74. Formation and Dissociation Mechanism of Amide Complexes. V[1]). Interconversion of Dimeric and Monomeric Cu^{2+}-Complexes with 1,8-Diamino-3,6-diaza-2,7-octanedione: Comparison between the Reactivity of Terminal and Internal Amide Groups

by **Andreas D. Zuberbühler** and **Thomas A. Kaden**

Institut für Anorganische Chemie, Spitalstr. 51, CH-4056 Basel

(7.XII.81)

Summary

The protonation and deprotonation rates of the coordinated amide groups in the Cu^{2+}-complexes of 1,8-diamino-3,6-diaza-2,7-octanedione (DED = L) have been studied by stopped-flow techniques. Starting at low pH from Cu^{2+} and DED the dimeric $Cu_2L_2^{4+}$-complex, fully formed within the mixing time of the stopped-flow instrument, reacts in two consecutive steps to yield the final product $CuLH_{-2}$. The rate constants of the forward and backward reactions have been determined and are given in *Table 1*. The intermediate was identified as $Cu_2L_2H_{-2}^{2+}$ by measuring its VIS.-absorption spectrum.

The rate constants for the interconversion of the amide groups from the *O*- to the *N*-coordinated form in the Cu^{2+}-complexes of DED, 2,10-dioxo-1,4,8,11-tetraaza-undecane (DANA) and triglycine (TRIGLY) are compared with each other. It is shown that these rate constants are similar, no matter whether the amide group is terminal or internal as long as the rotation is easily possible as is the case in the dimeric species $Cu_2L_2^{4+}$ and $Cu_2L_2H_{-2}^{2+}$. However, for $CuLH_{-2}$ the interconversion only takes place after opening of one of the chelate rings in a rapid protonation preequilibrium.

Introduction. – Cu^{2+} and Ni^{2+} are able to bind the amide group either through the carbonyl O-atom or after deprotonation through the amide N-atom *(Eqn. 1)* [2].

$$Cu^{2+} \ldots O{=}\overset{|}{C}{-}NH_2 \rightleftharpoons Cu^{2+} \ldots HN^{-}{-}\overset{|}{C}{=}O + H^{+} \qquad (1)$$

The kinetics of this interconversion have been studied in detail for oligopeptides [3][4] and for a series of synthetic ligands having the amide group in terminal position [1][5]. Whereas the first type of ligands is interesting because of its relation to biological systems, the second type is simpler to investigate on a molecular level,

[1]) Part IV, see [1].

0018-019X/82/3/0753-09$01.00/0

since the interconversion can take place without an additional change in the rest of the coordination sphere. Thus, no rapid preequilibria must be taken into account.

In order to compare the reactivity of an end-standing amide group as in 2,10-dioxo-1,4,8,11-tetraazaundecane (DANA, **1**) or its diethyl derivative **2** (DANEA) [1] with that of an amide included into the backbone of the ligand, we have studied

$$R{-}NH{-}CO{-}CH_2{-}NH{-}CH_2{-}CH_2{-}CH_2{-}NH{-}CH_2{-}CO{-}NH{-}R$$

1 R = H
2 R = Et

the complexes of 1,9-diamino-3,7-diaza-2,8-nonanedione with Cu^{2+} [6] and Ni^{2+} [7]. However, since the Cu^{2+}-complex of this ligand dissociates or adds the two amide protons in one step, no direct comparison was possible. 1,8-Diamino-3,6-diaza-2,7-octanedione (DED, **3**) seemed to us more suitable for comparison, since potentiometric measurements showed that the addition and dissociation of the amide

$$H_2N{-}CH_2{-}CO{-}NH{-}CH_2{-}CH_2{-}NH{-}CO{-}CH_2{-}NH_2$$

3

protons proceeds stepwise. However, the equilibria of the (Cu^{2+}/DED)-system were so complicated that only after a detailed spectrophotometric and potentiometric study [8] our kinetics results became interpretable.

