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The Functionalization of Saturated Hydrocarbons. Part 35.[+] On the Intermediates in an Fe^{III} Catalase Model in Pyridine. Relevance to the Catalase Enzyme.

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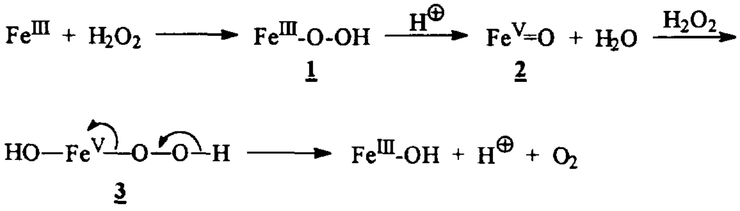
Abstract: Ferric chloride in pyridine behaves as an efficient model for the catalase enzyme. It converts H_2O_2 nearly quantitatively into water and oxygen ($2\ H_2O_2 \rightarrow 2\ H_2O + O_2$). The addition of Ph_2S to the model system affords Ph_2SO, the amount of which increases with the Ph_2S added. The inverse relationship between oxygen and Ph_2SO formation proves that there is an intermediate in the model catalase reaction. When di-*n*-butyl, di-*t*-butyl and diphenyl sulfides are reacted in pairs in competition for the intermediate a large steric effect of over 600 is found for the di-*n*-butyl versus di-*t*-butyl sulfoxide formation. In contrast the same number for per-acid oxidation is 8. It is concluded from this and other evidence that the intermediate is and Fe^V oxenoid, or equivalent, which reacts competitively with H_2O_2 to give oxygen and with sulfides to furnish sulfoxides. Comparison is made with the catalase enzyme in water and in water-acetonitrile. An unexpected by-product of this study is an efficient and economic procedure for the oxidation of sulfides to sulfoxides without further significant oxidation to sulfones.

The enzyme catalase [1,2] converts hydrogen peroxide very efficiently in water into oxygen and water according to the equation $2\ H_2O_2 \rightarrow 2\ H_2O + O_2$. Using a simple ferric salt as catalyst this catalase reaction can be carried out with comparable efficiency in water or in pyridine without the help of catalase. The reactions in pyridine do not involve hydroxyl radicals. There is little coupling to pyridine and the selectivity is not appropriate.[3]

This article supports the simple succession of events shown in Scheme 1. This involves the formation of a hydroperoxide of Fe^{III} $\underline{1}$ and the evolution of $\underline{1}$ to an Fe^V species $\underline{2}$. By reaction of $\underline{2}$ with H_2O_2 the intermediate $\underline{3}$ is formed which decays to Fe^{III} and oxygen.

[+] Part 34: D. H. R. Barton, B. Chabot and B. Hu, *Tetrahedron*, submitted for publication.

Scheme 1 is in agreement with the extensive kinetic analysis by Kremer and Stein for the reaction of H_2O_2 with ferric perchlorate in water.[4] In brief two intermediates, written here as 1 and 2, are deduced to exist in order to explain the observed kinetics. We add the third intermediate 3 to explain the final step in the formation of oxygen.



Scheme 1

In principle intermediates 1 and 2 could be captured by the addition of a suitable trap before the final step of oxygen formation. Extensive experience with what we call Gif chemistry has shown the value of traps.[5] We now report that diphenyl sulfide is a useful reagent for the detection of an intermediate in the catalase model reaction using $FeCl_3$ in pyridine. Table 1 summarizes the results.

Two blank experiments (Table 1, entries 1 and 2) showed that Ph_2SO was not formed in the absence of either Fe^{III} or H_2O_2. Furthermore, Ph_2SO was not oxidized to Ph_2SO_2 under comparable conditions (entry 3). With a very large amount of H_2O_2 (total 40 mmol) over two days only 3% of Ph_2SO_2 was formed (entry 4).

We then showed that there was a competition between oxygen formation and Ph_2SO formation (entries 5 through 8). As the ratio of Fe^{III} (1 mmol) to Ph_2S changed from 5 mmol Ph_2S to 20 mmol, Ph_2SO formation increased from 1.6 to 2.7 mmol and oxygen formation diminished. Allowing 1 H_2O_2 for sulfoxide formation and 2 H_2O_2 for oxygen production the total of the two was constant (3.8, 3.5, 3.7 and 3.7 respectively). Thus an iron species is formed from H_2O_2 which reacts either with Ph_2S to make Ph_2SO or with H_2O_2 to make oxygen.

