

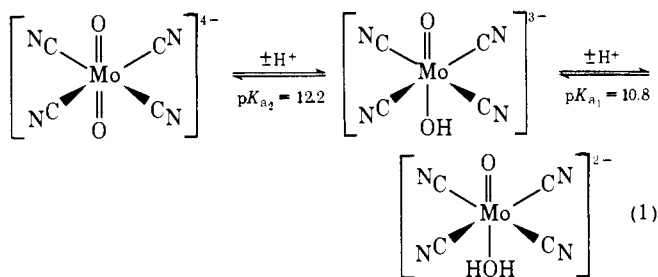
The Chemical Evolution of a Nitrogenase Model. X. Reduction of Coordinated Cyanide Ion in Cyano Complexes of Molybdenum(IV) and Their Use as Catalysts for the Reduction of Molecular Nitrogen and of Other Substrates

G. N. Schrauzer,* P. R. Robinson, E. L. Moorehead, and T. M. Vickrey

Contribution from the Department of Chemistry, The University of California, San Diego, Revelle College, La Jolla, California 92037. Received May 7, 1975

Abstract: The complex anions $[\text{Mo}(\text{O})_2(\text{CN})_4]^{4-}$ (1), $[\text{Mo}(\text{O})(\text{OH})(\text{CN})_4]^{3-}$ (2), and $[\text{Mo}(\text{O})(\text{H}_2\text{O})(\text{CN})_4]^{2-}$ (3) represent rare examples of defined monomeric complexes of oxomolybdate(IV) and may be regarded as models of substrate-catalyst complexes in the reduction of CN^- by molybdothiol catalyst systems. The reduction of coordinated CN^- in 3 to NH_3 , CH_4 , C_2H_6 , and C_2H_4 was demonstrated with BH_4^- , $\text{S}_2\text{O}_4^{2-}$, and reduced ferredoxin model compounds as reducing agents. Evidence for a significant stimulatory effect of ATP was also obtained. The reduction of coordinated CN^- is inhibited by O_2 , CO , and the known substrates of nitrogenase enzymes. The latter are reduced under the reaction conditions, involving coordinatively unsaturated cyano complexes of oxomolybdate(IV) as the catalysts. The reduction of molecular N_2 to NH_3 and traces of N_2H_4 was also observed and proceeds more efficiently than in the molybdothiol model systems of nitrogenase.

The molybdothiol model systems¹ of nitrogenase (N_2 -ase) in their simplest form consist of complexes of oxomolybdate ions with thiol ligands. In the presence of a reducing agent, these systems catalyze the reduction of all known substrates of N_2 -ase by a mechanism proposed to involve mononuclear oxomolybdate(IV) complexes of thiols as the catalytically active species.¹ Although their stationary concentrations under the conditions of substrate reduction is low, sufficient evidence has been accumulated during the past years to indicate that they possess the properties of a metallonucleophile, and that they form intermediate complexes of the substrates. However, since these reactive complexes, designated " Mo^{red} " cannot be isolated, all mechanistic evidence thus far rests on kinetic rather than direct synthetic evidence. It thus seemed desirable to test the validity of key mechanistic concepts through the study of the reaction of a well defined, monomeric complex of Mo(IV), in which the ligands are reducible substrates of N_2 -ase. Cyano complexes of oxomolybdate(IV) appeared to be particularly suitable for this purpose. We have for this reason studied the reduction of coordinated cyanide in the complex anions derived from the well-known² salt $\text{K}_4\text{Mo}(\text{O})_2(\text{CN})_4 \cdot 2\text{H}_2\text{O}$, which is one of the few examples of a mononuclear Mo(IV) derivative. In solution, the purple anion $[\text{Mo}(\text{O})_2(\text{CN})_4]^{4-}$ (1) exists only in the presence of strong alkali, i.e., in 1.5 *F* KOH.³ At pH 11-12, the predominant species in solution is the blue hydroxoanion 2, which is protonated further to the aquo derivative 3 in the pH range between 7 and 10 (eq 1).³



The use of these anions as models for substrate-catalyst intermediates in the molybdothiol systems is particularly attractive since the structures of all three species have been

elucidated by X-ray diffraction analysis of crystalline salts,^{3,4} and because their solution chemistry is well understood. Cyanide, on the other hand, is a substrate of N_2 -ase and is enzymatically reduced to NH_3 , CH_4 , and traces of C_2H_6 , C_2H_4 , and CH_3NH_2 .^{5,6} Since the formation of these products was also observed in the reduction of CN^- with molybdothiol catalyst systems,⁷ it appeared of interest to study the reduction of the coordinated CN^- in the anions 1-3 as well. In the following we report the results of this work and introduce a "molybdocyanide" model of nitrogenase which promises to become a valuable system for the continued study of simulated N_2 -ase reactions.

Results

Reduction of Ions 1-3 with NaBH_4 . Solutions of the salt $\text{K}_4[\text{Mo}(\text{O})_2(\text{CN})_4] \cdot 2\text{H}_2\text{O}$ were prepared in borate buffers of different pH and treated with an excess of NaBH_4 both in the absence and presence of ATP. The reduction of coordinated CN^- is very slow in solutions of pH 13, but becomes noticeable at pH 10 and significant in the pH range between 9 and 7 (Table I). The main reaction products were identified as NH_3 , CH_4 , C_2H_6 , and C_2H_4 . After long reaction times traces of C_3 -hydrocarbons, and traces of CH_3NH_2 were sometimes observed. For the actual study of the reaction we have monitored the concentration of hydrocarbons in the gas-phase by GLPC. Typical yield-time plots of reactions in the absence and presence of ATP are shown in Figures 1 and 2. It may be seen that CH_4 is the main product of the reactions in the absence of ATP. In the presence of ATP in substrate amounts, a significant stimulation of the hydrocarbon production is observed which is particularly noticeable for C_2H_4 . The hydrocarbon yield increases with increasing concentration of molybdenum complex up to the concentration of 0.006 *M* (without ATP); with ATP, the optimal concentration of molybdenum complex is observed at about 0.004 *M*; higher concentrations of complex cause a lowering of the hydrocarbon production (Figure 3). At optimal concentrations of molybdenum complex in the presence of ATP, 12% of the coordinated CN^- is converted to hydrocarbons until the available BH_4^- is fully consumed. The overall electron transfer efficiency thus is low and most of the BH_4^- is decomposed to yield H_2 .

