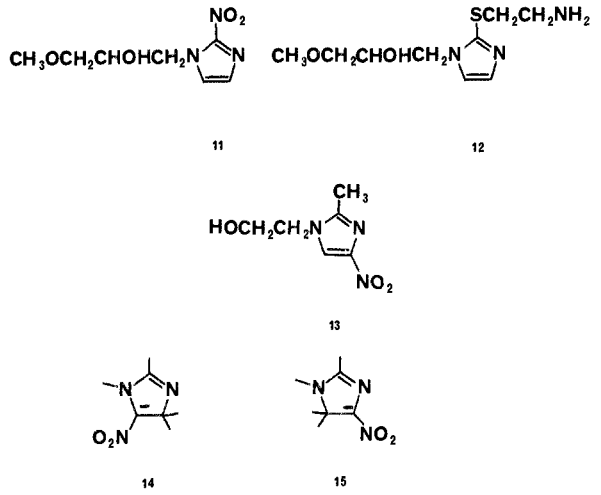

When either bacteria or xanthine oxidase are incubated with metronidazole (**1**) under anaerobic conditions, extensive fragmentation of the imidazole ring occurs.[20] This fragmentation probably does not involve thioimidazoles as intermediates, since compound **2** is inert to aqueous acid and base and even to aqueous mercuric chloride.[21] However, preliminary experiments with water and hydrazine[9] suggest that adducts formed when metronidazole reacts with other nucleophiles may be less resistant to fragmentation. Decomposition of these adducts may lead to many of the ultimate metabolites, while conversion of other important cellular nucleophiles to inert imidazoles may account for the biological activity of nitroimidazoles.

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Cryochemical Studies. 1. ESR Spectrum of Ag_3[1]

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There have been several ESR investigations of silver atoms isolated in rare-gas matrices at low temperatures.[2-5] Kasai and McLeod[2] found that EPR spectra in Ne, Ar, Kr, and Xe consist of two doublets of almost equal intensity from the two isotopes of silver with hyperfine interactions (hfi) up to 6% larger than the values found in the gas phase.[6] These workers also found that silver atoms react with ethylene and acetylene in rare-gas matrices to give a variety of metal atom–organic ligand complexes and pseudocomplexes.[3,4] For instance, Ag atoms and C_2H_4 give

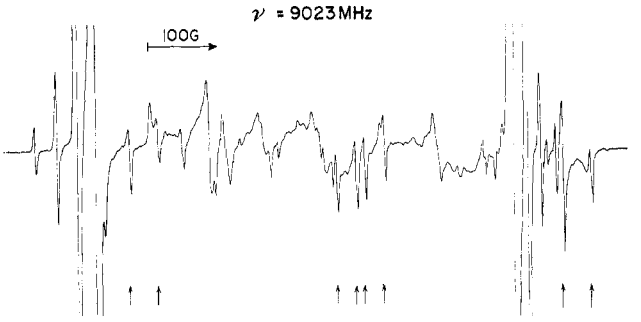

Figure 1. EPR spectrum of C_6D_6 containing ^{107}Ag at 103 K. The arrows indicate the resonance positions of Ag_3.

EPR spectra which were attributed to isolated Ag atoms, a pseudocomplex, Ag···C_2H_4, with a Ag hfi ~6% smaller than that of the free atom, and a complex, $Ag(C_2H_4)_2$, with a small Ag hfi of ~16.4 G. Ag atoms and C_2H_2 behaved somewhat differently, and EPR spectra have been assigned to the pseudocomplexes Ag···C_2H_2, Ag···$(C_2H_2)_2$, Ag···$(C_2H_2)_{n\geq 3}$, and the vinyl radical AgCH=CH. Recently, Ozin[5] using high concentrations of silver in Ar and Kr ($1:10^2$–$1:10^3$) and photoaggregation of matrices dilute in silver ($1:10^3$–$1:10^4$) obtained spectra which were weak in Ag atoms and strong in an isotropic feature centered at $g \sim 2$. Ozin concluded that the latter spectrum was a composite of two spectra, one consisting of sharp lines which were attributed to a range of silver aggregates with molecular cluster properties and a broader conduction EPR spectrum which was assigned to small silver microcrystallites.