The data presented above showed that sulfoxide and oxygen formation are in competition. It was, therefore, of interest to examine what other factors can influence the ratio of the reaction products. In the above experiments (entries 5 through 8) the Fe^{III} to H_2O_2 ratio was 1:4. Increasing the ratio to 1:1 (in mmol) did not change the sulfoxide to oxygen ratio or the efficiency of the reaction (entry 9). Increasing the amount of Fe^{III} to 4 mmol and keeping H_2O_2 as a 1:1 ratio (entry 10) again did not change the sulfoxide to oxygen ratio or the efficiency of the reaction. Lowing the temperature to -20°C (entry 11) again did not change the sulfoxide to oxygen ratio, or the efficiency of the reaction. However, the half-life for oxygen formation increased four-fold to about 15 min.

Up to this time competition between sulfoxide and oxygen formation was examined on a competitive basis from time zero. With the longer reaction times at -20 °C it was possible to add the sulfide at various times after the addition of the hydrogen peroxide. The entries 12 and 13 show the effect of adding the sulfide at 5 and 10 min respectively after the addition of the H_2O_2. There is a significant increase in oxygen formation, as would be expected, but there is still significant formation of sulfoxide in entry 13 even when the sulfide has been added at T= 10 min at about the half-life for oxygen formation. The overall efficiency of the reactions remained the same at 3.5-3.6 as for all the competitive experiments.

Table 1. Experiments with Ph_2S or Ph_2SO

Entry	$FeCl_3$ (mmol)	Ph_2S (mmol)	H_2O_2 (mmol)	Temp. (°C)	O_2 (mmol)	Ph_2SO (mmol)	Other products (mmol)	Ph_2SO/O_2
1	1	4	0	RT	---	n.d.	---	---
2	0	4	4	RT	---	n.d.	---	---
3(a)	1	---	4	0	1.607	3.889	n.d.	2.42
4(b)	1	10	20	0 °C	---	4.995	Ph_2SO_2 (0.131)	---
			40	to RT		6.629	Ph_2SO_2 (0.372)	
5	1	5	4	0	1.094	1.602	---	1.46
6	1	10	4	0	0.759	2.070	---	2.73
7	1	15	4	0	0.625	2.392	---	3.83
8	1	20	4	0	0.513	2.673	---	5.21
9	1	10	1	0	0.179	0.552	---	3.08
10	4	10	4	0	0.804	2.075	---	2.58
11	4	10	4	-20	0.759	2.080	---	2.74
12	4	10(c)	4	-20	1.116	1.382	---	1.24
13	4	10(d)	4	-20	1.406	0.695	---	0.49
14(e)	0.5	10	4	0	0.737	2.087	A (0.156)	2.83
15(e)	1	10	4	0	0.714	2.045	A (0.104)	2.86
16(e)	2	10	4	0	0.750	2.056	A (0.088)	2.74
17(e)	4	10	4	0	0.737	2.025	A (0.053)	2.75
18(e)	1	20	4	0	0.491	2.557	A (0.082)	5.21

Compounds: A = Cyclohexanone

Notes:
- (a) Ph_2SO (4 mmol) was added.
- (b) 20 mmol (10 mmol x 2 / 30min) of H_2O_2 was added at 0 °C, then the reaction was allowed to continue at RT over night. Another 20 mmol H_2O_2 (10 mmol x 2 / 30 min) was added and reaction was continued for one more day.
- (c) Ph_2S was added 5 min after H_2O_2.
- (d) Ph_2S was added 10 min after H_2O_2.
- (e) Cyclohexane (20 mmol) was added.

Although we have determined the $T_{1/2}$ for oxygen evolution, the data are not reliable kinetically, at least for short $T_{1/2}$ of a few minutes. A danger with oxygen determination is that all the oxygen is not released at the rate that it is formed. Even an oxygen flush of one hour before the experiment begins does not solve the problem. As Prof. K. U. Ingold has kindly informed us, there is a well marked supersaturation phenomenon in oxygen evolution which can only be handled by special procedures. However, the final values of oxygen produced are reliable when the evolution comes to a definite halt.

