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AMINO ACIDS AND PEPTIDES—V[1]

NOVEL PEPTIDE BOND FORMATION CATALYZED BY METAL IONS—III[1]

ELUCIDATION OF THE FORMATION MECHANISM*

M. WAGATSUMA, S. TERASHIMA and S. YAMADA*

Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo, Japan

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Abstract—Elucidation of the mechanism for peptide bond formation observed when an amino acid ester is treated with anhyd $CuCl_2$ in an anhyd alcoholic solvent, was attempted using results of IR and visible spectra measurements of the amino acid ester-$CuCl_2$ complex and accumulated experimental data.

This novel reaction proceeds through a mechanism in which the amino anion produced by elimination of the proton from the Cu(II)-coordinating amino group, attacks the non-activated ester CO group of the amino acid ester which shares a common Cu(II) ion. It differs completely from the peptide formation reaction featuring a Co(III)-amino acid ester complex.

Previously we reported,[1,2] that peptide bond formation occurs when amino acid esters are treated with Cu(II) ion in a completely anhydrous solvent, and that this novel discovery depends on several kinds of divalent metal ions accelerating the hydrolysis rate of the ester group.[2]

The following three mechanisms are possibly the key steps in the acceleration of the hydrolysis rate observed for the amino acid ester:

(a) Activation of the ester CO group by direct coordination of the ester group to the metal cation (mechanism A: $X = OH^-$),[3] (b) the attack of the metal-coordinating hydroxide anion to the non-activated ester CO group (mechanism B: $X = OH^-$),[3] and (c) the decrease in electron density of the ester CO group due to coordination of the amino group of the amino acid ester to the metal cation making the ester CO group susceptible to attack by the hydroxide anion (mechanism C: $X = OH^-$).[4] Accelerated hydrolysis of the amino acid ester by the metal cation proceeds exclusively through mechanism A,[3] and the peptide bond formation independently developed by Buckingham[5] and Collman,[6] using the Co(III) complex, is also due to an analogous kind of ester group activation (mechanism A: $X = NH_2CH(R)COOR'$).

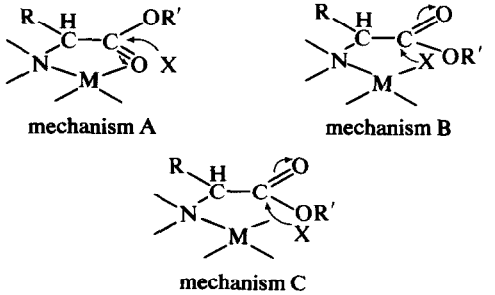

CHART 1

To understand the behaviour of the amino acid ester in the vicinity of Cu(II) ion, IR and visible spectra of the amino acid ester-cupric chloride ($CuCl_2$) complex were investigated. Based on these spectral measurements and on experimental data[1,2] collected for several kinds of amino acid esters, we determined that this novel peptide formation proceeds through mechanism B ($X = NH^-CH(R)COOR'$).†

I. *IR spectra of the amino acid ester*-$CuCl_2$ *complex*

The IR spectra in a solid state of 1:1 and 2:1 complexes of amino acid ester and Cu(II) ion, have been reported.[7,8] We also prepared these complexes[7,8] and measured their IR spectra in a solid state and in a chloroform solution.

In a solid state, dichloro-mono(methyl L-alaninato)-copper(II) (Cu(Ala-OMe)Cl_2) exhibited its $\nu_{C=O}$ absorption at 1660 cm^{-1} due to the Cu(II)-coordinating ester group. The IR spectrum of dichloro-bis(methyl L-alaninato)-copper(II) (Cu(Ala-OMe)$_2Cl_2$) showed two split $\nu_{C=O}$ absorptions of equal intensity at 1745 and 1715 cm^{-1}. Although the ester $\nu_{C=O}$ absorption of Cu(Ala-OMe)$_2Cl_2$

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†All amino acids except glycine, have L-configuration.

