mada, *ibid.*, **86**, C23 (1975); (h) *Tetrahedron Lett.*, 645 (1975); (i) H. Sakurai, Y. Kobayashi, and Y. Nakadaira, *J. Am. Chem. Soc.*, **93**, 5292 (1971); (j) H. Sakurai, Y. Kobayashi, and Y. Nakadaira, *ibid.*, **96**, 2656 (1974); (k) Y. Nakadaira, S. Kanouchi, and H. Sakurai, *ibid.*, **96**, 5623 (1974); (l) P. Boudjouk, J. R. Roberts, C. M. Golino, and L. H. Sommer, *ibid.*, **94**, 7926 (1972).

(2) Satisfactory elemental analyses were obtained for all new compounds reported here.

(3) In this reaction, 7% of an unidentified product was also obtained.

(4) Compound **4**: NMR δ (CCl_4) 0.35 (CH_3–Si, s, 9 H), 7.13–7.53 (ring protons, m, 4 H); ir (cm^{-1})(neat) 730, 750, 840 (lit.[5] 730, 750, 840).

(5) H. A. Clark, A. F. Gordon, C. W. Young, and M. J. Hunter, *J. Am. Chem. Soc.*, **73**, 3798 (1951).

(6) Compound **5**: NMR δ (CCl_4) −0.04 (CH_3–$SiMe_2$, s, 9 H), 0.31 (CH_3–SiMe, s, 6 H), 0.44–1.01 (CH_2CH_2, m, 4 H), 7.08–7.56 (ring protons, m, 4 H); ir (cm^{-1})(neat) 725, 745, 835.

(7) In addition to **4** and **5**, $C_6H_3Cl(SiMe_3)SiMe_2CH_2CH_2SiMe_3$ was also obtained in 60% yield; M^+ 342; NMR δ (CCl_4) −0.07 (CH_3–$SiMe_2$, s, 9 H), 0.31 (CH_3–SiMe, s, 6 H), 0.35 (CH_3–SiC_6H_3, s, 9 H), 0.45–0.95 (CH_2CH_2, m, 4 H), 7.12–7.62 (ring protons, m, 3 H).

(8) Compound **3b**: M^+ 264, *m/e* 207 (55.7), 191 (100); NMR δ (CCl_4) 0.36 (CH_3–$SiMe_2$, s, 9 H), 0.39 (CH_3–SiMe, s, 6 H), 0.83 (CH_2–C, d, 2 H, J = 6.8 Hz), 0.91 (CH_3–CMe, d, 6 H, J = 6.6 Hz), 1.81 (H–CMe_2, m, 1 H), 7.16–7.64 (ring protons, m, 4 H).

(9) Compound **3c**: M^+ 290, *m/e* 207 (17.9), 191 (59.2); NMR δ (CCl_4) 0.42 (CH_3–$SiMe_2C_6H_4$, s, 9 H), 0.44 (CH_3–SiMe, s, 3 H), 0.46 (CH_3–SiMe, s, 3 H), 0.91 (CH_2–CHMe, dd, 1 H, J_{gem} = 14.8 Hz, J_{vic} = 7.8 Hz), 1.13 (CH_2–CHMe, dd, 1 H, J_{gem} = 14.8 Hz, J_{vic} = 6.5 Hz), 1.05 (CH_3–CH, d, 3 H, J = 6.9 Hz), 1.71 (CH_3–C, s, 3 H), 2.43 (H–C(Me)–CH_2, sext, 1 H, J = 6.9 Hz), 4.58–4.74 (CH_2=C, m, 2 H), 7.18–7.68 (ring protons, m, 4 H). Irradiation of the sextet signal at 2.43 changed the two double doublets at 0.91 and 1.13 into two doublets.

