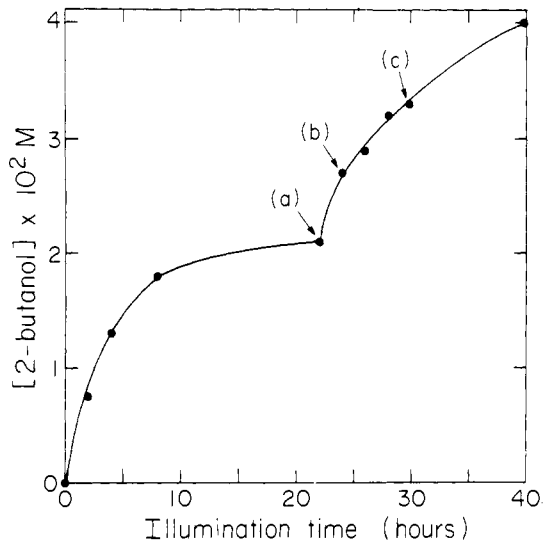

Figure 1. Rate of 2-butanol formation at different illumination time intervals. Initial $(NH_4)_3EDTA$ concentration 2×10^{-2} M. (a) Addition of $(NH_4)_3EDTA$, 2×10^{-2} M, (b) addition of $(NH_4)_3EDTA$, 1.7×10^{-2} M, (c) addition of $(NH_4)_3EDTA$, 1.7×10^{-2} M.

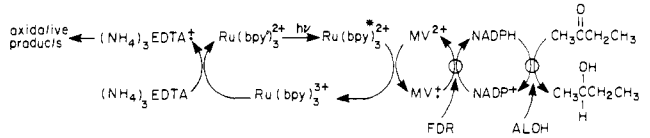

Figure 2. Cyclic scheme for the photosensitized reduction of 2-butanone by $(NH_4)_3EDTA$.

Table I. Turnover Numbers (TN) of Components Involved in the Photosensitized Reduction of 2-Butanone

	$Ru(bpy)_3^{2+}$	MV^{2+}	FDR[a]	$NADP^+$	ALDH[b]
TN	530	40	24000	40	6000

[a] FW ≃ 40000; cf.: Shin, M. *Methods Enzymol.* **1971**, *23*, 441. [b] FW ≃ 40000.[9]

and their different turnover numbers after 40 h of illumination are summarized in Table I.

The different steps involved in the photoinduced reduction of 2-butanone have been confirmed by separate experiments. Illumination of the aqueous system that includes only the sensitizer, $(NH_4)_3EDTA$, MV^{2+}, $NADP^+$ and the enzyme ferredoxin reductase, results in the formation of NADPH (followed spectroscopically at λ = 340 nm). When either MV^{2+} or ferredoxin reductase are excluded from the system no photosensitized formation of NADPH is observed. These results imply that $MV^{+\cdot}$ formed upon illumination mediates the reduction of $NADP^+$ to NADPH in the presence of the enzyme.[11] Introduction of 2-butanone to the photochemically produced NADPH does not lead to the disappearance of NADPH, and no alcohol is formed. Yet, upon introduction of the enzyme alcohol dehydrogenase the NADPH disappears and 2-butanol is observed as product. These facts confirm that NADPH mediates the reduction of 2-butanone in the presence of the second enzyme.[9] In view of these results we suggest the scheme outlined in Figure 2 as the cyclic photoinduced process leading to the reduction of 2-butanone. This cycle involves a photosystem that produces $MV^{+\cdot}$ and two subunits of enzyme-catalyzed reactions. In the first unit NADPH is formed in a process catalyzed by ferredoxin-$NADP^+$ reductase. The subsequent unit utilizes NADPH in the reduction of 2-butanone in the presence of alcohol dehydrogenase.

The net reaction accomplished in this system corresponds to the reduction of 2-butanone by EDTA (eq 1).[12] The thermodynamic balance of this process is endoergic by ca. 33 kcal per mol of EDTA.

$$R{-}N(CH_2CO_2^-)_2 + CH_3C(=O)CH_2CH_3 + 2H_2O \rightarrow R{-}NHCH_2CO_2^- + CH_2O + HCO_3^- + CH_3CHOHCH_2CH_3 \quad (1)$$

It should be noted that substitution of EDTA by other electron donors, i.e., cysteine and triethanolamine, similarly results in the photoinduced formation of $MV^{+\cdot}$ with the subsequent formation of NADPH. This demonstrates the wide applicability of various electron donors in the system.

