Table II. Rate Constants for Solvolyses[a] of *endo*- and *exo*-Dinitrobenzoates **1a,b**

Temp, °C[c]	Rate constants,[b] $10^5 \times k$, sec^{-1} k_7[d]	k_5[e]	k_{rac}[endo]
	endo-DNB **1a**		
75.73	2.98 ± 0.03	2.52	0.03 ± 0.20
75.73	2.97 ± 0.03	2.58	
83.52	7.55 ± 0.06	6.45	
83.88	7.84 ± 0.17	6.77	
Temp, °C[c]	Rate constants,[b] $10^5 \times k$, sec^{-1} k_8[f]	k_6[g]	k_{rac}[exo]
	exo-DNB **1b**		
100.00	2.14 ± 0.04	2.06	1.65 ± 0.18
100.00	2.27 ± 0.05	2.15	
109.50	4.99 ± 0.07	4.80	
109.50	5.05 ± 0.04	4.88	

[a] Unbuffered 80% aqueous acetone. [b] All errors are standard deviations. [c] Temperature error is ±0.05°. [d] $k_7 = (k_1 + k_3 + k_5)$. [e] $\Delta H^{\ddagger} = 28.9 \pm 0.4$ kcal/mol, $\Delta S^{\ddagger} = 2.9 \pm 1.1$ eu. [f] $k_8 = (k_2 + k_4 + k_6)$. [g] $\Delta H^{\ddagger} = 24.1 \pm 0.7$ kcal/mol, $\Delta S^{\ddagger} = -15.8 \pm 1.8$ eu.

Table III. Deuterium Scrambling in Solvolysis[a] of *exo*-D-DNB **1f**

Product	% deuterium scrambling[b]
exo-D-DNB **1f** (unreacted)	87.4 ± 0.3
Δ^3-DNB **2a**	83.6 ± 0.5
endo-OH **1c**	94.9 ± 1.1
exo-OH **1d**	98.4 ± 3.3
Δ^3-OH **2b**	90.4 ± 0.6

[a] Lutidine-buffered 80% aqueous acetone at 110° after 93% reaction. [b] Determined by nmr; all errors are standard deviations.

DNB **1f** at 93% reaction can be estimated from Table II. The calculated 87% racemization agrees with the observed scrambling and therefore demands that the mechanisms of racemization and deuterium scrambling are the same, namely, a degenerate cyclopropylcarbinyl rearrangement.

In summary, the results clearly show that the cation(s) or ion pairs from *endo*-DNB **1a** do not scramble or racemize while those from the *exo* system both scramble and racemize. These results are reasonable because the isomer which gives racemization has the cyclopropyl group *trans* to the leaving group. Unfortunately our results do not distinguish between conformationally isomeric bisected cations, bicyclobutonium ions, or other possible sets of intermediate cations. Nevertheless, studies such as we have described should be performed on the other bicyclo[*n*.1.0]alk-2-yl systems (n = 2–7)[1a-f] before firm conclusions can be made. Only the *endo*-bicyclo[4.1.0]hept-2-yl *p*-nitrobenzoate has been studied polarimetrically and gave no racemization.[1c] In light of our results, the failure to study the *exo* system is belatedly a serious one.

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Chemical Evolution of a Nitrogenase Model. I. Reduction of Acetylene and Other Substrates by a Molybdenum–Thiol Catalyst System

Sir:

Two of the most remarkable features of the nitrogenase enzyme of *Clostridium pasteurianum*, *Azotobacter vinelandii*, and other microbial nitrogen-fixing systems are the nonspecificity with regard to substrates that can be reduced and the ease with which acetylene is reduced to ethylene but not to ethane.[1] For this reason Hardy introduced acetylene as a convenient substrate for assaying nitrogenase activity.[2] Acetylene is a competitive inhibitor of N_2 fixation and is believed to be bound to the same active site.