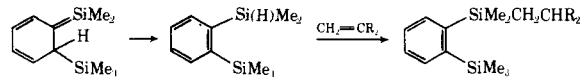
(10) Compound **7**: NMR δ (CCl_4) 0.35 (CH_3–$SiMe_2$, s, 9 H), 0.37 (CH_3–SiMe, s, 6 H), 0.81 (CH_2, d, 2 H, J = 6.8 Hz), 0.90 (CH_3–CMe, d, 6 H, J = 6.6 Hz), 1.79 (H–CMe_2, m, 1 H), 2.33 (CH_3–C_6H_3, s, 3 H).

(11) Ir spectrum of **8** was fully consistent with that reported[5] for the same substance.

(12) Compound **9**: NMR δ (CCl_4) 0.25 (CH_3–Si, s, 6 H), 0.74 (CH_2–C, d, 2 H), 0.90 (CH_3–CMe, d, 6 H), 1.80 (H–CMe_2, m, 1 H), 2.33 (CH_3–C_6H_4, s, 3 H), 7.08–7.52 (ring protons, m, 4 H); ir (cm^{-1})(neat) 745, 780, 830, 850.

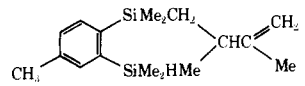
(13) Photolysis of **1** (2537 Å) in the absence of olefin gave a polymeric substance as a main product. When **1** was photolyzed in the presence of methyl alcohol, addition products (M^+ 240) consisting of two isomers were obtained in 33% yield. Attempts to isolate these in a pure form have been unsuccessful.

(14) For the production of **3**, one of the referees has suggested a possibility of an alternative mechanism outlined by the sequence as follows.

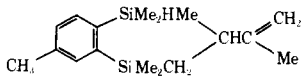


3

Photolysis of *p*-$CH_3C_6H_4SiMe_2SiMe_2H$ in the presence of 2,3-dimethylbutadiene under the same conditions gave compound **10** in 54% yield as a single product. If hydrosilylation reaction were involved leading to the observed products, compound **11** also should be formed. However, no evidence for the formation of **11** was obtained.



10



11

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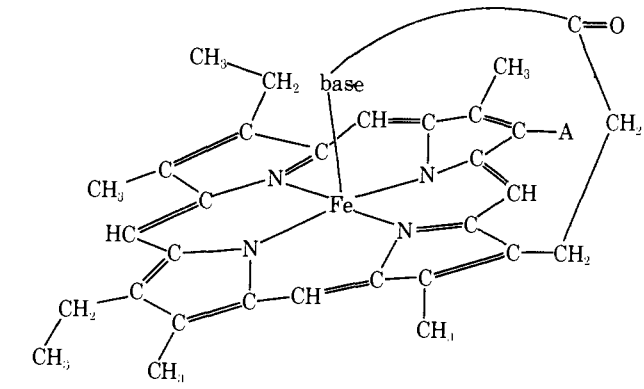
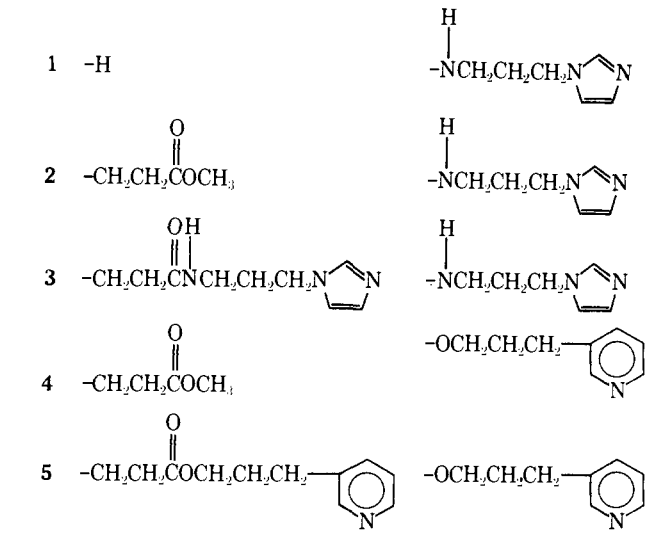
Coordination of Myoglobin Active Site Models in Aqueous Solution as Studied by Kinetic Methods[1]