In conclusion, we have demonstrated the feasibility of coupling enzymes as catalysts in artificial photosensitized electron-transfer reactions. This approach is useful as a synthetic tool for the light induced preparation of chiral alcohols, as well as a novel attitude for the development of energy conversion and storage systems. It is conceivable that under similar experimental conditions other NADPH-dependent enzymes, i.e., lactate dehydrogenase, glutamic dehydrogenase, and alanine dehydrogenase, would lead to the reduction of other prochiral ketones as well as to the preparation of optically active amino acids.

Acknowledgment. The support of the Schonbrunn Fund (Hebrew University) is gratefully acknowledged. This study was performed under the auspices of the Fritz Haber Molecular Dynamics Research Center, The Hebrew University of Jerusalem.

Registry No. $Ru(bpy)_3^{2+}$, 15158-62-0; $(NH_4)_3EDTA$, 15934-01-7; MV^{2+}, 4685-14-7; $NADP^+$, 53-59-8; FDR, 9029-33-8; ALDH, 9031-72-5; 2-butanone, 78-93-3; (-)-2-butanol, 14898-79-4.

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Spin Trapping of a Cobalt-Dioxygen Complex

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The spin-pairing model for binding O_2 to transition-metal complexes describes the bound O_2 as a fragment into which anywhere from 0.1 to 0.8 of an electron is transferred upon binding to the metal.[1-3] As the ligand field strength of the ligands attached to the metal is increased more extensive electron transfer occurs. In all instances the unpaired electron in this system is localized mainly on O_2 making it difficult to determine the actual charge on this fragment. This charge transfer increases the basicity of a coordinated O_2 molecule over that of molecular oxygen. For example, it has been shown that trifluoroethanol can hydrogen bond to the terminal oxygen of the O_2 adduct of [bis(salicylidene-γ-iminopropyl)methylamine]cobalt(II) (CoSMDPT-O_2).[4] The magnitude of the shift in the O-H stretching frequency indicates[5] that the enthalpy of hydrogen bond formation is 6.6 kcal mol^{-1} making the terminal oxygen of the bound O_2 about as basic as the carboxyl oxygen in *N,N*-dimethylacetamide.

This work was extended to a study of the Co(II) complex catalyzed oxidation by O_2 of 2,6-disubstituted phenols to quinones.[6] The mechanism proposed for this reaction[6] suggests that coor-

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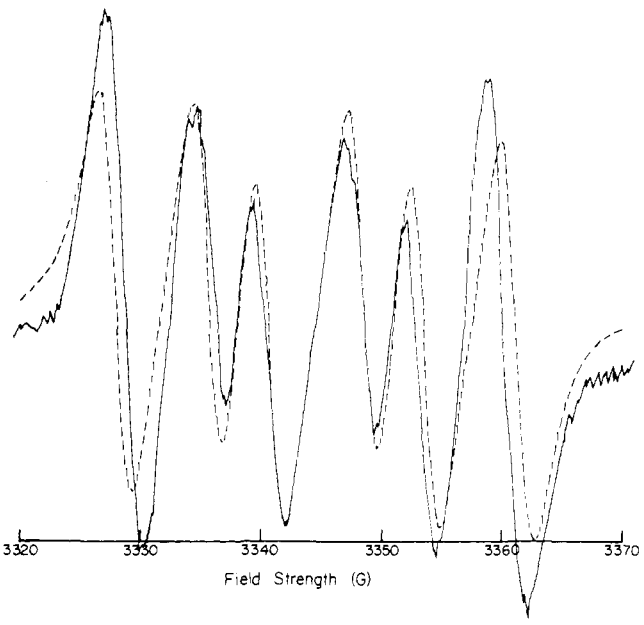
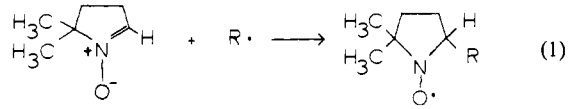


Figure 1. Solid line: room temperature EPR spectrum of CoSMDPT–O_2 plus DMPO in toluene. Dashed line: computer simulation of an EPR spectrum with g = 2.008, A_N = 12.8G and A_{H_β} = 7.68 G.

dination of O_2 to Co(II) not only enhances the basicity of the O_2 but also increases its tendency to undergo free radical reactions. We were thus interested in probing the radical reactivity of Co–O_2 adducts with spin traps.

