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Synthetic Studies of Amino Acids by the Use of Copper Complex. II.[1] The Condensation Reaction of *N*-Pyruvylideneglycinatocopper(II) Complexes with various Aldehydes

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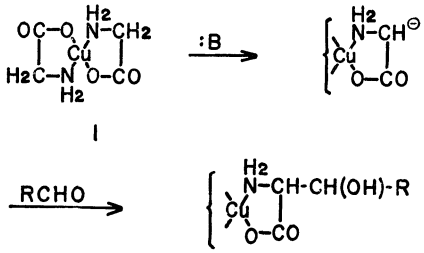
An investigation of the mechanism of the reaction which involves the base-catalyzed condensation of bis(glycinato)copper(II) with aldehydes was carried out with the purpose of improving it so that it can proceed even with lower molar equivalent ratios of aldehydes to the complex and under milder conditions. On the basis of the results reported in the literature and those of this investigation, the reaction has been carried out as expected and has been newly shown to be applicable to *p*-tolualdehyde and *p*-chlorobenzaldehyde, and, moreover, to such aldehydo sugar derivatives as 2,3-*O*-isopropylidene-D-glyceraldehyde by the use of *N*-pyruvylideneglycinatocopper(II) complexes in the reaction in place of bis(glycinato)copper(II). An attempt at further improvement will also be described in the present communication.

The base-catalyzed condensation reaction of bis(glycinato)copper(II) (**1**) and acetaldehyde (**2**) has been known to be important in the preparation of DL-threonine (**3**),[2] and the mechanism[3] involving the carbanion depicted in Scheme I has generally been accepted on the basis of the masking effect of the copper ion on the amino group[4] or of the electronic polarization effect of the copper ion on the methylene carbon.[3] However, no evidence for the former effect has been reported in connection with these systems.[5]



Scheme I

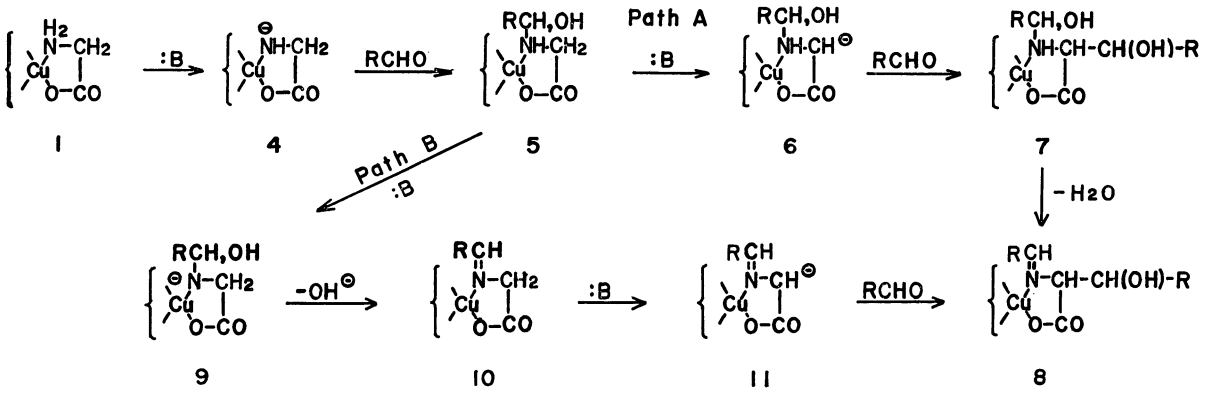
1) T. Ichikawa, S. Maeda, Y. Araki, and Y. Ishido, *J. Amer. Chem. Soc.*, **92**, 5514 (1970).

2) M. Sato, K. Okawa, and S. Akabori, This Bulletin, **30**, 937 (1957).

3) A. Nakahara, *Yuki Gosei Kagaku Kyokaishi*, **27**, 951 (1969).

4) F. P. Dwyer, "Chelating Agents and Metal Chelates," Academic Press, New York, N. Y. (1964), p, 347.

5) M. M. Jones, "Ligand Reactivity and Catalysis", Academic Press, New York, N. Y. (1968), p, 123.



Scheme II

From the synthetic standpoint of view, the reaction is usually carried out under strongly basic conditions in the presence of 2—5 mole equivalents of aldehydes to glycine; it is thus unsuitable for aldehydes which are susceptible to self-condensation or isomerization under basic conditions. The investigation of this series has been undertaken so as to improve the reaction so that it can proceed even under weakly basic conditions and can be used for the aldehydes above mentioned; we have examined the findings on this reaction reported by several groups of investigators.[2-6]

Results and Discussion

Reaction Mechanism. The preferred dissociation of the proton on the nitrogen atom of **1** to that on the methylene carbon atom was explicitly exhibited by the isolation of complexes, *i.e.*, $K[Ni(NHCH_2CO_2)(NH_2CH_2CO_2)]$ and $K_2[Ni(NHCH_2CO_2)_2]$,[7] and by a NMR study of the dissociation of similar complexes of cobalt under slightly alkaline conditions.[8] When mixtures of **1** and **2** (0—2.238 mole equivalents to glycine) were stirred in water at pH 9.5 at room temperature for 5 min, an almost linear relationship was observed between the recovery yield of **1** and the amount of **2** used, as may be in Fig. 1. In this case, it is noticeable that the formation of **3** could not be detected by thin-layer chromatography. This result can be considered to suggest that **2** is not subjected to the attack of the carbanion shown in Scheme I, but to that of the amino group of **1** in the first stage of the reaction. Consequently, the reaction was assumed to proceed *via* a mechanism involving the methylol-type intermediate (**5**), shown in Scheme II. The stability constants of *N*-substituted glycine[9] and ethylenediamine complexes[10] have been known to

