the geometry of the exchange pathways, the electronic structure and anisotropy of the M and X species, and the overlap of the different metal d orbitals with those on the XO_4 group. Clearly, these will be very sensitive to different M and X atoms, which accounts for the variety of magnetic structures seen in these compounds and the difficulty in rationalizing them.

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Supplementary Material Available: Table listing anisotropic thermal parameters (1 page); table of observed and calculated structure factors for β-$CrAsO_4$ (1 page). Ordering information is given on any current masthead page.

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Copper(I) Complexes with Bidentate Tertiary Phosphine Ligands: Solution Chemistry and Antitumor Activity

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Copper may play an important role in the antitumor activity of diphosphines. The tetrahedral bischelated copper(I) complexes $[Cu(dppey)_2]Cl$ and $[Cu(dppp)_2]Cl$, where dppey is $Ph_2PCH{=}CHPPh_2$ and dppp is $Ph_2P(CH_2)_3PPh_2$, were synthesized and characterized. The bridged dicopper(I) complex $(CuCl)_2(dppe)_3$, where dppe is $Ph_2P(CH_2)_2PPh_2$, underwent dissociative equilibria in $CDCl_3$ and CD_2Cl_2 solutions, as determined by temperature-dependent ^1H and ^{31}P NMR studies. One of the products was $[Cu(dppe)_2]^+$, the proportion of which increased on adding excess dppe. The complex was also a product from the reaction of Cu^{2+} with excess dppe in DMA. The complexes $[Cu(P\text{-}P)_2]Cl$, where P-P is dppey or dppp, and $(CuCl)_2(dppe)_3$ were all active against P388 leukemia, M5076 reticulum cell sarcoma, and B16 melanoma. $[Cu(eppe)_2]Cl$, where eppe is $Et_2P(CH_2)_2PPh_2$, was active only against P388 leukemia. The activities were comparable to those of the analogous Au(I) complexes, and complexes were more potent than the free ligands.

Introduction

1,2-Bis(diphenylphosphino)ethane (dppe) and related phenyl-substituted diphosphines exhibit antitumor activity in several murine tumor models.[2] The activities of the bridged digold(I) complexes $XAu(Ph_2P(CH_2)_nPPh_2)AuX$, where X is for example Cl or thioglucose,[2] and tetrahedral bischelated Au(I) complexes $[Au(Ph_2P(CH_2)_nPPh_2)_2]X$, where X is e.g. Cl,[3,4] are comparable to those of the free ligands but the Au(I) complexes are 5- to 10-fold more potent.[2-4] The bridged digold complexes readily undergo ring-closure reactions to form chelated complexes. For example, the formation of $[Au(dppe)_2]^+$ from XAu(dppe)AuX (where X is Cl or thioglucose) occurs in the presence of thiols or blood plasma.[5] The antitumor activity of the diphosphines and the bridged complexes is highest for ligands that are able to form five- or six-membered chelate rings (i.e. n = 2, 3 or cis CH=CH).

Copper may play an important role in the mechanism of the cytotoxicity and antitumor activity. Cu(II) salts potentiate the cytotoxicity of diphosphines,[6] and $[Au(dppe)_2]Cl$ reacts in solution with Cu(II) to give a Cu(I) complex.[3] The aim of the present work was to synthesize tetrahedral copper(I) diphosphine complexes in the hope that these might exhibit pronounced activity.

There have been relatively few previous investigations of copper(I) diphosphine complexes. More effort has been directed toward monodentate tertiary phosphine complexes. These exhibit a wide variety of coordination geometries and stoichiometries[7] of the general formula $L_m(CuX)_n$, where L is a monophosphine and X an anion. The structures include monomeric, dimeric, and tetrameric species that often undergo complicated dissociative equilibria in solution involving species with different m:n stoichiometries.[8,9]

For the bidentate diphosphines, dppe and dppm complexes with P:Cu ratios of 4:1, 3:1, 2:1, 3:2, 4:3, and 1:1 have been isolated.[10-20] Some have been structurally characterized. They often contain both bridging halide and diphosphine ligands.[11-15] These complexes may dissociate in solution, but there have been no detailed studies of their solution chemistry. The stability of Cl–Cu–Cl linkages is particularly relevant to biological studies in view of the high concentration of Cl^- ions in, for example, blood plasma. With analogous gold complexes such bridges are rare.

The only known example of a copper(I) tetrakis(phosphine) complex with a halide counterion appeared to be $[Cu(PMe_3)_4]Cl$.[21] However, we were recently able to characterize $[Cu(eppe)_2]Cl$ (where $Ph_2P(CH_2)_2PEt_2$ is eppe) as a complex with remarkably high thermodynamic and kinetic stability, similar to the analogous bischelated Ag(I) and Au(I) complexes.[22] In the present work

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we sought therefore to prepare further examples of bischelated copper(I) diphosphine complexes (using dppey, dppp, and dppe), to investigate their structures in solution by NMR methods, and to compare their antitumor activities with those of the analogous tetrahedral bischelated gold(I) diphosphine complexes.

