63. Complexation of Cu^{2+} by Binucleating Macrocycles Containing the N_6 Donor Set

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The hexadentate ligands 1,4,7,12,15,18-hexaazacyclododecosane ([22]aneN_6) and 1,4,7,14,17,20-hexaazacyclohexacosane ([26]aneN_6) both form eight complexes with Cu^{2+}, three of them being binuclear. The corresponding stability constants have been determined potentiometrically, and the electronic absorption spectra have been obtained from spectrophotometric data. Possible interactions of the Cu^{2+} pairs in the three binuclear complexes have, in addition, been investigated by ESR and linear-sweep voltammetry (LSV). The binuclear complex of [22]aneN_6 with one additional OH group is exceptionally stable, ESR-silent, and the results of the LSV-experiments are characteristically different from those of the other binuclear complexes with both ligands. This indicates that [22]aneN_6 forms a very stable hydroxo-bridged binuclear Cu^{2+} complex $Cu_2L(OH)^{3+}$, whereas in the case of [26]aneN_6 no bridged Cu^{2+} pair exists.

Introduction. – One main interest in binuclear copper complexes arises from the widespread occurrence of binuclear copper centers in enzymes such as hemocyanin [1], superoxide-dismutase [2], and cytochrome c-oxidase [3].

Macrocycles with two chelating *N*-(2-aminoethyl)ethylenediamine (dien) subunits, like 1,4,7,11,14,17-hexaazacyclocosane ([20]aneN_6) [4] [5], 1,4,7,12,15,18-hexaazacyclododecosane ([22]aneN_6) [4] [5], 1,4,7,13,16,19-hexaazacyclotetracosane ([24]aneN_6) [4–8], or 10,22-dioxa-1,4,7,13,16,19-hexaazacyclotetracosane ([24]aneN_6O_2) [4] [5] [7–14] can incorporate two Cu^{2+} ions. For the latter, the structures of imidazolato- [9] and hydroxo-bridged binuclear Cu^{2+} complexes [12] [13] have been solved. The Cu^{2+}-[24]aneN_6O_2 system has also been investigated in detail without additional ligands such as imidazole, and the stability constants of the mono- and binuclear complexes with Cu^{2+} have been determined [14].

The influence of the length of the chain between the two dien subunits is studied with the aid of two analogous ligands [15], 1,4,7,12,15,18-hexaazacyclododecosane ([22]aneN_6) and 1,4,7,14,17,20-hexaazacyclohexacosane ([26]aneN_6) containing C_4 and C_6 interconnecting chains, respectively. The stability constants and absorption spectra of the mono- and the binuclear complexes with Cu^{2+} were determined by potentiometric and spectrophotometric titrations. The binuclear complexes were investigated by ESR and linear-sweep voltammetry (LSV) to study any coupling between the Cu^{2+} centers.

Experimental. – The synthesis of the hydrochlorides [22]ane$N_6 \cdot 6$ HCl and [26]ane$N_6 \cdot 6$ HCl has been described in [15]. $CuCl_2 \cdot 2\, H_2O$ and $Cu(NO_3)_2 \cdot 3\, H_2O$ were used as the sources of Cu^{2+}. These salts and all other reagents were of anal. grade and used without further purification. The measurements were done at 25 ± 0.1° (LSV and ESR experiments at ~ 20°), and the ionic strength was adjusted to $I = 0.5$ by KCl (ESR and LSV: KNO_3). Potentiometric measurements were run using our fully automatic pH-titration unit, consisting of a combined glass electrode (*Metrohm UX*), a *Metrohm E600* digital pH-meter, a *Metrohm E655* digital burette, a *Dolphin* microprozessor, and a tape recorder (*Microcorder ZE601*) [16]. The pH electrode was precalibrated with two buffer solns. of pH 4 and pH 7. The exact calibration was achieved by titrating 25 ml of a stock soln. containing 3.2 mM tris(hydroxymethyl)methylamine (tris), 5.5 mM HNO_3 with 0.4M NaOH. The optimum values of the activity coefficient (α), the ion product of water (K_W), and the protonation constant (K^H_{LH}) of tris were predetermined by repeated titrations with an exactly calibrated electrode and were fixed to 0.914 for α, 13.878 for pK_W, and 8.252 for $\log K^H_{LH}$.