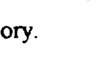
It was now time to see if the oxidation of cyclohexane could interfere with sulfide oxidation. The data (entries 14 through 17) are where the Fe^{III} varies from 0.5 mmol to 4 mmol whilst the H_2O_2 added stays always at 4 mmol, the Ph_2S at 10 and the cyclohexane at 20 mmol. Throughout the 0.5 to 4.0 Fe^{III} range the amounts of sulfoxide and oxygen (normalized) are 3.6, 3.5, 3.6 and 3.5. However, an interesting observation is that the formation of ketone, although small, varies inversely with the Fe^{III} used. The amounts of ketone produced contribute significantly to the efficiency of the reaction. For entry 14 about 9% of the oxidizing power is used to make ketone, but for entry 17 ketone formation only requires 3% of the oxidizing power used. Since ketone formation as a major reaction requires the presence of a suitable carboxylate ligand,[5] the production of significant amounts of ketone at low Fe^{III} to H_2O_2 ratio deserves further investigation.

When the amount of sulfide was doubled (entry 18) there was a minor decrease in oxygen formation, a small increase in sulfoxide and ketone formation did not change. The results are comparable with those in entry 8 (*vide supra*).

The data in Table 1 show that there is an intermediate in the model catalase reaction which reacts with Ph_2S to give the sulfoxide. The intermediate could be **1**. If this reacted fast with Ph_2S then as we doubled and redoubled the Ph_2S (entries 5 through 8) there should be only formation of Ph_2SO. This is not so and it seems more probable that intermediate **1** rearranges to **2** before reacting competitively with H_2O_2 or Ph_2S.

A similar conclusion can be reached from inspection of the data in entries 14 through 17. Here, the concentration of Fe^{III} over an eightfold variation does not change the amount of oxygen or of Ph_2SO produced. This again suggests that the sulfoxide can not be formed by the reaction of Ph_2S with intermediate **1**. At low concentrations of Fe^{III}, the sulfoxide formation should be more efficient. The results would be explained if there was a rapid formation of intermediate **2** which then partitioned into oxygen and sulfoxide.

However, the reaction of H_2O_2 with intermediate **2** is not the only way in which oxygen could be formed. If two H_2O_2 reacted with one Fe^{III} then complex **4** (see arrows) could explain the formation of the oxygen as could suitable fragmentation of complex **5**. The data in entries 14 through 17 are not compatible with this kind of theory.



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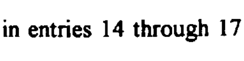

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A more precise analysis of the intermediate responsible for sulfoxide formation as compared with that for oxygen formation came from an analysis of steric effects in the two sulfides di-*n*-butyl and di-*t*-butyl sulfides. If the two sulfides were reacting with intermediate **1** then, accepting that it would be the terminal hydroxyl which would be responsible for sulfoxide formation then steric effects should be minor. However, if it were intermediate **2** that was involved then, allowing for all the pyridines (possibly 4) associated with intermediate **2**, the oxygen responsible for sulfoxide formation would be sensitive to steric effects. In the literature,[6] an electrophilic *tert*-hydroperoxide showed a ratio of about 6 for *n*-$Bu_2S=O$ versus *t*-$Bu_2S=O$ formation. Since these data were approximate, we decided to make precise direct competition experiments with *m*-chloroperoxybenzoic acid (Table 2). First (entries 1 and 2) we competed Ph_2S against *t*-Bu_2S and then against *n*-Bu_2S. The results showed that the latter sulfide was more reactive than Ph_2S whereas the former was somewhat less reactive. A ratio of 8.3 could be deduced from the two experiments. We then confirmed this value by a direct competition of the two butyl sulfides which gave a ratio of 7.8. Clearly a steric effect of a minor nature can be seen.