Spin trapping has been used to aid in the identification of short-lived radicals by EPR spectroscopy. This technique employs a diamagnetic compound which reacts with a free radical to form a relatively stable radical product referred to as a spin adduct.[7,8] This spin adduct is commonly a nitroxide free radical which has a characteristic EPR spectrum. Information about the type of free radical trapped may be obtained from the nitrogen and proton hyperfine coupling constants. The spin trap used in this study is 5,5-dimethyl-1-pyrroline N-oxide (DMPO), which functions as a trap as shown in eq 1. The product, a spin adduct of DMPO,


(1)

exhibits hyperfine splitting due to both the β-hydrogen and the nitroxide nitrogen. The values are sensitive to the type of radical trapped. DMPO has been used in several studies to trap HO_2 as well as other oxygen-centered radicals.[9-15] Since this spin trap does not form an adduct with free O_2, reactivity with a metal-bound O_2 would demonstrate enhanced radical reactivity of O_2 as a result of metal coordination.

At room temperature a toluene solution of CoSMDPT that has been exposed to oxygen shows no EPR signal, although at lower temperature (liquid nitrogen) a spectrum for the cobalt–dioxygen complex is observed.[16] When DMPO is added to this CoSMDPT solution an EPR signal at room temperature is immediately observed and is shown in Figure 1. DMPO added to a solution of

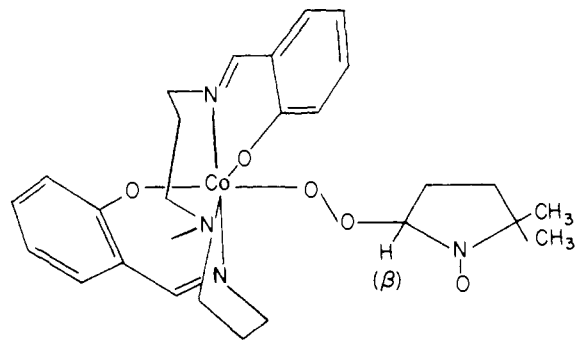


Figure 2. Structure of the spin adduct formed by CoSMDPT–O_2 with DMPO.

CoSMDPT prepared in an inert atmosphere does not produce a room temperature EPR spectrum. The CoSMDPT–O_2/DMPO spectrum is different from that of 5,5-dimethyl-2-pyrrolidone-1-oxyl (DMPOX), produced by oxidation of DMPO using PbO_2. This result rules out oxidation of DMPO by CoSMDPT–O_2 as the source of the EPR signal. The EPR spectrum of DMPOX in toluene consists of a triplet with g = 2.009 and A_N = 13 G (37 MHz). Prolonged exposure of the CoSMDPT/DMPO solution to oxygen does lead to formation of DMPOX, since a triplet with A_N = 13 G is seen.

Computer simulation[17] of the EPR spectrum, also shown in Figure 1, leads to a g value of 2.008 and hyperfine splitting constants as follows: A_N = 12.8 G (36.0 MHz) and $A_{\beta H}$ = 7.68 G (21.6 MHz). The relative magnitudes of the A values ($A_N > A_{\beta H}$) indicate that an oxygen-centered radical has been trapped.[18,19] The hyperfine splitting values are unlike those for radicals formed when DMPO reacts with either the phenyl (A_N = 13.76, $A_{\beta H}$ = 19.22 G) or benzyl radical (A_N = 14.16, $A_{\beta H}$ = 20.66 G).[18] This rules out the possibility that radicals arising from reactions of the toluene solvent are being trapped.

