Reactions of Amino-acids Co-ordinated to Metal Ions. Part 1. Investigation of the Condensation of Formaldehyde and Metal-co-ordinated Glycine

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The condensation reaction of formaldehyde with glycine or glycine Schiff bases co-ordinated to Co^{III}, Cu^{II}, and Ni^{II} has been studied. The reaction leads invariably to α-hydroxymethylserine. The influence of the metal ion, the charge of the complex, pH, and the ratio of the reactants are discussed, together with the effect of Schiff-base formation. With $[Cu(glyO)_2](glyO=glycinate)$ the condensation of formaldehyde at the α-carbon atom is preceded by condensation on the amino-group and is followed by cyclization to give an oxazolidine-type ring. No evidence for such a mechanism has been obtained in the case of $[Co(en)_2(glyO)]^{2+}$(en=ethylenediamine).

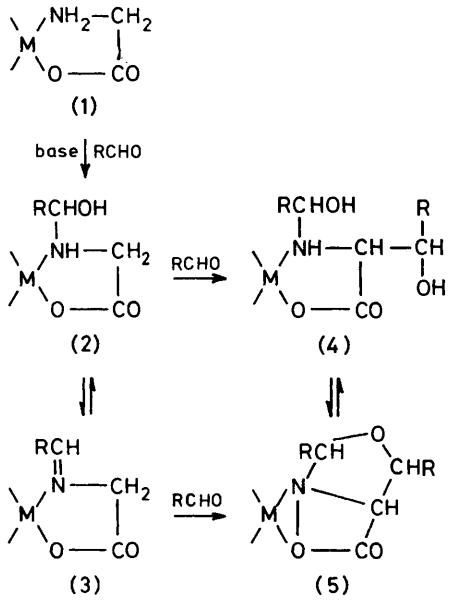
Co-ordination of α-amino-acids to metal ions is known to produce an increase in the nucleophilic reactivity at the α position through bond polarisation promoted by the the metal ion.[1] This chelation effect could also be considered as a simple model of the action of metal ions in some biological transformations of amino-acids themselves.[2]

Carbon–carbon bond formation, in basic media, at the α-carbon atom of amino-acids has been widely studied on *N*-protected amino-acid esters.[3] It was soon recognised, however, that the same reaction could be carried out more easily if the amino-acid was co-ordinated to particular metal ions. Since the pioneering work of Akabori and co-workers [4] a great deal of literature has appeared concerning the condensation reaction of metal-co-ordinated amino-acids [5–12] or small peptides [13] with various aldehydes. The reaction has also been carried out starting from chiral octahedral cobalt(III) complexes containing glycine [14] with relatively high asymmetric yields but low overall yields. Later it was discovered that the use of an amino-acid Schiff-base complex instead of a simple amino-acid metal complex increases the nucleophilicity at the amino-acid α-carbon atom and at the same time prevents the occurrence of *N*-alkylation.[15–20]

Such an increase in reactivity has been exploited for reactions with aldehydes susceptible to self-condensation since it is possible to operate under mild basic conditions. Recently, chiral bis(salicylideneaminoacidato)cobalt(III) complexes have been used to perform a reasonably good asymmetric condensation of acetaldehyde on glycine with high overall yields.[21,22]

Despite the large amount of work, general information, such as the effect of the nature of the metal ion, of its co-ordination sphere, of pH *etc.* (in short, the real mechanism of the reaction) is rather lacking. For copper complexes it has been proposed that the reactions between acetaldehyde and metal-co-ordinated glycine in basic medium proceed *via* the intermediate formation of a *N*-hydroxyethyl glycine derivative (2).

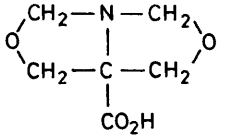
The bis(oxazolidine)copper(II) complex (5) ($R=CH_3$) has been characterized by *X*-ray analysis,[5] its treatment with hydrogen sulphide in acid medium leads to the recovery of threonine. A recent comment [14] suggests that the condensation of acetaldehyde with positively charged cobalt(III) glycine complexes also involves attack at the α-carbon atom subsequent to *N*-alkylation. The aldol condensation of formaldehyde with glycine complexes is much less well understood. Comparative studies show that formaldehyde is unable to form serine by reaction with bis(glycinato)copper(II), in the conditions in which acetaldehyde gives threonine in *ca.* 40% yield.[17] This reduced reactivity could be related to a slow attack at the α-carbon atom in the second step of the



Scheme

Scheme. In fact the addition of formaldehyde to the system acetaldehyde–bis(glycinato)copper(II) decreases the yields of threonine,[17] indicative of some competition between the two aldehydes in the first step (*N*-alkylation) of the Scheme. The same order of aldehyde reactivity is maintained in the reaction at the α carbon of the amino-acid moiety in *N*-pyruvylideneglycinatocopper(II). Moreover two recent observations complicate the under-

standing of the formaldehyde reaction at the glycine fragment: (a) bis(serinato)copper(II) reacts very easily with an excess of formaldehyde to give the copper(II) complex of (6);[9-12] (b) the reaction of formaldehyde with the positively charged bis(ethylenediamine)glycinatocobalt(III) ion has been reported [11] to give the corresponding complexes of α-hydroxymethylserine (7) and of a macrocycle formed by *N*-condensation of formaldehyde



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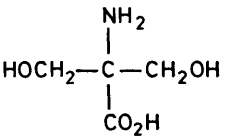

(7)

with the two ethylenediamines, *i.e.* formaldehyde reacts only at the α-methylene group of glycine to give (7) and not at the nitrogen atom to give (6).

We present here the results of an investigation into the many factors operating in the aldol condensation of formaldehyde with glycine metal complexes and with glycine Schiff-base complexes. Throughout the paper the following abbreviations will be used: glyO = glycinate, serO = serinate, hmserO = α-hydroxymethylserinate, thrO = threoninate, proO = prolinate, en = ethylenediamine, bipy = 2,2′-bipyridyl, salglyO and nisalglyO stand for the dianions of the Schiff bases derived from the condensation of salicylaldehyde and 5-nitrosalicylaldehyde with glycine respectively, and pyvglyO = the dianion of the Schiff base formed from pyruvic acid and glycine.