Table 2. Oxidation of sulfides with Gif catalase reaction and *m*CPBA method

Entry	Oxidant	Ph_2S (mmol)	Bu_2S (mmol)	Products (mmol)			Ph_2SO/ Bu_2SO	*n*-Bu_2SO/ *t*-Bu_2SO
				O_2	Ph_2SO	Bu_2SO		
1	*m*CPBA	5	(*t*-) 5	---	0.35	(*t*-) 0.25	1.40	8.3
2	*m*CPBA	5	(*n*-) 5	---	0.13	(*n*-) 0.77	0.17	
3	*m*CPBA	---	(*n*-) 5 (*t*-) 5	---	---	(*n*-) 0.70 (*t*-) 0.09	---	7.8
4	Fe^{III} + H_2O_2	10	(*t*-) 10	0.74	2.05	(*t*-) 0.10	20.5	684
5	Fe^{III} + H_2O_2	10	(*n*-) 10	0.11	0.11	(*n*-) 3.67	0.03	

Gif catalase experiments were carried out using $FeCl_3{\cdot}6H_2O$ (1 mmol) and H_2O_2 (4 mmol) in pyridine (33 mL) at 0°C under air for 1 h. Normal acidic work-up procedure was utilized, except that AcOEt was used instead Et_2O.

In spectacular contrast (Table 2, entries 4 and 5), the same competition experiments with the catalase model system gave a ratio for the *n*-butyl sulfide against the *t*-butyl sulfide of nearly 700. In comparison, this is a major steric effect. Since this result is important we give the detailed competitive experiments in Table 3. After 6 minutes in the case of the di-*n*-butyl sulfide (Expt. B), sulfoxide formation was complete. The final evolution of oxygen from the supersaturated solution took nearly 1 h. For the di-*t*-butyl sulfide (Expt. A), the sulfoxide formation was completed after 10 min and oxygen evolution took 30 min. What was also clear was that oxygen formation seven times greater for the hindered di-*t*-butyl sulfide than for the di-*n*-butyl sulfide, again in agreement with a competition between H_2O_2 and sulfides for intermediate 2. However, it also shows that the H_2O_2 has to attack the Fe^V oxenoid at the Fe^V center, which is a more hindered reaction than that between a sulfide and the oxygen of the Fe^V oxenoid.

Table 3. Gif catalase model reactions with two sulfides

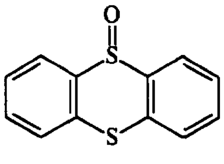
Time (min)	Products (Experiment A)				Products (Experiment B)			
	O_2	Ph_2SO	$(t\text{-}Bu)_2SO$	Ratio	O_2	Ph_2SO	$(n\text{-}Bu)_2SO$	Ratio
2	0.268	1.498	0.071	21.1	0.022	0.095	3.335	0.028
4	0.469	---	---	---	0.045	0.111	3.775	0.029
6	0.558	1.819	0.093	19.6	0.067	0.115	3.827	0.030
8	0.625	---	---	---	0.076	---	---	---
10	0.647	1.955	0.104	18.8	0.080	0.113	3.811	0.030
15	0.692	---	---	---	0.085	---	---	---
20	0.714	2.002	0.104	19.3	0.089	0.109	3.785	0.029
30	0.737	2.034	0.105	19.4	0.098	0.113	3.788	0.030
40	0.737	---	---	---	0.107	---	---	---
50	0.737	---	---	---	0.112	---	---	---
60	0.737	2.046	0.102	20.1	0.112	0.110	3.670	0.030

Table 4. Experiments with variable amount of $FeCl_3$ and H_2O_2 at various temperature

Entry	$FeCl_3$ (mmol)	H_2O_2 (mmol)	Temp.	O_2 (mmol)	Eff.
1	1	4	0 °C	1.696	84.8%
2	2	1	0 °C	0.402	80.4%
3	4	1	0 °C	0.446	89.2%
4	4	2	0 °C	0.826	82.6%
5	4	2	-20 °C	0.848	84.8%
6	4	1	-20 °C	0.446	89.2%
7	4	4	-20 °C	1.719	86.0%
8	0.1	4	0 °C	1.585	79.3%
9	0.2	2	0 °C	0.826	82.6%
10	5	2	0 °C	0.938	93.8%
11	10	2	0 °C	0.960	96.0%
12	2	4	0 °C	1.786	89.3%
13	1	2	0 °C	0.826	82.6%

We have also studied the efficiency of the model catalase reaction without any trap. The results are summarized in Table 4. For entries 1 through 4, the efficiency of oxygen formation is high and highest when the Fe^{III} concentration is highest. Entries 5 through 7 refer to initial temperature of -20 °C. Here the $T_{1/2}$ for oxygen evolution is 4-5 times longer.

