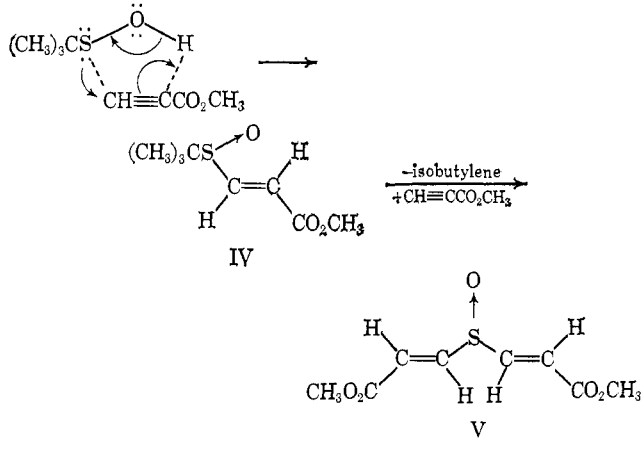
The adduct was identified as ethyl β-(2-methylpropyl-2-sulfinyl)propionate (III) and was identical with the material prepared by standard methods. The adduct

III

was stable to elimination of isobutylene, but the propionate group could be thermally exchanged with other olefins such as acrylonitrile, presumably through the sulfenic acid intermediate.

The sulfenic acid also adds to methyl propiolate to give the diadduct (V), β,β′-bis(*trans*-carbomethoxy)-divinyl sulfoxide, by a double elimination–addition reaction; mp 105–106° from ethanol. *Anal.* Calcd for $C_8H_{10}SO_5$: C, 44.04; H, 4.59; S, 14.67. Found:[10] C, 43.84; H. 4.43; S, 14.51. The infrared spectrum in KBr showed absorptions at 1092 (S→O) and 962 cm^{-1} (*trans*-disubstituted olefin). The nmr spectrum in C_6D_6 had peaks at τ 6.63 (singlet, six protons) and two AB doublets centered at τ 3.18 and 2.88 (four protons, J = 15 cps). The diadduct thus has both double bonds in the *trans* configuration. Studies with corresponding compounds possessing the *cis*/*cis* (J = 15 cps) and *cis*/*trans* (J_{cis} = 10 cps, J_{trans} = 15 cps) configurations showed they were stable toward rearrangement under the reaction conditions.

The stereochemistry of the adduct points to a *cis* addition of the sulfenic acid. The intermediate adduct,



IV

V

IV, can be observed in low concentration during the reaction. The stereoselectivity remains unchanged when polar solvents such as DMSO are employed; in no case was any *cis* product obtained.

The characterization of *t*-butylsulfenic acid by nmr and infrared spectral data and the demonstration of its presence by addition reactions with electrophilic olefins and acetylenes confirm the formation of this compound in the thermolysis of *t*-butyl sulfoxide. This is the first instance in which the existence of an aliphatic sulfenic acid has been demonstrated.

(10) Analysis by Galbraith Laboratories.
(11) National Science Foundation Cooperative Fellow, 1965–1967.

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Chloroperoxidase. IV. Evidence for an Ionic Electrophilic Substitution Mechanism[1,2]

Sir:

Chloroperoxidase catalyzes the peroxidative formation of the carbon–halogen bond according to eq 1, where X^- represents an oxidizable halogen anion (chloride, bromide, or iodide) and HA represents an acceptor molecule with a replaceable proton.[3] We have directed our efforts toward resolving the question

