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# Ternary Complexes in Solution. 25.[1] Influence of Alkyl Side Chains with Hydroxy or Thioether Groups on the Stability of Binary and Ternary Copper(II)–Dipeptide Complexes

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Equilibrium constants have been measured potentiometrically for protonation and $Cu^{2+}$ coordination of glycyl-L-serine, glycyl-L-threonine, glycyl-*S*-methylcysteine, or glycyl-L-methionine [=glycyl(α-alkyl-*S*/*O*)glycines] and L-serylglycine, L-threonylglycine, *S*-methyl-L-cysteinylglycine, or L-methionylglycine [=(α-alkyl-*S*/*O*-glycyl)glycines]. For two of these dipeptides, glycyl-*S*-methyl-L-cysteine and *S*-methyl-L-cysteinylglycine, synthetic routes have been developed. Besides the binary complexes $CuL^+$ and Cu(L–H), the mixed-ligand complexes with 2,2′-bipyridyl, viz., Cu(bpy)$L^+$ and Cu(bpy)(L–H), were also studied. The results were evaluated by comparison with data obtained earlier for glycyl(*N*- or α-alkyl)glycinates and (*N*- or α-alkylglycyl)glycinates. For the glycyl(α-alkyl-*S*/*O*)glycinates, the stability of $CuL^+$ depends only on the basicity of the amino group, while for the complexes with (α-alkyl-*S*/*O*-glycyl)glycinates, there is a considerable increase in stability attributable to an interaction of the hydroxy or thioether groups with an apical coordination position of $Cu^{2+}$. With the (α-alkyl-*S*/*O*-glycyl)glycinates, the ionization of the amide proton is also somewhat facilitated; i.e., Cu(L–H) is somewhat more stable for these dipeptides than it is with glycylglycinate, whereas those of the glycyl(α-alkyl-*S*/*O*)glycinates are of the same stability. However, from detailed considerations it is concluded that the donor atom of the side chain in both types of dipeptides coordinates in Cu(L–H). The results obtained for the ternary systems resemble those of the binary ones; the main difference is that deprotonation of the amide group in the complexes is shifted toward higher pH values. The possible structures of these binary and ternary complexes are discussed.

The deprotonated peptide nitrogen is considered important in protein–copper binding,[4,5] and thus the coordination of peptides has received considerable attention.[6–12] Complex formation between $Cu^{2+}$ and peptides in aqueous solution starts with the terminal amino group and not from the carboxylate end, forming chelates involving the terminal amino moiety and the oxygen of the neighboring amide group. These complexes are usually deprotonated at the amide group in the pH range 4–7, and chelates are formed by the coordination of $Cu^{2+}$ to the deprotonated nitrogen of the amide group. This holds usually not only for binary but also for ternary, i.e., mixed-ligand, complexes.[13–15]

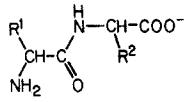
In order to learn how coordination may be altered by the kind of amino acids in the two terminal residues of such peptides or proteins, we have studied the simplest models—dipeptides; they contain all of the binding sites of interest. The first aim was to evaluate the influence of bulky alkyl groups on the stability and acidity of complexes formed in binary copper(II)–dipeptide and ternary 2,2′-bipyridyl–copper(II)–dipeptide systems.[15] Depending on the position of the alkyl group in the dipeptide, either the stability or the acidity of the complexes is altered: for the glycyl(*N*- or α-alkyl)glycinates, the stability of $CuL^+$ depends only on the basicity of the amino group, while for the complexes with (*N*- or α-alkylglycyl)glycinates, a considerable decrease in stability is observed. In contrast, with (*N*- or α-alkylglycyl)glycinates ionization of the amide proton is facilitated; i.e., Cu(L–H) is more stable for these dipeptides than with glycylglycinate, whereas the corresponding complexes of the glycyl-α-alkylglycinates are less stable.

In the present work, binary copper(II)–dipeptide and ternary 2,2′-bipyridyl–copper(II)–dipeptide systems with dipeptides containing alkyl side chains with hydroxy or thioether groups have been studied; the influence of these potentially weakly coordinating groups[16–18] on the stability of complexes was evaluated. The hydroxy- or thioether-substituted dipeptides studied, together with three dipeptides used for comparisons, are indicated in Figure 1.

## Experimental Section

**A. Synthesis. Materials.** Ionac A-310 was a gift from the Ionac Chemical Co., Birmingham, N.J. Carbobenzoxy chloride, carbobenzoxyglycine, glycine ethyl ester hydrochloride, and *N*,*N*′-dicyclohexylcarbodiimide were purchased from Sigma Chemical Co., St. Louis, Mo. Ethyl chloroformate, triethylamine, and trifluoroacetic acid were from Eastman Organic Chemicals, Rochester, N.Y. *S*-Methyl-L-cysteine, *N*-methylmorpholine, acetonitrile, and iodomethane were obtained from Aldrich Chemical Co., Milwaukee, Wis. Pyridine was from Allied Chemical Corp., Morristown, N.J., and Dowex 1-X2 and Dowex 50W-X8 were from Bio-Rad Laboratories, Richmond, Calif.

Thin-layer chromatography was performed on silica gel plates (N-HR) from Brinkman Instrument Co., Los Angeles, Calif. The following solvent systems were used: butanol–acetic acid–water (=BAW, 4:1:1 v/v/v), chloroform–methanol (=CM, 19:1 v/v), and chloroform–methanol–acetic acid (=CMA, 18:1:1 v/v/v). Paper chromatography was performed on Whatman No. 1 paper using ascending butanol–acetic acid–water (=BAW, 2:1:1 v/v/v).



$R^1 = R^2 = H$: glycylglycinate (gg)

$R^1 = H$; $R^2 = CH_2OH$: glycyl-L-serinate (gs)
$= CH(OH)CH_3$: glycyl-L-threoninate (gt)
$= CH_2SCH_3$: glycyl-*S*-methyl-L-cysteinate (gmc)
$= CH_2CH_2SCH_3$: glycyl-L-methioninate (gm)
$= CH_2CH(CH_3)_2$: glycyl-L-leucinate (gl)

$R^2 = H$; $R^1 = CH_2OH$: L-serylglycinate (sg)
$= CH(OH)CH_3$: L-threonylglycinate (tg)
$= CH_2SCH_3$: *S*-methyl-L-cysteinylglycinate (mcg)
$= CH_2CH_2SCH_3$: L-methionylglycinate (mg)
$= CH_2CH(CH_3)_2$: L-leucylglycinate (lg)

**Figure 1.** List of dipeptides used in this study.

*N*-Methylmorpholine and ethyl chloroformate were distilled before use and stored over silica gel. Acetonitrile was distilled from calcium hydride and pyridine from ninhydrin; triethylamine and trifluoroacetic acid were also distilled before use; all of these reagents were stored over desiccant.