Table I. Reduction of Coordinated Cyanide in 3 under Various Conditions^d

No.	Concn of variable	Concn of 1, 2, or 3	Initial concn of other reactants	Gas ^a	Initial rate ^b	Product ratios ^c
1	ATP, 0.0	3.7 mM	NaBH ₄ , 0.066 M; pH 7.5, 0.2 M borate buffer	Argon	9.3	100:16:12
2	ATP, 0.037 M				15.3	100:16:41
3	pH 13, borate	0.040 M	ATP, 0.061 M; NaBH ₄ , 0.132 M		0.022	100:trace:trace
4			ATP, 0.0		0.018	100:trace:trace
5	pH 10, borate		ATP, 0.061 M		0.15	100:6:6
6			ATP, 0.0		0.30	100:7:9
7	pH 7, borate		ATP, 0.061 M		15.3	100:16:20
8			ATP, 0.0		4.0	100:13:13
9	NaBH ₄ , 0.016 M	4.0 mM	ATP, 0.041 M; pH 7.5, 0.2 M borate buffer		3.7	100:6:81
10			ATP, 0.0		0.80	100:7:16
11	NaBH ₄ , 0.033 M		ATP, 0.041 M		4.9	100:10:62
12			ATP, 0.0		3.8	100:11:14
13	NaBH ₄ , 0.066 M		ATP, 0.041 M		15.7	100:17:40
14			ATP, 0.0		6.2	100:13:10
15	CO(g), 34 cm ³ , 1 atm	4.2 mM	ATP, 0.041 M; NaBH ₄ , 0.066 M; pH 7.5, 0.2 M borate buffer	CO	10.6	100:22:50
16			ATP, 0.0; same pH and NaBH ₄		7.1	100:15:8
17	Na ₂ S ₂ O ₄ , 0.03 M	2.3 mM	ATP, 0.054 M; pH 8.0	Argon	7 × 10 ⁻⁵	100:trace:trace
18			ATP, 0.0		1 × 10 ⁻⁴	100:trace:trace
19	Na ₂ S ₂ O ₄ , 0.03 M; FeSO ₄ , 2.5 mM		ATP, 0.054 M		3.7 × 10 ⁻⁴	100:trace:21
20			ATP, 0.0		6 × 10 ⁻⁵	100:trace:trace
21	K ₄ Mo(CN) ₈ , 0.024 M	—	ATP, 0.056 M; NaBH ₄ , 0.132 M; pH 7.5, borate buffer		0.08	100:trace:250
22		—	ATP, 0.0; same pH and NaBH ₄		Trace	—

^a (34 cm³, 1 atm). ^b (nmol of CN⁻ reduced to hydrocarbons)/min. ^c (CH₄:C₂H₆:C₂H₄). ^d All samples contained a total of 4 ml of solution; reaction temperature, 27°.

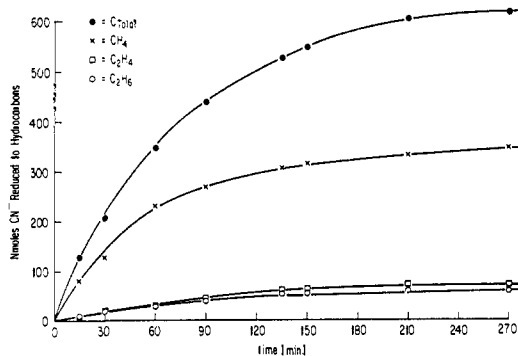


Figure 1. Time-yield plot of the reduction of 3 by BH₄⁻ in the absence of ATP. Reaction solutions contained, in a total volume of 4 ml: 3, 15.2 μmol; BH₄⁻, 0.265 mmol (initial concentrations). Solvent: 0.2 M borate buffer, pH 7.5. Reaction was complete after 4 hr at 27°.

The ratios between CH₄ and the C₂ hydrocarbons are somewhat time-dependent. As a rule, more C₂H₄ relative to CH₄ and C₂H₆ is produced toward the end of the reaction, i.e., at low concentrations of remaining BH₄⁻. Higher yields of C₂H₄ are also observed at lower initial concentrations of BH₄⁻ (see Table I). The rate of CN⁻-reduction increases with increasing temperature. In the range between 0 and 50° the apparent Arrhenius energy of activation was determined to 4.0 ± 0.5, 6.1 ± 0.3, and 6.3 ± 0.3 kcal/mol for CH₄, C₂H₆, and C₂H₄, respectively, in the absence of added ATP. In the presence of ATP, the apparent *E_a*'s were found to be 3.0 ± 0.5, 11 ± 0.5, and 12 ± 0.5 kcal/mol for CH₄, C₂H₆, and C₂H₄. Initial rates of reduction, hydrocarbon yields, and ratios under a variety of different reaction conditions are summarized in Table I. From the observed

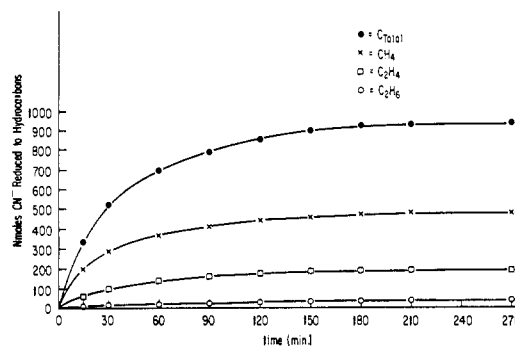


Figure 2. Time-yield plot of the reduction of 3 by BH₄⁻ in the presence of ATP. Reaction solutions contained the same components at the concentrations given in the legend to Figure 1 but also 0.215 mmol of ATP (initial concentrations). Reaction was essentially complete after 90 min at 27°.

pH dependence we conclude that 3 rather than 2 is the actually reacting species. The apparent inertness of 2 is attributed to the presence of the coordinated OH⁻-group, the higher negative charge of the complex anion, and the lower reactivity of BH₄⁻ at higher pH. The originally blue solutions of 3 attain a green color upon the addition of BH₄⁻ which eventually turns to brown as the reaction progresses. The solutions become nearly colorless after long reaction times. The terminal solutions contain MoO₄²⁻; a faint blue color (λ_{max} 620 nm) due to unreacted or reconstituted 2 or 3 is observed if the reactions are run at high concentrations of substrate or low levels of reductant.

