Table II. Effect of the Concentration of Isopropyl Alcohol on Oxidation Rates at 25° [a]

$[HClO_4]$, M	[ROH], M	$[PA_T]$, M	k_{exptl}, s^{-1}	$k_{exptl}/[ROH]$ (M^{-1} s^{-1})
1.85	0.117	0.0220	0.0162	0.14
1.85	0.0466	0.0220	0.0062	0.13
1.85	0.0230	0.0220	0.0033	0.14
1.85	0.00930	0.0220	0.0013	0.13
1.85	0.00465	0.0220	0.00058	0.12
0.102	0.371	0.0712	0.055	0.15
0.102	0.148	0.0712	0.0187	0.13
0.102	0.0742	0.0712	0.0101	0.14
0.102	0.0371	0.0712	0.0042	0.11
0.102	0.00371	0.0712	0.00043	0.11
0.102	0.00182	0.0712	0.000196	0.11
0.102	0.000910	0.0712	0.000121	0.13
0.102	0.000456	0.0712	0.000057	0.12
0.0118	0.131	0.118	0.0034	0.026
0.0118	0.0131	0.118	0.00033	0.025
0.0118	0.00652	0.118	0.000156	0.024

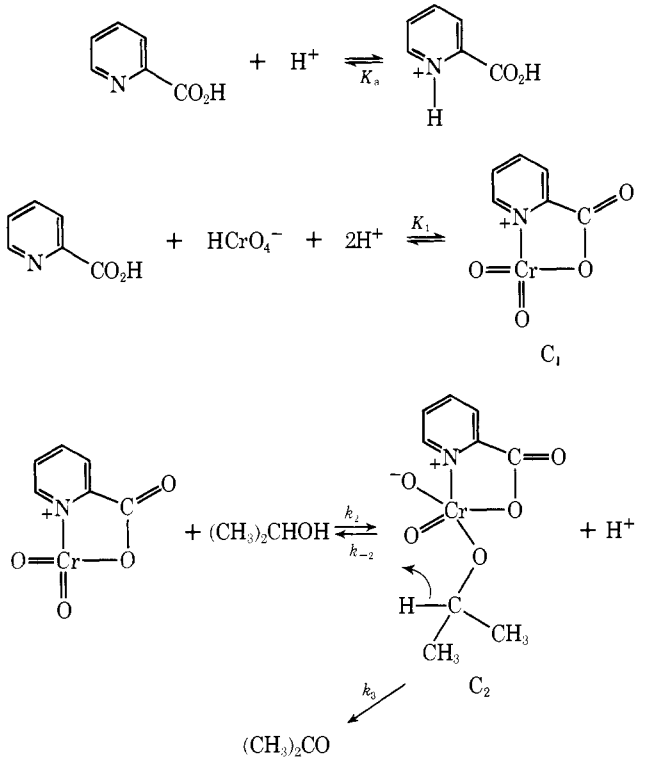
[a] Initial chromium(VI) = 6.24×10^{-4} M.

Table III. Isotope Effect in the Oxidation of 2-Deuterio-2-propanol with Different Acidities[a]

10^3H^+, M	k_H/k_D	10^3H^+, M	k_H/k_D
8.22	1.5	204	4.3
16.8	1.9	318	5.1
44.5	2.9	550	5.5
95.0	3.6		

[a] Picolinic acid (total) = 0.0472 M; isopropyl alcohol = 0.0261 M.

Scheme I



the oxidation, but nicotinic acid, isonicotinic acid, or 2-pyridylacetic acid are ineffective (Table I).

At constant acidity, the reaction is first order in chromium(VI), isopropyl alcohol, and in picolinic acid (Figure 1). It is also first order in isopropyl alcohol throughout the entire range of acidities (Table II). The acidity dependence is second order in hydrogen ions at low acidities and approaches a zero-order acidity dependency at high acid concentrations (Figure 2).

The deuterium isotope effect is also acidity dependent (Table III), indicating that the rate limiting step of the reaction involves the breaking of the carbon–hydrogen bond in the alcohol at high but not at low acidities.

These observations are consistent with the reaction mechanism shown in Scheme I, which involves the formation of a negatively charged termolecular complex (C_2) and its oxidative decomposition to reaction products. The carbon–hydrogen bond of the alcohol is broken in the latter step. The hydrolysis of the complex C_2 requires a proton and will therefore be acid catalyzed, while rate of the oxidative decomposition of C_2 is not. Consequently, the formation of C_2 will be reversible at high acidities but rate limiting at low acid concentrations.