Dowex 50W-X8 was washed with 1 N HCl until the pH of the effluent dropped to <1 and then washed with $H_2O$ until the pH rose to 4–5. Dowex 1-X2 was swollen in $H_2O$, washed with 1 N NaOH and 1 N sodium acetate until $Cl^-$ could not be detected in the effluent, and then washed with water. Ionac A-310 was swollen in $H_2O$, washed with 0.5 N NaOH until the pH of the effluent rose to 12, and then washed with $H_2O$ until the pH fell to about 5.

**Glycyl-*S*-methyl-L-cysteine.** *S*-Methyl-L-cysteine ethyl ester hydrochloride (I) was prepared by the method of Wilson and Cohen,[19] and *N*-carboxybenzoxyglycyl-*S*-methyl-L-cysteine ethyl ester (II) as follows. With the aid of 2.0 mL of *N*-methylmorpholine (15 mmol), 2.996 g of I (15 mmol) was dissolved in 30 mL of acetonitrile; 3.14 g of *N*-carboxybenzoxyglycine (15 mmol) was added. This solution was cooled to 0 °C with stirring, and 3.10 g of *N,N'*-dicyclohexylcarbodiimide was added; the system was stirred for 2 h at 0 °C and left at 5 °C without stirring for 18 h. The reaction mixture was cooled to 0 °C, and more *N,N'*-dicyclohexylcarbodiimide (0.15 g) was added. Stirring was continued for 4 h at 0 °C; subsequently, the solution was left at 5 °C without stirring for 18 h. Glacial acetic acid (2 mL) was then added to inactivate any remaining diimide. The reaction mixture was warmed to room temperature for 30 min, cooled to 0 °C, and filtered. The *N,N'*-dicyclohexylurea was washed with cold acetonitrile. The filtrate and washings were combined and evaporated to dryness in vacuo. The clear oil was dissolved in 100 mL of ethyl acetate. The solution was filtered and extracted successively with 5% $NaHCO_3$, $H_2O$, 1 N HCl, $H_2O$, and saturated NaCl; it was then dried over $Na_2SO_4$, filtered, and concentrated in vacuo to a clear oil (II).

*N*-Carbobenzoxyglycyl-*S*-methyl-L-cysteine (III) was prepared by dissolving II in 100 mL of methanol, and 10.6 mL of 1 N NaOH was added. The reaction mixture was left standing for 1.5 h at room temperature. The solution was then acidified to Congo Red with 2 N HCl and concentrated in vacuo to yield a slightly yellow oil, which was dissolved in 800 mL of ethyl acetate, filtered, and extracted with 1 N NaOH (3 × 500 mL). The combined aqueous extracts were back-extracted with 500 mL of ethyl acetate. The aqueous layer was then acidified to pH 2 with 2 N HCl and concentrated in vacuo until turbidity resulted. Crystallization commenced upon the addition of seed crystals. The white crystals were filtered off, and the filtrate was further concentrated, giving more crystals; two more crops of crystals were obtained this way.

The ethyl acetate layers were combined and concentrated in vacuo to impure unreacted II as a yellow oil (1.8 g), which was dissolved in 30 mL of methanol, and 3.8 mL of 1 N NaOH was added. The reaction mixture was left for 2 h at room temperature, acidified, and concentrated as before. The concentrate was dissolved in 200 mL of ethyl acetate, extracted with 0.1 N NaOH (3 × 100 mL), and worked up as before. The total yield was 3.51 g (10.8 mmol), with an uncorrected melting point of 165–167 °C. The compound (III) was characterized by IR and NMR and thin-layer chromatography ($R_f$ 0.6 [CMA]).

Glycyl-*S*-methyl-L-cysteine (IV) was obtained as follows. Liquid ammonia (300 mL) was dried over metallic sodium, and then distilled into a reaction vessel containing 3.03 g of III (9.3 mmol). The solution was stirred at −78 °C until the solid had dissolved. The temperature was allowed to rise to −31 °C, and pea-sized chunks of oxide-free sodium were added until the blue color persisted for 90 s. Ammonium chloride was added until the blue color disappeared; then 0.7 mL of iodomethane was added. The ammonia was evaporated at room temperature and the residue stored in vacuo. The oily solid was taken up in water, filtered, and neutralized with 6 N HCl. The solution was concentrated to about 20 mL and loaded onto a Dowex 50W-X8 ($H^+$) column (1.5 × 87 cm). The column was washed with 3 L of $H_2O$ and then eluted with 6 L of 0.05 M pyridinium acetate, pH 4. Fractions of 20 mL were collected and assayed by the quantitative ninhydrin method of Moore and Stein.[20] Fractions 296–320 were combined and concentrated. The crystalline product was triturated with hot 95% ethanol, filtered, and extensively dried in vacuo at 60 °C over $P_2O_5$. The dried product (1.06 g, 5.6 mmol) had a melting point of 219–222 °C dec (uncor). On thin-layer chromatography, the product migrated as a single spot ($R_f$ 0.27 [BAW]) and gave a positive test with ninhydrin (a test for primary amines) and chloroplatinic acid (a test for reduced sulfur) and a negative test with nitroprusside–cyanide (a test for thiols and disulfides). The product (IV) was further identified by IR and NMR and by elemental analysis. Anal. Calcd for $C_6H_{12}N_2O_3S$: C, 37.49; H, 6.29; N, 14.57; S, 16.68. Found: C, 37.40; H, 6.16; N, 15.09; S, 16.38.