In this communication we wish to report results of experiments performed toward the goal of developing plausible, catalytic nitrogenase model systems. The nitrogenase of *C. pasteurianum* consists of an "electron-activating system," which is ATP dependent, and the actual nitrogenase.[1] The active site is believed to contain molybdenum, nonheme iron, and labile sulfhydryl groups. In the absence of substrates the holoenzyme catalyzes hydrogen evolution.[1]

To develop an initial catalytic, nonprotein model for nitrogenase, we have approximated the active site with thiol complexes of transition metals, and the electron donor pool by reducing agents such as $NaBH_4$, $Na_2S_2O_4$, $NaBH(OCH_3)_3$, Na_2SnO_2, sodium ascorbate, metallic Zn, or Al. Table I shows the relative activity of various transition metals as catalysts for the reduction of acetylene to ethylene in an aqueous, alkaline solution containing thiol and excess $Na_2S_2O_4$.[3] While not the most vigorous reducing agent of those described above, sodium hydrosulfite was employed for this study because of its demonstrated ability as a reductant for the enzyme system.[1]

Table I. Relative Activities of Transition Metals in the Reduction of Acetylene to Ethylene at 27° [a]

Metal	Rel activity[b]	Metal	Rel activity[b]	Metal	Rel activity[b]
Ti	0	Y	0	Hf	...[c]
V	0	Zr	0	Ta	0.1
Cr	0	Nb	0.1	W	0
Mn	0	Mo	100.0	Re	0.1
Fe	0	Tc	...[c]	Os	0.1
Co	0	Ru	2.9	Ir	15.5
Ni	0	Rh	0.4	Pt	0.1
Cu	0	Pd	1.3	Au	0
Zn	0	Ag	0	Hg	0

[a] Reaction conditions: reaction solutions containing 1 mmol of transition metal salt (mostly chloride), 1 mmol of 1-thioglycerol, 2 mmol of $Na_2S_2O_4$, and 10 mmol of NaOH in 10 ml of H_2O were placed into glass vials of 15-ml volume and were sealed with rubber serum caps. The air was then replaced by water-washed acetylene at 1 atm of pressure. Relative rates are reported for 16-hr reaction periods. [b] Relative rate of Mo = 100.0. [c] Not determined.

The remarkable specific activity of molybdenum is one of the salient features of our system. Of all the

(1) (a) R. W. F. Hardy and R. C. Burns, *Ann. Rev. Biochem.*, 37, 331 (1968), and references cited therein; (b) K. Kuchynka, *Catalysis Rev.*, 3, 111 (1969), and references cited therein.

(2) R. W. F. Hardy and E. Knight, Jr., *Biochim. Biophys. Acta*, 139, 69 (1967).

(3) When $NaBH_4$ is used as the reducing agent, hydrogen evolution is observed at neutral or mildly alkaline pH. Thus, our model system also catalyzes hydrogen evolution.

other transition metals surveyed, only iridium demonstrated any appreciable activity, converting acetylene to ethylene at 15% of the rate of the molybdenum system.

Molybdenum compounds producing catalytically active systems with thiols include Na_2MoO_4, MoO_3, $MoCl_5$, $MoCl_3O$, and polyheteromolybdates. A wide variety of thiols has been tested. Especially high activity is displayed by dithioerythritol, 1-thioglycerol, 2-mercaptoethanol, and cysteine. The catalytic activity of the molybdenum–thiol systems passes through a maximum at a 1:1 ratio of the two components. The catalytically active species are thought to be reduced molybdenum–thiol complexes, some of which have been isolated and are presently under study. An all-inorganic model system, consisting of MoO_3, NaSH, and $NaBH_4$, in aqueous solution, proved to be moderately effective in reducing acetylene to ethylene. Acetylene reduction was also achieved in dimethylformamide and tetrahydrofuran as solvents. In all cases the acetylene was reduced almost exclusively to ethylene. Only traces of ethane were formed. Ethylene itself was not a substrate.