RESULTS

The Analytical Problem.—One of the major problems in the study of the condensation of formaldehyde with various metal complexes of glycine is the quantitative evaluation of the various amino-acids, in order to satisfy the mass balance of the reaction. This has never been done properly. Paper or thin-layer chromatography on cellulose F plates (80% pyridine–20% water as eluant) of the mixture of amino-acids recovered from the decomposition of the metal complexes after condensation of formaldehyde on co-ordinated glycine or glycine Schiff bases show the presence of two different reaction products: one spot is attributable to serine whereas the other, apparently the major and some times the only product, was previously attributed to an unidentified compound of empirical formula $C_4H_9NO_4$. The structure of this compound is readily assigned to α-hydroxymethylserine (7) by its ^{13}C n.m.r. proton-decoupled and single-frequency off-resonance decoupled spectra, in which signal multiplicity enables the distinction between methylene, methine, and quaternary carbon resonances (Table 1). Other analytical techniques are less effective in discriminating between serine and α-hydroxymethylserine.

It is known that the ^1H n.m.r. spectrum of serine in D_2O is typical of an ABC system [23] and that it depends on the pD, with a pseudo-first-order appearance in basic medium. The ^1H n.m.r. spectrum in D_2O of α-hydroxymethylserine has an AB pattern ($\Delta\nu$ = 8.06 Hz, J = −14.75 Hz) at any pD but with signal positions shifted to higher field with increasing pD. Unfortunately, ^1H n.m.r. spectra of serine and α-hydroxymethylserine have a wide area of superposition even in basic medium thus making impossible their quantitative evaluation by this technique when they are both present in the mixture.

TABLE 1

Carbon-13 n.m.r. spectra[a]

Compound	C-α	C-β	CO_2H	Others
Glycine	42.6 (t)		173.6 (s)	
Serine	57.7 (d)	61.4 (t)	173.2 (s)	
(7) α-Hydroxymethylserine	68.6 (s)	62.8 (t)	174.1 (s)	
(6) Dihydro-1*H*,3*H*,5*H*-oxazolo[3,4-*c*]oxazole-7a-carboxylic acid	78.1 (s)	76.2 (t)	180.1 (s)	N–CH_2O, 90.3 (t)
(±)[Co(en)$_2$(L-serO)]Cl_2	60.1 (d) [b] 60.4 (d) [b]	62.8 (t) [b] 63.1 (t) [b]	185.8 (s)	en, 44.2—47.2 [c]
(±)[Co(en)$_2$(L-serO)]Cl_2 + CH_2O	71.3 (s)	64.1 (t) [b] 64.6 (t) [b]		en, 44.2—47.2 [c] other weak peaks 78.2 (s), 88.2 (s)
(±)[Co(en)$_2$(hmserO)]Cl_2	71.2 (s) [b] 71.8 (s) [b]	64.3 (t) [d] 64.6 (t) [d] 64.8 (t) [d] 65.1 (t) [d]	186.5 (s) 187.1 (s)	en, 44.0—47.0 [c]
(±)[Co(en)$_2$(hmserO)]Cl_2 + CH_2O	71.0 (s) [b] 71.7 (s) [b]	64.3 (t) [d] 64.5 (t) [d] 64.7 (t) [d] 65.0 (t) [d]	186.8 187.5	en, 44.0—47.0 [c] other weak peaks 70.2, 90.4 (t)
(6)(+) *trans*-[Co(en)$_2$$Cl_2$]Cl	71.8 (s)	64.4 (t) [d] 64.7 (t) [d] 64.9 (t) [d] 65.1 (t) [d]	188.5	en, 44.0—47.0 [c]

[a] D_2O solution. δ values in p.p.m. relative to $SiMe_4$, with dioxane as internal reference. δ ($SiMe_4$) = δ (dioxane) + 67.5. Signal multiplicity in parentheses; s = singlet: d = doublet; t = triplet. [b] Two peaks due to the unequivalent moieties in the diastereoisomeric mixture. [c] Complex pattern. [d] Both enantiomers give rise to two different CH_2O groups.

Carbon-13 n.m.r. spectroscopy is not an acceptable analytical technique for the quantitative evaluation of these amino-acids. Likewise, gas chromatography of the *N*-trifluoroacetyl *O*-n-butyl esters of the amino-acids [24-26] cannot be applied since the chromatogram of (7) always shows more than one peak, one of which has a retention time very similar to that of the serine derivative although not attributable to serine because of the different mass spectra.

Similar results were obtained with the related *O*-methyl

esters. It appears that with α-hydroxyamino-acids many by-products are formed during the derivatization, or in the evaporation process in the injection chamber.[27] We are currently investigating this point through the full characterization by mass spectroscopy of the nature of the by-products.

Since it was not easy to solve the problem of the quantitative determination of a mixture of serine and α-hydroxymethylserine with common laboratory techniques, the condensation reaction of formaldehyde on metal-coordinated glycine was followed either by detection of the unreacted glycine by g.l.c. analysis or by evaluation of the ratio glycine : serine : α-hydroxymethylserine from integration of the corresponding signals in the ^1H n.m.r. spectra (the signal of glycine at δ 3.53 is well separated from that of the two α-hydroxyamino-acids) and from the elemental analyses of amino-acid mixtures isolated after decomposition of the reaction products. Carbon-13 n.m.r. spectra were also used to detect qualitatively the presence of serine in the reaction mixtures.

Since serine has been detected only in a minor number of experiments, the straightforward integration of the ^1H n.m.r. signals gave the ratio between unreacted glycine and α-hydroxymethylserine directly. In conclusion, from our analytical approach it follows that previous work [11,15,17] on the aldol condensation of formaldehyde must be considered only as a rather qualitative description.

TABLE 2

Reactions of glycine complexes

Complex [a]	Unreacted glycine [b] (mol %)
$[Co(bipy)(glyO)_2]Br$	0
$[Co(en)_2(glyO)]Cl_2$	2
α-$[Co(glyO)_3]$	9
$K[Co(NO_2)_2(glyO)_2]$	*ca.* 98
$[Cu(glyO)_2]$	37
$[Zn(glyO)_2]$	*ca.* 98

[a] Reaction conditions: concentration of the complex 0.01 mol dm^{-3}, mol ratio H_2O : glyO = 40 : 1; pH 10 ($K_2[CO_3]$); temperature 22 °C; reaction time 40 h. [b] Evaluated by quantitative g.l.c. analysis of the *N*-trifluoroacetyl *O*-n-butyl esters of the amino-acids. Glutamic acid was used as internal standard. With the exception of $K[Co(NO_2)_2(glyO)_2]$ peaks with the approximate retention time of serine were always found in the chromatogram (see Experimental section) but the area of this peak was always too small to reach the material balance.

