

## Reactions of Uridine with Copper(II) Acetate Dimer

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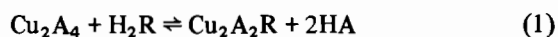
Two groups of *cis* diols may be categorized according to their reaction with the dimer  $\text{Cu}_2(\text{CH}_3\text{COO}^-)_4$  in dimethylsulfoxide. *Cis* diols of the ribose class destroy the dimer, as is revealed by a shift of the visible absorption to longer wavelengths with a loss of intensity and by an increase in magnetic moment. Addition of a uridine class *cis* diol also results in a loss of intensity but without a shift in the characteristic dimer 715 nm absorption maximum and by little change in magnetic moment. A quantitative analysis is presented of the changes in the visible absorption intensity upon uridine addition. The dimer to monomer equilibrium of copper acetate is taken into account. One molecule of uridine as a twice deprotonated *cis* diolate (*R*) reacts with one mol of copper acetate dimer ( $\text{Cu}_2\text{A}_2$ ) with expulsion of two acetates (*A*) as acetic acid to give  $\text{Cu}_2\text{A}_2\text{R}$ . A second product is also formed. Only upon inclusion of a product tetranuclear complex composed of  $\text{Cu}_4\text{A}_4\text{R}_2$  is there achieved a satisfactory quantitative description of the interactions over a 30 fold range of total Cu(II) concentration.

### Introduction

In the solid state and in non-aqueous solvents such as dimethylsulfoxide (DMSO), copper(II) acetate forms a dimer,  $\text{Cu}_2\text{A}_4$ , in which each of the four acetate ions donates one oxygen to each Cu(II) [1]. Each of the two Cu(II) is tetragonally coordinated to oxygens from four different acetates. Within the dimer the Cu(II)–Cu(II) distance is about 2.6 Å [2]. One axial donor such as solvent may also coordinate to each Cu(II). The presence of  $\text{Cu}_2\text{A}_4$  dimer is apparent from a relatively intense visible absorption band with a maximum near 715 nm.

The reaction of ribose sugar derivatives with  $\text{Cu}_2\text{A}_4$  in DMSO has aroused considerable interest and some controversy. Ribonucleosides such as uridine but not 2'-deoxyribonucleosides were reported to reduce the intensity of the 715 nm absorption band [3]. Experiments suggested that one molecule of ribonucleoside reacted with one molecule of  $\text{Cu}_2\text{A}_4$ . It was proposed that the two oxygen donors of a bridging acetate ion were replaced by the *cis* 2' and 3' hydroxy oxygens of the ribonucleoside sugar preserving the dimer which now bears a net positive charge [3]. This conclusion was criticized on the basis of ESR experiments on frozen DMSO solutions in which addition of uridine was interpreted to destroy the dimer and produce monomers [4]. The literature is replete, however, with ESR studies of Cu(II) complexes in frozen solutions which differ from conclusions of other techniques at room temperature. Because the  $\text{Cu}_2\text{A}_4$  dimer is less stable in hydroxylic solvents, it has been suggested that addition of a *cis* diol such as uridine will also disrupt the  $\text{Cu}_2\text{A}_4$  dimer [5]. However, this criticism ignores the apparent 1:1 stoichiometry of uridine addition to  $\text{Cu}_2\text{A}_4$  and the persistence of an absorption maximum near 715 nm. In the absence of acetate the absorption is much weaker, with a maximum near 800 nm.

Most recently the reaction of uridine with  $\text{Cu}_2\text{A}_4$  in DMSO was reinvestigated by several physical methods [6]. Addition of uridine to DMSO solutions of  $\text{Cu}_2\text{A}_4$  decreased both the magnetic moment and the intensity of the room temperature ESR spectrum. These results are inconsistent with destruction of the dimer to give monomeric Cu(II) of greater magnetic moment. On the basis of changes in the visible absorption spectrum it was proposed that addition of a ribonucleoside,  $\text{H}_2\text{R}$ , to  $\text{Cu}_2\text{A}_4$  results in coordination of the *cis* diol upon loss of the two hydroxylic protons, which are taken up by expulsion of two molecules of acetic acid, HA [6].



