The Chemical Evolution of a Nitrogenase Model. 12. Stoichiometric Reduction of Acetylene and of Molecular Nitrogen by Mononuclear Cyano Complexes of Oxomolybdate(IV)

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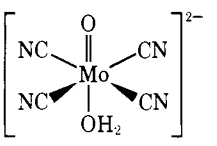
Abstract: The mononuclear complex anion $[Mo(O)(H_2O)(CN)_4]^{2-}$ decomposes in mildly acidic aqueous media to yield reactive cyanocomplexes of oxomolybdate(IV) which act as stoichiometric reductants of typical nitrogenase substrates such as acetylene and molecular nitrogen. The kinetic evidence is consistent with a mechanism in which one molecule of acetylene reacts with a mononuclear Mo(IV)-species to yield an intermediate complex which hydrolyzes to form ethylene. The stereochemical course of this reaction is interpreted in terms of a symmetrical side-on interaction of the substrate with the Mo(IV) ion, which is shown to act as a two-electron reducing agent. Molecular nitrogen is reduced to ammonia via diimide and hydrazie as the intermediate. The formation of diimide was demonstrated by trapping experiments. The effects of various inhibitors on the reduction of both acetylene and nitrogen are also described.

In parts 10 and 11 of this series we have shown[1,2] that the complex mononuclear anion of oxomolybdate(IV), **1**, may serve as a model for a catalyst-substrate complex in the reduction of cyanide by nitrogenase (N_2-ase) or the molybdothiol model systems of N_2-ase.[3,4] In the latter, the active reduced form of the catalyst was previously assumed to contain Mo(IV), but since this question was not firmly resolved we usually referred to the reduced form of the catalyst(s) as "Mo^{red}" and to the oxidized derivatives as "Mo^{ox}". Other authors[5] recently suggested that the oxidation state of molybdenum in "Mo^{red}" could actually be +III. In response to this suggestion we herein report the results of studies on the reduction of acetylene and of molecular nitrogen with mononuclear cyanocomplexes of oxomolybdate(IV) under *stoichiometric* conditions.



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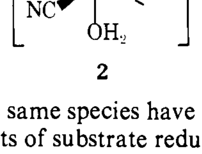
The anion **1** is known[6] to decompose in acidic media with the gradual release of HCN. It is reasonable to assume that the protolysis proceeds in a stepwise manner, giving rise to the formation of intermediate species such as **2–4**. Of these, **3** and **4** would resemble "Mo^{red}" in the molybdothiol systems:



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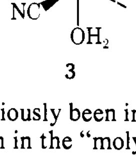

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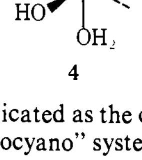

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The same species have previously been implicated as the catalysts of substrate reduction in the "molybdocyano" systems; for simplicity, we shall hereinafter designate them $[Mo^{4+}]$ wherever convenient. In the following we describe the results of experiments which demonstrate that these mononuclear complexes of oxomolybdate(IV) reduce acetylenes, nitrogen, and hydrazine in the absence of added external reductants.

Results

1. Reduction of Acetylenes. General Features. Aqueous solutions of **1** in the pH range of between 8.0 and 10 retain their characteristic blue color on exposure to 1 atm of C_2H_2 even on prolonged contact, and no reduction is observed at room temperature. The solutions turn green on acidification, which initiates a slow reduction of C_2H_2 which becomes conveniently measurable at 65–75 °C. At C_2H_4 pressures <0.3 atm, C_2H_4, C_2H_6 and H_2 are the main products for which a pH-yield profile is shown in Figure 1. At higher C_2H_2 concentrations, C_4-hydrocarbons (butadiene and presumably butene-1) are formed in addition to the C_2-hydrocarbons, but the formation of these products will not be discussed here. Figure 1 shows that H_2 evolution occurs at higher than the optimal pH for C_2H_4 production, suggesting that H_2 evolution and C_2H_2 reduction involve different reactive Mo(IV) species. On the other hand, maximum yields of C_2H_6 are found at somewhat lower pH, which indicates that C_2H_2 is reduced to C_2H_6 in a reaction also independent of that leading to C_2H_4. At the same initial pH, the C_2H_2 reduction is essentially unaffected by buffer type. There is also no stimulation by ATP or other nucleoside phosphates relative to other nonoxidizing acids at similar pH and ionic strength. In the presence of phosphates, the reaction solutions remain homogeneous due to the formation of soluble phosphate complexes of oxomolybdate(V). Reaction solutions that were acidified, e.g., with HCl, H_2SO_4, or CH_3COOH gradually become heterogeneous due to the precipitation of brown, insoluble Mo(V)-hydroxide,[7] but this has no noticable effect on the rate and yield of C_2H_2 reduction.

Kinetic Measurements. Because of the complexity of the reaction chemistry of **1** in acidic solution,[6] the reduction of C_2H_2 does not lend itself to a detailed kinetic study. Nevertheless, essential experimental information has been obtained and is summarized as follows: (1) The dependence of the yields of C_2H_4 from C_2H_2 on $[H^+]_{initial}$ is linear between pH 4 and 7 (see Figure 2A). (2) The yields of C_2H_4 increase linearly with the initial concentration of **1** (Figure 2B). (3) The yields of C_2H_4 at constant initial concentrations of **1** increase linearly with the concentration of C_2H_2 up to a partial pressure of about 0.1 atm (Figure 2C). At higher partial pressures the yield increments level off due to increased C_4-hydrocarbon production. (4) The reduction of C_2H_2 at temperatures below 65 °C is slow and proceeds with low overall yields (Figure 3). At 75 °C, the production of C_2H_4 at limiting concentrations of **1** follows a pseudo-first-order rate law during the first 20 h of reaction (Figure 4). (5) The apparent Arrhenius energy of activation as measured from the yields of C_2H_4 is 17.6 ± 0.5 kcal/mol in the temperature range between 8 and 80 °C. (6) In D_2O,

