where the $\delta g'_\alpha$ are defined in eq 2. Thus, it is possible to use the measured hfs tensor, A', to obtain the tensor characteristic of the (Fe^{IV}=O) moiety, A^{OT}. Analogous expressions hold for coupling to the iron.

To fully characterize the ^{17}O hyperfine splitting tensor, ENDOR spectra were obtained at fields across the entire envelope of the EPR spectrum. We show elsewhere[12] that two of the quantities of interest, A^{OT}_x and A^{OT}_y, are simply obtained from the ^{17}O ENDOR spectra observed when the field is set to the low-and the high-field edges of the EPR spectrum (Figure 1). The ν_+ ENDOR signal (Figure 1B,C), when corrected according to eq 1, gives $A'_x \simeq 17$ and $A'_y \simeq 19$ MHz. One treats the magnetic fields as corresponding to the extremal resonance fields of centers with corresponding g-tensor components, namely, $g'_x \simeq 1.5$ and $g'_y \simeq 2.5$, and uses the corresponding $\delta g'_i$ and A'_i in eq 3 to obtain the x and y components of A^{OT}: $A^{OT}_x \simeq 35$ MHz and $A^{OT}_y \simeq 36$ MHz. Thus, the triplet-spin ^{17}O hfs coupling tensor is seen to have no less than axial symmetry ($A^{OT}_x \simeq A^{OT}_y$); the same is true for the ^{57}Fe coupling parameters obtained from Mossbauer measurements.[7,12]

As H_0 approaches the field corresponding to $g_z \simeq 2$ from either above or below, the frequency of the ν_+ resonance is expected to approach $A'_z/2 + \nu_0$. Very close to $g = 2$ the frequency of the resonance approaches zero, indicating that $A'_z \sim 0$ as predicted by the first-order eq 3b, but the intensity also decreases, making it impossible to determine A'_z, and thus A^{OT}_z, with accuracy.

The axial symmetry of the observed ^{17}O and ^{57}Fe hfs tensors naturally suggests that we interpret the data in terms of a triplet oxyferryl (Fe^{IV}=O) center whose axis lies normal to the porphyrin cation plane. If we assume the two odd electrons of Fe^{IV}=O) to be in antibonding π-molecular orbitals

$$\Psi_x = (1 - c^2)^{1/2} d_{xz}{}^{Fe} - c p_x{}^{O}$$
$$\Psi_y = (1 - c^2)^{1/2} d_{yz}{}^{Fe} - c p_y{}^{O} \quad (4)$$

and utilize the previously determined reference hfs tensor for a single odd electron in an ^{17}O p-π orbital ,[14] one obtains an ^{17}O hfs tensor for the triplet system (Fe^{IV}=O) of $A^{OT} \simeq |c|^2$-[140,140,0] MHz. From the measured coupling constants and eq 3 one arrives at the estimate $c^2 \sim 0.25$, corresponding to an oxyferryl center whose unpaired odd electrons are substantially delocalized between the two atoms through d_π-p_π bonding, in excellent accord with theoretical expectations.[11]

Two alternatives to the symmetrical oxyferryl moiety may be considered, namely, Fe^{IV}-O-H and the recently proposed structure in which oxygen bridges the Fe and a porphyrin nitrogen.[15] However, the axial symmetry of both ^{57}Fe and ^{17}O hfs tensors is evidence against these alternative models. Furthermore, the failure to observe a large proton coupling in HRPI[5] argues against Fe^{IV}-O-H,[16] and the optical spectrum of HRPI is not reproduced by the carbenoid model for the protoporphyrin oxygen-bridged structure.[15] All this leads us to prefer the model discussed here for the oxyferryl moiety of the HRP compound I enzymic intermediate.

Acknowledgment. This work was supported by National Institutes of Health Grants HL-13531 and GM-07768 and National Science Foundation Grants PCM 76-81304 and PCM 79-10656. The measurements were performed in the magnetic resonance facility of Northwestern University's Material Research Center, supported in part under the National Science foundation NSF-MRL Program (Grant DMR76-80847).

(13) The second-order corrections give a nonzero value for A_z. One should also include the possible interaction of the doublet spin with nuclei of the oxyferryl center, A^{OP}, which simply augments eq 3: A' (eq 3) $\rightarrow A'$ (eq 3) + A^{OP}

(14) (a) Cohen, A. H.; Hoffman, B. M. *J. Phys. Chem.* **1974**, *78*, 1313-1321. (b) Symons, M. C. R.; Peterson, R. L. *Proc. R. Soc. London, Ser. B* **1978** *201*, 285-300.

