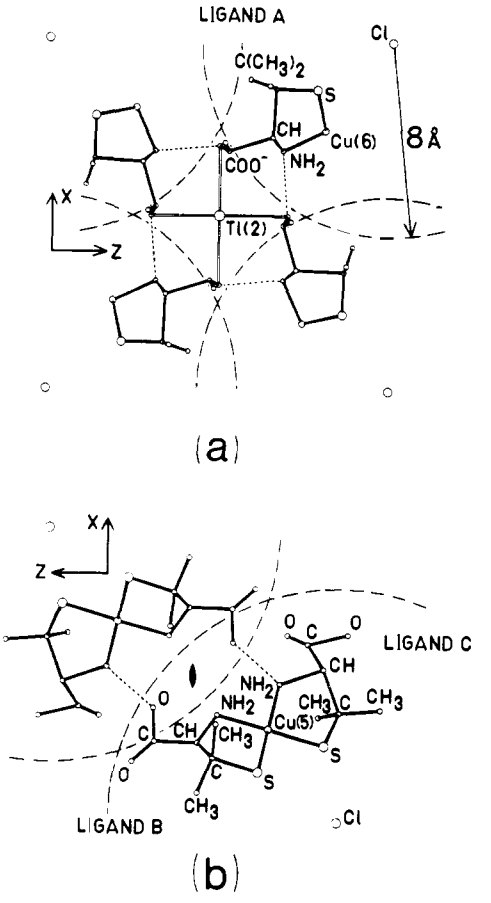


Figure 6. Hydrogen bonding between adjacent cluster complexes. (a) Projection parallel to y axis, showing $NH_2\cdots{}^-OOC$ hydrogen bonding and $Tl\cdots{}^-OOC$ interactions between complexes related by a fourfold axis. The boundaries of the 8 Å spheres in Figure 1 (c) are indicated by dashed lines. (b) Projection parallel to a twofold axis, showing $NH_2\cdots{}^-OOC$ hydrogen bonding between two complexes of the tetrahedral grouping shown in Figure 1(b).

$Cu^I_2Cu^{II}Pen_2\cdot 2H_2O$ *must* have contained Cl. A reasonable reinterpretation of the published analytical results for this compound leads to $Cu_{5/2}[Cu_{14}Pen_{12}Cl]\cdot 10H_2O$ or, better still, $Cu_{5/2}[Cu_{14}Pen_{12}Cl]\cdot CuCl_2\cdot 6H_2O$.[21] The compound described by Wright and Frieden[4] as $Na[Cu^ICu^{II}Pen_2]\cdot 6H_2O$ was said to contain 1.4% of Cl as "an impurity (as NaCl) or a polymeric end group". Their experimental value for the molecular weight of the purple complex in solution, 2600, is in excellent agreement with the calculated value 2691 for $[Cu_{14}Pen_{12}Cl]^{5-}$. We suggest that the solid product was either $Na_5[Cu_{14}Pen_{12}Cl]\cdot 25H_2O$ contaminated by some NaCl, or $Na_7[Cu_{13}Pen_{12}Cl]\cdot 36H_2O$.[22] (A low Cu content may have been caused by loss of Cu^{II} during purification on Sephadex G-25, as observed by others.[23]) The analytical results of Musker and Neagley[5] agree better with a formula $Na_3Cu[Cu_{14}Pen_{12}Cl]\cdot 10H_2O$ than with the formula $Cu^I_2Cu^{II}_2Pen_3\cdot 4H_2O$ suggested by Neagley.[6,24] Finally, it is clear that the purple solid complex which Rupp and Weser[23] recently isolated without proposing a formula for it was $Na_5[Cu_{14}Pen_{12}Cl]\cdot 14H_2O$.[25]

ESR Spectra of Cu-Pen and Related Complexes. The ESR spectrum of a frozen aqueous solution of the sodium salt of $[Cu^{II}_6Cu^I_8Pen_{12}Cl]^{5-}$, prepared as described in the Experimental Section, consists of a single broad band at $g \approx 2$ without Cu- or ligand-hyperfine splitting (Figure 7).[26–29] The spectrum is consistent with dipolar interactions between the paramagnetic centers on the six Cu^{II} atoms in the cluster. Each Cu^{II} atom is within 6.7 Å of four others. It has been shown that dipolar interactions between Cu^{II} atoms separated by 6.5 Å are

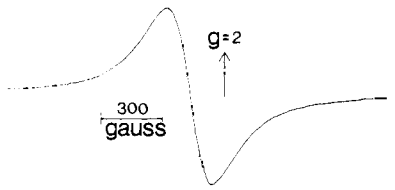


Figure 7. The ESR spectrum of $Na_5[Cu^{II}_6Cu^{I}_8(D\text{-}Pen)_{12}Cl]$ in frozen aqueous solution at 120 K.

capable of causing considerable broadening of ESR signals.[30]

