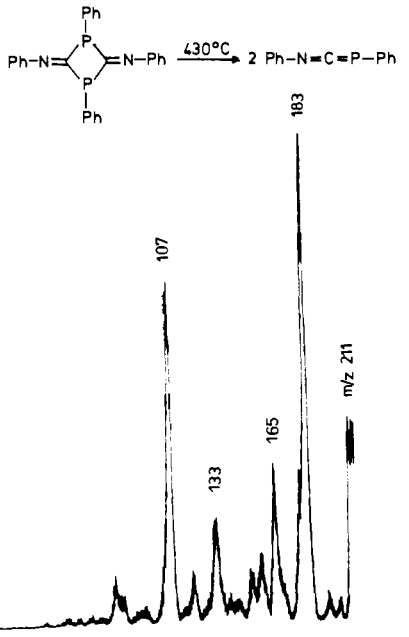

Figure 2. CID mass spectrum of m/z 211 from the pyrolysis of **3a** at 430 °C.

2e, the latter formed by the addition of phenyl isothiocyanate to the alkyl- or aryl(trimethylsilyl)phosphine.[10]

If the diphosphetanes **3** are, in fact, dimers of (iminomethylidene)phosphines, the monomers might be regenerated via a cycloreversion under suitable reaction conditions. This, indeed, is readily achieved by flash vacuum pyrolysis of **3**. Product formation was monitored by low-temperature IR spectroscopy, and a pyrolysis unit has also been attached to the ion source of a reversed-geometry Varian MAT 311A mass spectrometer[11] equipped with a collision chamber for obtaining CID (collisionally induced dissociation) spectra of initial pyrolysis products.

2,4-Bis(phenylimino)-1,3-diphenyl-1,3-diphosphetane (**3a**) (mp 139 °C) was vaporized at 139–145 °C (10^{-5} torr). When the pyrolysis temperature was increased to 400 °C, an IR band at 1853 cm^{-1} appeared in the pyrolyzate condensed at −196 °C. The maximum intensity of this band was observed at a pyrolysis temperature at 480 °C (Figure 1). On warming the pyrolyzate to −55 °C, the intensity of the 1853-cm^{-1} band started decreasing; at the same time, a band at 1560 cm^{-1} due to the starting material **3a** started increasing. This process was complete at −30 °C, and after warming to room temperature **3a** was recovered in better than 90% yield.[12]

These observations indicate that **3a** dissociates into two molecules of the (iminomethylidene)phosphine **4a** on gas-phase pyrolysis. The monomeric **4a** reverts to **3a** above −55 °C in the solid state. The strong band at 1853 cm^{-1} in the IR of **4a** is assigned to the asymmetric stretching vibration of the P=C=N moiety. For comparison, carbodiimides (RN=C=NR) absorb near 2100 cm^{-1}.

The analogous pyrolysis of **3b** (mp 220 °C) at 480 °C (sublimation temperature 110 °C, increasing to 200 °C in 35 min) gave rise to a strong IR band at 1839 cm^{-1} ascribed to **4b**. This material started redimerizing to **3b** at −25 °C, a process that was complete at 0 °C. Comparable results were obtained with the precursors **3c–e**.

The IR assignments were corroborated by using the mass spectrometry reactor. With increasing pyrolysis temperature, the $M^{+\cdot}$ peak due to **3a** decreased, while that of **4a** increased. The CID mass spectrum of **4a** obtained at 430 °C is shown in Figure 2. The base peak at m/z 183 may be ascribed to the dibenzophospholyl cation, $C_{12}H_8P^+$, which is typical of diphenylated phosphorus compounds.[13] In the pyrolysis of **3b** the parent peak of the precursor (m/z 382) disappeared at 490 °C, while that of **4b** (m/z 191) reached maximum intensity. Here, the spectrum is dominated by a loss of isobutene to give m/z 135, formally corresponding to $PhN{=}C{=}Ph^{+\cdot}$ or $PhNH{-}C{\equiv}P^{+\cdot}$.

We are continuing the studies of (iminomethylidene)phosphines, in particular cycloaddition reactions and attempts to obtain other phosphorus-containing cumulenes, e.g., R–P=C=O.

Registry No. 1a, 24103-42-2; **1b**, 87218-80-2; **1c**, 87729-47-3; **1d**, 87729-48-4; **1e**, 87729-49-5; **2b**, 87729-50-8; **2e**, 87729-51-9; **3a**, 87729-52-0; **3b**, 87218-81-3; **3c**, 87729-53-1; **3d**, 87729-54-2; **3e**, 87729-55-3; **4a**, 87729-56-4; **4b**, 87218-77-7; **4c**, 87729-57-5; **4d**, 87729-58-6; **4e**, 87729-59-7.

