



Ternary Copper(II) Complexes of the Anticonvulsant Drug Valproate with Diimines as Superoxide Dismutase Mimics

A. Latif Abuhijleh

Chemistry Department, Birzeit University, West Bank, Palestine

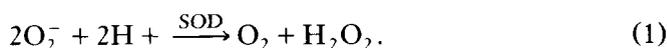
Abstract

Mononuclear complexes of the type $[\text{Cu}(\text{valp})_2(2,2\text{-bpy})]\text{H}_2\text{O}$ (2), $\text{Cu}(\text{valp})_2(\text{phen})$ (3), and $\text{Cu}(\text{valp})_2(\text{dmpb})$ (4), and polynuclear complexes of the type $[\text{Cu}(\text{valp})_2(\mu\text{-}4,4\text{-bpy})]_n$ (5), and $[\text{Cu}_2(\text{valp})_4(\mu\text{-}4,4\text{-bpy})]_n$ (6) [valp = valproate ion, bpy = bipyridine, phen = 1,10-phenanthroline, and dmpb = 2,9-dimethyl-1,10-phenanthroline], were synthesized and characterized by magnetic and spectroscopic measurements. Spectral and magnetic data for complexes 2, 3, and 4 and the preliminary x-ray measurements for complex 3 are consistent with mononuclear structure. The copper atom in 2 and 3 is coordinated to two nitrogen atoms from diimine, two asymmetric carboxylic oxygen atoms from each of a bidentate valproate ions to give a tetragonally elongated $\text{CuN}_2\text{O}_2 + \text{O}_2$ chromophore. The copper atom in complex 4 is coordinated to two nitrogen atoms from dmpb, two carboxylic oxygens of a bidentate valproate ion, and an oxygen atom of a monodentate carboxylic group of a second valproate ion to give a very distorted square pyramid or trigonal bipyramid CuN_2O_3 chromophore. The preceding data for 5 and 6 indicate that these complexes are polymeric in which the nitrogen atoms of 4,4'-bpy ligands bridge mononuclear $\text{Cu}(\text{valp})_2$ moieties, coordinating equatorially in complex 5, while bridge binuclear $\text{Cu}(\mu\text{-valp})_4\text{Cu}$ moieties, coordinating axially in complex 6. The superoxide dismutase-mimetic activity of the binary complex, $\text{Cu}_2(\text{valp})_4$ (1), and of the previous ternary complexes was measured and found to be in the order: $2 \approx 3 > 6 > 4 > 1 > 5$. *Journal of Inorganic Biochemistry* 68, 167-175 (1997) © 1997 Elsevier Science Inc.

Introduction

The reactive superoxide free radical anion (O_2^-), which is a product of the oxygen metabolic cycle [1], was implicated in a wide range of clinical disorders. For example, it is supposed to be implicated as a cause of tissue inflammation, aging, and some cancers [2]. Superoxide dismutases (SODs) are metalloenzymes that catalyze the dismutation of the superoxide anion to oxygen and hydrogen peroxide [Eq. (1)] and protect the living cells against the

toxicity of the superoxide anion [3],



Hydrogen peroxide formed in this reaction is destroyed *in vivo*, for example, by catalase. One of these metalloenzymes is Cu, Zn-SOD which is a dimeric protein (MW = 31200) with two identical subunits, each containing one Cu^{2+} and one Zn^{2+} ion. The direct utilization of this natural enzyme as a pharmaceutical agent is limited because of low membrane permeability as a consequence of its high molecular weight [5]. So considerable efforts were made in order to obtain nontoxic, low molecular weight biomimetic molecules which are able to catalyze the dismutation of superoxide anion and therefore to provide a suitable alternative to superoxide dismutase in clinical application [6]. A variety of low molecular weight complexes of transition metals, especially those of copper, were prepared and studied as SOD mimics [4, 7, 8, 9]. Examples of such complexes include derivatives of anti-inflammatory drugs salicylates, amino acids, peptides, and amines [7, 8, 9].

Valproic acid (2-propylpentanoic acid) in the form of its sodium salt, $(\text{CH}_3\text{CH}_2\text{CH}_2)_2\text{CHCOO}^- \text{Na}^+$, has a wide spectrum of activity as an anticonvulsant drug [10]. The observation that copper(II) complexes of anticonvulsant and anti-inflammatory drugs are more active agents than the free drugs led to the suggestion that the activity of such drugs may be due to the *in vivo* formation of metallic complexes [11]. Physical studies of copper(II) valproate [12] have shown that it contains binuclear units with bridging carboxylate ligands similar to other copper(II) carboxylates [13]. Several binuclear copper(II) carboxylates of anti-inflammatory drugs such as salicylates [8], indomethacin [14], and lonazolac [15] were studied as SOD mimics, but not of anticonvulsant drugs such as valproate. In addition, it was reported that the presence of coordination sites belonging to nitrogen heteroatomic rings such as imidazoles or pyridines is important for high SOD activity [16]. And since complexes with bipyridines and phenanthrolines are DNA intercalators, showing an ability to inhibit nucleic acid synthesis *in vivo* [17], these ligands are used in this study to form ternary copper(II) valproate complexes. In addition, we examined the SODlike activity of these ternary complexes and of the binary binuclear copper(II) valproate complex and they are compared to the activity of the native SOD enzyme.