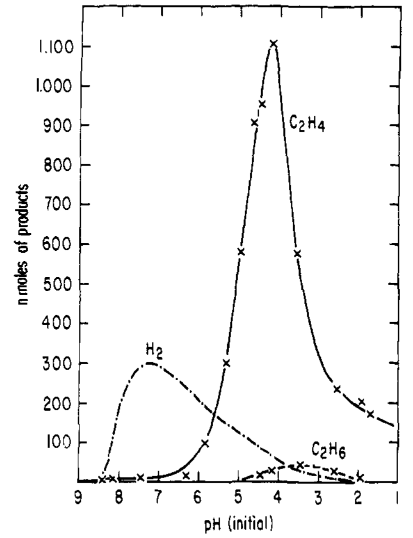


Figure 1. Stoichiometric reduction of C_2H_2 by **1** as a function of pH at 75 °C. Reaction solutions contained, in a total volume of 4.0 ml: 42.0 μmol of **1**, and 1 ml of 1% acetate buffer adjusted to different pH with either NaOH or HCl. The initial partial pressure of C_2H_2 was 0.3 atm, yields of hydrocarbons were measured after 1 h of reaction, the solution pH was determined after termination of the experiments.

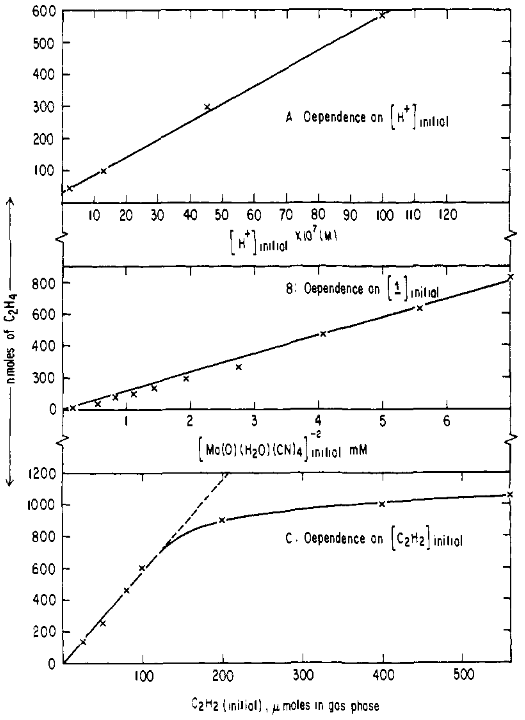


Figure 2. Dependence of the yields of C_2H_4 from the reduction of C_2H_2 by **1** as a function of $[H^+]$ (A), the initial concentration of **1** (B), and the partial pressure of C_2H_2 (C).

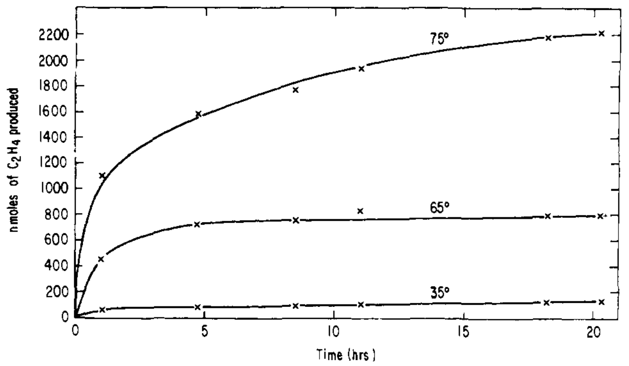


Figure 3. Yield-time curves for the production of C_2H_4 from **1** and C_2H_2 at different temperatures. Reaction solutions contained 42 μmol of **1** and 1 ml of 1% pH 3.7 acetate buffer. The initial pH of the reaction solutions was 4.2, the total solution volume, 4.0 ml. The initial pressure of C_2H_2 was 0.3 atm at 27 °C.

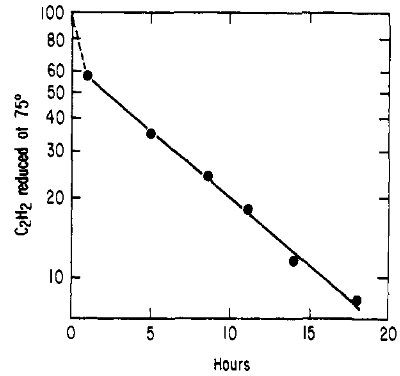


Figure 4. First-order time plot for the reduction of C_2H_2 to C_2H_4 by **1** at 75 °C. Experimental conditions as outlined in legend to Figure 3.

C_2H_4 is produced at about one-fourth of the rate observed in H_2O. According to ir analysis, the product is predominantly *cis*-1,2-dideuterioethylene. (7) The square roots of the yields of C_2H_6 depend linearly on the initial concentration of **1** (Figure 5). Ethylene is not reduced under the reaction conditions.

Stoichiometry. The summation of the yields of all hydrocarbon products formed from C_2H_2 at saturating pressures in addition to H_2 revealed that only about 50% of the available electrons in **1** are used for substrate reduction and/or H_2 evolution. Accumulation of Mo(V) hydroxide in the solutions as the reaction proceeds indicates that the Mo(VI) produced undergoes a secondary reaction with the remaining Mo(IV). Accordingly, the reduction of C_2H_2 to C_2H_4 is expressed in terms of eq 1-3 as the major pathway:

$$[Mo^{4+}] + C_2H_2 + 2H^+ \longrightarrow [Mo^{6+}] + C_2H_4 \quad (1)$$

$$[Mo^{4+}] + [Mo^{6+}] \longrightarrow 2[Mo^{5+}] \quad (2)$$

$$2[Mo^{4+}] + C_2H_2 + 2H^+ \longrightarrow 2[Mo^{5+}] + C_2H_4 \quad (3)$$

Evidence for reaction eq 2 as a Mo(IV)-consuming side-reaction was obtained by attempting the reduction of C_2H_2 with solutions of **1** containing equivalent amounts of MoO_4^{2-} Upon acidification, Mo(V) hydroxide precipitated rapidly and no reduction of C_2H_2 was observed. On the other hand, the consumption of Mo(IV) by Mo(VI) canbe effectively suppressed by increasing the concentration of reducible substrate. With a large excess of propargyl alcohol in 50% aqueous solution, a conversion to allyl alcohol was observed. The reaction solutions remained homogeneous and became colorless due to the oxidation of Mo(IV) to Mo(VI).