Abbreviations for Ligands: dppe = $Ph_2P(CH_2)_2PPh_2$; dppp = $Ph_2P(CH_2)_3PPh_2$; dppey = *cis*-$Ph_2PCH{=}CHPPh_2$; eppe = $Et_2P(CH_2)_2PPh_2$.

Experimental Section

Materials. 1,2-Bis(diphenylphosphino)ethane (dppe), 1,2-bis(diphenylphosphino)propane (dppp), and *cis*-1,2-bis(diphenylphosphino)-ethylene (dppey) were purchased from Strem Chemicals Inc., CuCl was obtained from Aldrich Chemicals, and $CuSO_4 \cdot 5H_2O$ was from BDH Chemicals.

NMR Measurements. ^1H NMR spectra were recorded at 199.5 MHz on a JEOL FX200 spectrometer and were referenced to Me_4Si. $^{31}P\{^1H\}$ NMR spectra were recorded in 10-mm tubes on a JEOL FX60 (24.2 MHz) or a Bruker WM250 (101.3 MHz) spectrometer. H_3PO_4/D_2O (85:15 v/v) was used as an external shift reference.

Preparations. [Cu(eppe)$_2$]Cl and [Au(dppe)$_2$]Cl were prepared as described previously.[22,23]

[Cu(dppey)$_2$]Cl. *cis*-Dppey (0.66 g, 1.66 mmol) was dissolved in 25 mL of $CHCl_3$, and solid CuCl (0.078 g, 0.79 mmol) was added. After 1 h of stirring under a steady stream of N_2, all the solid had dissolved. The volume was reduced to ca. 5 mL by rotary evaporation, and the product, which was obtained as a white crystalline solid on cooling to 3 °C, was recrystallized from aqueous methanol (50 mL); yield (recrystallized) 0.48 g (66%).

Anal. Calcd for $C_{52}H_{44}ClCuP_4 \cdot 2H_2O$: C, 67.32; H, 5.21; P, 13.35; Cl, 3.82. Found: C, 67.35; H, 5.30; P, 13.49; Cl, 3.89. Mass spectral analysis by fast atom bombardment (University of London Intercollegiate Research Service, VG Analytical ZAB-1F instrument) gave the parent ion at *m/e* 855.

[Cu(dppp)$_2$]Cl. Dppp (1.11 g, 2.68 mmol) was dissolved in 50 mL of $CHCl_3$, and solid CuCl (0.132 g, 1.33 mmol) was added. This dissolved after stirring for 1 h under a steady stream of N_2 to give a pale yellow solution. A small amount of insoluble material was filtered off and the solvent removed by rotary evaporation. The resultant gum, which was solidified by repeated scratching in ice-cold hexane (1 mL) and ice-cold diethyl ether (1 mL), was recrystallized from methanol (10 mL) after the addition of H_2O (10 mL); yield (recrystallized) 0.76 g (59%).

Anal. Calcd for $C_{54}H_{52}ClCuP_4 \cdot 3H_2O$: C, 66.32; H, 5.98; P, 12.67; Cl, 3.62. Found: C, 66.67; H, 5.48; P, 12.99; Cl, 4.10. Mass spectral analysis by fast atom bombardment gave the parent ion at *m/e* 887, with the correct isotopic substitution pattern for $[Cu(Ph_2P(CH_2)_3PPh_2)_2]^+$.

(CuCl)$_2$(dppe)$_3$. Dppe (0.88 g, 2.2 mmol) was dissolved in $CHCl_3$ (10 mL), and solid CuCl (0.0087 g, 0.88 mmol) was added. After 2 h of stirring under a steady stream of N_2, all the solid had dissolved. A white precipitate, obtained after the addition of hexane (ca. 5 mL) and cooling to 3 °C, was filtered off and dried in vacuo.

Anal. Calcd for $C_{78}H_{72}P_6Cu_2Cl_2 \cdot 2CHCl_3$: C, 58.88; H, 4.57; P, 11.39; Cl, 17.38. Found: C, 58.35; H, 4.52; P, 11.49; Cl, 17.10.

Reaction of $CuSO_4$ with Dppe. $CuSO_4 \cdot 5H_2O$ (6.8 mg, 0.027 mmol) in H_2O (ca. 100 μL) was added to a solution of dppe (43 mg, 0.108 mmol) in *N,N*-dimethylacetamide (1 mL). After the solution was shaken for a few minutes, a clear colorless solution was obtained. A series of $^{31}P\{^1H\}$ NMR spectra were recorded over the temperature range 330–253 K, by containing the sample in an 8-mm tube, with acetone-d_6 in an external 10-mm tube for a ^2D lock.