(15) Chevrier, B.; Weiss, R.; Lang, M.; Chottard, J.-C.; Mansuy, D. *J. Am. Chem. Soc.* **1981**, *103*, 2899-2901.

(16) It is reasonable to suppose that even a proton with a large and anisotropic coupling might be observable (Dalton, L. R.; Kwiram, A. L. *J. Chem. Phys.* **1972**, *57*, 1132-1145) and protons with couplings of moderate magnitudes and anisotropies typically are readily observed (e.g., ref 5).

Heterogeneous One-Electron Reduction of Metal-Containing Biological Molecules Using Molecular Hydrogen as the Reductant: Synthesis and Use of a Surface-Confined Viologen Redox Mediator That Equilibrates with Hydrogen

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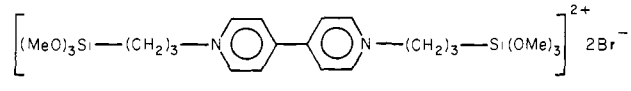
Study and use of metal-containing biological reagents often involves the need to manipulate the redox level. We report herein the synthesis of a heterogeneous catalyst system that allows the use of H_2 as a reductant for the one-electron reduction of horse-heart ferricytochrome *c* (cyt c_{ox}), sperm whale myoglobin, and stellacyanin from the lacquer of *Rhus vernicifera*. Application of the principles illustrated by our catalyst in other systems is possible inasmuch as the reducing power of H_2 is sufficiently great that many biological systems are thermodynamically reducible with H_2. An advantage in using H_2 as a source of reducing power is that the oxidation product is H^+ which is acceptable since most biological systems are studied in buffered media. A heterogeneous catalyst is desirable to facilitate the separation of the catalyst from the product.

A catalyst for one-electron reductions using H_2 must include functionality that will allow equilibration of the substrate with the H_2O/H_2 couple without the undesirable result of hydrogenating the substrate. The aim is to have a catalyst that equilibrates H_2 in such a way that two e^-'s and two H^+'s are available from H_2, not two H atoms. A heterogeneous catalyst must also include the functionality that overcomes the usual kinetic and adsorption problems typically encountered in heterogeneous electron-exchange processes involving large biological molecules.[1-3] For example, the rapid electrochemical reduction of cyc c_{ox} is only possible with certain types of electrodes.[4,5]

We have prepared the heterogeneous catalyst system represented by Scheme I. Basically, the catalyst is a redox-active polymer that (i) can be equilibrated with H_2O/H_2 via the dispersed Pt(0), (ii) reduces biological molecules when reduced, and (iii) can be anchored to a large number of surfaces including glass. Most of our work concerns the use of ordinary 13- × 100-mm Pyrex test tubes functionalized on the inside surface with the catalyst system. The catalyst could be anchored to higher surface area supports to achieve faster observed rates but the functionalized test tubes allow us to illustrate the principles of operation and synthesis. Synthesis of the surface-confined polymer begins with reaction of an *N,N'*-dialkyl-4,4'-bipyridinium (PQ^{2+}) derivative (I)[6-9] with pretreated Pyrex glass: (1) 13- × 100-mm



I

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(2) (a) Heineman, W. R.; Meckstroth, M. L.; Norris, B. J.; Su, C.-H. *Bioelectrochem. Bioenerg.* **1979**, *6*, 577. (b) Kuwana, T.; Heineman, W. R. *Acc. Chem. Res.* **1976**, *9*, 241.

(3) Margoliash, E.; Schejter, A. *Adv. Protein Chem.* **1966**, *21*, 113.

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(5) Lewis, N. S.; Wrighton, M. S. *Science (Washington, D.C)* **1981**, *241*, 944.