A similar low-temperature ESR spectrum consisting of a broad band without hyperfine structure and accounting for 40% of the total Cu has been reported for the violet complex prepared from Cu^{II} and thiomalate (mercaptosuccinate, $HSCH(COO^-)CH_2COO^-$).[31,32] Boas and co-workers[32] have invoked Cu^I-Cu^{II} charge-transfer interactions to account for this spectrum. The violet Cu-thiomalate complex has an unknown structure, but its slow rate of formation[33] suggests that it is a polymer.[20] Its other properties and mode of formation[33] resemble those of $[Cu^{II}_6Cu^{I}_8Pen_{12}Cl]^{5-}$ so closely that the two complexes are likely to be Cu^I,Cu^{II}-thiolate clusters of the same type (but not necessarily with the same stoichiometry, as we shall mention later). In the case of the Cu-Pen complex the grossly different coordination geometries of the Cu^I and Cu^{II} atoms (trigonal Cu^IS_3 and square-planar $Cu^{II}N_2S_2$) must make charge-transfer interactions energetically unfavorable. Such interactions may therefore be rejected as an explanation of the broadening of the ESR signal of the Cu-Pen complex. A dipolar coupling mechanism, which should be equally applicable to the Cu-Pen and Cu-thiomalate complexes, is to be preferred.

On the other hand, we question the claim[34] that there is an analogy between the violet Cu-thiomalate complex (prepared from Cu^{II} and thiomalate, RSH) and a violet Cu-cysteamine complex (prepared from Cu^I and cysteamine disulfide, R′SSR′). Both complexes have single broad ESR signals without hyperfine splitting. In each case the ESR spectrum is consistent with Cu^{II}-Cu^{II} interactions, but this fact alone does not prove that the complexes have analogous structures. We shall show in the second-last section of this paper that the thiomalate and cysteamine complexes probably belong to fundamentally different structural types.

UV-Visible and X-Ray Photoelectron Spectra. The violet color of the $[Cu^{II}_6Cu^{I}_8Pen_{12}Cl]^{5-}$ ion is due to an intense absorption with λ_{max} 518 nm.[3-5,20] At this wavelength the molecular absorbance of a purple solution prepared as described (see Experimental Section) is 25 500 mol^{-1} dm^3 cm^{-1}, corresponding to ϵ_{max} 4250 per Cu^{II}. Values of ϵ_{max} 550 and 1400 have been reported previously;[20,5] we assume (in the absence of explicit details) that these values represent absorbances per Cu and should be multiplied by a factor of $^{14}/_{6}$ to convert them to absorbances per Cu^{II}. A much lower published[29] value of ϵ 50 can be discounted on the previously mentioned grounds[26] that the authors were clearly dealing with a different type of complex. Schugar et al.[17] found ϵ 3400 per Cu^{II} at 518 nm for the structurally analogous Cu^I,Cu^{II} cluster complex of β,β-dimethylcysteamine. The absorption was ascribed by them to $S\rightarrow Cu^{II}$ charge transfer (LMCT), and its possible relevance to the anomalously high absorbances of the "blue" Cu proteins[35] was pointed out.

The x-ray photoelectron spectrum (XPS) recorded by Rupp and Weser for the violet Cu-Pen complex "shows the presence of a large portion of Cu(I), although signals attributable to Cu(II)··· are still detectable".[23] The present work provides the reason for this observation. We have already identified Rupp and Weser's product as the Na salt of $[Cu_{14}Pen_{12}Cl]^{5-}$ on the basis of its composition.[25]

Scheme for the Formation of the Purple Cu-Pen Complex. The fact that we now know that the purple Cu-Pen complex is a $[Cu_{14}Pen_{12}Cl]^{5-}$ cluster enables us to rationalize many observations concerning its formation. The first step in the reaction of Cu^{II} with H_2Pen in aqueous solution involves the reduction of Cu^{II} to Cu^I and the oxidation of H_2Pen to penicillamine disulfide, $(HPen)_2$.[3]

$$2Cu^{II} + 2H_2Pen \rightarrow 2Cu^{I} + (HPen)_2 + 2H^+ \quad (1)$$

A polymeric 1:1 Cu^I-HPen complex is then formed.

$$Cu^I + H_2Pen \rightarrow \frac{1}{q}[Cu^I(HPen)]_q + H^+ \quad (2)$$

This step is supported by polarographic evidence[36] and by the isolation of a yellow complex with an elemental composition corresponding to $[Cu^I(HPen)\cdot H_2O]_q$.[3] We have confirmed that the formation of this complex does *not* require the presence of Cl (see Experimental Section). The IR spectrum of the yellow complex indicates that the metal atoms are coordinated by the thiolate groups of the ligands but not by the amino groups, and that the amino groups are still in their protonated-NH_3^+ form.[3] We write a polymeric formula for the Cu^I-HPen complex since it is improbable that a monomeric species would be stable in solution.[20,37]

In the presence of Cl^- and excess Cu^{II}, the violet mixed-valence complex is now formed. This requires a structural reorganization with the incorporation of Cl^- and additional H_2Pen to give a $[Cu^I_8(HPen)_{12}Cl]^{5-}$ core, and the addition of six Cu^{II} ions and the displacement of 12 protons from the $-NH_3^+$ groups of the ligands:

$$\frac{8}{q}[Cu^I(HPen)]_q + 4H_2Pen + Cl^- \rightarrow [Cu^I_8(HPen)_{12}Cl]^{5-} + 4H^+ \quad (3)$$

$$[Cu^I_8(HPen)_{12}Cl]^{5-} + 6Cu^{II} \rightarrow [Cu^{II}_6Cu^I_8Pen_{12}Cl]^{5-} + 12H^+ \quad (4)$$

