ceptional stability of $FeC_2O_4^+$, reaction 2 can proceed in either direction depending upon the concentrations of $Co(C_2O_4)_3^{3-}$ and iron(III).[15] The $FeC_2O_4^+$ is produced in excess of its equilibrium concentration at high acidity and at low concentrations of $Co(C_2O_4)_3^{3-}$ and iron(III). At $[Co(C_2O_4)_3^{3-}] = 1.0 \times 10^{-3}$ *M*, $[Fe^{2+}] = 2.5 \times 10^{-2}$ *M*, and $[HClO_4] = 0.92$ *M* (the conditions used to obtain the traces shown in Figure 1), the half-life for the oxidation–reduction reaction is 0.85 sec, while the half-life for the disappearance of $FeC_2O_4^+$ is 2.4 sec. The latter half-life is in good agreement with the value of 2.1 sec calculated from the data of Moorhead and Sutin.[15,16] Moreover, t_{max}, the time required for the concentration of $FeC_2O_4^+$ to reach its maximum value, is 2.1 sec, while the yield of $FeC_2O_4^+$ at t_{max}, $[FeC_2O_4^+]_{max}/[Co(III)]_0$, is 0.51. These quantities are in good agreement with the values of 2.0 sec and 0.56, respectively, calculated for the above kinetic scheme.[17,18] We conclude, therefore, that the oxalate is bonded to both the cobalt and the iron in the transition state for the oxidation–reduction reaction. It is not known, however, whether chelation of the iron obtains in the transition state, since a monodentate oxalate intermediate would probably undergo chelation more rapidly than dissociation.[15] Additional information concerning this point is obtained by comparing the rates of reduction of $Co(NH_3)_5C_2O_4^+$ and $Co(NH_3)_5OCOCH_3^{2+}$ by iron(II). Since the oxalato complex reacts at least 10^4 times faster than the acetato complex,[8,19] it is reasonable to assume that the iron is chelated in the transition state for the former reaction.[20]

The Co(H·EDTA)Cl$^-$–Fe^{2+} Reaction. This reaction also proceeds in two stages.

$$Co(H\cdot EDTA)Cl^- + Fe^{2+} + 3H^+ \longrightarrow FeCl^{2+} + Co^{2+} + H_4EDTA \quad (3)$$

$$FeCl^{2+} \longrightarrow Fe^{3+} + Cl^- \quad (4)$$

The rate constant for reaction 3, obtained by following the disappearance of the cobalt(III) complex at 580 mμ (an absorption maximum of Co(H·EDTA)Cl$^-$), is 1.9 ± 0.2 M^{-1} sec^{-1} at 25°, $[HClO_4] = 2.2$–2.8 *M*, and ionic strength 3.0 *M*.[21] The formation of $FeCl^{2+}$ and its subsequent disappearance were followed at 336 mμ, an absorption maximum of $FeCl^{2+}$. In order to interpret the kinetics of the disappearance of $FeCl^{2+}$, it is necessary to compare the rate constant for reaction 3 with the rate constant for the dissociation of $FeCl^{2+}$.[22] In the range of iron(II) and hydrogen ion concentrations used in the present study ($[Fe^{2+}] = 0.05$–0.26 *M* and $[HClO_4] = 2.85$–2.25 *M*), the rate constant for the latter reaction varies from 2.9 to 5.9 sec^{-1}.[23] Thus, even at the highest iron(II) concentration used, the system eventually reaches a state of transient equilibrium in which the half-life for the disappearance of $FeCl^{2+}$ becomes equal to the half-life for the disappearance of the cobalt(III) complex.[18] For example, at $[Co(H\cdot EDTA)Cl^-] = 5.0 \times 10^{-4}$ *M*, $[Fe^{2+}] = 6.7 \times 10^{-2}$ *M*, $[HClO_4] = 2.8$ *M*, and ionic strength = 3.0 *M*, the half-lives for the disappearance of $FeCl^{2+}$ and of Co(H·EDTA)Cl$^-$ are 5.2 and 5.1 sec, respectively. The values $t_{max} = 1.15$ sec and $[FeCl^{2+}]_{max}/[Co(III)]_0 = 0.046$ determined in this experiment are in satisfactory agreement with the calculated values of 1.05 sec and 0.038, respectively.[17] We conclude, therefore, that the reaction of Co(H·EDTA)Cl$^-$ with iron(II) proceeds *via* a chloride-bridged transition state, a conclusion consistent with previous suggestions based on less direct evidence.[21]