The Effect of the Ion and the Charge.—In Tables 2 and 3 the amount of glycine recovered after reaction with formaldehyde for a series of glycine and glycine Schiff-base complexes is reported. It appears that the condensation takes place easily with cobalt(III) and copper(II) complexes whereas nickel(II) and zinc(II) complexes are rather unreactive. In the series of glycinatocobalt(III) complexes investigated, the formal charge of the complex ion plays an outstanding role, the greater reactivity of glycine being related to the presence of a positive charge while in the case of a complex carrying a negative charge {*e.g.* $[Co(NO_2)_2(glyO)_2]^-$} the reaction is almost completely inhibited.

These results are in agreement with some previous observations for the condensation of acetaldehyde [28] and would suggest a carbanion-type intermediate formed by cleavage of the glycine α-carbon–hydrogen bond. In fact we have observed a similar trend for the rate of hydrogen–deuterium exchange at the α-CH_2 group of glycine (followed by ^1H n.m.r in D_2O at pD 10) for the series: $[Co(en)_2(glyO)]^{2+} \simeq [Co(bipy)(glyO)_2]^+ > \alpha\text{-}[Co(glyO)_3 \gg [Co(NO_2)_2(glyO)_2]^-$. This trend has been recently confirmed for a similar series of cobalt(III) glycinato-complexes.[29]

The Effect of Schiff-base Formation.—The reactivity enhancement of the α carbon of the glycine fragment bound to a metal ion in the form of a chelate Schiff base [6-20] is confirmed; with these latter complexes the condensation reaction can be easily accomplished even with a complex carrying a negative charge, such as $[Co(salglyO)_2]^-$, whereas the low reactivity displayed by [Cu(salglyO)] and [Cu(nisalglyO)] is probably due only to their low solubility in water.[15]

TABLE 3

Reactions of glycine Schiff-base complexes

Complex [a]	Unreacted glycine [b] (mol %)
[Cu(salglyO)] [c]	83
[Cu(nisalglyO)] [c]	47
[Cu(pyvglyO)]	3
$Na[Co(salglyO)_2]$	31

[a] Reaction conditions: concentration of the complex 0.01 mol dm^{-3}, molar ratio CH_2O : glyO = 37 : 1; pH 9.5 ($K_2[CO_3]$); temperature 22 °C; reaction time 6 h. [b] Evaluated by quantitative g.l.c. analysis of the *N*-trifluoroacetyl *O*-n-butyl esters of the amino-acids (see Experimental section). [c] Not completely soluble in the reaction medium.

The Effect of Excess of HCHO *and the pH.*—With the exception of [Cu(pyvglyO)], which shows a very high reactivity towards formaldehyde, in all cases the reaction is controlled by the ratio of formaldehyde to glycine (Figure 1). Less reactive species such as $[Cu(glyO)_2]$ require a mol ratio of formaldehyde to glycine as high as 30 : 1—40 : 1 to obtain a reasonable conversion to the α-hydroxyamino-acids (see Table 4).

The reaction is affected by an increase in pH. Such an effect is higher for positively charged species {*e.g.* $[Co(en)_2(glyO)]^{2+}$} than for neutral complexes; for instance an increase in pH from 8 to 10 produces a nearly exponential increase in the glycine conversion with $[Co(en)_2(glyO)]^{2+}$ whilst the effect on the reactivity of $[Cu(glyO)_2]$ is almost linear. In Table 4 some significant results of the formaldehyde condensation on a series of metal complexes containing the glycine fragment are reported: the major product of the

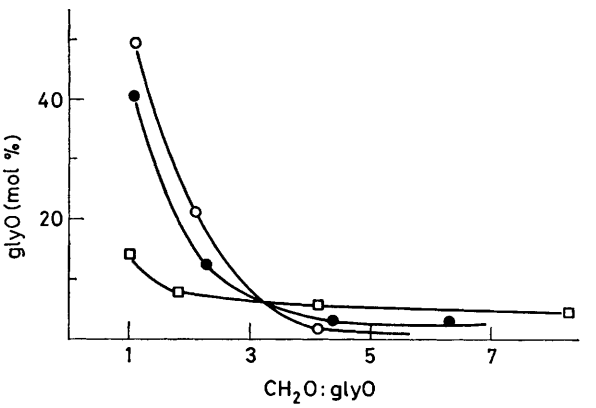

FIGURE 1 Recovery of unreacted glycine as a function of the mol fraction CH_2O: glyO for the reaction of formaldehyde with: $[Co(en)_2(glyO)]^{2+}$ (2.8×10^{-2} mol dm^{-3}, pH 10 at 22 °C for 70 h) (●); $[Co(bipy)(glyO)_2]^+$ (1.5×10^{-2} mol dm^{-3}, pH 10 at 22 °C for 43 h) (○); [Cu(pyvglyO)] (2.5×10^{-2} mol dm^{-3}, pH 8 at 22 °C for 8 h) (□)

condensation is α-hydroxymethylserine (7), which, for cobalt(III) complexes, is formed even under very mild conditions and with complexes of low reactivity. Any attempt to control the reaction towards the formation of serine failed because of the apparent higher reactivity of the serinato-complexes {compare the figures for $[Cu(serO)_2]$ with those for $[Cu(glyO)_2]$ in Table 4}. This behaviour seems to be peculiar to the formaldehyde condensation; for instance, the complex $[Co(salglyO)_2]^-$ leads to the exclusive formation of α-hydroxymethylserine in the same reaction conditions (pH and ratio of aldehyde to glycine) in which acetaldehyde respect to threonine or other α-hydroxyamino-acids when bound to a metal ion.