We wish to report here the first positive EPR identification of a neutral silver cluster (Ag_3) which has been produced at 77 K by cocondensation of ^{107}Ag atoms and C_6D_6 on the cold surface of a rotating cryostat.[7,8] Isotopically pure silver (98.22% ^{107}Ag from Oak Ridge National Laboratory, TN) was chosen for these experiments because of the anticipated complexity of the spectrum from natural silver. The EPR spectrum obtained from this experiment is shown in Figure 1. It is dominated by two doublets with large isotropic hyperfine splitting constants (a_{107}^{I} = 608.2 G, g_{iso} = 2.0004; a_{107}^{II} = 562.45 G, g_{iso} = 1.9926) which are associated with isolated atoms and the pseudocomplex Ag···C_6D_6.[9]

A more complex spectrum, again essentially isotropic, consists of four ~40-G doublets (arrows in Figure 1). The separation of the central pair of doublets is precisely that expected for a second-order splitting[10] associated with equal (~295 G) isotropic hyperfine interactions from two nuclear spins of magnitude $^1/_2$. We assign the spectrum to the cluster of three silver atoms Ag_3 in which the equivalent terminal nuclei show equal, larger hyperfine interactions.

An exact least-squares solution of an isotropic spin Hamiltonian for Ag_3 using all eight lines of the observed spectrum led to the following best-fit parameters: $a_{107}(2)$ = 295.0 ± 0.3 G, $a_{107}(1)$ = 38.5 ± 0.3 G, and g_{iso} = 1.9622 ± 0.0001. Closer inspection of the spectrum at high resolution suggested the presence of residual g anisotropy from an orthorhombic tensor with principal values ~1.960, 1.962, and 1.966. There was, however, no suggestion of ^{107}Ag hyperfine anisotropy.

Using the appropriate one-electron parameter for ^{107}Ag,[11] the isotropic hyperfine interactions in Ag_3 may be converted to spin populations of 44% for each of the terminal Ag(5s) and 6% for the central Ag(5s) atomic orbital. The composition of the semioccupied orbital (SOMO) of Ag_3 is thus strikingly similar to those of the alkali-atom clusters Na_3[12] and K_3.[13] In all three cases, the unpaired electron is more or less localized in valence s atomic

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0002-7863/81/1503-6226$01.25/0

orbitals of the terminal atoms. This suggests that the wave function of the unpaired electron has a node at the central atom and belongs to either the Σ_u^+ representation of $D_{\infty h}$ (linear species) or the B_2 representation of C_{2v} (nonlinear species). The occupation of such a nonbonding (or weakly antibonding) MO is predicted for three electrons placed in a simple Hückel MO scheme derived from linear combinations of three s atomic orbitals. The small *s* spin density at the central nucleus must then be due to spin polarization effects and is presumably negative.

The isotropic *g* shift of −0.04 for Ag_3 is surprisingly large, and it is difficult to reconcile such a shift with the established lack of silver hyperfine anisotropy in the spectrum. The sign of the *g* shift is consistent with matrix contributions to the spin–orbit interaction, as suggested for Na_3,[12] but the magnitude of the shift seems to be excessive for such a mechanism. It appears more likely to us that the *g* shifts in these triatomic clusters are due to intramolecular spin–orbit interactions. Because of the small anisotropic one-electron parameters expected for $^{107}Ag(5p)$,[11] considerable spin density present in such contributing atomic orbitals in Ag_3 would not give rise to resolved anisotropic hyperfine structure. It is possible, in principle, that the silver atoms contribute as much as 10% 5p character to the SOMO of Ag_3. Spin–orbit interaction between the ground state and very low-lying excited states having considerable Ag(5p) character would then give rise to negative *g* shifts.