The donor atoms replacing the second expelled acetate were not specified, nor were other details of the structure of the product  $\text{Cu}_2\text{A}_2\text{R}$ .

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The *cis* 2', 3' diol function of ribonucleosides is known to chelate metal ions [7]. The initial structure proposed for interaction of ribonucleosides with  $\text{Cu}_2\text{A}_4$  to give  $\text{Cu}_2\text{A}_3(\text{H}_2\text{R})^+$  bears a positive charge [3], perhaps an unstable situation. An attractive feature of the proposed  $\text{Cu}_2\text{A}_2\text{R}$  product of eq. 1 is its electroneutrality. In this paper we report experiments which furnish additional information on the products of the reaction of  $\text{Cu}_2\text{A}_4$  with ribonucleosides in DMSO. The presentation begins with the more qualitative results which provide the background for the choices made in the quantitative analysis that concludes the paper.

### Experimental

The monohydrate  $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$  from Baker Chemical Co. was used. For each  $\text{Cu}(\text{II})$  a water molecule is thereby introduced into the system. Several sources of dimethylsulfoxide including a spectrograde from Aldrich Chemical Co. were employed. Closely similar results were obtained in all cases except at lower concentrations of copper acetate (without uridine) than are reported in this paper. That the water introduced with the salt is not a variable was indicated by the insensitivity of the results to the addition of up to 40 mol water/mol  $\text{Cu}(\text{II})$ . Uridine was from Sigma Chemical Co. Copper(II) salts of propionate, butyrate, and pivaloate (trimethylacetate) were prepared from the acids by adding excess basic cupric carbonate. Absorption measurements were made on a Cary 14R spectrophotometer. Magnetic susceptibility was determined by an nmr method [8] with 10% benzene as the reference. ESR spectra were recorded on a Varian spectrometer.

### Results and Discussion

The room temperature paramagnetic characteristics of DMSO solutions of  $\text{Cu}_2\text{A}_4$  upon addition of *cis* diols were investigated. Addition of uridine to a solution 75 mM in  $\text{Cu}(\text{II})$  results in little change or in the presence of a 3-fold uridine excess to a slight decrease, in the magnetic moment of the solution as determined by a NMR method [8]. These results confirm those of a previous report [6]. Addition of the free sugar ribose increases the magnetic moment toward the values expected for monomeric  $\text{Cu}(\text{II})$ . We also confirm that the ESR signal of 2.5 mM  $\text{Cu}_2\text{A}_4$  dimer, already substantially weakened compared to monomeric  $\text{Cu}(\text{II})$ , becomes weaker still upon addition of uridine.

We confirm the results reported for changes in the visible absorption spectra upon addition of a variety of nucleosides to DMSO solutions of  $\text{Cu}_2\text{A}_4$  [3].

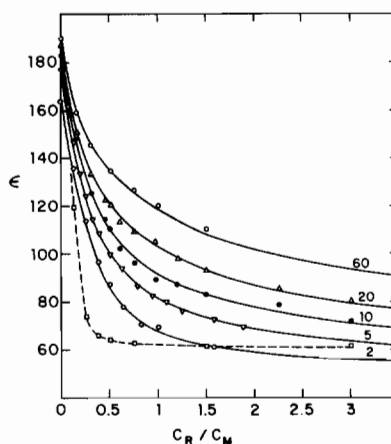


Fig. 1. Experimental points of observed molar absorptivities per mol of  $\text{Cu}(\text{II})$  at 715 nm versus ratio of total uridine to total  $\text{Cu}(\text{II})$  molar concentrations. Solid curves are drawn from eq. 4 with parameters described in text. The numbers at the right give the total  $\text{Cu}(\text{II})$  concentration (2 to 60 mM). The open squares with the dashed curve at the left depict the results for addition of D-ribose at 7.5 mM  $C_M$ . The intercept value for this solution is 179. The dashed curve is not theoretical but merely drawn through the experimental points.