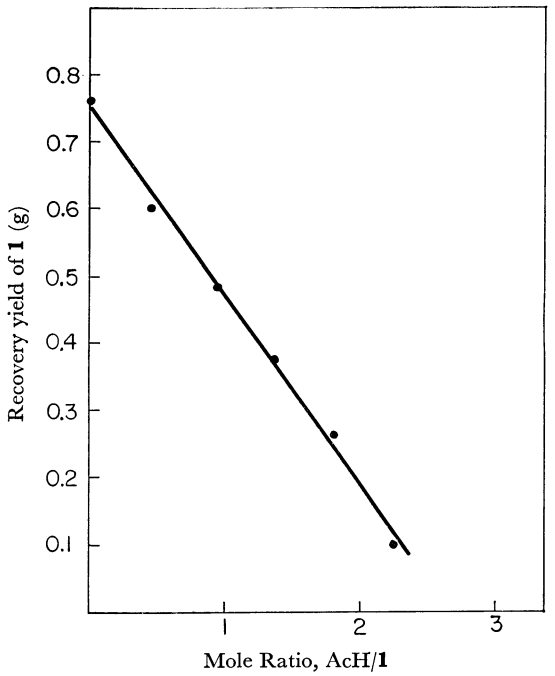


Fig. 1. Plot of recovery yield of bis(glycinato)copper(II)(**1**) *vs.* molar ratio of acetaldehyde (**2**) to glycine in **1** for the reaction of 5 mmol of **1** with **2** which were carried out at pH 9.5 for 5 min at room temperature.

be reduced in proportion to an increment in the bulkiness of *N*-substituent groups. The remarkable difference in reactivity between **2** and propionaldehyde or isobutyraldehyde, which are difficult to condense with **1** in the vicinity of pH 11.0 as far as we examined, may similarly be attributed to the stability or ease of the formation of the intermediate, **5**; *i.e.*, the difference may strongly suggest an important role of **5** in the reaction.

As to the subsequent process of the reaction, two paths, A and B, are conceivable. Path A involves the attack of the **6** carbanion, which is produced from **5** by the dissociation of the proton on the methylene carbon, on aldehydes to give **8** *via* **7**, with a simultaneous dehydration. On the other hand, Path B involves the attack of the Schiff base-type carbanion, **11**, which was produced *via* **9** and **10**, on aldehydes.

If the reaction is assumed to proceed *via* Path A, it can contradictorily be concluded that the dissoci-

6) a) K. Okawa and S. Akabori, Brit. Pat. 814063 (1959). b) Y. Ikutani, T. Okuda, and S. Akabori, This Bulletin, **33**, 582 (1960). c) S. Akabori, T. T. Otani, R. Marshall, M. Winiz, and J. P. Greenstein, *Arch. Biochem. Biophys.*, **83**, 1 (1959). d) T. T. Otani and M. Winitz, *ibid.*, **102**, 464 (1963). e) H. Mix and F. W. Wilke, *Z. Physiol. Chem.*, **337**, 40 (1964). f) M. Ohno and N. Kawabe, Japanese Pat. 6821286 (1968).

7) G. W. Watt and J. F. Knifton, *Inorg. Chem.*, **6**, 1010 (1967).

8) D. H. William and D. H. Busch, *J. Amer. Chem. Soc.*, **87**, 4644 (1965).

9) F. Basolo and Y. T. Chen, *ibid.*, **76**, 953 (1954).

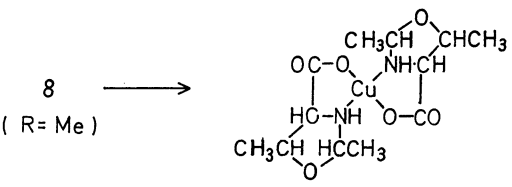
10) H. Irving and J. M. M. Griffiths, *J. Chem. Soc.*, **1954**, 213.

Table 1. An examination of the effect of formaldehyde on the condensation of bis(glycinato)copper(II) with acetaldehyde, and of the regeneration of acetaldehyde from the complex **12**[a)]

Run	$Cu(gly)_2$ (mmol)	CH_3CHO (mmol)	HCHO (mmol)	Complex **12** (mmol)	Yields(%)[12] of Recovered Glycine	Threonine
1	5	5	—	—	75	20
2	5	10	—	—	55	42
3	5	10	10	—	47	30
4	5	10	20	—	56	23
5	2.5	—	—	2.5	46	55

a) All the reactions were carried out by dissolving the reagents in water (50 m*l*) at room temperature for 4 hr in the presence of sodium carbonate (1 g).

ation of the proton on the methylene carbon atom takes preference over that of the proton on the nitrogen atom. However, so large an electron-attracting effect of the copper ion on the methylene carbon atom as to produce the **6** carbanion can not be expected from the structure of **5**. The mechanism *via* Path A can thus be discarded on the basis of these considerations. Consequently, the most important step of this reaction is considered to be that of the formation of **10**; hence, a sufficient electron-attracting effect of the copper ion on the methylene carbon atom can be expected from its structure. The structure of bis(2,5-dimethyloxazolidine-4-carboxylato)copper(II) (**12**),[11] which is easily prepared in a good yield by the reaction of **1** with **2** at pH 11, was confirmed by X-ray analysis; this fact suggests that the masking effect of the copper ion on the amino group is not so large as to prevent the reaction with **2**. **12** may be formed from **8** (R=Me) by the ring closure involving the addition reaction of a β-hydroxy group of threonine to the double bond of the *N*-ethylidene group (Scheme III). As can be seen from Table 1, the addition of formaldehyde (**13**) to the reaction system of **1** and **2** brought about a decrease in the yield of **3** as well as one in that of the total amino acids; the formation of DL-serine was not detected even by the use of an amino-acid analyzer.



