phobic amino acid side chains and protecting groups. Most of the improved deblocking procedures involve the use of more polar solutions. Unfortunately these solvents shrink the resin and tend to prevent reagent penetration into the resin. Therefore we have compromised to include CH_2Cl_2 in most reagents to minimize resin shrinkage. Our aim is to find a single deblocking reagent which succeeds on every step of the synthesis.

Since reporting on the difficulty in deblocking certain residues in I and III,[3] we have been informed of similar results in the synthesis of other peptides.[4] Our results illustrate the importance of measuring the efficiency of deblocking as well as coupling in solid phase peptide synthesis.

Acknowledgment. This work was supported by the National Multiple Sclerosis Society, Grants No. 464A and 464-D-8, and the National Institutes of Health, Grants No. NB 08278 and AM 13129.

(3) F. C.-H. Chou, R. K. Chawla, R. F. Kibler, and R. Shapira, 160th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1970.

(4) R. N. Piasio, Manager, Organic R&D, Schwarz/Mann, Orangeburg, N. Y., peronal communication.

Frank Chuen-Heh Chou, Rajender K. Chawla
Robert F. Kibler, Raymond Shapira*
Departments of Biochemistry and Medicine
Emory University, Atlanta, Georgia 30322

Received October 15, 1970

On Potential Model Systems for the Nitrogenase Enzyme

Sir:

Nitrogenase as isolated from *Azotobacter vinelandii*,[1] *Clostridium pasteurianum*,[2] and subsequently from other sources, consists of two proteins, one containing both molybdenum and iron (in the ratio of $\sim$1:15) and the second containing iron only. When combined, these two proteins are capable of catalyzing the reduction of N_2 and a variety of other substrates, *e.g.*, azide,[3] cyanide,[4] acetylene,[5] nitrous oxide,[6] and methylisonitrile.[7] In the absence of added substrate, and to a lesser extent in its presence, hydrogen gas is evolved.[8] All reactions involve the concomitant hydrolysis of ATP.

A recent communication,[9] in which the reduction of some of these same substrates has been demonstrated, has led to the conclusion that the essential chemical processes catalyzed by the N_2-reducing enzymes occur at a molybdenum-containing binding site. However, some of our results, reported herein, from attempts to produce model systems based on iron for the nitrogenase enzyme, emphasize that sufficient evidence is *not* yet available to distinguish between molybdenum, iron, or a multimetal center as the active site of the enzyme.

The reaction mixture usually employed in the present work consisted of 0.25 mmol of iron(II) complex and 1–2.5 mmol of both substrate and reductant (sodium borohydride or sodium dithionite) in 10 ml of solvent (ethanol, water, or buffer) in a 35-ml bottle fitted with a serum cap. For reactions involving gaseous substrates (acetylene, ethylene, etc.), the reaction bottle was purged with the particular gas for 4–5 min, while the solid (potassium cyanide, sodium azide) or liquid (acetonitrile) substrates were introduced under an argon atmosphere before the addition of solvent which initiated the reaction. Parallel series of reactions were used; one series was followed by gas–liquid chromatography[10] of samples of the gas phase and the other (not for the hydrocarbon reduction) by continuously flushing with a slow stream of argon and trapping evolved ammonia in 1 *M* hydrochloric acid. Ammonia was determined on aliquots spectrophotometrically using Nessler's reagent after isothermal distillation.[8] These reactions have lead to the reduction of azide, cyanide, acetonitrile, acetylene, methylacetylene, and ethylene and low levels of reduction of dinitrogen also. Control reactions were conducted in all cases.