In Vivo Antitumor Activity. This was measured for the four copper(I) diphosphine complexes in the ip P388 leukemia model in mice, under experimental conditions identical with those described in detail previously for $[Au(dppe)_2]Cl$.[3] Some of the compounds were also tested for antitumor activity against the ip tumors M5076 reticulum cell sarcoma and B16 melanoma by the same procedure as described for $[Au(dppe)_2]Cl$.

Results

Characterization of the Complexes and Solution Chemistry. Dppe Complexes. We reported previously[22] that the reaction of CuCl with a small molar excess of eppe produced the bischelated complex $[Cu(eppe)_2]Cl$. ^1H, ^{13}C, ^{31}P, and ^{65}Cu NMR studies all suggested that the complex adopted a tetrahedral geometry in solution. The reaction of CuCl with a slight excess of dppe was carried out under similar conditions. The isolated product was only moderately soluble in $CHCl_3$, CH_2Cl_2, and DMSO and was not significantly soluble in any other common solvent. The microanalytical data were consistent with the formulation $[(CuCl)_2(dppe)_3] \cdot 2CHCl_3$, and it seems likely that this has the same structure as the complex crystallized by Albano,[11] which has two CuCl moieties bridged by one dppe ligand and each chelated by another dppe ligand. The latter complex was isolated as a bis(acetone) solvate. The presence of a chloroform solvate in the present case was confirmed by the ^1H NMR spectrum in CD_2Cl_2. The structure of the complex in solution was investigated by ^1H and ^{31}P NMR.

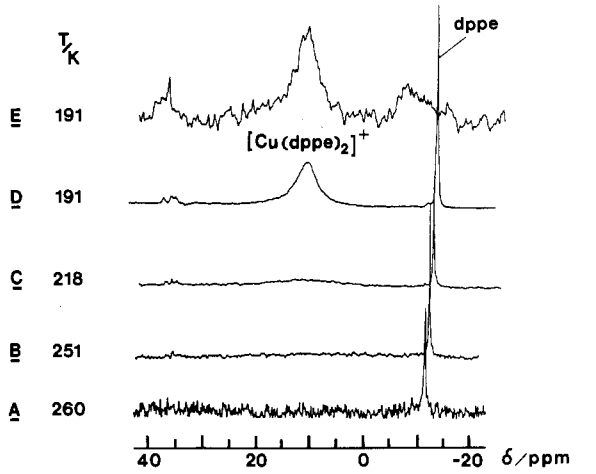

Figure 1. 24.2-MHz $^{31}P\{^1H\}$ NMR spectra: (E) a saturated solution of $(CuCl)_2(dppe)_3$ in CD_2Cl_2 at 191 K; (A–D) the same solution after the addition of 2.2 mol equiv of dppe at (A) 260, (B) 251, (C) 218, and (D) 191 K. The temperature dependence of the dppe resonance (−0.02 ppm/deg) is the same as that observed for dppe alone in this solvent. The broad resonance at 10.9 ppm in (D) is assigned to $[Cu(dppe)_2]^+$; see text.

The $^{31}P\{^1H\}$ NMR spectrum of $(CuCl)_2(dppe)_3$ displayed a temperature dependence indicative of a chemical exchange process. At 188 K the 101.2-MHz $^{31}P\{^1H\}$ NMR spectrum of a saturated solution of $[(CuCl)_2(dppe)_3]$ in CD_2Cl_2 consisted of two broad resonances at 10.0 and −9.1 ppm ($\Delta\nu_{1/2}$ = ca. 300 Hz) with an approximate intensity ratio of 2:1, together with minor broad resonances at −4.3 and 3.2 ppm ($\Delta\nu_{1/2}$ = ca. 250 Hz). As the temperature was raised, the peaks broadened and coalesced so that at 303 K the $^{31}P\{^1H\}$ NMR spectrum consisted of a single, very broad resonance, barely distinguishable from the base line noise.

The intensity ratio of the two major peaks in the spectrum at 188 K suggests that they may correspond to the nonequivalent phosphorus atoms in bridging and chelated environments. However, the chemical shift of the low-frequency resonance is close to that of free dppe (−13.0 ppm). Therefore, it seems likely that the solid-state structure of $(CuCl)_2(dppe)_3$ does not persist in solution and that dissociative equilibria occur with the release of free dppe. The minor resonances observed at −4.3 and 3.2 ppm may correspond to intermediate species in which the diphosphine ligand is monodentate.