An entirely analogous situation obtains in the *trans*-$Co(en)_2(OH_2)Cl^{2+}$–Fe^{2+} reaction. In one experiment at $[Fe^{2+}] = 0.26$ *M*, $[trans\text{-}Co(en)_2(OH_2)Cl^{2+}] = 5.0 \times 10^{-3}$ *M*, $[HClO_4] = 2.25$ *M*, and ionic strength = 3.0 *M*, the yield of $FeCl^{2+}$ reached a maximum value of 0.014 after 1.0 sec. The calculated values are 0.017 and 0.96 sec, respectively.[24,25] Again, the formation of $FeCl^{2+}$ in the oxidation–reduction reaction provides conclusive evidence for a chloride-bridged transition state and confirms previous speculations[25] concerning the mechanism of reduction of chloroamminecobalt(III) complexes by iron(II).

In order to explore the generality of the inner-sphere mechanism for cobalt(III)–iron(II) reactions, preliminary experiments were performed with $Co(NH_3)_3(OH_2)_2Cl^{2+}$, $Co(NH_3)_3(OH_2)_2N_3^{2+}$, and $Co(NH_3)_3(OH_2)C_2O_4^+$. In all these cases we observed the formation and subsequent disappearance of the iron(III) complex expected for a bridged transition state.[26] An inner-sphere mechanism has also been demonstrated for the $Co(OH_2)_5Cl^{2+}$–Fe^{2+} reaction.[11] This type of mechanism thus obtains in all the cobalt(III)–iron(II) reactions studied so far in which the primary products could be identified. On the basis of this evidence we conclude, therefore, that iron(II), like chromium(II), generally reacts with cobalt(III) complexes containing suitable ligands by an inner-sphere mechanism.

(15) E. G. Moorhead and N. Sutin, *Inorg. Chem.*, **5**, 1866 (1966).

(16) In this calculation, allowance is made for the contribution of the reverse of reaction 2.

(17) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," 2nd ed, John Wiley and Sons, Inc., New York, N. Y., 1961, p 166.

(18) G. Friedlander, J. W. Kennedy, and J. M. Miller, "Nuclear and Radiochemistry," 2nd ed, John Wiley and Sons, Inc., New York, N. Y., 1964, p 71.

(19) Espenson[8] reported a rate law of the form $-d \ln [Co(NH_3)_5C_2O_4H^{2+}]/dt = k[Fe^{2+}]/[H^+]$, where $k = 3.8 \times 10^{-3}$ sec^{-1} at 25° and ionic strength 1.0 *M*. Using the value 8.8×10^{-3} for the dissociation constant of $Co(NH_3)_5C_2O_4H^{2+}$ (C. Andrade and H. Taube, *Inorg. Chem.*, **5**, 1087 (1966)), we calculate a rate constant of 0.43 M^{-1} sec^{-1} for the $Co(NH_3)_5C_2O_4^+$–Fe^{2+} reaction.

(20) H. Taube, Advances in Chemistry Series, No. 49, American Chemical Society, Washington, D. C., 1965, p 107.

(21) Previously reported values are 2.2 M^{-1} sec^{-1} at 30°, $[HClO_4] = 2.0$ *M*, and ionic strength 3.0 *M* (A. Pidcock and W. C. E. Higginson, *J. Chem. Soc.*, 2798 (1963)) and 1.33 M^{-1} sec^{-1} at 20°, $[HClO_4] = 0.20$ *M*, and ionic strength 0.90 *M* (J. P. Candlin and J. Halpern, *Inorg. Chem.*, **4**, 1086 (1965)).

(22) The rate of disappearance of $FeCl^{2+}$ in the system is, of course, equal to the difference between the rates of its formation and decay.

(23) E. G. Moorhead and N. Sutin, submitted for publication.

(24) The rate constant for the *trans*-$Co(en)_2(OH_2)Cl^{2+}$–Fe^{2+} reaction at 3.0 *M* ionic strength was estimated from the value reported[25] at $[ClO_4^-] = 1.0$ *M*.

(25) P. Benson and A. Haim, *J. Am. Chem. Soc.*, **87**, 3826 (1965).

(26) The quantitative interpretation of the observations is complicated because the cobalt(III) complexes are mixtures of geometrical isomers which are reduced at different rates.

(27) (a) Fellow of the Alfred P. Sloan Foundation. (b) Visiting Chemist from the Department of Chemistry, State University of New York, Stony Brook, N. Y. 11790.