$$H_2O_2 + X^- + HA \longrightarrow AX + OH^- + H_2O \qquad (1)$$

of whether this reaction proceeds *via* a free-radical or ionic substitution mechanism. Firstly, several lines of evidence indicate that an initial step in the halogenation reaction involves the oxidation of the halogen anion by hydrogen peroxide rather than the oxidation of the halogen acceptor. For example, it has been established that crystalline chloroperoxidase preparations catalyze the peroxidation of iodide[3d] to form elemental iodine. This reaction, in which the halogen anion serves both as donor and acceptor, clearly indicates that chloroperoxidase can catalyze the oxidation of the halogen anion. In contrast, there are numerous compounds which can serve as acceptors in reaction 1, *i.e.*, tyrosine and monochlorodimedone, but are nevertheless completely resistant to oxidation by chloroperoxidase and hydrogen peroxide.[3d] Collectively, these results provide strong evidence for the hypothesis that chloroperoxidase catalyzes the oxidation of the halogen anion to an activated state prior to the reaction with the acceptor molecule. Secondly, chloroperoxidase exhibits a broad specificity with respect to halogen acceptors. A wide diversity of compounds, ranging from β-keto acids,[3b] cyclic β-diketones,[3d] phenol and substituted phenols,[3d] related aromatic compounds,[4] sulfides, and compounds containing the thiouracil grouping,[2] all serve in an acceptor capacity in the chloroperoxidase reaction. It occurred to us that we could utilize this nonspecificity of chloroperoxidase with respect to halogen acceptor in designing an experiment which could aid in resolving the mechanism question. If enzyme specificity is eliminated from playing a determinant role in the reaction of the oxidized halogen (presumably enzyme-bound) donor with the acceptor molecule, the products of the enzyme reaction should be strictly comparable to chemical halogenation by a free-radical or an ionic electrophilic substitution mechanism. Formally, the chloroperoxidase-catalyzed oxidation of the halogen anion could involve a one-electron oxidation ($X^- \rightarrow X\cdot + e$) to form a halogen radical species, or an over-all two-electron oxidation to the halogenium ion ($X^- \rightarrow X^+ + 2e$). These two halogenating species have quite different properties and yield different products with aromatic substrates. Therefore, we have compared the enzymatic chlorination of anisole with the halogenation of anisole by using hypochlorous acid as a prototype for the ionic reaction and

(1) This work was supported by a grant from the National Science Foundation (GB-2786).
(2) Part III: D. R. Morris and L. P. Hager, *J. Biol. Chem.*, **241**, 3582 (1966).
(3) (a) P. D. Shaw and L. P. Hager, *J. Am. Chem. Soc.*, 81, 6527 (1959); (b) P. D. Shaw and L. P. Hager, *J. Biol. Chem.*, **236**, 1626 (1961); (c) D. R. Morris and L. P. Hager, *ibid.*, **241**, 3582 (1966); (d) L. P. Hager, D. R. Morris, F. S. Brown, and H. Eberwein, *ibid.*, **241**, 1769 (1966).
(4) F. S. Brown and L. P. Hager, unpublished experiments.