Uridine and cytidine result in similar intensity decreases of the 715 nm absorption band. Adenosine and guanosine yield precipitates. Deoxythymidine and uracil- $\beta$ ,D-arabinofuranoside are without effect on the visible absorption spectrum. Thus a *cis*-diol configuration is required for the interaction with  $\text{Cu}_2\text{A}_4$  that maintains the absorption maximum near 715 nm but with reduced intensity. Addition of uridine to  $\text{Cu}_2\text{A}_4$  in DMSO reduces the intensity of the 715 nm absorption band, while the ratio of absorptivities at 715 nm to 800 nm remains constant at 1.50.

Not all *cis* diols, however, result in retention of the absorption maximum at 715 nm. Addition of D-ribose, D-glucose, and ethylene glycol (weak interaction) yields a marked decrease in intensity with a concomitant shift in the absorption maximum to longer wavelengths. The ratio of absorptivities at 715 nm to 800 nm decreases. Successive additions of fractional equivalents of *cis* diols to  $\text{Cu}_2\text{A}_4$  in DMSO reveal that for each mol of dimer only 0.5 mol of D-ribose destroy the dimer. The results for D-ribose appear as the dashed curve in Fig. 1. That only 0.5 mol of ribose are required might be accounted for by the availability of a second *cis* diol set in the  $\alpha$ -pyranose form and by the well known reduction of  $\text{Cu}(\text{II})$  by reducing sugars. In support of these suggestions is the time dependence observed in the reaction with ribose and 2'-deoxyribose. Substitution of D-ribofuranoside at the 1' position by a nucleic base precludes the last reactions in nucleosides.

Thus we are able to divide *cis* diols into two major classes, depending upon their effect on the  $\text{Cu}_2\text{A}_4$  dimer in DMSO. Addition of a *cis* diol of the ribose class, results in destruction of the dimer as revealed by loss of the visible absorption maximum near 715 nm, a decrease of the 715 nm to 800 nm absorptivity ratio, and an increase in magnetic moment. On the other hand, addition of a uridine class *cis* diol also results in a loss of intensity, but the maximum at 715 nm persists and the 715 nm to 800 nm absorptivity ratio is unaffected. The magnetic moment is changed little or reduced slightly. Recognition of two classes of *cis* diols in their effect on  $\text{Cu}_2\text{A}_4$  resolves some of the controversies in the literature that were mentioned in the introduction. The distinction between the two classes may not always be sharp: some *cis* diols may react in both ways or even switch their predominant mode of interaction with a change of conditions such as incorporation into a frozen solution.

Experiments were performed with varying ratios of  $\text{Cu}(\text{NO}_3)_2$ ,  $\text{CH}_3\text{COO}^-\text{K}^+$ , and uridine in DMSO solutions. The optimum  $[\text{CH}_3\text{COO}^-]/[\text{Cu}^{2+}]$  ratio for production of the 715 nm absorption band is 2.0. Excess acetate weakens the absorption. This optimum ratio of 2.0 holds even when uridine is present. With uridine present excess acetate weakens the intensity, but the absorption maximum remains at 715 nm. The 715 nm absorption band is not produced in a solution containing only  $\text{Cu}(\text{NO}_3)_2$  and uridine:acetate is required for the appearance of this band.

An extensive quantitative analysis of the species present at equilibrium upon addition of uridine to  $\text{Cu}_2\text{A}_4$  in DMSO was conducted. Spectrophotometric measurements were made at varying uridine to Cu(II) ratios at eight different total Cu(II) concentrations ( $C_M$ ). Values of the observed molar absorptivity at 715 nm per mol of total Cu(II) versus the mol ratio of uridine to Cu ( $C_R/C_M$ ) are plotted for five  $C_M$  concentrations in Fig. 1. Inspection of the curves in Fig. 1 reveals that the curves of higher  $C_M$ , yield greater final molar absorptivities than those of lesser concentration. This result qualitatively demands a greater number of particles on the right than on the left of the balanced chemical equation. It rules out the initial formulation [3] of simple substitution of a uridine for an acetate in the dimer. It is also inconsistent with the contrasting implied formulations [4, 5] of  $\text{Cu}_2\text{A}_4$  dimer destruction by uridine.