A typical model system, as employed for acetylene reduction, consisted of a 15-ml glass vial containing 10 ml of distilled water, 1 mmol of Na_2MoO_4, and 1 mmol of 1-thioglycerol and fitted with a rubber serum cap. The solution was purged with water-washed acetylene for 5 min, and the reaction was initiated by the addition from a syringe of 1 ml of an aqueous solution containing 25 mg of $NaBH_4$. The course of the reaction was followed by gas–liquid chromatography, using an F & M Model 700 chromatograph equipped with dual Porapak R 6 ft × 0.25 in. chromatography columns and with dual WX thermal conductivity detectors. The chromatography columns were operated at ambient temperature. Typically, red-brown, homogeneous solutions were produced, and the reduction by borohydride was complete within 15 min at 27° with about 75% of the initial acetylene converted to ethylene. With $Na_2S_2O_4$ as the reducing agent, the rate of reduction is slower by a factor of 5–10 than with $NaBH_4$.

In addition to acetylene, the model systems will reduce other substrates of nitrogenase. With $NaBH_4$ as the reducing agent, methylacetylene is reduced to propylene, and 2-butyne is slowly reduced to butenes. With $Na_2S_2O_4$ as the reducing agent, under the conditions described in Table I, methylacetylene is reduced to propylene, but 2-butyne is not acted upon. Methylacetylene is a substrate for nitrogenase, while 2-butyne is not.

Alkyl isocyanides are reduced by the model systems to products which are similar to those of the enzymatic reduction. Thus, cyclohexyl isocyanide was slowly converted by the 1-thioglycerol, Na_2MoO_4, $NaBH_4$ model system to methane, ethylene, ethane, and a C_3 hydrocarbon.

Since the nitrogenase enzyme also reduces N_3^-, sodium azide was employed as a substrate for the model system. A mixture composed of MoO_3, *o*-mercaptobenzoic acid, and $NaBH_4$ reduced sodium azide to nitrogen and ammonia, provided that the pH was kept close to neutrality. Ammonia was determined by the Kjeldahl method, and a 50% conversion of azide to ammonia was achieved after 16 hr of reaction at 27°. A slow reduction of N_2O to nitrogen and H_2O was observed under similar conditions.

Initial experiments have been conducted with molecular nitrogen at elevated pressure and room temperature, and detectable yields of ammonia have been observed. We are presently optimizing the reaction conditions. Previously, Haight and Scott[4] and Yatsimirskii and Pavlova[5] reported the formation of trace amounts of ammonia in solutions containing molybdate and various reducing agents (*e.g.*, activated hydrogen or zinc amalgam).

We conclude, therefore, that the reactions observed for the nitrogenase enzyme are typical for reduced molybdenum complexes, particularly with thiol-type ligands, and that the essential chemical processes catalyzed by the nitrogen-fixing enzymes occur at a molybdenum-containing binding site.[6] However, other cofactors, particularly iron, may be important to facilitate electron transfer to, and/or the binding of, molecular nitrogen. In forthcoming papers the mechanism of acetylene and nitrogen reduction will be discussed. Intensive studies are also under way to increase the catalytic efficiency of our model system.

Since some of the essential properties of nitrogenases can be mimicked by comparatively simple model systems, all potential nitrogenase models should be assessed with respect to their ability to reduce acetylene and the other substrates known to be reduced enzymatically.

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(4) G. P. Haight, Jr., and R. Scott, *J. Amer. Chem. Soc.*, **86**, 743 (1964).

(5) K. B. Yatsimirskii and V. K. Pavlova, *Dokl. Akad. Nauk SSSR*, **165**, 130 (1965).

(6) This site may contain two μ-oxo-bridged molybdenum ions. Thus, the known[7] molybdenum(V) cysteine complex $Na_2[Mo_2O_4(SCH_2CH(NH_2)CO_2)_2]\cdot 5H_2O$ is a catalyst of acetylene reduction with $Na_2S_2O_4$ or $NaBH_4$.

(7) (a) A. Kay and P. C. H. Mitchell, *Nature*, **219**, 267 (1968); (b) J. R. Knox and C. K. Prout, *Chem. Commun.*, 1227 (1968).

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