***S*-Methyl-L-cysteinylglycine.** *N*-Carbobenzoxy-*S*-methyl-L-cysteine (V) and *N*-carbobenzoxy-*S*-methyl-L-cysteinylglycine ethyl ester (VI) were prepared by the methods of Sokolovsky et al.[21] *N*-Carbobenzoxy-*S*-methyl-L-cysteinylglycine (VII) was obtained by dissolving VI (12.97 g, 36.6 mmol) in 236 mL of methanol and treating this solution with 60 mL of 1 N NaOH. The reaction mixture was left at room temperature without stirring for 100 min, cooled, acidified with 2 N HCl to Congo Red, and concentrated until it became cloudy. Crystallization commenced upon the addition of seed crystals. The crystals were collected and recrystallized from $CH_3OH$–$H_2O$. As the product was still chromatographically impure, it was dissolved in 200 mL of ethyl acetate, filtered, and extracted with 200 mL of 0.1 N HCl and then with 0.1 N NaOH (3 × 200 mL). The combined alkaline aqueous extracts were back-extracted with 200 mL of ethyl acetate. The aqueous phase was concentrated to about 100 mL. The pH was then adjusted to Congo Red with 2 N HCl, and seed crystals were added. The mixture was cooled and filtered. The precipitate was dried (9.26 g, 28 mmol) and recrystallized from $CH_3OH$–$H_2O$ (mp 129–130 °C (uncor)) giving VII, characterized by IR and NMR and thin-layer chromatography ($R_f$ 0.4 [CMA]).

Removing the carbobenzoxy-blocking group of VII by catalytic hydrogenation failed. The reaction of VII with sodium in liquid ammonia yielded a considerable amount of an unknown side product; extensive attempts at purification resulted in *S*-methyl-L-cysteinylglycine sulfoxides.[22] Therefore, the carbobenzoxy group was removed in boiling, anhydrous trifluoroacetic acid by the method of Weygand and Steglich.[24] Although the yield for this step was poor, the product was easily purified and crystallized. For this, 12.85 g of VII (39 mmol) and 7.8 g of phenol were dissolved in 39 mL of anhydrous trifluoroacetic acid. The solution was refluxed for 45 min, cooled, and evaporated to dryness in vacuo at 30 °C giving an oil which was dissolved in 100 mL of $H_2O$ and poured over a column (2.5 × 20 cm) of Ionac A-310 ($OH^-$ form). The column was washed with 100 mL of $H_2O$, and the total eluent was collected and extracted with ether. The aqueous solution was applied to a column (2.5 × 41 cm) of Dowex 1-X2 (acetate form), which was washed with 50 mL of $H_2O$ and then with 500 mL of pyridinium acetate buffer (0.01 M, pH 4.0). The total eluent was concentrated to dryness in vacuo, giving an oil which was dissolved in methanol and crystallized by the slow addition of ether. The crystalline product was triturated in methanol. The product (3.73 g, 19.5 mmol) had an uncorrected melting point of 179–181 °C dec. On thin-layer chromatography the compound migrated as a single spot ($R_f$ 0.23 [BAW]) with a trace of sulfoxides ($R_f$ 0.08 [BAW]). Similar results were observed on paper chromatography of the compound ($R_f$ 0.47 [BAW]) with a trace of sulfoxides ($R_f$ 0.31 [BAW]). The compound gave a positive result with the ninhydrin and chloroplatinic acid tests and a negative result with the nitroprusside–cyanide test. The compound was further identified by IR, NMR, and elemental analysis. Anal. Calcd for $C_6H_{12}N_2O_3S$: C, 37.49; H, 6.29; N, 14.57; S, 16.68. Found: C, 37.28; H, 6.18; N, 14.51; S, 16.60.

**B. Potentiometric Titrations. Materials and Measurements.** Glycyl-L-serine, glycyl-L-methionine, L-methionylglycine, 2,2'-bipyridyl,

Table I. Acidity Constants of Some Dipeptides and of Their Corresponding Binary $Cu^{2+}$ Systems ($I = 0.1$, $NaClO_4$; 25 °C)[a]

| Dipeptide | $pK^{H}_{H_2L}$ | $pK^{H}_{HL}$ | $pK^{2H}_{Cu+H_2L}$ | $pK^{3H}_{Cu+H_2L}$ |
|---|---|---|---|---|
| Glycylglycine[15] | 3.11 ± 0.01 | 8.15 ± 0.01 | 5.71 ± 0.07 | 9.70 ± 0.03 |
| Glycyl-L-leucine[15] | 3.13 ± 0.01 | 8.28 ± 0.01 | 5.52 ± 0.05 | 10.28 ± 0.06 |
| L-Leucylglycine[15] | 3.05 ± 0.01 | 8.10 ± 0.01 | 6.40 ± 0.22 | 9.66 ± 0.05 |
| Glycyl-L-serine | 2.99 ± 0.01 | 8.14 ± 0.01 | 5.47 ± 0.11 | 9.24 ± 0.03 |
| L-Serylglycine | 3.21 ± 0.01 | 7.33 ± 0.01 | 5.58 ± 0.06 | 9.18 ± 0.02 |
| Glycyl-L-threonine | 3.00 ± 0.02 | 8.14 ± 0.01 | 5.57 ± 0.06 | 9.71 ± 0.03 |
| L-Threonylglycine | 3.14 ± 0.01 | 7.34 ± 0.01 | 5.42 ± 0.14 | 8.97 ± 0.04 |
| Glycyl-*S*-methyl-L-cysteine | 2.90 ± 0.01 | 8.12 ± 0.01 | 5.32 ± 0.16 | 9.07 ± 0.07 |
| *S*-Methyl-L-cysteinylglycine | 3.10 ± 0.01 | 7.11 ± 0.01 | 5.21 ± 0.08 | 8.65 ± 0.03 |
| Glycyl-L-methionine | 2.93 ± 0.01 | 8.19 ± 0.01 | 5.35 ± 0.08 | 9.31 ± 0.05 |
| L-Methionylglycine | 3.13 ± 0.01 | 7.56 ± 0.01 | 6.02 ± 0.10 | 9.21 ± 0.03 |

[a] The range of error given is 3 times the standard deviation.