That a greater number of particles are produced as a result of a chemical reaction is confirmed by freezing point depression measurements. Because several kinds of molecules are present, comparisons were made with a control. To a DMSO solution of  $\text{Cu}_2\text{A}_4$  addition of one mol of uridine for one mol of dimer lowers the freezing point 20% more than

does addition of one mol of thymidine (2'-deoxy-nucleoside). This result is consistent with the quantitative analysis provided at the end of this paper.

The only literature formulation qualitatively consistent with Fig. 1 is that given in eqn. 1, where there are 2 particles on the left and 3 on the right of the balanced equation [6]. Also in agreement with eqn. 1 the observed molar absorptivity is driven back to its initial value at low  $C_R/C_M$  upon addition of acetic acid. An equilibrium condition exists. Acetate has the opposite effect as described above. We define the equilibrium constant for eqn. 1 as

$$K_E = [\text{Cu}_2\text{A}_2\text{R}][\text{HA}]^2/[\text{Cu}_2\text{A}_4][\text{H}_2\text{R}].$$

As already suggested [6], attempts to establish a constant value for  $K_E$  over a range of conditions were unsuccessful. The reaction of uridine with  $\text{Cu}_2\text{A}_4$  is more complex than indicated by eqn. 1.

One problem with eqn. 1 is the lack of a common ordinate intercept for the curves of Fig. 1: without uridine the molar absorptivity of  $\text{Cu}_2\text{A}_4$  decreases with decreasing total Cu(II) concentration. In order to allow for this effect, formation of mononuclear complexes is considered in eqn. 2.



We define the equilibrium constant  $K_D = [\text{Cu}_2\text{A}_4]/[\text{CuA}_2]^2$ . By considering eight DMSO solutions 2 to 60 mM in total Cu(II) without uridine and assuming a molar absorptivity  $\epsilon_M = 20$  for  $\text{CuA}_2$ , an equilibrium constant  $K_D = 6300 \text{ M}^{-1}$  satisfactorily fits the results. On this basis the molar absorptivity per mol of Cu(II)  $\epsilon_D = 198$  for the dimer  $\text{Cu}_2\text{A}_4$ . The value of  $K_D$  is dependent on the value chosen for  $\epsilon_M$ , but the two parameters work together so that the  $\epsilon_D = 198$  result is relatively firm. We have also employed a value of  $\epsilon_M = 40$  for which  $K_D = 4700 \text{ M}^{-1}$  and  $\epsilon_D = 198$ , identical to that above. The mol fraction of total Cu(II) in the form of  $\text{CuA}_2$  never exceeds 0.16 in our solutions with uridine and the choice of  $\epsilon_M = 20$  or  $\epsilon_M = 40$  is without consequence in our analysis. The former set of values is used throughout this presentation.

Consideration of eqns. 1 and 2 together with their associated equilibrium constants makes it even more difficult to discover parameters that yield a constant value of  $K_E$  over a range of conditions. Several alternative formulations with other binuclear product complexes including the coordinatively saturated  $\text{Cu}_2\text{A}_3\text{R}$  (with  $\text{HA} + \text{H}^+$ ) were also unsuccessful. The slight decrease observed in the magnitude of the magnetic moment and the intensity of the esr signal may be due to the shifting of the equilibria of eqns. 1 and 2 away from paramagnetic monomer on addition of uridine. The curves of Fig. 1 are most nearly fitted with a 1:1 reaction between  $\text{Cu}_2\text{A}_4$  and uridine.

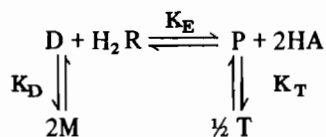
Since two acetates are replaced in the dimer  $\text{Cu}_2\text{A}_4$  by the *cis* diolate  $\text{R}^{2-}$ , one vacated tetragonal coordination position about each  $\text{Cu}(\text{II})$  remains to be filled by weakly coordinating solvent DMSO, product acetic acid, water, or excess neutral uridine. Coordination of any of the last three molecules no longer yields a greater number of particles on the right than on the left of the balanced chemical equation and is qualitatively inconsistent with the results portrayed in Fig. 1. We are thus forced to conclude that more than one product occurs as a result of the addition of uridine to DMSO solutions of  $\text{Cu}_2\text{A}_4$ . Testing of the two products  $\text{Cu}_2\text{A}_2\text{R}$  and  $\text{Cu}_2\text{A}_2\text{R} \cdot (\text{H}_2\text{R})$  together did not yield a constant value for  $K_E$ .