Address correspondence to: Dr. A. Latif Abuhijleh, Chemistry Department, Birzeit University, P.O. Box 14, West Bank, Via Israel; E-mail: latif@science.birzeit.edu.

Experimental

Reagents and Materials

All chemicals were of high purity grade (Aldrich or Sigma Chemical Co.) and were used without further purification. Tetrakis- μ -valproato dicopper(II), $\text{Cu}_2(\text{valp})_4$ (**1**), (valp = valproate ion) was prepared according to a published procedure [12] and recrystallized from absolute ethanol.

Preparation of Complexes

Bis(valproato)(2,2'-bipyridine)Copper(II) monohydrate, $[\text{Cu}(\text{valp})_2(2,2'\text{-bpy})\text{H}_2\text{O}]$, (2**).** A solution of 0.127 g (0.8 mmol) of 2,2'-bipyridine in 20 ml methanol was added to 0.283 g (0.4 mmol) of $\text{Cu}_2(\text{valp})_4$. The mixture was stirred at room temperature for 2 h. The blue solution was filtered and left in the hood to evaporate. The blue crystals that formed were recrystallized from chloroform and air dried. Anal. Calcd.: for $\text{C}_{26}\text{H}_{40}\text{N}_2\text{O}_5\text{Cu}$: C, 59.60; H, 7.64; N, 5.34. Found: C, 59.68; H, 7.56; N, 5.38%.

Bis(valproato)(1,10-phenanthroline) copper(II), $[\text{Cu}(\text{valp})_2(\text{phen})]$, (3**).** A solution of 0.144 g (0.8 mmol) of 1,10-phenanthroline in 20 ml methanol was added to 0.280 g (0.4 mmol) of $\text{Cu}_2(\text{valp})_4$. The mixture was stirred at room temperature for 1.5 h. The blue solution was filtered and left in the hood to evaporate. The bluish-green crystals that formed were recrystallized from chloroform and air dried. Anal. Calcd.: for $\text{C}_{28}\text{H}_{38}\text{N}_2\text{O}_4\text{Cu}$: C, 63.45; H, 7.17; N, 5.29. Found: C, 63.57; H, 7.08; N, 5.35%.

Bis(valproato) (2,9-dimethyl-1,10-phenanthroline) Copper(II), $[\text{Cu}(\text{valp})_2(\text{dmpb})]$, (4**).** A solution of 0.156 g (0.75 mmol) of 2,9-dimethyl-1,10-phenanthroline in 25 ml methanol was added dropwise to 0.258 g (0.37 mmol) of $\text{Cu}_2(\text{valp})_4$. The solution was protected from the light and stirred at room temperature for 2 h. The green solution was filtered and left in the hood to evaporate. The yellowish-green precipitate that formed was recrystallized from chloroform and air dried. Anal. Calcd.: for $\text{C}_{30}\text{H}_{42}\text{N}_2\text{O}_4\text{Cu}$: C, 64.57; H, 7.53; N, 5.02. Found: C, 63.96; H, 7.84; N, 5.01%.

Bis(valproato)(μ -4,4'-bipyridine)Copper(II), $[\text{Cu}(\text{valp})_2(\mu\text{-4,4'}\text{-bpy})_n]$, (5**).** A solution of 0.116 g (0.74 mmol) of 4,4'-bipyridine in 25 ml methanol was added to a solution of 0.269 g (0.37 mmol) of $\text{Cu}_2(\text{valp})_4$ in 25 ml chloroform. The blue solution was stirred at room temperature for 2 h. The solution was filtered and left in the hood to evaporate. The purple precipitate that formed was recrystallized from (dimethylformamide) DMF- CH_2Cl_2 (1:3) and air dried. Anal. Calcd.: for $\text{C}_{26}\text{H}_{38}\text{N}_2\text{O}_4\text{Cu}$: C, 61.72; H, 7.52; N, 5.54. Found: C, 61.91; H, 7.62; N, 5.32%.

Tetrakis(μ -valproato) (μ -4,4'-bipyridine)dicopper(II), $[\text{Cu}_2(\text{valp})_4(\mu\text{-4,4'}\text{-bpy})_n]$, (6**).** A solution of 0.58 g (0.37 mmol) of 4,4'-bipyridine in 20 ml methanol was added to a solution of 0.269 g (0.37 mmol) of $\text{Cu}_2(\text{valp})_4$ in 25

ml chloroform. The bluish-green solution was stirred at room temperature for 2 h. The solution was filtered over an anhydrous diethylether and left in the hood to evaporate. The green precipitate that formed was recrystallized from chloroform and air dried. Anal. Calcd.: for $\text{C}_{42}\text{H}_{68}\text{O}_8\text{N}_2\text{Cu}_2$: C, 58.95; H, 7.95; N, 3.27. Found: C, 58.91; H, 8.15; N, 3.15%.

Physical Measurements

Elemental analyses for C, H, and N were performed by Galbraith Laboratories, Knoxville, TN, U.S.A. Magnetic susceptibility measurements at 298 K of powdered samples were determined by the Gouy method, with $\text{HgCo}(\text{NCS})_4$ as a calibrant, and corrected for diamagnetism with appropriate Pascal constants. The effective magnetic moment was calculated from the expression: $\mu_{\text{eff}} = 2.84(\chi T)^{1/2}$.