The mechanism given in Scheme I leads to the rate law (eq 1)

$$k_{cat} = \frac{K_a K_1 k_2 k_3 [PA_T][ROH][H^+]^2}{k_3 K_a + (k_{-2}K_a + k_3)[H^+] + k_{-2}[H^+]^2} \quad (1)$$

for the catalyzed reaction; the rate law can be derived from eq 2–6:

$$-\frac{d[HCrO_4^-]}{dt} = k_{cat}[HCrO_4^-] = k_3[C_2] \quad (2)$$

$$[C_2] = \frac{k_2[C_1][ROH]}{k_{-2}[H^+] + k_3} \quad (3)$$

$$[C_1] = K_1[PA][HCrO_4^-][H^+]^2 \quad (4)$$

$$[PA] = \frac{[PA_T]K_a}{K_a + [H^+]} \quad (5)$$

$$[PA_T] = [PA] + [PAH^+] \quad (6)$$

where PA and PAH^+ represent picolinic acid and its protonated form; $K_a = 0.098$ is the acid dissociation constant of picolinic acid.[5] Equation 3 is obtained from applying the steady-state approximation to C_2. Figure 2 shows an excellent agreement between experimental points and the calculated curve.

We believe that the reason for the catalytic activity of picolinic acid depends on its ability to stabilize intermediate chromium valence states, probably chromium(IV), through complex formation.

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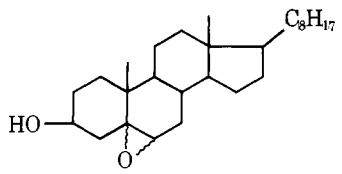
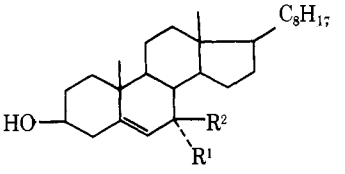
Singlet Molecular Oxygen from Hydrogen Peroxide Disproportionation[1]

Sir:

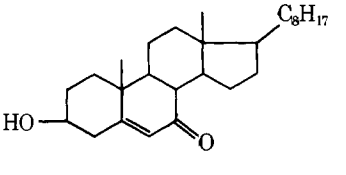
Despite the long recognized association of "active oxygen" with aqueous H_2O_2 solutions, the electronic excitation state of molecular oxygen evolved in base catalyzed (eq 1 and 2) or spontaneous (eq 3) disproportionation of H_2O_2



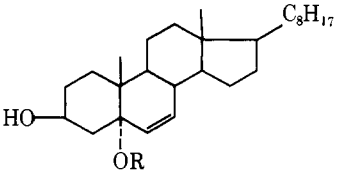
I, 5α,6α
II, 5β,6β



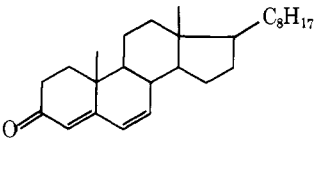
IIIa, R^1 = OOH; R^2 = H
IIIb, R^1 = OH; R^2 = H
IIIc, R^1 = H; R^2 = OOH
IIId, R^1 = H; R^2 = OH
IIIe, R^1 = $OCOC_{17}H_{35}$; R^2 = H



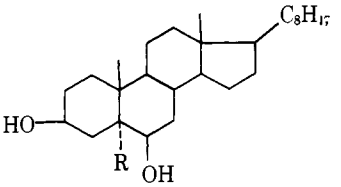
IV



Va, R = OH
Vb, R = H



VI



VIIa, R = H
VIIb, R = OH

has not been examined. Chemical and spectral data support singlet molecular oxygen ($^1O_2$) formation from H_2O_2 upon oxidation with hypohalite or halogen[2] and in certain other reactions[3,4] but not in the decomposition of H_2O_2 catalyzed by catalase[5] or horse-radish peroxidase[6] as speculated.[7] We now demonstrate $^1O_2$ formation in the base catalyzed disproportionation of H_2O_2.

$$H_2O_2 + HO^- \rightleftharpoons HOO^- + H_2O \quad (1)$$

$$H_2O_2 + HOO^- \longrightarrow H_2O + HO^- + \begin{Bmatrix} {}^1O_2 \\ {}^3O_2 \end{Bmatrix} \quad (2)$$

$$2H_2O_2 \longrightarrow 2H_2O + \begin{Bmatrix} {}^1O_2 \\ {}^3O_2 \end{Bmatrix} \quad (3)$$