Reduction of 2-Butyne. The observed formation of *cis*-1,2-dideuterioethylene from C_2H_2 upon reduction by **1** in D_2O/CH_3COOD indicates that the stereochemical course of the reduction is predominantly cis. This was confirmed by a study of the reduction of 2-butyne. In pH 3.7 acetate buffered 20% aqueous methanol (to promote solubility), 2-butyne is reduced at about 1% of the rate of C_2H_2, affording only *cis*-2-butene and traces of *n*-butane; *trans*-2-butene was not detectable.

Effects of Inhibitors. The reduction of C_2H_2 to C_2H_4 is inhibited by O_2, N_3^-, or ClO_4^-, by chelating agents, e.g., α,α-bipyridyl, as well as by CO, CN^-, and to some extent by H_2. The degree of inhibition depends upon the pH, temperature,

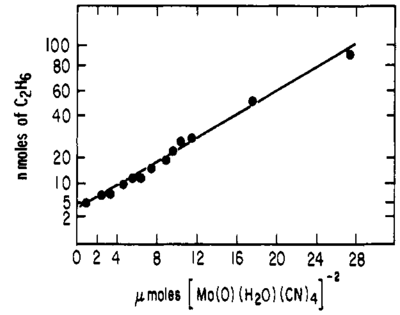


Figure 5. Yields of $[C_2H_6]^{1/2}$ plotted as a function of the initial concentration of **1** after 24 h of reaction at 75 °C, at pH 4.2 and $p_{C_2H_2} = 0.3$ atm.

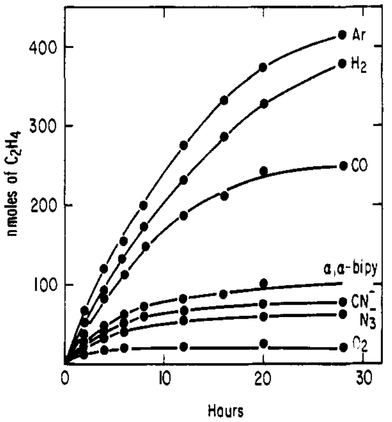


Figure 6. Inhibition of C_2H_2 reduction by H_2, CO, O_2, CN^-, N_3^-, and α,α-bipyridyl. The inhibiting gases were present at 1 atm, C_2H_2 at 0.3 atm, respectively. The concentrations of CN^- and of N_3^- were 0.025 N, all reaction solutions contained 42 μmol of **1** in a total volume of 4 ml of acetate buffer, the initial pH of each solution was 4.3, and the reaction temperature was 65 °C.

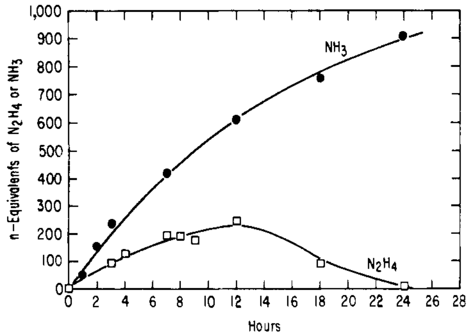


Figure 7. Time-yield plot of the reduction of N_2 by **1** at pH 4.3, 65 °C, and 1 atm of N_2. The reaction solutions contained 42 μmol of **1** at $t = 0$ in 4 ml of 0.25% acetate buffer. Ammonia and hydrazine were determined in separate experiments.

Table I. Yields of NH_3 in Nitrogen Fixation Experiments under Various Conditions

No.	$[1]_{init}$, mM	pH_{init}	Conditions	NH_3, μequiv
1	10.5	7.0	48 h at 65 °C, 0.5 atm $^{30}N_2$	0.30
2		5.1		1.73
3		4.3		2.05
4		4.0		1.84
5		2.0		0.68
6		4.3	at 75 °C	2.89
7			24 h at 65 °C, 1.0 atm N_2[a]	0.91
8			As in no. 7, +succinate[b]	0.84
9			As in no. 7, +fumarate[b]	0.36
10			As in no. 7, +CO (0.3 atm)	0.88
11			As in no. 7 +C_2H_2 (0.3 atm)	0.52
12			As in no. 7, +O_2 (0.3 atm)	0.16
13			As in no. 7, +N_2O (0.3 atm)	0
14			As in no. 7, +H_2 (0.3 atm)	0

[a] Natural abundance N_2. Yields of NH_3 determined colorimetrically; all are corrected for background. [b] 100 μmol, added in buffered aqueous solution.

substrate concentration, and the substrate-inhibitor ratio. Typical results are shown in Figure 6. Hydrogen is inhibitory only at low concentrations of substrate. At saturating pressures of C_2H_2 and low concentrations of H_2, C_2H_4 production is stimulated by up to 50% (see Figure 6). In the presence of H_2 nanomolar amounts of CH_4 are formed, both in the presence and absence of C_2H_2. the CH_4 formation is evidently due to the reduction of coordinated CN^- by H_2. The effect of O_2 is due to oxidation of Mo(IV) to Mo(VI). Similarly, N_3^- and also ClO_4^- act as oxidants. In the presence of these inhibitors, the reaction solutions become completely colorless and remain homogeneous; N_3^- is reduced to NH_3 and N_2, ClO_4^- to Cl^-, in analogy to previous work.[8] The inhibition of C_2H_2 reduction by α,α-bipyridyl, CO, and CN^- may be due to a competition of these ligands with the substrate for the Mo(IV) coordination sites. Finally, inhibition of C_2H_2 reduction by N_2 was observed at high N_2-C_2H_2 ratios (N_2, 0.97 atm; C_2H_2, 0.03 atm, percent inhibition 22%). This suggested that molecular nitrogen is reduced under the reaction conditions.