The stoichiometry for steps 1-3 is

$$8Cu^{II} + 20H_2Pen + Cl^- \rightarrow [Cu^I_8(HPen)_{12}Cl]^{5-} + 4(HPen)_2 + 20H^+ \quad (5)$$

and for the entire sequence 1-4 is

$$14Cu^{II} + 20H_2Pen + Cl^- \rightarrow [Cu^{II}_6Cu^I_8Pen_{12}Cl]^{5-} + 4(HPen)_2 + 32H^+ \quad (6)$$

A similar reaction scheme, differing from the preceding only in stoichiometry, was proposed by Sugiura and Tanaka.[3] Their stoichiometry, however, was influenced by slightly incorrect inferences about the composition of the final product.[21] Our revised scheme is entirely compatible with the spectroscopic data recorded by the cited authors for solutions prepared from Cu^{II} and H_2Pen in various proportions.[3] For a constant initial Cu^{II} concentration, the absorbance at 520 nm increases from zero to a maximum which is reached when 1.35 mol of H_2Pen has been added per mole of Cu^{II}. The absorbance/concentration curve (Figure 2 of ref 3) shows that the ratio 1.35 is within experimental error of the value 20/14 = 1.43 predicted by our eq 6. Above this ratio the absorbance at 520 nm decreases, evidently because the Cu^{II} required to form the mixed-valence cluster is reduced to Cu^I by excess H_2Pen. On the other hand, for a constant initial concentration of H_2Pen the formation of the purple complex commences when approximately 0.4 mol of Cu^{II} has been added per mol of H_2Pen, and is complete when 0.75 mol of Cu^{II} has been added (Figure 3 of ref 3). The ratios predicted by our eq 5 and 6 are 8/20 = 0.4 and 14/20 = 0.7, respectively.

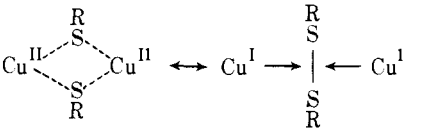
Another test of eq 6 is that the formation of the purple Cu-Pen complex from Cu^{II} and H_2Pen should be at a maximum when the mole fraction of Cu^{II} is 14/34 = 0.41. A Job's plot constructed (apparently with some experimental difficulties) by Musker and Neagley[5] leads to a value of about 0.44.

The solution at the end of the reaction is a mixture in which free Cu^{II} ions are in equilibrium with Cu^{II} ions chelated in the purple cluster complex, and with Cu^{II} ions chelated by penicillamine disulfide. The equilibrium is certain to favor the formation of the cluster complex (Cu^{II} chelated by N and S^-) at the expense of the penicillamine disulfide complex (Cu^{II} chelated by N and O^-[38]). As we shall show later, it is important from the point of view of the biological action of D-H_2Pen that the Cu^I atoms in the complex have effectively been removed from equilibrium with the surrounding medium.

Reactions of Other Thiols with Cu^{II}. The reaction of H_2Pen with Cu^{II} to form an intensely colored mixed-valence complex appears to be typical of a wide range of sulfhydryl compounds.[39] The violet to red-brown colors of the products correspond to intense absorptions between 520 and 480 nm. The first step in the reaction between the thiol RSH and Cu^{II} is generally observed to be the reduction of Cu^{II} (see eq 1). A white-yellow Cu^I-thiolate complex $[Cu^ISR]_q$ may frequently be isolated at this stage (see eq 2). Reaction with Cu^{II} then leads to the formation of a colored complex. It has been noted[40] that all thiols which behave in this way have structures which enable them to form five-membered chelate rings through a thiolate S^- atom and a second coordinating group such as $-NH_2$, $-COO^-$, or $-S^-$ In some cases (e.g., H_2Pen[4-7] and mercaptoisobutyrate[5]) the presence of Cl^-, Br^-, or one of a few other anions is essential for the formation of a violet-purple complex. In most other cases the presence of Cl^- is implicit in the published preparative procedures but there is no evidence to show whether the Cl^- is essential or not. In a few cases (e.g., mercaptosuccinate[32]) the development of a purple color has been observed even in the absence of Cl^- or Br^-.

The reported stabilities of the colored Cu–thiolate complexes cover a wide range: Buffered solutions containing the complexes of H_2Pen, β,β-dimethylcysteamine, and mercaptoisobutyric acid can be stored without detectable change for months.[3,6] The complexes of thiolactate, thioglycolate, cysteamine, and cysteine have lifetimes of only days, hours, minutes, and seconds, respectively, under ambient conditions.[6] The most stable of the violet to red-brown complexes are those which are formed in the presence of halide ions and which have ligands with two substituents on the carbon atom adjacent to the thiolate group. A unifying hypothesis which accounts for the common mode of formation, the intense electronic absorption bands, the requirement for two functional groups on the ligands, and the range of stabilities of the complexes is that the complexes are all Cu^I, Cu^{II} mixed-valence clusters in which Cu^{II} atoms are chelated on the exteriors of Cu^I-thiolate cores. The Cu^I-thiolate cores may belong to the $Cu^I_8S_{12}X$ type found in the present work, or to other polyhedral types such as $Cu^I_4S_6$, $Cu^I_5S_6$, and $Cu^I_5S_7$.[14,19] The substituents adjacent to the thiolate groups of the ligands are required to protect the Cu^I atoms sterically against oxidation. The function of the additional coordinating groups on the ligands is to make possible the chelation of Cu^{II}.