True catalytic activity was found in these iron systems, *i.e.*, more than 1 mol of substrate/mol of iron complex is reduced, a phenomenon not yet demonstrated in the molybdenum–thiol system.[9] For example, even using the known compound dichlorotetrapyridinoiron(II) as catalyst, sodium borohydride as reductant, and sodium azide as the substrate in ethanol, 4.5 mol of ammonia/mol of catalyst was obtained at 25° in 5 hr, at which time the reaction was stopped. It was also found that CN^- was reduced to methane plus a trace of ethane and ammonia (0.4 mol/mol of catalyst), and acetonitrile was reduced to ethane and ammonia under similar conditions by, for example, dichlorobis(*N*-phenyl-*S*-methyl-2-aminoethanethiol)iron(II).[11] The rate of the latter reaction was slower by a factor of 10 than that for cyanide. Slower reductions of acetonitrile were also observed with the enzyme,[7] but here the difference was 500-fold and methane was the product. Acetylene and methylacetylene were slowly reduced to ethylene and propylene, respectively, with 5 and 3% of the initial gas converted to products in 4 hr at 25°, respectively. Ethylene was also utilized as a substrate (this hydrocarbon is not reduced enzymatically). Using a system consisting of 0.25 mmol of the same iron complex and 2.5 mmol of sodium borohydride in 10 ml of ethanol, ethylene (1 mmol) was completely reduced to ethane in 2 hr at 25°. When smaller amounts ($\sim$0.7 mmol) of $NaBH_4$ were used, only 40% of the ethylene was reduced in this time, but addition of a further quantity of reductant completed the reduction. If the system was then purged with ethylene, reduction would begin again at a similar rate to that observed initially. With a mixture of acetylene and ethylene, it was observed

---

(1) W. A. Bulen and J. R. LeComte, *Proc. Nat. Acad. Sci. U. S.*, **56**, 979 (1966).

(2) L. E. Mortenson, J. A. Morris, and D. Y. Jeng, *Biochim. Biophys. Acta*, **141**, 516 (1967).

(3) R. Schöllhorn and R. H. Burris, *Proc. Nat. Acad. Sci. U. S.*, **57**, 1317 (1967).

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

(5) M. J. Dilworth, *ibid.*, **127**, 285 (1966).

(6) R. W. F. Hardy and E. Knight, Jr., *Biochem. Biophys. Res. Commun.*, **23**, 409 (1966).

(7) M. Kelly, J. R. Postgate, and R. L. Richards, *Biochem. J.*, **102**, 1C (1967).

(8) W. A. Bulen, R. C. Burns, and J. R. LeComte, *Proc. Nat. Acad. Sci. U. S.*, **53**, 532 (1965).

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

(10) An F and M 700 chromatograph with a 10 ft × 0.25 in. 80–100 mesh alumina column at 144° and a flame ionization detector was used.

(11) This and other new compounds used in this work have satisfactory elemental analyses.

that only 0.2% of the product was ethane after 5 hr. This suggests that the complexing of acetylene is so strong that it is bound exclusively and that as soon as reduction to ethylene occurs (a relatively slow process), this ethylene molecule is immediately replaced by acetylene. These reductions are accompanied by the evolution of much hydrogen gas produced by the decomposition of $NaBH_4$.[12] This decomposition is *not* to be considered as a model for the ATP-dependent hydrogen evolution catalyzed by nitrogenase,[8] as previously suggested.[9]

Experiments involving molecular nitrogen have been carried out at ambient temperature and pressure in systems similar to those described above. Small yields of ammonia of the order of 5% based on catalyst concentration have been obtained in both static and flow systems over 5-hr periods, and we are presently involved in attempts to optimize the catalytic activity of these systems.

The reactions of other types of iron complexes were also studied. For example, the ability of the compounds[13] dihydridotris(ethyldiphenylphosphine)-iron(II) (FeH_2L_3) and its dinitrogen adduct ($FeH_2(N_2)L_3$) to take part in reduction reactions similar to those described above was examined. Both of these compounds, without added reductant in benzene or ethanol, reduced *ca.* 0.4 mol of acetylene/mol of complex in 20 hr at 25° producing ethylene (0.35 mol), ethane (0.04 mol), and some unidentified polymeric material. With azide ion in ethanol FeH_2L_3, in the presence of sodium borohydride, produced *ca.* 2 mol of ammonia/mol of iron complex in 5 hr at 25°. Ammonia was also formed in 1 *M* HCl–ethanol (1:1) solutions containing iron(II) chloride, ethyldiphenylphosphine, and azide only, *i.e.*, without borohydride, the yield leveling off after 3–4 days at 25° at an ammonia to phosphine ratio of one.

