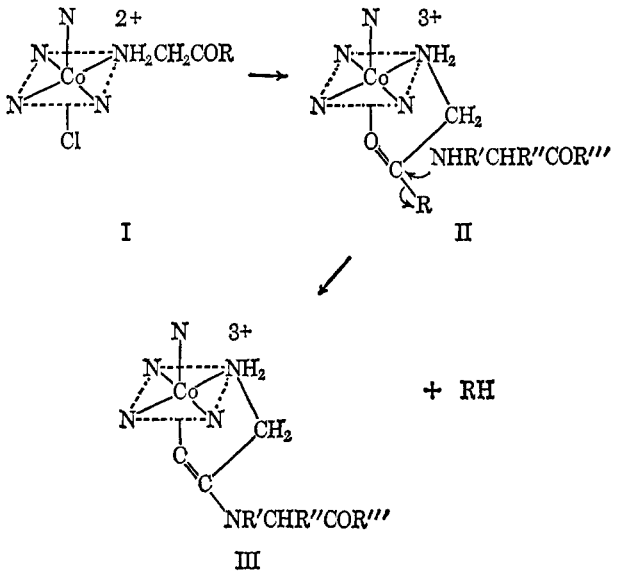
N-Terminal Addition of Glycine to Amino Acid and Peptide Esters Activated by the Cobalt(III) Ion

Sir:

The rapid condensation of glycine ethyl ester to form glycylglycine ethyl ester using the $Co(trien)^{3+}$ moiety (trien = triethylenetetramine) as an N-terminal protecting group and as an activating reagent[1,2] was consistent with, but did not establish, the following mechanism for peptide formation catalyzed by the CoN_4^{3+} unit since the intermediate complex II was not detected.



We now report the rapid and quantitative formation of chelated peptide esters using the isolated intermediate II, $[Co(en)_2(GlyOCH_3)](ClO_4)_3$ ($Co(en)_2^{3+}$ = CoN_4^{3+}; R = OCH_3), for the addition of the glycyl residue to amino acid or peptide esters. Treatment of $[Co(en)_2(GlyOCH_3)](ClO_4)_3$ with amino acid or peptide esters in anhydrous sulfolane, dimethyl sulfoxide, or acetone solutions resulted in the formation of the $[Co(en)_2$peptide-OR$]^{3+}$ ion (1). All reactions were complete within 1 min at 20°. The reactive nature of $[Co(en)_2(Gly$-

$$[Co(en)_2(GlyOCH_3)](ClO_4)_3 + \text{peptide-OR (amino acid-OR)} \longrightarrow [Co(en)_2(Gly\text{-peptide-OR})](ClO_4)_3 + CH_3OH \quad (1)$$

$OCH_3)](ClO_4)_3$ and β_2-$[Co(trien)(GlyOC_2H_5)](ClO_4)_3$ (also isolated) is consistent with the formation of II prior to the formation of the peptide bond in the reactions described.[1]

Table I. Reactions of $[Co(en)_2(GlyOCH_3)](ClO_4)_3$ with Amino Acid and Peptide Esters in Acetone

Ester	Product complexes
$GlyOC_2H_5$	$[Co(en)_2(GlyGlyOC_2H_5)](ClO_4)_3$
dl-$AlaOC_2H_5$	$[Co(en)_2(Gly\text{-}dl\text{-}AlaOC_2H_5)](ClO_4)_3$
$SarOC_2H_5$	$[Co(en)_2(Gly\text{-}dl\text{-}SarOC_2H_5)](ClO_4)_3$
dl-$PheOC_2H_5$	$[Co(en)_2(Gly\text{-}dl\text{-}PheOC_2H_5)](ClO_4)_3$
l-$HisOC_2H_5$	$[Co(en)_2(Gly\text{-}l\text{-}HisOC_2H_5)](ClO_4)_3$
l-$ProOC_2H_5$	$[Co(en)_2(Gly\text{-}l\text{-}ProOC_2H_5)](ClO_4)_3$
$GlyGlyOC_2H_5$	$[Co(en)_2(GlyGlyGlyOC_2H_5)](ClO_4)_3$
$(Gly)_3OC_2H_5$	$[Co(en)_2(GlyGlyGlyGlyOC_2H_5)](ClO_4)_3$

(1) D. A. Buckingham, L. G. Marzilli, and A. M. Sargeson, *J. Am. Chem. Soc.*, 89, 2772 (1967).

(2) Abbreviations used in this communication are: $GlyOC_2H_5$, $GlyOCH_3$, GlyOR: glycine ethyl, methyl, and alkyl esters; $GlyNH_2$, glycine amide; peptide-OR, peptide alkyl esters.