Albert Haim,[27] Norman Sutin

Department of Chemistry, Brookhaven National Laboratory
Upton, New York 11973

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A Solid-State Edman Degradation

Sir:

The Edman degradation,[1] in which amino acids are removed sequentially from the N terminus of

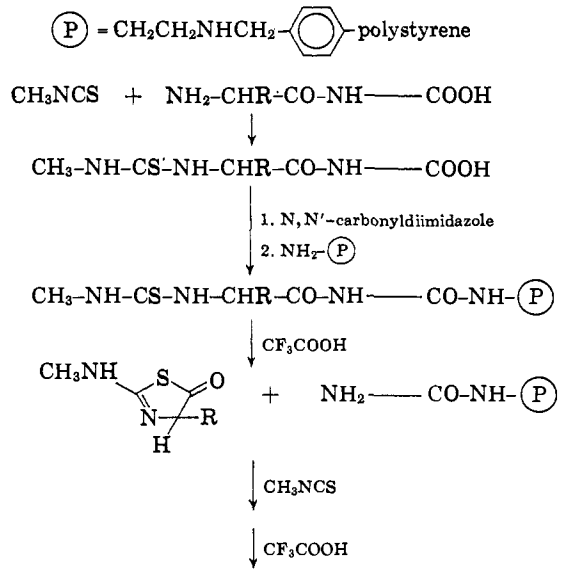

Figure 1.

peptides, is one of the most valuable chemical tools available to the protein chemist. However, a factor which has limited its use, particularly in the case of small peptides, has been the difficulty of separating the phenylthiohydantoin (or anilinothiazolinone[2]) from the residual peptide during each cycle of the degradation.

The success of Merrifield[3] in synthesizing peptides on a solid polymeric support, and of automating[4] the process, suggests the obvious analogy of degrading peptides on a polymeric support. The virtue of using solid supports lies in the ease of separating (*i.e.*, by filtration) the reaction products, and, in fact, this principle has been used by Stark[5] for the stepwise degradation of peptides. Stark's procedure involves attaching the peptide by its N terminus to a support of polystyryl isothiocyanate, cyclizing, isolating and analyzing the residual peptide, and reattaching the peptide to the resin.

In the method outlined in Figure 1, the peptide is attached by its *C-terminal* amino acid, and the degradation is performed in the usual manner. The liberated thiazolinone is removed in each cycle simply by filtration. In theory, the amount of peptide required is limited only by the amount of thiazolinone required for analysis.

Protection of the peptide is accomplished with methyl isothiocyanate, and activation with N,N′-carbonyldiimidazole.[6] The activated peptide is coupled with poly[(2-aminoethyl)aminomethylstyrene], cyclization is accomplished with trifluoroacetic acid, and the residual peptide is separated from the thiazolinone. The polymer is then treated with methyl isothiocyanate and the cycle is repeated.

In initial experiments the N-protected synthetic heptapeptide, BOC-Gly-Phe-Val-Ala-Pro-Leu-Gly-OH, labeled in the C-terminal glycine with ^{14}C to facilitate analytical procedures, was attached to the polymer, and the *t*-butyloxycarbonyl group (which proved to be more stable than the methylthiocarbamyl group) was removed

(1) P. Edman, *Acta Chem. Scand.*, **4**, 283 (1950).
(2) D. Ilse and P. Edman, *Australian J. Chem.*, **16**, 411 (1963).
(3) R. B. Merrifield, *Biochemistry*, **3**, 1385 (1964).
(4) R. B. Merrifield and J. M. Stewart, *Nature*, 207, 522 (1965).
(5) G. R. Stark, *Federation Proc.*, **24**, 225 (1965).
(6) R. Paul and G. W. Anderson, *J. Am. Chem. Soc.*, **82**, 4596 (1960).

