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Oxidative Decomposition of Methyl Linoleate by a Lipoxygenase Model Iron Complex $Fe((pz)_3BH)_2$

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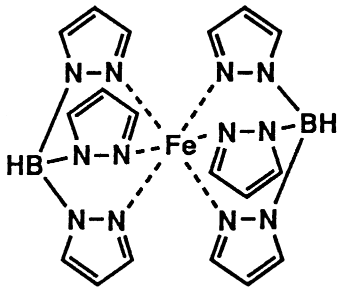
Highly effective aerobic oxidation of methyl linoleate was induced by ferrous pyrazolylborate complex in dichloromethane solution for the mimesis of lipoxygenase reaction.

In biological systems, unsaturated fatty acids react with molecular oxygen to give hydroperoxides by the catalysis of a group of enzymes called lipoxygenases in the first process of complicated transformation pathway leading to many biologically active compounds such as green odour in plants or prostaglandins in animals.1) Information of the enzyme structure has been accumulated for Soy Bean Lipoxygenase I (SBL-I), where iron coordinated by three or four imidazoles of histidine residues plays a crusial role.2) For the mimesis of the metal-coordination sphere composed of several histidine-imidazoles, polypyrazolylborate ligands are frequently used in constructing model complexes.3)



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We recently found that the oxidative decomposition of methyl linoleate is highly accelerated in the presence of bis(hydrotrispyrazolylborato)-iron(II) ($Fe((pz)_3BH)_2$, 1), as follows. Into a dichloromethane solution of methyl linoleate purified by silica gel chromatography just before use, a catalytic amount (1/100 molar ratio to methyl linoleate) of $Fe((pz)_3BH)_2$ synthesized according to Trofimenko's method4) was added under the O_2

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atmosphere and the mixture was stirred at room temperature. Soon after the addition of the complex, the pale purple solution characteristic of divalent $Fe((pz)_3BH)_2$ gradually turned dark red. The change of absorption spectrum of the solution was followed as shown in Fig. 1, where the increase of absorption at the wavelength of 450 nm was observed. The newly appeared 450-nm band was analogous to that observed in the $AgClO_4$-oxidation product of $Fe((pz)_3BH)_2$, reported by Armstrong and Lippard.[3a] It should be emphasized that the oxidation of the ferrous complex did not occur under an inert atmosphere even in the presence of the substrate. The complex itself is stable to molecular oxygen under the same conditions in dichloromethane when methyl linoleate is not present; oxidation of the complex occurs only when the three components, i.e. the complex, molecular oxygen, and methyl linoleate, are present in the system.

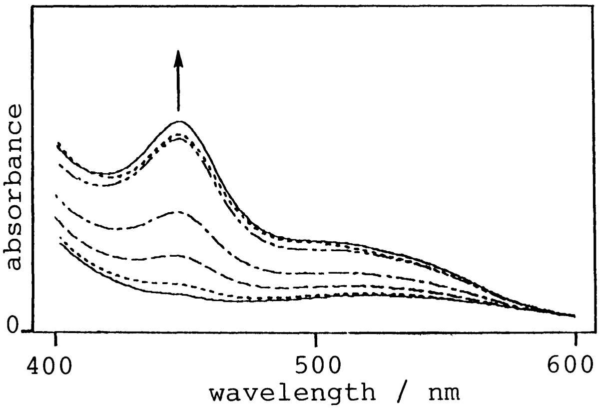


Fig. 1. Visible spectral change observed during the addition of methyl linoleate to dichloromethane solution of $Fe((pz)_3BH)_2$ in O_2 atmosphere.

Complex concentration: 5 mM, Complex/Substrate ratio = 1/100, Plotted every 15 minutes.

After 24 h of stirring at room temperature, the resulting solution was analysed by HPLC. The substrate was recovered in a yield of 66%. The percentage of conversion after 24 h was increased to 80% when much larger quantity of the catalyst (1/20 molar ratio) was present in the system. This system is far more effective for the linoleate decomposition compared with the similar systems in which the substrate is treated with molecular oxygen in the presence or absence of other iron complexes as summarized in Table 1.