Abbreviations denoting amino acid and peptide derivatives are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature; *Biochemistry* 5, 2485 (1966)

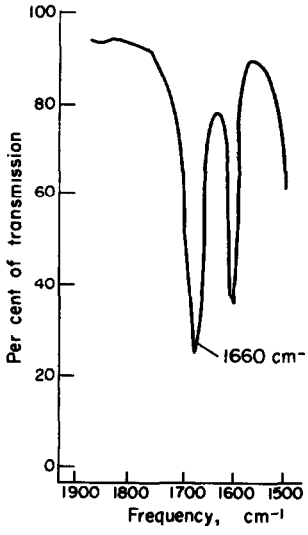


Fig 1. IR spectrum of $Cu(Ala\text{-}OMe)Cl_2$ in a solid state (KBr).

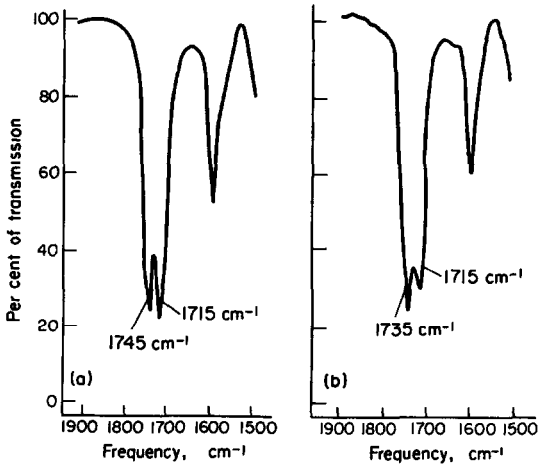


Fig 2. IR spectra of $Cu(Ala\text{-}OMe)_2Cl_2$ in a solid state (KBr) (a) and in a chloroform solution (b).

appearing at 1745 cm^{-1} is reasonably assigned to the non-coordinating ester group, as explained,[7,8] it is not clear whether the absorption at 1715 cm^{-1} can be explained as the presence of the weakly Cu(II)-coordinating ester group, or whether it has some other source. The IR spectrum of the 2:1 complex in a chloroform solution, exhibited a similar double split $\nu_{C=O}$ absorption at 1735 and 1715 cm^{-1}. These were the same phenomena as previously observed with dichloro-bis(ethyl glycinato)-copper(II) ($Cu(Gly\text{-}OEt)_2Cl_2$).[2]

To understand the environmental change of Cu(II) ion in the course of peptide bond formation, IR spectra measurements were periodically made on a chloroform solution of $Cu(Ala\text{-}OMe)_2Cl_2$ after 6·0 equivs of Ala-OMe was added. Just after the addition of Ala-OMe, the absorption at 1715 cm^{-1} observed for $Cu(Ala\text{-}OMe)_2Cl_2$ in a solid state and in a chloroform solution, suddenly disappeared, and the IR spectrum showed a single $\nu_{C=O}$ absorption at 1735 cm^{-1}, which was almost the same as that measured for the non-coordinating ester group. When the chloroform solution was kept at room temperature and its IR spectrum was periodically measured after 1·0, 3·0 and 20·0 hr reaction, amide $\nu_{C=O}$ absorption was clearly observed in addition to the single ester $\nu_{C=O}$ absorption at 1735–1740 cm^{-1}. These results show that peptide bond formation gradually occurred in the chloroform solution, and that there was no interaction such as coordination between the ester group of the amino acid ester and Cu(II) ion during peptide formation. IR spectra of the reaction mixture definitely exhibited two sets of amide $\nu_{C=O}$ absorption at 1625 and 1680 cm^{-1}, and the intensity of the latter became somewhat stronger than that