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(12) The band at 2120 cm^{-1} in Figure 1 is due to phenyl isocyanide, formed in a competing thermal fragmentation of **3a**. This material evaporates during warm-up and thus does not contaminate the final product. A yield of ca. 10% of phenyl isocyanide was obtained by distilling it into a cold trap and subsequently identifying it by comparison with an authentic sample.
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Enzyme System Generation of Singlet ($^1\Delta_g$) Molecular Oxygen Observed Directly by 1.0–1.8-μm Luminescence Spectroscopy

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Since the discovery of the chemical generation of singlet oxygen,[1,2] numerous attempts have been made to demonstrate the generation of singlet oxygen in biological systems. Using our ultrasensitive 1.2-μm region spectrometer, we now report the observation of a strong singlet Δ oxygen luminescence emission in the IR (Figure 1) produced in the decomposition of hydrogen peroxide by the enzymes *lactoperoxidase*, *catalase*, and *chloroperoxidase*. Preliminary reports on kinetic studies of lactoperoxidase-generated singlet oxygen[3] and a spectroscopic study of chloroperoxidase-generated singlet oxygen[4] have been given recently.

The *lactoperoxidase* system consists of a 0.01 μM/mL solution of lactoperoxidase (from milk, lyophilized; Sigma) in 0.01 M acetate buffer at pH + pD of 4.5 and 0.80 M KBr as a cofactor (Mallinckrodt, Analytical Reagent) at room temperature. The solvent is a 1:1 ratio of H_2O and D_2O. The experimental conditions are chosen to optimize singlet molecular oxygen emission; see the chemical scavenger studies of singlet molecular oxygen by Piatt et al.[5] and by Rosen and Klebanoff.[6] The enzyme and the hydrogen peroxide solutions are mixed in a 1:1 ratio under argon pressure into an optical cell with an overflow.

The luminescence emission spectrum of the lactoperoxidase/H_2O_2 consists of a single emission band (Figure 1A) with a peak at 1.28 μm (1280 nm), and a full bandwidth at half-maximum of 251 cm^{-1}. There is also the suggestion of a broad underlying band which will not be discussed further here. Other singlet molecular oxygen luminescence sources have been characterized

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(2) Cf.: "Singlet Molecular Oxygen"; Schaap, A. P., Ed.; Dowden, Hutchinson and Rose: Stroudsburg, PA, 1976.
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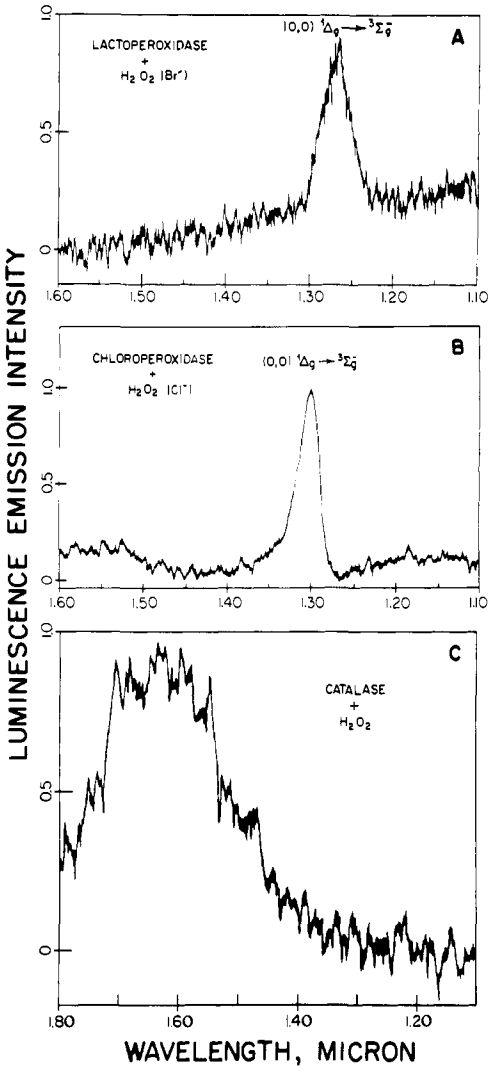