Sir:

The covalent attachment of the "proximal" base to simple hemes,[2-4] as in **1**, has made possible studies of both equilibria[4a-d] and kinetics[4e,f] of reversible heme oxygenation. Although some of the qualitative aspects of reversible



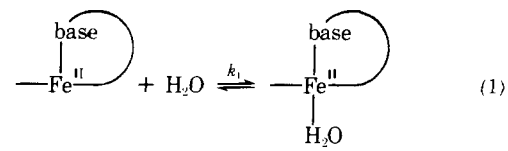
Compound A base



oxygenation of **1** and **2** have been duplicated with simple heme–base mixtures,[5-8] quantitative equilibria and kinetic studies of such mixtures have met with limited success[4e,9,10] due to the interference of the competing external bases.[11]

Because our "isolated site" models **1** and **2** showed oxygenation kinetics and equilibria at 20° in water similar to those of myoglobin,[4] and because **3** also binds oxygen reversibly in solution, it seemed interesting to investigate the coordination of **2** and **3** in aqueous solution. We report evidence that **2** is present in aqueous solution *almost entirely as the five-coordinate species* shown, whereas **3** exists as a mixture of five- and six-coordinate species in water.

We have previously reported that **1**, **2**, and **4** react with carbon monoxide as rapidly in water as in anhydrous solvents.[4d,f] This is evidence that, even in aqueous solution, water is not coordinated to the iron in **1** or **4** at room temperature (K_1 is small) (eq 1). However, as the temperature



(1)

of solutions of **1** or **2** in methanol–water or wet methylene chloride is lowered to <0° the broad band at 530 nm splits in α,β bands,[4a] typical of hexacoordinate hemes ($\epsilon_{555}/\epsilon_{528} \approx 1.5$ at −60°C in filtered wet methylene chloride). This indicates that K_1 becomes significant at low temperatures.[15]

Because there is still some disagreement concerning unequivocal correlations of visible spectra with axial ligation in hemes,[13b] we have developed an alternative kinetic meth-

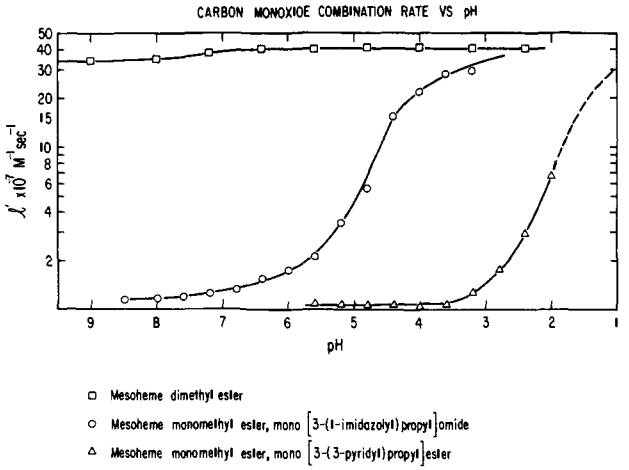
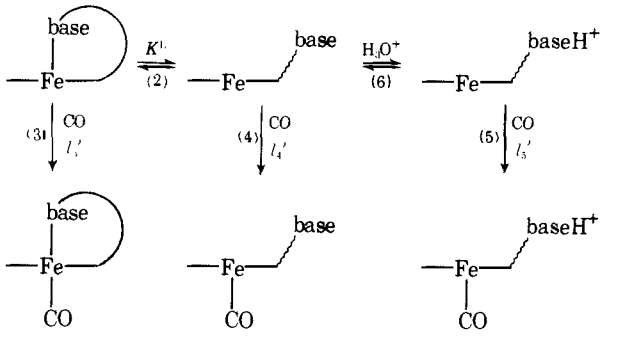


