ax isomers, the ^{11}B NMR spectra were quite simple and showed the expected downfield singlet[15] and two upfield doublets of relative intensity 1:4:4. However, as previously alluded to, the singlet was unusually hard to detect.

In the case of 10-X-1-SB_9H_8 (X = Cl, Br, I) we observe only two doublets of equal intensity when the ^{11}B NMR spectrum is determined with a short delay (10–100 ms) between $\pi/2$ pulses. The expected low-field singlet for the halogenated B site gradually grows in as the delay time is increased above 100 ms and reaches a limiting intensity (1 relative to 4 for the two doublets) at 8–10 s.[16] The intensity recovery for the singlet as the delay time increases follows first-order kinetics and was used to estimate[17] T_1 = 2.8, 3.9, and 2.6 s for X = Cl, Br, I, respectively. The π, τ, $\pi/2$ pulse sequence method of Vold[18] was used to determine the much shorter T_1's for the other B environments (ub = 10, 14, 8 ms; and lb = 24, 22, 19 ms for X = Cl, Br, I, respectively. For comparison, the T_1's for 1-SB_9H_9 are ax = 34, ub = 21, and lb = 40 ms, respectively. The halogen has a decided effect on the relaxation time of the axial B site. This seems even more remarkable in view of the T_1 results when the halogen is in a lb position, such as for 6-Br-1-SB_9H_8 where T_1 = 35 ms for the substituted boron and ranges from 11 to 22 ms for the other positions.

We do not feel that this effect is related to anisotropic molecular reorientation. For symmetric rotors, the relaxation time of a nucleus on the symmetry axis (such as the axial B site of 1-SB_9H_9) depends only on motion perpendicular to that axis.[19] As estimated from moments of inertia, such motion would give rise to longer correlation times (shorter T_1's) for 10-X-1-SB_9H_8 (X = Cl, Br, I) in comparison with 1-SB_9H_9. Moreover, for 6,10-Br_2-1-SB_9H_7, where the symmetric-top nature of 10-Br-1-SB_9H_8 has been removed by substitution of a second halogen on the lb, the ax- and lb-substituted borons still manifest the same large disparity in T_1 values.

The antipodal position of sulfur may somewhat influence T_1; however, we have found no saturation effects when running the spectrum of 12-Br-1-$SB_{11}H_{10}$, the icosahedral closo thiaborane with bromine substituted antipodal to the sulfur.[20] One perhaps salient difference between the axial positions in 1-SB_9H_9 and 1-$SB_{11}H_{11}$ is the coordination number of the deltahedral vertex, five in 1-SB_9H_9 and six in 1-$SB_{11}H_{11}$. However, we have no other axially halogenated 1-heterodecaboranes for comparison ((ax-I-$B_{10}H_9$)$^{2-}$ shows no saturation problems[21] but perhaps the unreported (10-X-1-PB_9H_8)$^-$ or (10-X-1-CB_9H_9)$^-$ would).

A comparison of T_1 values for ^{10}B and ^{11}B led Allerhand, Odom, and Moll to conclude that all contributions other than quadrupolar were negligible for B_2H_6, B_5H_9, and $B(C_2H_5)_3$; it was also estimated that electric field gradients at boron are relatively small.[6] It is our estimation that the electric field gradient is accidentally very small at the axial B site in 10-X-1-SB_9H_8 molecules even though this is not corroborated by EMHO calculations.[22]

In the absence of effective quadrupolar relaxation, it is entirely possible that scalar relaxation, caused by spin–spin interaction of the axial boron and the attached halogen, is dominant here. Since our present experiments only show that T_2 (effective) $\ll T_1$ rather than T_2 (actual) $\ll T_1$, the scalar mechanism cannot be established.[17]

Acknowledgment. We thank the National Science Foundation for partial support of this research (MPS74-0047 A01) and R. R. Sharp for helpful discussions.

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(27) On leave from Colby College, Waterville, Maine 04901.