The protonation constants of the ligands and the stability constants of the complexes were determined by titration with 0.4M NaOH under N_2. Titrating solns. of 0.93 mM (50 ml; 0.98 mM HCl), 1.12 mM (25 ml; 1.18 mM HCl), and 1.87 mM (25 ml; 1.99 mM HCl) [22]ane$N_6 \cdot 6$ HCl, and 0.91 mM (50 ml; 0.84 mM HCl), 1.02 mM (50 ml; 0.93 mM HCl), and 2.03 mM (25 ml; 1.86 mM HCl) [26]ane$N_6 \cdot 6$ HCl without copper gave the protonation constants. Metal-containing titration curves were obtained at overall ligand concentrations C_L of 1.61 mM (25 ml; 1.58 mM HCl) and 0.81 mM (50 ml; 0.79 mM HCl) for [22]ane$N_6 \cdot 6$ HCl, and 1.60 mM (25 ml; 1.67 mM HCl) and 0.80 mM (50 ml; 0.84 mM HCl) for [26]ane$N_6 \cdot 6$ HCl. The total metal concentrations (C_M) were 93% and 186% of C_L for [22]aneN_6, and 94% and 187% for [26]aneN_6. All experiments were run in duplicates.

The activity coefficient and the ion product of water in 0.5M KCl were determined by titrating three times a soln. containing HCl and tris (25 ml; 11.3 mM HCl; 6.49 mM tris; $I = 0.5$ (KCl): $\alpha = 0.918$ (2); $pK_W = 13.885$ (3)) and held constant. With the calculated α, all pH-readings were converted into proton concentrations, and concentration constants are given throughout this paper. The calculation of the pK_H values and the stability constants of the complexes was achieved on a *Hewlett-Packard HP310* desk-computer using the TITFIT program [17].

Spectrophotometric titrations were run using an automatic titration setup (*Cary 118C*) [18]. Eight solns. of each ligand with different C_L (0.75 mM to 3.45 mM for [22]aneN_6, 0.80 mM to 3.20 mM for [26]aneN_6) and Cu^{2+} (23% to 198% of C_L) were titrated with 0.1M NaOH. The calculations were achieved on a *Hewlett-Packard HP310* desk-computer using the SPECFIT program [19].

The ESR measurements were done on a *Varian E-9 ESR* spectrometer under N_2. Solns. of 25.44 mM [22]aneN_6 and 47.35 mM Cu^{2+}, or 27.65 mM [26]aneN_6 and 48.77 mM Cu^{2+} were adjusted with 0.1M KOH to pH values where the individual binuclear complexes are dominant (*cf. Fig. 1*).

The LSV measurements were done using an automatic voltammetry setup designed by *Kissner* [20]. The scan speed was 10 V/s. A polarograph (*Bruker E310*), a sitting Hg-drop electrode (modified *Metrohm 6.0335.000*) as working electrode, a Ag/AgCl/KCl (3M) electrode as reference electrode, and a Pt wire as auxiliary electrode were controlled by a μ-prozessor (*Rockwell AIM65*). The data were stored by the μ-processor and plotted on a *HP 7044A*. Solns. containing 0.15 – 0.18 mM ligand and *ca.* 1.9 equiv. of Cu^{2+} per ligand were adjusted with 0.1M KOH to values where binuclear complexes are dominant (*cf. Fig. 1*). All measurements were carried out under N_2.