Effects of Nucleoside Phosphates, Acid Anions, and of Mg²⁺. The effects of ATP, ADP, AMP, and of several anions of protic acids on the rate of hydrocarbon production

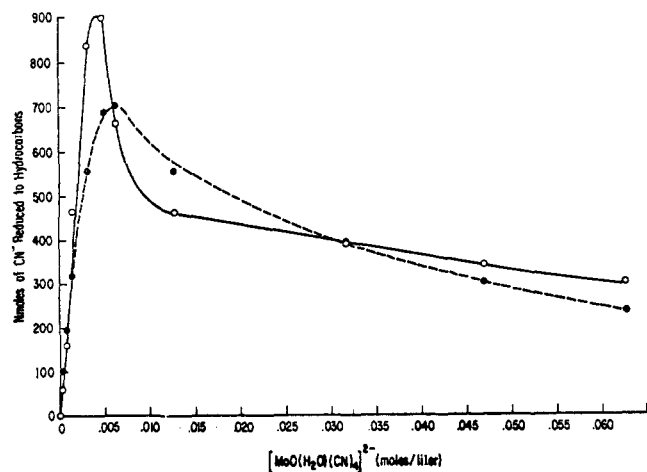


Figure 3. Dependence of the yields of hydrocarbons on the concentration of **3**. Reaction solutions contained, in a total reaction volume of 3.4 ml: **3**, as indicated; BH_4^- , 0.265 mmol (initial concentrations). Solvent, 0.2 F, pH 7.5 borate buffer: (---) without ATP; (—) with 0.26 mmol of ATP (initial concentrations).

Table II. Comparison of Hydrocarbon Yields and Ratios from the Reduction of **3** by BH_4^- in the Presence of Various Additives at Two Different Concentrations^b

Added component ^a	0.0026 M $\text{C}_2\text{H}_6/\text{C}_2\text{H}_4$	0.054 M $\text{C}_2\text{H}_6/\text{C}_2\text{H}_4$
ATP	19.0/17.3	17.2/33.0
ADP	18.2/18.1	13.9/30.1
AMP	20.1/18.4	14.0/26.5
HPO_4^{2-}	13.5/12.5	12.9/38.1
ClO_4^-	14.3/8.5	13.1/8.0
Cl^-	12.6/10.0	12.1/11.4
SO_4^{2-}	15.0/10.1	13.3/9.5
None	16.3/12.3	16.3/12.3

^a The entries at the concentration of the components of 0.0026 M represent examples for runs at lower than optimal ATP concentrations, those at 0.054 correspond to runs at higher than optimal ATP levels. ^b Experimental conditions as outlined in the legend to Figure 4. Yields are relative to $\text{CH}_4 = 100$.

from **3** were investigated in solutions buffered to the same initial pH of 7.5 to eliminate the effect⁸ of variations in the H^+ concentration. With ATP evidence for a significant stimulatory effect was observed; with ADP and AMP the effects were much weaker (Figure 4). All other acids tested proved to be inhibitory. The most significant inhibition was observed with phosphate ion. The nucleoside phosphates behave very much as in the molybdothiol model systems;⁸ ATP again shows an apparently specific effect which must be attributed to its direct interaction with the anions **2** or **3**. Qualitative experiments indicate that ATP is in part hydrolyzed to ADP and inorganic phosphate under the reaction conditions. Quantitative data on the extent of ATP hydrolysis could not yet be obtained due to difficulties with assays for phosphate; all methods investigated to-date failed to produce reproducible results due to the presence of the reduced molybdenum species. A comparison of the hydrocarbon product ratios reveals a similarity of the effects of ATP and PO_4H_2^- , and the absence of significant influences of the other anions studied (Table II). Since N_2 -ase requires Mg^{2+} ion for activity, we have also studied the effect of Mg^{2+} in the present system. The results in Figure 5 demonstrate that Mg^{2+} causes a slight stimulation of the CH_4 production and a diminution of the yields of the C_2 -hydrocarbons up to the Mg^{2+} -ATP ratio of 1:2; higher concentrations of Mg^{2+} are inhibitory.

Reductions in D_2O . The total hydrocarbon yields in D_2O in the absence of ATP are significantly higher when

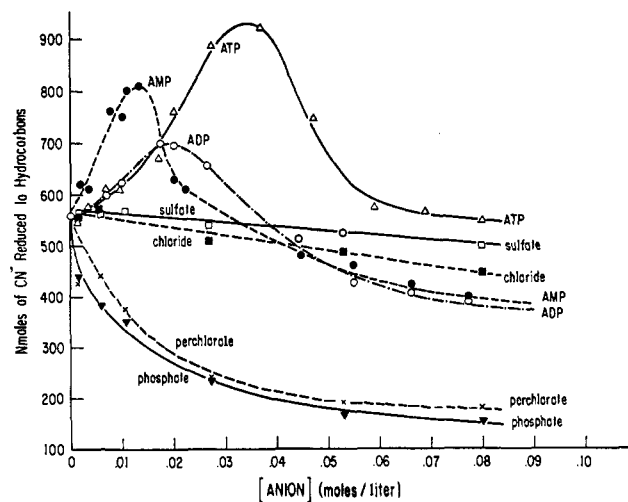


Figure 4. Effects of ATP, ADP, AMP, and of four different acid anions on the yields of hydrocarbons produced in the reduction of **3**. The reaction solutions contained, in a total volume of 4.0 ml: **3**, 15.2 μmol ; BH_4^- , 0.265 mmol, and the remaining additives as indicated. Solvent: 0.2 F borate buffer (pH 7.5). All test samples were made from stock solutions of the Na salts which were prepared in 0.2 F pH 7.5 borate buffer. Differences in the initial pH were eliminated by the addition of the appropriate acids and/or NaOH.

