hydroxide, which would require greater than 10 cm^{-1} D$_2$O sensitivity. *We assign the band instead to an Mn=O manganyl stretch, with the small D$_2$O downshift reflecting involvement of H-bonding from a distal protein residue.*

The dramatic 135-cm^{-1} lowering of this frequency relative to the typical value in 5-coordinate Mn^{IV}=O porphyrins[1b] is attributed to the imidazolate character of the proximal histidine ligand in HRP[10] and to strong distal H-bonding, possibly from Arg 38, which is indicated by NMR analysis to lie above pyrrole ring III.[11]   For compound II of FeHRP, the H-bonding group is believed to be a distal histidine because the Fe^{IV}=O stretching RR band shifts to a higher frequency as the pH is raised through 6.9 (isoenzyme A) or 8.8 (isoenzyme C).[10] In FeCCP compound I, which is insensitive to pH (4–8), strong H-bonding to distal Arg 48 is most likely responsible for its 22-cm^{-1}-lower Fe^{IV}=O stretching frequency than that of neutral pH FeHRP-II.[12]   We have found no change in the RR spectrum of MnHRP-I (isoenzyme C) between pH 6.4 and 10.5. Perhaps the enhanced basicity of Mn^{IV}=O, a consequence of its weak bond, induces a switch in the H-bond donor group in MnHRP-I.

Thus, compound I of MnHRP is shown to be an oxomanganese(IV) protein radical species with an unusually weak but exchange-resistant Mn=O bond.

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First Observation of Alkene Radical Anions by Electron Spin Resonance Spectroscopy: Electron and Hole Trapping in Hexene/*n*-Hexane Mixed Crystals Irradiated at 4.2 K

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In the present work, we report the first observation of alkene radical anions and their geometrical and electronic structures clarified by electron spin resonance (ESR) spectroscopy. Recently, considerable interest has been taken in the electronic structures of ethylene and alkene radical cations.[1-7] Alkene radical anions may play an important role in the radiolysis of organic compounds as well as the cations. However, the anions have never been observed by ESR and optical spectroscopies, because they have very large negative electron affinities (EA $\approx$ -2.3 eV[8]) and are unstable. Previously, we found that the radiation-induced electrons were trapped in the defects of the mixed crystals of binary *n*-alkanes with different molecular chains.[9]   This observation predicted the possibility of formation of the alkene anions by scavenging the electrons, if the mixed crystals of alkene and alkane

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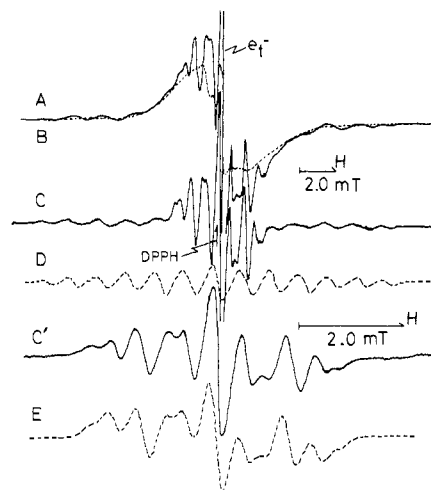

Figure 1. The first-derivative absorption ESR spectra observed for *trans*-3-hexene (4.0 mol %)/*n*-hexane-d_{14} mixed crystals (A) irradiated at 4.2 K and (B) after illuminated by visible light. (C) The difference spectrum obtained by subtracting spectrum B from spectrum A. (C') The expanded spectrum of the difference spectrum observed for the *trans*-3-hexene (8.0 mol %)/*n*-hexane-d_{14} mixed crystals. The ESR spectral simulations for the *trans*-3-hexene (D) cation and (E) anion radicals.

were irradiated at a very low temperature. Irradiation of the mixed crystals at 4.2 K has led to the successful ESR detection of the radical anions together with the corresponding cations.