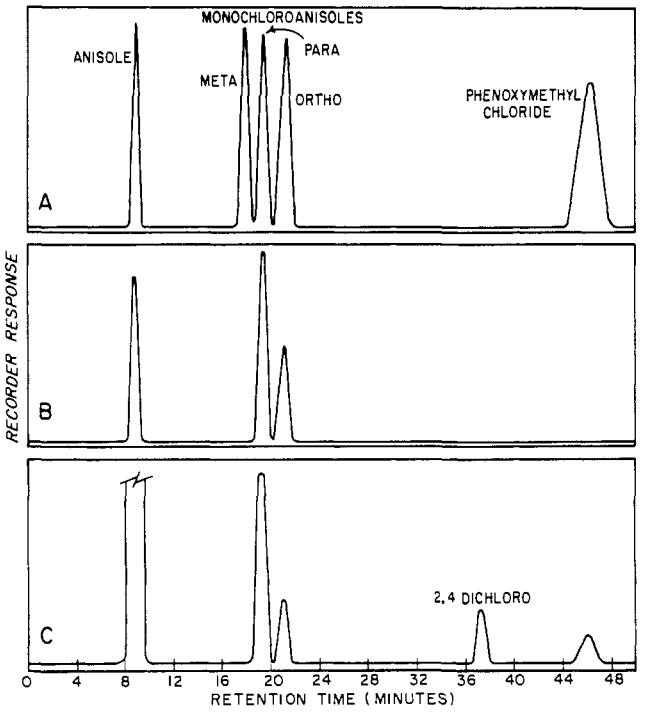

Figure 1. Gas chromatographic separation of anisole and its monochlorinated derivatives. (A) Anisole and its four monochloro isomers were separated from each other on an Aerograph Model A-350 B gas chromatograph equipped with a thermal conductivity detector using a 20 ft by 0.25 in. column with 30% FFAP (free fatty acid packing obtained from Wilkens Instrument and Research, Inc., Walnut Creek, Calif.) on acid-washed Chromosorb W 60–80 mesh. Chromatograph conditions: column, 213°; detector, 300°; injector, 280°; detector current, 225 ma; flow rate, 75 cc of helium/min. (B) Elution pattern of the products of the enzyme reaction. The reaction mixture contained 825 μmoles of anisole, 450 μmoles of hydrogen peroxide, 7.5 mmoles of sodium chloride, 75 μg of crystalline chloroperoxidase, and 10.5 mmoles of potassium phosphate buffer, pH 2.8, in a total volume of 105 ml. Chloroperoxidase was added to the incubation mixture at 5-min intervals in 16-μg aliquots. After 30-min incubation, the reaction mixture was extracted three times with 100-ml portions of ether. The ether extracts were combined, dried over magnesium sulfate, filtered, and evaporated to dryness under a stream of nitrogen. The residue was analyzed directly by gas chromatography as in A. (C) Elution pattern of the products of the free-radical halogenation reaction. The reaction mixture contained 2 ml of anisole in 100 ml of 0.1 *M* potassium phosphate buffer, pH 2.8, in a 500-ml, three-necked, round-bottom flask fitted with two dropping funnels and a reflux condenser. Five milliliters of sulfuryl chloride and 250 mg of benzoyl peroxide were added simultaneously from the two dropping funnels. The flask was illuminated by two 350-w photoflood lamps located 15 cm from either side of the flask. The flask was stirred constantly and heated to boiling while the additions were made and for 30 additional min after the completion of the additions. The products were then extracted with ether and analyzed as described in B and A.

with the halogenation of anisole using sulfuryl chloride, benzoyl peroxide, and light to generate chlorine free radicals. All of the halogenation reactions were carried out in aqueous medium at the same pH under essentially identical conditions. Figure 1A shows the gas chromatographic separation of anisole from its four monochlorinated derivatives, *o*-chloroanisole, *m*-chloroanisole, *p*-chloroanisole, and phenoxymethyl chloride. Figure 1B shows the same gas chromatographic separation of the products of the enzymatic reaction. The chloroperoxidase reaction produces only the *para* and *ortho* isomers at a *para*:*ortho* ratio of 1.9 (calculated from the area under each peak). The reaction of hypochlorous acid with anisole (0.5 HOCl:1 anisole)[5] under conditions identical with those employed in the enzymatic reaction yielded essentially the same results as found in the enzyme reaction. The reaction of hypochlorous acid with anisole yielded only the *ortho*- and *para*-monochlorinated anisole derivatives at a *para*:*ortho* ratio of 1.8. In contrast to these results, the reaction of chlorine free radicals with anisole under the conditions of the enzymatic reaction forms 2,4-dichloroanisole and *phenoxymethyl chloride* in addition to the *o*- and *p*-chloroanisoles (Figure 1C). The ratio of *para*:*ortho* chloroanisole formation in the free-radical reaction is 4.1. Thus, both the isomer distribution and the products, especially the formation of phenoxymethyl chloride, differ substantially between the enzyme and the free-radical reaction while the enzymatic chlorination and chlorination with hypochlorous acid are completely analogous.

We conclude that these results indicate that the gross details of the chloroperoxidase reaction correspond to the chemical ionic electrophilic substitution model and add support to our hypothesis that halogen ions undergo an over-all two-electron oxidation in the formation of the active halogenating species in the chloroperoxidase reaction.

(5) The incubation conditions for the chlorination of anisole with hypochlorous acid were identical with those used in the enzyme reaction (Figure 1B) except for the replacement of enzyme, hydrogen peroxide, and chloride ion with 412 μmoles of sodium hypochlorite.

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The Structure of Two Seven-Coordinate Complexes of Iron(III)

Sir:

We report here preliminary results of three-dimensional X-ray analyses of two seven-coordinate iron(III) complexes of a pentadentate macrocyclic ligand, 2,13-dimethyl-3,6,9,12,18-pentaazabicyclo[12.3.1]-octadeca-1(18),2,12,14,16-pentaene (structure I).[1] Compound I, the dimer $[(H_2O)BFe\text{–}O\text{–}FeB(H_2O)](ClO_4)_4$, where B = macrocyclic ligand, was reported earlier[2] as $[FeB(OH)](ClO_4)_2$. This compound crystallizes as red-orange orthorhombic crystals with unit cell dimensions a = 22.82, b = 21.35, c = 19.75 ± 0.05 A. From the extinctions observed on precession photographs, the space group was determined to be Pbca with eight molecules per unit cell. The intensity data were collected on the General Electric XRD-5 diffractometer, and 914 independent reflections were observed by the stationary–counterstationary crystal technique using Mo Kα radiation. The iron and chlorine positions were determined from a Patterson synthesis and the structure was solved from a Fourier map computed from the resulting phases. Owing to the large number of atoms per asymmetric unit (65

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(2) S. M. Nelson, P. Bryan, and D. H. Busch, *Chem. Commun.*, 641 (1966).