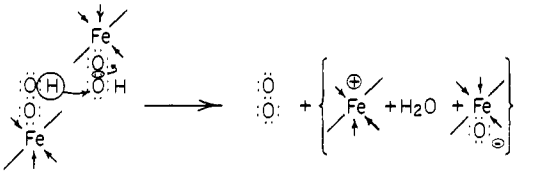
Figure 1. IR luminescence emission of singlet Δ molecular oxygen at 298 K generated by (A) lactoperoxidase (0.01 μM/mL) with H_2O_2 (0.25 mM) at pH + pD of 4.5, acetate buffer, Br^- (0.80 M), solvent 1:1 ratio of H_2O:D_2O; (B) chloroperoxidase (20 μM/mL) with H_2O_2 (10%) phosphate buffer, pH 2.85, Cl^- (0.07 M); (C) Catalase (1.00 mM/mL) with H_2O_2 (5%) in phosphate buffer (0.10 M, pH 7.6).

with the monochromator-germanium photodetector used in this experiment; e.g., photosensitized oxygen emits a narrow strong band at 1.28 μm,[7b] and the inorganic chemiluminescence reaction of H_2O_2/OCl^- yields a sharp band at 1.29 μm.[8] It is important to note that apart from the singlet molecular oxygen electronic state there is *no* other molecular electronic state capable of emitting a narrow single band emission in the 1.28-μm region. (Instrumental sensitivity characteristics shift the 1.268-nm gas-state emission band of singlet Δ oxygen to 1.28 nm in this apparatus.) Thus the strong emission at 1.28 μm shown in Figure 1A, originating in the luminescence reaction of lactoperoxidase with hydrogen peroxide, can be assigned with confidence to the (0,0) vibronic band of the $^1\Delta_g \rightarrow {}^3\Sigma^-_g$ transition of molecular oxygen.

Figure 1B shows the luminescence emission spectrum of the reaction of *chloroperoxidase* with hydrogen peroxide. There are three bands—a strong band at 1.30 μm identified positively as the (0,0) $^1\Delta_g \rightarrow {}^3\Sigma^-_g$ transition of molecular oxygen in a previous communication[4] and a possible weak band extending from the long-wavelength edge of the monochromator at 1.60 μm to 1.45 μm. It is suggested here that the latter weak infrared emission has the same origin as the 1.64-μm emission band of catalase seen in Figure 1C.

Figure 1C is the luminescence emission spectrum of *catalase* (Boehringer, Mannheim, GmbH) with hydrogen peroxide. To observe the emission beyond 1.60 μm, the monochromator was fitted with a high-intensity Bausch and Lomb grating blazed for 2.0 μm, in place of the grating blazed for 1.0 μm used for the two previous spectra. This modification extended the monochromator range from 1.6 to 1.8 μm. Figure 1C shows only one band with a peak at 1.64 μm and a full bandwidth at half maximum of 755 cm^{-1}, with very little indication of the 1.28-μm singlet Δ molecular oxygen emission. A number of possible molecular electronic origins can be suggested for this luminescence emission on consideration of the reaction specifics.

Catalase is a ferric heme tetramer with Fe^{3+} at the active site. The active-site geometry is not known in detail; however, kinetic studies of the relative decomposition rate of H_2O_2, CH_3OOH, and CH_3CH_2OOH indicate that catalase is a sequestered enzyme with stringent size limitation on substrate access to the active site.[9,10] Chance and Herbert[11] suggest the involvement of two Fe^{3+} heme sites in hydrogen peroxide decomposition. Isotopic studies by Jarnagin and Wang[12] that show no ^{18}O scrambling occurs in the decomposition; the molecular oxygen evolved originates from a *single* H_2O_2 molecule. Dounce and others[9] propose a hydride ion transfer from one hematin-bound H_2O_2 to the other:



This mechanism requires the release of spin-paired O_2, thus a singlet molecular oxygen.

One interpretation of the chemiluminescence emission shown in Figure 1C is therefore that the observed band at 1.64 μm arises from an environmentally perturbed singlet molecular oxygen emission (0,0) $^1\Delta_g \rightarrow {}^3\Sigma^-_g$. A parallel spectral shift is observed in solvation of singlet oxygen. Recently Chou and Khan[7a] have found very weak but distinct red-shifted emission bands of solvated singlet ($^1\Delta_g$) oxygen in a series of halogenated hydrocarbon solvents.

Another interpretation of the 1.64-μm emission of catalase is that the emitting species is *not* singlet ($^1\Delta_g$) oxygen but that the emission originates instead from an energy transfer from $^1\Delta_g$ molecular oxygen to the Fe-heme coordination complex at the active site; thus the Fe-heme complex would become the light-emitting species. Extended research on the spectroscopic properties of the Fe-heme complex would be needed to make this interpretation concrete.