Entries 8 through 13 are a study of the effect of varying the Fe^{III} to H_2O_2 ratio. The highest efficiency and the fastest rate are shown when the Fe^{III} concentration is a maximum (entry 11).



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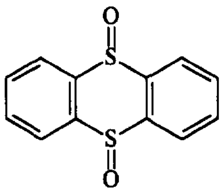

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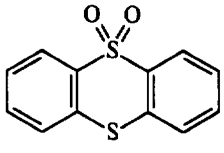

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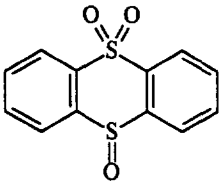

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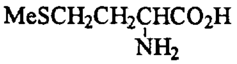
Thianthrene-5-oxide 6 has been used by Adam as a mechanistic probe to distinguish the nucleophilic versus electrophilic nature of oxygen transfer agents.[7] Electrophilic oxidants produce mostly thianthrene-5,10-doxide 7, while significant amounts of thianthrene-5,5-dioxide 8 and thianthrene-5,5,10-trioxide 9 are formed with nucleophilic oxidants. However, this compound 6 is not very reactive in the Gif catalase model system. Thus, only trace amounts of thianthrene-5,10-dioxide were detected under the normal conditions (see experimental part for details), while almost all the oxidation power forms O_2. Thianthrene-5,10-dioxide (0.11 mmol) could be quantified when an excess amount of H_2O_2 (5 equivalents with respect to 6) was added. Neither thianthrene-5,5-dioxide nor thianthrene-5,5,10-trioxide were detected in this reaction. These results confirm that an electrophilic oxidant is involved in this sulphoxidation reaction.

We have also made a preliminary study of the catalase enzyme itself (Table 5). In the standard buffer, the enzyme is very fast and efficient (entry 1). Addition of 50% CH_3CN has little effect on the enzyme (entry 2). On the other hand, 50% of dimethyl sulfoxide (entry 3) slows down the enzyme and makes it inefficient. However, 25% of CH_3CN + 25% dimethyl sulfoxide slows down the enzyme (entry 4) but does not change its efficiency. When PhSMe (5 mmol) was added to the enzyme in buffer + 50% CH_3CN, the oxygen formation was fast (entry 5) and quantitative. The PhSMe was soluble and was not oxidized. The same was found for (±)-methionine (10 mmol) and $MeSCH_2CH_2OH$ (10 mmol) (entries 6 and 7) and both were perfectly soluble in the buffer. The catalase reaction was inihibited in the presence of NaCN or NaN_3 (entries 8 and 9); on the other hand, the addition of aniline or 3-amino-1,2,4-triazole 10 had no effect on the oxygen formation (entries 10 and 11).

Table 5. Experiments with Catalase enzyme

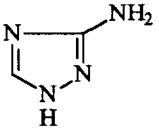
Entry	Solvents (mL)	Substrate (mmol)	O_2 (mmol)	$T_{1/2}$ (min)
1[(a)]	Buffer (33)	---	2.165	<< 2
2	Buffer (16.5) + CH_3CN (16.5)	---	2.143	< 2
3	Buffer (16.5) + DMSO (16.5)	---	0.580 1.116	0 °C, 1 hr RT, 4 hr
4	Buffer (16.5) + DMSO (9) + CH_3CN (9)	---	2.143	8
5[(b)]	Buffer (16.5) + CH_3CN (16.5)	PhSMe (5)	2.188	< 2
6	Buffer (33)	(±)-Methionine (10)	2.098	<< 2
7	Buffer (33)	$MeSCH_2CH_2OH$ (10)	1.920	<< 2
8	Buffer (33)	NaCN (10)	0.558	6
9	Buffer (33)	NaN_3 (10)	0.045	---
10[(b)]	Buffer (33)	Aniline (10)	2.232	~ 3
11	Buffer (33)	**10** (10)	2.143	~ 2

Notes: (a) With 15 mg catalase enzyme.
(b) Substrate does not dissolve very well in this solvent system.