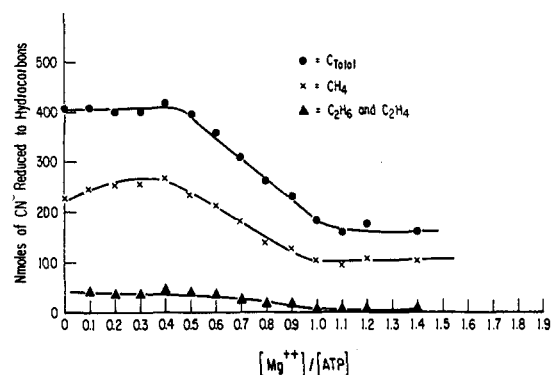


Figure 5. Effect of Mg^{2+} on the ATP-stimulated reduction of **3**. The reaction solutions (solvent: H_2O) contained, in a total volume of 4.0 ml: **3**, μmol ; BH_4^- , 0.265 mmol; ATP, 0.232 mmol (initial concentrations); $[\text{Mg}^{2+}]$ as indicated. Yields were measured in the gas phase after 60 min of reaction at 27°.

Table III. Hydrocarbon Yields and Ratios from the Reduction of **3** in D_2O and H_2O and with either NaBH_4 or NaBD_4 , in the Absence and Presence of ATP^a

Solvent	ATP	Reductant	Yields (nmol)			H_2 (cm ³)	Ratios $\text{CH}_4/\text{C}_2\text{H}_6/\text{C}_2\text{H}_4$
			CH_4	C_2H_6	C_2H_4		
H_2O	—	NaBH_4	8.4	0.4	0.4	9	100/5/5
D_2O	—	NaBH_4	6.5	1.0	0.7	7	100/15/11
H_2O	—	NaBD_4	10.1	0.4	0.4	8	100/4/4
D_2O	—	NaBD_4	25.0	1.0	0.6	8	100/4/2
H_2O	+	NaBH_4	161.	29.	31.	14.5	100/18/19
D_2O	+	NaBH_4	11.4	0.5	2.	11.0	100/4/18
H_2O	+	NaBD_4	110.	18.	29.	13.0	100/16/26
D_2O	+	NaBD_4	8.3	0.8	2.8	13.0	100/10/34

^a Reaction solutions contained, in a total volume of 4.0 ml: molybdenum complex, 0.0152 mmol; NaBH_4 (NaBD_4) 0.264 mmol (initial concentrations); ATP (where present), 0.18 mmol. Yields determined after 60 min of reaction at 27°.

NaBD_4 rather than NaBH_4 is employed as the reducing agent, and lower with NaBH_4 (Table III). In the presence of ATP, the hydrocarbon production is diminished in D_2O relative to H_2O , and lower still when NaBD_4 rather than

Table IV. Inhibitory Effects of O₂, CO, and of a Variety of Substrates of Nitrogenase on the Reduction of Coordinated CN⁻ in 3^d

Inhibitor of substrate	Concn	% inhibition relative to runs under argon, with (without) ATP				Substrate reduced to products
		H ₂	CH ₄	C ₂ H ₆	C ₂ H ₄	
O ₂	1 atm	62 (87)	95 (99)	97 (99)	99 (99)	(H ₂ O)
CO	1 atm	32 (9)	25 (46)	72 (58)	71 (60)	—
C ₂ H ₂	1 atm	85 (81)	95 (92)	(a)	(a)	C ₂ H ₄ , trace C ₂ H ₆
c-C ₆ H ₁₁ NC	0.02 M	74 (86)	98 (99)	96 (99)	99 (99)	CH ₄ , C ₂ H ₆ , C ₂ H ₄ , c-C ₆ H ₁₁ NH ₂
CN ⁻	0.02 M	47 (53)	94 (93)	98 (97)	96 (87)	NH ₃ , CH ₄ , C ₂ H ₆ , C ₂ H ₄
CH ₃ CN	0.50 M ^b	23 (66)	90 (63)	(a)	95 (58)	NH ₃ , C ₂ H ₆
CH ₂ =CHCN	0.02 M	72 (91)	91 (97)	98 (99)	99 (99)	NH ₃ , C ₃ H ₆ , C ₃ H ₈
N ₃ ⁻	0.02 M	64 (77)	63 (94)	81 (99)	93 (97)	NH ₃ , N ₂
N ₂ O	1 atm	55 (92)	99 (92)	66 (80)	99 (95)	N ₂ , H ₂ O
N ₂	1 atm	26 (0)	36 (0)	52 (0)	58 (0)	NH ₃ ^c

^a Per cent inhibition was not determined since substrate is reduced to the same product(s). ^b This substrate inhibits only weakly at lower concentrations. ^c NH₃ detected by colorimetric procedure; yields of NH₃: 4.8 μmol after correction for NH₃ produced through CN⁻ reduction, in runs with ATP. In the absence of ATP, corrected NH₃ yield was 0.2–0.7 μmol. ^d Reaction solutions contained, in a total volume of 4.0 ml: [Mo(O)(OH₂)(CN)₄]²⁻, 0.008 mmol; BH₄⁻, 0.264 mmol; ATP, 0.23 mmol (initial concentrations). Solvent: H₂O. The initial pH of the ATP stock solution was adjusted to 7.5 with NaOH; yields were determined after 60 min of reaction at 27°.

NaBH₄ is the reductant. The relative yields of C₂ hydrocarbons are generally higher in H₂O.

Inhibitors, and Reactions in the Presence of Nitrogenase Substrates. The reduction of coordinated cyanide in 3 is substantially inhibited by O₂ and CO, both in the absence and presence of ATP. Added CN⁻ is also inhibitory, as are all known alternate substrates of N₂-ase and molecular nitrogen itself (Table IV). The effect of the nitrogenase substrates may be explained by assuming their interaction with 3 or with coordinatively unsaturated fragments thereof. This interaction provides conditions favorable for the catalytic reduction of the substrates. Acetylene, for example, causes 90%+ inhibition of CN⁻ reduction but is itself reduced to C₂H₄. The reduction of this substrate proceeds at 1000 times the rate of reduction of coordinated cyanide, as evidenced from the observed CH₄:C₂H₄ ratios. Other substrates, including N₂, are also reduced. Under the reaction conditions, the Mo(IV)-cyanide complexes are probably converted into fragments which still contain coordinated cyanide, and these fragments can serve as catalysts of the reduction of the other substrates. Most interestingly, N₂ causes significant inhibition of cyanide reduction only in the presence of substrate amounts of ATP. A careful comparison of the yields of NH₃ under N₂ with those from experiments under argon reveal that nitrogen reduction to ammonia occurs in these systems. The Mo(IV)-cyanide complexes thus can serve as alternate models of N₂-ase. In forthcoming papers we shall discuss the reactions of N₂-ase substrates in these systems. It is sufficient at this point to mention that the reduction of N₂ with the Mo-cyanide complexes has been firmly established, both by measurements of the yields of NH₃ by a colorimetric procedure and by the use of ³⁰N₂ as the substrate (Table V).