Our analysis of the spectrum of Ag_3 in terms of an orthorhombic *g* tensor having all three principal values appreciably less than the free-spin value suggests that Ag_3 is probably bent, with a $^2B_2$ (C_v) ground state. A linear $^2\Sigma_u^+$ species would have an axial **g** tensor with $g_\parallel$ quite close to 2.0023. This conclusion does of course conflict with a recent laser Raman spectroscopic study of small silver clusters in Kr, from which it was concluded that Ag_3 is linear.[14]

In conclusion, we might add that all the $Ag–C_6D_6$ species formed in this experiment have not yet been positively identified, but it does appear that a $Ag_3\cdots C_6D_6$ pseudocomplex is formed.

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Enzyme-Catalyzed Organic Synthesis: NAD(P)H Regeneration Using Dihydrogen and the Hydrogenase from *Methanobacterium thermoautotrophicum*

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This paper describes several practical systems for the in situ regeneration of NAD(P)H from NAD(P), using dihydrogen as the ultimate reducing agent, in reactions catalyzed by the hydrogenase (H_2ase, EC 1.12.1.2) from *Methanobacterium thermoautotrophicum* (Scheme I). The development of simple and economical methods for regenerating the reduced nicotinamide cofactors represents an important intermediate step in the adaptation of enzymic catalysis to problems in practical organic synthesis.[1–5] Dihydrogen has the advantages as a reactant that it is inexpensive and a strong reducing agent and that its consumption leaves no byproducts. Previous hydrogenase-catalyzed reductions have been carried out on a small scale and have not provided the information concerning the stability and ease of manipulation of the enzymes involved that is required to judge the usefulness of these schemes for organic synthetic applications.[6–8]

Scheme I

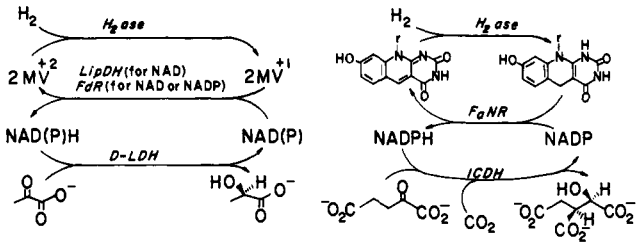

Table I. Synthesis of D-Lactate and Isocitric Acid

enzyme or cofactor	MV; lactate TN[a]	MV; lactate recovery, %	F_0; isocitrate TN[a]	F_0; isocitrate recovery, %
H_2ase	1.5×10^7[b]	78	6×10^5	76
LipDH	6×10^5	35[c]		
D-LDH	2×10^7	81		
F_0NR			1.5×10^7[b]	62
ICDH			3×10^5	78
NAD(P)(H)	1700[d]	68	1000[b]	40

[a] TN ≡ moles of product isolated per mole of enzyme or cofactor. [b] These turnover numbers are calculated by assuming the crude protein mixture used contained ~10% each of H_2ase and F_0NR by weight. [c] Calculated on the basis of the *total* LipDH added. [d] These numbers are calculated based on *isolated* product.

We have explored two redox cycles based on H_2ase. In one, H_2ase catalyzes the reduction of MV^{2+} to MV^+ (MV = methyl viologen), and MV^+ is used to reduce NAD(P) to NAD(P)H in reactions catalyzed by the flavoenzymes lipoamide dehydrogenase (LipDH, EC 1.6.4.3) or ferredoxin reductase (FdR, EC 1.6.99.4).[7–9] In the second, H_2ase catalyzes the reduction of cofactor F_0 to F_0H_2, and this soluble flavin analogue is used to reduce NADP to NADPH in a reaction catalyzed by F_0-NADP reductase (F_0NR, EC not assigned).[10,11] In the first cycle, FdR can accept either NAD or NADP as substrate; LipDH is specific for NAD.

The H_2ase and F_0NR required are present in quantity in the same preparation and are used in crude form. *M. thermoautotrophicum* was grown as described previously[12] and harvested, and the cells were broken in a French press (4-g wet cells, 0 °C, in 25 mL of 50 mM Tris, pH 7.5, 19000 psi). The resulting suspension was centrifuged at 14000*g* for 25 min and the supernatant passed through a DEAE column (2.2 × 3.5 cm). The resulting crude mixture of proteins (3.3 mg of protein[12] per mL of Tris buffer, ~38 mL) was immobilized in PAN gel[14] (20 g of polymer)

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