of the P-bonded Se atoms are bonded to only one P atom, whereas at 20 atom % P the fraction of P–Se–P units is substantially increased, as expected from probability considerations. Both the results of Table I[11] and the compositional evolution apparent in Figure 1B argue against the cluster models previously suggested for the structure of these glasses.[12] A more detailed interpretation, in conjunction with ^{31}P spin-echo and MAS-NMR studies will be published elsewhere.

The results presented here suggest that heteronuclear "*X–Y*" double resonance experiments have considerable potential for elucidating the structure of inorganic ceramics, semiconductors, and glasses. Further applications to other inorganic systems are currently in progress.

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[10] The sparse crystallographic literature on P–Se compounds indicates bond lengths ranging from 2.2 to 2.4 Å. SEDOR studies of such models have been impeded by extremely long ^{77}Se T_1's and other difficulties. However, the ^{113}Cd–^{31}P SEDOR of CdP_2, a model compound with similar spin dynamics, yields a dipolar second moment close to that calculated from the crystal structure.

[11] In spite of the potential errors arising from the back-extrapolation, Table I shows that for all three glasses the Se_P fraction is significantly higher than predicted from the cluster model (scenario II). This result is noteworthy, as incomplete inversion of the ^{31}P nuclei by imperfect 180° pulses would lead to underestimates.

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"Outer-Sphere" Oxidation of Nonconjugated Dienes by Simple Iron(III) Complexes: A New Mechanistic Consideration for Oxidation of Arachidonic Acid by Lipoxygenase

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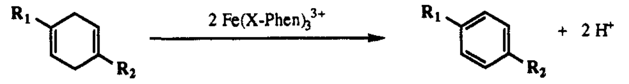
The design of effective inhibitors of the lipoxygenases, critical enzymes for the biosynthesis of medically important eicosanoids from arachidonic acid,[1] would be abetted by knowledge of the catalytic mechanism(s) of these non-heme iron dioxygenases.[2] Recent mechanistic proposals for this enzymatic reaction have centered on "inner-sphere" oxidation, invoking first an Fe(III)-olefin complex and then an organoiron intermediate.[3] However, "outer-sphere" oxidation of arenes[4] and olefins[5] by simple iron complexes has been observed, suggesting that dienes, too, might be oxidized by a noncoordinative process, one that should be kinetically enhanced by structural factors that favor one-electron oxidation of a double bond. For a 1,4-diene, oxidation should be facilitated by structural effects that maximize π interactions between the formally unconjugated double bonds. For example, arachidonic acid, conformationally constrained through enzyme binding to provide maximum π interaction between Δ^5 and Δ^8 of the tetraene ("homoconjugation"), might undergo facile noncoordinative oxidation. We report that simple 1,4-dienes, "conformationally locked" by incorporation into a ring, are in fact oxidized by simple Fe(III) complexes in a process fully consistent with outer-sphere oxidation in which interaction between the formally nonconjugated double bonds is clearly indicated.

In a definitive series of studies, Kochi has demonstrated outer-sphere oxidation of arenes[4,5] and simple olefins[5] by various $Fe(X\text{-phen})_3^{3+}$ species (X-phen = substituted 1,10-phenanthroline) by showing a correlation between the second-order rate constant for substrate oxidation and the difference between the standard oxidation and reduction potentials of the Fe(III) complex and the organic substrate, respectively (ln k_2 vs $E°_{ox} - E°_{red}$), and by demonstrating rate-limiting electron transfer in the overall oxidation process. Using his paradigm, we demonstrated outer-sphere oxidation for a series of simple 1,4-dienes by $Fe(X\text{-phen})_3^{3+}$ (Scheme I).

Scheme I



1 $R_1 = R_2 = H$
2 $R_1 = H; R_2 = CH_3$
3 $R_1 = H; R_2 = OCH_3$
4 $R_1 = R_2 = OCH_3$

Table I. Rates for Oxidation of Dienes by $Fe(X\text{-phen})_3^{3+}$ Complexes

complex[a]	*T*, °C	olefin[a]	k_2,[b] s^{-1} M^{-1}	rel rate
$Fe(phen)_3^{3+}$	+23	1,4-CHD (**1**)	$5.2\ (1.0) \times 10^{-4}$	1.0
	+23	1,3-CHD	9.2 (1.9)	1.8×10^4
	+23	1-Me-1,4-CHD (**2**)	$4.3\ (0.7) \times 10^{-3}$	8.2
$Fe(phen)_3^{3+}$	−30	1-Me-1,4-CHD (**2**)	$4.3\ (1.6) \times 10^{-4}$	1.0
	−30	1-MeO-1,4-CHD (**3**)	0.28 (0.07)	6.6×10^2
$Fe(4,7\text{-}Ph_2\text{-}phen)_3^{3+}$	−30	1-MeO-1,4-CHD (**3**)	$2.3\ (0.9) \times 10^{-3}$	1.0
	−30	$1,4\text{-}(MeO)_2\text{-}1,4$-CHD (**4**)	$3.7\ (1.7) \times 10^1$	1.6×10^4