In order to investigate further these dissociative processes, the effect on the NMR spectra of adding dppe to the solution of $(CuCl)_2(dppe)_3$ was studied. The 24.2-MHz $^{31}P\{^1H\}$ NMR spectrum of a saturated solution of $(CuCl)_2(dppe)_3$ in CD_2Cl_2 at 191 K is shown in Figure 1E. Two very broad resonances are just distinguishable at ca. 10.5 and ca. −9 ppm. This initial solution contained some undissolved compound, which may partly account for the poor resolution. On addition of 2.2 molar equiv of dppe, the solid dissolved to give a clear, colorless solution. The two broad peaks disappeared and were replaced by a single broad resonance at 10.9 ppm ($\Delta\nu_{1/2}$ = 140 Hz) and a sharp resonance at −12.6 ppm attributable to free dppe (Figure 1D). On warming of the solution, the dppe resonance remained sharp but the 10.9 ppm resonance gradually broadened, so that at 260 K the signal was too broad to be detected (Figure 1A). We assign the peak at 10.9

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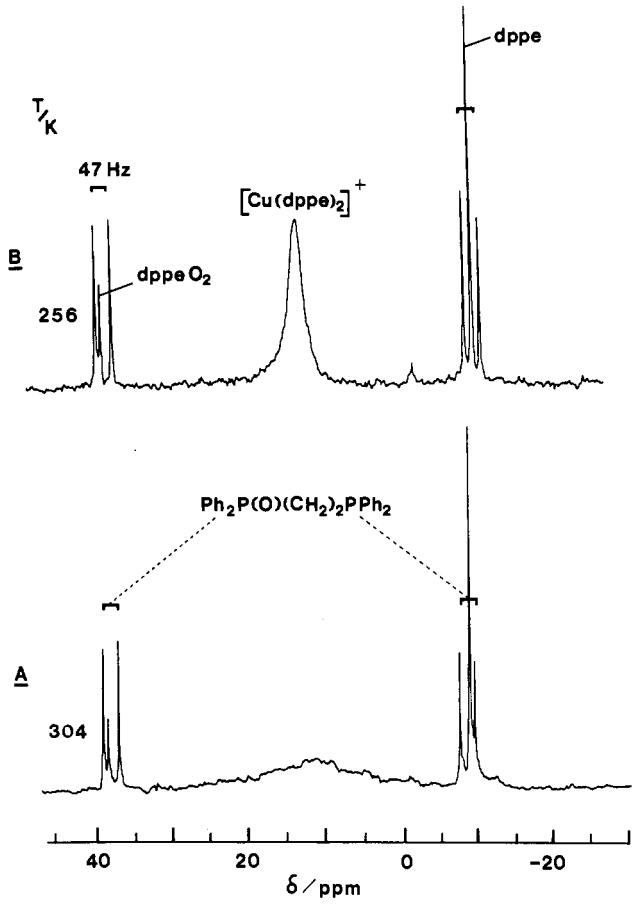

Figure 2. $^{31}P\{^1H\}$ NMR spectrum of dppe in dimethylacetamide with 0.25 mol equiv of $CuSO_4$ added at (A) 304 and (B) 256 K. The peak at 13 ppm is assigned to $[Cu(dppe)_2]^+$; see text.

ppm to the bischelated cation $[Cu(dppe)_2]^+$ since the chemical shift is similar to that of the tetrahedral complex $[Cu(eppe)_2]Cl$.[22] The variation in ^{31}P NMR line shape with decreasing temperature is consistent with the line shapes calculated by Marker and Gunter[24] for a CuP_4 complex undergoing fast quadrupolar relaxation.

The ^1H NMR spectra of solutions of $(CuCl)_2(dppe)_3$ in $CDCl_3$ and CD_2Cl_2 exhibited temperature dependences consistent with the occurrence of dissociative equilibria (see figure in supplementary material). In CD_2Cl_2 a peak at 2.42 ppm assignable to the CH_2 protons of $Cu(dppe)_2^+$ was present over the range studied (225–300 K). After the addition of dppe, only two resonances attributable to CH_2 protons were present in the aliphatic region: a sharp triplet at 2.10 ppm corresponding to free dppe and a slightly broadened peak at 2.42 ppm with a line shape that suggested the presence of unresolved ^{31}P–^1H coupling. The latter is assigned to the CH_2 protons of $[Cu(dppe)_2]^+$ since both the chemical shift and the line shape are similar to those of the previously characterized Au(I) species $[Au(dppe)_2]^+$.[23]

Reaction of Cu(II) with Dppe. To gain insight into the likely products of the reaction of dppe with a Cu(II) salt, we investigated the reaction of $CuSO_4$ with 4 molar equiv of dppe in dimethylacetamide. The color of the solution changed from blue to colorless within a few minutes of adding the phosphine, indicating that Cu(II) had been reduced to Cu(I). The ^{31}P NMR spectrum of this colorless solution at 304 K is shown in Figure 2A. The sharp peaks at −8.6 ppm and 38.4 were assigned to dppe and the bis(oxide) $Ph_2P(O)(CH_2)_2P(O)Ph_2$, respectively, and the pair of doublets at 38.0 and −7.9 ppm ($^3J(^{31}P–^{31}P)$ = 47 Hz) to the mono(oxide) $Ph_2P(O)(CH_2)_2PPh_2$. These assignments were made by comparison with the ^{31}P NMR spectra of the authentic oxides. An additional very broad peak was just visible at 13 ppm. On cooling of the solution, this peak sharpened, and at 256 K the line width at half-height was 60 Hz (Figure 2B). The chemical shift of the peak and the temperature dependence of its line shape are similar to that observed in the ^{31}P NMR spectrum of $(CuCl)_2(dppe)_3$ (Figure 1) when appropriate allowances are made for solvent differences, and it seems reasonable therefore to assign it to $[Cu(dppe)_2]^+$. It is apparent from Figure 2 that exchange reactions of free dppe with Cu(I) are very slow on the NMR time scale because the dppe resonance is very sharp at all temperatures studied.