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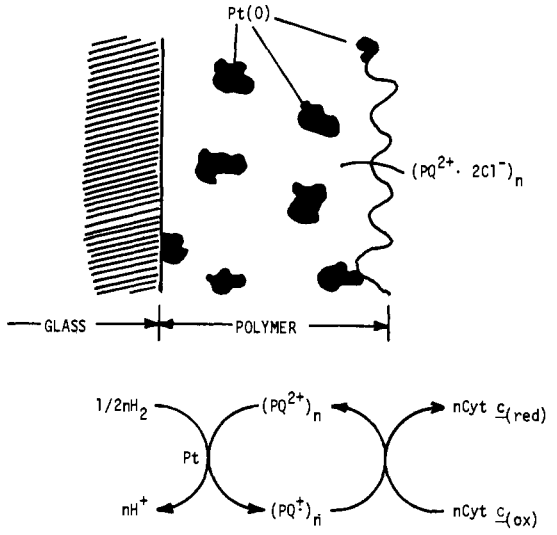
Scheme I. Representation of a Glass-Confined Heterogeneous Catalyst for the One-Electron Reduction of Cyt c_{ox} Using H_2



Pyrex test tubes are filled with 10 M NaOH and allowed to stand for 5 min at 25 °C, then rinsed liberally with distilled H_2O, and dried in an oven at 90 °C; (ii) 3 mL of CH_3CN solution of ~3 mM I with trace H_2O is introduced into the test tube, the test tube is corked, and allowed to react for ~3 days at 25 °C; (iii) the CH_3CN solution of I is removed and the derivatized test tube is washed liberally with distilled H_2O and heated in an oven at 80 °C for ~1 day to dry and further cross-link the polymer consisting of PQ^{2+} centers derived from hydrolysis of the Si–OMe bonds in I; (iv) the $(PQ^{2+}\cdot 2Br^-)_n$-bearing test tubes are then filled with an aqueous solution of ~3 mM K_2PtCl_4 to yield a $PQ^{2+}\cdot PtCl_4^{2-}$-bearing surface by ion exchange,[7-9] and the aqueous solution is removed and the test tube is again rinsed with distilled H_2O (at this point the test tube generally has a distinct yellow coloration due to $PtCl_4^{2-}$ introduction); (v) the test tube is then filled with distilled H_2O and exposed to 1 atm of H_2 that reduces $PtCl_4^{2-}$ to Pt(0), and the Pt(0) then equilibrates the H_2O/H_2 with the $PQ^{2+/+\cdot}$ redox centers to reduce the colorless $(PQ^{2+})_n$ to the intensely purple $(PQ^{+\cdot})_n$;[7,8] (vi) the synthesis of the functionalized test tube is completed by addition of 0.1 M HCl to oxidize $(PQ^{+\cdot})_n$ to $(PQ^{2+})_n$ with evolution of H_2 followed by liberal rinsing with distilled H_2O.[7,8] The resulting glass surface is represented by glass/$[(PQ^{2+}\cdot Pt(0)\cdot 2Cl^-)_n]_{surf}$ and the synthesis described above is represented by the sequence 1–4. The chemistry used to prepare glass/$[(PQ^{2+}\cdot Pt(0)\cdot 2Cl^-)_n]_{surf}$ follows directly from the functionalization of conducting materials such as Au, Pt, and Si.[6-9] Indeed, the chemistry for Si is very similar to that for glass inasmuch as the surface of Si is covered with an air oxide that should resemble glass.[10] The chemistry associated with the synthesis of glass/$[(PQ^{2+}\cdot Pt(0)\cdot 2Cl^-)_n]_{surf}$ has been previously proven by a combination of electrochemical and spectroscopic techniques.[6-9] The range of coverages found from the synthesis is $\sim 10^{-9}$–10^{-7} mol of PQ^{2+} centers per cm^2 of projected surface area.[11]

We have previously shown that glass/$[(PQ^{2+}\cdot Pt(0)\cdot 2Cl^-)_n]_{surf}$ on p-type Si is able to catalyze the evolution of H_2 via a mechanism that involves first reduction of the PQ^{2+} centers followed by equilibration with H_2O via the Pt.[7,8] In the present application we are simply using the catalyst in reverse to generate a strong, outer-sphere, one-electron reducing agent, namely, $PQ^{+\cdot}$ centers. Importantly, we have also previously shown that electrochemically generated $(PQ^{+\cdot})_n$ on a conducting surface (Au, Pt, or Si) will reduce cyt c_{ox} at a rate that is only limited by the rate of mass transport.[5]