Infrared spectra of nujol or hexachlorobutadiene mulls sealed between polyethylene sheets were obtained in the 4000 to 200 cm^{-1} region with a Perkin-Elmer model 843 infrared spectrophotometer. Electronic spectra of dichloromethane or methanol solutions were obtained with a Bausch and Lomb spectronic 2000. X-band electron spin resonance (ESR) spectra of powdered samples and of methanol/toluene (or DMF/ CHCl_3 (3:1) for complex (**5**) solutions at liquid nitrogen temperature were taken with a JEOL Jes-PE-1X spectrometer. Diphenylpicrylhydrazide (DPPH, $g = 2.0036$) was used as the calibrating field marker.

Superoxide Dismutase Assay

A standard assay method [8, 18] using alkaline dimethyl sulfoxide as a source of a superoxide generating system and nitro blue tetrazolium chloride (NBT) as a scavenger for superoxide was performed to measure the superoxide dismutase-mimetic activity of the copper(II) complexes. In a typical experiment 0.4 ml dimethyl sulfoxide (Me_2SO) solution of a copper(II) complex to be assayed was added to a solution containing 2.1 ml of 0.2 M potassium phosphate buffer (pH 8.6) and 1 ml of 75 μM NBT. The mixture was kept in ice for 15 min and then 1.5 ml alkaline Me_2SO solution (5mM NaOH and 0.55 M deionized water) containing superoxide ions was added with stirring. The absorbance of the violet color developed (diformazane formation) was monitored at 560 nm against a sample prepared under similar conditions except that NaOH was absent in the Me_2SO solution. The amount of copper complex which gives a 50% inhibition of NBT reduction was obtained from a plot of percent inhibition versus complex concentration.

Results and Discussion

Magnetic and Spectroscopic Characterization

The effective magnetic moments and electronic and IR spectral data are summarized in Table 1. The room temperature magnetic moments for complexes **2**, **3**, **4**,

Table 1. Magnetic Moments and Electronic and IR Spectral Data for Cu(II) Valproate Complexes

Compound	$\mu_{\text{eff}}(\text{BM})$ (298 K)	$\lambda_{\text{max}}^{(\text{nm})}$ in CH_3OH ($\epsilon = \text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$)	$\lambda_{\text{max}}^{(\text{nm})}$ in CH_2Cl_2 ($\epsilon = \text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$)	$\nu_{\text{asym}}(\text{CO}_2)$ (cm^{-1})	$\nu_{\text{sym}}(\text{CO}_2)$ (cm^{-1})
$\text{Cu}_2(\text{valp})_4$, (1)	1.33	694(320)	671(410) 375(146)	1580	1422
$\text{Cu}(\text{valp})_2(2,2\text{-bpy})$, (2)	1.90	670(60)	700(90) 390(78)	1588	1401
$\text{Cu}(\text{valp})_2(\text{phen})$, (3)	1.90	680(65)	700(90) 400(74)	1582	1400
$\text{Cu}(\text{valp})_2(\text{dmph})$, (4)	1.95	750(75)	820(195)	1610 1595	1380 1420
$\text{Cu}(\text{valp})_2(4,4\text{-bpy})$, (5)	1.85	680 ^a	704(210) 388(270)	1572	1415
$\text{Cu}_2(\text{valp})_4(4,4\text{-bpy})$, (6)	1.30	688 ^a 370(sh) ^b	695(430) 383(270)	1620	1419

^a These complexes are not completely soluble in methanol and ϵ values are not included.

^b sh = shoulder.

and 5 are in the range 1.85–1.95 BM. These values are consistent with the presence of one unpaired electron in a mononuclear copper(II) complexes. The room temperature magnetic moment for complex 6 is 1.30 BM. This subnormal value is lower than the spin-only magnetic moment of 1.73 BM, suggesting that coupling between copper(II) ions occurs. This value is comparable to the magnetic moment values of binuclear or polynuclear copper(II) carboxylate adducts of the type $[\text{Cu}(\text{RCOO})_2\text{L}]_2$ [13], including those reported for binuclear copper(II) valproate adducts [19, 20].

The electronic spectra for complexes 2, 3, and 5 obtained in methanol solutions exhibit one very broad absorption band in the 670–680 nm region (Table 1). This band is assigned to the copper(II) *d-d* transitions. The position of this band falls within the range expected for mononuclear copper(II) complexes that contain a $\text{CuN}_2\text{O}_2 + \text{O}_2$ chromophore in a distorted tetragonal geometry [20–22]. It is comparable to those found for mononuclear copper(II) valproate complexes with imidazoles [21] which contain the *trans*- or *cis*- $\text{CuN}_2\text{O}_2 + \text{O}_2$ chromophore, including that for structurally known mononuclear copper(II) valproate with 2-methylimidazole [21]. We recently determined by single crystal x-ray structure analysis that this bis-adduct contains the *trans*- $\text{CuN}_2\text{O}_2 + \text{O}_2$ chromophore in a tetragonal arrangement. The electronic spectra of these complexes in CH_2Cl_2 solutions exhibit a shift in the broad copper(II) *d-d* transitions to about 700 nm. In addition there is an additional band at 390 to 400 nm (Table 1), which appears to be of ligand-to-metal charge-transfer origin. A similar band (shoulder) around 400 nm with comparable molar absorptivity also attributed to charge transfer was previously observed for copper(II) carboxylate adducts [13, 23].