Since coordinatively unsaturated fragments of the cyano complexes are likely to function as catalysts of the reduction of any of the substrates studied, the yield of NH₃ from N₂ must be based on the number of moles of active catalyst rather than the total molybdenum concentration. From the yields of hydrocarbons listed in Table V it is estimated that 1 mol of active molybdenum catalyst reduced 50–100 molecules of N₂ under the reaction conditions employed. The reaction thus appears to be genuinely catalytic, even though it is as yet not fully optimized. Traces of N₂H₄ are also formed, as evidenced from the observed yields of ³⁰N₂ after hypobromite oxidation of the reaction solutions. The reaction thus appears to proceed via the diimide mechanism as in the molybdothiol systems, but with a better electron transfer efficiency.

Reduction with Reduced Ferredoxin Model Compounds. Reduced ferredoxin model compounds,⁹ i.e., anions of the

Table V. Reduction of ³⁰N₂ with Cyanomolybdate (IV) Complexes as the Catalysts^b

Gas phase	ATP	Total CN ^{-a} reduced, μmol	Yields, μmol ^a		% ³⁰ N ₂ reduced
			NH ₃	N ₂ H ₄	
Ar	—	0.070	0	0	
Ar	+	0.220	0	0	
Ar + 609 μmol of N ₂	—	0.076	1.11	0.13	0.10
Ar + 203 μmol of N ₂	+	0.160	1.32	0.15	0.50
Ar + 406 μmol of N ₂	+	0.170	1.52	0.18	0.25
Ar + 609 μmol of N ₂	+	0.176	5.96	0.52	0.57

^a Yield measurements after 2 hr of reaction at 27°. ^b Reaction solutions contained, in a total volume of 4 ml: [Mo(O)(OH₂)(CN)₄]²⁻, 15.2 μmol; ATP (where present), 0.096 mmol; BH₄⁻, 0.264 mmol (initial concentrations). Solvent: H₂O, initial pH prior to NaBH₄ addition was adjusted to 7.5.

type [Fe₄S₄(SR)₄]^{z-}, z = 3, 4 can be generated in CH₃OH solution by the combination of equivalent amounts of Fe-salt, Li₂S, and LiSR.¹⁰ The resulting solutions possess sufficiently high reducing power for the use as stoichiometric reductants of N₂-ase substrates in molybdothiol model systems. It therefore was of interest to establish their reactivity in the reduction of coordinated CN⁻ as well. Using the cluster anion with z = 4 as generated in situ in a tenfold excess over 3, reduction of coordinated CN⁻ could be demonstrated. It is of interest that the CN⁻ under these conditions is reduced primarily to CH₄ and C₂H₆, and that smaller amounts of C₂H₄ are formed. The reaction is also stimulated by substrate amounts of ATP (Table VI).

Reduction with S₂O₄²⁻. Experiments with this reducing agent in solutions buffered from pH 7 to 9 produced a small yield of hydrocarbons from the coordinated CN⁻ in 3 after very long reaction times (see Table I). This agent is evidently too unreactive to merit application in the present system.

Discussion

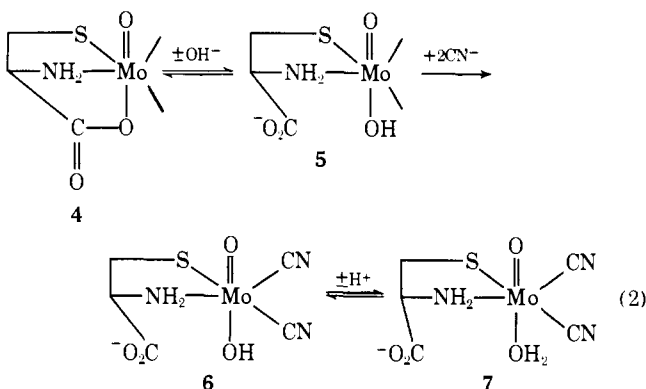
General Conclusions. The present work confirms some of the key postulates of the previous studies with molybdothiol catalyst systems¹ and permits further substantiation of the proposed structure and reactivity of the intermediates in the reduction of N₂-ase substrates. It is now clear that coordinated CN⁻ is indeed reduced in mononuclear complexes of Mo(IV), and since the structures of the complexes are known it is also possible to make more detailed predictions concerning the structure of the intermediates in molybdothiol catalyzed reactions. We previously suggested that the catalytically active species in the latter systems are thiol complexes of oxomolybdate(IV).¹ These highly reactive species could not be investigated directly, but the available

Table VI. Reduction of 3 by Reduced Ferredoxin Model Compounds in the Presence and Absence of ATP^a

[Fe ₄ S ₄ (SR) ₄] ⁴⁻ (μmol)	ATP	Fe/Mo ratio	nmol CN ⁻ reduced to hydrocarbons	Yield ratios CH ₄ :C ₂ H ₆ :C ₂ H ₄
5	-	0.5	6.5	100/trace/trace
20	-	1.8	7.5	100/30/trace
100	-	9.0	25.0	100/135/25
200	-	18	41.6	100/190/50
300	-	27	46.8	100/250/45
5	+	0.5	6.4	100/trace/50
20	+	1.8	12.2	100/30/30
100	+	9.0	26.7	100/110/20
200	+	18	47.1	100/105/20
300	+	27	66.5	100/145/40