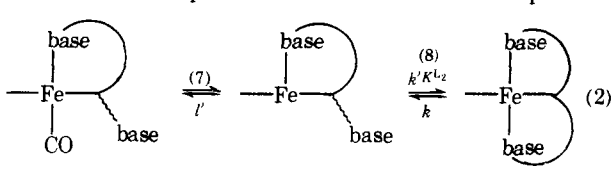
Figure 1. Kinetics were followed by flash photolysis as previously described.[4e] Heme solutions ($<5 \times 10^{-6}$ *M* in heme, $>2 \times 10^{-5}$ *M* in CO) were prepared in 0.06 *M* phosphate buffer, pH 9.00, suspended in 2.5% CTAB detergent.[4f] pH changes were effected by incremental additions of degassed 10% H_3PO_4 as measured in a separate experiment. Buffer concentrations ranged from 0.06 to 0.18 *M*.

od to demonstrate 0, 1, or 2 axial ligation of hemes. This method, besides being more definitive for coordination than are spectra, provides direct evidence concerning ligation of heme proteins. While mesoheme dimethyl ester in aqueous suspension (CTAB) reacts with carbon monoxide with a rate constant $l' = 4 \times 10^8$ l./(mol sec),[16] compounds **1, 2,** and **4** exhibit rate constants about 1×10^7 l./(mol sec),[4f] more nearly like those of some heme proteins. At low pH, where the base cannot be coordinated to the iron, compounds **1, 2,** and **3** display CO combination rates equivalent to the faster rate of mesoheme dimethyl ester. Thus, a



pH-CO rate profile plus the pK_a of the bases involved should reveal the pK^L for the binding of the covalently attached base to the iron. Figure 1 reveals a pH for half-conversion from slow to fast CO combination rates at ~4 and 1.4 for compounds **2** and **4**, respectively.[22] This corresponds to half-conversion from coordinated base to uncoordinated protonated base. If we assume that $l_5' = l_4'$ and that the pK_a's for the uncoordinated bases are the same as those of similar alkyl bases (pK_a = 6.9 for *N*-methylimidazole[17a] and pK_a = 5.7 for 3-methylpyridine),[17b] then we can calculate p$K^L \simeq$ 2.9 and 4.3 for **2** and **4**, respectively. Thus, $K_{Im}{}^L \simeq 800$ and $K_{Pyr}{}^L \simeq 20000$.

A study of the rates of reaction of **3** and **5** with CO at various concentrations allows the calculation[18] of K^{L_2}. Biscoupled hexacoordinate hemes readily react with CO and can be studied via eq 2. At concentrations of CO requisite[19]



(2)

for calculation of K^{L_2}, the return to carboxy-heme is slowed due to competing rapid coordination of the second axial base. The K^{L_2} for **3** is 5 and that for **5** is 130 in water and both are about eight times larger in DMF-water mixtures, indicating that heme-base complexes are more stable in nonaqueous solvents.[20]

The oxygen and carbon monoxide binding constants of **2, 3, 4,** and **5** are in agreement with these results. Thus **4** binds both oxygen and carbon monoxide much better than does **5**, whereas the differences in binding between **2** and **3** are very small.

The finding that pyridine is a better fifth and sixth ligand on hemes than is imidazole whereas the opposite is true for hemins suggests that the Fe^{II} in heme is a π-electron donor. That the fifth base binds more strongly than the sixth is consistent with the synergistic π bonding or π repulsion through the iron.[4b] This effect serves both to destabilize the bis(imidazole) complex and to stabilize the imidazole-oxygen complex.