It is an essential part of our hypothesis that the Cu^I and Cu^{II} atoms in the Cu–thiolate clusters have different coordination geometries and are bonded to different types of donor atoms. The colored complexes are accordingly true "mixed valence" complexes ("type I" in the classification of Robin and Day[41]). An alternative hypothesis widely advocated by Hemmerich[34,37,42] is that the reaction of Cu^{II} with thiols leads to the formation of thiolate-bridged complexes in which electron delocalization renders the oxidation states of the Cu atoms indeterminate and indistinguishable from one another:



In other words, the complexes are regarded not as "mixed valence" but as "indeterminable valence" complexes (Robin and Day's "type III-A"[41]). We shall now show that the two descriptions are both valid but that they apply to different types of reaction products.

The strongest evidence in support of Hemmerich's hypothesis is provided by an experiment[34,32] in which a colored complex (ϵ 5 × 10^3 mol^{-1} dm $^3cm^{-1}$ per Cu at 480 nm[43]) was obtained by titrating NaOH into a mixture of Cu^I and cystamine (cysteamine disulfide, RSSR). The solution of the complex had a maximum absorbance at 480 nm under conditions where the integrated ESR signal was at a minimum and represented only 10% of the total Cu. The ESR spectrum of the solution was a single broad signal consistent with Cu^{II}–Cu^{II} interaction in a $S = 1$ system and inconsistent with $Cu^I \leftrightarrow Cu^{II}$ interaction in a $S = \frac{1}{2}$ system. A solid complex isolated under "suitable" (but unspecified) conditions had an elemental composition corresponding to the formula $[(Cu^{II}SR)_2](NO_3)_2$.[34] It was not shown that either the same ESR spectrum or the same solid complex could be obtained by starting with Cu^{II} and cysteamine (RSH). Nevertheless, the published facts are consistent with the formation of a species $[(Cu^{II}SR)_2]^{2+} \leftrightarrow [Cu^I_2(RSSR)]^{2+}$ from Cu^I and RSSR. The intense color of such a species would be due to charge transfer from S to Cu^{II}, just as in $[Cu^{II}_6Cu^I_8Pen_{12}Cl]^{5-}$.

We believe that the "type III" complex fits into the reaction scheme for Cu–thiol interaction as an *intermediate* product in the initial redox reaction:

$$\begin{array}{ccc} 2Cu^{II} + 2RSH & & 2Cu^I + RSSR + 2H^+ \\ & \searrow \quad \swarrow & \\ & (Cu^{II}SR)_2 + 2H^+ \leftrightarrow Cu_2{}^I(RSSR) + 2H^+ & \\ & \text{"type III", labile} & \end{array} \qquad (7)$$

This accounts for the frequently reported observation that a transient purple color is developed immediately after Cu^{II} and thiol solutions are mixed, even in cases where a stable mixed-valence complex is ultimately formed. The presence of halide ions is not essential for this reaction. Furthermore, the "type III" complex is likely to be obtained in high concentration *only* when it is formed by the reaction of Cu^I and disulfide: when the equilibrium is approached from the Cu^{II} side, reaction 7 is driven to the right by the formation of the more stable Cu^I-thiol complex:

$$Cu^I + RSH \rightarrow \frac{1}{q}[Cu^ISR]_q + H^+ \qquad (8)$$

The generalized scheme for the formation of a Cu^I,Cu^{II}-thiolate cluster then continues as in reactions 3–6:

$$pCu^{II} + [Cu^ISR]_q + rRSH + X \rightarrow Cu^{II}_pCu^I_q(SR)_{q+r}X + rH^+ \qquad (9)$$

X represents a halide ion or nothing. The overall stoichiometry is

$$(p+q)Cu^{II} + (2q+r)RSH + X \rightarrow Cu^{II}_pCu^I_q(SR)_{q+r}X + (q/2)RSSR + (2q+r)H^+$$

"type I", potentially stable (10)

In principle it is possible to form the "type 1" complex from Cu^I and RSSR instead of Cu^{II} and RSH:

$$(2q + r)Cu^{I} + \tfrac{1}{2}(q + r)RSSR + X \rightarrow Cu^{II}_{p}Cu^{I}_{q}(SR)_{q+r}X + (q + r - p)Cu^{II} \quad (11)$$

The "type I" complexes of H_2Pen and α-mercaptoisobutyrate have in fact been prepared by both routes (eq 10 and 11).[5,6] For the α-mercaptoisobutyrate complex, Job's plots have been published for both the Cu^{II} + RSH and the Cu^{I} + RSSR reactions. In these cases the characterization of the products leaves no doubt that they are "type I" mixed-valence complexes. The general correctness of our reaction scheme, in which "type III" complexes play an important but hitherto unexplained role, is thereby confirmed.