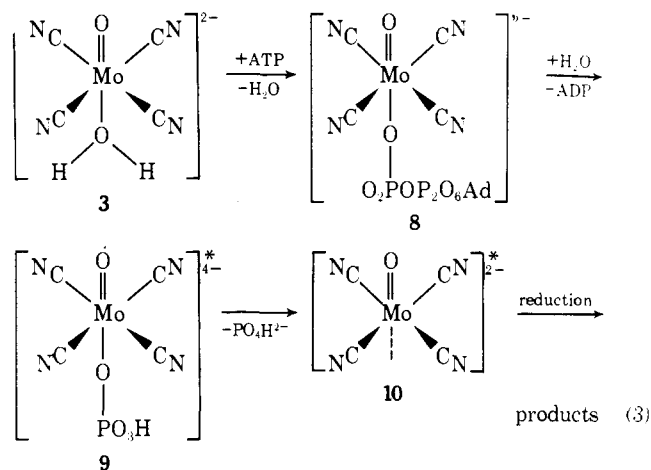
^a Reaction solutions contained, in a total volume of 3.6 ml: 3 0.011 mmol; ATP, where present, 0.18 mmol. Ferredoxin model compounds, presumably species of composition [Fe₄S₄(S-*n*-C₃H₇)₄]⁴⁻, were generated by combining solutions of FeCl₂, Li₂S, and of LiS-*n*-C₃H₇ in equivalent amounts, at concentrations indicated. Solvent: 80% CH₃OH-H₂O. Hydrocarbon yields were determined after 60 min of reaction at 27°.

kinetic data indicated that they are monomeric, and other studies demonstrated that they contain one molecule of the thiol ligand. With L-(+)-cysteine as the thiol component, the catalytically active reduced species Mo^{red} was proposed to have either of the structures 4 and 5, and we may now conclude that its reaction with CN⁻ could form intermediates of structure 6 and 7 (eq 2).



The anion 2, which closely resembles the hypothetical intermediate complex 6, is as such reduced only slowly. The aquo derivative 3 is reduced, but even in this reaction the removal of the coordinated H₂O is necessary to permit interaction with the reductant. Since the removal of coordinated H₂O is possible by a displacement reaction, this process may also be facilitated by ATP. Assuming the initial displacement of H₂O by ATP, we formulate the subsequent reaction steps as shown in eq 3, where Ad denotes the adenosyl moiety. Intermediates 9 and 10 are presumed to be in an activated state due to the energy release through ATP hydrolysis, indicated by []*.

In accord with similar conclusions derived from studies in molybdothiol systems,⁸ we assume that ATP forms a complex with the deaquated anion of 3 via the terminal phosphate group. This complex formation could well lead to an acceleration of ATP hydrolysis, giving rise to the formation of ADP and a complex containing a molybdenum-bound phosphate group. The fact that phosphate ion could interact with the molybdenum complex in the manner indicated in eq 3 is supported by the observed significant inhibitory effect of phosphate, and the similarity of the hydrocarbon product ratios in the ATP-stimulated and phosphate-inhibited reduction of 3. Since the hydrolysis of ATP to ADP and inorganic phosphate is molybdate-catalyzed, we fur-



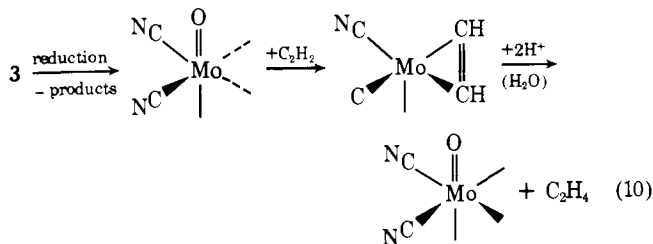
thermore postulate that some of the energy released upon the hydrolysis of the phosphate bond is transferred to 9, which thus could well be expected to have enhanced reactivity as compared to a complex of similar constitution which results if 3 interacts with PO₄H²⁻. This could explain why ATP stimulates and phosphate inhibits the reduction of coordinated CN⁻ in our system. We have shown in part IX of this series⁸ that simple protic acids have as a rule significantly lower stimulatory effects than ATP. In the present system all acid anions studied were found to act as inhibitors, presumably because they compete for, or block the molybdenum binding site. The observed effect of Mg²⁺ is also consistent with the mechanism in eq 3. The results in Figure 5 show that Mg²⁺ increases the formation of CH₄ at the expense of the C₂-hydrocarbons. It is possible that Mg²⁺ facilitates the removal of PO₄H²⁻ from 9, or that it forms an inactive complex with ATP. The net effect of Mg²⁺ could be that the concentration of available ATP is lowered, and that the formation of the reactive species 10 from 9 is favored. The combination of both effects will affect the electron-transfer efficiency from the reductant to the molybdenum complex and thus influence the hydrocarbon product distribution. It is interesting that the optimal Mg²⁺:ATP ratio is 1:2 and that the optimal ATP:Mo ratio is about 12:1, just as with N₂-ase. The inhibitory effect of higher concentrations of Mg²⁺ is plausibly attributed to the formation of inactive Mg-ATP complexes in the system. It remains to be seen, how the extent of ATP hydrolysis correlates with the amount of coordinated substrate reduced. We must defer detailed studies of this point until we find a reliable assay for PO₄H²⁻ in the presence of reduced molybdenum species and nucleoside phosphates.

Since the CH₄-C₂H₆ ratio remains nearly the same in the presence or absence of ATP, a direct involvement of ATP in reactions leading to the formation of these hydrocarbons need not be postulated. However, the formation of C₂H₄ may well be specifically ATP stimulated, as will be outlined below.

The reduction of 3 by reduced ferredoxin model compounds confirms that Fe²⁺ derivatives of mercaptoiron sulfides possess sufficiently high reducing power and affinity for molybdenum to effect the efficient transfer of electrons into the molybdenum-bound cyanide ion. Similar conclusions were reached from studies in the molybdothiol systems,¹⁰ but the present work demonstrates for the first time that the reduction of a substrate-molybdenum complex by reduced ferredoxin model compounds is ATP-stimulated.

Mechanism of Cyanide Reduction. The actual mechanism of the reduction of coordinated CN⁻ in 3 may be formulated in analogy to the mechanism proposed for the reduction of CN⁻ in the molybdothiol system.⁷ Neglecting, for sim-

but they are also reduced under the reaction conditions. The partial reduction of **3** evidently produces coordinatively unsaturated fragments which function as catalysts of the reduction of the added substrates as exemplified for C_2H_2 (eq 10). The observations thus lead us to introduce a "molybdo-



cyano" model of N_2 -ase which will be described in greater detail in forthcoming publications. Although the ligands in the new model system are unrelated to those present in the enzyme, it has the advantage over the molybdothiol systems inasmuch as the catalytically active species show less tendency to form inactive dimers. The demonstrated reduction of N_2 to ammonia in these systems is of particular interest, since we have found that this substrate can be reduced more efficiently than in the molybdothiol systems. From the NH_3 yields in Table V the turnover number for N_2 reduction is estimated to 2–6% of that of N_2 -ase (per mole of active molybdocyano catalyst).