We propose that the dioxygen adduct of CoSMDPT, which has one unpaired electron, is trapped by DMPO to yield the spin adduct shown in Figure 2. Since magnetic nuclei more than three bond lengths away from the nitroxide usually do not cause resolvable splitting,[10,18,19] hyperfine splitting due to the cobalt nucleus is not expected.

The superoxide adduct of DMPO in benzene has been reported to have hyperfine splitting values of A_N = 12.9 G and $A_{\beta H}$ = 6.9 G.[13] This reported species may be either the O_2^- or HO_2 adduct.[10] The values in toluene are expected to be the same because the dielectric constants for the two solvents, which would influence the A values, are about the same. It is unlikely that the EPR spectrum observed here is that of the superoxide adduct of DMPO. Not only is $A_{\beta H}$ different, but the generation of free superoxide from CoSMDPT–O_2 is difficult. Walker and co-workers[20] estimated the equilibrium constant for dissociation of CoTPP–O_2 to form superoxide and Co^{III}(TPP) to be less than 10^{-12}. Furthermore, in a study of the reaction of Co(acacen)py–O_2 with acids, a bimolecular reaction in cobalt was observed and no superoxide detected.[21]

Comparison of the superoxide and Co(SMDPT)–O_2 adduct hyperfine splittings shows that although the A_N values are about the same (12.9 vs. 12.8 G) there is a significant difference in the $A_{\beta H}$ values (6.9 G for the superoxide adduct vs. 7.68 G). The β-hydrogen hyperfine coupling depends on the dihedral angle between the C–H_β bond and the nitrogen p orbital.[19] Since the

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size of the trapped radical influences this angle, the difference in $A_{\beta H}$ is probably due to the steric requirements of CoSMDPT-O_2. Clearly, we have obtained further support for our earlier conclusions about cobalt(II) coordination enhancing the basicity and reactivity of molecular oxygen.

Acknowledgment. We thank the National Science Foundation for financial support of this research. Helpful discussions with Professor Barry B. Corden, Tufts University, are gratefully acknowledged.

Registry No. CoSMDPT-O_2, 91279-12-8; CoSMDPT-O_2/DMPO, 91210-84-3; O_2, 7782-44-7.

Steric Inhibition of Hyperconjugation. Vanishing Equilibrium Isotope Effect in Bridgehead-Deuterated 2,3-Dimethylbicyclo[2.2.2]octyl Cation

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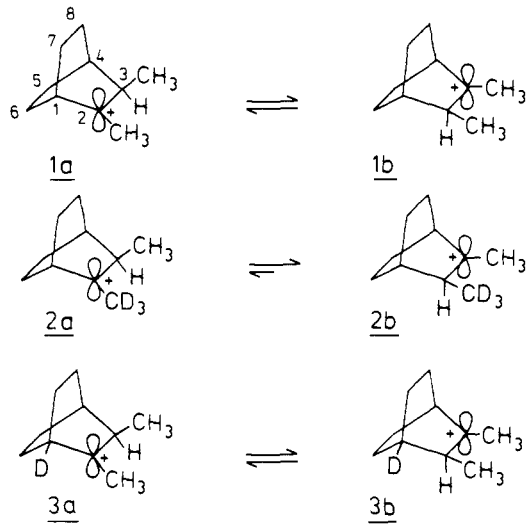
Secondary isotope effects on reaction rates arise from force-field changes at the isotopic position between the reactant and the transition state.[1] A significant array of experimental results indicate that hyperconjugation makes at least the dominant if not the sole contribution to the β-deuterium isotope effect in carbenium ion solvolysis reactions.[2] Shiner[3] furnished the first data relating the dihedral angle between an adjacent C–H or C–D bond and the vacant p orbital of the cation center with the magnitude of the isotope effect. The magnitude of kinetic isotope effects in solvolysis may be distorted by possible multiple reaction pathways like competing elimination, participation, ion pairing, rearrangements, and other factors.[4] Equilibrium isotope effects in degenerate rearrangements of stable carbocations involve only the properties of well-defined species that can be measured by NMR spectroscopy.[5]

We have investigated the angular dependence of the β-deuterium equilibrium isotope effect in a stable model cation with fixed stereochemistry to prove the hyperconjugational origin unambigiously.