2. Reduction of Molecular Nitrogen. Using $^{30}N_2$-enriched N_2 as the substrate, the formation of NH_3 and N_2H_4 was demonstrated and was found to proceed in weakly acidic solutions at temperatures between 60 and 80 °C. With normal N_2, the reduction to NH_3 and N_2H_4 was confirmed colorimetrically. A typical time-yield plot is shown in Figure 7. As with C_2H_2 as the substrate, the reaction solutions become heterogeneous as a function of time due to the formation of Mo(V) hydroxide. Disregarding the formation of traces of H_2, it is calculated that 0.07 mol of N_2 is reduced to NH_3 per mole of Mo(IV), corresponding to an electron transfer-efficiency of 21%. The results of other nitrogen fixation experiments are summarized in Table I.

Kinetic Measurements. For reasons outlined above, a full kinetic study of the reduction of N_2 in acidic solutions of **1** was not feasible. However, the salient features of the reaction are as follows: (1) The yields of NH_3 produced from N_2 reach a maximum at about the pH optimal for C_2H_4 production from C_2H_2 (see Table I). (2) A linear dependence of the yields of NH_3 on the concentration of **1** is shown in Figure 8. (3) The reduction of N_2 proceeds optimally at temperatures between 60 and 80 °C little or no reaction is observed at room temperature. At 75 °C, the formation of NH_3 from N_2 at limiting concentrations of **1** follows a pseudo-first-order rate law. The observed rate of NH_3 production is 10-20% slower than the rate of C_2H_4 formation from C_2H_2 under similar conditions (Figure 9). (4) Hydrazine accumulates at intermediate reaction times (see Figure 7). Independent experiments show that N_2H_4 is reduced by **1** under the conditions of N_2 reduction. Its reduction to NH_3 follows a pseudo-first-order rate law (Figure 10). In the presence of excess **1**, the reduction of N_2H_4 is quantitative and is ca. 30 times faster than the reduction of N_2 to NH_3.

Detection of Diimide. Our previous work indicated that diimide, N_2H_2, either free or complexed, is an intermediate in the reduction of N_2 in the molybdothiol model systems of N_2-ase,[9] as well as in the molybdocyano systems.[2] Employing fumarate as a diimide trapping agent, evidence for the inter-

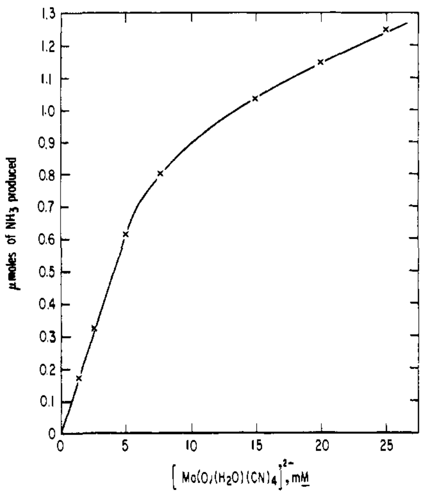


Figure 8. Dependence of the yields of NH_3 from N_2 at 1 atm on the initial concentration of **1**, at pH 4.3, after 24 h of reaction at 65 °C. Total solution volume was 4.0 ml, 0.25% acetate buffer.

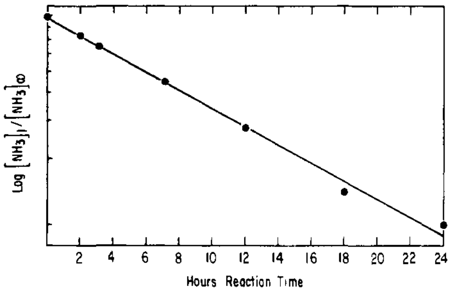


Figure 9. Pseudo-first-order rate plot of reduction of N_2 to NH_3. Data from Figure 7.

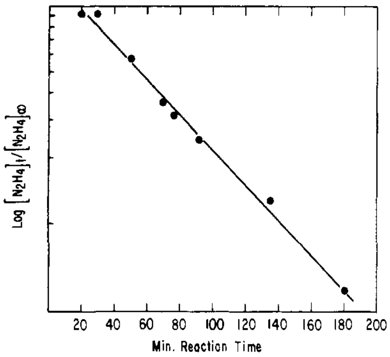


Figure 10. Pseudo-first-order rate plot of the reduction of N_2H_4 to NH_3 by **1** in pH 4.3 acetate buffered solution. The solutions contained 42 μmol of **1**, and 3.1 μmol of N_2H_4 at $t = 0$, in 4.0 ml of 0.25% acetate buffer. Reaction was followed by monitoring the disappearance of N_2H_4 at 26 °C.

mediate formation of N_2H_2 under stoichiometric conditions of N_2 reduction was obtained. We have found that neither fumarate nor succinate inhibit C_2H_2 reduction to C_2H_4. However, Table I shows that N_2 reduction is clearly diminished in the presence of fumarate. Experiments with ^{14}C-labeled fumarate revealed that succinate is formed in the presence of N_2 and in amounts corresponding to the expected concentrations of N_2H_2 generated under the reaction conditions (see Experimental Section). No succinate was detected in control experiments under argon instead of N_2 or in the presence of C_2H_2.