We note incidentally that the Job's plots for the α-mercaptoisobutyrate complex suggest that the complex has the same stoichiometry $[Cu^{II}_6Cu^{I}_8(SR)_{12}Cl]^{5-}$ as the D-Pen complex. The maxima in the Job's plots occur when the mole fractions of Cu^{II} and Cu^{I} are 0.41 and 0.77 (±0.02), respectively. These are precisely the values (14/34 and 20/26) predicted from eq 10 and 11 when the stoichiometric parameters are $p = 6$, $q = 8$, and $r = 4$. The elemental composition of the solid complex is represented better by $Na_5Cu_{14}(SR)_{12}Cl\cdot 10H_2O$ than by the formula $Cu_4(SR)_3\cdot 4H_2O$ proposed by the original authors.[44]

Chemotherapeutic Action of Penicillamine. The present work provides the following additional insights into the chemotherapeutic action of D-penicillamine, especially in relation to Wilson's disease: (1) D-Penicillamine is seen to be capable of forming a type of complex in which Cu is bound partly as Cu^{II} and partly as Cu^{I}. The Cu^{II} is still in equilibrium with the surrounding aqueous medium but is strongly chelated through N and S donor atoms. The Cu^{I} is removed from equilibrium with the surrounding aqueous medium. (2) The $-CH_3$ groups of the penicillamine side chain are essential for the steric protection of the Cu^{I} atoms against oxidation. (3) The complex owes its high solubility in water to the 12 charged $-COO^-$ groups on its "surface". (4) Chloride, which was previously known to be essential for the formation of the complex but whose function was not understood,[4,5] is seen to be an important structural component of the complex. (5) The complex, while stable for long periods under physiological conditions of pH and saline concentration, decomposes relatively rapidly in urine under aerobic conditions. The importance of the C_β substituents of penicillamine in protecting the drug itself against metabolic degradation by, e.g., L-cysteine desulfhydrase or amino acid oxidases has been recognized previously.[45]

Before we discuss the probable mode of action of D-penicillamine we recall some relevant facts concerning Wilson's disease. The explanation of this disease in molecular terms is due largely to Walshe.[2] In normal humans, 95% or more of the Cu in plasma is bound to the protein ceruloplasmin. In Wilson's disease patients the proportion of ceruloplasmin-bound Cu is generally decreased, and a correspondingly larger proportion of Cu is attached as Cu^{II} to other carriers. The most important nonceruloplasmin Cu carrier is serum albumin.[46] Albumin-bound Cu is much more labile than ceruloplasmin-bound Cu. The failure to incorporate Cu into ceruloplasmin accounts for the greatly enhanced lability of plasma Cu in Wilson's disease. This enhanced lability, in turn, accounts not only for the increased urinary excretion of Cu but also for the ability of the metal to combine with intracellular proteins which happen to have a high affinity for Cu.[47,48] It has been shown that the mode of action of D-penicillamine depends upon the ability of the drug to reduce the plasma concentration of Cu by rendering it more readily available for diffusion across the glomerular membrane of the kidney. There is a consequent rise in the renal clearance of the metal.[49]

It is not possible to state with certainty that the purple $[Cu^{II}_6Cu^{I}_8Pen_{12}Cl]^{5-}$ anion described in the present paper is the form in which Cu is excreted by Wilson's disease patients during penicillamine therapy. The chemical efficacy of D-penicillamine demonstrates that the drug competes successfully for Cu^{II} against serum albumin and other nonceruloplasmin Cu^{II} carriers in vivo. The reaction between Cu^{II} albumin and D-penicillamine in vitro has been the subject of an elegant study by Sugiura and Tanaka,[50] who showed that the reaction leads to the formation of a purple Cu^{I},Cu^{II}-penicillamine complex. Wright and Frieden[4] recovered significant amounts of the purple complex intact from the urinary tracts of rabbits after intravenous infusion, thus providing that the complex is able to be transported across the kidney membrane. We have already established with high probability that the purple solutions and solids prepared by the cited authors had the $[Cu^{II}_6Cu^{I}_8Pen_{12}Cl]^{5-}$ anion as a common component.

There appears at the time of writing to be only one published experimental result which is in direct conflict with the hypothesis that the in vivo reaction leads to the formation of the same Cu^{I},Cu^{II}-penicillamine complex as the in vitro reaction. Gel-filtration patterns and electrophoretograms of urine from Wilson's disease patients have led McCall and co-workers[51] to the conclusion that the Cu excreted during penicillamine therapy is not associated with D-penicillamine at all. This discordant finding may have been due to delays between the collection and examination of the specimens, since we have observed that the violet Cu^{I},Cu^{II}-penicillamine complex is unstable in urine solutions under normal laboratory conditions (see Experimental Section).