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Trivalent Copper as a Probable Intermediate in the Reaction Catalyzed by Galactose Oxidase[1]

Sir:

Recently we reported[2] preliminary evidence that Cu(III), or Cu(II) in close proximity to an enzymic radical, may be an important catalytic intermediate in the reaction (eq 1) catalyzed by galactose oxidase (D-galactose: O_2 oxidoreductase; 1.1.3.9), and that a Cu(II) form of the enzyme is inactive catalytically. These conclusions were based especially on the following observations: (1) superoxide dismutase inhibits the galactose oxidase reaction and causes an increase in the intensity of the EPR signal given by the galactose oxidase,[3] and (2) the addition of superoxide or ferricyanide increases the rate of the galactose oxidase reaction, and ferricyanide causes an almost complete disappearance

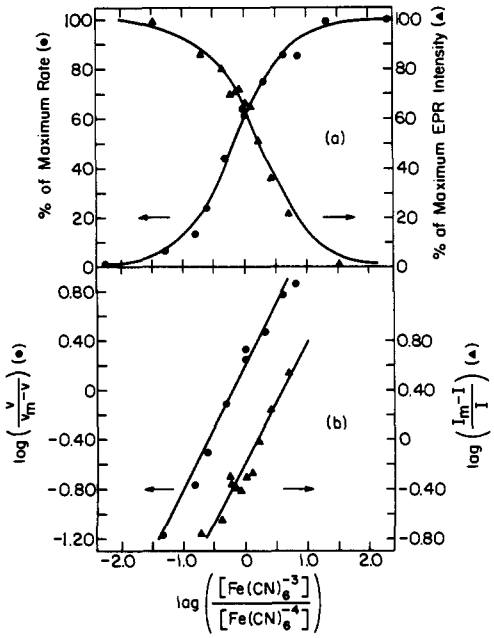
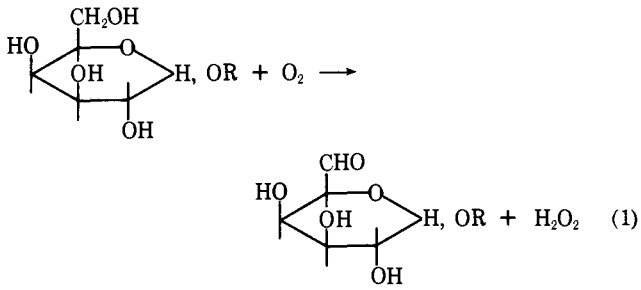


Figure 1. Effects of different ratios of ferricyanide to ferrocyanide concentrations on the rate (●) of the galactose oxidase reaction and on the intensity (▲) of the EPR signal given by the enzymic Cu(II). All solutions had 0.10 M phosphate, pH 7.0, air atmosphere, and had differing ferricyanide and ferrocyanide concentrations varying from 0 to 1 mM (total iron concentration 1–2 mM). The kinetic results were obtained using 0.10 M galactose and 2.7 nM galactose oxidase. For the EPR experiments the galactose oxidase concentration was 30 μM. In the bottom figure (b) the ordinates are essentially log ([E_{ox}]/[E_{red}]); v is the initial rate of O_2 uptake for a particular ratio of ferricyanide to ferrocyanide, and v_m the rate obtained with 1 mM ferricyanide alone. Similarly, I is the EPR signal intensity with a particular ratio of ferricyanide to ferrocyanide and I_m the intensity obtained with 1 mM ferrocyanide. The curves in the upper part (a) and the lines in the lower part (b) of the figure are theoretical calculated for a one-electron change and using the oxidation–reduction potentials given in the text.



(1)

of the EPR signal due to the enzymic copper. An alternate explanation for the earlier EPR results with ferricyanide present is that the enzyme binds ferricyanide close enough to the Cu(II) that its EPR spectrum is eliminated. Although this seemed unlikely (the kinetics indicate that such a species would have to be catalytically active) and is unprecedented in enzymic work, the earlier results could not eliminate such a possibility, and others[4] have suggested this as a possible explanation for our observations. The results reported here show conclusively that such an explanation is not correct.

