Chemical Evolution of a Nitrogenase Model. III. The Reduction of Nitrogen to Ammonia

Sir:

In parts I and II of this series[1,2] we described catalytic systems based on complexes of molybdenum with thiols which are capable of reducing most of the substrates of nitrogenases of, *e.g.*, *Clostridium pasteurianum* or of *Azotobacter vinelandii*,[3] such as C_2H_2, isocyanides, N_2O, N_3^-, HCN, acetonitrile, and nitrogen. In the present communication we outline the optimal reaction conditions by which the reduction of nitrogen to ammonia was reproducibly achieved with a molybdate–thioglycerol system, using $NaBH_4$ as the reducing agent and iron as cocatalyst.

Sodium molybdate (5 mmol) and 2.5 mmol of 1-thioglycerol were dissolved in 50 ml of doubly distilled H_2O.[4] The solution was transferred into a 300-ml glass-lined rocking autoclave. Immediately before the start of the experiment, 28 mg (0.1 mmol) of $FeSO_4 \cdot 5H_2O$ was added to the reaction solution. A dry test tube containing one pellet of $NaBH_4$[5] (0.25 g) was placed into the autoclave in such a fashion as to prevent contact of the pellet with the reactor solution. The autoclave was sealed and deaerated by flushing five times with N_2,[6] and subsequently pressurized to 2000 psi. To initiate the reaction the $NaBH_4$ pellet was brought into contact with the reactor solution by rocking the autoclave. Rocking at room temperature was maintained for the duration of the experiment. After 5 days of reaction the nitrogen in the autoclave was slowly purged through 5 ml of a 0.1 *N* HCl solution. Subsequent analysis of this solution with Nessler's reagent indicates the presence of NH_3 corresponding to approximately 10% of the total amount produced. The ammonia in the reactor solution was assayed by a modified microdiffusion technique,[7] yielding between 3 and 5 μmol of NH_3. The concentration of NH_3 and of NH_3-producing contaminants in the reagents was shown to be $\simeq$0.3 μmol.

In numerous experiments it was established that the highest yields of NH_3 are obtained in the presence of molybdate, thiol, and catalytic amounts of iron (supplied as $FeSO_4 \cdot 5H_2O$). The optimal molar ratio of molybdate to thiol is 2:1 (Figure 1). No NH_3 was detected in systems containing $FeSO_4$, thioglycerol, and $NaBH_4$. Replacement of the iron cocatalyst with salts of copper, palladium, or nickel also abolished the nitrogen-fixing ability of the molybdate–thioglycerol system, indicating a specific cocatalyst effect of iron.

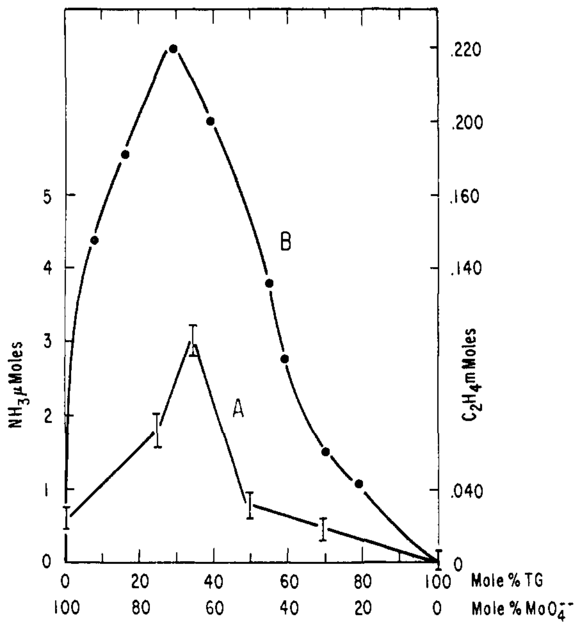

Figure 1. Catalytic activity in the system MoO_4^{2-}–Tg (reducing agent, $NaBH_4$) with (A) yields of ammonia formed by the reduction of nitrogen (total molybdate–thioglycerol concentration, 7.5 mmol in 50 ml of H_2O; N_2 pressure, 2000 psi; reaction time, 5 days) compared with (B), the yield of ethylene from acetylene (total molybdate–thiolglycerol concentration, 0.75 mmol in 4 ml of H_2O; C_2H_2 pressure, 1 atm; reaction time, 2.5 hr).