Table II. Equilibrium Constants of Some Binary Copper(II)–Dipeptide and Ternary 2,2′-Bipyridyl–Copper(II)–Dipeptide Systems ($I = 0.1$, $NaClO_4$; 25 °C)[a]

| Dipeptide | $\log K^{Cu}_{CuL}$ | $pK^{H}_{CuL}$ | $\log K^{Cu(bpy)}_{Cu(bpy)L}$ | $pK^{H}_{Cu(bpy)L}$ | $\Delta \log K$ |
|---|---|---|---|---|---|
| Glycylglycine[15] | 5.55 ± 0.07 | 3.99 ± 0.06 | 5.09 ± 0.10 | 7.77 ± 0.04 | −0.46 |
| Glycyl-L-leucine[15] | 5.89 ± 0.05 | 4.76 ± 0.06 | 5.75 ± 0.03 | 8.58 ± 0.03 | −0.14 |
| L-Leucylglycine[15] | 4.75 ± 0.22 | 3.26 ± 0.23 | 4.13 ± 0.10 | 6.33 ± 0.04 | −0.6 |
| Glycyl-L-serine | 5.66 ± 0.10 | 3.77 ± 0.11 | 5.56 ± 0.01 | 7.71 ± 0.10 | −0.10 |
| L-Serylglycine | 4.96 ± 0.06 | 3.60 ± 0.05 | 4.32 ± 0.29 | 5.81 ± 0.08 | −0.6 |
| Glycyl-L-threonine | 5.57 ± 0.06 | 4.14 ± 0.06 | 5.56 ± 0.04 | 8.05 ± 0.02 | −0.01 |
| L-Threonylglycine | 5.06 ± 0.14 | 3.55 ± 0.16 | 4.17 ± 0.29 | 5.97 ± 0.07 | −0.9 |
| Glycyl-*S*-methyl-L-cysteine | 5.70 ± 0.16 | 3.75 ± 0.17 | 5.65 ± 0.06 | 7.61 ± 0.09 | −0.05 |
| *S*-Methyl-L-cysteinylglycine | 5.00 ± 0.08 | 3.44 ± 0.10 | 4.24 ± 0.11 | 5.36 ± 0.17 | −0.76 |
| Glycyl-L-methionine | 5.77 ± 0.08 | 3.96 ± 0.09 | 5.66 ± 0.03 | 7.92 ± 0.01 | −0.11 |
| L-Methionylglycine | 4.67 ± 0.10 | 3.19 ± 0.12 | 3.94 ± 0.20 | 5.35 ± 0.17 | −0.7 |

[a] The range of error given is 3 times the standard deviation. For the constants of the binary $Cu^{2+}$–2,2′-bpy system, see ref 27.

$NaClO_4$, and $Cu(ClO_4)_2 \cdot 6H_2O$ were purchased from Fluka AG, Buchs, Switzerland. Glycyl-L-threonine and L-threonylglycine were from Cyclo Chemical, Division Travenol Laboratories Inc., Los Angeles, Calif., and L-serylglycine from Sigma Chemical Co., St. Louis, Mo.

The measurements were performed under $N_2$ by potentiometric titrations ($I = 0.1$, $NaClO_4$; 25 °C) as described previously.[13,25]

**Equilibrium Constants.** The acidity constant $K^{H}_{H_2L}$ of 2,2′-bipyridyl was taken from Linnell and Kaczmarczyk,[26] while the acidity constant $K^{H}_{HL}$ and the stability constants of the copper(II)–2,2′-bipyridyl 1:1 and 1:2 complexes, which were used for the evaluation of the mixed-ligand systems, were from Anderegg.[27]

The acidity constants ($K^{H}_{H_2L}$ and $K^{H}_{HL}$) of the dipeptides and the stability and acidity constants of the binary ($K^{Cu}_{CuL}$ and $K^{H}_{CuL}$) and ternary complexes ($K^{Cu(bpy)}_{Cu(bpy)L}$ and $K^{H}_{Cu(bpy)L}$) were determined exactly (including concentrations and numbers of titrations) as recently.[15] The pH range for evaluation was such that hydroxo complexes could be ignored, and this was checked by separate titrations,[15] especially in those cases where an excess of $Cu^{2+}$ or $Cu^{2+}$/2,2′-bpy was used.

## Results and Discussion

The acidity constants ($I = 0.1$, $NaClO_4$; 25 °C) of the dipeptides (eq 1 and 2) and of their binary $Cu^{2+}$ systems (eq 3 and 4) are summarized in Table I. Table II contains the equilibrium constants determined for the binary $Cu^{2+}$–dipeptide (eq 5–8) and ternary 2,2′-bipyridyl–$Cu^{2+}$–dipeptide complexes (eq 9 and 10), together with the values for $\Delta \log K$.[28] This latter constant is defined by eq 11 and corresponds to equilibrium 12; it characterizes the stability of ternary complexes.[29,30]

$$H_2L^+ \rightleftharpoons HL + H^+$$
$$K^{H}_{H_2L} = [HL][H]/[H_2L] \quad (1)$$

$$HL \rightleftharpoons L^- + H^+$$
$$K^{H}_{HL} = [L][H]/[HL] \quad (2)$$

$$Cu^{2+} + H_2L^+ \rightleftharpoons CuL^+ + 2\,H^+$$
$$K^{2H}_{(Cu+H_2L)} = [CuL][H]^2/[Cu][H_2L] \quad (3)$$

$$Cu^{2+} + H_2L^+ \rightleftharpoons Cu(L\text{-}H) + 3H^+$$
$$K^{3H}_{(Cu+H_2L)} = [Cu(L\text{-}H)][H]^3/[Cu][H_2L] \quad (4)$$

$$Cu^{2+} + L^- \rightleftharpoons CuL^+$$
$$K^{Cu}_{CuL} = [CuL]/[Cu][L] \quad (5)$$

$$CuL^+ \rightleftharpoons Cu(L\text{-}H) + H^+$$
$$K^{H}_{CuL} = [H][Cu(L\text{-}H)]/[CuL] \quad (6)$$

$$\log K^{Cu}_{CuL} = pK^{H}_{H_2L} + pK^{H}_{HL} - pK^{2H}_{(Cu+H_2L)} \quad (7)$$

$$pK^{H}_{CuL} = pK^{3H}_{(Cu+H_2L)} - pK^{2H}_{(Cu+H_2L)} \quad (8)$$

$$Cu(bpy)^{2+} + L^- \rightleftharpoons Cu(bpy)L^+$$
$$K^{Cu(bpy)}_{Cu(bpy)L} = [Cu(bpy)L]/[Cu(bpy)][L] \quad (9)$$

$$Cu(bpy)L^+ \rightleftharpoons Cu(bpy)(L\text{-}H) + H^+$$
$$K^{H}_{Cu(bpy)L} = [H][Cu(bpy)(L\text{-}H)]/[Cu(bpy)L] \quad (10)$$

$$\Delta \log K = \log K^{Cu(bpy)}_{Cu(bpy)L} - \log K^{Cu}_{CuL} = \log K^{CuL}_{CuL(bpy)} - \log K^{Cu}_{Cu(bpy)} \quad (11)$$

$$CuL^+ + Cu(bpy)^{2+} \rightleftharpoons Cu(bpy)L^+ + Cu^{2+} \quad (12)$$

**Stability and Structure of the Binary Complexes $CuL^+$ and Cu(L–H).** As mentioned, the initial complex formation between $Cu^{2+}$ and a peptide results in a chelate involving the terminal amino moiety and the oxygen of the neighboring amide group. Thus, the second (bifunctional) amino acid in a peptide should have little influence on the stability of the complexes. Indeed, Rabin[31] has observed a straight line by plotting $\log K^{Cu}_{CuL}$ vs. $pK^{H}_{HL}$ for a number of glycyl peptides; i.e. the stability of the complexes depends mainly on the basicity of the terminal amino group. Our recent results[15] on