For all experiments, essentially homogeneous enzyme, prepared by slight modifications[5a] of methods previously described,[5b] was used. Enzyme concentrations were determined from the absorption at 280 nm assuming a molar extinction coefficient of 105 000.[6] Kinetic results were obtained by following oxygen uptake at 25 °C with a Gilson Oxygraph equipped with a Clark electrode. EPR spectra were taken on a Varian E-9 spectrometer under the following conditions: frequency, 9 GHz; microwave power, 30 mW; modulation amplitude, 12.5 G; time constant, 0.3 s; scanning rate, 125 G/min; temperature, 100 K.

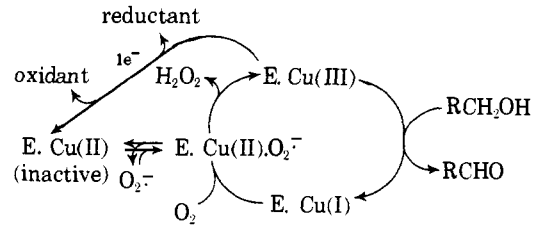
If the kinetic activation by ferricyanide is due to oxidation of some enzymic group it should be replaceable by other oxidants. Under conditions (temp 25 °C, 0.1 M sodium phosphate, pH 7.0, air atmosphere, 0.10 M galactose, and 2.7 nM galactose oxidase) where a rate of oxygen uptake of 0.5 katals/kg[7] is observed in the absence of any activator, the initial rate in the presence of 2 μM Na_2IrCl_6 or 1 mM ferricyanide is 2.6 katals/kg.[8] Thus, the kinetic activation is not due to some specific effect of ferricyanide.

In the earlier studies using ferricyanide alone it was found[2,5] that the amount of activation increases as the concentration of ferricyanide is increased up to a maximum of 1 mM. Subsequently we observed that ferrocyanide inhibits the enzymic reaction and the ferricyanide–ferrocyanide effects are reversible. Thus, if the enzymic reaction is initiated with 1 mM ferricyanide present, the addition of 1 mM ferrocyanide about a minute later causes a decrease in the rate of O_2 consumption. Correspondingly, if the reaction is initiated with 1 mM ferrocyanide present a very slow uptake of O_2 is observed but the addition of 1 mM ferricyanide increases the rate to the same final value as above. As the results in Figure 1a indicate, the amount of activation and the intensity of the EPR signal due to enzymic Cu(II) depend only on the *ratio* of ferricyanide to ferrocyanide concentrations. For any given ratio the same results within experimental error (10%) are obtained regardless of the total concentrations of ferricyanide and ferrocyanide (in the range 0.5–2 mM). Such results are not consistent with the observed effects being due to an enzyme–ferricyanide complex, but they are completely consistent with the oxidation and reduction of some enzymic group which is only catalytically active in the oxidized state. The results shown in Figure 1a can be used to calculate an oxidation–reduction potential for the enzymic group (Figure 1b). Assuming the ferricyanide–ferrocyanide couple has a potential of 0.424 V[9] one obtains a potential at pH 7 for the enzymic group of 0.44 V from the EPR data and 0.41 V from the kinetic data.[10] The fact that the lines in Figure 1b have a slope of 1 indicates that the catalytically active but EPR inactive oxidized form of the enzyme and the catalytically inactive but EPR active reduced form differ by only one electron.

An optical difference spectrum obtained using a solution of galactose oxidase, which had been dialyzed against 1 mM ferricyanide, in one cuvette vs. native enzyme and the dialysate in separate cuvettes showed peaks at 318 and 443 nm with molar extinction coefficients of approximately 5000–7000 at each wavelength. The fact that these peaks are found in the native enzyme as well (but with extinction coefficients of only about 1000)[11] is further evidence for our earlier conclusion[2] that the native enzyme exists partially (15–25%) in the active oxidized state.