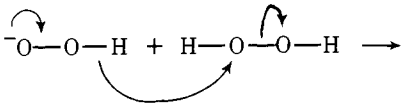
Aqueous sodium stearate dispersions (pH 8.5–9.5) of cholesterol (1 mg/ml) containing 4 mM H_2O_2 heated at 50° for 6 h under nitrogen gave the isomeric 5,6-epoxycholestan-3β-ols I (0.2% on unrecovered cholesterol) and II (1.5%) in the same manner observed using cumene or sterol hydroperoxides as oxidants.[8] However, we isolated other products including the cholesterol 7-hydroperoxides IIIa and IIIc (0.4%) and their thermal decomposition products IIIb (6.1%), IIId, (5.9%), and IV (9.0%),[9] 5α-cholest-6-ene-3β,5-diol (Vb) (1.3%), cholesta-4,6-dien-3-one (VI) (1.0%), 7α-stearatoxycholest-5-en-3β-ol (IIIe) (4.3%), 5α-cholestane-3β,6β-diol (VIIa) (0.9%), and 5α-cholestane-3β,5,6β-triol (VIIb) (0.4%).[10] The 7-hydroperoxides IIIa and IIIc were the first products detected.

By monitoring reaction progress and reintroduction of sterols I–VII into the system it was demonstrated that the products resulted from four competing reactions of cholesterol and several sequential reactions thereafter: (i) epoxidation to I and II with their hydration to VIIb, (ii) formal hydration to the 3β,6β-diol VIIa (not formed from I or II), (iii) free radical oxidation by ground-state molecular oxygen ($^3O_2$) to IIIa and IIIc with their subsequent decomposition to IIIb, IIId, and IV, and (iv) attack of $^1O_2$ yielding putatively the 5α-hydroperoxide Va (undetected) and its unique alteration products Vb, VI, and IIIe.

Reaction of cholesterol with air in these dispersions also yielded the 7-hydroperoxides IIIa and IIIc as initial products in a typical free radical oxidation[8,11] with no 5α-hydroperoxide Va (inferring participation of $^1O_2$)[12] nor its thermal decomposition products Vb or VI[9] nor IIIe detected. Pure 7α-hydroperoxide IIIa in the system epimerized to IIIc,[9a,13] and both IIIa and IIIc decomposed to IIIb, IIId, and IV with no 5α-hydroperoxide Va nor its derivatives Vb, VI, or IIIe detected. Pure 5α-hydroperoxide Va in the system was rapidly isomerized to the 7α-hydroperoxide IIIa (thereby yielding also IIIb, IIIc, IIId, and IV), degraded to Vb and VI, and transformed to the 7α-stearate ester IIIe, thus providing a distinctive pattern of products not formed from other sterols I, II, III, IV, or VII.

Accordingly, the first formed products IIIa and IIIc in the H_2O_2 system must derive from disproportionation of H_2O_2 to $^3O_2$ and water with the $^3O_2$ then oxidizing cholesterol as previously established.[8,11] The products Vb, VI, and IIIe unique to Va infer formation of Va in the H_2O_2 system and thereby the attack of $^1O_2$ on cholesterol. Were the hydroperoxides IIIa, IIIc, and Va indeed not formed by attack of $^3O_2$ and $^1O_2$ derived from H_2O_2 disproportionation, alternative unprecedented attack of peroxide anion on the cholesterol B-ring must be posited, with ultimate extrusion of hydride ion, a matter unlikely in these systems.[14]

Derivation of $^1O_2$ from H_2O_2 may be via a concerted two-electron transfer process (eq 4) as previously suggested for the oxidation of H_2O_2 by bromine or hypohalite.[3d] From



$$^1O_2 + H_2O + HO^- \quad (4)$$

the amounts of Vb, VI, and IIIe relative to all products III, IV, V, and VI attributed to H_2O_2 dismutation, a ratio of $^1O_2$:$^3O_2$ of 1:3 was indicated.[15] Even higher $^1O_2$ yields may obtain, for isomerization of Va to IIIa and of Vb to IIIb reduced the amounts of Va and Vb which could be isolated.