Our qualitative experiment on the reaction between D-penicillamine and the Cu^{II} complex of diglycyl-L-histidine[52] pinpoints several features which we assume the biological reaction to have. The tripeptide is recognized as being a good model for the NH_2-terminal Cu^{II}-binding sites in human, bovine, and rat serum albumins.[53] The structure of its Cu^{II} complex is known.[54] The coordination of the metal is square planar. The ligand is quadridentate, the donor atoms being the nitrogen of the terminal NH_2 group, the deprotonated nitrogens of the two peptide groups, and the 1 nitrogen of the imidazole group. Reaction with D-penicillamine leads to the development of the high absorbance at 518 nm which is characteristic of the Cu^{I},Cu^{II}-penicillamine complex. Clearly D-penicillamine has acted not only as a competitive chelator but also as a reducing agent. The reduction of Cu^{II} weakens its binding to a protein because the square-planar disposition of donor atoms which favors the coordination of Cu^{II} is unfavorable for Cu^{I},[28] and because the amino and peptide nitrogen donor atoms have lower affinities for Cu^{I} than for Cu^{II}.[55] As noted by Peisach and Blumberg,[28] it is the ability to act in the dual capacity of reductor and chelator which makes D-penicillamine an effective drug.

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Supplementary Material Available: Listings of (1) observed and calculated structure amplitudes for $Tl_5[Cu^{II}_6Cu^{I}_8(D\text{-}Pen)_{12}Cl]$, (2) observed and calculated structure amplitudes for 34 reflections corrected for contributions from the disordered Tl atoms and H_2O molecules, and (3) bond angles at the Cu, S, and Cl atoms in the

$[Cu^{II}_6Cu^{I}_8(D\text{-Pen})_{12}Cl]^{5-}$ cluster (14 pages). Ordering information is given on any current masthead page.