The electronic spectra of complex 4 display a very broad *d-d* band at 750 nm in methanol solution and shifted to about 820 nm in CH_2Cl_2 solution. It is similar to that seen for structurally known mononuclear $[\text{Cu}(\text{dmc})_2(\text{dmph})]$ (dmc = 2,5-diethoxycinnamic acid an-

ion) [24] and other copper(II) carboxylates with dmph ligand [25, 26]. These complexes have rhombic structure, intermediate between square pyramidal and trigonal bipyramidal. The copper(II) ion is five coordinated, contains the CuN_2O_3 chromophore, and complexes by a chelating dmph, one bidentate and one monodentate carboxylate groups. The much lower energy of the *d-d* electronic transitions in this complex compared to the other diimine adducts indicates that the basal plane must be greatly distorted due to steric congestion.

The electronic spectrum of complex 6 in CH_2Cl_2 solution exhibits two bands analogous to the spectra of other binuclear copper(II) adducts with bridging carboxylates [13, 19]. Band I at about 695 nm can be assigned to copper(II) *d-d* transitions, whereas band II at about 380 is referred to as a characteristic of the bridging system [13]. The positions of the bands are indeed comparable with those found for structurally known binuclear copper(II) valproate with pyridine [19].

The assignments of IR frequencies for the antisymmetric stretch, $\nu_{\text{asym}}(\text{CO}_2)$, and the symmetric stretch, $\nu_{\text{sym}}(\text{CO}_2)$, of the valproate group are given in Table 1. For complexes 2, 3, and 5 the $\nu_{\text{asym}}(\text{CO}_2)$ frequencies occur between 1572 and 1588 cm^{-1} , and $\nu_{\text{sym}}(\text{CO}_2)$ frequencies occur between 1400 and 1415 cm^{-1} . The band positions and the separation $\Delta\nu$ ($\nu_{\text{asym}}(\text{CO}_2) - \nu_{\text{sym}}(\text{CO}_2)$), between 157 and 188 cm^{-1} , when compared with those of sodium valproate ($\nu_{\text{asym}}(\text{CO}_2)$, 1570; $\nu_{\text{sym}}(\text{CO}_2)$, 1417; $\Delta\nu$, 153 cm^{-1}) are in the range expected for carboxylate groups that act as asymmetrical bidentate ligands [21, 27]. These parameters are comparable to those reported for mononuclear copper(II) valproate complexes that contain imidazoles having *trans*- or *cis*- $\text{CuN}_2\text{O}_2 + \text{O}_2$ chromophore, including the structurally known mononuclear $\text{Cu}(\text{valproate})_2(2\text{-methylimidazole})_2$ complex [21]. The stretching vibrations corresponding to those typical of coordinated 2,2'-bpy (complex 2) occur at 740 and 770 cm^{-1} , and those of phen (complex 3) occur at 730, 860, and 1510 cm^{-1} [28]. The stretching vibrations corresponding to the coordina-

tion mode of 4,4⁻-bpy as bidentate bridging ligand in complex **5** occur at 1610, 1490, 1410(sh), 1225, 1080, 1015, 830, 730, and 647 cm⁻¹. These vibrations are comparable to those reported recently for the structurally known [CuX(4,4⁻-bpy)]_n (X = Cl or Br) complexes in which 4,4⁻-bpy ligands bridge Cu(II) atoms [29]. A very broad band due to a water molecule was observed at 3440 cm⁻¹ in complex **2**. When this complex was heated at 110°C for 10 to 15 min this band only disappeared from the IR spectral while the rest of the spectrum retained its features. This observation indicated that the water molecule in this complex is not coordinated to the copper atom but is present as a lattice molecule which was lost on heating.

The IR spectra for complex **4** exhibit two $\nu_{\text{asym}}(\text{CO}_2)$ and two $\nu_{\text{sym}}(\text{CO}_2)$ stretching bands (Table 1), indicating the presence of two valproate ligands involved in different coordination modes. The 1610 and 1380 cm⁻¹ ($\Delta\nu = 230$ cm⁻¹) pair are assigned to the carboxyl group that acts as monodentate ligand, and the 1595 and 1420 cm⁻¹ (shoulder) ($\Delta\nu = 175$ cm⁻¹) pair are assigned to the other carboxyl that acts as asymmetric bidentate ligand [27]. These results are consistent with the proposed mononuclear structure containing CuN₂O₃ chromophore.

The IR stretching frequencies for $\nu_{\text{asym}}(\text{CO}_2)$ and $\nu_{\text{sym}}(\text{CO}_2)$ of the valproate groups in complex **6** occurred at 1620 and 1419 cm⁻¹, respectively. The positions of these frequencies and the separation between them (201 cm⁻¹) when compared with those of sodium valproate (as shown previously); are in the range expected for the carboxylate group that acts as a bridging bidentate ligand [13a, 20, 26]. These parameters are comparable to those reported for the structurally known binuclear copper(II) valproate complex that contains pyridine [19]. The stretching vibrations corresponding to the coordination mode of 4,4⁻-bpy as bidentate bridging ligand in this complex occur at 1610, 1490, 1220, 1070, 1010, 853, 810, 725, 655, and 630 cm⁻¹. These vibrations are comparable with those reported recently for 4,4⁻-bpy acting as bidentate bridging ligand [29].