Dppey and Dppp Complexes. In contrast to the reaction of CuCl with dppe, the products isolated from the reactions of CuCl with 2 molar equiv of either dppey or dppp, were very soluble in a variety of solvents including methanol, ethanol, acetone, and $CHCl_3$. The mass spectral and analytical data indicated that the products were the bischelated complexes $[Cu(dppey)_2]Cl$ and $[Cu(dppp)_2]Cl$, respectively. The ^1H NMR and $^{31}P\{^1H\}$ NMR spectra of $[Cu(dppey)_2]Cl$ were fully consistent with a tetrahedral coordination geometry. The $^{31}P\{^1H\}$ NMR spectrum of the complex in $CDCl_3$ at 302 K consisted of a very broad peak at 8.4 ppm ($\Delta\nu_{1/2}$ = 800 Hz). $^{63/65}Cu$–^{31}P couplings were not resolved. On cooling of the solution to 223 K, the peak shifted to slightly higher frequency (13.6 ppm) and sharpened ($\Delta\nu_{1/2}$ = 170 Hz). This sharpening is indicative of an increase in the rate of the quadrupolar relaxation.[24] The ^1H NMR spectrum gave no evidence for any dissociation of the complex in either $CDCl_3$ or acetone-d_6 solution. The CH=CH protons gave rise to a second-order multiplet pattern similar to that previously observed for $[Au(dppey)_2]Cl$.[23] This was attributed to spin–spin coupling of each proton with all four phosphorus atoms. The chemical shift and multiplet pattern of the phenyl protons in $[Cu(dppey)_2]Cl$ are very similar to those of the Au(I) complex, but the CH=CH protons are slightly deshielded with respect to $[Au(dppey)_2]Cl$ so that in $CDCl_3$ the CH=CH multiplet pattern is not obscured by overlapping resonances from the phenyl protons. In acetone-d_6 the methine protons were deshielded further, consistent with the trend observed for $[Au(dppey)_2]Cl$.

The ^1H NMR spectrum of $[Cu(dppp)_2]Cl$ in methanol-d_4 was very similar to that of the previously characterized[23] $[Au(dppp)_2]Cl$. Only one set of resonances was observed for the two types of CH_2 environments (see table in supplementary material: ^1H NMR data for $[Cu(dppey)_2Cl]$ and $[Cu(dppp)_2]Cl$). ^{31}P–^1H couplings were not resolved, and the line shapes were similar to those of the tetrahedral Au(I) complex, indicating that they correspond to the same second-order spin systems. There was no evidence of significant dissociation of dppp in methanol, but the behavior appeared to be more complicated in $CHCl_3$. Although only a single set of CH_2 resonances at 2.34 and 1.61 ppm were observed in the spectrum at 310 K, the peaks were broadened with respect to those of the Au(I) analogue. In addition, when the solution was cooled slightly to 294 K, the low-frequency resonance broadened further and an additional peak appeared at 2.47 ppm. There were also significant changes in the appearance of the phenyl resonances. This suggests that the bischelated complex dissociates to some extent in $CDCl_3$. Curiously, the 24.2-MHz ^{31}P NMR spectrum of $[Cu(dppp)_2]Cl$ did not appear to be dependent on the solvent. In methanol-d_4 the spectrum at 302 K consisted of a peak at −10.7 ppm, which, although broad ($\Delta\nu_{1/2}$ = 270 Hz), was sharper than that observed for $[Cu(dppey)_2]Cl$ at 302 K. On cooling of the solution to 218 K, the peak shifted slightly to higher frequency (−4.8 ppm) but the line width did not change significantly. Similar behavior was observed for a solution of $[Cu(dppp)_2]Cl$ in $CDCl_3$ (δ −13.1 at 302 K). Comparison with the ^{31}P NMR line shapes calculated by Marker and Gunter for CuP_4 complexes indicated that the observed line shapes were consistent with those expected for very fast rates of quadrupolar relaxation, but the ^{31}P NMR spectra alone did not confirm a tetrahedral geometry in solution.