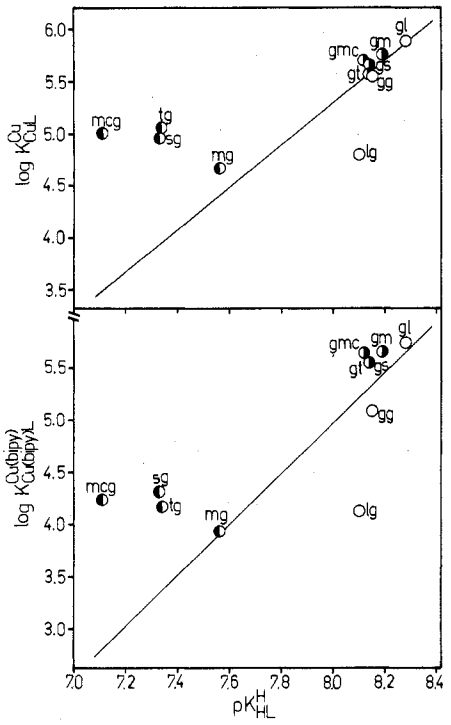



**Figure 2.** Relation between log $K^{Cu}_{CuL}$ and $pK^{H}_{HL}$ for the *binary* 1:1 complexes, $CuL^+$ (*upper part*), and between log $K^{Cu(bpy)}_{Cu(bpy)L}$ and $pK^{H}_{HL}$ for the *ternary* 1:1:1 complexes, $Cu(bpy)L^+$ (*lower part*), where L = dipeptide without a potential binding group in a side chain (○), dipeptide with a potential binding group in the side chain of the first half of the molecule (◐), or dipeptide with a potential binding group in the side chain of the second half of the molecule (◑) (for abbreviations and data see Figure 1 and Tables I and II, respectively). The straight reference lines are taken from earlier work;[15] they represent the results obtained for the corresponding $Cu^{2+}$ complexes with L = glycyl(*N*- or α-alkyl)glycinates.

copper(II)–glycyl(*N*- or α-alkyl)glycinate systems confirm this, giving the straight line used as the reference line in Figure 2: in the upper half, the data of the binary systems from Tables I and II are plotted.

Evidently the data for the copper(II) dipeptides where the glycine moiety contains alkyl side chains with hydroxy or thioether groups [=glycyl(α-alkyl-*S*/*O*)glycines] fit within experimental error the straight line of Figure 2 (upper half), indicating that the substituent with the potential binding side has no significant influence on complex stability; i.e., it does not coordinate. However, the (α-alkyl-*S*/*O*-glycyl)glycinate–copper(II) complexes are about 0.3–1.5 log units more stable than expected on the basis of the basicity of the terminal amino group of the dipeptides, showing that hydroxy and thioether groups substituted at the glycyl moiety enhance the complex stability and thus participate in complex formation. Contrary, steric effects cause simple (*N*- or α-alkylglycyl)-glycinate–copper(II) complexes to be *less* stable than is expected on the basis of the basicity of the terminal amino group.[15] In the case of copper(II) L-leucylglycinate, for example, this accounts for a *decrease* in stability of about 0.7 log unit (cf. Figure 2, upper part).

To elucidate the structure of the (α-alkyl-*S*/*O*-glycyl)-glycinate–copper(II) complexes, which show increased stability, it is necessary to consider first the coordination sphere of the metal ion: "$Cu^{2+}$ takes up a distorted octahedral structure with four short planar metal ion–ligand distances and two longer axial ones. Stronger ligand field donors appear in the planar positions and with a sufficient number of nitrogen donors in planar positions, axial interactions become weak and even nonexistent."[32,33] The stabilities[34] and catalytic behavior[35] of $Cu^{2+}$ complexes in aqueous solution also show that $Cu^{2+}$ usually prefers a square-planar structure. There is evidence, however, that some potentially tridentate amino acetates, e.g., aspartate, do interact with one of the apical $Cu^{2+}$ positions.[36,37] On the other hand, the hydroxy group of serinate or threoninate does not seem to complex with $Cu^{2+}$,[38-41] although it does with $Mn^{2+}$,[38] $Co^{2+}$, $Ni^{2+}$, or $Zn^{2+}$,[40] which have a regular octahedral coordination sphere. For (α-alkyl-*S*/*O*-glycyl)-glycinate–copper(II) complexes, assuming that the stronger donors bind to the planar positions, the hydroxy and thioether groups can coordinate only apically to the metal ion. The three binding sites, i.e., terminal amino group, amide oxygen, and thioether or hydroxy group, cannot all coordinate to the square plane of $Cu^{2+}$ for steric reasons.

On the basis of the suggested structures for the $CuL^+$ complexes, one can easily rationalize why the complex with L-methionylglycinate is stabilized less than those with *S*-methyl-L-cysteinylglycinate, L-serylglycinate, or L-threonylglycinate (cf. Figure 2, upper part): in the latter dipeptides the donor atom of the side chain is positioned such that a five-membered chelate results from coordination, whereas with the thioether group of L-methionylglycinate a six-membered chelate is formed. Studies of binary[42] and ternary[43] complexes show that five-membered rings are favored over six-membered ones.[44]

At higher pH, the $Cu^{2+}$ complex where, e.g., glycylglycinate is bound in a bidentate manner undergoes deprotonation of the amide group and rearrangement to the tridentate chelate Cu(L–H) in which the dipeptide is coordinated via the amino group, the amide nitrogen, and the carboxylate group.[8,13,33] For the $CuL^+$ complexes with the (α-alkyl-*S*/*O*-glycyl)-glycinates, in which the donor atom of the side chain coordinates, the "steric effects" of a noncoordinating alkyl side chain,[15] as in L-leucylglycinate, are expected to diminish. Indeed, the deprotonation tendency of $CuL^+$ for L-seryl-, L-threonyl-, and *S*-methyl-L-cysteinylglycinate ($pK^{H}_{CuL} \simeq 3.5$; *cf.* Table II) is between those for L-leucylglycinate ($pK^{H}_{CuL}$ = 3.26) and glycylglycinate ($pK^{H}_{CuL}$ = 3.99), while that of Cu(L-methionylglycinate)$^+$, where the coordination tendency of the thioether group is smaller, corresponds closely ($pK^{H}_{CuL}$ = 3.19) to the value of Cu(L-leucylglycinate)$^+$.