The ESR parameters, g and A , for frozen solutions and solid-state spectra of complexes under investigation are summarized in Table 2. The ESR spectra for frozen solutions of complexes **2**, **3**, and **5** exhibit resolved structure with $g_{11} > g_{\perp}$ and are consistent with a tetragonally elongated structure [30]. A representative spectrum is that of **5** shown in Figure 1(A). The g_{\perp} signal and the lowest field component of the g_{\parallel} signal exhibit ¹⁴N superhyperfine structure that consists of five equally spaced lines attributed to the presence of two nitrogen atoms in the copper(II) ion plane. ESR spectral parameters for these complexes are comparable to those previously reported for tetragonally distorted copper(II) complexes that contain a CuN₂O₂ + O₂ chromophore, including those reported for copper(II) carboxylates with diimine [21–23, 30, 31]. In complexes for which structural data are available, the copper(II) atom is bonded to two nitrogen donors and one oxygen atom from each of the two carboxylate ligands. The second oxygen atom of

each carboxylate ligand is weakly bonded in a pseudo axial arrangement [21, 24, 25].

The room temperature ESR spectra of solid-state samples of **2**, **3** and **5** are anisotropic and contain g_{\parallel} and g_{\perp} components. The spectral data for these complexes are consistent with the presence of a tetragonally elongated CuN₂O₂ + O₂ chromophore [21, 30]. Our preliminary x-ray data for complex **3** clearly indicated that in the solid state, Cu(II) atom is in a tetragonal co-ordination environment with in-plane bonds to two nitrogens from phenanthroline and one carboxylate oxygen atom from each of the two valproates with weaker off-axial bonds to other carboxylate oxygens (Scheme I). The structure of this compound is similar to that determined recently for the mononuclear copper(II) complex of 3,5-diisopropylsalicylate with 1,10-phenanthroline [25]. Based on similar ESR spectral properties along with other spectral data for complexes **2** and **3**, the structure of **2** can be described in the same manner except the 2,2'-bipyridine ligand replaced the 1,10-phenanthroline ligand (Scheme I).

The g_{\perp} region of the solid-state ESR spectrum for **5** is partially resolved into its x and y components and this may be attributed to slight distortion in the basal plane which contains a CuN₂O₂ chromophore. A similar spectrum and spectral data are exhibited by the structurally known bis-adduct of copper(II) valproate with 2-methylimidazole [21]. In this complex the copper(II) ion is bonded to two imidazole nitrogen atoms and one oxygen atom from each of the two valproate ligands. The second oxygen atom of each valproate ligand is weakly bonded in a pseudo axial arrangement. The structure of the mononuclear units of complex **5** can be described in the same manner except that two pyridine nitrogens of 4,4⁻-bipyridine bridging ligands replaced equatorially the two monodentate 2-methylimidazole ligands and bridging Cu(valp)₂ moieties (Scheme II). In addition, elemental analysis for this complex confirmed the formula [Cu(valp)₂(4,4⁻-bpy)].

The solid-state and the frozen solution ESR spectra (Figure 1(B)) of complex **4** are clearly of rhombic type (Table 2) suggesting a distortion from square symmetry. While the g_x and g_y components exhibit copper(II) hyperfine structure, the g_x component exhibits ¹⁴N superhyperfine structure consisting of five lines. This structure is attributed to the presence of two nitrogen atoms coordinated to the copper(II) ion. The structure deviation from square symmetry is attributed to the presence of methyl groups in the 2 and 9 positions on the phenanthroline moiety, close to the coordinated nitrogens to copper(II). The ESR and other spectral parameters for this complex are comparable to those of ternary mononuclear copper(II) carboxylate complexes with 2,9-dimethyl-1,10-phenanthroline, [24–26], including those reported for the structurally known copper(II) complex of 2,5-dimethoxycinnamic acid [24]. The geometry of these complexes was described as a very distorted square pyramid or a trigonal bipyramid. Accordingly, the copper(II) ion in complex **4** is five coordinated and complexes by two nitrogen atoms from dmph, two carboxylic oxygen atoms