12

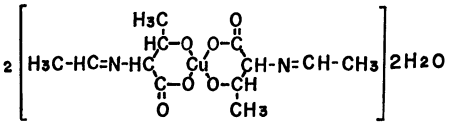
Scheme III

Moreover, the regeneration of **2** from **12** on treatment with **1** under the same conditions was experimentally confirmed. It can be thus stated that the formation of carbanion **11** is prevented by the addition of **13**. It is well known, on the other hand, that **13** can not be condensed with **1** so easily as **2**.[6c)] These facts can be explained on the assumption that the product in the first step of the condensation reaction of **1** with **13** is the *N*-methylol-type complex **5** (R=H) and that it remains as it is on account of the instability of the corresponding Schiff base-type intermediate, **10**, under the such conditions. According to Levy[13] and Tomiyama,[14] the monoformyl compound predominates in the pH range above 8.0 in the case of the formol titration. The above consideration is strongly supported by a recent study in which a Schiff base-type intermediate is also assumed in the racemization reaction of bis(L-alaninato)- and bis(*N*,*N*-dimethyl-L-alaninato)copper(II) under basic conditions.[15]

Attempts at Improvement. From the above point of view, it is natural to consider that the application of a complex such as **10**, whose amino group is protected by a proper carbonyl compound, to this reaction in place of **1** may bring about a considerable improvement. An attempted use of *N*-pyruvylideneglycinatoaquocopper(II) (**14**)[16] in the reaction expectedly brought about an improvement in the yields of amino acids and, moreover, made it feasible to condense various aldehydes with **14**. The reaction conditions

11) a) J. P. Aune and P. Maldonado, *Chem. Commun.*, **1970**, 135: the α-form crystal of **12** was analyzed. b) The corresponding β-form crystal of **12** was also analyzed recently by X-ray crystal structure analysis; it was confirmed to have the structure reported in the previous paper. The detailed results will be published elsewhere.

Although Imado reported the preparation of **12** from bis(DL-threoninato)copper(II) and acetaldehyde in the presence of sodium bicarbonate in water, and that it had the structure of bis(*N*-ethylidene-DL-threoninato)copper(II), as is depicted here, this structure can be considered sceptically because **12** does not react with sodium carbonate but nevertheless should be acidic; moreover,



the same complex is obtained from **1** and **2** under strongly basic conditions (pH 11), while it is well known that usual Schiff bases, which are composed of amino acids and aliphatic aldehydes, are unstable under such alkaline conditions [*cf.* S. Imado, *Yakugaku Zasshi*, **81**, 828, 832, and 837 (1961)].

12) No distinct explanation can be given for the drifting change of the yields of total amino acids; they may be arisen from the formation of a structurally-unestablished polymer [*cf.* G. O. Kalland, *Acta Chem. Scand.*, **19**, 2200 (1965)] or other unexpected factors.

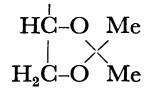
13) M. Levy, *J. Biol. Chem.*, **99**, 767 (1933).

14) T. Tomiyama, *ibid.*, **111**, 51 (1935).

15) V. M. Belikov, S. V. Vitt, N. I. Kuznetsova, M. G. Bezrukov, and M. B. Saporovskaya, *Chem. Abstr.*, **72**, 67248s (1970) (*Izv. Akad. Nauk. SSSR, Ser. Khim.*, **1969**, 2536).

16) A. Nakahara, H. Yamamoto, and H. Matsumoto, This Bulletin, **37**, 1137 (1964); the complex could not be obtained in a good yield according to the method described in this article. By the use of cupric hydroxide in place of cupric acetate, it was, however, obtained in a good yield, as has been described in the Experimental section.

Table 2. Reactions of *N*-pyruvylideneglycinatoaquocopper(II) (**14**) with some aldehydes[a]

R (R-CHO)	Molar ratio (R-CHO:**14**)	Solvent system	pH	Period (hr)	β-Hydroxy amino acids yields(%) under air[b]	β-Hydroxy amino acids yields(%) under N_2[c]
H-	1.1	H_2O	8.0	6	28[d]	—
Me-	1.5	H_2O	9.8	2	61	—
Et	3.0	MeOH	NaOMe[e]	4	30	76
n-Pr-	3.0	MeOH	NaOMe[e]	5	48	75
iso-Pr-	3.0	MeOH	NaOMe[e]	7	—	75
p-NO_2-Ph-	1.0	H_2O - MeOH	9.0	2	80	80
o-NO_2-Ph-	1.0	H_2O - MeOH	9.0	0.5	—	66
Ph-	3.0	MeOH	NaOMe[e]	9	—	67
p-Cl-Ph-	3.0	MeOH	NaOMe[e]	10	—	44
p-Me-Ph-	3.0	MeOH	NaOMe[e]	14	26	41
	1.1	H_2O	9.5	1	70	—

a) All the reactions were carried out by the use of 10 mmol of **14** [except in the case of 2,3-*O*-isopropylidene-D-glyceraldehyde (9 mmol)] at room temperature (23—26°C) except in the case of *p*-nitrobenzaldehyde (30°C).
b) These results were obtained under atmospheric condition.
c) These results were obtained under nitrogen atmospheric condition.
d) The yield was determined by amino acid analysis with Hitachi KLA 3B amino acid analyzer; An extra peak was observed in this case with almost the same strength in the vicinity of the position where the peak of aspartic acid should be observed.
e) To methanol suspension (40 ml), 1N methanolic sodium methoxide solution (7 ml) was added.

were greatly improved in the following three ways: 1) The reaction is sufficiently induced in the pH range of 9.0—9.5, in contrast with the above pH value, 10.8, which was used in the reaction of **1**. 2) the reaction satisfactorily proceeds in the presence of 1.0—3.0 mol equivalents of aldehydes to **14**, in comparison with the fact that 2—5 mol equivalents have been utilized in the reaction of **1**. 3) It has come to be possible to condense aldehydes which could not be condensed with glycine in **1** with glycine by the use of **14**. The results, along with the reaction conditions, are summarized in Table 2. The reactions of **14** with **2**, propionaldehyde, *n*-butyraldehyde, and *p*-tolualdehyde were carried out under atmospheric conditions. Except for the reaction with **2**, however, the reactions needed a long period to complete, and not very good results were obtained. In the case of formaldehyde, structurally unestablished amino acid, along with DL-serine, was obtained in a low yield. On the basis of the fact that no glycine could be detected by testing the resultant reaction mixtures thin-layer-chromatographically, and the suggestive report of Nyilasi and Vargha[17] that glycine in **1** can be subjected to oxidative degradation to give glyoxylic acid and ammonia, it was considered possible that the degradation of glycine in **14** can take place similarly; hence, the above reactions were attempted under a nitrogen atmosphere. The yields of the corresponding β-hydroxy amino acids were thus improved greatly, as may be seen by comparing the data in the 6th and the 7th columns of the table. In the case of highly-reactive *p*-nitrobenzaldehyde (**15**), no difference was observed in the yields of β-(*p*-nitrophenyl)-DL-serine with respect to the atmospheres used. In this connection, the reactions with benzaldehyde and *p*-chlorobenzaldehyde were both carried out under a nitrogen atmosphere. It is remarkable, moreover, that 2,3-*O*-isopropylidene-D-glyceraldehyde (**16**) also gave the corresponding 2-amino-2-deoxypentoaldonic acid in a good yield, even under atmospheric conditions, in spite of the short reaction period, although free sugars are generally known to be susceptible to isomerization or polymerization[18a,b] under weakly basic conditions. These findings promise to make possible the application of the reaction to the field of carbohydrate chemis-