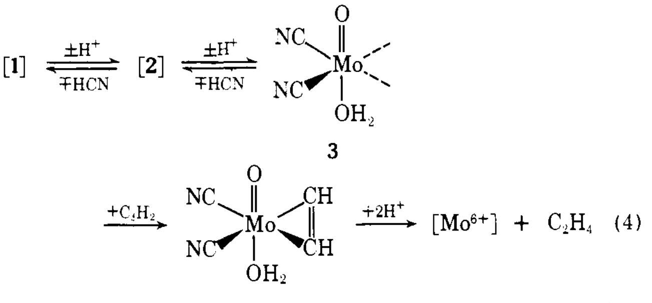
Effects of Inhibitors. The reduction of N_2 is inhibited by C_2H_2, O_2, N_2O, and H_2. Under the conditions employed thus far, CO showed only a slight inhibitory effect (Table I). As is the case for C_2H_2 reduction, the degree of inhibition of N_2 fixation depends upon a number of variables. Hence, in-depth investigations of these effects would be necessary. However, we can at least conclude that the reduction of N_2 is apparently inhibited by H_2, under conditions where C_2H_2 reduction is inhibited only slightly.

H_2-D^+ Exchange Experiments. Solutions of **1** in D_2O, under the conditions of substrate reduction at 65 °C, promote H_2-D^+ exchange. The observed HD-D_2 ratios were 2.0 under argon, 0.5 in the presence of substrate amounts of C_2H_2, and 2.09 after 1 h at 1 atm of N_2. Whereas functional N_2-ase catalyzes D_2-H^+ exchange only in the presence of N_2,[10] a similar effect has not been observed under the conditions of stoichiometric N_2-reduction employed.

Discussion

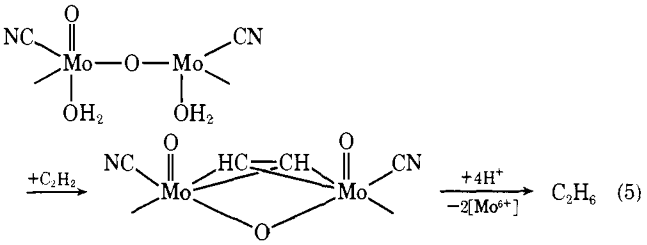
The present work demonstrates that the complex anion **1** yields reactive cyanocomplexes of oxomolybdate (IV) on protolysis, which are capable of reducing typical substrates of N_2-ase. Recent studies,[1,2] in which **1** was used for the catalytic reduction of substrates, suggested that species such as **3** and **4** are the reactive forms of the catalyst. The same species are undoubtedly also generated during the acid degradation of **1**, which is known to proceed with the gradual release of HCN.[6] Since N_2-ase substrates are reduced more rapidly under catalytic conditions and with lower apparent Arrhenius energies of activation, our present results indicate that the rates of the reduction of the substrates in the stoichiometric reactions are determined primarily by the slow rate with which equilibrium concentrations of the reactive species **3** or **4** are generated under the conditions employed.

Reduction of Acetylene. The reduction of C_2H_2 to C_2H_4 in weakly acidic solutions of **1** occurs through the interaction of one molecule of C_2H_2 with a mononuclear cyanocomplex of oxomolybdate(IV). The observed stereochemical course of the reaction of C_2H_2 in D_2O and the exclusive formation of *cis*-2-butene from 2-butyne are consistent with a mechanism via a symmetrically side-on bonded organomolybdenum intermediate, whose hydrolysis gives rise to C_2H_4. The linear dependence of C_2H_4 production on $[H^+]$ in the pH range between 4 and 7 suggests that the removal of one CN^- ion from a Mo(IV) species is the rate-determining process. Assuming **3** to be the reactive complex, the overall process of C_2H_2 reduction to C_2H_4 may be formulated according to eq 4. While



a similar reaction of **4** with C_2H_2 could also be formulated, **2** should have little or no reactivity as it has only one coordination site available for substrate binding. The Mo(VI) reaction product, which is probably molybdic acid, undergoes secondary reaction with Mo(IV) species to yield Mo(V) according to eq 2, as evidenced by the formation of insoluble Mo(V) hydroxide. The overall electron transfer efficiency of reduction f C_2H_2 is for this reason only about 50%, and since the Mo(IV) consuming reaction **2** occurs rapidly, no reduction of C_2H_2 takes place if Mo(VI) is added to the reaction solutions. Reaction eq 2 can be effectively suppressed by increasing the concentration of reducible substrate. Propargyl alcohol, for example, oxidizes Mo(IV) to Mo(VI) almost quantitatively. It is therefore concluded that Mo(IV) complexes act as two-electron reductants in reactions with acetylenic substrates, in accord with the previous mechanistic postulates.[11]

The formation of C_2H_6 from C_2H_2 is due to an independent side-reaction and presumably involves binuclear, oxobridged cyanocomplexes of Mo(IV) as the reactive species, which are generated in equilibrium amounts during the protolysis of **1**. Since C_2H_4 is not reduced, the reduction of C_2H_2 to C_2H_6 is postulated to occur according to eq 5. A similar mechanism of C_2H_6 production in the molybdothiol system has been proposed.[11]

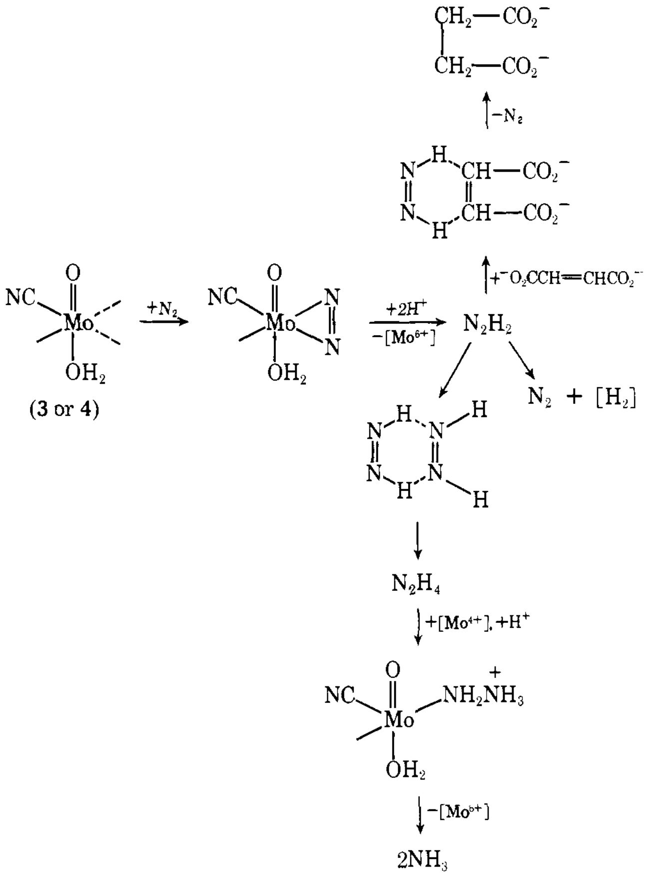


(5)