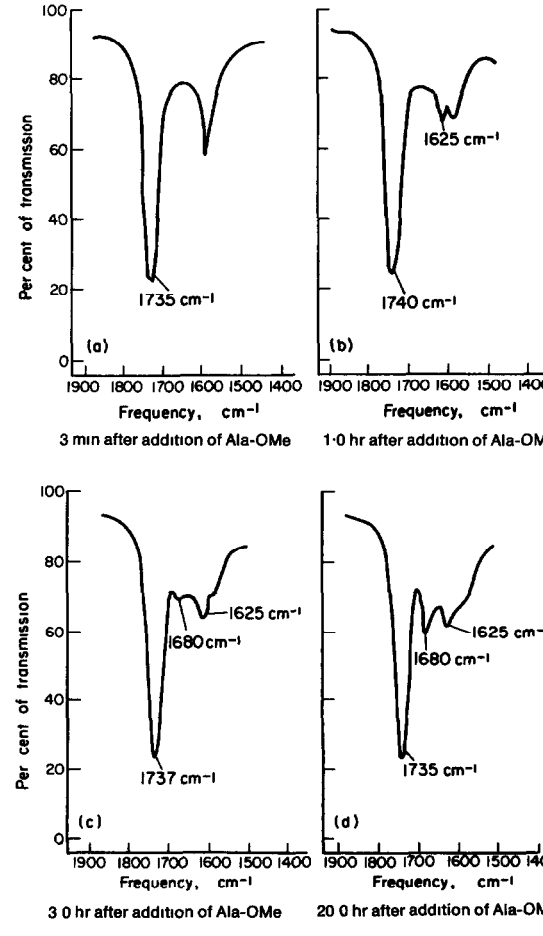


Fig 3. IR spectra of a mixture of $Cu(Ala\text{-}OMe)_2Cl_2$ and AlaOMe (6·0 eq.) in chloroform, periodically measured at room temperature.

of the former after 20·0 hr. The absorption at 1680 cm^{-1} is that of the normal amide $\nu_{C=O}$ absorption value, but it is not clear if the absorption at 1625 cm^{-1} is due to coordination of the amide oxygen (Chart 2, 1), or the amide nitrogen (Chart 2, 2) to Cu(II) ion. However, the above observation on amide $\nu_{C=O}$ absorption may correlate with the

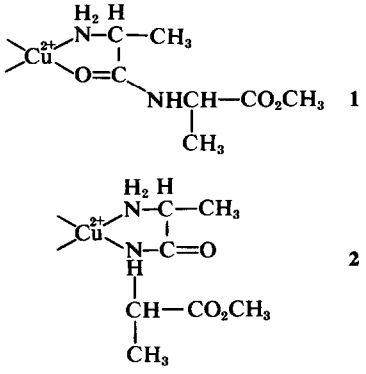


CHART 2

speculation that the peptide bond formed in the vicinity of Cu(II) ion is gradually freed from Cu(II) ion by ligand exchange with unreacted Ala-OMe, which is in excess in the reaction mixture.

II. *Visible spectra on the amino acid ester*-$CuCl_2$ *complex*

To obtain more information on peptide bond formation, measurements of visible spectra were made for a mixture of ethyl L-alaninate, (Ala-OEt) and $CuCl_2$ in anhyd ethanol, in which the concentration of Cu(II) ion was kept constant (5·0 mM) and the equivalent of Ala-OEt was varied. When the equivalent of Ala-OEt to $CuCl_2$ was varied from 2, 3, 4, 6, and 10, the following respective maximum absorptions were observed: $\lambda_{max}753_{m\mu}$ (ϵ 130), $\lambda_{max}750_{m\mu}$(ϵ 138), $\lambda_{max}745_{m\mu}$(ϵ 130), $\lambda_{max}737_{m\mu}$(ϵ 122), and $\lambda_{max}733_{m\mu}$(ϵ 110). These values clearly show that a small but definite hypsochromic shift in maximum absorption occurred with an increase in Ala-OEt concentration. This hypsochromic shift could be due to an increase in the concentration of Ala-OEt which enhanced the $d \rightarrow d^*$ transition energy of Cu(II) ion, i.e. it stabilized the Cu(II) complex. Analogous spectral changes were also observed for a similar mixture prepared with ethyl L-phenylalaninate (Phe-OEt) and $CuCl_2$.*

*When visible spectra measurements were carried out using samples in which the equivalent of Phe-OEt to $CuCl_2$ was changed from 2, 3, 4, 6, 10 and 15; the following respective maximum absorptions were observed. λ_{max} 765 mμ (ϵ 161), λ_{max} 750 mμ (ϵ 136), λ_{max} 745 mμ (ϵ 134), λ_{max} 742 mμ (ϵ 132), λ_{max} 740 mμ (ϵ 128), and λ_{max} 730 mμ (ϵ 124).