Antitumor Activity. The activities of the isolated and characterized chelated copper(I) diphosphine complexes against ip P388 leukemia in mice are shown in Table I. The complexes were administered by ip injection on days 1–5 following tumor im-

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Table I. Antitumor Activity of the Copper(I) Diphosphine Complexes in Mice Bearing ip P388 Leukemia, ip M5076 Reticulum Cell Sarcoma, and ip B16 Melanoma

compd	MTD,[a] μmol/(kg day)	ILS,[b] % P388	M5076	B16
$[(CuCl)_2(dppe)_3]$	2	100, 115	60	54
$[Cu(dppp)_2]Cl$	2 (3)	89 ± 14[d]	31	42
$[Cu(dppey)_2]Cl$	1 (2)	66 ± 20[d]	40	32, 47
$[Cu(eppe)_2]Cl$	2	48, 45	neg[c]	neg
$[Au(dppe)_2]Cl$	3	86 ± 25[e]	57 ± 15[f]	38 ± 9[f]
$[Au(eppe)_2]Cl$	4	54 ± 16[d]	neg	neg

[a] Maximally tolerated dose for B6D2F mice on an ip qD × 10 regimen (ip qD × 5 for P388). [b] Maximum increase in lifespan produced in mice bearing the ip tumor with respect to nontreated control animals; the mean is given ± standard deviation; figures separated by a comma represent data generated in separate experiments. A compound is considered to be active in these systems if it produces >40% ILS against P388 leukemia and >25% ILS in the other two tumor models. [c] Inactive. [d] Based on 4 experiments. [e] Based on 33 different experiments. [f] Based on 5 experiments.

plantation, and the degree of antitumor activity was assessed by the percent increase in lifespan (ILS) with respect to untreated control animals, at the maximum dose (MTD) tolerated by the animal. The Cu(I) complexes containing only phenyl-substituted ligands (i.e. dppe, dppp, and dppey) exhibited antitumor activities comparable to that of $[Au(dppe)_2]Cl$ at a similar MTD (2–3 μmol/kg).[3] $[Cu(eppe)_2]Cl$, although equally toxic to the animal, showed reduced antitumor activity in P388 leukemia and was inactive in mice bearing M5076 reticulum cell sarcoma and B16 melanoma. We observed a similar reduction in activity for Au(I) analogues of $[Au(dppe)_2]Cl$, when the phenyl substituents were exchanged for ethyls (Table I).

The Cu(I) complexes containing only phenyl-substituted diphosphines were also evaluated against the ip implanted tumors M5076 reticulum cell sarcoma and B16 melanoma (Table I). All exhibited activity comparable to that of $[Au(dppe)_2]Cl$.

Discussion

The stability of bischelated diphosphine complexes of copper(I) chloride appears to be highly dependent on the nature of the phosphine ligand. We were unable to isolate $[Cu(dppe)_2]Cl$ by reaction of CuCl with an excess of dppe. Instead, the dppe-bridged complex $(CuCl)_2(dppe)_3$ was obtained. However, the bischelated complexes were isolated for the ligands eppe, dppey, and dppp. As judged by NMR, $[Cu(dppp)_2]Cl$ appeared to undergo solvent-dependent dissociation in solution, but $[Cu(eppe)_2]Cl$[22] and $[Cu(dppey)_2]Cl$ did not appear to dissociate significantly in solution.

Tetrakis(tertiary phosphine) complexes of Cu(I) halides are rare. To the best of our knowledge $[Cu(PMe_3)_4]Cl$ is the only other example of a complex of this type.[21] Generally, halide binds with the exclusion of the fourth phosphine ligand. For instance, Lippard[8] reported that $Cu(PR_3)_3Cl$ was isolated from reaction of CuCl with 4 or more molar equiv of PPh_3, $PMePh_2$, or PMe_2Ph. It seems likely that steric effects play a role in these reactions. Tetrakis complexes of the type $[CuP_4]X$ where X is a noncoordinating anion have been isolated. For instance, $[Cu(PPh_3)_4]ClO_4$ has been prepared from the reaction of $Cu^{II}(ClO_4)_2$ with an excess of PPh_3,[25] and $[Cu(dppe)_2]^+$ has been isolated with CF_3COO^-, $[Cu(mesityl)_2]^-$, and NO_3^- counteranions.[17,19,20] The higher stability of the bischelated complex of dppey may arise because the rigid cis geometry of the ligand favors chelate ring formation. For dppp, steric interactions in the bischelated complex might be expected to be less severe than for dppe as a result of the increased size of the chelate rings. However, the NMR data indicate that in chloroform solution, Cl^- displacement of the dppp ligands occurs to some extent.