The reduction of C_2H_2 is inhibited by CO, CN^-, or α,α-bipyridyl obviously because these agents compete with C_2H_2 for Mo(IV) coordination sites. The effects of oxidants are due to the oxidation of Mo(IV) to Mo(VI) and were observed with O_2, ClO_4^-, and N_3^-. Azide, a substrate of N_2-ase, is reduced to NH_3 and N_2. The observed effects of H_2 are of interest as it inhibits or stimulates C_2H_4 production from C_2H_2. The reactive molybdenum species are evidently capable of interaction with molecular hydrogen, a feature which will be discussed below.

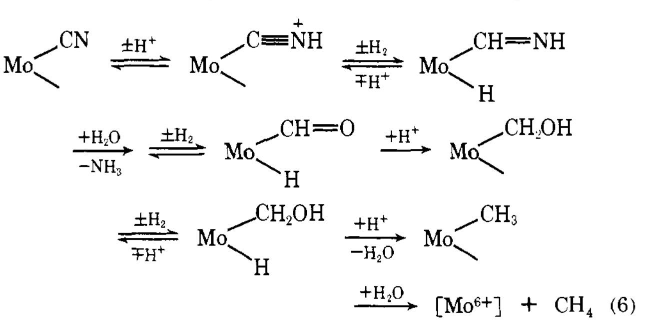
Reduction of Nitrogen. The demonstrated reduction of N_2 to NH_3 and N_2H_4 by **1** in weakly acidic solutions shows that cyanocomplexes of oxomolybdate(IV) possess affinity for N_2 and a sufficiently high reduction potential to accomplish this reaction even in the absence of external reductants. Since the Mo(IV) species are mononuclear and act as two-electron reducing agents, diimide, N_2H_2, must be the initial product of N_2 reduction. Its formation was clearly demonstrated by trapping experiments, and since it has been possible to reduce fumarate to succinate under the conditions of N_2 reduction, we conclude that N_2H_2 has the cis configuration and is generated in the free state. Its subsequent reduction to NH_3 proceeds via N_2H_4, the disproportionation product of N_2H_2, as outlined in Scheme I. A fraction of the N_2H_2 undoubtedly also decomposes into N_2 and H_2, or is lost through secondary oxidation processes. The extent to which these N_2H_2-consuming side-reactions occur is difficult to ascertain quantitatively. They are expected to be strongly pH and concentration dependent. Although gaseous N_2H_2 is stable in the gas phase (in the presence of NH_3) for several minutes,[12] its lifetime in aqueous solution is not known but must be definite in view of the ease with which it can be used for the reduction of unsaturated organic compounds. However, we also suspect that certain molybdenum compounds or complexes are either reduced by N_2H_2 or catalyze its decomposition into the elements in our systems. This also applies to N_2H_4, whose oxidation to N_2 is catalyzed by molybdate. Huang and Spence[13] have shown that N_2H_2 is an intermediate in this reaction. In the molybdothiol systems in the presence of ATP, N_2H_2 decomposition (or oxidation) occurs as soon as excess reducing agent (BH_4^-) is consumed. In the presence of CN^- or CO, the N_2H_2-destroying side reactions are either retarded or inhibited, which explains why the reduction of N_2 proceeds more efficiently in the molybdocyano systems,[1,2] and possibly accounts for the fact that CO inhibits N_2 reduction only to a minor extent. Assuming that the reduction of N_2 to NH_3 occurs as a six-electron process, the maximum electron transfer efficiency in the stoichiometric reduction by **1** is calculated to 21%; this low efficiency is a also suggestive of N_2H_2 losses during N_2 reduction. In Scheme I, the reduction of N_2 to NH_3 and the reactions of N_2H_2 are summarized. We assume that N_2 binds to Mo(IV) species in the side-on-fashion in view of the analogous behavior of acetylenic substrates.

Scheme I. Reduction of Molecular Nitrogen by Cyanocomplexes of Oxomolybdate (IV) in Protic Media



(3 or 4)

Reactions Involving Hydrogen. The observed evolution of H_2 during the reduction of C_2H_2 (see Figure 1) demonstrates that cyanocomplexes of oxomolybdate(IV) reduce protons of the medium. As in the molybdothiol systems, this suggests the existence of intermediate hydridomolybdenum species. We note in passing that H_2 is evolved from solutions of **1** also in the absence of C_2H_2, more so in neutral or alkaline than in acidic solutions. The observed reactions of cyanomolybdates with H_2 are of interest, particularly since they are accompanied by a reduction of coordinated CN^- to CH_4 and NH_3. Some of the reactions which may occur are summarized in eq 6. The formation of hydridomolybdenum species in eq 6 was assumed to be reversible to accommodate the fact that **1** catalyzes H_2-D^+ exchange reactions under the conditions of substrate reduction. The higher HD/D_2 ratios observed in the presence of N_2 as compared to those under C_2H_2 could well be due to the intermediate formation and subsequent partial decomposition of N_2HD. The inhibition of N_2 reduction by H_2, finally,



(6)

suggests that H_2 competes with N_2 for molybdenum coordination sites.