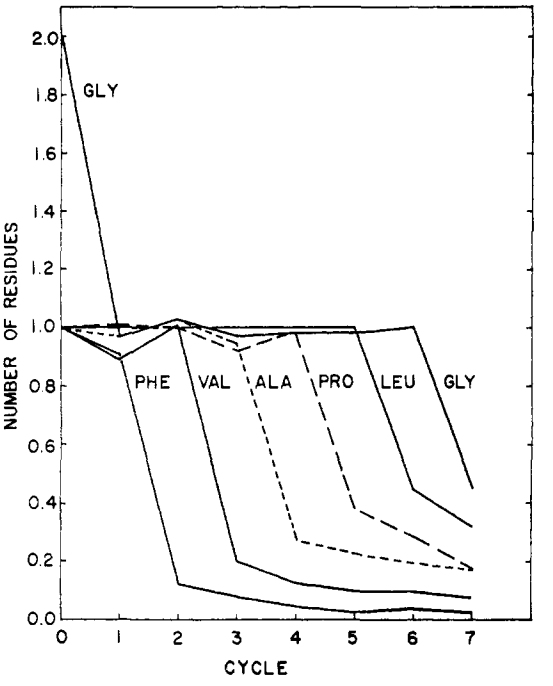

Figure 2. Solid-state Edman degradation of 0.5 μmole of a heptapeptide. Amino acid analysis of polymer-bound residual peptides.

with trifluoroacetic acid. The polymer, prepared by the reaction of polychloromethylstyrene[3] (cross-linked with 2% divinylbenzene) with ethylenediamine, contained about 0.7 (2-aminoethyl)aminomethyl groups/styrene unit.

In a typical experiment, 0.7 μmole of the BOC-heptapeptide was activated with a 5- to 20-fold excess of N,N′-carbonyldiimidazole at room temperature in dry dimethylformamide. The activated peptide was then coupled (in yields up to 70%) with 150 mg of the amino polymer in dimethylformamide over a 3-hr period. This and all subsequent operations were carried out in a special cell designed to allow stirring, filtration of the polymer, and introduction of reagents, all under a nitrogen atmosphere. At the end of the reaction, excess reagents were removed by filtration, the polymer was washed with methanol and dried under a stream of nitrogen, and the peptide was deblocked with trifluoroacetic acid. The resin was then neutralized with triethylamine and treated with an excess of methyl isothiocyanate in pyridine–triethylamine. Cyclization to the thiazolinone was accomplished with trifluoroacetic acid. After filtration to remove the thiazolinone, the polymer was treated with triethylamine and methyl isothiocyanate and the cycle repeated.

A complete degradative cycle required about 3.5 hr for the reaction with methyl isothiocyanate, 0.5 hr for cyclization, and 0.5 hr for manipulations. After each cycle a sample of the resin was removed and hydrolyzed in 6 *N* hydrochloric acid for 24 hr at 110°, and the hydrolysate was subjected to amino acid analysis (see Figure 2).

In a similar manner, the heptapeptide blocked with the methylthiocarbamyl group could be attached to the resin. A 1.6-μmole sample of the deblocked heptapeptide in a pyridine buffer was evaporated to dryness, and 0.1 ml of pyridine–triethylamine–methyl isothiocyanate (9:1:1) was added. After 2.5 hr, the excess reagents were evaporated under vacuum and the

methylthiocarbamyl peptide was activated and coupled to the resin as described above. After the first trifluoroacetic acid treatment (which removed the N-terminal amino acid), the residual peptide had the analysis: $Phe_{0.98}Val_{0.93}Ala_{0.99}Pro_{0.93}Leu_{1.00}Gly_{0.99}$.

The data in Figure 2 show that it is possible to determine the sequence of 0.5 μmole (or less) of a simple heptapeptide in a relatively short time (in fact, the rate-determining factor in this case was amino acid analysis of the residual peptides), even though the amino acids were not liberated quantitatively at each stage. The reason for these nonquantitative results may be that the carboxyl end of the peptide has become buried in the resin, and that reaction is inhibited because of steric hindrance. However, it should be possible to obtain more unambiguous results by analysis of the thiazolinones, since the nonreactive peptides will not release large amounts of contaminating thiazolinones.

The reaction conditions described here are not optimal, and it is clear that more work is required to find the proper reaction times, solvents, polymer characteristics, etc. Furthermore, the peptide model used in these experiments contains only amino acids with nonfunctional side chains, so other models will have to be examined. The problem of blocking the carboxyl groups of peptides containing glutamic and aspartic acids can probably be overcome in many cases by total esterification followed by deesterification, specifically, of the C-terminal carboxyl with a proteolytic enzyme (*e.g.*, in the case of peptides obtained by digestion of a protein with trypsin or chymotrypsin) prior to attachment of the peptide to the resin. These problems are under investigation.