Figure 1 shows a summary of two key experiments using the glass/$[(PQ^{2+}\cdot Pt(0)\cdot 2Cl^-)_n]_{surf}$ system. The first experimentation relates to the equilibration of the PQ^{2+} redox centers with H_2 as a function of pH (Figure 1a). The $(PQ^{2+})_n \rightarrow (PQ^{+\cdot})_n$ reduction is associated with a large visible spectral change that can be used to show that H_2 will effect the reduction.[12] However, the $E^{\circ\prime}$-$[(PQ^{2+/+\cdot})_n]_{surf}$ is pH independent on Pt or Au at -0.31 ± 0.05 V vs. NHE is aqueous electrolytes,[7,8] while the reducing power of 1 atm of H_2 in H_2O varies 59 mV/pH. Thus, only above a certain pH will H_2 be able to reduce PQ^{2+}; the spectroscopic data in Figure 1a show that glass/$[(PQ^{2+})_n]_{surf}$ is 50% reduced at a pH of ~5.5. The formal reducing power of 1 atm of H_2 at pH 5.5 is –0.33 V vs. NHE, and thus the $E^{\circ\prime}$ for glass/$[(PQ^{2+/+\cdot})_n]_{surf}$ is the same, within experimental error, as when the $[(PQ^{2+/+\cdot})_n]_{surf}$ is on Au or Pt. This firmly supports the conclusion that H_2O/H_2 can be equilibrated with the $[(PQ^{2+/+\cdot})_n]_{surf}$ system. The Pt(0) is essential since the reduction of the polymer does not occur unless the Pt is incorporated into the polymer. The $[(PQ^{2+/+\cdot}\cdot Pt(0))_n]_{surf}$ system is durable under 1 atm of H_2 at pH 7 and is not hydrogenated on the time scale of 2 weeks at 25 °C, as established by the constancy of the optical spectrum.

Using the glass/$[(PQ^{2+}\cdot Pt(0)\cdot 2Cl^-)_n]_{surf}$ catalyst, H_2 will effect the one-electron reduction of cyt c_{ox} as illustrated by the optical spectral changes shown in Figure 1b, and the approximate relative initial rate depends on pH in a manner consistent with the intermediacy of $(PQ^{+\cdot})_n$.[13] The reduction of cyt c_{ox} has been done at cyt c_{ox} concentrations from ~5 μM to ~1 mM, and we typically find that the $t_{1/2}$ for the formation of cyt c_{red} is in the vicinity of 10 min with complete reaction (>95%) over in approximately 100 min at pH 7. During all stages of the reaction the functionalized test tube is purple in color, indicating that the equil-

$$n\mathrm{I} + \underset{\text{colorless}}{\text{pretreated glass}} \xrightarrow[\substack{\text{3 days,}\\ \text{CH}_3\text{CN,}\\ \text{trace H}_2\text{O}}]{\text{25 °C}} \underset{\text{colorless}}{\text{glass/}[(PQ^{2+}\cdot 2Br^-)_n]_{surf}} \quad (1)$$

$$\underset{\text{colorless}}{\text{glass/}[(PQ^{2+}\cdot 2Br^-)_n]_{surf}} + n\mathrm{K_2PtCl_4} \xrightarrow[\substack{\text{5 min,}\\ \text{H}_2\text{O}}]{\text{25 °C}} \underset{\text{yellow}}{\text{glass/}[PQ^{2+}\cdot PtCl_4^{2-})_n]_{surf}} + 2n\mathrm{KBr} \quad (2)$$

$$\underset{\text{yellow}}{\text{glass/}[(PQ^{2+}\cdot PtCl_4^{2-})_n]_{surf}} + {}^3/_2 n\mathrm{H_2} \xrightarrow[\substack{\text{10 min,}\\ \text{H}_2\text{O}}]{\text{25 °C}} \underset{\text{purple}}{\text{glass/}[(PQ^{+\cdot}\cdot Pt(0)\cdot Cl^-)_n]_{surf}} + 3n\mathrm{HCl} \quad (3)$$

$$\underset{\text{purple}}{\text{glass/}[(PQ^{+\cdot}\cdot Pt(0)\cdot Cl^-)_n]_{surf}} \xrightarrow[\substack{\text{fast,}\\ \text{0.1 M HCl}}]{\text{25 °C}} \underset{\text{gray}}{\text{glass/}[(PQ^{2+}\cdot Pt(0)\cdot 2Cl^-)_n]_{surf}} + {}^1/_2 n\mathrm{H_2}$$