The reaction did not proceed without complexes as well as the pyrazolylborate ligand itself (Entries 1,2). In the five iron complexes we have examined, all ferric complexes (Entries 6-8) were ineffective for the oxidation of the substrate. Phthalocyaninatoiron(II) (Fe(PC), Entry 5) showed some effect but the conversion was low even when the concentration of the complex was high. The efficiency of $Fe((pz)_3BH)_2$ is unusual compared with other ferrous and ferric complexes.

Examination of products by HPLC revealed the presence of at least 30 compounds in the reaction mixture. Separation and purification of the major products were attempted in a preparative chromatography: After stirring 590 mg (2 mmol) of the substrate with 1/20 equiv. of $Fe((pz)_3BH)_2$ for 10 h, 210

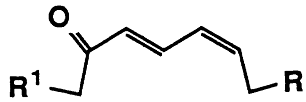
Table 1. Oxidative Decomposition of Methyl linoleate by various Iron Complexes[a)]

Entry	Complex[b)]	[Complex]/[Substrate]	Conversion/%
1	none	-----	trace
2	$KHB(pz)_3$	1 / 100	trace
3	$Fe((pz)_3BH)_2$	1 / 100	34
4		1 / 20	80
5	Fe(PC)	1 / 20	5
6	Fe(TPP)Cl	1 / 100	trace
7	$Fe(acac)_3$	1 / 100	trace
8	$[Fe((pz)_3BH)_2]ClO_4$	1 / 100	trace

a) Solvent: CH_2Cl_2, Reaction condition: room temp, 24 h, Substrate concentration: 5 mM.
b) Fe(PC) : Phthalocyaninatoiron(II),
Fe(TPP)Cl : Tetraphenylporphinatoiron(III)chloride,
$Fe(acac)_3$: Tris(acetylacetonato)iron(III),
$[Fe((pz)_3BH)_2]ClO_4$: see text.

mg (35%) of the substrate, 49 mg of ketones and aldehydes, 59 mg of epoxyenones, 76 mg of alcohols, and 60 mg of diols were collected in the order of increasing elution time. We were able to determine partial structures ofthe second and the third fractions by NMR and mass spectroscopies as those indicated by 2 for the second fraction and 3 for the third fraction, respectively.[5)]

It is well known that unsaturated fatty acids are oxidized by oxygen to give hydroperoxides having two conjugated double bonds next to the hydroperoxide carbon as the result of 1,3-shift.[6)] 2 and 3 are involved in the compounds found in the decomposition reaction of linoleic acid hydroperoxide by $FeCl_3$-cysteine complex.[7)] These facts suggest that the reaction pathway in this system is similar to that of autoxidation or Fenton-like reaction.



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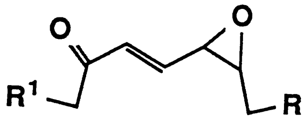

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Though it has not been well established how lipoxygenase produces hydroperoxides, no evidence has directed that it produces "activated oxygen". Instead, a pathway in which "activated substrate" reacts with molecular oxygen has been postulated as a better description of the reaction in a native enzyme.[8)] Our results seem to be in agreement with the latter postulation judging from the fact that the model complex, which alone is inert to molecular oxygen, is co-oxidized with the substrate.

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5) Data for 2: ^{1}H-NMR; 7.48(1H, dd, J = 15.8, 11.4 Hz), 7.12(1H, dd, J = 15.8, 9.8 Hz), 6.0-6.2(2H, m), 3.67(3H, s) 2.53(2H, t, J=7.4 Hz), 2.29(2H, t, J = 7.3 Hz), 1.3-1.6(18H, br), 0.90(3H, br), MS; 308(M^+), 277(M^+-OCH_3), 237(M^+-C_5H_{11}), 151(M^+-$(CH_2)_7CO_2CH_3$).
Data for 3: ^{1}H-NMR; 6.30-6.57(2H, m), 3.63(3H, s), 3.19(1H, dd, J = 6.4, 2.0 Hz), 2.88(1H, tt, J = 5.4, 2.0 Hz), 2.51(2H, t, J = 7.3 Hz), 2.29(1H, td, J = 7.5, 1.5 Hz), 1.4-1.7(6H, br), 1.2-1.4(12H, br), 0.88(3H, br).

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