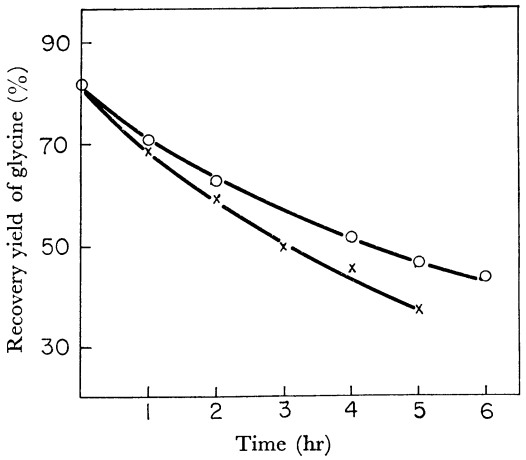


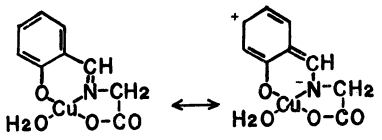
Fig. 2. Plot of recovery yield of glycine *vs.* time for the degradation reaction of *N*-pyruvylideneglycinatoaquocopper(II) (**14**) under nitrogen stream (○) and under atmospheric condition(×).

17) a) J. Nyilasi and E. Vargha, *Acta Chim. Sci. Hung.*, **14**, 113 (1958). b) J. Nyilasi, *ibid.*, **38**, 261 (1963).

18) a) In the reaction with **1**, the corresponding 2-amino-2-deoxyaldonic acid was obtained in a very small yield in spite of the use of 6 equivalents of **15** to each one of **1**. b) C. D. Gutsche, D. Redmore, R. S. Buriks, K. Nowotony, H. Grassner, and C. W. Armbruster, *J. Amer. Chem. Soc.*, **89**, 1235 (1967). c) T. Ichikawa, T. Okamoto, S. Maeda, Y. Ohdan, Y. Araki, and Y. Ishido, *Tetrahedron Lett.*, **1971**, 79.

try as a procedure of synthesizing 2-amino-2-deoxy-aldonic acids involving a chain lengthening by two carbons on aldehydo-sugars.[18c]

To confirm the degradation of glycine in **14**, it was stirred alone in water at pH 9.4—9.6 under both atmospheric and nitrogen atmospheric conditions for comparison, and the recovery yields of glycine were plotted against the reaction period. The results are shown in Fig. 2. The degradation of glycine in **14** may also be explained by the mechanism which was proposed by Nyilasi and Vargha[17] for the degradation of **1**. The observation of a considerable degradation of glycine under nitrogen atmospheric conditions, on the other hand, may suggest the existence of other degradation paths. In the case of the less reactive aldehydes, accordingly, it may be considered that the lower yields of the β-hydroxy amino acids were brought about by the competitive degradation of glycine, especially under atmospheric conditions. The extent of the degradation in the course of the reactions, however, can not be discussed in parallel with that in the examination since the reactions were carried out in methanol in the presence of sodium methoxide. In addition, the degradation test of **14** in methanol could not be performed on account of its insolubility in methanol in the absence of aldehydes.



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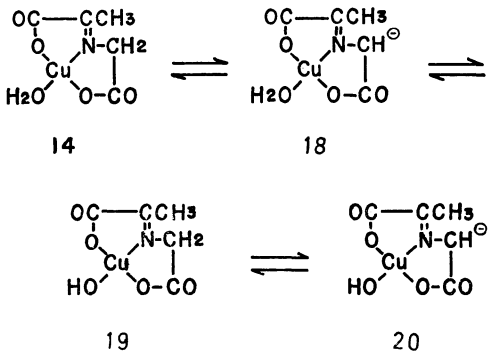
On the other hand, *N*-salicylideneglycinatoaquocopper(II) (**17**),[19] which belongs to the same category as **14**, unexpectedly gave no condensation product, even in the reaction with **15**, under the conditions used for the reaction of **14**, although the reaction of **17** with aldehydes[20] is known to proceed provided that 30 mol equivalents of aldehydes are utilized at pH 8.0. These results may be attributed to the resonance effect on the copper ion arising from the inflow of the electron from the benzene ring, as is depicted; hence, protecting the amino group with salicylaldehyde was concluded not to be so advantageous as has been described by Nakahara.[3]

In spite of an attempt to prepare other copper-glycine complexes whose amino groups were protected with acetylacetone or aldol, the corresponding complexes could not be isolated in a pure form; therefore, attempts to use them to the reaction in place of **1** were abandoned.

An attempt at further improvement was undertaken in view of the behavior of **14** upon treatment with an aqueous sodium hydroxide solution. When the alkali solution was added, drop by drop, an aqueous methanolic suspension of **14** and **15**, the mixture turned from blue to bluish green at about pH 6.0, and a gelatinous mass began to separate from the solution.

19) A. Nakahara, This Bulletin, **32**, 1195 (1959).
20) K. Harada and J. Oh-hashi, *J. Org. Chem.*, **32**, 1103 (1967).