(7) Bookbinder, D. C.; Bruce, J. A.; Dominey, R. N.; Lewis, N. S.; Wrighton, M. S. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 6280.
(8) Dominey, R. N.; Lewis, N. S.; Bruce, J. A.; Bookbinder, D. C.; Wrighton, M. S. *J. Am. Chem. Soc.*, in press.
(9) Bruce, J. A.; Wrighton, M. S. *J. Am. Chem. Soc.*, in press.
(10) (a) Schmidt, P. F.; Michel, W. *J. Electrochem. Soc.* **1957**, *104*, 230. (b) Raider, S. I.; Flitsch, R.; Palmer, M. J. *Ibid.* **1975**, *122*, 413.
(11) The surface coverage of a test tube is 545-nm by measuring the optical density of the fully reduced form at 545 nm ($\epsilon = 1.0 \times 10^7$ cm^2/mol). The ϵ value was determined by measuring the 550-nm absorption for a known coverage of $(PQ^{+\cdot})_n$ prepared electrochemically on an optically transparent SnO_2 electrode.
(12) The optical spectrum of $(PQ^{+\cdot})_n$ in the visible is very similar to that for the dimeric form of $MV^{+\cdot}$, $\lambda_{max} = 545$ nm ($\epsilon = 10000$ cm^{-1} M^{-1} or 1.0×10^7 cm^2/mol). Kosower, E. M.; Cotter, J. L. *J. Am. Chem. Soc.* **1964**, *86*, 5524.
(13) All optical determinations and rate measurements were made by using a Cary 17 UV–VIS–near-IR spectrophotometer. The measurements were made by attaching a standard, optical quality absorption cell to the functionalized test tube using rubber tubing. The reactant solution was then simply airlessly poured from the reaction chamber to the absorption cell and reaction stops because the sample is removed from the catalyst anchored to the test tube. The solutions are purged with H_2 via syringe needles through the rubber tubing, and reaction is carried out under 1 atm of H_2. Cyt c_{ox} was studied in buffered solution with 1.0 M KCl added and was obtained from Sigma Chemical Co. as their Type VI (highest purity) material.