An attractive way to fill the vacated tetragonal coordination positions about each  $\text{Cu}(\text{II})$  with a more strongly coordinating group than solvent DMSO is to form a tetranuclear complex according to



We define the associated equilibrium constant  $K_T = [\text{Cu}_4\text{A}_4\text{R}_2]^{1/2} / [\text{Cu}_2\text{A}_2\text{R}]$ . A possible structure for the tetramer consists of two dimers stripped of two acetates that occupied *cis* positions which are now linked in the tetramer by alcoholate bridges from the two ribose molecules. Each ribose molecule furnishes two alcoholate bridges and is coordinated to all four  $\text{Cu}(\text{II})$ . Alcoholate bridging of  $\text{Cu}(\text{II})$  has been suggested before [9]. Consideration of a tetranuclear complex as the sole  $\text{Cu}(\text{II})$  containing product yields a constant value for  $K_E$ , but only under the condition that the molar absorptivity per  $\text{Cu}(\text{II})$  in the tetranuclear complex  $\epsilon_T < 20$ , which is considered unreasonable. The trends found in  $K_E$  for a tetranuclear complex are just the opposite of those realized for binuclear  $\text{Cu}_2\text{A}_2\text{R}$  as the sole  $\text{Cu}(\text{II})$  containing product.

The most successful fitting of experimental points, like those presented in Fig. 1, was achieved by considering simultaneously eqns. 1–3 and their associated equilibrium constants. The dimer  $\text{Cu}_2\text{A}_4$  is in equilibrium with a small amount of monomer  $\text{CuA}_2$  and reacts with uridine  $\text{H}_2\text{R}$  to give acetic acid and binuclear  $\text{Cu}_2\text{A}_2\text{R}$  and tetranuclear  $\text{Cu}_4\text{A}_4\text{R}_2$  products. The equilibrium reactions in abbreviated form become



where M represents monomer, D the reactant dimer  $\text{Cu}_2\text{A}_4$ ,  $\text{H}_2\text{R}$  the *cis* diol, P the binuclear product  $\text{Cu}_2\text{A}_2\text{R}$ , and T the tetranuclear product complex. The three already defined equilibrium constants become

$$K_D = [\text{D}] / [\text{M}]^2; K_E = [\text{P}] [\text{HA}]^2 / [\text{P}] [\text{H}_2\text{R}];$$

$$K_T = [\text{T}]^{1/2} / [\text{P}]$$

where  $K_D = 6300 \text{ M}^{-1}$  and  $K_E$  and  $K_T$  are to be determined. From conservation of mass we write for the total molar concentration of acetate

$$C_A = 2[\text{M}] + 4[\text{D}] + 2[\text{P}] + 4[\text{T}] + [\text{HA}]$$

for the total molar concentration of *cis* diol

$$C_R = [\text{H}_2\text{R}] + [\text{P}] + 2[\text{T}]$$

and for the total molar concentration of  $\text{Cu}(\text{II})$

$$C_M = [\text{M}] + 2[\text{D}] + 2[\text{P}] + 4[\text{T}]$$

Since we are measuring the absorption properties of  $\text{Cu}(\text{II})$ , it is convenient to define the copper weighted mole fractions

$$\alpha_M = [\text{M}] / C_M; \alpha_D = 2[\text{D}] / C_M; \alpha_P = 2[\text{P}] / C_M;$$

$$\alpha_T = 4[\text{T}] / C_M$$

The observed molar absorptivity per mol of  $\text{Cu}(\text{II})$  is then expressed as

$$\epsilon = \alpha_M \epsilon_M + \alpha_D \epsilon_D + \alpha_P \epsilon_P + \alpha_T \epsilon_T$$

where at 715 nm  $\alpha_M$  is taken as 20,  $\epsilon_D$  is known to be 198, and  $\epsilon_P$  and  $\epsilon_T$  are to be determined. All molar absorptivities are per mol of  $\text{Cu}(\text{II})$  even in binuclear and tetranuclear complexes.