A thin-layer chromatographic test of the solution obtained by treating the supernatant of the reaction mixture with hydrogen sulfide showed that it contains only the complex of the resultant amino acid; a corresponding test of the precipitate, on the other hand, showed that it contains only the complex of glycine. It was thus confirmed that the reaction is already induced at about pH 6.0; however, it failed to proceed, presumably on account of the predominant gel formation. The amount of the gel was increased with the addition of the alkali solution, and the mixture was finally turned into gel at about pH 7.5. Even at this point, it was thin-layer-chromatographically confirmed that the condensation reaction made little progress in comparison with the mixture at about pH 6.0. It was confirmed, in addition, that the reaction was not induced by the addition of **15** to the completely-gelled mixture of **14** in aqueous methanol of about pH 7.5. However, the gel began to dwindle over about pH 8.8 upon the further addition of the alkali solution, and the mixture came to be loose enough at pH 9.0 as the reaction proceed smoothly. On the basis of this phenomenon, an equilibrium composed of the species in Scheme IV was assumed; *i.e.*, the entity of the reactant to aldehyde in the vicinity of pH 6.0 may be **18**, and that in the vicinity of pH 9.0 may be **20**, the latter being produced from **14** *via* the salt of hydroxo *N*-pyruvylideneglycinatocopper(II) (**19**).



14 18

19 20

Scheme IV

Accordingly, **14** was titrated in water with a 2N aqueous sodium hydroxide solution under nitrogen atmospheric conditions in order to secure evidence of its behavior toward alkali, especially in the pH range of 6.0—9.0. The degradation of glycine in **14** may thus offer no problem since the period required for the titration is short and the pH of the titration mixture is under 9.5. The pH value of the mixture were plotted against the molar ratios of sodium hydroxide to **14** to give the curve shown in Fig. 3. However, the inflection point observed was not clear enough for us to explain the postulated equilibrium. This may arise from a rapid equilibrium among the species in Sheme IV and/or other potential ones. An alteration of the molar concentration of **14** from 5 to 10 mmol had practically no effect on the molar ratio of about 0.15 where the gel formation takes place. These facts presumably suggest the existence of an equilibrium for **14**, although a decisive discussion of this phenomenon is impossible. The dissociation of the α-methylene

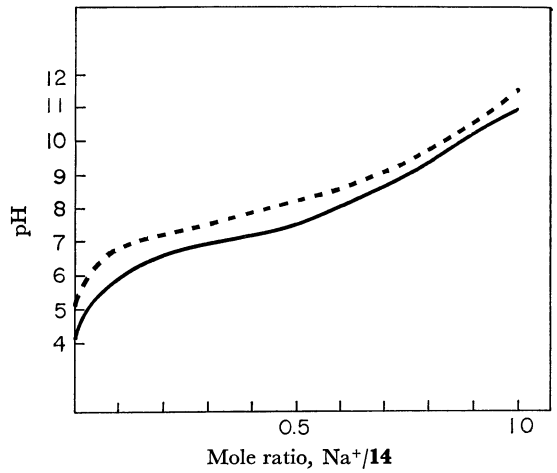


Fig. 3. Titration curves of *N*-pyruvylideneglycinatoaquocopper(II) (**14**) with 2N sodium hydroxide solution in water (50 m*l*) [5 mmol: dotted line; 10 mmol: straight line].

proton of **19** may occur with a certain degree of difficulty compared with that of **14** because of the greater electron density on the copper ion in **18**. However, the active methylene proton of **19** is considered yet to be reactive enough with aldehydes, since an NMR study of sodium chloro *N*-pyruvylideneglycinatopalladium(II) (**21**)[21] established that the α-methylene protons of glycine exclusively dissociate when **21** is dissolved in deuterium oxide. On the basis of these results, it was considered to be favorable for the condensation reaction to replace the aquo ligand in **14** with that which occupies no proton on its coordinating atom; the latter ligand could participate in $p\pi$-$d\pi$ conjugation with copper atom and so minimize the electron density on the copper atom. Pyridine was used as one of the most simple and typical species. *N*-Pyruvylideneglycinatopyridinecopper(II) (**22**) was newly prepared in a good yield by treating equimolar amounts of **14** and pyridine in acetone under vigorous stirring. The equimolar base-catalyzed condensation of **22** with **15** at pH 7.3—7.5 at room temperature was completed within 20 min; it gave β-(*p*-nitrophenyl)-DL-serine in a 67% yield. The reaction conditions in this case are in marked contrast to those used in the reaction of **14**, *i.e.*, at pH 9.5 for 2 hr. Therefore, it is now confirmed that the use of **22** in place of **14** in the condensation reaction with aldehydes brings about so favorable an effect that the reaction proceeds with great ease, even in the vicinity of pH 7.0.

Experimental

All the melting points are uncorrected. Amino acid analyses were carried out by means of a Hitachi KLA-3B Amino Acid Analyzer.

*An Examination of the Recovery of Bis(glycinato)copper (II) (**1**) in Relation to the Mole Equivalents of Acetaldehyde (**2**) Used in the Condensation Reaction.* To a suspension of **1** (1.150 g, 5 mmol) in water (50 m*l*), were added 0—2.238 mol equivalents of **2**; the mixture was then stirred at pH 9.5 for 5 min at room temperature. After the reaction, the undissolved **1** was gathered by filtration, washed with ethanol, and dried by heating *in vacuo*. The melting points (dec) and IR spectra of the recovered **1** were identical with those of the authentic specimen. The results shown below were also presented in Fig. 1.