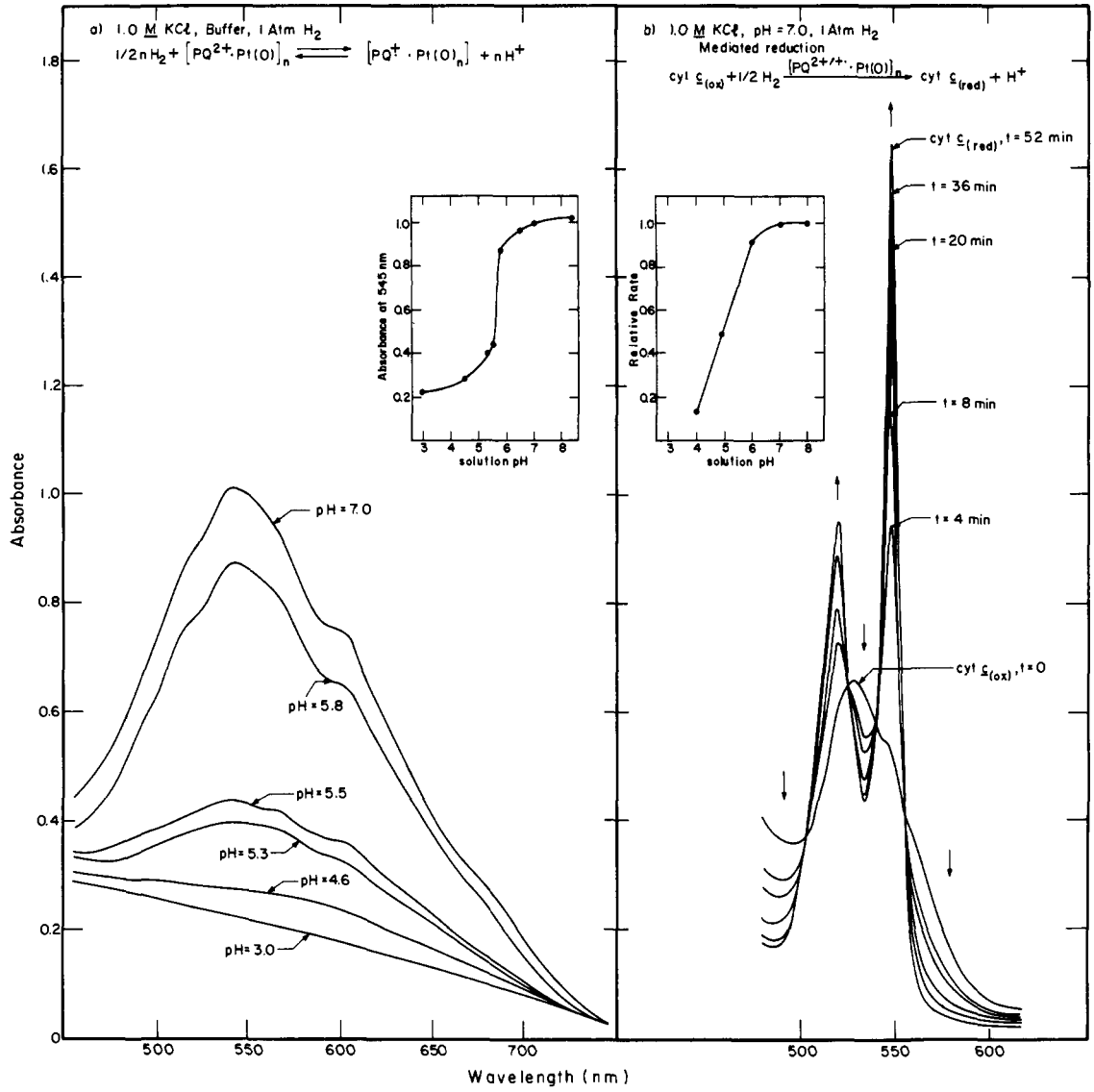


Figure 1. Visible absorption spectra of (a) a 13- × 100-mm test tube containing $[(PQ^{2+/+\cdot}Pt(0))_n]_{surf}$, 1.0 M KCl, buffer, 1 atm of H_2. Spectra taken at various pH's and a plot of absorbance at 550 nm vs. pH is shown in the inset. Polymer coverage = 5×10^{-8} mol/cm^2.[11] A similar test tube containing the buffer but no polymer was used in the reference compartment of the spectrophotometer. (b) 50 μM cyt c in 1.0 M KCl, pH 7.0, under 1 atm of H_2. The test tube in (a) was used to heterogeneously reduce the cyt c_{ox}. The inset shows initial relative rate of cyt c reduction catalyzed by $[(PQ^{2+}\cdot Pt(0))_n]_{surf}$ vs. solution pH. Spectra taken through a 1.0-cm quartz cell.

ibration of the polymer with H_2 is not the rate-limiting step. This observation, with earlier electrochemical measurements,[5] is then consistent with the assertion that the reduction of the cyt c_{ox} is in fact limited by diffusion of the cyt c_{ox} up to the surface of the catalyst.

An important consideration in the use of our catalyst is whether the PQ^{2+} centers and Pt(0) actually remain presistently anchored to the glass, since even very small amounts of such species are at least a nuisance and could provide a dominant mechanism for the reduction of the biological molecule. We do not find any leaching of PQ^{2+} centers into the solution as determined by UV–VIS spectroscopy where submicromolar quantities could be detected. Another test is to place a typical cyt c_{ox}/buffer solution into a glass/$[(PQ^{2+}\cdot Pt(0)\cdot 2Cl^-)_n]_{surf}$ catalyst and let it stand for 1 h. The solution containing any leached PQ^{2+} or Pt(0) is then added to a test tube functionalized with [3-(trimethoxysilyl)-propyl]trimethylammonium chloride, ion exchanged with $PtCl_4^{2-}$ and reduced under H_2 to form glass/$[(TAA^{+\cdot}{}_{1/2}Pt(0)\cdot Cl^-)_n]_{surf}$. As for functionalization with I, the test tube is pretreated with 10 M NaOH. Such a test tube will catalyze the reduction of *N,N'*-dimethyl-4,4'-bipyridinium (MV^{2+}) to $MV^{+\cdot}$ using H_2. The $MV^{2+/+\cdot}$ is a well-known redox mediator for biological systems[14] and is capable of mediating reduction of cyt c_{ox},[15] obviating our interest in polymers derived from I. The glass/$[(TAA^{+\cdot}{}_{1/2}Pt(0)\cdot Cl^-)_n]_{surf}$ catalyst does not reduce fresh solutions of ~50 μM cyt c_{ox} under conditions where MV^{2+} can be reduced and importantly we find no cyt c_{ox} reduction when the solution is first placed against glass/$[(PQ^{2+}\cdot Pt(0)\cdot 2Cl^-)_n]_{surf}$ for 1 h. In a typical experiment 4-h reaction of 50 μM cyt c_{ox} is reduced <5% with a glass/$[(TAA^{+\cdot}{}_{1/2}Pt(0)\cdot Cl^-)_n]_{surf}$ catalyst where the catalyst derived from I gives complete reduction in <1 h and $t_{1/2}$ of <10 min under the same conditions. These results show that insignificant (for mediation) amounts of PQ^{2+} are leached from the surface and also show that Pt alone in the polyion will not equilibrate H_2 with cyt c_{ox}.