$MeSCH_2CH_2\underset{NH_2}{CH}CO_2H$

(±)-Methionine



10 3-Amino-1,2,4-triazole

Although the catalase enzyme is considered to form the equivalent of an Fe^V oxenoid bonded to a porphyrin, it is the fifth ligand (tyrosine) which makes catalase so special.[1,8] Thus the tyrosine by elimination and addition controls the ease of formation of the oxenoid and hence the formation of oxygen for the attack of the second hydrogen peroxide. Thus, the catalase enzyme does not oxidize simple methyl sulfides (entries 5, 6 and 7). The catalase enzyme was also reported to be able to oxidize alcohols, such as ethanol, in the presence of very low concentrations of H_2O_2.[9] In fact, this reaction is stereospecific in that only pro-R-hydrogen in ethanol is removed to form acetaldehyde.[10]

An unexpected by-product of this study is an efficient procedure for the oxidation of sulfides to sulfoxides without further significant oxidation to sulfones. Many oxidizing agents can oxidize sulfides.[11] However, usually the oxidation of a sulfide will produce the corresponding sulfoxide and/or sulfone. Very mild and highly selective methods have to be used to yield sulfoxides alone. On the other hand, complete oxidation to the sulfone is much easier. For example, diphenyl sulfide was oxidized to produce a mixture of 30% diphenyl sulfoxide and 70% diphenyl sulfone with oxone ($2KHSO_5{\bullet}KHSO_4{\bullet}K_2SO_4$);[12] while 32% sulfoxide and 42% sulfone were obtained with ruthenium tetroxide (RuO_4) oxidation.[13] H_2O_2 is the most

Table 6. Preparation of sulfoxides by Fe^{III}-H_2O_2 in pyridine

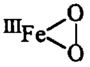
H_2O_2 (mmol)	Products from Ph_2S (mmol)	Products from *n*-Bu_2S (mmol)	Products from *t*-Bu_2S (mmol)
20	Ph_2SO (10.96) Ph_2SO_2 (traces) Ph_2S (9.73)	*n*-Bu_2SO (17.67) *n*-Bu_2SO_2 (traces) *n*-Bu_2S (2.73)	*t*-Bu_2SO (2.60) *t*-Bu_2SO_2 (n.d.) *t*-Bu_2S (17.57)
25	---	*n*-Bu_2SO (18.80) *n*-Bu_2SO_2 (0.79) *n*-Bu_2S (traces)	---
40	Ph_2SO (16.88) Ph_2SO_2 (0.49) Ph_2S (2.54)	---	*t*-Bu_2SO (4.55) *t*-Bu_2SO_2 (n.d.) *t*-Bu_2S (15.32)
50	Ph_2SO (17.47) Ph_2SO_2 (0.80) Ph_2S (1.45)	---	---
60	Ph_2SO (17.91) Ph_2SO_2 (1.06) Ph_2S (0.95)	---	*t*-Bu_2SO (6.49) *t*-Bu_2SO_2 (n.d.) *t*-Bu_2S (13.21)
70	Ph_2SO (18.24) Ph_2SO_2 (1.21) Ph_2S (0.73)	---	---
80	---	---	*t*-Bu_2SO (7.95) *t*-Bu_2SO_2 (n.d.) *t*-Bu_2S (11.35)
100	---	---	*t*-Bu_2SO (9.22) *t*-Bu_2SO_2 (n.d.) *t*-Bu_2S (9.78)

$FeCl_3 \cdot 6H_2O$ (5 mmol), sulfides (20 mmol) and pyridine (33 mL) were used in each experiment.

widely used oxidizing agent for the oxidation of organic sulfides. It can be either used alone or associated with various catalysis or solvents. The oxidation of sulfides with H_2O_2 alone is a relatively slow reaction. Thus, with four equivalents of H_2O_2 (with respect to the sulfide), a 50% yield of diphenyl sulfoxide (32% of the starting sulfide was recovered) was obtained from the corresponding sulfide after 170 h at room temperature in methanol.[14] By employing Fe^{III}-H_2O_2 in pyridine in the absence of any carboxylic acids, sulfides could be oxidized to sulfoxides efficiently and selectively. Further oxidation to sulfones was not significant. Among the three sulfides studied (Table 6), *n*-Bu_2S was the most reactive. Slightly more than one equivalent of H_2O_2 (with respect to sulfide used) was needed to oxidize *n*-Bu_2S completely to *n*-Bu_2SO (almost 95%). The corresponding sulfone was formed in less than 5%. As expected, Ph_2S was less reactive than *n*-Bu_2S. However, using more H_2O_2, the sulfoxide was still formed in approximately 90%, while only 5% sulfone was produced. The oxidation of *t*-Bu_2S was the least efficient. Even with 5 equivalents of H_2O_2, only 50% of the sulfide was oxidized. But, once again the corresponding sulfone formed was negligible.