Comparisons with Nitrogenase Reactions. The present work continues to support our proposal that all substrate reactions of N_2-ase are characteristic of a molybdenum active site. It is now well established that a protein-bound mononuclear oxomolybdate(IV) residue would indeed exhibit the required reactivity for the reduction of N_2-ase substrates. The observed inhibition of N_2 reduction by H_2 in the stoichiometric system employed is also of interest in view of similar effects of H_2 in the enzymatic N_2 reduction. Since the reduction of all N_2-ase substrates has been accomplished in model systems containing molybdenum as the only metal component and non-heme iron model compounds have been shown to catalyze the transfer of electrons from external reductants to molybdenum, the roles of both elements in biological N_2 fixation are now clearly delineated; cooperative bimetallic substrate binding as a prerequisite of reduction need no longer be invoked.[14,15]

Experimental Section

Reagents and Chemicals. The salt $K_2[Mo(O)(H_2O)(CN)_4]$ was prepared by the reaction of Mo(V) hydroxide with KCN as outlined in ref 16. The complex was stored in a vacuum desiccator containing $Mg(ClO_4)_2$ and KOH as the drying agents. Stock solutions of the complex were prepared by dissolving weighed amounts in water. The solutions were usually 0.14 M in complex and were diluted to 0.014 M immediately prior to the experiments. The 0.14 M stock solution can be stored in the frozen state for several weeks. All other chemicals required for the present work were of analytical or reagent grade purity and were used without further purification.

Typical Experimental Procedures for C_2H_2 Reduction. Aliquots (usually 3.0 ml) of a freshly prepared 0.014 M stock solution of $K_2[Mo(O)(H_2O)(CN)_4]$ were injected into sealed reaction bottles which had been flushed for 20 min with pure argon. The reaction flasks and silicone rubber seals are marketed by Pierce Chemical Co., Rockford, Ill., and have a capacity of 38 ml. Subsequently, measured volumes of C_2H_2 at 1 atm of pressure were withdrawn from a storage flask by means of a syringe and injected into the reaction flasks. If larger volumes of C_2H_2 than 10 ml are to be added, it is preferable to withdraw a similar amount of argon prior to the injection of C_2H_2, to prevent pressure buildup in the flask, particularly since the experiments are as a rule run at temperatures between 60 and 80 °C. To initiate the reaction, 1 ml of 1% pH 3.7 acetate buffer is injected, and the flasks are placed into a thermostatically controlled water bath. Addition of the buffer changes the pH of the solution to ca. 4.2; pH changes also occur during the reaction. It is necessary, therefore, to measure the pH of the reaction solutions before and after the experiment. At various time points, 0.2–0.5-ml gas samples were withdrawn. The yields of hydrocarbons (i.e., C_2H_4) and remaining C_2H_2 were determined by gas–liquid phase chromatography (GLC), using a Hewlitt-Packard Series 1200 Aerograph instrument, equipped with a 6 ft Durapak phenylisocyanate–porasil 80–100 mesh column. The reproducibility of such measurements has previously been determined to be in the order of ±1.5%.[1] The experiments were modified by changing the concentrations of reactants and variation of pH or temperature as outlined in the legends to the figures and tables. Hydrogen evolution during the reaction was monitored by GLC as well, using a column of 6 ft length, filled with molecular sieve (5 A), operating at 27 °C.

Experimental Procedures for N_2 Reduction. The N_2 reduction experiments were performed as outlined above for acetylene except that measured amounts of N_2 (isotopically labeled or unlabeled) were injected into the reaction flasks. A number of experiments were also conducted at 1 atm of pressure. In these, the reaction flasks were previously flushed with 99.998% pure N_2 for 20 min instead of argon.

Ammonia Assays. In nitrogen fixation experiments with isotopically labeled N_2 the reaction solutions were carefully vacuum degassed prior to hypobromite oxidation. The nitrogen released was collected by means of a Toepler pump and analyzed mass spectrographically for $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$. 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 with samples of air. The absolute yields of NH_3 produced from labeled N_2 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 $^{15}NH_4Cl$. The yields of N_2H_4 were calculated from the observed $^{30}N_2$ peaks, corrections for natural abundance are insignificant in this case.

For the colorimetric determination of NH_3 the procedure outlined in ref 17 was employed. Since CN^- interferes with this assay, the reaction solutions were first treated with 10 ml of saturated aqueous $HgCl_2$ solution, made alkaline with 5 ml of 50% NaOH and subsequently distilled in a Kjeldahl apparatus into 10 ml of 1% pH 3.7 acetate buffer. Since NH_3 is also formed from CN^- under the reaction conditions, simultaneous blank experiments under argon instead of N_2 were performed in each case. The yields of NH_3 were corrected for background and agreed with those obtained in experiments with isotopically labeled N_2 to within ±10%. Hydrazine was determined colorimetrically according to the method of Watt and Chrisp,[18] as previously modified.

Identification of *cis*-1,2-Dideuterioethylene and of *cis*-2-Butene. The reduction of C_2H_2 by **1** in D_2O/CH_3COOD at pD 4.2 was performed as outlined above. After 24 h of reaction at 65 °C, a gas-sample was transferred into an evacuated 10-cm ir gas cell. The presence of *cis*-1,2-dideuterioethylene was demonstrated by measuring the absorbance at 842.1 cm^{-1} and compared to an internal standard.

The products of the reduction of 2-butyne by **1** were identified by GLC. Structural assignments for *cis*-2-butene and *n*-butane were confirmed by cochromatography with authentic samples of the hydrocarbons.