Experimental Part. – 1,8-Diamino-3,6-diaza-2,7-octanedione (DED) was synthesized as described in [9]. All other reagents were of analytical grade and used without purification except 2-picoline, 2,6-lutidine, and 2,4,6-collidine which were distilled before use.

The kinetics of the protonation and deprotonation of the coordinated amide groups were followed on a *Durrum D110* stopped-flow spectrophotometer with *KelF* blocks and a 2 cm cell. The signal was captured by a *Datalab 901* transient recorder on line with an *A1M 65* computer with 4 *kbytes* memory. The computation of the rate constants was based on the non-linear least-squares method described previously [10]. In some cases the concentration *vs.* time curves were also photographed from a storage oscilloscope, and the rate constants were calculated manually. In general the two consecutive steps observed during these experiments were distinctly separated so that each reaction could be evaluated independently. In a few cases the wavelength at which the reaction was followed was chosen so that only one reaction could be measured.

The deprotonation was followed at 470 nm and 530 nm starting from pH 4.8 and 7.3. Typical concentrations were: $[DED] = 2.5 \cdot 10^{-4} - 5 \cdot 10^{-2}$M, $[Cu^{2+}] = 2.3 \cdot 10^{-4} - 7.2 \cdot 10^{-4}$M and the pH was varied from 6 to 9.7 using 0.05M 2,4,6-collidine and 0.035M borate as buffers. The protonation was followed at 470 nm and 600 nm starting from pH 9.4 or 7.2. Typical concentrations were: $[DED] = 2.5 \cdot 10^{-4} - 7.5 \cdot 10^{-4}$M, $[Cu^{2+}] = 2.3 \cdot 10^{-4} - 7.2 \cdot 10^{-4}$M and the pH was varied from 8 to 5 with 0.05M 2,4,6-collidine, 2,6-lutidine or 2-picoline as buffers. All measurements were done at I = 0.5 (KCl) and 25°.

In a separate set of experiments the spectra of the starting solution, of the intermediate, and of the final product were determined by working at wavelenghts between 400 and 800 nm taking a point every 30 nm. Concentrations were: $[Cu^{2+}] = 4.6 \cdot 10^{-4}$M, $[DED] = 5 \cdot 10^{-4}$M adjusted to pH 9.4 or 7.3 and mixed with 0.1M 2,4,6-collidine (I = 0.5) of pH 6.2 for the protonation and the same Cu^{2+}- and DED-solutions adjusted to pH 4.7 or 7.3 mixed with 0.07M borate (I = 0.5) of pH 8.9 for the deprotonation.

Results and discussion. – To understand the kinetics of the (Cu^{2+}/DED)-system, which is complicated because up to seven species can occur at equilibrium condi-