There are several examples in the literature of complexes with a 2:3 Cu(I):dppe stoichiometry. Complexes of the type $(CuX)_2(dppe)_3$ where X = halide,[11] acetate,[16] NO_3,[17] and azido[18] have been isolated. Crystal structure determinations of the Cl^- and azido complexes have shown that they have similar dinuclear structures, each Cu(I) being bound to one chelated and one bridged dppe. The NMR studies described here suggest that $[(CuCl)_2(dppe)_3]$ is involved in a series of solvent- and temperature-dependent dissociative equilibria in solution, involving release of dppe. Consideration of possible dissociation mechanisms leads to the proposal of the equilibria

$$[(CuCl)_2(dppe)_3] \rightleftharpoons [\{CuCl(dppe)\}]_2 + dppe \quad (1)$$

$$[(CuCl)_2(dppe)_3] \rightleftharpoons [Cu(dppe)_2]^+ + [CuCl_2]^- + dppe \quad (2)$$

Edwards and Richards[16] proposed similar schemes for dissociation of the analogous bis(acetate) complex in solution on the basis of molecular weight and conductivity measurements. In our study the ^1H and ^{31}P NMR spectra both provide strong evidence that $[Cu(dppe)_2]^+$ is one of the dissociation products. In the presence of excess dppe, the bischelated complex was the only Cu(I) dppe species observed in solution, suggesting that the equilibrium

$$[(CuCl)_2(dppe)_3] + dppe \rightleftharpoons 2[Cu(dppe)_2]Cl$$

lies well to the right. It is evident from Figures 1 and 2 that $[Cu(dppe)_2]^+$ undergoes slow exchange with free dppe on both the ^1H and ^{31}P NMR time scales, indicating a similarly high *kinetic* stability compared to that previously observed for the tetrahedral Au(I)[26] and Ag(I)[27] $(dppe)_2$ complexes.

Our ^{31}P NMR studies provide strong evidence that the Cu(I) species $[Cu(dppe)_2]^+$ is formed by reaction of $Cu^{II}SO_4$ with an excess of dppe. However, we did not attempt to isolate the product from solution. Anderson and co-workers[17] isolated a colorless product formulated as $(CuNO_3)_3(dppe)$ by a similar reduction of $Cu(NO_3)_2$ with dppe. This was converted to $[Cu(dppe)_2]NO_3$ by bubbling O_2 through a solution of the compound in hot ethanol.

The tetrahedral $[Cu(P\text{-}P)_2]^+$ complexes might be expected to give rise to two overlapping 1:1:1:1 quartets in their ^{31}P NMR spectra due to spin–spin coupling to the two spin-$^3/_2$ nuclei ^{63}Cu and ^{65}Cu. However, these have been resolved only for highly symmetrical $[Cu(PR_3)_4]^+$ complexes, such as $[Cu(P(OR)_3)_4]^+$ where R = Me, Et, and nBu.[24,28,29] For complexes containing bulkier phenyl-substituted phosphites or phosphines, the line widths are too large to resolve ^{31}P–$^{63/65}Cu$ couplings.[29] Presumably, steric interactions within these molecules give rise to significant distortions from tetrahedral symmetry, nonzero electric field gradients, and, consequently, efficient quadrupolar relaxation. Steric interactions within the bischelated complexes are likely to be very severe.

Anticancer Activity. The copper(I) diphosphine complexes exhibit antitumor activities against ip P388 leukemia in mice comparable to those of the bischelated gold(I) diphosphine complexes.[3,4] Both the Au(I) and Cu(I) complexes are at least 20-fold more potent than the ligands alone.[2] The maximum tolerated dose of dppe, for example, is 50 μmol/kg. At this dose dppe gives an ILS of 107% for P388 leukemia. The preliminary results of activities against the other ip tumors B16 melanoma and M5076 reticulum cell sarcoma suggest that the Cu(I) complexes may exhibit a spectrum of antitumor activity similar to that of $[Au(dppe)_2]Cl$. Dppe is itself active in these tumor models also.[2] The potent activity of the Au(I) complex may be due to a subtle combination of thermodynamic and kinetic stabilities so that the ligand is delivered to critical cellular sites with protection from unfavorable oxidation reactions, and in addition, there is sufficient lability in the Au–P bond so that ring opening can readily occur. These studies suggest that $[Cu(dppe)_2]^+$ has an enhanced kinetic stability similar to that of $[Au(dppe)_2]^+$.[23,26] We observed pre-

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viously[22] that the Au(I), Ag(I), and Cu(I) complexes $[M(eppe)_2]^+$ underwent an intramolecular inversion process in solution involving facile breaking of at least one M-P bond. The kinetic stability of the Cu(I) complex was slightly higher than that of the Ag(I) and Au(I) complexes.

The effect of exchanging phenyl substituents for ethyls in $[Cu(eppe)_2]Cl$ is to increase water solubility and decrease antitumor activity. A similar trend was observed for the Au(I) series, and the reduced activity was attributed partly to a greater reactivity of $[Au(eppe)_2]Cl$ toward protein disulfide bonds.[4] These were cleaved in model reactions with release of the phosphine oxide.