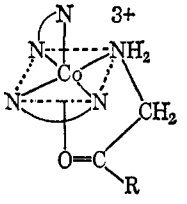
Figure 1. Pmr spectra of (A) $[Co(en)_2(GlyOCH_3)](ClO_4)_3$ in acetone-d_6 (solvent impurity δ 2.15), (B) $[Co(en)_2(GlyGlyGlyGly$-$OC_2H_5)](ClO_4)_3$ in D_2O–D_2SO_4 (HOD signal, δ 5.65), (C) $[Co(en)_2$-$gly]^{2+}$ product + CH_3OD on hydrolyzing $[Co(en)_2(GlyOCH_3)]$-$(ClO_4)_3$ in D_2O (HOD signal, δ 4.85), and (D) $[Co(en)_2(glyNH_2)]$-$(NO_3)_2ClO_4$ in D_2O–D_2SO_4 (HOD signal, δ 5.2, acetone trace, δ 2.25). Internal reference, sodium trimethylsilylpropane sulfonate.

The products isolated are given in Table I. Pmr spectra for $[Co(en)_2(GlyOCH_3)](ClO_4)_3$ and $[Co(en)_2$-$((Gly)_4OC_2H_5)](ClO_4)_3$ (98% yield) (*Anal.* Calcd: C, 21.84; H, 4.71; N, 14.56. Found: C, 21.81; H, 4.44; N, 14.64) are given in Figure 1. Chromatography of the reaction solution showed quantitative formation of the complex peptide ester. Similar results were obtained for the other species.

$[Co(en)_2(GlyOCH_3)](ClO_4)_3$ is one of several novel complexes $[Co(en)_2(GlyOR)](ClO_4)_3$ (R = CH_3, C_2H_5, *t*-Bu, Bz, *n*-Bu) isolated in these laboratories; infrared: C=O stretch, 1630 cm^{-1}; ester I band, 1315 cm^{-1}. *Anal.* Calcd: C, 14.84; H, 4.09; N, 12.36. Found: C, 15.04; H, 4.00; N, 12.51. This compound rapidly forms $[Co(en)_2Gly]^{2+}$ and methanol in water (Figure

1c) and reacts rapidly with amines and alcohols to form the $[Co(en)_2(GlyNHR)]^{3+}$ and $[Co(en)_2(GlyOR)]^{3+}$ ions, respectively. For example, $[Co(en)_2(GlyOCH_3)](ClO_4)_3$ in acetone was treated with NH_3 (20 sec, 20°). The amide, precipitated with anhydrous ether and recrystallized from water (pH 4, $LiNO_3$) as $[Co(en)_2(GlyNH_2)](NO_3)_2ClO_4$ (*Anal.* Calcd: C, 15.12; H, 4.65; N, 23.51. Found: C, 15.11; H, 4.99; N, 23.61), was identical with that isolated from aqueous reaction 2, pmr spectrum given in Figure 1d.

$$[Co(en)_2Br(GlyNH_2)](ClO_4)_2 + Hg^{2+} \xrightarrow{pH\ 1} [Co(en)_2(GlyNH_2)]^{3+} + HgBr^+ + 2ClO_4^- \quad (2)$$



IV

The X-ray crystallographic study of $[Co(trien)(GlyGlyOC_2H_5)](ClO_4)_3 \cdot H_2O$,[3] its rapid formation from $[Co(trien)(GlyOC_2H_5)](ClO_4)_3$, and the rapid amination of $[Co(en)_2(GlyOCH_3)](ClO_4)_3$ suggest that all three classes of compounds (R = OR, NHR, $NHCHR'CO_2C_2H_5$) are analogous and have structural unit IV. Intermediates of this nature have been proposed,[4-6] and infrared evidence for the carbonyl coordinated chelated ester intermediate $[Co(en)_2(GlyOR)]^{3+}$ was recently obtained[5] in reaction 3. The properties of the isolated

$$[Co(en)_2X(GlyOR)]^{2+} + Hg^{2+} + H_2O \longrightarrow [Co(en)_2(Gly)]^{2+} + HOR + H^+ + HgX^+ \quad (3)$$
$$X = Cl, Br;\ R = CH_3, C_2H_5, i\text{-}C_3H_7$$

chelated ester complexes support this claim as does isolation of the amide intermediate $[Co(en)_2(GlyNH_2)](NO_3)_2ClO_4$ from reaction 2.

We propose that one of the steps in the mechanism for peptide formation catalyzed by the CoN_4^{3+} unit is II → III. It is apparent that the cobalt(III) atom protects the NH_2 group of the chelated ester and also markedly activates the carbonyl carbon toward nucleophilic attack. Hydrolysis of a monodentate ester is much slower[5] than for the chelated ester and no Br^- is bound when the complex chelated ester is hydrolyzed in 7 *N* HBr. These results also suggest that chelation is essential for this activation of the ester moiety and that the chelate ring remains intact during the formation of the peptide bond.