The optimalization of the reaction conditions for the reduction of acetylene to ethylene revealed parallels with the behavior of nitrogen as the substrate. Thus, the optimal molybdate:thioglycerol ratio for acetylene reduction coincides with that for nitrogen reduction (Figure 1). Although the reduction of acetylene to ethylene occurs in the absence of added iron salt, it is significantly stimulated by it.[2] Nitrogen fixation was inhibited in the presence of 10% acetylene in the reactor nitrogen but was not appreciably inhibited by CO under similar conditions. We ascribe the lack of inhibition by CO to the availability of an additional coordination site on molybdenum in the model system, which is occupied in the enzyme. The catalytic activity of the molybdate–thioglycerol system for the reduction of acetylene to ethylene is 10^4 times greater than for nitrogen as the substrate and approaches 0.3% of the enzymatic activity.[2] The comparatively low activity of the model system for nitrogen reduction may indicate an inefficiency of the iron-catalyzed electron transfer from borohydride to the molybdenum–thiol complex in the model systems. We currently favor the view that the molybdenum-containing active site of nitrogenase is similarly reduced by electrons transferred by the iron protein portion in the enzyme. The higher activity of nitrogenase in nitrogen reduction suggests that the enzymatic electron-transfer system is much more efficient than the iron cocatalyst used in our model studies. However, we are presently concentrating our efforts to improve the electron-transfer efficiency and to study the effect of ATP. The principal similar-

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(1) G. N. Schrauzer and G. Schlesinger, *J. Amer. Chem. Soc.*, **92**, 1808 (1970).

(2) G. N. Schrauzer and P. A. Doemeny, *ibid.*, **93**, 1608 (1971).

(3) See R. W. F. Hardy, *Ann. Rev. Biochem.*, 37, 331 (1968), and references cited therein.

(4) Sodium molybdate (Baker Analyzed reagent) was recrystallized from ammonia-free, boiled distilled water twice before use. 1-Thioglycerol from commercial sources occasionally contains considerable amounts of NH_3 in form of ammonium salts and therefore must be carefully purified before use. Purification is effected by boiling 100 ml of commercial thioglycerol with 50 ml of 20% methanolic NaOH for 8 hr. After careful acidification with analytical grade HCl the crude thioglycerol is fractionally distilled. The main fraction, bp 122–124° (3 mm), was collected and redistilled.

(5) Ventron Corp., Metal Chemicals Division, used without further purification.

(6) Commercial purified nitrogen of 99.999% purity was used for all experiments.

(7) *Caution!* Thioglycerol is sufficiently volatile and in larger amounts inhibits NH_3 detection by Nessler's reagent. The NH_3 out of the reaction solutions was therefore carefully fractionally distilled and collected in 0.1 *N* HCl. The reaction solution was heated to 65° during the distillation. The NH_3 was quantitatively removed and collected in the receiver by blowing a stream of acid-washed, pure argon through the distillation apparatus for 120 min.

ity between the behavior of acetylene and nitrogen in nitrogenase and the model systems indicates that both are reduced at a molybdenum-containing active site.

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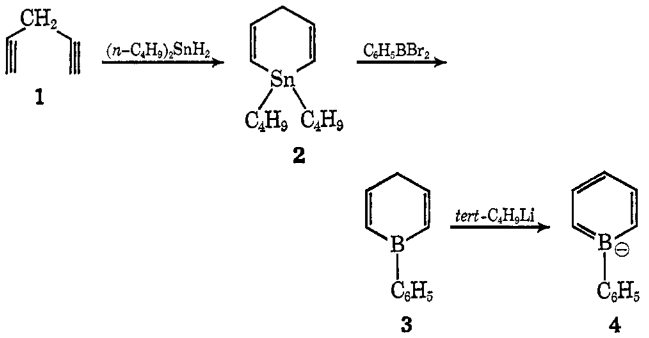
The 1-Phenylborabenzene Anion

Sir:

The extensive investigations of the chemistry of borazarenes have shown that replacement of carbon atoms of "normal aromatic compounds" by the isoelectronic B^- and N^+ leads to aromatic systems of considerable stability.[1] However, there have been no known aromatic compounds in which B^- alone replaces a carbon atom. The simplest of such systems, the 1-phenylborabenzene anion **4**, was recently reported as the ligand of a transition metal π complex.[2] We now wish to report the synthesis and some properties of the parent anion.