The cytotoxic potency of dppe in vitro and its toxicity in vivo are significantly increased when dppe is incubated in the presence of noncytotoxic concentrations of Cu(II) salts.[6] It is possible that complexation to the metal protects the ligand from oxidation and promotes its uptake into cells. In addition, delivery of Cu(I) into a cell, or its translocation within cells, could play an additional important role in the cytotoxicity of the copper(I) diphosphine complexes. Copper has been implicated in the anticancer activity of a number of potential chelating agents, such as thiosemicarbazones,[30] 2,9-dimethyl-1,10-phenanthroline,[31] 1,10-phenanthroline,[32] and 4'-(9-acridinylamino)methanesulfon-*m*-anisidide.[33] For the latter two agents there is evidence that DNA strand cleavage results from a Cu(II)-dependent production of oxygen free radicals. We have observed[34] by agarose gel electrophoresis that $(CuCl)_2(dppe)_3$, not $[Au(dppe)_2]Cl$, produced single-strand breaks in supercoiled plasmid-DNA. Further experiments are required to investigate whether molecular O_2 is involved in the strand scission.

There are clearly many problems in identifying whether copper(I) diphosphine complexes play a key role in the cytotoxicity and antitumor activity of diphosphine ligands. The Cu(I) dppe system is particularly complicated. The studies reported here have shown that Cu(II) will react with an excess of dppe to form the bischelated species $[Cu(dppe)_2]^+$. However, we have not investigated the effect of Cl^- ions on this reaction, and from the above discussion it is clear that chloride could successfully compete as a ligand and a number of diphosphine- and halide-bridged species may be present in solution. The position of equilibria involving Cl^- could change greatly on passage of the complex from outside cells, where the Cl^- concentration is high (ca. 104 mM), to inside cells, where the concentration is much lower (ca. 3 mM). In addition, copper(II) phosphine oxide complexes could be formed. Anderson et al. have reported[17] that $(CuNO_3)_2(dppe)_3$ readily converts into a copper(II) bis(phosphine oxide) complex in chlorinated solvents.

These studies have shown that the bischelated complex $[Cu(dppey)_2]Cl$ exhibits good antitumor activity in animal models, and in contrast to the Cu(I) dppe complexes, it has a well-defined structure in solution. These properties make it highly suitable as a probe for investigating the effect of copper(I) diphosphines on critical cellular processes, with the aim of elucidating the possible role of copper in the antitumor activity of diphosphine ligands and complexes. The mixed-ligand complex $[Cu(eppe)_2]Cl$ is less active in vivo, but it is still potently cytotoxic. It also has a well-defined solution chemistry, and its high water solubility may make it more suitable for some model studies.

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Supplementary Material Available: A figure showing 200-MHz ^1H NMR spectra of saturated solutions of $(CuCl)_2(dppe)_3$ at various temperatures and a table listing ^1H NMR data for $[Cu(dppey)_2]Cl$ and $[Cu(dppp)_2]Cl$ (2 pages). Ordering information is given on any current masthead page.

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Electron Transfer between Hemoglobin and Arenediazonium Salts. Mechanism of Heme Aryl-Iron Complex Formation

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Two distinct processes characterize the electron-transfer reactions between hemoglobin and arenediazonium tetrafluoroborate salts. In the first, oxidation of hemoglobin by arenediazonium ions that possess polar substituents (*p*-NO_2, *p*-CN, *p*-CH_2COO^-, and *p*-PhNH) results in the formation of methemoglobin and arene products obtained by hydrogen abstraction. Their reaction rate constants correlate with those from ferrocyanide oxidation and with Hammett σ values (ρ = 3.0). In the second, reactions of hemoglobin with arenediazonium ions whose substituents are more hydrophobic (*p*-Cl, *p*-F, *p*-CH_3, *p*-Et, *p*-Me_2CH, *p*-Me_3C, *p*-$CH_3(CH_2)_4CH_2O$, *p*-CH_3O) form σ-bonded aryliron(III) complexes. Their rate constants are greater than predicted from the Hammett plot, but there is good correlation with the hydrophobicity parameter π. These results are explained by a mechanism in which electron transfer either takes place in the aqueous medium surrounding the hemoprotein, where hydrogen atom abstraction from a hydrogen donor solvent is the preferred process, or at the hydrophobic surface of hemoglobin, after which the neutral aryl radical enters the heme pocket to form the σ-arylheme adduct.

Introduction

Arylhydrazines react with hemoglobin and myoglobin in the presence of dioxygen to form σ-bonded aryliron(III) complexes whose thorough characterization by ^1H NMR spectroscopy in the intact protein[1,2] and by X-ray crystallography of phenylmyoglobin[3] has recently been reported. Although stable within the protein, these heme adducts normally rearrange to their corresponding *N*-arylprotoporphyrin IX complexes when the prosthetic group is extracted aerobically from the inactivated hemoprotein,[2,4,5] and

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