Run	Acetaldehyde (m*l*)	Acetaldehyde mole equiv. to glycine in **1**	Recovery yield of **1** (g)
1	0	0	0.758
2	0.25	0.448	0.603
3	0.50	0.895	0.485
4	0.75	1.343	0.375
5	1.00	1.790	0.263
6	1.25	2.238	0.100

*An Examination of the Degree of Reactivity between Acetaldehyde (**2**) and Formaldehyde (**13**) with Bis(glycinato)copper(II) (**1**).* A solution of **1** (1.15 g, 5 mmol), **2** (0.5—1.0 mol equivalents), **13** (0—2.0 mol equivalents), and sodium carbonate (1.0 g, 10 mmol) in water (50 m*l*) was stirred at 27°C for 4 hr. After the reaction, concentrated aqueous ammonia (25 m*l*) was added, and the mixture was treated on a column (2.5×9 cm) of Amberlite IR-120B (NH_4 form) and then washed with distilled water (100 m*l*). The effluent and washings were then combined and concentrated to a volume of about 20 m*l* to remove the aldehydes and ammonia. The content of amino acids in the concentrate was determined by amino-acid analysis. The results are summarized in Table 1.

*An Examination of the Regeneration of Acetaldehyde (**2**) from Bis(2,5-dimethyloxazolidine-4-carboxylato)copper(II) (**12**).* A solution of **12** (0.97 g, 2.5 mmol), **1** (0.56 g, 2.5 mmol), and sodium carbonate (1 g, 10 mmol) in water (50 m*l*) was treated in the way described in the previous experiment. The content ratio of the amino acids in the resultant solution was determined by amino-acid analysis; the results are shown in Table 1.

*N-Pyruvylideneglycinatoaquocopper(II) Dihydrate (**14**).* To a suspension of glycine (21.3 g, 284 mmol) in aqueous ethanol (H_2O - EtOH=2:1; 80 m*l*), were added pyruvic acid (25. 0g, 284 mmol). The resultant mixture was stirred at 25°C for 30 min until it become homogeneous. On the addition of freshly-prepared cupric hydroxide, which had been obtained by treating cupric sulfate pentahydrate (71.0 g, 284 mmol) with sodium hydroxide (22.6 g, 568 mmol), a light blue precipitate was formed; the mixture was then allowed to stand overnight with stirring at room temperature. The resultant precipitate was gathered by suctional filtration and dried *in vacuo* over phosphorus pentoxide to give **14** (74.5 g, 75%), mp 195°C(dec).

Found: C, 22.97; H, 4.30; N, 5.32%. Calcd for $C_5H_{11}NO_7Cu$: C, 22.09; H, 4.64; N, 5.39%.

DL-*Serine.* A mixture of **14** (2.60 g, 10 mmol) and formaldehyde (0.9 ml of a 37% aqueous solution, 11 mmol) in aqueous methanol (H_2O - MeOH=3:1; 40 m*l*) was stirred at pH 8.0 for 6 hr at 30°C. After the reaction, the pH of the solution was adjusted to 4 with 3N aqueous acetic acid and the solution was treated with hydrogen sulfide gas. The resultant cupric sulfide was filtered off, and the filtrate was treated on a column (2.5×7 cm) of Amberlite IR-120B (H form) to adsorb the amino acids. After washing with distilled water (5 *l*), the column was eluted with 1N aqueous ammonia (500 m*l*) and the content of amino acids in the eluate was determined by amino-acid analysis. The yield of DL-serine was estimated to be 28%; moreover, an extra peak, one

21) H. Yoneda, Y. Morimoto, Y. Nakao, and A. Nakahara, This Bulletin, **41**, 255 (1968).

observed with almost the same strength, was in the vicinity of the position where the peak of aspartic acid should be observed.

DL-*Threonine.* A mixture of **14** (2.60 g, 10 mmol) and **2** (0.85 m*l*, 15 mmol) in water (50 m*l*) was stirred at pH 9.8 at room temperature for 2 hr. After the reaction, the pH of the resultant mixture was adjusted to 4.5 with 3N aqueous acetic acid; the mixture was then treated with hydrogen sulfide gas and the cupric sulfide was filtered off. The filtrate was concentrated to a volume of about 30 m*l*, and the concentrate was treated on a column (2.5×7 cm) of Amberlite IR-120B (H form), followed by washing with distilled water (5 *l*) and then elution with 1N aqueous ammonia (500 m*l*). The effluent was concentrated almost to dryness, and the residue was treated with ethanol (50 m*l*) to give white crystals of DL-threonine (0.7 g, 61%), mp 216°C (dec).

Found: C, 40.48; H, 7.55; N, 11.51%. Calcd for $C_4H_9NO_3$: C, 40.33; H, 7.62; N, 11.76%.

*β-Hydroxy-*DL*-norvaline.* To a mixture of **14** (2.6 g, 10 mmol) and propionaldehyde (2.1 m*l*, 30 mmol) in methanol (40 m*l*), were added 1N methanolic sodium methoxide (7 m*l*), the mixture was then stirred under a nitrogen atmosphere at 26°C for 4 hr. After the reaction, the resultant mixture was acidified with 3N aqueous acetic acid (5 m*l*), concentrated to a volume of 15 m*l*, and diluted with distilled water (50 m*l*). The solution was washed with diethyl ether (15 m*l*×3 portions) and then with methylene chloride (10 m*l*×2 portions). Then, the aqueous layer was treated with hydrogen sulfide gas, and the resultant cupric sulfide was filtered off. The filtrate was concentrated to a volume of 30 m*l*, and the concentrate was poured onto a column (2.5×7 cm) of Amberlite IR-120B (H form). The column was washed with distilled water (5 *l*) and then eluted with 1N aqueous ammonia (500 m*l*). After the concentration of the effluent *in vacuo* almost to dryness, the residue was treated with ethanol (25 m*l*); the resultant white crystals were gathered by suctional filtration to give *β*-hydroxy-DL-norvaline (1.0 g, 77%), mp 219°C (dec).

Found: C, 44.89; H, 8.14; N, 10.33%. Calcd for $C_5H_{11}NO_3$: C, 45.10; H, 8.33; N, 10.52%.

*β-Hydroxy-*DL*-norleucine, β-Hydroxy-*DL*-leucine, and* DL*-Phenylserine.* These amino acids were obtained by the reaction of *n*-butyraldehyde, isobutyraldehyde, and benzaldehyde respectively with **14** under the conditions described in Table 2 and by the treatment described in the case of propionaldehyde. The melting points and the results of their elemental analyses are shown below:

β-Hydroxy-DL-norleucine, mp 229°C (dec)

Found: C, 48.79; H, 9.20; N, 9.27%. Calcd for $C_6H_{13}NO_3$: C, 48.96; H, 8.90; N, 9.52%.