These results show that the heme pocket need not enforce five coordination[12] by holding the proximal imidazole on iron or preventing water from binding to iron. Both are properties of the heme molecule and can be achieved even in water. It is further demonstrated that the failure of hexacoordinate heme proteins such as cytochrome b_5[21] to bind carbon monoxide cannot be attributed to the coordinating ability of the heme, but is brought about by the rigid heme environment which prevents the sixth (imidazole) ligand from leaving the iron. Such a rigid environment effect was previously demonstrated by placing **5** in a polystyrene film where it does not bind carbon monoxide.[4c]

References and Notes

(1) We wish to thank the National Institutes of Health (Grant HL-13581) for financial support of this research and (Grant RR-00,708) for support of the NMR facilities which were used.
(2) P. K. Warme and L. P. Hager, *Biochemistry*, **9**, 1606 (1970).
(3) Compounds **2** and **4** are mixtures having the groups A and base interchanged.
(4) (a) C. K. Chang and T. G. Traylor, *Proc. Nat. Acad. Sci. U.S.A.*, **70**, 2647 (1973); (b) *J. Am. Chem. Soc.*, **95**, 8475 (1973); (c) *ibid.*, **95**, 8477 (1973); (d) Priestley Centennial, Pennsylvania State University, University Park, Pa., July 1974; (e) *Biochem. Biophys. Res. Commun.*, **62**, 729 (1975); (f) *Proc. Nat. Acad. Sci. U.S.A.*, **72**, 1166 (1975).
(5) W. S. Brinigar and C. K. Chang, *J. Am. Chem. Soc.*, **95**, 5595 (1974).
(6) G. C. Wagner and R. J. Kassner, *J. Am. Chem. Soc.*, **96**, 5593 (1974).
(7) D. L. Anderson, C. J. Weschler, and F. Basolo, *J. Am. Chem. Soc.*, **96**, 5599 (1974).
(8) J. Almog, J. E. Baldwin, R. L. Dyer, J. Huff, and C. J. Wilkerson, *J. Am. Chem. Soc.*, **96**, 5600 (1974).
(9) (a) Oxygen complexes of certain hindered iron porphyrin compounds oxidize more slowly in solution than do those of, e.g., **1**; (b) J. P. Collman, R. R. Gagne, C. A. Reed, T. R. Halbert, G. Lang, and W. T. Robinson, *J. Am. Chem. Soc.*, **97**, 1427 (1975); (c) J. Almog, J. E. Baldwin, and J. Huff, *ibid.*, **97**, 227 (1975).
(10) C. J. Weschler, D. L. Anderson, and F. Basolo, *J. Chem. Soc., Chem. Commun.*, 835 (1974).
(11) Even the mixture of the hindered base 2-methylimidazole with a heme,[12] which apparently forms predominantly five-coordinate heme,[12,13] does not behave like myoglobin because the carbon monoxide *on* rates in this system are inversely proportional to the imidazole concentration from 0.01 to 2 *M*, the same behavior displayed by unhindered imidazoles.[14]
(12) J. P. Collman and C. A. Reed, *J. Am. Chem. Soc.*, **95**, 2048 (1973).
(13) D. Brault and M. Rougee, *Biochem. Biophys. Res. Commun.*, **57**, 654 (1974); (b) *Biochemistry*, **13**, 4598 (1974).
(14) J. Cannon, unpublished results.
(15) The effect of this water coordination upon low temperature kinetics[14] will be described elsewhere. However, the splitting of the α,β bands at low temperatures also occurs in anhydrous solvents such as toluene.
(16) M. H. Smith, *Biochem. J.*, **73**, 90 (1959), reported somewhat slower rates than those we observed, but he used sodium hydroxide rather than CTAB. Comparisons of these kinetics in aqueous CTAB with those in 50% aqueous methanol indicate that CTAB suspensions behave as aqueous solutions of the hemes.
(17) D. D. Perrin, "Dissociation Constants of Organic Bases in Aqueous Solution", Butterworths, London, 1965, (a) p 193; (b) p 167.
(18) Because $k > l'$(CO) for our CO concentrations, the Gibson equation[19] (1/R = (1/*k*) + (*k'*/*kl'*(CO))) can be used.
(19) R. W. Noble, Q. H. Gibson, M. Brunori, E. Antonini, and J. Wyman, *J. Biol. Chem.*, **244**, 3905 (1969).
(20) The concentration of *N*-methylimidazole which must be added to slow