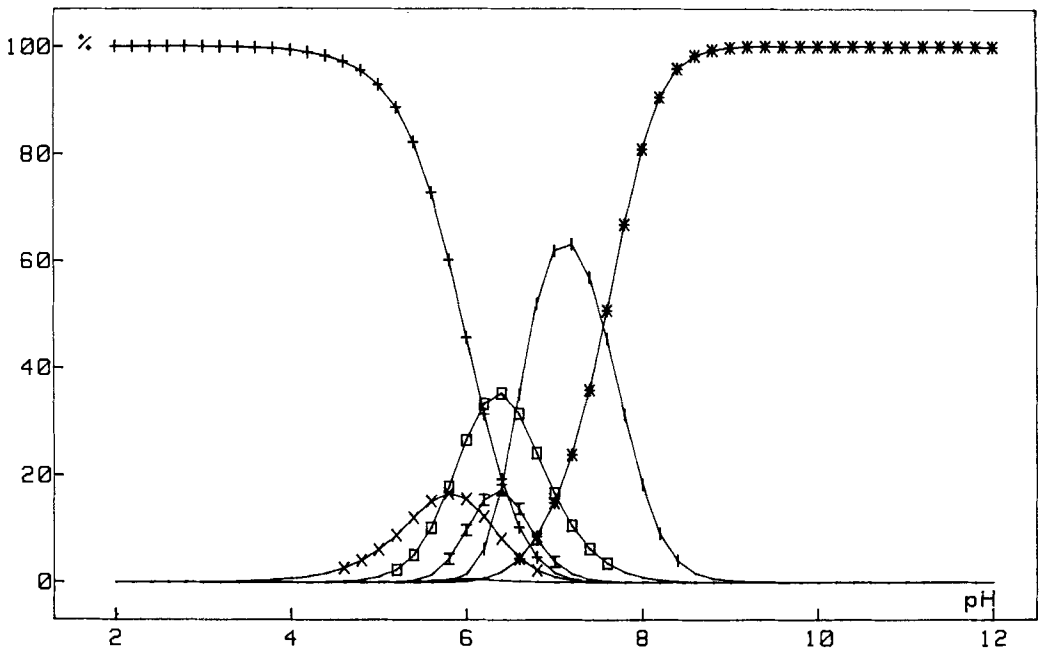


Fig. 1. *Species distribution for the Cu^{2+}/DED system with [DED] = 2.5 · 10^{-4} M and [Cu^{2+}] = 2.3 · 10^{-4} M as a function of the pH* (+: Cu^{2+}, X: $CuLH^{3+}$, □: CuL^{2+}, I: $Cu_2L_2^{4+}$, I: $Cu_2L_2H_{-2}^{2+}$, *: $CuLH_{-2}$)

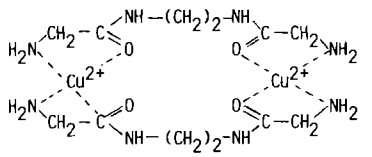
tions [8], it is important to know how the different complexes are distributed as a function of pH. With the equilibrium constants given in [8] we have calculated this for $[Cu^{2+}] = 2.3 \cdot 10^{-4}$M and $[DED] = 2.5 \cdot 10^{-4}$M. The results are plotted *in Figure 1.*

The overall reaction can be described as follows: mixing a solution of pH 4, in which practically only Cu^{2+} and LH_2^{2+} are present with a buffer of pH higher than 9 one obtains $CuLH_{-2}$ quantitatively *(Fig. 1)*. The kinetics, however, shows that this takes place in two consecutive reactions and the question is whether the equilibrium species are also the reactive ones. We have therefore followed the reaction at wavelengths between 400 and 800 nm in order to obtain the spectra of the different species during the reaction. At time $t = 0$ we observe a spectrum with $\lambda_{max} = 670$ nm and $\varepsilon = 38\ M^{-1}\ cm^{-1}$, which by comparison with the spectral data given in [8] can be identified as the spectrum of $Cu_2L_2^{4+}$ with structure **4**. This means that Cu^{2+} and LH_2^{2+} react within the dead-time of the stopped-flow spectrophotometer to give $Cu_2L_2^{4+}$. This is expected since the formation of Cu^{2+}-complexes is very fast [11] as long as no amide group is deprotonated.

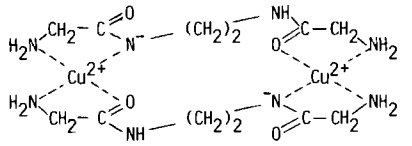
The spectrum of the intermediate with $\lambda_{max} = 585$ nm and $\varepsilon = 56\ M^{-1}\ cm^{-1}$ corresponds to that of $Cu_2L_2H_{-2}^{2+}$ with structure **5**. Thus the first step of the

$$Cu_2L_2^{4+} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} Cu_2L_2H_{-2}^{2+} + 2\,H^+ \qquad (2)$$

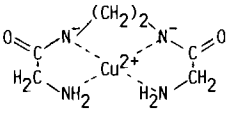
Scheme. *Structure of the complexes* $Cu_2L_2^{4+}$ (**4**), $Cu_2L_2H_{-2}^{2+}$ (**5**), $CuLH_{-2}$ (**6**) *and* $CuLH_{-1}^{+}$ (**7**)