Results. – The protonation constants calculated from potentiometric titrations are listed in *Table 1*. For comparison, the $\log K^H_{LH_n}$ values of 3,6,9-triazaundecane (ded) [21] and [24]aneN_6O_2 [14] are also included. We chose ded rather than dien as the model, since ded, like the investigated ligands, contains only secondary amino groups. Only a slight influence of the different chain lengths between the dien subunits in [22]aneN_6 and [26]aneN_6 can be observed. Assuming that protonation of the ligands occurs alternatively

Table 1. *Weighted Means and Standard Deviations of the Ligand Protonation Constants at 25° and* I = *0.5 (KCl)*

	[22]aneN_6	[26]aneN_6	[24]aneN_6O_2[a])	ded[b])
$\log K^H_{LH}$	10.64(1)	10.73(1)	9.65	10.44
$\log K^H_{LH_2}$	10.12(1)	10.31(1)	8.92	9.78
$\log K^H_{LH_3}$	9.37(1)	9.93(1)	8.30	4.24
$\log K^H_{LH_4}$	8.86(1)	9.47(1)	7.64	
$\log K^H_{LH_5}$	3.44(1)	3.82(1)	3.81	
$\log K^H_{LH_6}$	3.42(1)	3.57(1)	3.26	

[a]) [14]; $I = 0.1$ (KNO_3), 25°.

[b]) [21]; $I = 0.5$ (KCl), 25°, converted to concentration constants.

Table 2. *Weighted Means and Standard Deviations of the Formation Constants of the* Cu^{2+} *Complexes at 25° and* $I = 0.5$ *(KCl)*

	[22]aneN_6	[26]aneN_6	[24]aneN_6O_2[a])	ded[b])
$\log K^{H}_{MLH_3}$	3.51(2)	3.86(1)	3.45	
$\log K^{H}_{MLH_2}$	8.57(5)	9.23(1)	7.46	
$\log K^{H}_{MLH}$	9.25(5)	9.90(1)	8.01	3.39
$\log K^{M}_{ML}$	16.65(2)	16.20(1)	16.46	15.01
$\log K^{H}_{ML}$	10.90(2)	10.64(1)	10.63	9.39
$\log K^{ML}_{M_2L}$	11.47(2)	13.06(2)	10.84	
$\log K^{H}_{M_2L}$	7.14(1)	8.65(1)	6.51	
$\log K^{H}_{M_2LH_{-1}}$	11.34(2)	10.39(2)	10.40	

[a]) [14]; $I = 0.1$ (KNO_3), 25°.

[b]) [21]; $I = 0.5$ (KCl), 25°, converted to concentration constants.

in the two subunits, the two ligands behave essentially like two separated molecules of ded. The greater acidity of LH_6^{6+} and LH_5^{5+} of both ligands and LH_4^{4+} and LH_3^{3+} of [22]aneN_6 can easily be explained by weak interactions of the positive charges.

The potentiometric titrations of both ligands in the presence of Cu^{2+} give the same species, as established by *Martell* and coworkers [14], for [24]aneN_6O_2. The stability constants of the complexes are given in *Table 2,* again together with the data for [24]aneN_6O_2 and ded. It was not possible to obtain all constants from a single titration curve. Depending on the relative ratio of ligand to Cu^{2+}, some species are dominant and others are practically inexistent, and, therefore, the corresponding stability constants are not well defined. Accordingly, titration curves with different total concentrations and different ligand-to-metal ratios had to be calculated together. In this way, all experimental titration curves can be explained satisfactorily using the stability constants given in *Table 2;* overall standard errors were in the range of $2 \cdot 10^{-3}$ ml (0.2% in added base). Because of our specific interest in the binuclear complexes, the distribution curves of both ligands with a Cu^{2+}-to-ligand ratio of 2:1 are shown in *Fig. 1.*

The complex-formation constants could not be calculated from the spectrophotometric pH-titration curves because of the great similarity of the species spectra. Using the stability constants obtained from the potentiometric data, the spectrophotometric curves could be fitted, however, and the spectra of all copper complexes were calculated. The λ_{max} values and the molar absorptivities $\varepsilon_{\lambda(max)}$ of the spectra are given in *Table 3,* together with data for related systems. The binuclear species (M_2L, M_2LH_{-1}, M_2LH_{-2}) are only well defined in titration curves with metal-to-ligand ratios of 2:1, and the corresponding data in *Table 3* were obtained by using these curves only.