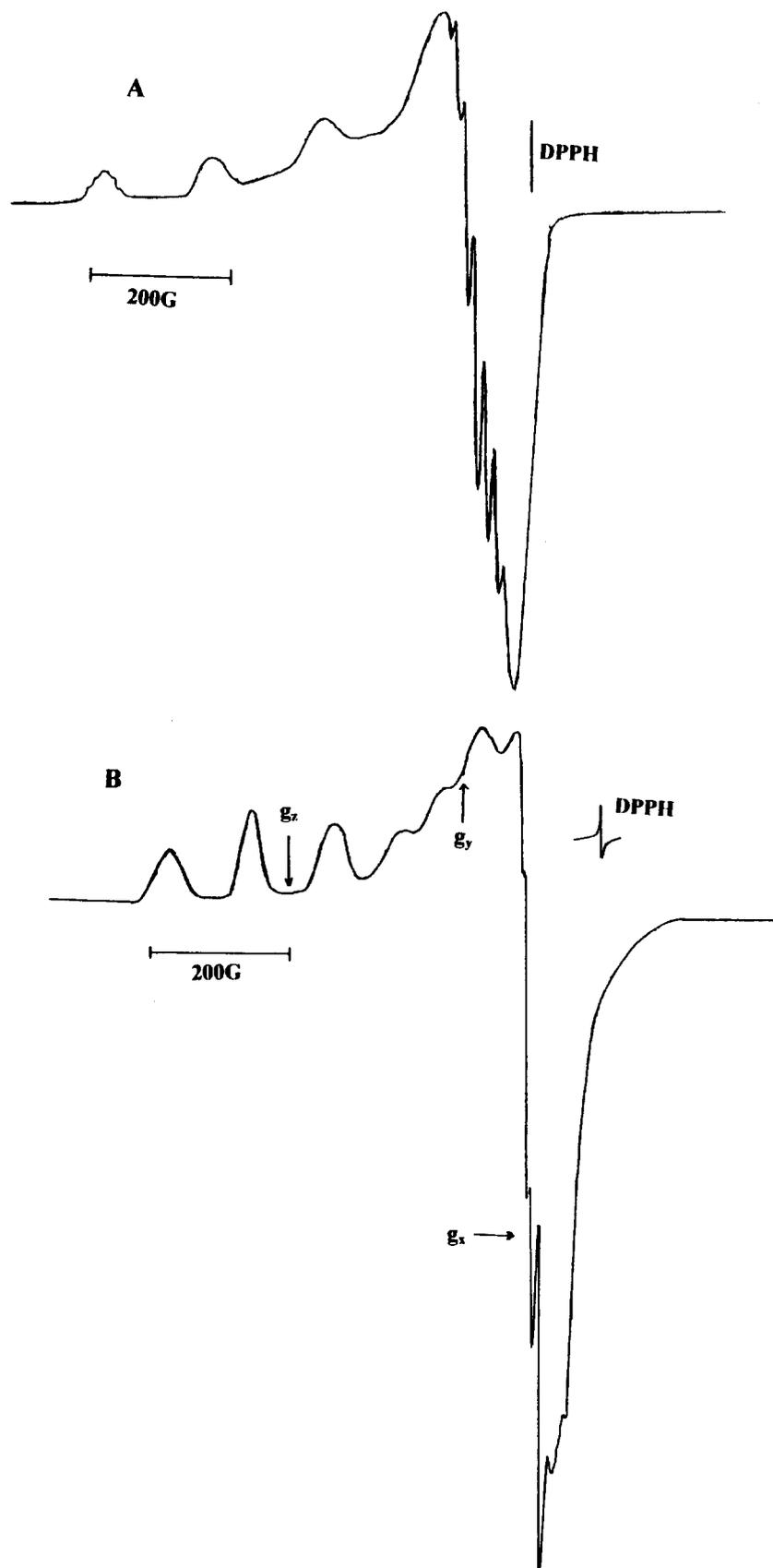


Figure 1. Frozen-solution ESR spectra of compound 5 (A) and compound 4 (B).

Table 2. ESR Data for Cu(II) Valproate Complexes

Compound	State (temperature)	g_0^a	g_{\perp}	g_{\parallel}	$A_{\parallel} \text{Cu}$ ($\times 10^4 \text{ cm}^{-1}$)	$A_{\perp} \text{N}$ ($\times 10^4 \text{ cm}^{-1}$)
$\text{Cu}_2(\text{valp})_4$, (1) ^b	Solid (room)	2.130	2.016	2.341 ($D = 0.344 \text{ cm}^{-1}$)	—	—
$\text{Cu}(\text{valp})_2(2,2\text{-bpy})$, (2)	Solid (room)	2.144	2.080	2.273	—	—
	Frozen (77 K)	2.130	2.069	2.270	170	14.5
$\text{Cu}(\text{valp})_2(\text{phen})$, (3)	Solid (room)	2.151	2.090	2.273	—	—
	Frozen (77K)	2.135	2.065	2.277	170	14.5
$\text{Cu}(\text{valp})_2(\text{dmp})$, (4)	Solid (room)	2.176	$g_x = 2.089$ $g_y = 2.132$	$g_z = 2.308$	—	—
	Frozen (77 K)	2.178	$g_x = 2.073$ $g_y = 2.137$	$g_z = 2.324$	$A_x = 18.8$ $A_y = 55.4$ $A_z = 130$	12.0
$\text{Cu}(\text{valp})_2(\mu\text{-}4,4\text{-bpy})$, (5)	Solid (room)	2.120	$g_x = 2.055$ $g_y = 2.075$	2.230	—	—
	Frozen (77 K)	2.131	2.062	2.270	165	14.0 ($A_{\parallel} \text{N} = 10.0$)
$\text{Cu}_2(\text{valp})_4(\mu\text{-}4,4\text{-bpy})$, (6)	Solid (room)	2.147	2.041	2.360 ($D = 0.369 \text{ cm}^{-1}$)	—	—

^a g_0 Values are calculated from $g_0 = \frac{1}{3}(2g_{\perp} + g_{\parallel})$.

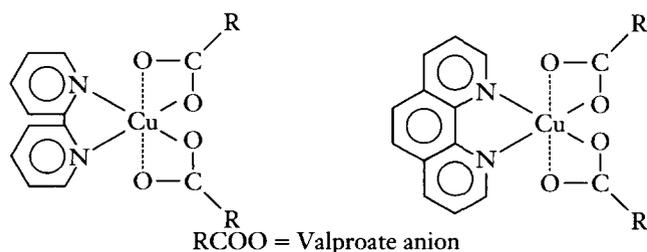
^b ESR data are taken from Ref. 19.

of a bidentate valproate, and one oxygen atom of a monodentate carboxyl group of a second valproate molecule (Scheme III).