These results revealed that the oxidation of sulfides to sulfoxides by Gif Fe^{III}-H_2O_2 system is a cheap and practical method for making these compounds.

CODA

A number of helpful Referees find the idea of an $Fe^{V}=O$ species, as in 2, to be unacceptable. Indeed, the evidence for this entity remains circumstantial. However, in the presence of a suitable carboxylate ligand the selective attack (regiochemical for ketone formation and chemoselectivity for ketonization in preference to oxidation of diphenylsulfide) on saturated hydrocarbons does require novel iron species. Of course a cyclic Fe^{III} peroxide  might be more acceptable. It could rearrange on contact with the hydrocarbon to give the real reagent and would thus explain the "Sleeping Beauty" effect.[5] It would also explain the model catalase studies here reported. The oxygen used for sulfoxide formation would be directly attached to the iron to provide the major steric effect that we have observed.

EXPERIMENTAL

General:

Chemicals were purchased from Aldrich Chemical Co., except for pyridine (Mallinckrodt); diethyl ether, $MgSO_4$ and H_2O_2 (Fisher Scientific Co.); pH 7 yellow phosphate buffer (EM Science). Catalase from Bovine Liver (2800 units/mg solid, 3700 units/mg protein) was purchased from Sigma. Unless otherwise stated, all solvents and chemicals were after verification used as purchased. H_2O_2 was used as 30% in H_2O. 3-Chloroperoxybenzoic acid was used as 57-86% (titrated for exact purity before use). Thianthrene-5-oxide was prepared by the oxidation of thianthrene with *m*CPBA, and purified by flash chromatography. Other thianthrene oxides were prepared according to the literature methods.[15]

Gas chromatography analysis was performed on a Hewlett Packard 5890 series II instrument equipped with flame ionization detector and Hewlett Packard 3396A integrator. Purified N_2 was used as the carrier gas. The columns used were DB-WAX (30 m, 0.32 mm i.d., 25 μm film thickness), DB-5 (30 m, 0.32 mm i.d., 25 μm film thickness) or DB-1 (15 m, 0.32 mm i.d., 25 μm film thickness) capillary columns from J&W Scientific.

Gas chromatography-mass spectrometry (GC-MS) analysis was carried out on a Hewlett Packard 5890 series II gas chromatograph coupled with a Hewlett Packard 5971 series quadropole mass-selective detector

(40 eV, electron impact). Helium was used as the carrier gas. The column used in the GC-MS was a HP-5MS (30 m, 0.25 mm i.d., 0.25 μm film thickness).

^{1}H-NMR and ^{13}C-NMR spectra were performed on Varian XL-200E or Varian Gemini 200 with tetramethylsilane (TMS) as the internal reference. Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Ultraviolet (UV) spectra were determined on a Beckman DU-7 spectrophotometer. Infrared (IR) spectra were recorded on a Perkin Elmer Model 881 instrument.

HPLC analyses were carried out on a Perkin-Elmer Series 410 Bio system equipped with a C-18 reversed-phase column (250 x 4.1 mm i.d.). Solvent A was a mixture of 95% water, 5% CH_3CN and 0.1% TFA; solvent B was a mixture of 5% water, 95% CH_3CN and 0.1% TFA. The ratio of solvent A and B was changed from 65:35 to 35:65 and the flow rate was 1 mL/min. Detection was performed at $\lambda = 254$ nm.

General quantification procedures:

1) Typical acidic work-up (normal work-up). An aliquot (1 mL) was taken from the reaction mixture and added to 2 mL 25% H_2SO_4 at 0 °C, and extracted 3 times with diethyl ether (5 mL each time). The combined organic extracts were washed with saturated solution of $NaHCO_3$ and water, dried over $MgSO_4$ and added with 1 mL naphthalene solution (0.08 M in diethyl ether) as an internal standard. The products were analyzed by gas chromatography.