β-Hydroxy-DL-leucine, mp 225°C (dec)

Found: C, 48.98; H, 9.05; N, 9.61%. Calcd for $C_6H_{13}NO_3$: C, 48.96: H, 8.90; N, 9.52%.

DL-Phenylserine, mp 194.5°C (dec)

Found: C, 60.15; H, 6.47; N, 8.17%. Calcd for $C_9H_{11}NO_3$: C, 59.66; H, 6.47; N, 7.73%.

*β-(*p*-Nitrophenyl)-*DL*-serine.* A suspension of **14** (2.6 g, 10 mmol) and *p*-nitrobenzaldehyde (**15**) (1.5 g, 10 mmol) in aqueous methanol (H_2O - MeOH=1:2; 30 m*l*) was stirred at pH 9.0 for 2 hr at 30°C under a nitrogen stream. After the reaction, water (100 m*l*) was added, the pH of the mixture was adjusted at 5.0 by the addition of 3N aqueous acetic acid, and the resultant suspension was treated with hydrogen sulfide gas and the cupric sulfide was filtered off. The filtrate was treated on a column (2.5×9 cm) of Amberlite IR-120B (H form), washed with distilled water (5 *l*), and then eluted with 1N aqueous ammonia (500 m*l*). The effluent was concentrated almost to dryness and then treated with ethanol (20 m*l*) to give slightly brownish crystals (1.8 g, 80%), mp 181°C (dec). One recrystallization from water gave a pure specimen, mp 182°C (dec).

Found: C, 47.75; H, 4.47; N, 12.30%. Calcd for $C_9H_{10}N_2O_5$: C, 47.79; H, 4.46; N, 12.39%.

*β-(*o*-Nitrophenyl)-*DL*-serine.* *o*-Nitrobenzaldehyde (1.5 g, 10 mmol) and **14** (2.6 g, 10 mmol) were treated in the way described in the previous experiment; the reaction period was 30 min, and the reaction temperature was 20°C. Silghtly brownish crystals (1.5 g, 66%), mp 179°C (dec) were thus obtained.

Found: C, 47.79; H, 4.53; N, 12.52%. Calcd for $C_9H_{10}N_2O_5$: C, 47.79; H, 4.46; N, 12.39%.

*β-(*p*-Chlorophenyl)-*DL*-serine.* To a mixture of **14** (2.6 g, 10 mmol) and *p*-chlorobenzaldehyde (4.2 g, 30 mmol) in methanol (40 m*l*), were added 1N methanolic sodium methoxide (7 m*l*); the mixture was then stirred at 23°C under a nitrogen atmosphere for 10 hr. After the reaction, the mixture was treated with 3N aqueous acetic acid (5 m*l*) and then diluted with distilled water (100 m*l*). The resultant greenish precipitate was filtered off by suction and then washed with benzene (15 m*l*). The filtrate was concentrated *in vacuo* to a volume of 100 m*l*; the concentrate was then washed with diethyl ether (25 m*l*×2 portions) in a separating funnel. The greenish precipitate mentioned above and the aqueous layer were treated together with hydrogen sulfide gas, and the precipitated cupric sulfide was removed by filtration. The filtrate was concentrated to a volume of about 30 m*l*, and the concentrate was treated on a column (2.5×7 cm) of Amberlite IR-120B (H form). Then, the column was washed with distilled water (5 *l*) and eluted with 1N aqueous ammonia (500 m*l*). The effluent was evaporated *in vacuo* almost to dryness, and the residue was triturated with ethanol (10 m*l*). The resultant white crystals were filtered to give *β*-(*p*-chlorophenyl)-DL-serine (0.95 g, 44%), mp 186°C (dec).

Found: C, 50.13; H, 4.65; N, 6.52%. Calcd for $C_9H_{10}NO_3Cl$: C, 50.12; H, 4.67; N, 6.50%.

*β-(*p*-Tolyl)-*DL*-serine.* To a mixture of **14** (2.6 g, 10 mmol) and *p*-tolualdehyde (3.54 m*l*, 30 mmol) in methanol (40 m*l*), were added 1N methanolic sodium methoxide (7 m*l*); the mixture was then stirred at 26°C under a nitrogen atmosphere for 14 hr. After the reaction, the resultant reaction mixture was acidified with 3N aqueous acetic acid (5 m*l*) and diluted with distilled water (100 m*l*). Then, a small amount of the greenish mass washed with diethyl ether (15 m*l*). The filtrate was concentrated *in vacuo* to a volume of 20 m*l*, and the concentrate was washed with diethyl ether (25 m*l*×2 portions) in a seperating funnel. The greenish precipitate and the aqueous layer were treated in the way described in the previous experiment to give *β*-(*p*-tolyl)-DL-serine (0.8 g, 41%), mp 186°C (dec), as white crystals.

Found: C, 61.68; H, 6.98; N, 7.03%. Calcd for $C_{10}H_{13}NO_3$: C, 61.52; H, 6.71; N, 7.18%.