In the cases of lactoperoxidase and chloroperoxidase, free singlet oxygen is generated. In vitro chloroperoxidase uses hydrogen peroxide as an oxygen source, with Cl^- anion as a cofactor in a typical singlet oxygen type of reaction, e.g., methionine oxidation.[13] Lactoperoxidase, a constituent of milk and saliva, with hydrogen peroxide and Br^- or I^- cofactor is a highly effective antimicrobial agent against bacteria, fungi, and viruses[14-16] consistent with previous speculation concerning the physiological use of singlet oxygen. These peroxidase reactions requiring halogen ion cofactors are highly suggestive of the inorganic H_2O_2/OCl^- reaction where the scission of the Cl^- anion from the chloroperoxy reaction in-

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(10) Chance, B. *J. Biol. Chem.* **1949**, *179*, 1341.
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termediate $OOCl^-$ leaves a spin-paired oxygen molecule, thus a singlet excited state.[17] Catalase, however, functions chiefly in the decomposition of hydrogen peroxide to molecular oxygen without the presence of halogen ion cofactors, and in this system, a bound singlet molecular oxygen is generated predominantly, which then radiatively decays either directly as a perturbed species or via an energy-transfer mechanism.

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Rearrangement of Bridging Alkylidyneiron Complexes to Bridging Alkenyliron Complexes

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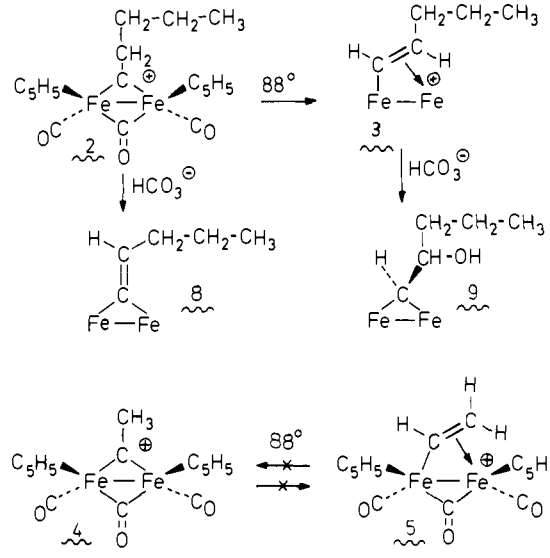
The electrophilic diiron methylidyne complex $[(C_5H_5)_2(CO)_2Fe_2(\mu\text{-}CO)(\mu\text{-}CH)]^+PF_6^-$ (**1**)[1] reacts rapidly with alkenes by adding the methylidyne C–H bond across the C=C double bond to produce μ-alkylidyne complexes.[2] In the course of examing the possibile reversibility of this hydrocarbation reaction, we found that μ-alkylidyne complexes rearrange to μ-alkenyl complexes in a reaction whose rate is extremely sensitive to the degree of alkyl substitution at the carbon α to the carbyne carbon.

When the μ-pentylidyne complex $[(C_5H_5)_2(CO)_2Fe_2(\mu\text{-}CO)(\mu\text{-}C\text{–}CH_2CH_2CH_2CH_3)]^+PF_6^-$ (**2**)[3,4] was heated in the solid state or in dilute CD_2Cl_2 solution, no (<5%) reversal to 1-butene and **1** was detected. Instead, upon heating to 88 °C for 29 h, solid



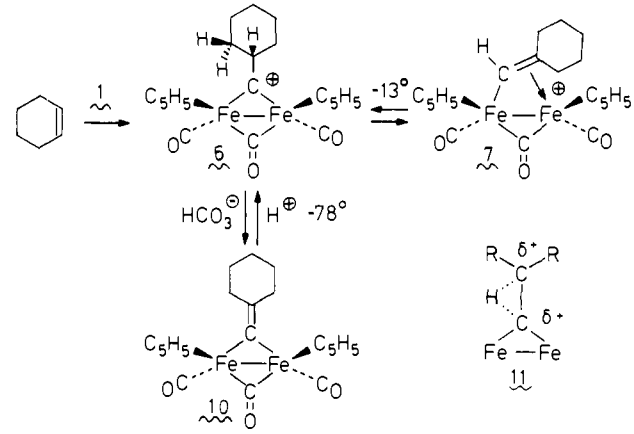
2 rearranged to the μ-1-pentenyl complex $[(C_5H_5)_2(CO)_2Fe_2(\mu\text{-}CO)(\mu\text{-}\eta^1,\eta^2\text{-}(E)\text{-}CH{=}CHCH_2CH_2CH_3)]^+PF_6^-$ (**3**)[4] in 89% yield after recrystallization.[7] The rearrangement of **2** to **3** in CD_2Cl_2 at 88.0 ± 0.1 °C was followed by ^1H NMR observation of the Cp resonances; the first-order rate constant was found to be 2.9 ± 0.5 × 10^{-4} s^{-1} which corresponds to $\Delta G^{\ddagger}$ = 27.1 ± 0.2 kcal. Rearrangement of the related α-deuterated compound $[(C_5H_5)_2(CO)_2Fe_2(\mu\text{-}CO)(\mu\text{-}C\text{–}CD_2CH_2CH_2CH_3)]^+CF_3SO_3^-$ (**2**-d_2)[8] gave $[(C_5H_5)_2(CO)_2Fe_2(\mu\text{-}CO)(\mu\text{-}\eta^1,\eta^2\text{-}(E)\text{-}CD{=}DCCH_2CH_2CH_3)]^+CF_3SO_3^-$ (**3**-d_2) in which >95% of the deuterium was located in the vinylic sites as established by ^2H NMR; this indicates that the net 1,2-hydride shift involves only the protons on the carbon α to the carbyne carbon of **2**.