Detection of Diimide. Six reaction flasks were flushed with the following gasses: two each with N_2, argon, and C_2H_2, all at 1 atm of pressure. To one of each duplicate set was added 3 ml of a 0.014 M solution of $K_2[Mo(O)(H_2O)(CN)_4]$, to all flasks, 1 ml of 1% pH 3.7 aqueous acetate buffer, and 1 ml of 0.1 M ^{14}C-labeled fumaric acid solution. Each flask contained 0.05 μCi ^{14}C activity. The reactions were run for 80 h at 65 °C. The reaction solutions were treated with 1 ml of 12 N HCl, evaporated to near dryness after removal of solids by centrifugation. The residues were taken up with 3 ml of CH_3OH, the resulting solutions were evaporated to about 0.5 ml volume. Aliquots of the solutions were spotted on Whatman No. 1 chromatography paper and chromatographed by the ascending method using ether, acetic acid, water (13:3:1) as the eluting solvent. The ^{14}C radioactivity profiles were obtained by counting the ^{14}C radioactivity with a strip counter. Radioactivity due to ^{14}C succinate was observed only in experiments using N_2 as the substrate, the identification of succinate was confirmed by comparison of the R_f value with that of authentic succinate chromatographed under identical conditions. Control experiments were also performed with unlabeled fumarate. After chromatography, succinate and fumarate were identified on the strips with Altman's reagent.[19] From the observed ^{14}C radioactivity, the amounts of succinate produced in typical experiments varied from 3 to 4 μmol.

Effects of Inhibitors. Gaseous inhibitors were added to the reaction flasks by means of a syringe prior to initiation of the reaction. The other inhibitors were injected in solution. Product yields were determined as described above. In the experiments with N_2 as the substrate, yields of NH_3 were corrected for background from simultaneous experiments under argon in the presence of the specified inhibitors. This was necessary, since certain inhibitors (e.g., O_2) enhance NH_3 production from CN^-.

H_2-D^+ Exchange Experiments. Reaction flasks were flushed with argon. After the addition of 3 ml of 0.014 M stock solution of $K_2[Mo(O)(H_2O)(CN)_4]$ in D_2O, 10 ml of N_2 and C_2H_2 (both at 1 atm), respectively, were injected. Subsequently, 1 ml of H_2 gas was injected, and the reactions were initiated by the addition of 1 ml of 1% pD 3.7 acetate buffer in D_2O, after the reaction solutions had reached 65 °C. After 60 min of reaction, gas samples were withdrawn by means of a gas-lock syringe for mass spectrographic determination of HD and D_2.

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Organometallic Reaction Mechanisms. 14. Role of Transition Metal Catalysts in the Formation of Aromatic Pinacols and Hydrols during Grignard Reagent Addition to Ketones[1]

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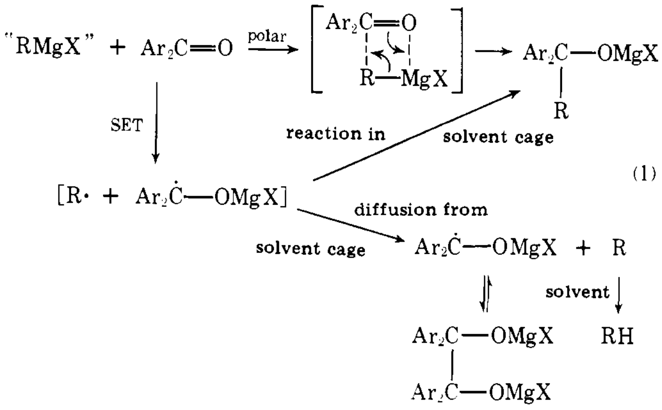
Abstract: The reaction of 2-methylbenzophenone with methylmagnesium bromide was studied under a variety of conditions. Methylmagnesium bromide prepared from magnesium samples containing significant amounts (>20 ppm) of iron and other first-row transition metals yielded substantial amounts of 2,2′-dimethylbenzopinacol at high Grignard to ketone ratios. 2,2′-Dimethylbenzopinacol was also produced in high yield when catalytic amounts of iron and other first-row transition metal salts were added to the Grignard–ketone reaction mixture in nearly equal molar amounts. Multiple regression and correlation analysis shows a direct relationship between the amount of transition metal salt added to the Grignard reagent and the amount of pinacol formed. In reactions with 2-methylbenzophenone, both the erythro and threo pinacols were formed. The threo pinacol (isolated in substantial yield early in the reaction at low temperature) was shown to be the kinetic product which quickly converts to the thermodynamic erythro pinacol (95:5) at room temperature. A mechanism describing the transition metal catalyzed formation of pinacols is presented which is consistent with the known facts about this reaction. The formation of 2-methylbenzhydrol at high Grignard to ketone ratios was found to be due to a minor amount, ca. 0.2%, of a very reactive magnesium hydride species formed during the reaction of methylbromide with magnesium metal in diethyl ether. The relationship between the grade of magnesium used to prepare the Grignard reagent and the amount of 2-methylbenzhydrol formed was found to be due solely to the size of the magnesium crystals and the rate at which methyl bromide was added to the magnesium.

Introduction

The importance of the Grignard reaction in synthetic organic chemistry is well recognized; however, the mechanism whereby Grignard reagents react with organic substrates (and particularly ketones) is not well understood. The exact nature of alkyl transfer from the Grignard reagent to the ketone, whether it proceeds by a polar or a single-electron transfer (SET) mechanism has been a source of considerable speculation. As a result of previous studies,[2] we have discussed in detail the polar mechanism whereby methylmagnesium bromide ("CH_3MgBr") reacts with 2-methylbenzophenone[3] (2-MBP) and benzonitrile.[4] However, while this work was being carried out, evidence was presented by several other research groups to indicate that the reaction of Grignard reagents with ketones could and does proceed in some cases by a SET pathway.

In 1968, Blomberg and Mosher presented evidence supporting SET pathways in Grignard reactions.[5] In the reaction of "neopentylmagnesium chloride" with benzophenone in THF, not only did they observe 1,2 addition, but they also found benzopinacol and neopentane both in 20% yield. Presumably the neopentane arose from hydrogen abstraction of the solvent by a neopentyl radical. In this study, Blomberg and Mosher also reported observing an ESR signal which they assigned to the ketyl. They suggested the mechanism below (eq 1) to explain their data.



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