Experimental Section

Reagents and Chemicals. All standard chemicals were of analytical or reagent grade purity and were used without further purification. The salts $K_4[Mo(O)_2(CN)_4] \cdot 2H_2O$, $K_3[Mo(O)(OH)(CN)_4]$, and $K_2[Mo(O)(H_2O)(CN)_4]$ were prepared according to the procedures given in ref 2 and 3b. The complexes were stored in a vacuum desiccator containing $Mg(ClO_4)_2$ and KOH as the drying agents. Stock solutions of the salts were prepared by dissolving weighed amounts in water or buffer immediately prior to the experiments. The complex $K_4[Mo(CN)_8] \cdot 2H_2O$ was synthesized as described in ref 11.

Typical Experimental Procedure. Aliquots (e.g., 0.4 ml) of a freshly prepared 0.037 *F* stock solution of $K_4[Mo(O)_2(CN)_4] \cdot 2H_2O$ in pH 7.5, 0.2 *F* borate buffer (pH of solution was readjusted to 7.5 with HCl) were injected into sealed reaction bottles which had been flushed for 20 min with 99.995% pure argon. The reaction flasks and silicone rubber seals are marketed by Pierce Chemical Co., Rockford, Ill. and have a capacity of 38 ml. A freshly prepared solution of ATP (0.54 *F*, usually 0.4 ml) was injected next. The pH of the ATP stock solution was as a rule first adjusted to pH 7.5 with 1 *F* $NaOH$. To suppress ATP hydrolysis during this operation it is preferable to add the (previously predetermined) amount of 1 *F* $NaOH$ to the solution of complex **1** prior to the addition of ATP. Immediately thereafter, enough 0.2 *F* pH 7.5 borate buffer was added to bring the total reaction volume to 3.6 ml. At $t = 0$, 0.4 ml of a freshly prepared 0.66 *F* solution of $NaBH_4$ in H_2O was injected. Since H_2 is evolved during the reaction, a syringe of 50 ml capacity with fully depressed piston was attached to a vent-needle to permit gas-volume expansion as the reaction proceeds. At various time-points the total gas volume was recorded by reading the position of the piston in the 50 "pressure equalizing" syringe. Since the movement of the piston is occasionally impeded, and to assure good mixing of the gases evolved, the piston of the syringe was repeatedly depressed and allowed to be pushed back to the equilibrium position. After the total gas volume was determined in this manner, 0.2- or 0.5-ml gas samples were withdrawn by means of a 1-ml syringe. The yields of hydrocarbons in the gas phase were determined by gas-liquid-phase chromatography (GLPC), using a Hewlett-Packard Series 700 laboratory gas chromatograph or a Varian Series 1200 Aerograph instrument, equipped with a 6 ft Durapak phenylisocyanate-porasil 80–100 mesh column. The reproducibility of the experiments was tested in separate experiments with standard hydrocarbon gas samples to be on the order of $\pm 1.5\%$ (average of ten injections) and was as a rule

checked by a second injection of a gas-sample at the experimental time-points. The yields of hydrocarbons in the gas-phase were calculated by considering variations in the total gas volume. The experiments were modified by changing the concentrations of reactants, variation of the reducing agent or by adding other components; details are given in the legends to the figures and tables, as well as in Table 1. The pH of all samples was measured upon the completion of the experiment and was found to vary by no more than ± 0.1 pH units from the average value of 8.80.

Inhibition and Substrate Reduction Experiments. The experiments in the presence of gaseous inhibitors were performed as described above, except that the reaction vessels were filled with the inhibiting gases at 1 atm instead of argon. Experiments with other inhibitors or reducible substrates were run by adding them immediately before the addition of $NaBH_4$.

Nitrogen Reduction. The reduction of molecular nitrogen in the present systems is strongly dependent on the concentration of the reactants, particularly of **3**. A typical experiment which reproducibly demonstrated N_2 reduction to NH_3 was conducted as follows.

Into a N_2 -filled reaction flask of the type described above, 0.4 ml of a 0.037 *F* solution of **3** at pH 7.5 was injected, followed by 2.7 ml of 0.2 *F* pH 7.5 borate buffer and 0.5 ml of a freshly prepared 0.5 *F* ATP solution (initial pH adjusted to 7.5 with $NaOH$). The solutions were freed from dissolved O_2 by passing a stream of N_2 (pyrogallol- and acid-washed, 99.995% purity) through the solutions. At $t = 0$, 0.4 ml of a freshly prepared $NaBH_4$ solution (0.66 *F* in H_2O) was injected. The gas pressure was kept at 1 atm by means of a 50-ml syringe as described above. A simultaneous control experiment, using the same solutions and reactants, but argon instead of gaseous N_2 , was also performed. At $t = 60$ min, gas samples were withdrawn for hydrocarbon analysis. The reaction solutions were analyzed immediately thereafter. First, CN^- and other interfering anions were removed by adding 2.5 g of anion exchange resin (Dowex 1-X). After filtration, the reaction solutions were acidified to pH 3.7 with acetic acid, and the ammonia was determined colorimetrically employing the method of Kruse and Mellon.¹² If the CN^- is not completely removed, the aqueous assay solutions turn dark purple. A part of the colored complex extracts into the CCl_4 layer. To remove this interference, the CCl_4 layer must then be repeatedly extracted with 1% pH 3.7 aqueous acetate buffer until the extracts become colorless. The amount of NH_3 observed in the experiment with argon was subtracted from the amount found under N_2 . As a cross-check, the amount of NH_3 produced by the reduction of CN^- was calculated from the observed hydrocarbon yields. The corrected NH_3 yields varied between 3 and 6 μmol . Definite proof for N_2 reduction was obtained from experiments with $^{30}N_2$ as described below.