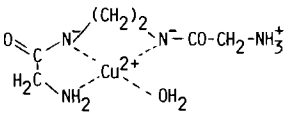
4



5



6



7

observed reaction, in which the deprotonation of the first amide group takes place, can be described by *Equation 2*. The final product of the reaction is $CuLH_{-2}$ (structure **6**) with $\lambda_{max} = 520$ nm and $\varepsilon = 150\ M^{-1}\ cm^{-1}$ ([8]: 513 nm and 160 $M^{-1}\ cm^{-1}$) so that we can state *Equation 3* for the second step. It is interesting to note that the

$$Cu_2L_2H_{-2}^{2+} \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} 2\ CuLH_{-2} + 2\ H^+ \tag{3}$$

second reaction with the rate constant k_{+2} can also be measured starting from pH 7.3 at which $Cu_2L_2H_{-2}^{2+}$ is already formed *(Fig. 1)*. Thus, after the first reaction step the solution contains the same species which are present under equilibrium conditions.

The rate law of the first step is given by *Equation 4* and the results are shown in

$$k_{1,obs} = k_{1,0} + k_{1,OH}\,[OH^-] \tag{4}$$

Figure 2. This reaction is independent of the excess of DED and of the total concentration and is first-order in $[OH^-]$. The second step has a similar although more complicated rate law *(Eqn. 5)*, where [L] is the concentration of free DED, *i.e.* the

$$k_{2,obs} = k_{2,L}\,[L] + k_{2,OH}\,[OH^-] \tag{5}$$

excess of ligand over Cu^{2+}. The pH- and L-dependences are given in *Figure 3* and *4*. The increase in the rate of deprotonation *(Eqn. 5)* by the excess of DED is unusual. However, this deprotonation takes place in $Cu_2L_2H_{-2}^{2+}$ (structure **5**), a dimeric species which must undergo a striking change to become $CuLH_{-2}$ (structure **6**)

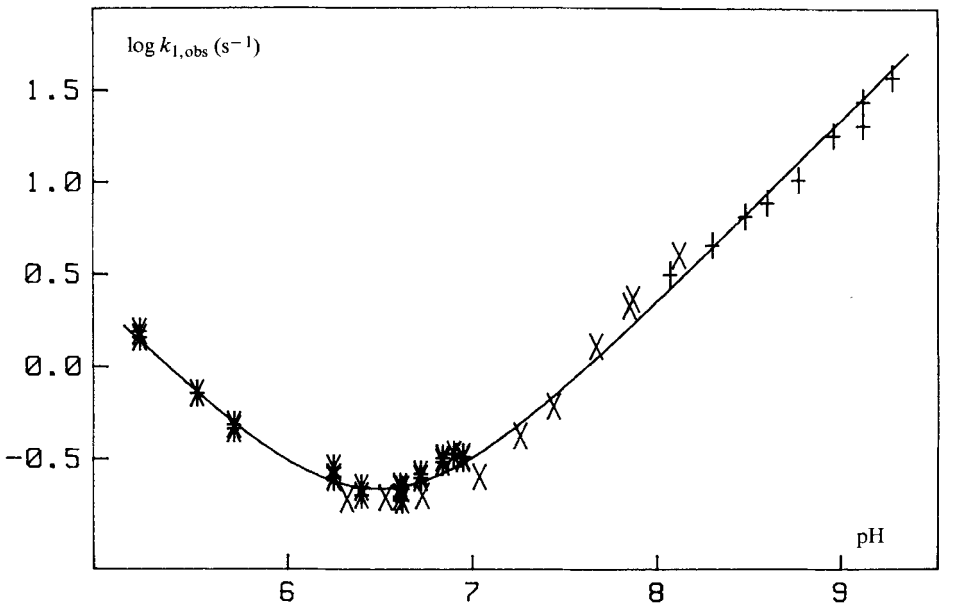


Fig. 2. *pH-Dependence of* Reaction 2 (The curve is calculated with $k_{1,0}+k_{1,OH}[OH^-]+k_{-1,H}[H^+]$ and the constants given in *Table 1*. Protonation of $Cu_2L_2H_{-2}^{2+}$ in 2-picoline buffer (*), deprotonation of $Cu_2L_2^{4+}$ in 2,4,6-collidine (X) and borate (+) buffers).