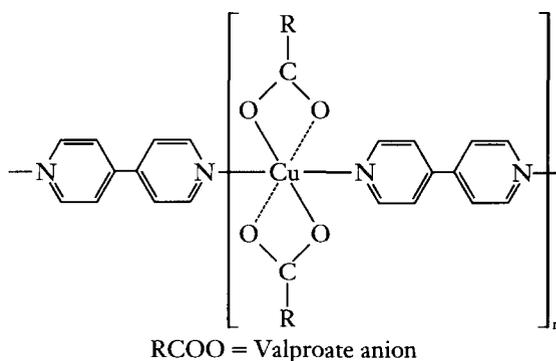
Solid-state ESR spectrum of complex 6 at room temperature exhibits three transitions: a moderately strong absorption at 500 G, a weak absorption at 6188 G (parallel H_{Z1} , and H_{Z2} components, respectively), and a strong absorption at 4854 G (perpendicular component H_{\perp}). Using these absorptions typical for the randomly oriented triplet state ($S = 1$) of axial symmetry and the available literature methods [13a, 22], the ESR parameters for this complex are calculated (Table 2). The parameters g_{\parallel} , g_{\perp} , and D values along with other spectral properties are comparable to those reported for other binuclear or polynuclear copper(II) carboxylate adducts with nitrogen donor ligands [13, 19, 20, 22], including those reported for the structurally known binuclear copper(II) valproate adduct with pyridine [19]. On these bases the formulation $[\text{Cu}_2(\text{valp})_4(\mu\text{-}4,4\text{-bpy})_n]$ can be proposed for the binary complex 6, in which 4,4'-bipyridine molecules bridge binuclear $\text{Cu}(\mu\text{-valp})_4\text{Cu}$ moieties, coordinating axially (Scheme IV). In addition, elemental analysis for this complex confirmed the formula $[\text{Cu}_2(\text{valp})_4(4,4\text{-bpy})]$.

Superoxide Dismutase-Mimetic Activity

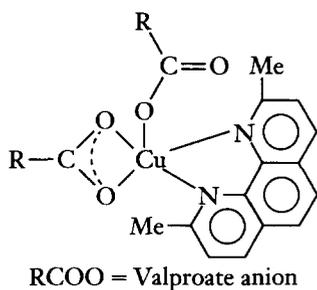
The superoxide dismutase-mimetic activity of the binary $\text{Cu}_2(\text{valp})_4$ complex and their ternary complexes with diimines was measured using the alkaline $\text{Me}_2\text{SO-NBT}$ method [8, 18]. A unit superoxide dismutase activity is the concentration of complex or enzyme which causes 50% inhibition of alkaline Me_2SO mediated reduction of NBT; this concentration is termed IC_{50} for comparative purposes. One representative example of a plot of percent inhibition with increase in concentration is that of complex 2 shown in Figure 2. The data for SOD mimetic activity of the complexes under investigation are given in Table 3. In addition, to ascertain the effectiveness of the present complexes as functional SOD mimics, we compared the IC_{50} of several known Cu(II) complexes (Table 3), which were previously demonstrated as SOD mimics [8], by the NBT method under the same conditions. The data suggest that the activities of the present complexes are higher than those of other copper(II) complexes.



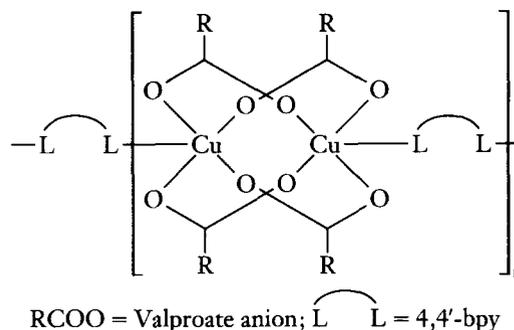
Scheme I



Scheme II



Scheme III



Scheme IV

The mechanism believed to be operating in both Cu, Zn-SOD and Cu(II) complexes involves the initial binding of superoxide to the axial Cu(II) site, with subsequent redox cycling of the Cu(II) ion [4, 32],



Some factors were proposed which may discriminate among the dismutation features of the copper(II) complexes *in vitro*, and these may include: (1) A fast exchange of molecules axially linked to the center and limited steric hindrance to the approach of the superoxide anion are considered essential requirements for the successful binding of the O_2^- radical [33]. (2) The flexibility of the copper(II) arrangement, which facilitates the interaction of the O_2^- radical, followed by the rapid electron transfer reaction which results in reduction to copper(I) species [34]. (3) The favorable response of π -electrons of the coordinated ligands in stabilizing the Cu(II)- O_2^- interaction [7e, f, 8b]. These requirements are satisfied in the tetragonally distorted complexes 2, 3, and 5 and in the