The same product patterns (I, II, III, IV, Vb, VI, VII) were obtained at 70, 50, and 37° but no products were detected at 25° within the times studied. In that cholesterol dispersions at 37° simulate the state of cholesterol in animal tissue, our results suggest a new direction for consideration of oxygen toxicity in tissue, that of fortuitous release of $^1O_2$ from H_2O_2 not rapidly destroyed by catalase or peroxidases. Furthermore, our findings provide a basis for rationalizing numerous controversial reports of $^1O_2$ arising from superoxide radical anion by spontaneous[16] or enzyme catalyzed[17] dismutation. The contribution of base catalyzed disproportionation of H_2O_2 (formed as the second product

of superoxide anion dismutation) to $^1O_2$ levels in systems containing superoxide anion should be carefully assessed.

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Susceptibility Studies of Laccase and Oxyhemocyanin Using an Ultrasensitive Magnetometer. Antiferromagnetic Behavior of the Type 3 Copper in *Rhus* Laccase

Sir:

The laccases are enzymes (*p*-diphenol: O_2 oxidoreductase, EC 1.10.3.2) that contain four copper atoms in three distinct sites.[1] In addition to the two EPR-detectable centers (types 1 and 2), there are two EPR-nondetectable (or type 3) copper atoms. The type 3 coppers, which are responsible for an absorption band at 330 nm, together function as a two-electron acceptor in the enzyme. Most investigators have considered a type 3 site to contain either an antiferromagnetically coupled Cu(II) pair[2] or two Cu(I) units associated with another two-electron acceptor (e.g., a disulfide[3]). Another possibility that suggests itself from work[4] on D-galactose oxidase is a low-spin Cu(III)–Cu(I) unit.

The temperature dependence of the magnetic susceptibility is the best method for obtaining direct evidence on the state of the type 3 copper atoms. For an antiferromagnetically coupled Cu(II) dimer the variation of the susceptibility with temperature will not follow the Curie law ($\chi \propto 1/T$) for paramagnetism, but will exhibit a maximum at a position dependent on the strength of the coupling.[5] Both low-spin Cu(III)–Cu(I) and binuclear Cu(I) units will be diamagnetic and exhibit no temperature dependence of the susceptibility. Previous measurements of χ vs. T have been made for *Rhus vernicifera* laccase, but antiferromagnetic components were not resolved.[6] As very high sensitivity would be required for such resolution, we have measured the temperature dependence of the volume susceptibility of *Rhus* laccase over a wide range with a magnetometer utilizing quantum flux detection methods.

Rhus vernicifera laccase was purified to an A_{280}/A_{614} ratio of 15.4 by a standard method.[7] One sample was dialyzed extensively against distilled, deionized water and then concentrated by ultrafiltration to 1.25 mM with a ratio of 15.6. A second sample was dialyzed for 24 h against 0.001 M EDTA, 25 mM Tris buffer (pH 8.1). This sample was then dialyzed against buffer alone for another 24 h and concentrated to 3.4 mM. At a concentration of 3.4 mM, A_{280}/A_{614} increased to 19, but A_{280}/A_{330} remained constant. Apo laccase was prepared by extensive dialysis against 0.01 M NaCN in Tris buffer. Copper content was checked by atomic absorption and found to be negligible.

The magnetic susceptibilities were measured using an oscillating sample[8] superconducting magnetometer.[9] The instrument was calibrated with a 17 mM $NiCl_2$ solution using $\chi_M^{Ni} = 4434 \times 10^{-6}$ cgs/mol at 20 °C. The high sensitivity of this instrument allows changes in volume susceptibility of 0.5% of the diamagnetism of water to be resolved. Sample volumes of 0.12 ml were measured over a 30–210 K range with a reproducibility of 1.5% of water diamagnetism between runs. Several measurements of protein susceptibilities were made at each temperature and the error was taken as the maximum deviation from the mean. Compensation for sample rod contraction was made by repositioning the sample after every temperature change. Experimental difficulties with the high temperature system prevented obtaining data above 140 K for the 3.4 mM laccase sample.

The measured volume susceptibilities of *Rhus* laccase samples as a function of $1/T$ are presented in Figure 1. Paramagnetism is observed as a decrease from solution diamagnetism. The intersection of the extrapolated low temperature lines gives $\chi_{cc}^{\infty} = -0.669 \times 10^{-6}$ cgs, which is close to the volume susceptibility of water ($\simeq -0.68 \times 10^{-6}$ cgs at 223 K). The slope of the best-fit line for the apo