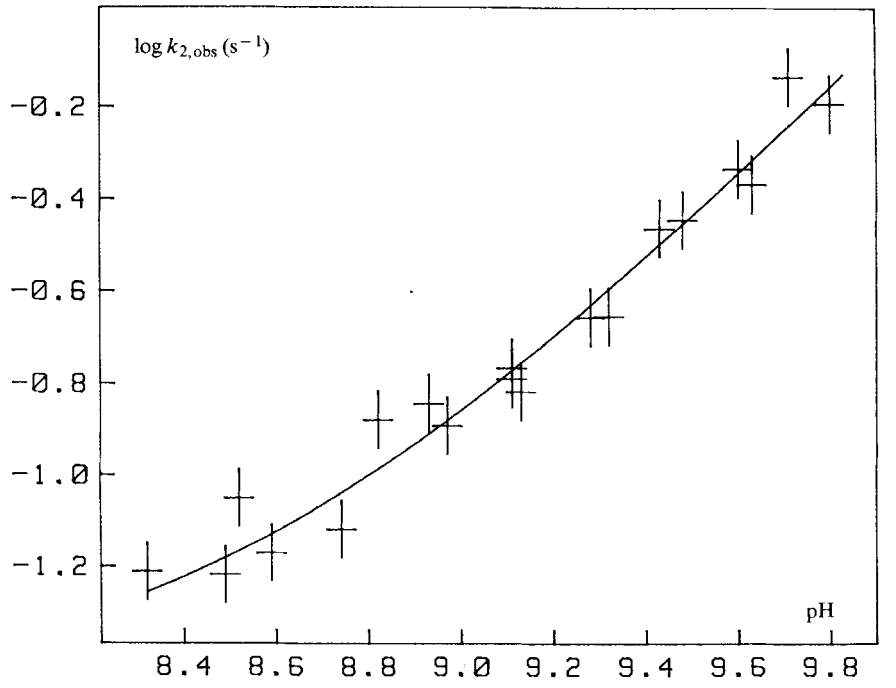


Fig. 3. *pH-Dependence of* $k_{2,obs}$ *in borate buffer* (The curve is calculated with *Equation 5* and the constants of *Table 1*)

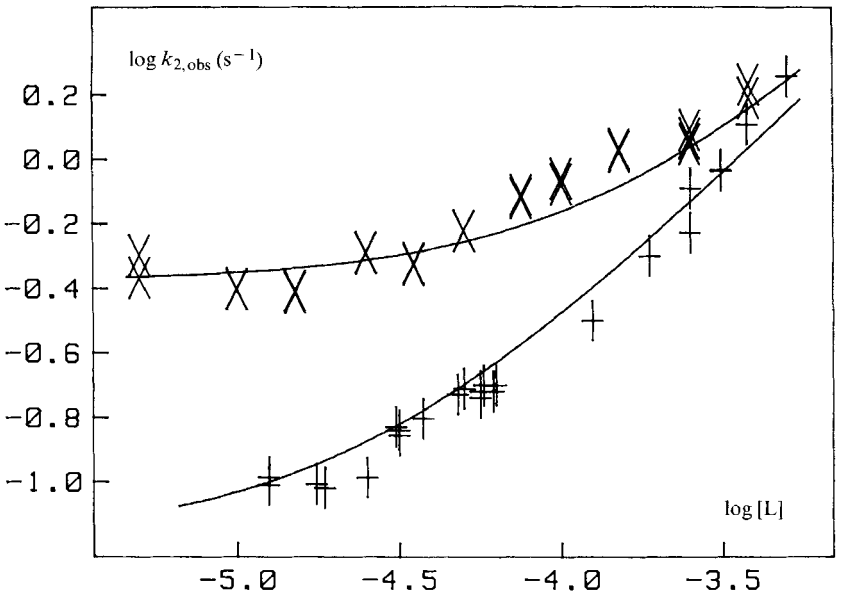


Fig. 4. *Ligand excess dependence of* $k_{2,obs}$ *at pH 8.80* (+) *and pH 9.60* (x) (The curves are calculated with *Equation 5* and the rate constants of *Table 1*)