Experiments with $^{30}N_2$. Reaction flasks of 38-ml capacity were filled with argon. Fresh, 0.037 *F* solutions of **3** in pH 7.5, 0.2 *F* borate buffer (0.4 ml) were injected, followed by 0.5 ml of ATP solution (0.5 *F* in H_2O , pH adjusted to 7.5 with $NaOH$). Borate buffer (0.2 *F*, pH 7.5), 2.7 ml, was added to bring the total reaction volume to 3.6 ml. Control experiments were run under argon, both in the presence and absence of added ATP. Nitrogen reduction experiments were performed by adding varying amounts of N_2 containing 49.4% of $^{30}N_2$ relative to $^{28}N_2$ by means of a syringe, e.g., 5, 10 or 15 ml, after previous withdrawal of the same amount of argon. At $t = 0$, 0.4 ml of a freshly prepared 0.66 *F* $NaBH_4$ solution in H_2O was injected. Pressure-equalizing syringes of 50 ml capacity were employed as described above. After 2 hr of reaction at 27°, 0.5-ml gas samples were withdrawn for hydrocarbon analysis by GLPC. For the determination of NH_3 , 1-ml aliquots of the reaction solutions were withdrawn and subjected to hypobromite oxidation in an evacuated Rittenberg flask as outlined in ref 13. The N_2 formed was collected by means of a Toepler pump and analyzed for $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$. The numerical results as measured with a LKB 9000 mass spectrograph from which the data listed in Table V were obtained are given in Table VII. All yields of $^{29}N_2$ were corrected for natural abundance of ^{15}N from the observed $^{28}N_2$ peaks, assuming the natural abundance of $^{29}N_2$ to be 0.74%. The latter value was confirmed by measurements of samples of air. The yields of NH_3 were determined from the observed corrected $^{29}N_2$ yields by comparison with the $^{30}N_2$ yields from the hypobromite oxidation of standard solutions of 53.6% $^{15}NH_4Cl$. The yields of N_2H_4 were calculated from the observed $^{30}N_2$ peaks (corrections for natural abundance are insignificant in this case).

Table VII. Results of the Mass Spectrographic Analyses of the N₂ Released upon Hypobromite Oxidation of Reaction Solutions of Experiments in Table V, Expressed in mm Peak Heights. (Analysis was performed with 1-ml aliquots of a total of 4 ml)

No.	Gas phase	ATP	²⁸ N ₂	²⁹ N ₂ corr	³⁰ N ₂
1	Ar	—	10000	0.01 ^a	0
2	Ar	+	11680	0.02 ^a	0
3	Ar, +609 μmol of N ₂	—	12000	44.4	Trace
4	Ar, +203 μmol of N ₂	+	13000	49.5	3.0
5	Ar, +406 μmol of N ₂	+	13650	53.0	3.5
6	Ar, +609 μmol of N ₂	+	13290	220.0	10 ^b
Standard			12780	119.0	2200 ^c

^a Background. ^b The yield of ³⁰N₂ was 9 mm when a second aliquot of this solution was subjected to hypobromite oxidation after the addition of 10 μmol of ¹⁴NH₄Cl. ^c This standard corresponds to the yield of ³⁰N₂ formed by the hypobromite oxidation of 14.4 μmol of ¹⁵NH₃Cl of 96% isotopic purity.

However, the presence of ³⁰N₂ in our experiments per se does not demonstrate the formation of N₂H₄ in view of the high concentration of ¹⁵NH₃. A control experiment was therefore performed in which an additional aliquot of ¹⁴NH₄Cl was added to the reaction solutions prior to hypobromite oxidation. The results in Table VII (see experiment no. 7) show that the addition of ¹⁴NH₄Cl did not lower the yield of ³⁰N₂ appreciably. This is consistent with the presence of N₂H₄ containing the original ¹⁵N-¹⁵N bond of ³⁰N₂.

Reduction of 3 with Ferredoxin Model Compounds. Solutions containing [Fe₄S₄(SR)₄]⁴⁻ (R = *n*-C₃H₇) or equivalent *n*-propylmercapturon sulfide species were prepared exactly as outlined in ref 10, by combining equivalent amounts of FeCl₂, Li₂S, and LiS-*n*-C₃H₇ in anhydrous CH₃OH. The *n*-C₃H₇ derivatives of the ferredoxin-like species were used in the experiments due to their tendency to decompose to C₃H₆ and C₃H₈ under the reaction condi-

tions. The C₃-hydrocarbons do not interfere with the assay for CH₄, C₂H₆, and C₂H₄. The concentrations of the systems components employed in several experiments are given in the legend to Table VI.

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Synthesis of Tricarbonyliron Complexes of Functional 1,2-Disubstituted Cyclobutadienes

George Berens, Fred Kaplan, Ronald Rimerman,¹
Bryan W. Roberts,* and Allan Wissner

Contribution from the Departments of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19174, and University of Southern California, Los Angeles, California, 90007. Received July 18, 1974

Abstract: The synthesis of a series of 1,2-disubstituted cyclobutadieneiron tricarbonyl complexes is described. The key intermediate, diester **4**, was prepared in 35-40% yield via reaction of sodium tetracarbonylferrate(-II) with tetrachlorodiester **3a**. The latter was obtained via benzophenone sensitized photochemical addition of dichloromaleic anhydride to *trans*-1,2-dichloroethylene, followed by hydrolysis and esterification. Variation of the vicinal functionality in **4** has led to tricarbonyliron complexes of 1,2-divinylcyclobutadienes, 1,2-di-*tert*-butylcyclobutadiene, "push-pull" cyclobutadienes, and a number of cyclobutadienes possessing electron-withdrawing groups. The assignment of **4** and complexes derived therefrom as authentic cyclobutadiene complexes is based on conversion of **4** to the known 1,2-dimethylcyclobutadieneiron tricarbonyl and on the consistency of a variety of data with this formulation.

The discovery in 1965 that oxidative degradation of cyclobutadieneiron tricarbonyl gave rise to free, transient cyclobutadiene^{2a,3} provided new direction to organic chemists' historical quest of this elusive species.⁴ Transition metal complexes have since proved to be one of the most convenient sources of cyclobutadienes for mechanistic and synthetic studies.²

A few years ago we became interested in utilizing cyclobutadiene transition metal complexes to generate substituted cyclobutadienes, including, in particular, systems in

which the cyclobutadiene ring is fused to other polyenic rings of various sizes. As a strategy for achieving these objectives, we envisaged synthesis of transition metal derivatives of appropriate functional 1,2-disubstituted cyclobutadienes, utilization of functionality for construction of a complexed fused-ring system, and release of the cyclobutadienoid system via decomplexation procedures. We now report full details⁵ of the realization of the first of these goals, namely the synthesis of tricarbonyliron complexes of a series of 1,2-disubstituted cyclobutadienes.