†These complexes are insoluble to ethanol.

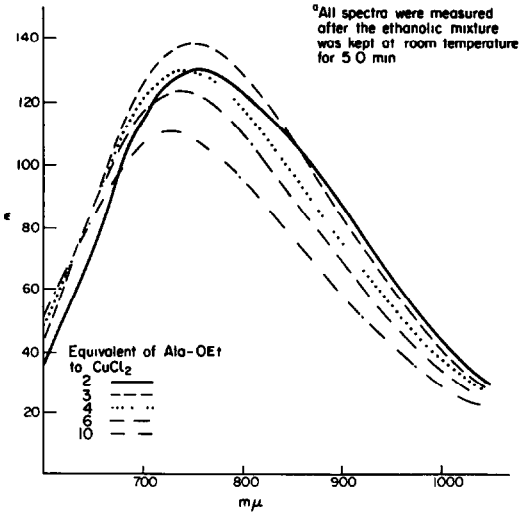


Fig 4. Visible spectra of a mixture of Ala-OEt and $CuCl_2$ in anhyd ethanol.

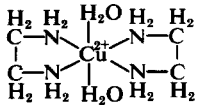
A clear hypsochromic shift observed when more than six equivs of Ala-OEt was added to the ethanolic solution of $CuCl_2$ in spite of the fact that the maximum coordination number of Cu(II) ion was six, suggests the presence of the following rium in a mixture of Ala-OEt or Phe-OEt and $CuCl_2$.

$$CuCl_2 \overset{L}{\rightleftharpoons} Cu(L)Cl_2 \overset{L}{\rightleftharpoons} Cu(L)_2Cl_2 \overset{L}{\rightleftharpoons} Cu(L)_3Cl_2 \overset{L}{\rightleftharpoons} Cu(L)_4Cl_2$$

L = Ala-OEt or Phe-OEt

CHART 3

Correlation between the increase in the number of coordinated amino groups and the hypsochromic shift in the maximum absorption value for the formed Cu(II) complex was ascertained from experiments using Cu(II) complexes such as $[Cu(en)_2]SO_4$[(en=$NH_2CH_2CH_2NH_2$) (3)[9] and [Cu(dn)Cl]Cl(dn=$NH_2CH_2CH_2NHCH_2NH_2$) (4).[10] No change in the maximum absorption value was observed when Phe-OEt (3·0 eq) was added to an aqueous solution of 3† being established to form a stable coordination structures shown in Chart 4 in which four amino groups were coordinated to the central Cu(II) ion in a common plane. On the other hand, the addition of Phe-OEt (4·0 eq) to an aqueous solution of 4, in which three amino groups



3

CHART 4

and one water molecule are coordinated to the central Cu(II) ion, showed the same hypsochromic shift of maximum absorption, i.e. λ_{max} 615 mμ (ε 176) → λ_{max} 608 mμ (ε 189) as observed for Ala-OEt and Phe-OEt. These observations show that the added Phe-OEt molecule expelled the water molecule weakly coordinated to the Cu(II) ion and afforded a stable structure where four amino groups were chelated to the central cation (**5**).

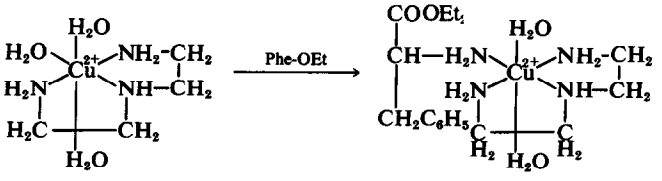
The Cu(II) complex prepared with ammonia and Cu(II) ion in an aqueous solution has been reported to have a structure of four amino groups in coordination,[11] and that it is very difficult to prepare a complex of six amino groups in coordination even if the maximum coordination number of the Cu(II) ion is six and a large excess of ammonia is present.