Similarly, for the $CuL^+$ complexes with the glycyl(α-alkyl-*S*/*O*)glycinates, a facilitated deprotonation of the amide nitrogen is expected if the donor atom of the side chain coordinates in Cu(L–H). Indeed, Cu(glycyl-L-leucinate)$^+$, with a noncoordinating alkyl side chain, is less acidic ($pK^{H}_{CuL}$ = 4.76) than are the copper(II)–glycyl(α-alkyl-*S*/*O*)glycinate complexes ($pK^{H}_{CuL} \simeq 3.9$; cf. Table II), whereas their acidity is close to that of Cu(glycylglycinate)$^+$ ($pK^{H}_{CuL}$ = 3.99).

**Stability and Structure of the Ternary Complexes $Cu(bpy)L^+$ and Cu(bpy)(L–H).** From the results in Tables I and II, one may conclude that the influence of alkyl substituents containing hydroxy or thioether groups on the stability of the ternary complexes, $Cu(bpy)L^+$, parallels the observations made with the binary $CuL^+$. A more detailed evaluation of the data shown in the lower part of Figure 2, where log $K^{Cu(bpy)}_{Cu(bpy)L}$ is plotted vs. $pK^{H}_{HL}$, confirms this conclusion. The reference line is again taken from our earlier work[15] and refers to the corresponding complexes formed with glycyl(*N*- or α-alkyl)glycinates. In $Cu(bpy)L^+$, the glycyl(α-alkyl-*S*/*O*)-glycinates coordinate bidentately using the terminal $NH_2$ group and the amide oxygen. For the (α-alkyl-*S*/*O*-glycyl)-glycinate–copper(II)–2,2′-bipyridyl complexes, we assume the stronger ligand field donors to be equatorial. This, with the evidence from the stability data (lower part of Figure 2) that these dipeptides act as tridentate ligands, leads to the conclusion that the thioether or hydroxy groups coordinate apically.

The stability of $Cu(bpy)L^+$ may also be characterized by $\Delta \log K$ (eq 11; Table II), which corresponds to equilibrium 12. Commonly, for mixed-ligand $Cu^{2+}$ complexes formed by 2,2′-bipyridyl and a ligand with an O and N donor, $\Delta \log K$ is usually[30] about −0.4. This is also true for the systems with glycinamide[15] and glycylglycinate (Table II), while for the glycyl(*N*- or α-alkyl)glycinates, $\Delta \log K \simeq -0.1$ and for the (*N*- or α-alkylglycyl)glycinates, $\Delta \log K \simeq -0.8$.[15] These latter values are practically identical with the ones observed for the ternary systems with glycyl(α-alkyl-*S*/*O*)glycinates and (α-alkyl-*S*/*O*-glycyl)glycinates where $\Delta \log K \simeq -0.1$ and −0.7, respectively (Table II). The result for the glycyl(α-alkyl-*S*/*O*)glycinates is not surprising, because the potential donor atoms in the side chain of the glycinate part cannot participate in complex formation for steric reasons.[13] Indeed this agrees with the results obtained for complex stability in Figure 2. However, the corresponding result for the (α-alkyl-*S*/*O*-glycyl)glycinates must mean that the alterations in complex stability, resulting from the presence of coordinating atoms in the side chain of the glycyl part, are approximately the same for the binary and ternary complexes and do therefore not appear in $\Delta \log K$. These results are thus further support for the structures of the complexes outlined in the preceding paragraph.

Comparison of the stability of the mixed-ligand complex Cu(bpy)(L–H) reveals that the deprotonation of the amide proton occurs in the glycyl(α-alkyl-*S*/*O*)glycinates at somewhat lower pH than with glycyl-L-leucinate (cf. Table II), and the acidity constants of the glycyl(α-alkyl-*S*/*O*)-glycinate complexes are rather close to that of glycylglycinate. It is uncertain whether this indicates a reduction in the "steric effects" of the side chain[15] by the coordinating atom in these chains or if the glycyl(α-alkyl-*S*/*O*)glycinates coordinate just as glycylglycinate. If the latter is true, the somewhat greater acidity constant of the glycyl-L-leucinate complex could be due to a hydrophobic interaction between the bulky alkyl moiety of the leucinate part and 2,2′-bipyridyl, which would make the rearrangement connected with the deprotonation more difficult. Similar hydrophobic interactions are known;[46] they correspond to the metal ion bridged stacking adducts as observed, for example, between 2,2′-bipyridyl[47] or indole derivatives[1] and purine nucleotides.[30] The ternary complexes with (α-alkyl-*S*/*O*-glycyl)glycinates are more acidic than the ones with glycylglycinate or even with L-leucylglycinate (Table II), indicating an increased stability of Cu(bpy)(L–H) and possibly also participation of the donor atoms of the side chains in complex formation.

We have already concluded[13,15] that in the mixed-ligand complexes Cu(bpy)(L–H) the square-planar coordination sphere of $Cu^{2+}$ consists of bipyridyl, the amino moiety, and the deprotonated nitrogen of the neighboring amide group, leaving the carboxylate group uncoordinated. This suggestion is based on the observation that the values of $pK^{H}_{Cu(bpy)L}$ for the ternary $Cu^{2+}$ complexes with glycinamide and glycylglycinate are identical within experimental error (=7.7)[15] and that those of other oligoglycines are very similar (≃8).[13] In contrast to this, the values of $pK^{H}_{CuL}$ differ for the binary complexes with glycinamide (=7.01) and glycylglycinate (=3.99)[15] by 3 log units, due to the participation of an extra binding site, viz., carboxylate, in the glycylglycinate complex when $Cu^{2+}$–N(amide) coordination occurs. The sensitivity of the acidity of the amide proton in these complexes to the participation of a further binding site also follows from results with other oligoglycines ($pK^{H}_{CuL} \simeq 5.3$),[13] which offer as an additional binding site the carbonyl oxygen of the second amide group. It should also be noted that $pK^{H}_{CuL}$ of the binary and $pK^{H}_{Cu(bipy)L}$ of the ternary complexes differ by 3.7 log units for glycylglycinate, while for the bidentate glycinamide, the