Our synthesis has relied heavily on the elegant conversions of 1,5-diacetylenes to borepin derivatives through organotin intermediates.[3] The necessary starting material, diethynylmethane (**1**), was prepared by the coupling of propargyl bromide with acetylenemagnesium bromide.[4] This highly reactive liquid could be isolated in 20% yield from the tetrahydrofuran solution. The structure follows from spectroscopic evidence: nmr (CCl_4) τ 6.7 (t, J = 2.5 Hz, 2 H), 8.16 (t, J = 2.5 Hz, 2 H); ir (CCl_4) 3350 (s), 2970 (s), 2150 cm^{-1} (m); mass spectrum M^+ 64 (base peak).

Stannohydration of **1** with dibutyltin hydride in refluxing heptane followed by heating to 200° gave 40% of 1,4-dihydro-1,1-dibutylstannobenzene (**2**). The molecular ion was not observed in the mass spectrum of **2**. The base peak at *m/e* 243 (M − 57) corresponded to loss of a butyl group from $C_{12}H_{24}{}^{120}Sn$. Divinyldibutylstannane shows similar behavior.[5] The pmr spectrum showed a broad peak due to the 18 protons of the *n*-butyl groups at τ 7.5–9.0, a 2-proton multiplet at τ 6.55 due to the diallylic protons and the 4 vinylic protons as an AB pattern (J_{AB} = 14 Hz) at τ 3.48 and 3.06 (each peak was split into a triplet; J_{AC} = 2 Hz and J_{BC} = 3.5 Hz, respectively).

Reaction of **2** with phenylboron dibromide gave dibutyltin dibromide and 1-phenyl-1,4-dihydroborabenzene (**3**), which could be separated by distillation in 50% yield. The mass spectrum of **3** gave a base peak at *m/e* 154 (the molecular ion for $C_{11}H_{11}{}^{11}B$). Nmr (THF-d_8, external TMS) showed a two-proton diallylic signal at τ 6.52 split by the two groups of two vinyl protons (J_{AX} = 3 Hz, J_{BX} = 1.5 Hz) and half of a four-vinyl-proton AB pattern (J_{AB} = 13 Hz) at τ 2.56, which was split into triplets (J_{BX} = 1.5 Hz) (the other half of the AB pattern and three of the phenyl protons occur as a complex pattern at τ 2.2–2.45; the two remaining phenyl protons occur as multiplet at τ 1.6–1.75). The ^{11}B nmr shows a signal +37 ppm from external trimethylborane, close to that of trivinylborane at +31.2 ppm.[6] Treatment of **3** with acetic acid gave benzene and 1,4-pentadiene.

3 could be deprotonated by lithium triphenylmethide in THF or more conveniently by the method of Bates using *tert*-butyllithium in pentane–THF.[7] Treating these solutions of **4** with excess acetic acid gave 1,4-pentadiene, *cis*-1,3-pentadiene, and benzene. The presence of anion **4** was established by examining the products of deuteration by acetic acid-d_1. The mass spectrum of the *cis*-1,3-pentadiene showed a molecular ion at *m/e* 71, indicating incorporation of three deuterium atoms. The nmr spectrum of **4** in THF-d_8 (external TMS) is quite characteristic: the proton at C_4 occurs as a triplet (J = 6.5 Hz) at τ 3.66; the protons at C_2 and C_6 occur as half of an AB pattern at τ 3.05 (J_{AB} = 10 Hz), while the protons at C_3 and C_5 as well as three of the phenyl protons form a complex pattern at τ 2.4–2.8 The remaining two phenyl protons are a multiplet at τ 1.9–2.

The chemical shift values of protons of aromatic systems are sensitive to the charge density at adjacent carbon and to ring-current effects.[8] Peak positions for **4** occur at relatively low field compared to those of the corresponding pentadienyl anions.[9] This is consistent with substantial ring-current effects and/or diminished negative charge at carbon. In either case the result suggests considerable electron donation to boron. Additional evidence for this comes from the ^{11}B nmr spectrum of **4**, which consists of a broad band

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