In summation, it is obvious that at most four amino acid esters will coordinate to the Cu(II) ion when peptide bond formation is examined using $CuCl_2$ and a large excess of amino acid ester in an anhydrous solvent.

III. *Elucidation of the formation mechanism*

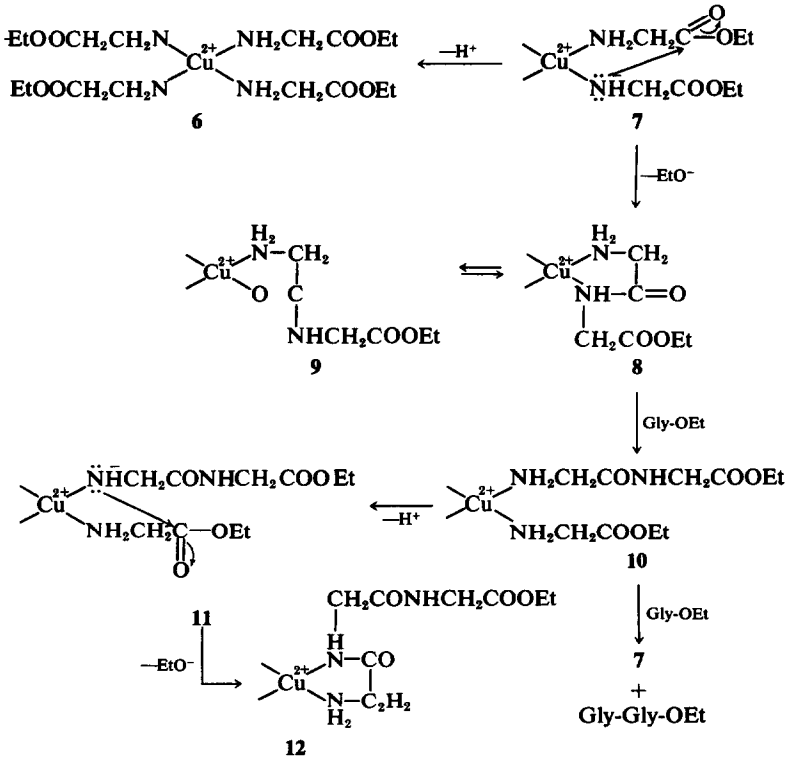
From studies of IR and visible spectra of the Cu(II) complex, it is evident that only the amino nitrogen of the amino acid ester coordinates to Cu(II) ion when peptide bond formation is examined using a large excess of amino acid ester, as compared with the amount of Cu(II) ion used, and that at most coordination of four molecules of amino acid esters is possible even if ligand exchange by equilibrium cannot be neglected in solution.

These arguments definitely exclude the possibility that peptide bond formation proceeds through mechanism A (X=$NH_2CH(R)COOR'$). That no formation[1] of peptide esters with ethyl L-prolinate (Pro-OEt) and methyl L-histidinate (His-OMe) occurs also excludes the possibility of the



4 **5**

CHART 5



6 **7** **9** **8** **10** **11** **12**

SCHEME 1

intervention of mechanism C (X=$NH_2CH(R)$-COOR'). Consequently, the mechanism B (X=$NH^-CH(R)COOR'$) seems the most likely mechanism. Scheme I illustrates the proposed mechanism using ethyl glycinate (Gly-OEt) as an example of coordinating ligand to Cu(II) ion.

As shown in the scheme, the amino anion (**7**) produced by elimination of the proton from the coordinating amino group would attack the non-activated ester CO group present in the vicinity of the formed anion, to afford a 5-membered chelation structure (**8**).