Figure 1A shows the first-derivative absorption ESR spectrum observed for *trans*-3-hexene (4.0 mol %)/*n*-hexane-d_{14} mixed crystals irradiated at 4.2 K by X-rays (45 kV, 40 mA, for 15 min). Figure 1B is the remaining ESR spectrum after photobleaching with visible light. It was the same spectrum as that observed for the irradiated pure *n*-hexane-d_{14}. The difference spectrum obtained by subtracting the spectrum in Figure 1B from that in Figure 1A is shown in Figure 1C, indicating that two kinds of photobleachable radicals were formed from the solute 3-hexene molecules in addition to a small amount of trapped electrons. The trapped electrons were no longer produced by increasing the solute concentration to 8.0 mol % (Figure 1C'). A similar difference ESR spectrum was obtained in the protiated hexane matrix except for slightly broader line widths. One species gives a wide resonant magnetic field spread over $\sim$18 mT, and the other has a narrow spread of $\sim$5 mT. The double integration of the difference spectrum indicated that the amounts of the two species were nearly equivalent. The former species was assigned to the 3-hexene radical cation by the spectral simulation, which is shown in Figure 1D. The ESR parameters obtained are listed in Table I. Two pairs of C–H β-proton hyperfine couplings are 4.6 and 2.9 mT, and a pair of C–H α-proton couplings is 1.3 mT. The cations are known to be stabilized by hyperconjugation and to have large β-proton hyperfine couplings compared with neutral delocalized radicals.[1-7] The large size of the proton hyperfine couplings observed in the present study agrees well with those reported for alkene cations[1-5] and supports the assignment.

The other species gives a strong seven-line ESR spectrum at the center in Figures 1C and 1C'. This species would be assigned to the 3-hexene radical anion, since it was formed as the counter radical to the 3-hexene cation with an equivalent yield and since both radicals were simultaneously photobleached. This assignment was confirmed by a competitive reaction of electron capture between 3-hexene and 1-chloro-*n*-hexane-h_{13} molecules in the C$_6$D$_{14}$ matrices. Instead of the 3-hexene anion, the chain-end alkyl radical (CH$_2$CH$_2$–C$_4$H$_9$) was selectively formed by dissociative electron attachment to the 1-chlorohexane molecules along with the 3-hexene cation.[10] The ESR spectral simulation of the anion is shown in Figure 1E, and the ESR parameters obtained are listed in Table I. The shoulders of the outermost lines of the spectrum

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Table I. Proton Hyperfine Couplings Observed for the Anion and Cation Radicals of Hexene Isomers in *n*-Hexane Matrices

molecule		coupling (mT)	
		anion	cation
trans-3-hexene	αH × 2	|0.5, 0.1, −0.3| ± 0.1	1.3
(in C$_6$D$_{14}$)	βH × 2	1.38	4.6
	βH × 2	0.56	2.9
trans-2-hexene	αH × 2	|0.7, 0.2, −0.3| ± 0.2	1.3
(in C$_6$H$_{14}$)	βH × 2[a]	1.45	3.9
	βH × 2[a]	0.9	3.9
1-hexene	αH × 2	|0.7, 0.2, −0.3| ± 0.2	–
(in C$_6$H$_{14}$)	αH × 1	|1.3, 0.7, 0.1|	
	βH × 1	1.6	
	βH × 1	0.8	

[a]The methyl rotation is frozen at 4.2 K. The third β-proton coupling of the methyl group was not resolved and is estimated to be less than 0.2 mT from the ESR line widths.
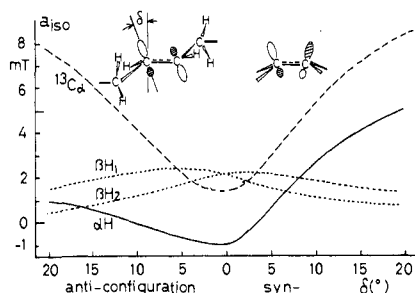
Figure 2. The proton hyperfine couplings for the *trans*-3-hexene radical anion obtained by INDO molecular orbital calculation (see text).

could be reproduced only by a pair of hyperfine coupling tensors of equivalent α-protons (|A|(H$_\alpha$×2) = |(0.5, 0.1, −0.3)| ± 0.1 mT) and two pairs of isotropic couplings of equivalent β-protons (*a*-(H$_{\beta1}$×2) = 1.38 mT, *a*(H$_{\beta2}$×2) = 0.56 mT; Table I). The three pairs of equivalent proton couplings obtained indicate that the radical has a C_2 symmetry of the parent molecules (−CH$_2$CH=CHCH$_2$−). The dipolar tensors of the two α-protons are not small (**B** = 0.4, 0, −0.4 mT), suggesting that each half of the unpaired electron spin density resides on the equivalent C=C α-carbon atoms. The small isotropic couplings for β- and α-protons cannot be explained by alkyl, allyl, or vinyl radicals or planar π ion radicals. The small α-proton couplings (*a*(H$_\alpha$) = ±0.1 mT) suggest that the species has a pyramidal radical structure with a σ-character. σ-type radicals are known to have small α-proton couplings by cancelling the contributions of spin polarization and spin delocalization.[11] The irradiation of the mixed crystals of hexane containing 1- or *trans*-2-hexene yielded also the corresponding alkene anions with similar pyramidal radical structures. The preliminary results obtained are listed in Table I.