The structure of **3** was established spectroscopically.[4] Separate signals are seen for the nonequivalent C_5H_5 rings of **3** in the low-temperature ^1H NMR at δ 5.83 and 5.62 and in the low-temperature ^{13}C NMR at δ 92.6 and 89.8; at room temperature a fluxional process leads to single coalesced peaks.[9] The proton on the α-vinyl carbon of **3** appears characteristically downfield at δ 12.06 (d, J_{trans} = 11.8 Hz) and the proton of the β-vinyl carbon appears as a multiplet at δ 3.66. In the ^{13}C NMR of **3**, the α- and β-vinyl carbons appear at δ 175.4 and 96.7. Similar spectra for μ-vinyl compounds have been observed by Pettit[6] and Dyke.[10]

In contrast, attempted rearrangement of the parent ethylidyne complex **4**[5,6] by heating at 88 °C for 100 h gave no detectable isomerization (<5%) to μ-vinyl complex **5** but led to 50% decomposition. When the potential rearrangement product **5**[6,10] was independently synthesized and heated at 88 °C for 20 h, no ethylidyne complex **4** was observed but 80% decomposition of **5** had occurred. Apparently, an α-alkyl substituent on the μ-alkylidyne complexes can greatly accelerate the rearrangement to a μ-alkenyl complex.

The possibility that two α-alkyl substituents might further accelerate the rearrangement of μ-alkylidyne complexes to μ-alkenyl complexes caused us to reassess our interpretation of the reaction of **1** with 1,2-disubstituted alkenes. Earlier we had found that cyclohexene, cyclopentene, and *cis*- and *trans*-2-butene all reacted with **1** to give mixtures of μ-alkylidyne complexes and μ-alkenyl complexes. We postulated that the μ-alkylidyne complexes were formed by direct 1,2-addition of the CH bond of **1** to the alkene and that the μ-alkenyl complexes were formed via a hydrogen migration of an intermediate carbocation.[11] If these two products rapidly interconvert either might be the initial product of reaction of the 1,2-disubstituted alkene with **1**.

When the 1.4:1.0 mixture of μ-alkylidyne **6** and μ-alkenyl **7** complexes obtained from reaction of **1** with cyclohexene was heated at 88 °C in the solid state or in CD_2Cl_2 solution, no change in isomer ratio was seen. Even when partial decomposition



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(3) **2** was prepared by the reaction of **1** with 1-butene in 76% yield or by reaction of *n*-BuLi with $(C_5H_5)_2(CO)_4Fe_2$ followed by acidification with aqueous HPF_6 in 26% yield.[5,6]

(4) See supplementary material for full spectral and analytical characterization.

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(7) The analogous $[(C_5H_5)_2(CO)_2Fe_2(\mu\text{-}CO)(\mu\text{-}CCH_2CH_3)]^+PF_6^-$ complex[2] underwent a similar rearrangement to $[(C_5H_5)_2(CO)_2Fe_2(\mu\text{-}CO)(\mu\text{-}\eta^1,\eta^2\text{-}(E)\text{-}CH{=}CHCH_3)]^+PF_6^-$ (84% yield, ≥95% conversion) upon heating at 88 °C for 30 h in the solid state.

(8) **2**-d_2 was prepared by deuterium exchange of the vinylic protons of **8** using CF_3COOD/D_2O. The product **8**-d_1 was protonated with CF_3SO_3D yielding **2**-d_2 in 49% yield for the two steps.

(9) Casey, C. P.; Marder, S. R., unpublished results.

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(11) In contrast, 1-methylcyclohexene reacts with **1** to give a μ-alkenyl compound via exclusive carbon migration.[2]

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