References and Notes

(1) D-H_2Pen = D-penicillamine, $HSC(CH_3)_2CH(COO^-)NH_3^+$.
(2) J. M. Walshe in "The Biochemistry of Copper", J. Peisach, P. Aisen, and W. E. Blumberg, Ed., Academic Press, New York, N.Y., 1966, pp 475–498.
(3) Y. Sugiura and H. Tanaka, *Chem. Pharm. Bull.*, **18**, 368–373 (1970).
(4) J. R. Wright and E. Frieden, *Bioinorg. Chem.*, **4**, 163–175 (1975).
(5) W. G. Musker and C. H. Neagley, *Inorg. Chem.*, **14**, 1728–1732 (1975).
(6) C. H. Neagley, Ph.D. Thesis, University of California at Davis, 1974; *Diss. Abstr. Int. B*, **35**, 2597 (1974).
(7) P. J. M. W. L. Birker and H. C. Freeman, *J. Chem. Soc., Chem. Commun.*, 312–313 (1976).
(8) G. F. Bryce and F. R. N. Gurd, *J. Biol. Chem.*, **241**, 122–129 (1966).
(9) "International Tables for X-Ray Crystallography", Vol. III, Kynoch Press, Birmingham, England, 1962, pp 157–170.
(10) The following computer programs were used in this analysis: direct methods, MULTAN by P. Main, M. M. Woolfson, and G. Germain; Fourier and Patterson syntheses, FORDAP by A. Zalkin; full-matrix least-squares refinement, NUCLS5, the revision by J. A. Ibers of ORFLS by W. R. Busing, K. O. Martin, and H. A. Levy; molecular geometry, ORFFEC, the revision by J. A. Ibers of ORFFE by W. R. Busing and H. A. Levy; molecular diagrams, ORTEP by C. K. Johnson. Locally written programs were used for data reduction (J. M. Guss) and the Patterson search (P.J.M.W.L. Birker). Absorption corrections were made by the method of P. Coppens, L. Leiserowitz, and D. Rabinovitch, *Acta Crystallogr.*, **18**, 1035 (1965).
(11) "International Tables for X-Ray Crystallography", Vol. IV, Kynoch Press, Birmingham, England, 1974, pp 71–102.
(12) A. D. Mighell and R. A. Jacobson, *Acta Crystallogr.*, **16**, 443–445 (1963).
(13) See paragraph at end of paper regarding supplementary material.
(14) I. G. Dance and J. C. Calabrese, *Inorg. Chim. Acta*, **19**, 141–142 (1976).
(15) L. E. McCandish, E. C. Bissell, D. Coucouvanis, G. P. Fackler, and K. Knox, *J. Am. Chem. Soc.*, **90**, 7357–7359 (1968).
(16) F. G. Hollander and D. Coucouvanis, *J. Am. Chem. Soc.*, **96**, 5646–5648 (1974).
(17) H. Schugar, C. Ou, G. A. Thich, J. A. Potenza, R. A. Lalancette, and W. Furey, Jr., *J. Am. Chem. Soc.*, **98**, 3047–3048 (1976).
(18) H. B. Bürgi, H. Gehrer, P. Strickler, and F. K. Winkler, *Helv. Chim. Acta*, **59**, 2558–2565 (1976).
(19) (a) I. G. Dance, *J. Chem. Soc., Chem. Commun.*, 103–107 (1976); (b) *ibid.*, 68–69 (1976).
(20) E. W. Wilson and R. B. Martin, *Arch. Biochem. Biophys.*, **142**, 445–454 (1971).
(21) Found: Cu, 36.6; C, 23.5; H, 4.7; Cl, 3. Calcd for $Cu_3Pen_2{\cdot}2H_2O$: Cu, 36.1; C, 23.1; H, 4.3; Cl, 0. Calcd for $Cu_{16.5}Pen_{12}Cl{\cdot}10H_2O$: Cu, 34.6; C, 23.8; H, 4.2; Cl, 1.2. Calcd for $Cu_{17.5}Pen_{12}Cl_3{\cdot}6H_2O$: Cu, 36.0; C, 23.3; H, 3.9; Cl, 3.4. We have repeated Sugiura and Tanaka's preparation[3] and find that the solid isolated under the conditions described by them indeed contains Cl. We have included our value for Cl among the cited analytical results.
(22) Found: Na, 5.0; Cu, 21.6; C, 22.4; H, 4.9; N, 5.1; S, 11.6; Cl, 1.4. Calcd for $NaCu_2Pen_2{\cdot}6H_2O$: Na, 4.2; Cu, 23.0; C, 21.7; H, 5.5; N, 5.1; S, 11.6; Cl, 0. Calcd for $Na_5Cu_{14}Pen_{12}Cl{\cdot}25H_2O$: Na, 3.5; Cu, 27.3; C, 22.1; H, 4.9; N, 5.2; S, 11.8; Cl, 1.1. Calcd for $Na_7Cu_{14}Pen_{12}Cl_3{\cdot}25H_2O$: Na, 4.8; Cu, 26.4; C, 21.4; H, 4.7; N, 5.0; S, 11.4; Cl, 3.1. Calcd for $Na_7Cu_{13}Pen_{12}Cl{\cdot}36H_2O$: Na, 4.7; Cu, 24.0; C, 21.0; H, 5.2; N, 4.9; S, 11.2; Cl, 1.0.
(23) H. Rupp and U. Weser, *Biochem. Biophys. Res. Commun.*, **72**, 223–229 (1976).
(24) Found: Cu, 32.2; C, 23.9; H, 4.5; N, 5.3; S, 12.8; Cl, 1.6. Calcd for $Cu_4Pen_3{\cdot}4H_2O$: Cu, 33.2; C, 23.5; H, 4.6; N, 5.5; S, 12.6; Cl, 0. Calcd for $Na_3Cu_{15}Pen_{12}Cl{\cdot}10H_2O$: Cu, 31.7; C, 24.0; H, 4.3; N, 5.6; S, 12.8; Cl, 1.2.
(25) Found: Na, 1.2; Cu, 29.3; C, 23.3; H, 4.3; N, 6.6; S, 12.9; 0, 20.4; Cl, 1.8. Calcd for $Na_5Cu_{14}Pen_{12}Cl{\cdot}14H_2O$: Na, 3.8; Cu, 29.1; C, 23.5; H, 4.4; N, 5.5; S, 12.6; 0, 19.9; Cl, 1.1.
(26) The ESR spectrum of $[Cu^{II}_6Cu^{I}_8Pen_{12}Cl]^{5-}$ bears no resemblance to the spectra reported by Blumberg and Peisach,[27] Peisach and Blumberg,[26] and Laurie, Lund, and Raynor[29] for frozen solutions prepared by adding D-penicillamine to $CuSO_4$. The absence of Cl^- from the reaction mixtures would in all three cases have precluded the formation of a $[Cu_{14}Pen_{12}Cl]^{5-}$ cluster complex. The ESR spectrum reported by Blumberg and Peisach[27,26] was recorded at high pH. Although the color of the solution was blue, the ESR spectrum appears to have originated from a yellow complex species, $Cu^{II}(Pen)_2^{2-}$, which is formed at pH >8.[20] The composition of the complex is supported by the fact that the same stoichiometry has been established experimentally for a yellow Cu^{II}–cysteine complex with a similar ESR spectrum and UV absorption at high pH (D. Cavallini, C. de Marco, S. Dupre, and G. Rotilio, *Arch. Biochem. Biophys.*, **130**, 354–361 (1969)).