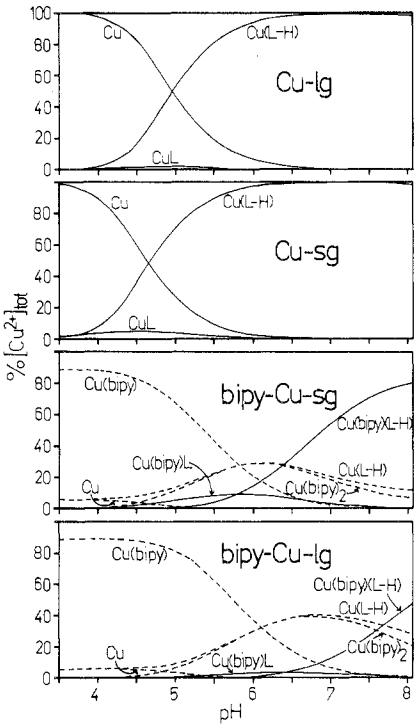



**Figure 3.** Influence of pH on the concentrations of the species present in aqueous solutions of the systems copper(II)–L-leucylglycine (Cu–lg), copper(II)–L-serylglycine (Cu–sg), 2,2′-bipyridyl–copper(II)–L-serylglycine (bpy–Cu–sg), and 2,2′-bipyridyl–copper(II)–L-leucylglycine (bpy–Cu–lg) (from top to bottom). Results are given as the percentage of the total $Cu^{2+}$ present, computed with the equilibrium constants of Tables I and II for concentrations of $10^{-3}$ M for each reactant. The concentrations of $CuL^+$ in the ternary systems are <1%; the concentrations of dipeptide–hydroxo complexes are <4% in the binary and ternary systems at pH ≤8.

difference is only 0.7 log unit,[15] suggesting that the carboxylate group plays an important role in the binary complex but not in the ternary one.

This interpretation of the structure of these ternary complexes in solution contrasts with a recent crystal structure analysis, done by Martin et al.,[48] of the ternary 1,10-phenanthroline–copper(II)–glycylglycinate complex. In the solid state, the glycylglycine dianion coordinates with all three binding sites, i.e., including the carboxylate group, to the square-plane around $Cu^{2+}$. While one of the phenanthroline nitrogens completes the square, the other occupies a tilted apical position, giving a distorted square-pyramidal geometry about $Cu^{2+}$. On the basis of visible spectra, it is suggested[48] "that an appreciable, perhaps predominant, fraction of the complexes in aqueous solution adopts the structure found in the crystal" and that this holds also for the 2,2′-bipyridyl complex, although an evaluation of absorption spectra of such systems at higher pH is difficult because of the many species present.[49]

Though this somewhat conflicting situation remains unsolved, we still favor our earlier conclusion[13,15] because, based on the structure suggested for aqueous solution, the different acidities and stabilities of the binary and ternary complexes can easily be rationalized. For a structure of the ternary complex Cu(bpy)(L–H), which allows a threefold coordination of the dipeptide to the square plane of $Cu^{2+}$, the nature of the third binding site (i.e., a carboxylate, carbonyl, or neither) should also be reflected in the acidity constants of $Cu(bpy)L^+$, as it is the case in the binary systems for $CuL^+$. Of course, (i) should a dynamic Jahn–Teller effect involving fluxional motion of the coordination sphere[53,54] occur in Cu(bpy)(L–H) in solution or (ii) should the fraction of the complex having

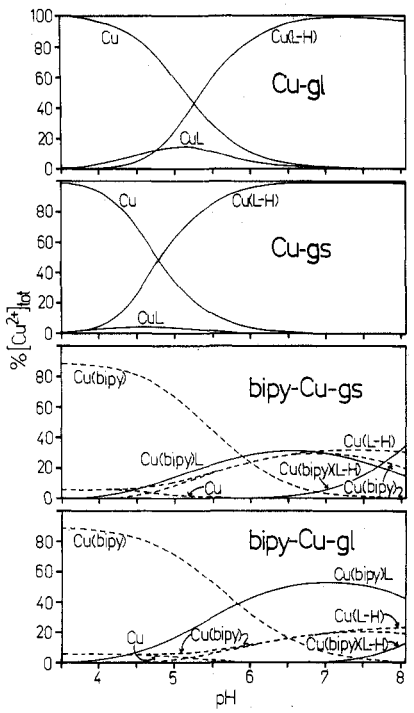



**Figure 4.** Influence of pH on the concentrations of the species present in aqueous solutions of the systems copper(II)–glycyl-L-leucine (Cu–gl), copper(II)–glycyl-L-serine (Cu–gs), 2,2′-bipyridyl–copper(II)–glycyl-L-serine (bpy–Cu–gs), and 2,2′-bipyridyl–copper(II)–glycyl-L-leucine (bpy–Cu–gl) (from top to bottom). Results are given as the percentage of the total $Cu^{2+}$ present, computed with the equilibrium constants of Tables I and II for concentrations of $10^{-3}$ M for each reactant. The concentrations of $CuL^+$ in the ternary systems are <1.5%; the concentrations of dipeptide–hydroxo complexes are <4% in the binary and ternary systems at pH ≤8.

in solution the structure found in the crystal[48] be only 30% or less,[55] then the apparent conflict would largely be solved.

**General Considerations.** Though the interpretation of some of the results with regard to the structure of the complexes is presently not unambiguously possible, it is clear that donor atoms present in the alkyl side chains of amino acid residues can participate in complex formation. This is especially evident from the binary and ternary complexes $CuL^+$ and $Cu(bpy)L^+$ that are formed with the ($\alpha$-alkyl-*S*/*O*-glycyl)glycinates. This means that hydroxy and thioether groups present in the side chains of proteins must be taken into account in considering potential binding sites, not only for $Cu^{2+}$ but also for other metal ions. Obviously, "borderline" $Cu^{2+}$ will complex with both the "hard" hydroxy and the "soft" thioether groups, while the "harder" metal ions like $Mn^{2+}$ and $Zn^{2+}$ prefer hydroxy groups, and the "softer" $Ag^+$ and $Hg^{2+}$ exhibit a higher affinity to thioether moieties.[17,18,56]

Finally, it is worthwhile to correlate the stability of some complexes with their concentration in solution. In Figure 3, the systems with L-serylglycinate are compared with the corresponding ones with L-leucylglycinate, while in Figure 4, the distribution of species is given for the glycyl-L-serinate and glycyl-L-leucinate systems. At first sight, the various systems appear rather similar, but a closer inspection reveals differences in the formation degree of species as well as in the pH range in which they are formed.