[a] Phen = 1,10-phenanthroline; CHD = cyclohexadiene. [b] Standard deviations in parentheses.

Table II. Oxidation of 1-Me-1,4-CHD (**2**) by Fe(III) Complexes of Varying Reduction Potential

complex	$E_{1/2}$,[a] V	k_2,[b] s^{-1} M^{-1}	rel rate
$Fe(5\text{-}NO_2\text{-}phen)_3^{3+}$	1.18	$1.2\ (0.6) \times 10^{-1}$	28
$Fe(phen)_3^{3+}$	0.98	$4.3\ (0.7) \times 10^{-3}$	1.0
$Fe(4,7\text{-}Ph_2\text{-}phen)_3^{3+}$	0.91	$1.3\ (0.4) \times 10^{-3}$	0.30

[a] For reduction, vs SCE. [b] Standard deviations in parentheses.

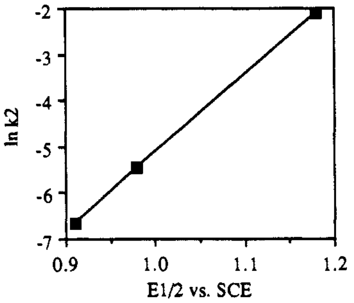


Figure 1. Plot of ln k_2 for oxidation of 1-Me-1,4-CHD (23 °C) vs $E_{1/2}$ of the Fe(III) complex.

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In a typical procedure,[6] 0.1 g of $[Fe(phen)_3](ClO_4)_3$ was dissolved in 50 mL of rigorously dried acetonitrile. Exactly 2.0 mL of this stock solution was placed in a quartz cuvette, which was then sealed with an air-tight septum. Initial UV absorbances (HP 8452 diode array spectrophotometer) were obtained for Fe(III) (586 nm, ϵ = 500) and background Fe(II) (510 nm, ϵ = 10300) (due to trace water). The sample was then cooled to the appropriate temperature by using a recirculating cooling bath, and 10–20 μL of diene was added by syringe (1,4-dimethoxy-1,4-cyclohexadiene, a solid, was added from a stock solution in acetonitrile). Absorption spectra for Fe(III) and Fe(II) were measured automatically as a function of time. Oxidation of the diene to the arene was confirmed by quantitative GC analysis. Second-order rate constants for oxidation of a series of dienes are shown in Table I. In a corollary series of experiments, the effect of base, pyridine, on the rate of oxidation of 1,4-cyclohexadiene by $Fe(phen)_3^{3+}$ was measured,[7] and it was shown[5a] that electron transfer was rate determining for the overall process. (In typical experiments, no base other than the solvent was present.[8]) Oxidation of 1-methyl-1,4-cyclohexadiene (1-Me-1,4-CHD) by Fe(III) complexes of varying reduction potential[4] was effected, second-order rate constants were measured (Table II), and the correlation obtained between ln k_2 and $E_{1/2}$ was excellent (Figure 1).

Although half-wave oxidation potentials have not been measured for all of the 1,4-dienes studied[9,10] (Table I), the general trend of increasing rate constant with increasing π-donor ability of the substituent group on a single double bond is obvious. Most significant is the observed 10^4 increase in k_2 for **4** vs **3**, effected by replacement of hydrogen on the "unactivated" double bond of 1-MeO-1,4-CHD (**3**) by another donor methoxy group. This large rate enhancement, of the same magnitude as that observed for 1,4-CHD vs its 1,3-isomer,[10] strongly suggests a π interaction between the double bonds of the 1,4-diene in the rate-determining step for the oxidation reaction.[11] (Were the effect of placement of this second substituent merely inductive in nature, an *opposite*, and likely smaller, difference in rates would have been noted.) If not in the isolated molecule, at least in the presence of the Fe(III) oxidant, "homoconjugation" exists for this constrained system. Models for metalloenzyme-catalyzed oxidation of formally nonconjugated substrates must, therefore, consider outer-sphere oxidation to be a viable possibility when conformational mobility exists, which, by enabling "homoconjugative" interactions, lowers the oxidation potential of the conformer below that of its individual components.