(27) W. E. Blumberg and J. Peisach, *J. Chem. Phys.*, **49**, 1793–1802 (1968).
(28) J. Peisach and W. E. Blumberg, *Mol. Pharmacol.*, **5**, 200–208 (1969).
(29) S. H. Laurie, T. Lund, and G. B. Raynor, *J. Chem. Soc., Dalton Trans.*, 1389–1394 (1975).
(30) J. R. Pilbrow, T. D. Smith, and A. D. Toy, *Aust. J. Chem.*, **23**, 2287–2296 (1970).
(31) D. C. Gould and A. Ehrenberg in "Physiology and Biochemistry of Hemocyanins", F. Ghiretti, Ed., Academic Press, New York, N.Y., 1968, pp 95–112. An ambiguity on p 660 of ref 34 is open to the erroneous interpretation that Gould and Ehrenberg studied the Cu–cysteamine system instead of the Cu–thiomalate system.
(32) J. F. Boas, J. R. Pilbrow, J. G. Troup, C. Moore, and T. D. Smith, *J. Chem. Soc. A*, 965–971 (1969).
(33) I. M. Klotz, G. H. Czerlinski, and H. A. Fiess, *J. Am. Chem. Soc.*, **80**, 2920–2923 (1958).
(34) P. Kroneck, C. Naumann, and P. Hemmerich, *Inorg. Nucl. Chem. Lett.*, **7**, 659–666 (1971).
(35) See, e.g., J. A. Fee, *Struct. Bonding*, **23**, 1–60 (1975).
(36) J. J. Vallon and A. Badinand, *Anal. Chim. Acta*, **42**, 445–454 (1968).
(37) P. Hemmerich in "The Biochemistry of Copper", J. Peisach, P. Aisen, and W. E. Blumberg, Ed., Academic Press, New York, N.Y., 1966, pp 15–32.
(38) J. A. Thich, D. Mastropaolo, J. Potenza, and H. J. Schugar, *J. Am. Chem. Soc.*, **96**, 726–731 (1974).
(39) These include thioglycolate (mercaptoacetate), $HSCH_2COO^-$;[33] thiolactate, $HSCH(CH_3)COO^-$;[8] α-mercaptoisobutyrate, $HSC(CH_3)_2COO^-$;[5,6] thiomalate (mercaptosuccinate), $HSCH(COO^-)CH_2COO^-$;[33] α,β-dimercaptosuccinate, $HSCH(COO^-)CH(SH)COO^-$;[33] cysteamine (β-mercaptoethylamine), $HSCH_2CH_2NH_2$;[3,33,35,42] β,β-dimethylcysteamine, $HSC(CH_3)_2CH_2NH_2$;[6,17] cysteine, $HSCH_2CH(COO^-)NH_3^+$;[3,6,20,42] β-methyl-β-ethylcysteine, $HSC(CH_3)(C_2H_5)CH(COO^-)NH_3^+$;[3] N-acetylpenicillamine, $HSC(CH_3)_2CH(COO^-)NHCOCH_3$;[3] penicillamine methyl ester, $HSC(CH_3)_2CH(COOCH_3)NH_2$.[3]
(40) H. Sakurai, A. Yokoyama, and H. Tanaka, *Chem. Pharm. Bull.*, **18**, 2373–2385 (1970).
(41) M. B. Robin and P. Day, *Adv. Inorg. Chem. Radiochem.*, **10**, 247–457 (1967).
(42) P. Hemmerich, H. Beinert and T. Vänngard, *Angew. Chem., Int. Ed. Engl.*, **5**, 422–423 (1966).
(43) There is an inconsistency between the values $\epsilon \sim 5 \times 10^2\ M^{-1}\ cm^{-1}$ and $\epsilon \sim 5 \times 10^3\ M^{-1}\ cm^{-1}$ in ref 34 and 42.
(44) Found: Cu, 33.9; C, 22.2; H, 3.9; S, 14.7; Cl, 1.8. Calcd for $Cu_4(C_4H_6SO_2)_3{\cdot}4H_2O$: Cu, 37.4; C, 21.2; H, 3.8; S, 14.1; Cl, 0. Calcd for $Na_5Cu_{14}(C_4H_6SO_2)_{12}Cl{\cdot}10H_2O$: C, 33.7; C, 21.8; H, 3.5; S, 14.6; Cl, 1.4. The low molar conductances reported for aqueous solutions of the complex do not prove that the complex is a nonelectrolyte as deduced by Musker and Neagley.[5,6] A Debye–Hückel–Onsager calculation for a complex Na_5B with a reasonable but guessed value for the ion conductance of B^{5-} leads to molar conductances which are close to the observed values. If the complex is a 5:1 electrolyte, then the molecular weight of 790 derived from vapor phase osmometry measurements on the assumption that the complex is nonionic must be multiplied by a factor between 1 and 6. The calculated formula weight of $Na_5Cu_{14}(C_4H_6SO_2)_{12}Cl$ is 2458.
(45) (a) H. V. Aposhian, "Metal Binding in Medicine", M. J. Seven, Ed., J. B. Lippincott Co., Philadelphia, Pa., 1960, pp 290–295; (b) H. V. Aposhian, *Drug Intell. Clin. Pharm.*, **4**, 351 (1970).
(46) R. P. Agarwal and D. D. Perrin, *Agents Actions*, **6**, 667–672 (1976).
(47) J. M. Walshe, *Clin. Sci.*, **25**, 405–411 (1963).
(48) S. B. Osborn and J. M. Walshe, *Clin. Sci.*, **26**, 213–217 (1964).
(49) J. M. Walshe, *Clin. Sci.*, **26**, 461–469 (1964).
(50) Y. Sugiura and H. Tanaka, *Mol. Pharmacol.*, **8**, 249–255 (1972).
(51) J. T. McCall, N. P. Goldstein, R. V. Randall, and J. B. Gross, *Am. J. Med. Sci.*, **254**, 35/13–45/23 (1967).
(52) A detailed kinetic study of the mechanism of this reaction is in progress (D. W. Margerum, personal communication).
(53) (a) E. Breslow, *J. Biol. Chem.*, **239**, 3252–3259 (1964); (b) T. Peters and F. A. Blumenstock, *ibid.*, **242**, 1574–1578 (1967); (c) W. T. Shearer, R. A. Bradshaw, F. R. N. Gurd, and T. Peters, *ibid.*, **242**, 5451–5459 (1967); (d) R. A. Bradshaw, W. T. Shearer, and F. R. N. Gurd, *ibid.*, **243**, 3817–3825 (1968).
(54) N. Camerman, A. Camerman, and B. Sarkar, *Can. J. Chem.*, **54**, 1309–1316 (1976).
(55) M. W. Whitehouse, *Agents Actions*, **6**, 201–206 (1976).