Discussion. – The differences in the stability constants of the mononuclear complexes of [22]aneN_6 and [26]aneN_6 can, with the exception of ML, be explained by the higher basicity of the amino groups in [26]aneN_6. The fully deprotonated [22]aneN_6 forms the more stable 1:1 complex with Cu^{2+} than [26]aneN_6. The same is true with [24]aneN_6O_2. Also, the absorption maxima of the two compounds are different, $\lambda_{max} = 650$ and 620 for [22]aneN_6 and [26]aneN_6, respectively. The absorption maxima of the remaining 1:1 species are much more similar, and the same is true for the molar absorptivities. This indicates a special arrangement of the Cu^{2+} for [22]aneN_6. Molecular models show that this smaller ligand can easily occupy all six places in the octahedral coordination sphere

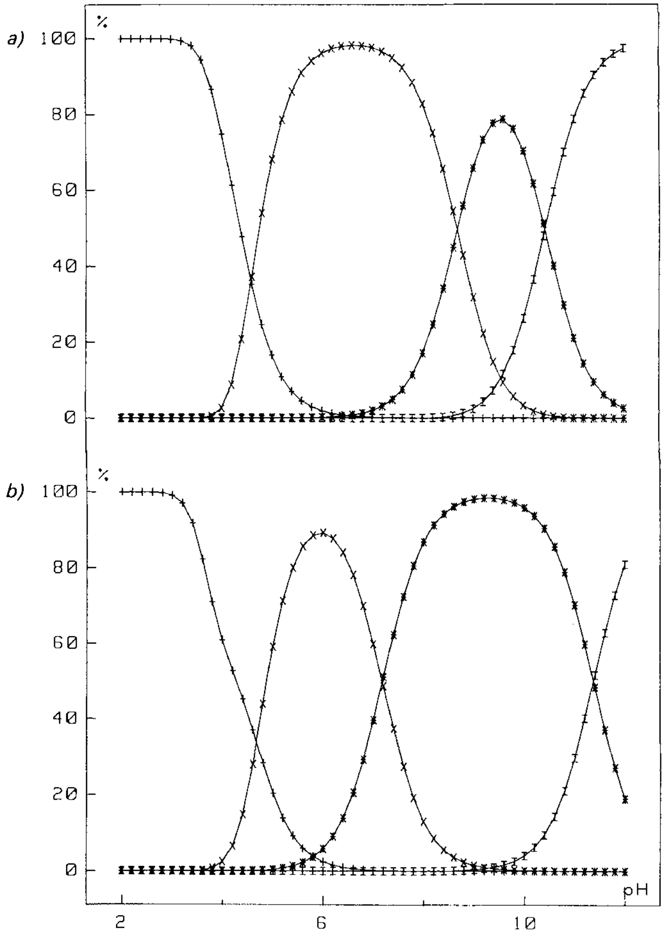

Fig. 1. *Species-distribution curves of the binuclear* Cu^{2+} *complexes with [26]ane*N_6 (a) *and [22]ane*N_6 (b) *as a function of pH* (C_L = 1mM; C_M = 2mM). +: M; ×: M_2L; ∗: M_2LH_{-1}; I: M_2LH_{-2}.

Table 3. *Absorption Maxima* (λ_{max}) *and Molar Absorptivities* ($\varepsilon_{\lambda(max)}$) *for the* Cu^{2+} *Complexes* (standard deviations in brackets)

	[22]aneN_6		[26]aneN_6		ded[a])	
	λ_{max} [nm]	$\varepsilon_{\lambda(max)}$ [$M^{-1} \cdot cm^{-1}$]	λ_{max} [nm]	$\varepsilon_{\lambda(max)}$ [$M^{-1} \cdot cm^{-1}$]	λ_{max} [nm]	$\varepsilon_{\lambda(max)}$ [$M^{-1} \cdot cm^{-1}$]
MLH_3	646(2)	163(7)	639(2)	178(25)		
MLH_2	650(1)	160(1)	636(4)	163(2)		
MLH	613(2)	123(3)	620(5)	136(8)	645	126
ML	650(2)	161(1)	620(3)	142(6)	631	158
MLH_{-1}	613(2)	117(3)	609(2)	122(2)	614	120
M_2L	643(1)	160(2)	635(1)	159(2)		
M_2LH_{-1}	635(1)	146(1)	632(1)	137(2)		
M_2LH_{-2}	619(1)	124(2)	608(1)	121(1)		

[a]) [21]; $I = 0.5$ (KCl), 25°.