l_3'(CO) down to that of **3** at the same CO concentration is 0.11 *M* compared to 10^{-5} *M* present in the usual solutions of **3**. This means that aggregation through imidazole–iron bonding is no problem below about 0.01 *M* in **1** or **2**.

(21) F. S. Mathews and P. Strittmatter, *J. Mol. Biol.*, **41**, 295 (1969).

(22) NOTE ADDED IN PROOF. Titration of an aqueous suspension of **2** with acid brings about an isosbestic change of the Soret band from that of five-coordinate[4a] to that of four-coordinate (or aquo) heme[13] with a half point at pH 3.6, in agreement with the kinetic results.

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The Basis for Chemical Attack by Active Nitrogen on Liquid Methanol. Induction of Molecular Decomposition by Recombination Events

Sir:

Although $(CH_2OH)_2$ and CH_2O are, in addition to HCN, the only organic compounds formed in significant yield by the reaction at 9°C of "active nitrogen" with aqueous methanol,[1] neither the ratio of their yields[2] ($(CH_2OH)_2/CH_2O$ = 0.25) nor the large increase in production of $(CH_2OH)_2$ when tetranitromethane is used as a scavenger[3] is consistent with their formation via combination and disproportionation of free $\cdot CH_2OH$ radical. We now report results of an investigation of the reaction of "active nitrogen" with neat liquid methanol at −79°. These results show that $\cdot CH_2OH$ radicals and H atoms are formed but that free radicals homogeneously distributed in bulk solution are precursors of only a minor portion of the observed stable products. It is proposed that chemical changes are initiated by decomposition of methanol molecules by deposition of energy of recombination of nitrogen atoms; part of the products are produced by direct dissociation to stable molecules, part via the intermediacy of free radicals.

"Active nitrogen" was produced in a fast-flow, low vacuum system[4] by irradiation with 2450-MHz microwaves. The flow rate of N(^{4}S) was determined by NO titration.[5] The reaction flask was cooled by a Dry Ice–acetone bath and the solution temperature was measured during reaction by means of a thermocouple. Yields of HCN,[6] CH_2O,[7] and $(CH_2OH)_2$[8] were determined by standard methods. H_2 was sampled by Toepler pumping and analyzed by mass spectrometry or gas chromatography. Phenyl-*tert*-butyl nitrone (PBN) was used to trap free radicals and identify them by EPR.[9]

$\cdot CH_2OH$ and H atom were the only radicals detected by spin trapping (see below). This technique cannot give quantitative data because trapping efficiencies are not known. The radicals were monitored conveniently by means of their reactions with Fe(III).[10]

$$\cdot CH_2OH + Fe(III) \rightarrow CH_2O + Fe(II) + H^+ \quad (1)$$

$$H + Fe(III) \rightarrow Fe(II) + H^+ \quad (2)$$

The isotopic composition of molecular hydrogen from CH_3OD was used as a probe of precursors of CH_2O and glycol.

The only detectable products of the reaction with neat CH_3OH were $(CH_2OH)_2$, CH_2O, HCN, and H_2. (See Table I for yields in the absence and presence of $FeCl_3$.) Yields were not affected significantly by 0.001 *M* $MgCl_2$. Molecular hydrogen obtained with CH_3OD as substrate did not contain a significant amount of D_2 and was composed of approximately equal amounts of HD and H_2. The only significant components of the EPR spin adduct spectrum were a triplet of triplets, a_N = 15.7 and a_H = 8.4 g, and a triplet of doublets, a_N = 15.2 and a_H = 3.6 g. These can be assigned respectively to the adducts of H atom[11] and $\cdot CH_2OH$ or $\cdot CH_3$.[11] $\cdot CH_3$ can be excluded as a significant intermediate because CH_4 could not be detected in the gaseous products by either gas chromatography or mass spectrometry.