Since galactose oxidase contains only one atom of Cu per molecule, the results reported here and earlier[2] require that an enzyme form at an oxidation state higher than the Cu(II) enzyme is a catalytically active species. This is probably a Cu(III) species but Cu(II) in close proximity to some enzymic radical is an alternate possibility. The facts that various nonenzymic Cu(III) compounds are known,[12] and that some[12b,13] carry out reactions closely related to the oxidation of an alcohol to an aldehyde, indicate that the previously postulated mechanism[2] (Scheme I) for the enzymic reaction is reasonable. Thus, in the usual catalytic cycle the enzyme apparently oscillates between the Cu(III) and Cu(I) forms but once in several thousand turnovers superoxide leaks out to give the inactive Cu(II) form. The inactive Cu(II) form and the active Cu(III) form can be inter-

Scheme I



converted by one-electron redoxants such as the iron hexacyanides. The oxidation of the alcohol by the enzymic Cu(III) presumably occurs by a mechanism similar to the oxidation of alcohols by Cr(VI)[2,14] and Cr(V).[14]

The ease by which the Cu(III) form of galactose oxidase is formed suggests that Cu(III) may well be an intermediate in other cuproenzymic reactions also. This seems especially likely in the case of tyrosinase; recent work with a tyrosinase containing only one atom of Cu per molecule[15] indicates that it exists in two redox states (differing by two electrons), neither of which is EPR active. Very possibly these are the Cu(I) and Cu(III) forms as in the case of galactose oxidase.

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(1) This research was supported by a research grant (AM 13448) from the National Institute of Arthritis, Metabolism, and Digestive Diseases, Public Health Service. The EPR spectrometer used in this research was purchased using funds partially supplied by an equipment grant from the National Science Foundation to the Chemistry Department.

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Benzo[1,2:3,4]dicyclobutene

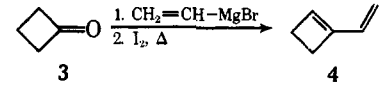
Sir:

We wish to report the synthesis of benzo[1,2:3,4]dicyclobutene (**1**), a benzene molecule upon which two cyclobutene rings are fused meta to one another. The isomeric benzo[1,2:4,5]dicyclobutene (**2**) has been reported by Cava and co-workers who prepared this system by the thermal extrusion of two molecules of sulfur dioxide from the corresponding disulfone.[1]

1 2

Lawrence and MacDonald have determined the x-ray structure of **2**.[2] They found little variation in the bond lengths of the six-membered ring. The two bridging bonds measured 1.35 Å while the remaining bonds in the benzene ring were found to be 1.38 Å. The interior benzene bond angles were substantially distorted from the normal 120°, measuring 108° at the four bridgehead carbons and 126° at the two remaining positions. A theoretical consideration of the hybridization of **2** utilizing the maximum overlap method has lent further support to the highly strained nature of **2**.[3] The potential for similar comparisons between **1** and **2** prompted us to synthesize the meta-fused isomer.

The key reaction employed in the synthesis of **1** is the Diels–Alder addition of 1-vinylcyclobutene (**4**) to dimethyl cyclobutene-1,2-dicarboxylate (**5**). The preparation of diene **4** is as yet unreported but could be accomplished in a straightforward manner. The addition of vinyl magnesium bromide to cyclobutanone provided 1-vinylcyclobutanol in 66% yield. When this alcohol was heated in the presence of a small amount of iodine crystals, dehydration occurred and 1-vinylcyclobutene was distilled from the mixture in 72% yield, bp 82° (760 mm). The NMR spectrum of **4** showed an ABX pattern at δ 6.5–4.9 for the three vinyl protons, an olefinic resonance at δ 5.83, and the allylic ring protons as a multiplet at δ 2.5.



3 4

The 2 + 4 cycloaddition of **4** and **5** provided 1,8-dicarbomethoxytricyclo[6.2.0.0^{2,5}]dec-5-ene (**6**) which showed two overlapping peaks at long VPC retention time. This product mixture could be explained by competing exo and endo modes of Diels–Alder addition which should provide a molecule which is epimeric at the tertiary carbon, C-2. An NMR spectrum of the mixture exhibited three groups of signals in the ratio of 1:6:11, lending support to the gross overall structure of **6**. Hydrolysis of **6** with potassium hydroxide in refluxing aqueous methanol provided the corresponding diacid **7** whose NMR spectrum showed a two proton signal at δ 9.8 as well as the disappearance of the methyl ester singlets.