The glass/$[(PQ^{2+}\cdot Pt(0)\cdot 2Cl^-)_n]_{surf}$ catalyst is useful for the H_2 reduction of 50 μM sperm whale myoglobin[16] or 0.12 mM stellacyanin[17] under the same conditions as for cyt c_{ox}. For ~50 μM myoglobin the reduction appears to occur as rapidly as for cyt c_{ox} at ~50 μM as evidenced by optical spectral changes. However, attempts to reduce myoglobin at ~0.6 mM fail, and the presence

(14) Summers, L. A. "The Bipyridinium Herbicides"; Academic Press: London, 1980; pp 122–124.

(15) Land, E. J.; Swallow, A. J. *Ber. Bunsenges. Phys. Chem.* **1975**, *79*, 436.

of the high concentration myoglobin inhibits the reduction of cyt c_{ox}. Thus, it appears that high concentrations of myoglobin block reduction, presumably due to surface adsorption. Stellacyanin at 0.12 mM, that is even reducible at Pt electrodes, can be reduced with H_2 using the glass/$[(PQ^{2+}\cdot Pt(0)\cdot 2Cl^-)_n]_{surf}$ catalyst. The rate is at least as good as with cyt c_{ox} at the same concentration and conditions, and we observe no complications from surface adsorption.

We have illustrated the principles of a heterogeneous catalyst for the reduction of biological molecules using H_2 as the reductant.[18] Additional applications of the catalyst are presently being elaborated in these laboratories.

Acknowledgment. N.S.L. acknowledges support as a John and Fannie Hertz Fellow, 1977–1981. D.C.B. acknowledges partial support as an M.I.T. NPW Fellow, 1981. Partial support from the United States Department of Energy, Basic Energy Sciences, Division of Chemical Sciences, is acknowledged. We appreciate the generous assistance of Professor Edward I. Solomon and his research group and the use of their purified stellacyanin. Helpful discussions with Professors William H. Orme-Johnson, Alexander Klibanov, and George M. Whitesides are gratefully acknowledged.

(16) Sperm whale myoglobin was obtained from Sigma Chemical Co. as their Type II material and reduction was monitored at pH 7.0 buffered with phosphate buffer in 1.0 M KCl under 1 atm of H_2. The ~450–700-nm region of the optical absorption was monitored as described in ref 13. The oxidized form shows absorption maxima at 503 (ϵ ~9000) and 634 nm (ϵ ~3670), and the reduced form shows a peak at 555 nm (ϵ ~11 700). There are four isosbestic points at 463, 521, 612, and 660 nm just as when $S_2O_4^{2-}$ is used as a reductant. Our measured extinction coefficients are within 5% of those given above from the literature: Ray, D. K.; Gurd, F. R. N. *J. Biol. Chem.* **1967**, *242*, 206. Willick, G. E.; Schonbaum, G. R.; Kay, C. M. *Biochemistry* **1969**, *8*, 3729.

(17) Purified stellacyanin from the lacquer of *Rhus vernicifera* was generously provided by Professor Edward I. Solomon. Reduction of the stellacyanin results in the decline of the visible feature at 604 nm (ϵ 4030). Exposure of reduced material to O_2 in air regenerates the 604-nm feature. The stellacyanin was studied at 0.12 mM in 0.2 M phosphate buffer, pH 7.0. Purity was established by the ratio of 604 to 280-nm absorption, 1–5.6, as in the literature: Reinhammas, B. *Biochem. Biophys. Acta* **1970**, *205*, 35.

(18) An important control experiment using naked, clean, smooth Pt as a heterogeneous catalyst shows that ~50 μM concentrations of cyt c_{ox} are reducible at a rate approaching that of our catalyst but at high concentration; ~1 mM naked Pt does not work whereas our catalyst does work as well as at 50 μM. Myoglobin is not reducible (<5% in 1 h) using naked Pt. Stellacyanin is reducible using the naked Pt as expected from electrochemical experiments using a Pt electrode. However, using Pt alone in any situation may lead to hydrogenation and hydrogenolysis reactions unrelated (and undetected by optical methods) to the redox reactions.