At this time, it seems appropriate to include some words of caution about the indiscriminate application of the results from model chemistry to enzyme systems. In agreement with recent work,[9] we have also found that the unidentified complexes produced by iron(II) and simple thiol compounds have very little activity in these reductions. However, the environment at the active site of the enzyme has not been shown to consist exclusively of thiol ligands. Thus, taking into account the recent report[9] on Mo chemistry and the work on Fe detailed above, conclusions excluding either Mo or Fe from a catalytic role at the active site of the enzyme cannot be drawn at this time. It must be pointed out that model experiments carried out under one particular set of conditions can easily lead to erroneous conclusions when applied to the enzyme, even though the model chemistry itself is valid. Finally, the very sensitive acetylene reduction assay for nitrogenase activity is well known and extensively used, but its use as the *sole* criterion for N_2-reducing ability is invalid. The crucial test of a nitrogenase model is its ability to catalyze the reduction of molecular nitrogen at ambient temperature and pressure. The ability to satisfy the requirements with respect to the reduction of the other substrates of nitrogenase may be used, in a supportive role, as a measure of the closeness of the chemistry of the model system to that of the enzyme and indeed may help to elucidate the mechanism(s) of the enzymatic reactions.

W. E. Newton,* James L. Corbin
Peter W. Schneider, William A. Bulen
Contribution No. 410
Charles F. Kettering Research Laboratory
Yellow Springs, Ohio 45387

Received September 22, 1970

---

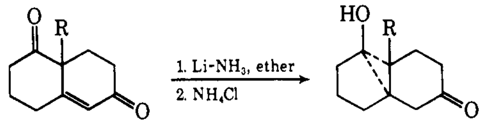
[12] "Sodium Borohydride; Handling, Properties and Uses," Metal Chemical Division, Ventron Corp., Beverly, Mass., p 25, and references therein.

[13] A. Sacco and M. Aresta, *Chem. Commun.*, 1223 (1968).

Transformations of Cyclopropanol Intermediates. I. Synthesis of Angularly Substituted Perhydroindan Systems *via* a Stereospecific Cyclopropanol Rearrangement

Sir:

We recently described a novel reductive cyclization of angularly substituted bicyclo[4.4.0]dec-1-ene-3,7-diones (I and II) to 1-substituted 2-hydroxytricyclo-[4.4.0.0^{2,6}]decan-8-ones (III and IV, respectively).[1] Subsequent study has demonstrated that these cyclopropanols can be transformed into several isomeric bicyclic ring systems, and in this and the following communication the synthetic versatility of these remarkable intermediates will be illustrated using the cyclopropanol III derived from the Wieland–Miescher ketone I.[2,3]



I, R = CH_3
II, R = $CH_2CH{=}CH_2$

III, R = CH_3
IV, R = $CH_2CH{=}CH_2$

Reduction of I by a solution of lithium in ammonia and ether yielded up to 80% of the cyclopropanol III:[4] mp 98–100°; ν_{max} 3590 and 1705 cm^{-1}; nmr signals at δ 0.9 (3 H, singlet), 1.4–2.5 (12 H, m), and 4.55 (1 H, singlet, rapidly exchanged with D_2O); molecular ion at *m/e* 180 in the mass spectrum. Although III is unstable, it can be stored for several days in a refrigerator and has been converted to a stable methyl ether semicarbazone derivative, mp 212–213°. Treatment of III with aqueous ferric chloride regenerated I in 70% yield.

We were surprised to find that reaction of III with a solution of *p*-toluenesulfonic acid in refluxing benzene (1 hr) or a methanolic potassium hydroxide solution at room temperature (3 hr) did not produce either of the bicyclic isomers (V or VI) expected from simple ring cleavage of the cyclopropanol moiety.[5] The major product (up to 60% from both of these reactions) was a colorless solid, mp 166–167°, which was identified

---

[1] P. S. Venkataramani and W. Reusch, *Tetrahedron Lett.*, 5283 (1968).

[2] S. Ramachandran and M. S. Newman, *Org. Syn.*, 41, 38 (1961).

[3] Part of this work was reported at the 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969.

[4] With the exception of X, all new compounds described in this communication have elemental compositions confirmed by combustion analysis (Spang Microanalytical Laboratory, Ann Arbor, Mich.) to within 0.3% in carbon, 0.1% in hydrogen, and 0.15% in nitrogen.

[5] C. H. DePuy, *Accounts Chem. Res.*, 1, 33 (1968).