Although we have observed (^{1}H n.m.r. spectroscopy in D_2O) that both $[Co(en)_2(alaO)]^{2+}$ (alaO = alaninate) and $[Co(en)_2(thrO)]^{2+}$ react with formaldehyde with rates comparable but lower than that of $[Co(en)_2(serO)]^{2+}$, it is quite probable that steric effects could also be involved in the selective mono-condensation of aldehydes other than formaldehyde. In fact steric effects have recently been proposed as an explanation of the stereoselectivity

TABLE 4

Amino-acids recovered after the reaction of formaldehyde with some metal complexes of glycine at 22 °C

Complex	Concentration (10^{-2} mol dm^{-3})	$[CH_2O]/[gly]$	pH	Time/h	Amino-acids recovered (%) [a] glycine	serine	α-hydroxymethylserine
$[Co(en)_2(glyO)]Cl_2$	6.6	9	8	360	45		30
	5.5	9	10	68	5		70
$[Co(bipy)(glyO)_2]Br$	4.3	35	10	33			70
α-$[Co(glyO)_3]$	3.1	8	10	40	5		55
$K[Co(NO_2)_2(glyO)_2]$	5.0	8	10	46	69		4
$Na[Co(salglyO)_2]$	1.3	8	9.5	42	12		56
$[Cu(glyO)_2]$ [b]	5.2	8	10	41	75	9	10
	4.0	65	9.7	264			60
$[Cu(serO)_2]$	10.0	8	9	14			82
[Cu(pyvglyO)]	16.0	1.1	8	7	12	45	24
	20.0	8	8	10			74
[Cu(salglyO)]	11.0 [c]	30	8.2	8	88		
	2.5 [c]	8	10	42	62		30
$[Ni(glyO)_2]$ [b]	5.0	8	10	40	90		

[a] Analysed through ^{1}H n.m.r. and elemental analysis (see text). [b] These complexes have been decomposed by ion-exchange chromatography (see Experimental section). [c] Not completely soluble.

gives only the mono-condensation product (the diastereoisomeric threonines).[22] This trend is quite general since di-condensation of formaldehyde is the main reaction for all the complexes which give products of mono-condensation with acetaldehyde: *viz.* $[Cu(glyO)_2]$,[4,8] [Cu(pyvglyO)],[17] α-$[Co(glyO)_3]$,[4] and $[Co(en)_2(glyO)]^{2+}$.[14] Interestingly, [Cu(pyvglyO)] is so reactive that condensation occurs even with the stoicheiometric amount of formaldehyde; under these conditions some serine can be recovered, but (7) is still formed in a rather high yield, in accordance with the very high reactivity of the serinato-complexes.

DISCUSSION

Our investigation has shown that the aldol condensation of formaldehyde on metal-co-ordinated glycine proceeds quite differently from that of other aldehydes. It has been reported that aldehydes always give rise only to mono-condensation products,[4,12] whilst we have observed that formaldehyde with a large series of different glycine or glycine Schiff-base metal complexes produces preferentially the bis(hydroxymethyl) derivative of glycine (7), even under rather mild conditions or with very low ratios of formaldehyde to glycine. This is surprising since it is known that the attack of formaldehyde on co-ordinated glycine is much slower than that of other aldehydes such as acetaldehyde.[17]

The rather selective formation of α-hydroxymethylserine (7) could be ascribed either to some steric effect present with other aldehydes in the mono-condensation intermediates, or to a higher reactivity of serine with of the mono-condensation of acetaldehyde with glycine co-ordinated to Co^{III}.[14] The origin of these steric effects is attributed to the preliminary condensation of the aldehyde on the N–H group of glycine, since these *N*-alkylated intermediates can readily produce rather rigid cyclic oxazolidine structures, as in (5).[14] The *X*-ray structure of copper(II) bis(2,5-dimethyloxazolidine-4-carboxylate dihydrate),[8] isolated in the reaction between $[Cu(glyO)_2]$ and acetaldehyde, confirms that steric hindrance can make the condensation of another two molecules of acetaldehyde to produce a second oxazolidine ring difficult. This is not the case with formaldehyde.[9]

We have also tried to confirm the existence of an initial attack on the amino-group in the case of formaldehyde, as proposed by other authors.[8,17] By addition of 1 mol of formaldehyde for each mole of metal-co-ordinated glycine to both $[Cu(glyO)_2]$ and $[Co(en)_2(glyO)]^{2+}$ at pH 8.5—10 we have observed in the electronic spectra a drastic increase in the intensity of the charge-transfer bands, while the *d*–*d* transitions remain unaffected, both in position and intensity. In fact the new spectra are quite different from those of $[Cu(serO)_2]$ and $[Co(en)_2(serO)]^{2+}$ which have electronic spectra rather similar to those of $[Cu(glyO)_2]$ and $[Co(en)_2(glyO)]^{2+}$ respectively.

Since both the changes in intensity and position of the charge-transfer bands can be ascribed to a change of the optical electronegativity of some donor atoms in the co-ordination sphere,[30] it is possible that formaldehyde

condensation occurs first on the nitrogen donor atom of the metal-co-ordinated glycine. Such a condensation should produce the required change in the optical electronegativity of the N donor atom of glycine, but not its ligand-field strength. However, for $[Co(en)_2(glyO)]^{2+}$, the observed spectral change could also originate from the condensation of formaldehyde to the NH groups of ethylenediamine. Such a reaction, however, has been reported [11] to occur at higher pH than that at which the spectral change can be observed (pH 8.5).

With copper complexes it is likely that the condensation of formaldehyde at the α-carbon atom of *N*-hydroxymethylglycine (2) leads to the cyclic oxazolidine ring (10), since identical electronic spectra have been obtained for the products of addition of 2 or 4 mol of formaldehyde to $[Cu(serO)_2]$ and $[Cu(glyO)_2]$ respectively. This assumption is also supported by circular dichroism (c.d.) evidence (see Figure 2). The c.d. spectrum of $[Cu(L\text{-}serO)_2]$ at pH 10.5 is stable over a period of days [spectrum (*a*)] (*i.e.* little or no racemization occurs under these conditions). Upon addition of a large excess (40-fold) of formaldehyde spectrum (*b*) is immediately obtained. A slower reaction finally leads to achiral products [spectrum (*c*)].