We have reported[2] that ease in peptide bond formation among the α-, β-, and γ-amino acid esters has the following order $\alpha > \beta > \gamma$. This result is compatible with the above mechanism, since α-amino ester affords the most stable 5-membered chelate ring at stage **8**.

As two different types of amino anions may be formed by elimination of the proton from the coordinating amino group when two different kinds of amino acid esters are used in peptide formation, production of a mixture of different kinds of peptide esters is a reasonable expectation, moreover, it is compatible with the observed results.[1]

Since two molecules of amino acid ester should be located adjacent to each other to afford the chelating intermediate (**8**), a fairly large steric hindrance arises in the formation of **8**. This expected steric hindrance is reflected in the absence of peptide ester formation from methyl L-isoleucinate (Ile-OMe),[1] and in the extremely low reactivity of the secondary amine as compared to that of the primary one in amino acid amide formation with amino acid ester.[12]

The inability of His-OMe and Pro-OEt to form intermediates **6** and **8**, due to electro- and stereochemical factors are considered to be responsible for the lack of formation of the expected peptide esters.[1] Using the proposed mechanism one can also explain why treatment of ethyl N-carbobenzoxy-glycinate (Z-Gly-OEt) with $Cu(Ala\text{-}OEt)_2Cl_2$ gave no desired dipeptide ester.[1]

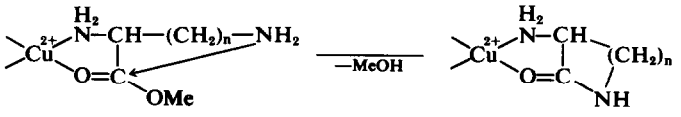
Formed dipeptide ester which originally coordinates to Cu(II) ion by its terminal amino group and the amide nitrogen (**8**), or by its terminal amino group and the amide oxygen (**9**) after intramolecular ligand exchange, will be liberated from Cu(II) ion by intermolecular ligand exchange with Gly-OEt, or will again be involved in peptide bond formation affording tripeptide ester by way of the intermediates **10** and **11**. The formation of ethyl L-alanyl-glycyl-glycinate(Ala-Gly-Gly-OEt) instead of ethyl glycyl-glycyl-L-alaninate(Gly-Gly-Ala-OEt) when ethyl glycyl-glycinate(Gly-Gly-OEt) was treated with Ala-OEt,[1] clearly suggests that tripeptide formation occurs at the N-terminus of the dipeptide ester, i.e. tripeptide ester can be produced by way of intermediates **10** and **11**, to afford the 5-membered chelation structure (**12**).

Steric hindrance in the vicinity of Cu(II) ion should increase as the long peptide chain builds up. Increasing steric hindrance will possibly stop peptide bond formation at some stage. Then, even if Gly-OEt with the smallest steric hindrance among the amino acid esters examined, is used for peptide formation, the reaction is interrupted at the stage of the tetra-peptide ester.[2] Phe-OEt, whose steric hindrance due to its side chain is clearly larger than that of Gly-OEt, affords dipeptide ester as the sole reaction product.[1]

As stated, peptide ester produced by the Cu(II) ion and liberated from the coordination sphere, might again coordinate to Cu(II) ion by ligand exchange or by simple coordination; thus, it is almost impossible for Cu(II) ion to exhibit a complete catalytic action in this peptide bond formation. This is one of the main reasons why prolonged reaction time had no effect on improving the yield of peptide ester formation.

Since this peptide bond formation does not proceed through the mechanism for activating the ester CO function by Cu(II) ion, almost no racemization would occur during this reaction, which differs completely from the peptide formation reactions discovered by Buckingham[5] and Collman.[6]