The influence of a ligand like 2,2′-bipyridyl on the distribution of complexes formed in a copper(II)–dipeptide system is evident from both figures. While in a binary system in certain pH regions the concentration of one complex strongly dominates, this is no longer true in the presence of 2,2′-bipyridyl where at all pH values a number of species are in equilibrium. This holds especially for the physiological pH range at about 7 and is of interest regarding exchange reactions in a "floating" biological system. In addition, it should be noted that in the mixed-ligand systems, the $Cu(bpy)L^+$ ternary complexes, which contain a dipeptide with an un-ionized amide group, occur in larger concentrations than the corresponding $CuL^+$ species in the binary system (see Figure 4). This demonstrates how mixed-ligand complex formation may increase or decrease the concentration of species containing a certain ligand form.

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**Registry No.** I, 52799-91-4; II, 61586-99-0; III, 61587-00-6; IV, 61587-01-7; V, 61587-02-8; VI, 61587-03-9; VII, 61587-04-0; glycyl-L-serine, 687-63-8; L-serylglycine, 7361-43-5; glycyl-L-threonine, 686-44-2; L-threonylglycine, 7093-70-1; glycyl-L-methionine, 14486-03-4; L-methionylglycine, 554-94-9; $Cu(gs)^+$, 61587-50-6; $Cu(sg)^+$, 61587-51-7; $Cu(gt)^+$, 61587-52-8; $Cu(tg)^+$, 61587-53-9; $Cu(gmc)^+$, 61587-54-0; $Cu(mcg)^+$, 61587-55-1; $Cu(gm)^+$, 61587-56-2; $Cu(mg)^+$, 61587-57-3; $Cu(bpy)(gs)^+$, 61587-58-4; $Cu(bpy)(sg)^+$, 61587-59-5; $Cu(bpy)(gt)^+$, 61587-60-8; $Cu(bpy)(tg)^+$, 61587-61-9; $Cu(bpy)(gmc)^+$, 61587-62-0; $Cu(bpy)(mcg)^+$, 61587-63-1; $Cu(bpy)(gm)^+$, 61618-17-5; $Cu(bpy)(mg)^+$, 61618-18-6; *N*-carboxybenzoxyglycine, 1138-80-3; *S*-methyl-L-cysteinylglycine, 61587-05-1.

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# Electronic Spectrum of Hydroxopentaamminechromium(III)

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Absorption, luminescence, and luminescence excitation spectra of single crystals and powders of $[Cr(NH_3)_5OH](ClO_4)_2$ have been measured between 7 K and room temperature. $[Cr(NH_3)_5OH]^{2+}$ takes a unique position within the series $[Cr(NH_3)_5X]^{n+}$ (X = $I^-$, $Br^-$, $Cl^-$, $F^-$, $H_2O$, $OH^-$). The great capacity of the $OH^-$ ligand to destabilize $z^2$, $xz$, and $yz$ orbitals leads to a large tetragonal crystal field component. This causes a large tetragonal splitting of the second spin-allowed band as well as a reversal of the order of the lowest lying doublet states. The lowest excited state, from which luminescence is observed, is not a component of $^2E_g$ as in "normal" chromium(III) complexes but is the $^2E$ component ($C_{4v}$ notation) of $^2T_{1g}$. A progression in the totally symmetric Cr–O stretching frequency of 570 $cm^{-1}$ is observed in the luminescence spectrum. The positions of nine experimentally located electronic states could be rationalized by a crystal field calculation.

## 1. Introduction

There have been a number of spectroscopic investigations of complexes of the type $[CrN_5X]^{n+}$ and $[CrN_4X_2]^{n+}$ (N = nitrogen ligand; X = $I^-$, $Br^-$, $Cl^-$, $F^-$, $H_2O$, $OH^-$) in recent years.[1–5] Low-temperature single-crystal absorption spectra, luminescence spectra, and EPR spectra have led to a better understanding of the electronic structure in these classes of compounds.

No single-crystal study of the complex $[Cr(NH_3)_5OH]^{2+}$ has been performed so far, and the powder luminescence and diffuse reflectance spectra of $[Cr(NH_3)_5OH)](ClO_4)_2$ have been measured at 77 K under rather poor resolution.[6] On the other hand, as far as its electronic properties are concerned, the hydroxopentaamminechromium(III) complex is expected to take quite a unique position within the series $[Cr(NH_3)_5X]^{n+}$. This expectation is based on the great capacity of the hydroxo ligand for both $\sigma$ and $\pi$ bonding,[7,8] which should lead to a large tetragonal crystal field component. A large splitting of the second spin-allowed band is observed in a solution and diffuse reflectance spectrum of this complex. Our main interest in the hydroxopentaamminechromium(III) complex arose from our studies of hydroxo- and oxo-bridged polynuclear chromium(III) complexes.[9,10] In order to understand the spectroscopic properties of dinuclear $[(NH_3)_5CrOHCr(NH_3)_5]^{5+}$ (acid rhodo chromium) and $[(NH_3)_5CrOCr(NH_3)_5]^{4+}$ (basic rhodo chromium) it is necessary to get a feeling about the electronic properties of the constituent single ions. Since exchange interactions cannot simply be switched off, some suitable mononuclear complex has to be used as a reference. It was thought that, as far as the strength of both $\sigma$ and $\pi$ bonding within the Cr–O bond was concerned, $[Cr(NH_3)_5OH]^{2+}$ would lie in between the two dinuclear complexes $[(NH_3)_5CrOHCr(NH_3)_5]^{5+}$ and $[(NH_3)_5CrOCr(NH_3)_5]^{4+}$. In $[(NH_3)_5CrOHCr(NH_3)_5]^{5+}$ $^2E_g$ is the lowest lying excited state, from which luminescence is observed.[9] On the other hand it was not possible to detect any luminescence from $[(NH_3)_5CrOCr(NH_3)_5]^{4+}$ even at very low temperatures.[10] This behavior is quite unusual for a chromium complex, and one possible explanation is that due to the very large tetragonal crystal field component the $^2E$ component ($C_{4v}$ notation) of the $^2T_{1g}$ state has been pushed way below $^2E_g$, thus leading to a situation where luminescence is expected to be rather broad and lie in the infrared region where detection becomes a problem.

## 2. Experimental Section

$[Cr(NH_3)_5OH](ClO_4)_2$ was prepared by the method of Linhard.[11]