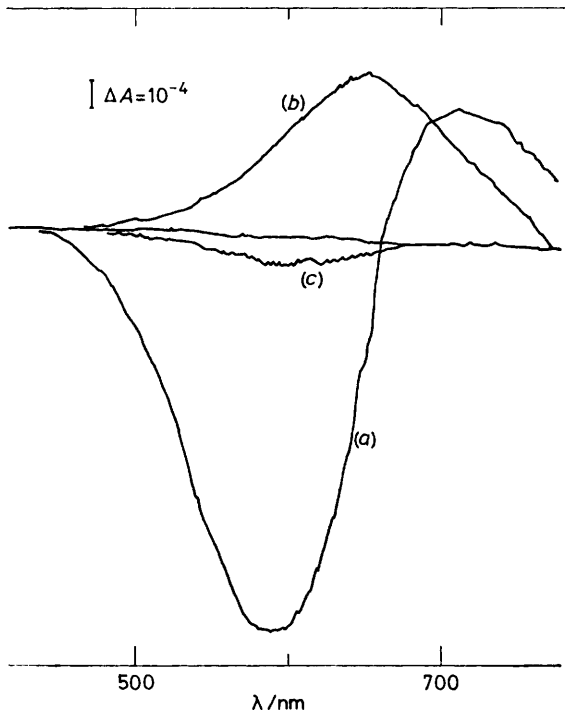

FIGURE 2 Circular dichroism spectra of (*a*) a solution of $[Cu(L\text{-}serO)_2]$ in water (8.1×10^{-3} mol dm^{-3}) at pH 10; this spectrum is stable for a few days; (*b*) immediately after the addition of CH_2O (0.625 mol dm^{-3}); and (*c*), after 24 h at 22 °C. Cell path = 1 cm, sensitivity for (*a*) and (*b*) spectra is as shown, for spectrum (*c*) the sensitivity is twice as large

The inversion of the trend of the c.d. spectrum from (*a*) to (*b*) can be interpreted as an inversion of the conformation of the chelate ring of the amino-acid which occurs upon *N*-alkylation followed by rapid cyclization to give (10) (see above). The five-membered ring in (10), which has the same absolute configuration as at the α-carbon atom of L-serine, is rather similar to that of L-proline. Since it has been observed that $[Cu(L\text{-}proO)_2]$ shows a c.d. spectrum, in the *d–d* transition region, opposite to that of the derivatives of other amino-acids with the same absolute configuration,[31] we propose that the observed inversion of the Cotton effect is attributable to the formation of (10). Reaction of (10) with an excess of formaldehyde leads eventually to the copper complex of (6) which is obviously optically inactive. With $[Co(en)_2(glyO)]^{2+}$, evidence for similar intermediates is not so straightforward.

When the isomeric mixture of $[Co(en)_2(L\text{-}serO)]^{2+}$ (as

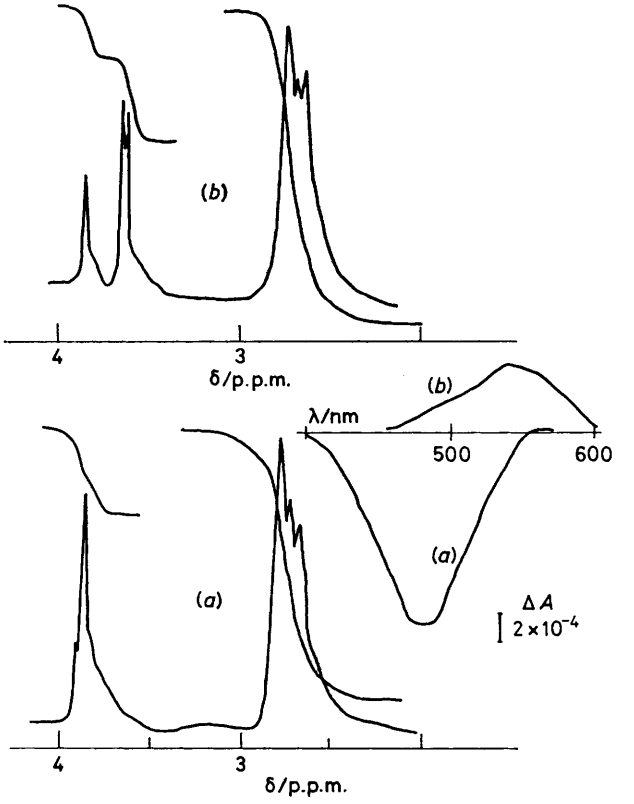

FIGURE 3 Circular dichroism and ^1H n.m.r. spectra of $[Co(en)_2(L\text{-}serO)]^{2+}$ (0.095 mol dm^{-3} in D_2O) at pD 8.5 with 0.099 mol dm^{-3} CH_2O, (*a*), beginning of the reaction and (*b*), after 20 days

obtained by standard preparation, see Experimental section) was treated with an equimolar amount of formaldehyde at pH 8.5 a similar inversion of sign, in the *d–d* transition region occurs although at a much lower rate (Figure 3). This inversion of the c.d. spectrum could be attributed to the formation of a cyclic oxazolidine ring, but the ^{1}H n.m.r. evidence does not support such an assignment (Figure 3 and Table 5). In fact, as a result of the addition of formaldehyde at the same pD, the complex peak in the ^{1}H n.m.r. spectrum of co-ordinated serine at δ *ca.* 3.8 is reduced in intensity while a broad new doublet at δ 3.68 appears. These changes occur at a rate comparable to those of the c.d. spectrum. A similar behaviour was observed when formaldehyde was added to $[Co(en)_2(glyO)]^{2+}$ at pD = 9 (Table 5). First there is a slow decrease in intensity of the peak at δ 3.36 due to the methylene group of glycine; in the meantime a new broad signal is observed at δ 3.72. Gradually, the glycine signal disappears whilst the broad signal at δ 3.72 becomes a doublet. This final spectrum is not that of $[Co(en)_2(serO)]^{2+}$, but it is superimposable not only to that obtained from this latter complex by addition of formaldehyde, but also to the spectrum of $[Co(en)_2(hmserO)]^{2+}$ (see Table 5). The integration of the doublet at δ *ca.* 3.7 is always consistent with four protons per molecule of amino-acid, being half as intense as the signal from the CH_2 ethylenediamine protons.