In recent years Edman has developed an automated version[7] of the phenyl isothiocyanate degradation in which it has been possible to remove as many as 60 amino acids[8] from the N terminus of a protein. Thiazolinones are separated from the protein by an elegant extraction procedure. However, as with the more classical extraction procedures, problems of solubility cause the method to be less satisfactory with small peptides.[8] It is hoped that the solid-state modification will help to compensate for this deficiency. Alternatively, it should be possible to use the chemistry discussed here to attach a lipophobic "tail" which will prevent the peptide from being extracted into organic solvents, and in this way make the analysis of small peptides amenable to the automated procedure of Edman.

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(7) P. Edman, *Thromb. Diath. Haemorrhag., Suppl.*, 13 (1963).
(8) P. Edman, personal communication.
(9) Address to which requests for reprints should be sent.

Richard A. Laursen

James Bryant Conant Laboratories of Harvard University Cambridge, Massachusetts, and Department of Chemistry Boston University, Boston, Massachusetts 02215[9]

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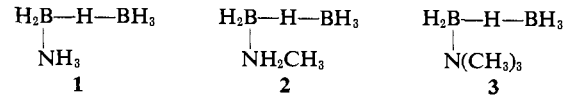
Structural Evidence for Singly Hydrogen-Bridged Boranes. Their Relationship to Symmetrical and Unsymmetrical Cleavage Reactions of Diborane

Sir:

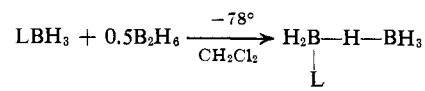
The existence of the singly hydrogen-bridged borane (**1**) as an intermediate in the reaction of diborane with ammonia has been suggested by chemical[1] and cryoscopic[2] evidence. Using procedures similar to those of Brown, Stehle, and Tierney,[3] who report a series of compounds which are believed to be singly bridged structures, we have prepared **1–3** and have obtained



1 **2** **3**

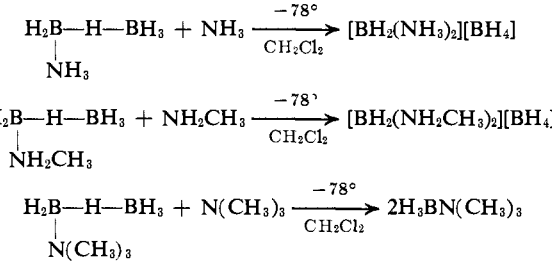
boron-11 nmr spectra which support the proposed structural formulations.

Singly hydrogen-bridged species were formed by adding diborane to appropriate amine boranes by means of tensiometric titrations at −78° in methylene chloride



where L = amine. In each case the break in the titration curve corresponded to the molar ratio of 1 amine borane/0.5 B_2H_6, and the boron-11 nmr spectrum established the presence of a BH_2 and BH_3 unit.

Addition of 1 mole of amine per mole of singly hydrogen-bridged species gave the products cited in the equations below. X-Ray powder diffraction data confirmed the formation of $[BH_2(NH_3)_2][BH_4]$ and $H_3BN(CH_3)_3$, while $[H_2B(NH_2CH_3)_2][BH_4]$ was identified by its boron-11 nmr spectrum.[4] The products formed in these reactions are the same products formed when diborane reacts with an excess quantity of



amine.[4–6] Thus, so-called "unsymmetrical cleavage"[5] products are obtained in the first two reactions while a "symmetrical cleavage" product is observed in the latter case.

In view of the results presented above, plus the fact that there exists evidence for analogous intermediates in related systems,[5,7–10] it is not unreasonable to sup-

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(2) B. Z. Egan and S. G. Shore, *ibid.*, **83**, 4717 (1961).
(3) H. C. Brown, P. F. Stehle, and P. A. Tierney, *ibid.*, **79**, 2020 (1957).
(4) S. G. Shore, C. W. Hickam, Jr., and D. Cowles, *ibid.*, **87**, 2755 (1965).
(5) R. W. Parry, in collaboration with D. R. Schultz, S. G. Shore, and P. R. Girardot, *J. Am. Chem. Soc.*, **80**, 4, 8, 15, 20 (1958).
(6) O. T. Beachley, Jr., *Inorg. Chem.*, **4**, 1823 (1965).
(7) A. B. Burg and G. W. Campbell, *J. Am. Chem. Soc.*, **74**, 3744 (1952).
(8) S. H. Bauer, J. V. Martinez, D. Price, and W. D. Jones, Advances in Chemistry Series, No. 42, American Chemical Society, Washington, D. C., 1964, p 35.