and it is very plausible that DED helps to attack and disrupt **5**. The protonation of the different (Cu^{2+}/DED)-complex systems also takes place in two steps. Starting from pH 9.4, at which $CuLH_{-2}$ is the only species present, $Cu_2L_2H_{-2}^{2+}$ ($\lambda_{max} = 590$ nm and $\varepsilon - 45$ M^{-1} cm^{-1}) again is formed as an intermediate when the pH is lowered. It then reacts further to give a mixture of $Cu_2L_2^{4+}$ and $CuLH^{3+}$-complexes and Cu^{2+} depending on the final pH-value *(Fig. 1)*. Again, as in the deprotonation, the two slow steps involve the rearrangement of the amide groups, whereas the equilibria from $Cu_2L_2^{4+}$ to $CuLH^{3+}$ and Cu^{2+} are fast. Thus the overall protonation reaction can be described by *Equations 2* and *3* from right to left.

The first step of the protonation has an unusual rate law given by *Equation 6*

$$v_{-2} = (k_{-2,0} + k_{-2,H}[H^+]^2)[CuLH_{-2}]^2 \tag{6}$$

with a bimolecular dependence on $CuLH_{-2}$. The experimental points are given in *Figure 5*. To explain the second-order in $[H^+]$ and $[CuLH_{-2}]$ we assume the sequence of reactions given in *Equation 7* whereby the species $CuLH^{+}_{-1}$ is formed in

$$2\,CuLH_{-2} + 2\,H^+ \overset{\text{fast}}{\rightleftharpoons} 2\,CuLH_{-1}^{+} \xrightarrow{\text{slow}} Cu_2L_2H_{-2}^{2+} \tag{7}$$

a fast preequilibrium. We have seen that in general the slow steps involve a rearrangement of the amide group so that $CuLH^{+}_{-1}$ has probably the structure **7** in which a chelate ring has been opened and the amide group remains unchanged.

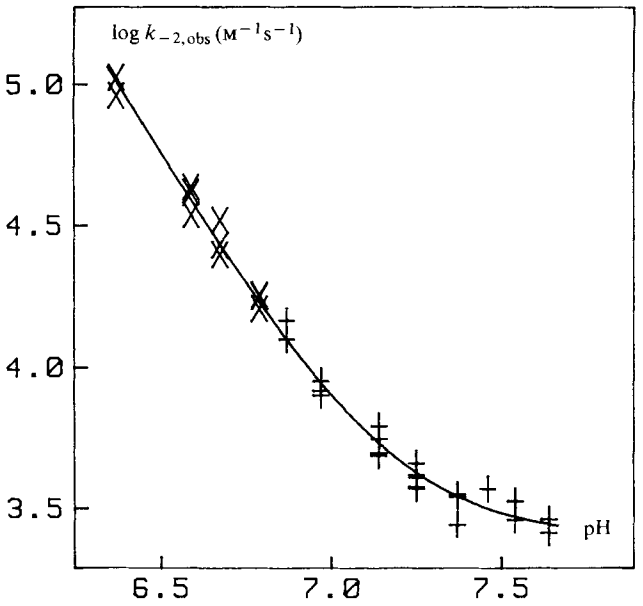


Fig. 5. *pH-Dependence of* $k_{-2,obs}$ *in 2,6-lutidine* (X) *and 2,4,6-collidine* (+) (The curve is calculated with *Equation 6* and the rate constants of *Table 1*)

The fast protonation of the terminal amine is apparently necessary before the amide group can react.