The 2,3-bicyclo[2.2.2]octyl cation **1** combines both prerequisites, a fast degenerate 2,3-hydride shift **1a** $\rightleftharpoons$ **1b**, which might be perturbed by β-deuterium, and a fixed dihedral angle of 90° between the vacant p orbital of the cation center and the C–D bond in the bridgehead-deuterated cation **3**. The methyl-deuterated cation **2**, which should show a conformation-independent isotope effect,[6] can serve as a model for the other limiting conformation with a dihedral angle of 0°.

Solutions of **1** in SO_2ClF/SO_2F_2 were prepared from the corresponding chloride[7] by reaction with SbF_5 using standard methods.[8] At −122 °C the ^{13}C NMR spectrum of **1** (Figure 1a)[9]



shows four peaks (56.12 ppm averaged bridgehead carbons C_1, C_4; 29.43 ppm averaged methylene carbons C_7, C_8 endo to CH_3; 24.98 ppm averaged methyl carbons; 23.78 ppm averaged methylene carbons C_5, C_6 exo to CH_3). The peak of the averaged cation and methine carbons C_2 and C_3 is not observable due to very large kinetic line broadening at these conditions. Assignments were made from different temperature-dependent line broadening and ^1H-coupled and ^1H-specific-decoupled ^{13}C NMR spectra and were confirmed by analysis of the ^{13}C NMR spectra of the deuterated cations **2** and **3**. The time-averaged symmetry in the spectrum proves the fast 2,3-hydride shift in cation **1a** $\rightleftharpoons$ **1b**. The energy barrier for the 2,3-hydride shift was determined as $\Delta G^{\ddagger} = 4.7 \pm 0.15$ kcal/mol[10] at −122 °C.

Cation **2** was prepared analoguously to **1** from 2-chloro-2-(trideuteriomethyl)-3-methylbicyclo[2.2.2]octane.[7] The ^{13}C NMR spectrum (Figure 1b) shows typical splittings of the averaged peaks due to the lifting of the degeneracy of the equilibrium **1a** $\rightleftharpoons$ **1b**. As expected the largest isotope splittings are observed for those signals showing the largest kinetic line broadening. At −122 °C the bridgehead-carbon signals are split into two peaks by 6.6 ppm, the C_7, C_8 carbons by 4.4 ppm, and the C_5, C_6 carbons by 0.3 ppm. The isotope splitting of the CH_3/CD_3 groups is 6.4 ppm. The upfield peak is of lower intensity because of extra broadening due to unresolved deuterium coupling, longer relaxation time, and unfavorable NOE. The upfield shift of the CD_3 peak is 0.9 ppm larger than the downfield shift of the CH_3 peak. This shows the direction of the isotope effect. The CD_3 peak at higher field means the isomeric cation with the CD_3 group further removed from the positive charge, i.e., **2b**, is favored in the equilibrium **2a** $\rightleftharpoons$ **2b**.

Hyperconjugative stabilization of the positive charge decreases the force constants of the C–H bonds in the adjacent methyl group. The zero point energy of this methyl group is lower than that at the remote position. A CH_3 group has a priori a higher zero point energy than a CD_3 group, thus CH_3 is lowered more in energy when attached adjacent to the charged carbon, hence the equilibrium **2a** $\rightleftharpoons$ **2b** is shifted toward **2b**. The size of the equilibrium constant is calculated from the isotope splitting. Using the equation[5] $K = (\Delta + \delta)/(\Delta - \delta)$ we obtain $K_{CD_3} = 1.73$ at −120 °C.

Equilibrium isotope effects and thus the isotope splittings δ are temperature dependent.[2] ln K was found to vary lineary with $1/T$ in the temperature region studied (−137 to −92 °C). The thermodynamic parameters for the equilibrium **2a** $\rightleftharpoons$ **2b** were determined from $\ln K = -\Delta H/(RT) + (\Delta S/R)$ via regression analysis as $\Delta H = 65 \pm 2.5$ cal/mol per D and $\Delta S = 0.06 \pm 0.007$

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