Ferric chloride has little, if any, effect on the yield of HCN; 3×10^{-4} *M* $FeCl_3$ alters the yield of $(CH_2OH)_2$ and CH_2O by amounts which are not changed on increasing the concentration of scavenger up to 1×10^{-1} *M*. In contrast, the yield of H_2 is affected equally by 3×10^{-4} and 1×10^{-3} *M* scavenger but is reduced further by 1×10^{-2} *M* and somewhat more by 1×10^{-1} *M* $FeCl_3$. The decrease in yield of H_2 by up to 1×10^{-3} *M* $FeCl_3$ is equal within experimental error to twice the observed decrease in glycol yield. This approximate equality indicates that the precursor of most of the *scavengeable* glycol is $\cdot CH_2OH$ radical formed in bulk solution by reaction 3, rather than formed directly by action of N atoms upon CH_3OH.

$$H + CH_3OH \rightarrow H_2 + \cdot CH_2OH \quad (3)$$

Apparently, even at the lowest concentration of $FeCl_3$ used, all H atoms which diffuse into bulk solution are scavenged. It can be further noted that if free homogeneously distributed $\cdot CH_2OH$ were the precursor of $(CH_2OH)_2$ and CH_2O, reaction 1 would suppress $(CH_2OH)_2$ completely and increase the yield of CH_2O by an amount equal to twice the yield of glycol plus the yield of CH_2O in the absence of scavenger, i.e., by 27.5 ± 1 in the units of Table I. The observed small increase in yield of CH_2O in the presence of 0.0003–0.1 *M* $FeCl_3$, 3.7 ± 0.6, apparently reflects a minor yield (i.e., 3.7) of free $\cdot CH_2OH$. The observed large yield of HD from CH_3OD is also not consistent with formation of all or most of the glycol and CH_2O by combination and disproportionation of $\cdot CH_2OD$. It is consistent with unimolecular dissociation of a highly energetic CH_3OD molecule to give CH_2O and HD. Reduction of yields of H_2, but not of CH_2O, by high concentrations of $FeCl_3$ can be explained by reaction 4.

$$CH_3OH^* + 2Fe(III) \rightarrow CH_2O + 2Fe(II) + 2H^+ \quad (4)[12]$$

Presumably, unscavengeable glycol results from a bimolec-

Table I. Product Yields[a,b]

	(10^2 × moles of product[c])/(g atoms of incident N)			
$(FeCl_3)$, *M*	$(CH_2OH)_2$	CH_2O	HCN	H_2
0	7.0 ± 0.4 (10)	13.5 ± 0.5 (10)	5.6 ± 0.4 (10)	28.7 ± 1.5 (4)
3×10^{-4}	2.6 ± 0.1 (2)	17.2 ± 0.3 (2)	6.4 (1)	18.0 ± 1.5 (2)
1×10^{-3}	2.5 ± 0.2 (2)	17.2 ± 1.5 (2)	6.2 (1)	18.3 ± 2 (3)
1×10^{-2}	2.9 ± 0.5 (2)	17.0 ± 1.4 (2)	6.4 (1)	9.6 ± 1 (2)
1×10^{-1}	2.9 ± 0.2 (2)	17.3 ± 1.2 (2)	6.3 (1)	6.5 ± 1 (2)

[a] Flow rate of atomic nitrogen 1.3×10^{-6} mol sec^{-1}. [b] Indicated uncertainties are standard deviations. [c] Numbers in parentheses indicate numbers of complete replicate experiments. Analyses were performed in triplicate for every replicate experiment.