The second protonation step of the amide is the same whether one starts from pH 9.3 or 7.3. Thus, as in the case of the deprotonation, the species found at equilibrium conditions are also present after the first amide group has been protonated. The rate law for the second protonation step is given by *Equation 8*, where $k_{1,0}$ is the same as the one found for the opposite reaction. This is expected since in the

$$k_{-1,obs} = k_{1,0} + k_{-1,H}[H^+] \tag{8}$$

equilibrium region k_{obs} is equal to the sum of the rate constants for the forward and the back reaction [12]. The measurements are also plotted in *Figure 2*.

The rate constants of all these reactions are given in *Table 1* together with their standard errors as obtained from the non-linear least-squares fitting of the different dependences. The curves calculated with these rate constants are shown in

Table 1. *Rate constants for the protonation and deprotonation of the amide groups in the DED-complexes of* Cu^{2+} *at 25° and* $I = 0.5$ M

$Cu_2L_2^{4+}$ ⇌ $Cu_2L_2H_{-2}^{2+}$	$Cu_2L_2H_{-2}^{2+}$ ⇌ $CuLH_{-2}$
$k_{1,0} = (8.3 \pm 1.1) \cdot 10^{-2}\ s^{-1}$	$k_{2,0} = (2.5 \pm 0.1) \cdot 10^{3}\ M^{-1}\ s^{-1}$
$k_{-1,H} = (2.1 \pm 0.2) \cdot 10^{5}\ M^{-1}\ s^{-1}$	$k_{-2,H} = (5.5 \pm 0.2) \cdot 10^{17}\ M^{-3}\ s^{-1}$
$k_{1,OH} = (1.4 \pm 0.1) \cdot 10^{6}\ M^{-1}\ s^{-1}$	$k_{2,OH} = (6.9 \pm 0.2) \cdot 10^{3}\ M^{-1}\ s^{-1}$
	$k_{2,L} = (2.7 \pm 0.1) \cdot 10^{3}\ M^{-1}\ s^{-1}$

Table 2. *Comparison of the protonation and deprotonation rate constants of terminal and internal amide groups in different Cu^{2+}-complexes.*

	DED	DANA [1]	DANEA [1]	TRIGLY [3]
$k_{-1,H}(M^{-1}s^{-1})$	$2.1 \cdot 10^5$	$2.8 \cdot 10^6$	$9.1 \cdot 10^5$	–
$k_{1,OH}(M^{-1}s^{-1})$	$1.4 \cdot 10^6$	$1.3 \cdot 10^5$	$3.2 \cdot 10^4$	–
$k_{-2,H}(M^{-1}s^{-1})$	–	$4.1 \cdot 10^7$	–	$4.9 \cdot 10^6$
$k_{2,OH}(M^{-1}s^{-1})$	$6.9 \cdot 10$	$9.3 \cdot 10^3$	–	$2.4 \cdot 10^4$

Figures 2–5. Our results together with those of previous investigations [1] and those of *Margerum et al.* [3] for triglycine (TRIGLY) are given in *Table 2.* A direct comparison of the protonation and deprotonation of the amide group in the different Cu^{2+}-complexes is difficult since for DED the reacting species are dimeric. In these (structures **4** and **5**) the amide group, although internal, is not enclosed between two chelate rings and is still relatively free to rotate from the *O*- to the *N*-coordinated form or *vice versa.* It is therefore understandable that the rate constants $k_{-1,H}$, $k_{1,OH}$ and $k_{2,OH}$ of DED are similar to those of DANA or DANEA with external amide groups. The slightly lower value of $k_{-1,H}$ for DED probably reflects steric and charge effects whereas the higher value of $k_{1,OH}$ can be explained by the high positive charge (4+) of the complex. The values of $k_{2,OH}$ however, are similar to each other, although the charges are 2+ for DED, 1+ for DANA and 0 for TRIGLY.