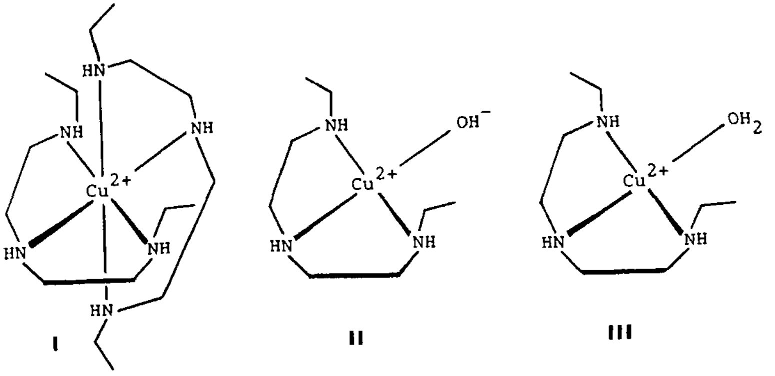

Fig. 2. *Suggested main structures of the Cu^{2+} complexes.* **I:** ML (L = [22]aneN_6), ML_2 (L = dien); **II**: MLH_{-1} (L = ded, [22]aneN_6, [26]aneN_6), MLH, M_2LH_{-1}, M_2LH_{-2} (L = [22]aneN_6, [26]aneN_6), ML (L = [26]aneN_6); **III**: ML (L = ded), M_2L (L = [22]aneN_6, [26]aneN_6), M_2LH_{-1} (L = [26]aneN_6).

of Cu^{2+} (**I** in *Fig. 2*). The longer bridges between the two dien subunits hinder the formation of an analogous complex with [26]aneN_6. The change from three N-atoms to six N-atoms in the coordination sphere of Cu^{2+} with dien leads to a shift in the absorption maxima from 615 nm for Cu(dien)$^{2+}$ to 631 nm for Cu(dien)$_2^{2+}$ [22].

The absorption maxima and the extinction coefficients of ML for [26]aneN_6 as well as of MLH and MLH_{-1} for both ligands are rather similar to the analogous spectrum of MLH_{-1} with ded. This implies a coordination sphere containing one unit of dien and one OH^- ion (**II** in *Fig. 2*). In this case, the other dien unit has to be protonated with the exception of MLH_{-1}. Such structures are not unreasonable in view of the corresponding deprotonation constants with log *K* values between 8 and 11 as is appropriate for both the hydrolysis of an aquo ion coordinated to Cu^{2+} and the deprotonation of the two terminal amino groups of the dien substructure. In fact, comparing with the results for ded, the best description for MLH and ML ([26]aneN_6 only) seems to be a mixture of the hydroxo species **II** and the aquo complex **III** for the macrocyclic ligands.

The spectra of MLH_3 and MLH_2 with both ligands are almost the same and very close to that of ML with ded. The additional protonation of MLH_2 to MLH_3 occurs in the other, uncoordinated, part of the ligand. The complexes, thus, correspond to structure **III**.

The stability constants of the binuclear complexes (M_2L, M_2LH_{-1}, and M_2LH_{-2}) show a rather remarkable effect of the different chain lengths. In line with its greater basicity, the larger ligand ([26]aneN_6) forms the more stable species M_2L and M_2LH_{-2} than the smaller ligand ([22]aneN_6). Contrary to this expected behavior, the species M_2LH_{-1} is more than 10 times more stable with [22]aneN_6. The distribution curves (*cf. Fig. 1*) of the binuclear complexes illustrate this difference between the two ligands. With [22]aneN_6, M_2LH_{-1} is the dominant species in the pH range of 7 to 11. Only between pH 9 and 10, the same species with [26]aneN_6 is present in a significant amount. Practically the same distribution curves as with [22]aneN_6 are obtained with the log *K* values of [24]aneN_6O_2 system published by *Martell* and coworkers [14]. On the other hand, the spectra of the binuclear complexes of both ligands show no striking dissimilarity (*cf. Table 3*). Grossly different coordination spheres can, thus, in all probability be excluded. Consulting molecular models indicates easy bridging of the two Cu^{2+} centers by an OH group in the species M_2LH_{-1} with [22]aneN_6. The distance between the metallic centers is at most 6 Å, and any distortion from this position brings the coordinated OH group close to the uncomplexed Cu^{2+} ion, normally under a favorable angle. In the [26]aneN_6 system, however, the two Cu^{2+} centers are widely separated (~ 8.5 Å) in favorable conformations. To form a hydroxo bridge between them would lead to a loss of entropy and also to steric interaction. Therefore, the additional stabilization of M_2LH_{-1} with [22]aneN_6 (and also [24]aneN_6O_2) can be explained by a hydroxo bridge between the two metallic centers.