Attention should also be paid to the lactam formation observed when methyl L-lysinate (Lys-OMe) and methyl L-ornitinate (Orn-OMe) were treated with $CuCl_2$.[1] This abnormal reaction might be construed to proceed through the mechanism for activation of the ester CO function with Cu(II) ion, followed by intramolecular nucleophilic attack by ω-amino group as shown in Scheme 2, because no lactam formation was achieved at room temperature with only Lys-OMe, or by the reaction of methyl 6-aminocaproate with $CuCl_2$. A faster lactam formation rate than that observed in normal peptide bond formation catalysed by Cu(II) also lends support to the proposed mechanism. Why the reactions of Lys-OMe and Orn-OMe with $CuCl_2$ proceeded through the above mechanism, instead of that proposed for ordinary peptide ester formation, may be the result of faster and easier intra-



n = 3 or 4

SCHEME 2

molecular nucleophilic attack by the ω- or δ-amino group rather than exclusion of the ester group from Cu(II) ion by the amino group of other amino acid ester molecules through ligand exchange.

EXPERIMENTAL

All m.p s are uncorrected. IR spectra measurements were made using spectrometers, Model 402, Japan Spectroscopic Co., Lit. and Grating Infrared Spectrophotometer, Hitachi Co., Ltd. Visible spectra were measured with spectrometers, Model EPS-3T, Hitachi Recording Spectrophotometer, Hitachi Co., Ltd and Model Cary 11, Recording Spectrophotometer, Cary Ltd

Dichloro-mono(methyl L-*alaninato)-copper*(II) (Cu(Ala-OMe)Cl_2).[7] A clear dark-green soln prepared by the addition of anhyd $CuCl_2$ (740 mg, 5·5 mmole) in anhyd MeOH (7·0 ml) to an anhyd methanolic soln (5·0 ml) containing Ala-OMe (570 mg, 5·5 mmole) was concentrated below 20°, to precipitate the desired green Cu(II) complex, which was collected and dried *in vacuo*. It weighed 1·17 g (89·5%) and showed m.p. 160–161·5°. IR ν_{max}^{KBr} cm^{-1}: 1660 ($\nu_{C=O}$).

Dichloro-bis(methyl L-*alaninato)-copper*(II) (Cu(Ala-OMe)$_2Cl_2$)[7] A mixture of Ala-OMe (760 mg, 7·4 mmole) and anhyd $CuCl_2$ (500 mg, 3·7 mmole) in anhyd MeOH (8·0 ml) precipitated a blue complex, which was collected, washed with MeOH, and dried *in vacuo*. It weighed 820 mg (65·0%), and showed m.p. 115–116·5° (dec); IR ν_{max}^{KBr} cm^{-1}: 1745, 1715 ($\nu_{C=O}$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}. 1735, 1715 ($\nu_{C=O}$).

Bis(ethylenediamine)-copper(II) *sulfate* ([Cu(en)$_2$]SO_4)[9] Addition of ethylenediamine (1·2 g, 20 mmole) in water (5·0 ml) to an aqueous soln (20 ml) of cupric sulfate hydrate (2·0 g, 8·0 mmole) at room temp, afforded a deep-violet soln. [Cu(en)$_2$]SO_4 was separated as violet crystals by adding EtOH to the aqueous soln, and was collected, and dried *in vacuo*. It weighed 2·30 g (92·0%) and showed $\lambda_{max}^{H_2O}$ 545 mμ (ε 171). (Found: C, 17·32; H, 5·72; N, 19·92. Calcd. for $C_4H_{16}O_4N_2CuS$: C, 17·16; H, 5·76; N, 20·02%.)

Chloro-diethylenetriamine copper(II) *chloride* ([Cu(dn)Cl]Cl)[10] A mixture of dethylenetriamine (2·06 g, 20·0 mmole) in a 95% aqueous EtOH (20 ml) was added at room temp to 95% aqueous EtOH (30 ml) containing cupric chloride dihydrate (3·41 g, 20·0 mmole), to precipitate a deep-blue complex, which was collected and washed with EtOH, then dried over P_2O_5; $\lambda_{max}^{H_2O}$ 615 mμ (ε 176). (Found: C, 18·40; H, 6·10; N, 15·74. Calcd. for $C_4H_{13}N_3Cl_2Cu$-1½H_2O: C, 18·16; H, 6·10; N, 15·89%.)

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