To further confirm that the observed final spectrum is really that of $[Co(en)_2(hmserO)]^{2+}$, and not that of (9) or (10) (where the α-hydrogen has exchanged with D_2O), similar by accidental degeneracy, we have followed by ^{1}H n.m.r. the condensation of formaldehyde on $[Co(en)_2(L\text{-}alaO)]^{2+}$ at pD 9 (Figure 4). As soon as formaldehyde is added the two doublets centred at δ *ca.* 1.4, due to the methyl group of L-alanine co-ordinated in a diastereoisomeric environment, decrease in intensity while a broad single signal appears in the same region. In the meantime the pseudo quintet corresponding to the CH group of L-alanine co-ordinated in a diastereoisomeric environment (δ *ca.* 3.7) decreases in intensity while the new broad signal at δ 3.72 slowly changes into a doublet. At the end of the reaction the spectrum shows only one singlet at δ 1.38 (CH_3 group) and a doublet centred at δ 3.72 (presumably a CH_2O group) with an integration consistent with two protons per molecule of alanine. Since under the same conditions no H–D exchange occurs at the α-carbon atom of the co-ordinated alanine the observed spectral change must be attributed to the attack of

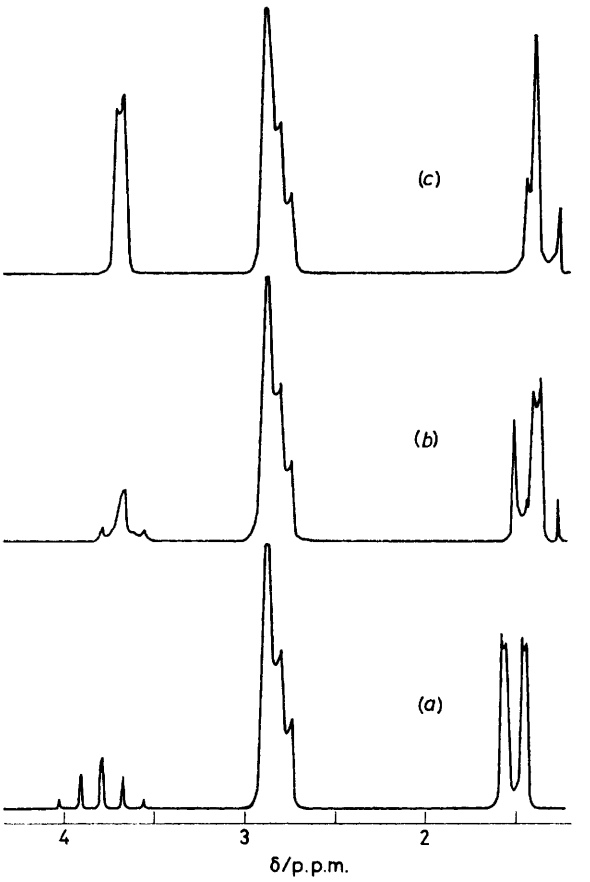

FIGURE 4 (*a*) ^{1}H n.m.r. spectrum of (±) $[Co(en)_2(L\text{-}alaO)]^{2+}$ in D_2O at pD 9; (*b*) after the addition of a four-fold excess of formaldehyde and (*c*) after 20 days at 22 °C

TABLE 5

Hydrogen-1 n.m.r. spectra [a]

Compound	α-CH	α-CH_2O	en	Others
(±)$[Co(en)_2(glyO)]Cl_2$	3.55 (2, s)		2.8 (8, m)	
(±)$[Co(en)_2(glyO)]Cl_2$ [b] + CH_2O		3.72 (4) [c]	2.8 (8, m)	
(±)$[Co(en)_2(L\text{-}serO)]Cl_2$		3.82 (3) [d]	2.8 (8, m)	
(±)$[Co(en)_2(L\text{-}serO)]Cl_2$ [e] + CH_2O		3.70 (4) [c]	2.8 (8, m)	
(±)$[Co(en)_2(L\text{-}alaO)]Cl_2$	3.73, 3.85 [f] (1, m)		2.9 (8, m)	CH_3, 1.50 1.52 (3, d) [f]
(±)$[Co(en)_2(L\text{-}alaO)]Cl_2$ [g] + CH_2O		3.66 (~2) [c]	2.8 (8, m)	CH_3, 1.37 (3, s)
(±)$[Co(en)_2(hmserO)]Cl_2$		3.70 (4) [c]	2.8 (8, m)	
(±)$[Co(bipy)(glyO)_2]Br$	3.69 (4) [h]			bipy, 7.8, 8.3 (8, m)

[a] D_2O solution, at pD between 8.5 and 10. δ Values in p.p.m. with dss as internal reference. Number of protons and signa multiplicity is given in parentheses: s = singlet, d = doublet, m = multiplet. [b] After ten days of reaction at room temperature, at pD *ca.* 10 with six-fold excess of formaldehyde. [c] AB pattern analogous to that of free α-hydroxymethylserine. [d] Unresolved multiplet. [e] After ten days of reaction at room temperature, at pD *ca.* 8.5 with stoicheiometric formaldehyde. [f] Two different alaninato-groups in the diastereoisomeric mixture (see Figure 5). [g] After one month of reaction at room temperature at pD *ca.* 8.5 with three-fold excess of formaldehyde. [h] AB pattern expected for the *trans*-(*N*) isomer.[35] On addition of formaldehyde this complex gave unresolved spectra.

HCHO at the α-carbon atom of the alanine, and the doublet centred at δ 3.72 cannot be attributed to two different CH_2O or CH_2OH residues but only to one of these groups in which the two hydrogen atoms are not equivalent (an AB system).

In conclusion, unlike $[Cu(glyO)_2]$, ^1H n.m.r. evidence does not show the formation of oxazolidine rings in the final condensation product of $[Co(en)_2(glyO)]^{2+}$, although c.d. and especially visible spectroscopy do not disprove some interaction of the formaldehyde with the amino-groups in the co-ordination sphere of cobalt. To settle this point we have prepared a solution of compound (6);[12] its ^{13}C n.m.r. spectrum is reported in Table 1. We have also attempted to prepare its complex with the '$Co(en)_2$' moiety, by reaction with *trans*-$[Co(en)_2Cl_2]Cl$ (see Experimental section). The final solution has an electronic spectrum which shows the presence of a $[Co(en)_2(aa)]^{2+}$ (aa = amino-acid anion) species, but its ^{13}C n.m.r. spectrum is very different from that of (6), and very similar to that of $[Co(en)_2(hmserO)]^{2+}$. Interestingly, the signals that we assigned to $-O-CH_2-N-$ in the spectrum of (6) have disappeared completely.