The question whether an internal amide group which is part of two chelate rings can interconvert between the *O*- and the deprotonated *N*-form without opening of one of the chelate rings can only be studied in the protonation step of $CuLH_{-2}$. In the case of L = DED this seems not possible, since we have to postulate a rapid preequilibrium (7) in which the terminal amino group is protonated and the ring must be opened. Similarly *Margerum et al.* [3] have also proposed for L = TRIGLY that the carboxylate dissociates prior to the protonation of the amide group. Finally, in the case of L = 1,9-diamino-3,7-diazanonanedione a preequilibrium with the formation of the species **7** was postulated to explain the protonation of $CuLH_{-2}$ [6]. Thus, in all cases in which an internal amide group is blocked in its rotation by two chelate rings, the protonation of the coordinated amide group seems only possible if one of the chelate rings opens so that free rotation can occur.

Another interesting question is why the dimeric species $Cu_2L_2H_{-1}^{3+}$ and $Cu_2L_2H_{-3}^{+}$ were neither observed in the equilibrium [8] nor in this kinetics study, although, *a priori*, they represent reasonable intermediates between $Cu_2L_2^{4+}$ and $CuLH_{-2}$. Models of $Cu_2L_2^{4+}$ (**4**) and $Cu_2L_2H_{-2}^{2+}$ (**5**) show that in both cases favorable structures exist with essentially no steric interactions. The two coordination squares around the Cu^{2+} do not lie in the same plane, but are displaced relatively to each other so that a two-steps stair results. The examination of the models of $Cu_2L_2H_{-3}^{+}$ and $Cu_2L_2H_{-1}^{3+}$, however, shows that their structures are very unfavorable because of strong steric interaction of the methylene protons which are pushed towards the center of the macroring connecting the two Cu^{2+}. The strain which makes the existence of the asymmetric species very improbable can be released when the coordination sphere of both Cu^{2+} changes at the same time to

give the symmetric complexes $Cu_2L_2^{4+}$ or $Cu_2L_2H_{-2}^{2+}$ or the monomeric $CuLH_{-2}$.

The technical assistance of Ms. *L. Siegfried-Hertli* is gratefully acknowledged. This work was supported by the *Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung.*

REFERENCES

[1] *A. D. Zuberbühler & Th. A. Kaden,* Helv. Chim. Acta *57,* 1897 (1974).
[2] *D. W. Margerum & G. R. Dukes,* Met. Ions Biol. Syst. *1,* 157 (1974); *Th. A. Kaden & A. D. Zuberbühler,* Helv. Chim. Acta *54,* 1361 (1971).
[3] *G. Pagenkopf & D. W. Margerum,* J. Am. Chem. Soc. *90,* 6963 (1968).
[4] *E. J. Billo & D. W. Margerum,* J. Am. Chem. Soc. *92,* 6811 (1970); *H. Hauer, G. R. Dukes & D. W. Margerum,* J. Am. Chem. Soc. *95,* 3515 (1973).
[5] *A. D. Zuberbühler & Th. A. Kaden,* Helv. Chim. Acta *55,* 623 (1972).
[6] *D. Wagnerova, Th. A. Kaden & A. D. Zuberbühler,* Helv. Chim. Acta *52,* 1776 (1969).
[7] *Th. A. Kaden,* Helv. Chim. Acta *54,* 625 (1971).
[8] *M. Briellmann & A. D. Zuberbühler,* Helv. Chim. Acta *65,* 46 (1982).
[9] *A. D. Zuberbühler & S. Fallab,* Helv. Chim. Acta *50,* 889 (1967).
[10] *H. Gampp, M. Maeder & A. D. Zuberbühler,* Talanta *27,* 1037 (1980).
[11] *D. W. Margerum, G. R. Cayley, D. C. Weatherburn & G. K. Pagenkopf* in 'Coordination Chemistry' Ed. A. E. Martell, Vol. 2, American Chemical Society, Washington 1978, p. 1.
[12] *A. Frost & R. Pearson,* 'Kinetics and Mechanisms', Wiley, New York 1963, p. 186.