The extension of this reaction to the incorporation of N-terminal amino acids other than glycine, and detailed kinetic studies of the hydrolysis reactions

$$\left.\begin{matrix}[Co(en)_2(GlyOR)]^{3+} \\ [Co(en)_2(GlyNR_1R_2)]^{3+} \\ [Co(en)_2(dipeptide\text{-}OR)]^{3+}\end{matrix}\right\} \xrightarrow{H_2O} [Co(en)_2Gly]^{2+} + \left\{\begin{matrix}ROH \\ NR_1R_2 \\ amino\ acid\text{-}OR\end{matrix}\right.$$

and the esterification and amination reactions

$$[Co(en)_2(GlyOR)]^{3+} + R_1OH \rightarrow [Co(en)_2(GlyOR_1)]^{3+} + ROH$$
$$[Co(en)_2(GlyOR)]^{3+} + NHR_1 \rightarrow [Co(en)_2(GlyNHR_1)]^{3+} + ROH$$

(3) M. Fehlmann, H. Freeman, D. A. Buckingham, and A. M. Sargeson, to be published.

(4) H. Kroll, *J. Am. Chem. Soc.*, **74**, 2036 (1952); (b) M. L. Bender and B. W. Turnquest, *ibid.*, **79**, 1889 (1957).

(5) M. D. Alexander and D. H. Busch, *ibid.*, **88**, 1130 (1966).

(6) D. A. Buckingham, J. P. Collman, D. A. R. Happer, and L. G. Marzilli, *ibid.*, **89**, 1082 (1967).

are at present in progress. Also the recovery of the peptide, its optical purity, and the degree of stereospecificity incorporated in the syntheses are being examined.

D. A. Buckingham, L. G. Marzilli, A. M. Sargeson
Research School of Chemistry, Australian National University
Canberra, A. C. T., Australia
Received April 17, 1967

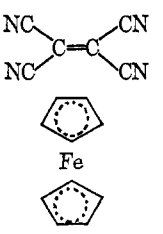
Structure of the Ferrocene–Tetracyanoethylene Complex

Sir:

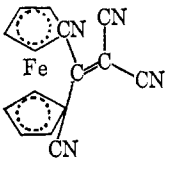
Recently[1] evidence was presented for the formulation of the complex formed by ferrocene with tetracyanoethylene (TCNE) as a charge-transfer complex rather than a salt.[2,3] With regard to the detailed structure of this substance, it was noted that, aside from the more classical formulation (**1**) involving electron donation from cyclopentadienyl ring orbitals, an alternative structure (**2**) was admissible on theoretical grounds but that the evidence was insufficient to decide in its favor.[4]

Collins and Pettit[5] have very recently confirmed the charge-transfer nature of the complex on the basis of Mössbauer experiments and have suggested **1** for its structure.

We now present the results of a single-crystal X-ray diffraction study which show definitively that the complex in the crystalline state is best represented by structure **1**, and that no apparent interaction between the metal atom and TCNE exists in this state.



1



2

The structure determination was complicated by decomposition of the substance on exposure to air and on irradiation with X-rays. In addition, crystals of the complex tended to deform easily and as a consequence led to poorly shaped spots on the diffraction photographs.[6]

Weissenberg and precession photographs revealed a triclinic cell with dimensions $a = 7.77$, $b = 7.87$, $c =$

(1) M. Rosenblum, R. W. Fish, and C. Bennett, *J. Am. Chem. Soc.*, **86**, 5166 (1964).

(2) O. W. Webster, W. Mahler, and R. E. Benson, *ibid.*, **84**, 3678 (1962).

(3) Several other complexes of ferrocene, nickelocene, and cobaltocene with a variety of acceptor molecules have been isolated, but these have been shown to be salts rather than charge-transfer complexes: J. C. Goan, E. Berg, and H. E. Podall, *J. Org. Chem.*, **29**, 975 (1964); L. R. Melby, *J. Am. Chem. Soc.*, **84**, 3374 (1962); R. L. Brandon, J. H. Osiecki, and A. Ottenberg, *J. Org. Chem.*, **31**, 1214 (1966).

(4) The general question of the involvement of the metal atom in the reactions of the iron-group metallocenes has recently been reviewed: M. Rosenblum and F. W. Abbate, Advances in Chemistry Series, No. 62, American Chemical Society, Washington, D. C., 1967, p 532.

(5) R. L. Collins and R. Pettit, *J. Inorg. Nucl. Chem.*, **29**, 503 (1967).

(6) To minimize some of these difficulties, the crystals used in the analysis were immediately placed in thin-wall capillaries filled with Fluorolube (Hooker Chemical Corporation, Niagara Falls, N. Y.). The intensity data were obtained from equiinclination Weissenberg photographs of two different crystals using Cu Kα radiation and a Ni foil placed between the crystal and the film to cut down fluorescent radiation.