Addition of a large excess of formaldehyde to $[Co(en)_2(hmserO)]^{2+}$ produces a very weak signal in the ^{13}C n.m.r. spectrum at δ 90.4 which could be attributed to a $-O-CH_2-N-$ group, but it is possible that under these conditions formaldehyde has partially condensed with the NH_2 groups of ethylenediamine as found by Sargeson and co-workers;[11,32] similarly, the observed changes of the c.d. and visible spectra can be attributed to such a partial interaction of formaldehyde with the amino-groups of ethylenediamine. In conclusion, we have found that the major product of the condensation of formaldehyde with metal-co-ordinated glycine is α-hydroxymethylserine (7), under any conditions. Serine is formed only under specific conditions, such as a very low mol ratio of formaldehyde to glycine with [Cu(pyvglyO)]. With glycine, the condensation reaction proceeds *via* an oxazolidine ring for copper complexes, while for $[Co(en)_2(glyO)]^{2+}$ we have no evidence for condensation of the aldehyde on the amino-group of glycine. Although the presence of a small, but kinetically very active, amount of (2) or (3) (R = H, M = Co) cannot be ruled out, it is quite possible that in simple copper and cobalt complexes of glycine the condensation of formaldehyde takes place with two different mechanisms.

EXPERIMENTAL

All chemicals were reagent grade unless otherwise stated. Natural abundance ^{13}C n.m.r. spectra were recorded on a Varian XL-100A spectrometer operating at 25.2 MHz, in pulsed Fourier-transform, proton-noise decoupled, and single-frequency off-resonance decoupled mode. The field frequency was locked to internal D_2O. Peak positions were measured relative to $SiMe_4$, with dioxane as internal reference; δ ($SiMe_4$) = δ (dioxane) + 67.5;[33] ^1H n.m.r. spectra at 60 MHz were recorded on a Varian NV-14 spectrometer, sodium 4,4-dimethyl-4-silapentanesulphonate (dss) was used as an internal reference for D_2O solutions. Electronic spectra were obtained on a Beckman DK-2A instrument, circular dichroism spectra were recorded on a Jobin Yvonne mark III instrument. Quantitative gas-chromatographic analyses of the mixture of amino-acids were performed on their *N*-trifluoroacetyl *O*-n-butyl esters with a Carlo Erba Fractovap GT 200 gas chromatograph with flame ionization detectors (H_2–air), equipped with a disc integrator. The glass column (1 m × 2 mm) was packed with 1% neopentylglycolsuccinate on Chromosorb G (80—100 mesh). Conditions were: injector temperature 200 °C, 11 min initial isotherm at 120 °C, heating rate 20 °C min^{-1}, final isotherm 170 °C. The carrier gas was nitrogen at a flow rate of 60 cm^3 min^{-1}. Quantitative evaluation of the amino-acids was performed by adding a known amount of glutamic acid as internal standard. Retention times (min): glycine, 5; serine, 8; glutamic acid, 21. Under these conditions α-hydroxymethylserine gave two main peaks, one with the approximate retention time of serine and the second with a retention time of *ca.* 1.5 min, presumably because of some decomposition in the flash heater. Mass spectra (m.s.) and combined g.l.c.–m.s. experiments were carried out on a Varian MAT 112 spectrometer equipped with Varian Aerograph 144010 gas chromatograph; 1.2 m × 3 mm glass column packed with 1.3% neopentylglycolsuccinate on Chromosorb G, 80—100 mesh, at 140 °C; He, flow rate 20 cm^3 min^{-1}.

Paper (Whatman 3M) or thin-layer (Merck cellulose F plates) chromatography was used for a qualitative evaluation of the amino-acids present in the reaction mixture. A 1-cm^3 sample of the reaction solution was decomposed with hydrogen sulphide (see later), the insoluble metal sulphide removed by filtration, and a few drops of the filtrate chromatographed with 80% pyridine as eluant. The chromatograms were developed with ninhydrin (0.25% in acetone) and heated at 100 °C for 5 min; R_f values: glycine, 0.23; serine, 0.34; α-hydroxymethylserine, 0.45.

Preparation of the Metal Complexes.—All the following complexes were prepared according to literature methods, and gave satisfactory elemental analyses: $[Co(en)_2(glyO)]I_2$;[34] $[Co(en)_2(L\text{-}alaO)]I_2$;[34] $[Co(bipy)(glyO)_2]Br$;[35] $K[Co(NO_2)_2(glyO)_2]$;[36] α-$[Co(glyO)_3]$;[37] $[Ni(glyO)_2]$;[38] $[Cu(glyO)_2]$;[39] $Na[Co(salglyO)]$;[22] [Cu(pyvglyO)];[17] and [Cu(salglyO)].[40]

The complexes $[Co(en)_2(glyO)]Cl_2$ and $[Co(en)_2(L\text{-}alaO)]Cl_2$ were obtained from their iodides by ion-exchange chromatography on Amberlite IRA-400 (Cl form); the solutions thus obtained were evaporated to dryness under reduced pressure (Found: C, 19.3; H, 6.35; N, 18.25. Calc. for $C_6H_{20}Cl_2CoN_5O_2 \cdot 3H_2O$: C, 19.05; H, 6.90; N, 18.5%. Found: C, 23.45; H, 7.20; N, 20.7. Calc. for $C_7H_{22}Cl_2CoN_5O_2 \cdot H_2O$: C, 23.6; H, 7.10; N, 20.7%).

The complex $[Co(en)_2(L\text{-}serO)]Cl_2$ was obtained by a variation of the literature method.[41] An equimolar amount of solid *trans*-$[Co(en)_2Cl_2]Cl$ was added to a warm solution of sodium L-serinate, the red solution thus obtained was neutralized and kept at 40 °C for 2 h, filtered hot, concentrated under reduced pressure, and chromatographed on a column of Sephadex G-10. The fraction with $\lambda_{max.}$ 490 nm was collected and taken to dryness *in vacuo*, giving a red hygroscopic solid. Circular dichroism spectroscopy showed that the diastereoisomeric mixture is not enriched with the Λ isomer, as it is in the case when the iodide is crystallized from water[41] (Found: C, 20.3; H, 7.00; N, 17.25. Calc. for $C_7H_{22}Cl_2CoN_5O_3 \cdot 3H_2O$: C, 20.6; H, 6.85; N, 17.15%).

The complex $[Co(en)_2(L\text{-}thrO)]Cl_2$ was prepared by the same method (Found: C, 24.0; H, 6.90; N, 17.25. Calc for $C_6H_{24}Cl_2CoN_5O_3 \cdot 2H_2O$: C, 23.75; H, 6.95; N, 17.35%).