distorted square pyramid or trigonal bipyramid complex 4. In these complexes which contain the $\text{CuN}_2\text{O}_2 + \text{O}_2$ chromophore (in complexes 2, 3 and 5) or the CuN_2O_3 chromophore (in complex 4), the axial atoms are the weakly coordinated second carboxylate oxygen atoms. The weakly interacting oxygen atoms are readily dissociated to provide sites on Cu(II) for O_2^- bonding. Dissociation of the weakly bonding donors would also facilitate any necessary geometrical changes induced by O_2^- bonding. The SOD-like activity of the binary binuclear complex 1 is explained in terms of both a fast exchange of axial solvent molecules and a limited steric hindrance to the approach of the superoxide anion in the complex. Polynuclear complex 6 is likely to dissociate in solution into its binuclear units $[\text{Cu}_2(\text{valp})_4(4,4^- \text{-bpy})]$. The relatively high catalytic activity of this complex may be due to axial coordination of the $4,4^- \text{-bpy}$ ligand on one Cu(II) atom which activates the axial site of the other Cu(II) atom for O_2^- bonding and further stabilizes this interaction through $d-\pi$ interaction between the copper(II) and the π -system of the $4,4^- \text{-bpy}$ ligand.

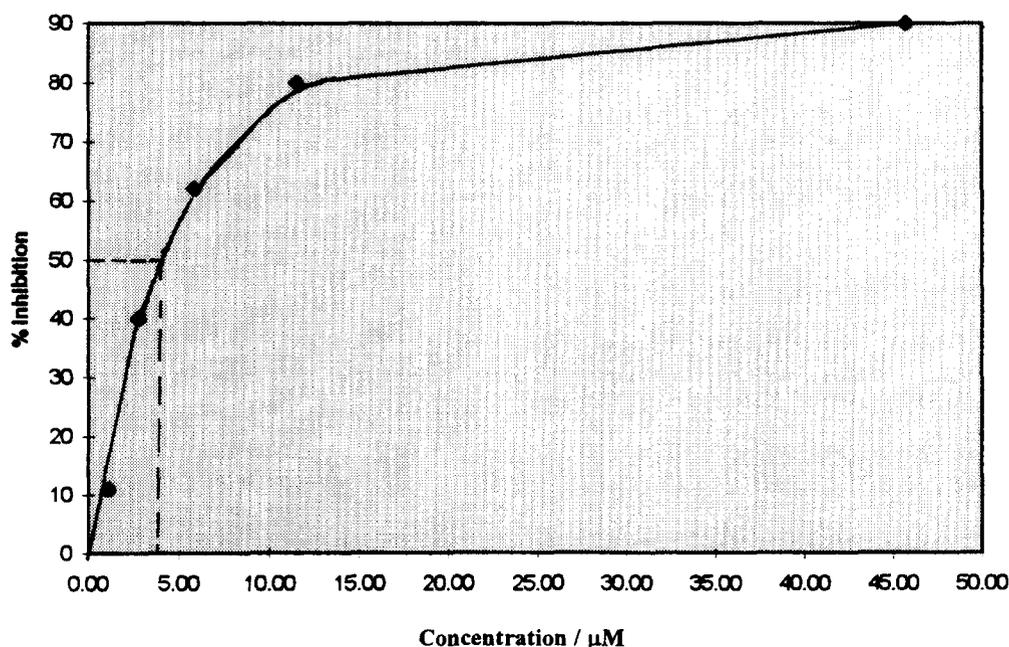


Figure 2. A plot of percentage of inhibiting NBT reduction with an increase in the concentration of compound 2.

Table 3. Superoxide Dismutase-Mimetic Activity

Compound	IC ₅₀ ^a /μM
Cu ₂ (valp) ₄ , (1)	10.4
[Cu(valp) ₂ (2,2'-bpy)], H ₂ O, (2)	4.2
Cu(valp) ₂ (phen), (3)	4.5
Cu(valp) ₂ (dmp), (4)	6.3
[Cu(valp) ₂ (μ-4,4'-bpy)] _n , (5)	18.3
[Cu ₂ (valp) ₄ (μ-4,4'-bpy)] _n , (6)	5.0
Cu(salicylate) ₂ ^b	44
Cu(aspirinate) ₂ (pyridine) ₂ ^b	13
Cu(glycylglycinate)(2,2'-bpy), 3H ₂ O ^c	25
Cu(glycylglycinate)(phen), 3H ₂ O ^c	32
Cu(cimetidine) ₂ (ClO ₄) ₂ ^d	4.0
Cu,Zn-SOD	0.72

^a IC₅₀ is defined as the concentration of complex or enzyme which produces 50% inhibition of NBT reduction.

^b Ref. 8a.

^c Ref. 8b.

^d SODlike activity was determined by the xanthine-xanthine oxidase system as described in Ref. 7b.

In conclusion, the SODlike activity of binary and ternary copper(II) complexes of the anticonvulsant drug valproate with diimines is relatively lower than the activity of the native SOD enzyme (Table 3). The IC₅₀ values measured for the most active ternary complexes **2**, **3**, and **6** are among the highest available in the literature for copper(II) complexes showing SOD-mimetic activity studied under similar conditions [8], and are only about six times lower than that determined for the native enzyme on a molar basis by the NBT method [8]. On the other hand, the least active adduct **5** is about 25 times less active than the native enzyme on a molar basis. These complexes are potent SOD mimics, especially the most active ones, considering the very low molecular weights of these complexes when compared to that of the native SOD enzyme.

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