The pyramidal structure in the anti configuration of alkene anions was supported by INDO MO calculations, which results are shown in Figure 2. The MO calculations gave proton hyperfine couplings agreeable with the observed values in the case of the anti configuration of a pyramidal radical structure with a 10–15° deviation from the plane. The minimum of the total energy was also obtained in the anti configuration, although the angle of the nonplanarity is small (~3°). On the other hand, the MO calculations for the syn conformation and for the twist radical structure did not result in small α-proton and β-proton couplings, respectively, excluding the occurrence of their forms.[10]

Alkene radical cations (RCH=CHR)$^{•+}$ have been reported to have a planar structure except for vinyl derivatives (CH$_2$=CR$_1$R$_2$) with a twisted structure and to be stabilized by hyperconjugation to the CH β-bonds and/or CH α-bonds,[5-7] while the anions may be stabilized by mixing [2s;C] atomic orbital with a larger core integral to the [2p;C] orbitals, resulting in a pyramidal radical structure.
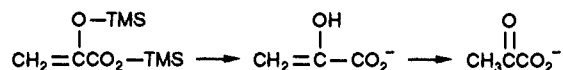
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Preparation and Properties of Enolpyruvate

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The enolic form of pyruvate is considered to be a key intermediate in enzymatic reactions catalyzed by pyruvate kinase, phosphoenolpyruvate carboxylase, pyruvate-phosphate dikinase, malic enzyme, and oxalacetate decarboxylase,[1] but few direct studies have examined the reactions or kinetic competency of enolpyruvate.[2] Enolpyruvate has been formed by the action of acid phosphatase on phosphoenolpyruvate in acidic solutions[3-6] and found to have reasonable kinetic stability in D$_2$O at low buffer concentrations ($t_{1/2}$ = 3.6 min at 20 °C in D$_2$O, pD = 6.4). However, this method is not ideal for detailed study of enolpyruvate because the solution necessarily contains protein, phosphoenolpyruvate, phosphate, buffer, and other undesirable materials, and only a limited range of pH is accessible. In this paper we present a simple, clean, and effective method for generating and studying enolpyruvate that is free of these limitations. The material thus generated has the expected kinetic properties and permits direct estimation of the rate of water-catalyzed ketonization of enolpyruvate.

We synthesized bis-TMS-enolpyruvate (i.e., 2-((trimethylsilyl)oxy)propenoic acid trimethylsilyl ester), in 51% yield by the deprotonation of pyruvic acid with triethylamine and silation with trimethylsilyl chloride in dimethylformamide.[7] The compound showed the expected NMR, IR, and mass spectra and was stable for several weeks at −20 °C.

When bis-TMS-enolpyruvate was injected directly into buffer solutions in D$_2$O (final concentration 0.5 mM) and the time-dependent change in the absorbance at 225 nm recorded, biphasic kinetics were observed, showing an initial rapid absorbance increase followed by a slower decrease, presumably because of relatively rapid hydrolysis of the two TMS groups, followed by slow ketonization. Similar biphasic kinetics have been reported for the hydrolysis and tautomerization of vinyl acetals.[8]

$$\underset{\underset{\text{CH}_2=\text{CCO}_2-\text{TMS}}{|}}{\text{O}-\text{TMS}} \longrightarrow \underset{\underset{\text{CH}_2=\text{C}-\text{CO}_2^-}{|}}{\text{OH}} \longrightarrow \underset{\text{CH}_3\text{CCO}_2^-}{\overset{\text{O}}{\|}}$$

When fluoride was used to effect cleavage of the TMS groups, the initial phase was eliminated, presumably because cleavage of the TMS groups became fast.[9] The absorbance change observed then followed clean first-order kinetics, with a rate constant essentially the same as that of the second phase observed above. In the absence of added buffer, the ketonization half-life at 20 °C was estimated to be about 7 min in D$_2$O. As originally ob-

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