Dihydro-1H-, 3H-, 5H-*oxazolo*[3,4-c]*oxazole*-7a-*carboxylic acid* (6) was prepared [12] by reaction (ten days at 40 °C) of bis(serinato)copper(II) (2.2 g) with an excess of formaldehyde (polymeric, 30 g) in 0.5 l of water at pH 8.5 (sodium bicarbonate). The solution was concentrated under reduced pressure below 40 °C to *ca.* 150 cm^3 and the blue precipitate was collected by filtration, washed with water, ethanol, and diethyl ether, and dried *in vacuo*. The blue crystals were suspended in 10 cm^3 of cold water and solid sodium tetrahydroborate was added until a black precipitate was formed. At this stage an ammonia-like smell evolved. The slurry was centrifuged, filtered, and the filtrate evaporated to dryness *in vacuo*, in the cold, to give a white solid mixture. Any attempt to separate the compound from sodium borate led invariably to decomposition to α-hydroxymethylserine.

α-Hydroxymethylserine (7) was prepared by treating the above prepared white solid with acids or destroying the reaction mixture of $[Cu(serO)_2]$ with an excess (50-fold) of formaldehyde, with a column of Amberlite IR 120 (H^+ form) as described later (Found: C, 35.5; H, 6.70; N, 10.4. Calc. for $C_4H_9NO_4$: 35.55; H, 6.65; N, 10.35%).

Reaction between trans-*Dichlorobis(ethylenediamine)cobalt*(III) *Chloride and* (6).—The white solid containing (6) was dissolved in water, the pH was adjusted to 8, and solid *trans*-dichlorobis(ethylenediamine)cobalt(III) chloride was added; the solution was kept at 30 °C for four days and evaporated to dryness *in vacuo*. The quantity of the cobalt used was calculated from the quantity of $[Cu(serO)_2]$ used to prepare (6), assuming an overall yield of less than 50%; in any case, however, the cobalt ion was in excess with respect to (6), as judged from the intensity of the peaks in the ^{13}C n.m.r. spectrum of the final product.

Bis(ethylenediamine)α-hydroxymethylserinatocobalt(III) *Chloride*.—This was prepared using the same procedure as for $[Co(en)_2(serO)]Cl_2$ from pure α-hydroxymethylserine and *trans*-dichlorobis(ethylenediamine)cobalt(III) chloride (Found: C, 23.05; H, 6.60; N, 17.4. Calc. for $C_8H_{24}Cl_2CoN_5O_4 \cdot H_2O$: C, 23.9; H, 6.45; N, 17.4%).

Quantitative Evaluation of the Data reported in Tables 2 *and* 3.—The reactions were carried out in air and at room temperature. Formaldehyde (either polymeric or 35% water solution, no difference in reactivity was ever found) was added to a solution of the appropriate complex in water; the pH was adjusted and kept constant throughout the experiment by addition of sodium carbonate. At the end of the reaction the solution was taken to a known volume. In the case of [Cu(salglyO)] and [Cu(nisalglyO)] the reaction mixture was acidified with a few drops of concentrated hydrochloric acid in order to obtain a homogeneous solution. To an aliquot of solution containing a known weight (*ca.* 10 mg) of the amino-acids a known quantity of a standardized solution of glutamic acid (5—10 mg of the amino-acid) was added. The samples containing complexes of cobalt, nickel, and zinc were treated directly with gaseous hydrogen sulphide for 5 min. Samples of copper complexes were previously acidified with a few drops of hydrochloric acid. The metal sulphides were filtered off and the filtrate, acidified if necessary, was evaporated to dryness to give the crude amino-acid mixture. The *N*-trifluoroacetyl *O*-n-butyl esters of the amino-acids were prepared according to a known literature method.[27] The amino-acids mixture was dissolved in 30 cm^3 of a freshly prepared solution of hydrogen chloride (3 mol dm^{-3}) in anhydrous n-butanol and heated in an oil bath at 110 °C for 30 min with stirring. The solution was then evaporated to dryness under reduced pressure below 60 °C, and the residue was dissolved in 3 cm^3 of methylene chloride, filtered, treated with 1 cm^3 of trifluoroacetic anhydride, and heated in sealed tubes at 150 °C for 5 min. The solution was evaporated to dryness *in vacuo*, and the residue was dissolved in 1 cm^3 of methylene chloride and injected into the gas chromatograph.

Quantitative Evaluation of the Data reported in Table 4.—All the reactions were carried out in air and at room temperature. Polymeric formaldehyde was added to a slurry of the appropriate complex in water; the pH was adjusted and kept to a constant value throughout the experiments by addition of sodium carbonate. With the exception of [Cu(salglyO)] all the reaction mixtures gave a complete solution in basic medium within a few hours. At the end of the reaction the cobalt complexes were destroyed with hydrogen sulphide in the original basic solution, while, with copper, the solution was first taken to pH 4 with concentrated acetic acid. With [Cu(salglyO)] the mixture was acidified to pH 2 with concentrated hydrochloric acid and salicylaldehyde was repeatedly extracted with diethyl ether prior to treatment with hydrogen sulphide. The metal sulphide was removed by filtration and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in 200 cm^3 of water and filtered, if necessary. The solution was adjusted to pH 4 with acetic acid and poured onto a column (23 × 3.8 cm) of Amberlite IR 120 (H^+ form). The column was then washed with water (2 l) and eluted with 1 mol dm^{-3} aqueous ammonia until the eluate became alkaline. The eluate was evaporated to dryness under reduced pressure and the residue treated with 20 cm^3 of ethanol; the amino-acids were recovered by filtration and dried *in vacuo*. For the reaction mixtures of $[Ni(glyO)_2]$ and $[Cu(glyO)_2]$ a more straightforward decomposition procedure was followed.[42] The solution of the metal complex was adjusted to pH 4 with concentrated acetic acid and poured directly onto a column (23 × 3.8 cm) of Amberlite IR 120 (H^+ form). The column was washed with water and eluted with 0.5 mol dm^{-3} aqueous ammonia; the eluate was evaporated to dryness *in vacuo* to obtain the crude amino-acids. The composition of the mixture of amino-acids was evaluated both by the integration of ^1H n.m.r. signals corresponding to glycine, and serine plus hydroxymethylserine, and the data from elemental analysis of the mixture. The qualitative evaluation of the three amino-acids was performed by ^{13}C n.m.r. or paper or thin-layer chromatography. When serine was absent the composition of the mixture was evaluated only by ^1H n.m.r. spectroscopy.

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