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Supplementary Material Available: General reaction scheme, UV spectrophotometric data for oxidation of the 1,4-diene to the arene, and data enabling calculation of rate constants for oxidation of the dienes (9 pages). Ordering information is given on any current masthead page.

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(6) For example, Fe(III), 1.34 × 10^{-3} M; 1,4-cyclohexadiene, 0.177 M; k_{obsd} = 9.94 × 10^{-5} s^{-1}; k_2 = 5.62 × 10^{-4} s^{-1} M^{-1}; average of four runs, k_2 = 5.2 (±1.0) × 10^{-4} s^{-1} M^{-1}.

(7) For the complete reaction scheme, see supplementary material Figure 1, for which rate constants are given in supplementary material Table 3.

(8) For the use of acetonitrile solvent as a base in an electrochemical oxidation (of an arene), see: (a) Bewick, A.; Edwards, G. J.; Mellor, J. M.; Pons, B. S. *J. Chem. Soc., Perkin Trans. 2* **1977**, *2*, 1952. (b) Bewick, A.; Mellor, J. M.; Pons, B. S. *Electrochim. Acta* **1980**, *25*, 931. Interestingly, electrochemical oxidation of 1,4-cyclohexadiene in methanol or acetic acid yields only benzene, and no trapping products are observed.[9] Thus, apparently, a base even as weak as acetic acid can deprotonate the intermediate cation radical of 1,4-cyclohexadiene oxidation, and a nucleophile as good as methanol cannot intercept it.

(9) The oxidation potentials of a series of double bond substituted norbornadienes correlate with σ_p^+. See: Shono, T.; Ikeda, A.; Hayaishi, J.; Hakozaki, S. *J. Am. Chem. Soc.* **1975**, *97*, 4261.

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(11) A similar transannular interaction has been proposed,[9] based on electrochemically measured oxidation potentials for several nonconjugated dienes.

Detection of Nuclear Overhauser Effects between Degenerate Amide Proton Resonances by Heteronuclear Three-Dimensional Nuclear Magnetic Resonance Spectroscopy

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The key to protein structure determination by NMR lies in the identification of as many ^1H–^1H nuclear Overhauser effects (NOEs[1]) as possible in order to obtain a large set of approximate interproton distance restraints.[2] With the advent of a range of heteronuclear three-dimensional (3D) NMR experiments,[3–6] it has now become possible to obtain complete ^1H, ^{15}N, and ^{13}C assignments and to determine the 3D structures of proteins in the 15–25-kDa molecular weight range.[6,7] Despite these advances, it has remained impossible to observe NOEs between protons with degenerate chemical shifts. Such interactions occur repeatedly, both among aliphatic or aromatic protons and between sequential amide protons in helical proteins. Here we describe a 3D heteronuclear experiment that allows the observation of these NOEs and demonstrate its applicability for calmodulin, a protein of 148 residues and molecular weight 16.7 kDa.

Provided that the chemical shifts of the directly bonded heteronuclei are not degenerate, NOEs between degenerate protons can be detected in a 3D spectrum in which the heteronuclear chemical shifts are labeled in the F_1 and F_2 dimensions and the ^1H chemical shift is labeled in the F_3 dimension. In the case of NOEs involving NH protons, a ^1H–^{15}N HMQC–(^1H–^1H NOESY mixing)–^1H–^{15}N HMQC 3D experiment yields the necessary information. The pulse scheme is shown in Figure 1. This type of experiment is related to the triple-resonance $^{13}C/^{15}N$-edited 4D NOESY experiment[8] which permits observation of NOEs between degenerate ^{15}N and ^{13}C attached protons. Briefly, in the new 3D experiment heteronuclear multiple quantum coherence[9] is generated during the t_1 period, which is subsequently converted back into transverse ^1H magnetization. Thus, at the end of the t_1 period, ^1H magnetization is modulated by the shift of its attached ^{15}N nucleus. During the subsequent NOESY mixing period, τ_m, ^1H magnetization is transferred to its immediate spatial

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(1) Abbreviations used: NOE, nuclear Overhauser effect; 3D, three-dimensional; HMQC, heteronuclear multiple quantum coherence; NOESY, nuclear Overhauser enhancement spectroscopy.

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