Simultaneous solution of the above equations leads to the following expressions for the mol fractions of each  $\text{Cu}(\text{II})$  containing species:

$$\begin{aligned} \alpha_P^2 K_T^2 C_M (\epsilon_D - \epsilon_T) + (\epsilon_D - \epsilon_P) \alpha_P - \\ (\epsilon_D - \epsilon) + (\epsilon_D - \epsilon_M) \alpha_M = 0 \end{aligned}$$

This equation may be solved quadratically for  $\alpha_P$ .

$$\alpha_T = \alpha_P^2 K_T^2 C_M$$

$$\alpha_D = 1 - \alpha_P - \alpha_T - \alpha_M$$

$$\alpha_M = (\alpha_D / 2K_D C_M)^{1/2}$$

The procedure used was to substitute the prepared values of  $C_M$ , the observed value for  $\epsilon$ , and trial values of  $\epsilon_P$ ,  $\epsilon_T$ , and  $K_T$  into the  $\alpha$  equations and iterate in the above order on a computer until a constant value of the four  $\alpha$  values was obtained. Only a few iterations were necessary to achieve constancy. The results for the  $\alpha$  values were then substituted into the derived equation for  $K_E$ .

TABLE I. Values of Equilibrium Constant  $K_E$  for Reaction of Uridine with Copper Acetate.

$C_M, mM$	$-\log K_E$
2.00	$2.02 \pm 0.06$
3.33	$1.97 \pm 0.05$
3.75	$2.02 \pm 0.04$
5.00	$2.00 \pm 0.03$
10.0	$1.95 \pm 0.05$
20.0	$1.98 \pm 0.03$
30.0	$2.00 \pm 0.03$
60.0	$2.02 \pm 0.05$
Average	$2.00 \pm 0.03$

$$K_E = \frac{\alpha_P(\alpha_P + \alpha_T)^2 C_M}{\alpha_D[F - (\alpha_P + \alpha_T)/2]} \quad (4)$$

where  $F$  is the molar ratio  $C_R/C_M$ .

The procedure was repeated for 89 experimental points at 8 total Cu(II) concentrations  $C_M$  from 2 to 60 mM and with values of the molar ratio  $F$  up to 3. Results of 5 of the 8  $C_M$  concentrations (including the two extreme ones) are shown as the experimental points in Fig. 1. The curves of Fig. 1 are the theoretical ones with the parameters that are judged to provide the most constant value to  $K_E$  over the 89

points. The most constant  $K_E$  was obtained with  $\epsilon_P = 49$ ,  $\epsilon_T = 60$ , and  $K_T = 12 M^{-1/2}$ . These parameters yield the calculated values listed in Table I for  $-\log K_E$  with standard deviations (units of  $M$ ). There is close agreement both among different points at each of 8  $C_M$  and among the average values over a 30 fold range of total Cu(II) concentration.

Two molecules of acetic acid appear as a product in eq. 1 and increases in the 715 nm molar absorptivity upon addition of acetic acid to produce reactant  $Cu_2A_4$  in a back reaction were treated quantitatively. In a solution with  $C_M = 20$  mM with molar ratios of  $C_R/C_M = 0.25, 0.5, 1, \text{ and } 2$ , the addition of acetic acid up to  $C_M$  was tested in a form of eqn. 4 modified to allow for external acetic acid addition. For 12 experimental points the calculated equilibrium constant  $-\log K_E = 1.99 \pm 0.08$ . This result is in excellent agreement with those in Table I and strongly supports the appearance of two mol of acetic acid product in eqn. 1.