Formation of Monocyclic and Bicyclic Aza-β-lactams and Other Novel Heterocycles from 1-(Diphenylmethylene)-3-oxo-1,2-diazetidinium Inner Salt[1]

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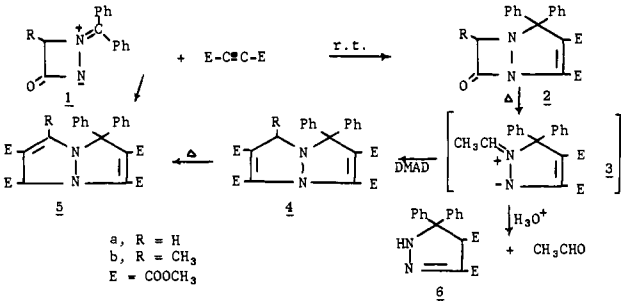
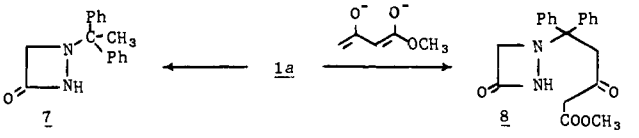
Received August 24, 1981

Several years ago we described the intramolecular dehydrohalogenation of the α-chloroacyl hydrazones of diaryl ketones to give 1-(diarylmethylene)-3-oxo-1,2-diazetidinium inner salts (e.g., **1**).[2] We now report some reactions of these readily accessible azomethine ylides which provide novel entries into a variety of heterocyclic systems, including monocyclic and bicyclic aza-β-lactams.[3]

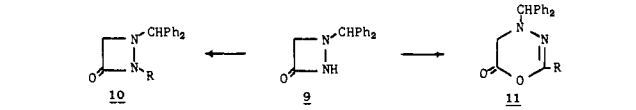
Scheme I



Scheme II



Scheme III



R	mp, °C	Yield, %
a, $COC(CH_3)_3$	112-113	78
b, CH_3	131-132	72
c, CH_2CH_3	75-77	91
d, CH_2Ph	98-100	37
e, $CH(CH_3)_2$	119-121	84
f, CH_2COOH	136-138	41
g, Ph	114	70
h, CH_2CH_2COOH	oil	46

R	mp, °C	Yield, %
a, CH_3	109-112	75
b, Ph	203-204	76
c, OCH_2Ph	113-114	74
d, NHC_6H_4-CH_3-p	158-159	67

Reaction of **1a** with dimethyl acetylenedicarboxylate (DMAD) in methylene chloride at 100 °C gives **4a** (a 2:1 cycloadduct with loss of CO), mp 135.2–136 °C (90%), which isomerizes upon melting to **5a**, mp 117.4–117.5 °C (98%). The course of this transformation was elucidated by examining the reaction of **1b** with DMAD. After 5 days at room temperature, a 1:1 cycloadduct (**2b**) was obtained as yellow crystals, mp 138 °C (56%, IR 1840 cm^{-1}). This compound loses CO upon warming to 70 °C; the ylide **3b** is a possible intermediate, since hydrolysis with dilute hydrochloric acid gives acetaldehyde (isolated as its 2,4-DNP) and 5,5-diphenyl-3,4-bis(carbomethoxy)-Δ^2-pyrazolidine (**6**)[4] (60%), and reaction with additional DMAD gives **5b**, mp 127–128 °C (80%).

Certain organometallic reagents add to the iminium bond of **1a**, providing 1-substituted 1,2-diazetidin-3-ones. Thus, reaction of **1a** with methylmagnesium bromide gives 1-(1,1-diphenylethyl)-1,2-diazetidin-3-one (**7**) as a gum (61%), and addition of the dianion of methyl acetoacetate to **1a** gives **8**, mp 123–125 °C (51%).

We reported previously[5] that selective reduction of the iminium bond in **1a** to give 1-benzhydryl-1,2-diazetidin-3-one (**9**), mp 173–174 °C (99%), could be effected by treatment with a stoichiometric amount of sodium borohydride in methanol. We now report that **9** undergoes a remarkable series of substitution and ring-expansion reactions. Thus, treatment of **9** with pivaloyl chloride in the presence of triethylamine results in the formation of the 2-pivaloyl derivative **10a**. Reaction of **9** with acetic anhydride, however, leads to ring expansion with the exclusive formation of 4-benzhydryl-2-methyl-4,5-dihydro-1,3,4-oxadiaz-

(1) We are indebted for partial support of this work to Eli Lilly and Company and the National Science Foundation (Grant CHE-7918676). Acknowledgment is also made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this research.

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0002-7863/81/1503-7659$01.25/0