To further investigate Cu^{2+}–Cu^{2+} interaction in the binuclear complexes, we adjusted solutions with a Cu^{2+}/ligand ratio of 2:1 to pH values where the individual species are at their maximum concentrations (for [22]aneN_6 pH = 5.99, 9.03, and 11.68; for [26]aneN_6 pH = 6.60, 9.51, and 11.25; *cf. Fig. 1*). The ESR spectra clearly support our assumption. All three binuclear complexes with [26]aneN_6 show a broad ESR signal with roughly full intensity. The Cu^{2+} centers in these complexes are thus paramagnetic and not coupled. With [22]aneN_6, only the species M_2L and M_2LH_{-2} give an ESR signal and indicate paramagnetic Cu^{2+} centers. On the contrary, M_2LH_{-1} with [22]aneN_6 is ESR silent. This can only be interpreted as coupled and probably hydroxo-bridged Cu^{2+} centers.

Additional information concerning the binuclear complexes was obtained from electrochemical experiments (LSV). Again, solutions with a Cu^{2+}-to-ligand ratio of nearly 2 and a pH corresponding to a region where one of the binuclear compounds is dominant were used (for [22]aneN_6 pH = 6.03, 9.03, and 11.47; for [26]aneN_6 pH = 6.51, 9.41, and 11.62). All three solutions containing [26]aneN_6 show reduction peaks; the two most negative ones are almost invariant (~ −420 mV and ~ −560 mV *vs.* Ag/AgCl/KCl (3M)). The other two reduction peaks are shifted to more negative potentials by increasing the pH (from −54 mV to −167 mV and from −123 mV to −245 mV), which can be understood by the stabilization of the Cu^{2+} ion through the exchange of a H_2O molecule by an OH^- ion. In the [22]aneN_6 system, the pattern changes in the case of the solution where M_2LH_{-1} is dominant. Beside the two reduction peaks, which are as with [26]aneN_6 invariant over the pH region investigated (~ −370 mV and ~ −600 mV *vs.* Ag/AgCl/KCl (3M)), the solution with pH = 9.03 shows only one additional peak near −255 mV. The other two solutions have the same characteristics as in the [26]aneN_6 system: two reduction peaks which are shifted from +24 mV and −140 mV at a pH = 6.03 to −173 mV and −278 mV *vs.* Ag/AgCl/KCl (3M) at pH = 11.47.

Conclusions. – Macrocycles with two chelating dien units are useful models for binuclear Cu^{2+} centers, as found in some redox enzymes. Of the investigated ligands, [26]aneN_6 forms in all likelihood no significantly interacting Cu^{2+} pairs. No result from potentiometric, spectrophotometric, ESR, and LSV data would indicate such an interaction. On a molecular model, the reason is easily recognized. The two Cu^{2+} ions are wide apart (8.5 Å), and the space between them is crowded with the two chains linking the two dien units together. A molecular model of the [22]aneN_6 system shows a significant difference. The two Cu^{2+} ions, each coordinated by a dien unit have a distance of approximatively 6 Å in the fully stretched form, and contrary to the case of [26]aneN_6 the chain between these subunits can easily be rearranged to a conformation suitable for bridging. An OH^- group coordinating to one of the Cu^{2+} ions is very close to the other Cu^{2+} ion. Combining these model considerations with the experimental data, the occurence of a hydroxo-bridged Cu^{2+} couple with [22]aneN_6 is strongly indicated.

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