A smaller number of similar experiments were also performed on the analogous propionate, butyrate, and pivaloate (trimethylacetate) salts of Cu(II). With parameters similar to those given for acetate, the average equilibrium constant values  $-\log K_E$  analogous to those in Table I for acetate are as follows: propionate, 1.88; butyrate, 2.4; pivaloate, 2.8 (with  $K_T = 8$ ). Thus the tendency to react with uridine decreases down the series with the equilibrium constant  $K_E$  for the acetate salt being about 75% that of propionate and almost 3 times greater than that for butyrate. The smaller  $K_E$

TABLE II. Equilibrium Mol Fractions of Copper Containing Species.

$C_M, mM$	$C_R/C_M$	Mol Fraction			
		$CuA_2$	$Cu_2A_4$	$Cu_2A_2R$	$Cu_4A_4R_2$
2	0.50	0.20	0.26	0.51	0.04
	1.00	0.15	0.14	0.64	0.06
	1.56	0.12	0.08	0.72	0.07
5	0.50	0.15	0.35	0.44	0.07
	1.09	0.12	0.21	0.56	0.11
	1.56	0.10	0.16	0.61	0.12
10	0.50	0.12	0.42	0.37	0.09
	0.98	0.10	0.28	0.48	0.15
	1.50	0.09	0.23	0.51	0.17
20	0.50	0.09	0.50	0.29	0.11
	0.98	0.08	0.41	0.35	0.16
	1.50	0.08	0.32	0.40	0.20
60	0.50	0.06	0.62	0.19	0.13
	1.00	0.06	0.53	0.22	0.19
	1.50	0.05	0.47	0.25	0.23

and  $K_T$  equilibrium constants for pivaloate suggest that steric hindrance may be a factor with this salt.

Formation of  $Cu_2A_2R$  and inclusion of the tetranuclear complex  $Cu_4A_4R_2$  is the only combination of two Cu containing products tested that produced a fairly constant value for  $K_E$  over the 89 experimental points with chemically reasonable values of input molar absorptivities. This result does not, of course, prove the existence of the two complexes as products. We can attest, however, to the difficulty of finding any single product or any other combination of two products that yield constant  $K_E$  values. Other Cu containing products almost certainly occur, and combinations of three or more products in significant amounts can almost surely be made to fit the points with reasonable parameters. We feel the presence of  $Cu_2A_2R$  is highly likely and that of  $Cu_4A_4R_2$  reasonable.

An alternative informative presentation of some of the calculations is given by the values of the equilibrium mol fractions among copper containing species. Some results for the reaction of copper acetate and uridine in DMSO solutions at the same total Cu(II) concentration,  $C_M$ , and mol ratio  $C_R/C_M$  of experimental points plotted in Fig. 1 are shown in Table II. Inspection of Table II reveals the truly equilibrium nature of the reaction. Mol fractions of reactants appear in columns 3 and 4 and of products in columns 5 and 6. As  $C_M$  increases the mol fractions of reactant binuclear and product tetranuclear complexes increase and those of reactant mononuclear and product binuclear complexes decrease. The mol fraction of monomer  $CuA_2$  is significant enough to justify its inclusion in the analysis. More unreacted dimer,  $Cu_2A_4$ , of high molar absorptivity appears at higher  $C_M$  concentrations consistent with the greater observed  $\epsilon$  values in Fig. 1. It is not until 60 mM  $C_M$  that the mol fractions of binuclear and tetranuclear products become comparable. Since the latter complex contains twice as

much copper, the metal ion becomes approximately evenly divided between the two products at about 20 mM  $C_M$ . At 60 mM  $C_M$  more than half the molecules are those of reactants even at high  $C_R/C_M$  mol ratios. However, because the tetranuclear complex contains twice as much Cu as the binuclear complex and four times as much as the mononuclear complex, most of the Cu is contained in products at  $C_R/C_M > 1$ . The mol fractions in Table II differ from the copper weighted mol fractions ( $\alpha$ s) defined previously in this paper. To obtain the latter from the concentration mol fractions in Table II, it is necessary to halve the values for  $CuA_2$ , double the value for  $Cu_4A_4R_2$ , add to the values given for the two binuclear species, rescale the sum to unity, and calculate the copper weighted mol fractions.

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