*2-Amino-2-deoxy-4,5-*O*-isopropylidene-*D*-pentoaldonic Acid.* To a solution of 1,2;5,6-di-*O*-isopropylidene-D-mannitol[22] (1.3 g, 5 mmol) in water (50 m*l*), were added sodium metaperiodate (1.1 g, 5 mmol); the solution was then stirred at room temperature for 60 min. The sodium iodate thus precipitated was filtered off, and the filtrate was concentrated *in vacuo* below 40°C almost to a sirup. When the sirup was then treated with ethanol (20 m*l*), the sodium iodate further precipitated was removed by filtration. This

22) D. Horton, J. B Hughes, and J. K. Thompson, *J. Org. Chem.*, **33**, 728 (1968).

operation was repeatedly carried out in order to remove a small amount of the sodium iodate remaining in the sirup. A solution of the sirup thus obtained and an equimolar amount of **14** (2.3 g, 9 mmol) in water (30 m*l*) was adjusted at pH 9.5 by the addition of a 1N aqueous sodium hydroxide solution, and then the mixture was stirred at room temperature for 1 hr. After the reaction, the solution was treated with sodium sulfide ($Na_2S \cdot 9H_2O$: 2.7 g, 11 mmol); the resultant precipitate was filtered off. The filtrate was immediately treated on a column (2.5×9 cm) of Amberlite IR-120B (NH_4 form), after which the column was washed with distilled water (100 m*l*). The combined effluent was concentrated to a volume of 50 m*l* and treated on a column (2.5×5 cm) of Amberlite IR-45 (OH form), after which the column was washed with distilled water (100 m*l*). The effluent was concentrated to a volume of 50 m*l*, and the concentrate was washed with methylene chloride (10 m*l*×3 portions). The aqueous layer was then concentrated to a volume of about 5 m*l*. The white crystals thus precipitated were gathered by filtration and washed with several milliliters of methanol. The filtrate and washings were combined and concentrated almost to a sirup. The white crystals separated on the addition of several milliliters of methanol were similarly gathered by filtration. This procedure was repeated three times more, and then all the crystals were combined and recrystallized from aqueous methanol to give 2-amino-2-deoxy-4,5-*O*-isopropylidene-D-pentoaldonic acid (1.3 g, 70%), mp 198°C (dec).

Found: C, 46.53; H, 7.18; N, 6.96%. Calcd for $C_8H_{15}NO_5$: C, 46.82; H, 7.37; N, 6.83%.

*An Examination of the Degradation of **14** in an Aqueous Alkaline Solution.* Into water (100 m*l*) in a two-necked Erlenmeyer flask (20 m*l*) equipped with a Toa Electronic, Ltd., GC-125 Electrode, were bubbled nitrogen gas more than 1 hr under mechanical stirring; **14** (2600 g, 10 mmol) which had been allowed to stand for 24 hr under a nitrogen atmosphere was then added to the flask. The solution was stirred at 23°C for several hours, its pH value kept at 9.5 by the addition of a 2N aqueous sodium hydroxide solution. After the reaction, the resultant solution was treated in the way described in the reaction of **14** with acetaldehyde. The recoveries of glycine, which were also presented in Fig. 2, were as follows:

Run	Period (hr)	Recovery of glycine (g)	(%)
1	0	0.616	82.1
2	1	0.530	70.8
3	2	0.465	62.0
4	4	0.386	51.5
5	5	0.345	46.0
6	6	0.330	43.5

The recoveries of glycine in the examination, obtained under atmospheric conditions in the same way as above, were as follows:

Run	Period (hr)	Recovery of glycine (g)	(%)
1	0	0.616	82.1
2	1	0.516	68.8
3	2	0.434	57.9
4	3	0.376	50.1
5	4	0.337	44.9
6	5	0.276	36.8

*Titration of N-Pyruvylideneglycinatoaquocopper(II) (**14**) with an Aqueous Sodium Hydroxide Solution.* *Reagent*: A 2N aqueous sodium hydroxide solution was prepared by the Sörensen procedure to remove the carbon dioxide.

Conditions: A Toa Electronics, Ltd., HM-5A model pH meter with glass and reference electrodes was used to record the hydrogen-ion concentration. The titrations were carried out in a 100 m*l* multi-necked flask with a flat bottom to accommodate gas inlet and outlet tubes, a buret delivery tube, and electrodes. A magnetic stirrer was used for the stirring. In order to obtain a carbon dioxide-free system, nitrogen which had been passed through a 5N aqueous sodium hydroxide solution was bubbled into the system. All the measurements were made at 26°C, and pH values were read 1 min after the addition of one drop of the sodium hydroxide solution.

Titrations: A solution of **14** (1.300 g, 5 mmol and 2.600 g, 10 mmol) in distilled water (50 m*l*) was treated with the 2N aqueous sodium hydroxide solution (f=1.160) under a nitrogen stream; the results are presented in Fig. 3.

*N-Pyruvylideneglycinatopyridinecopper(II) Trihydrate (**21**).* Into a mixture of pyridine (0.8 m*l*, 10 mmol) and acetone (12 m*l*), were stirred **14** (2.6 g, 10 mmol), portion by portion, after which the suspension was stirred at room temperature for 2 hr. Then, blue crystals were precipitated, filtered, and dried to give **21** (3.1 g, 91%), mp 133°C, (a slight coloration began at about 133°C, but the crystals merely turned dark brown without any evolution of gas).

Found: C, 34.98; H, 4.52; N, 7.92%. Calcd for $C_{10}H_{16}N_2O_7Cu$: C, 35.35; H, 4.76; N, 8.24%.

*Preparation of β-(p-Nitrophenyl)-DL-serine by the Use of **21**.* A solution of **21** (3.4 g, 10 mmol) and *p*-nitrobenzaldehyde (1.5 g, 10 mmol) in aqueous methanol (H_2O - MeOH=1:1; 40 m*l*) was stirred at pH 7.3—7.5 for 20 min at room temperature. The resultant reaction mixture was treated in the way described in the reaction of **14** with the aldehyde; it thus gave slightly brownish crystals. The crystals were recrystallized from water to give β-(*p*-nitrophenyl)-DL-serine (1.5 g, 67%), mp 162°C (dec). The amino acid obtained here was confirmed by paper as well as by thin-layer chromatography to be composed of two amino acids of R_f (paper chromatog) 0.51 and 0.61 (*n*-BuOH: EtCOMe: concd. aq. NH_3: H_2O=5:3:3:1) in a ratio of about 2:3, although it is uncertain which of them corresponds to the *threo* isomer and which to the *allo* isomer.

Found: C, 46.06; H, 4.92; N, 12.37%. Calcd for $C_9H_{10}N_2O_5 \cdot 1/2\ H_2O$ (dried at 65°C over phosphorus pentoxide): C, 45.96; H, 4.72; N, 11.91%.