2) Typical basic work-up. An aliquot (1 mL) was taken from the reaction mixture and added to 2 mL 5% NaOH solution at 0 °C. After extraction with diethyl ether(5 mL, 3 times) and dried over $MgSO_4$, 1 mL naphthalene solution (0.08 M in diethyl ether) was added as an internal standard. The products were analyzed by gas chromatography.

3) Measurement of O_2 evolved. The reaction system was made gastight and connected to a manometric burette filled with saturated brine solution which was saturated with oxygen (air) prior to use. The volume of O_2 gas evolved from the Gif reactions was measured. During the readings, the pressure was always equilibrated using a separation funnel by adjusting the brine levels to the same heights. Also, the appropriate temperature and atmospheric pressure were taken into account before each reading and considered in the calculations using the ideal gas law.

Typical experiment for Fe^{III}-H_2O_2 model system:

$FeCl_3 \cdot 6H_2O$ (270 mg, 1 mmol) was dissolved in 33 mL pyridine. The solution was cooled to 0 °C (or -20 °C) and the reaction was initiated by the addition of H_2O_2 (0.4 mL, 4 mmol) dropwise (30 s to 1 min). Oxygen formed was measured as described in the general quantification procedures. The solution was kept at this temperature for 1 h, then allowed to gradually come to room temperature. The reaction was usually left over night for the final oxygen measurement.

Typical experiment for oxidation of sulfide by Fe^{III}-H_2O_2 model system:

$FeCl_3 \cdot 6H_2O$ (270 mg, 1 mmol) and sulfide (10 mmol) were dissolved in 33 mL pyridine. The solution was cooled to 0 °C (or -20 °C) and the reaction was initiated by the addition of H_2O_2 (0.4 mL, 4 mmol) dropwise (30 s to 1 min). Oxygen formed was measured as described in the general quantification procedures. The solution was kept at this temperature for 1 h, then allowed to gradually come to room temperature. The reaction was usually left over night for the final oxygen measurement. The products were quantified by typical acidic work-up procedure but using AcOEt instead of Et_2O to do the extraction. For a kinetic study, two same experiments were set up: one for O_2 quantification and the other for sulfoxide quantification.

Typical experiment for oxidation of sulfide by *m*CPBA method:

Suitable sulfides (5 mmol each) were dissolved in 33 mL CH_2Cl_2. The solution was cooled to 0 °C and the reaction was initiated by the addition *m*CPBA (1 mmol) slowly. The reaction mixture was then allowed to come to room temperature and the reaction continued for over night. 3 mL of solution was taken and added with 1 mL naphthalene standard solution (0.08 M in Et_2O). The products formed were then quantified by GC.

Typical experiment with catalase enzyme:

Catalase (10 mg) was dissolved in 33 mL pH 7 phosphate buffer (or appropriate mixture of solvents). The reaction was initiated by the addition of H_2O_2 (0.4 mL, 4 mmol) dropwise (30 s to 1 min) at room temperature. Oxygen formed was measured as described in the general quantification procedures. The reaction was usually continued at room temperature for 1 hour. When other substrates were used, the products formed were quantified by following the typical work-up procedure except that no acid or base was used and AcOEt was used as extraction solvent.

The oxidation of thianthrene-5-oxide by Fe^{III}-H_2O_2 model system:

$FeCl_3 \cdot 6H_2O$ (135 mg, 0.5 mmol) and thianthrene-5-oxide (464 mg, 2 mmol) were dissolved in 16.5 mL pyridine. The solution was cooled to 0 °C and the reaction was initiated by the addition of H_2O_2 (0.2 mL, 2 mmol) dropwise (30 s to 1 min). Oxygen formed was measured as described in the general quantification procedures. The solution was kept at this temperature for 1 h, then allowed to gradually come to room temperature. The reaction was left over night for the final oxygen measurement. 0.78 mmol of oxygen was produced in this reaction. 1 mL of solution was taken for a typical acidic work-up procedure but using CH_2Cl_2 instead of Et_2O to do the extraction. CH_2Cl_2 was evaporated on a rotavapor and replaced by CH_3CN for HPLC analysis. A trace amount of thianthrene-5,10-dioxide was detected. The reaction mixture was then cooled to 0 °C again, and more H_2O_2 (2 mmol x 4) was added. The oxidation products were quantified by the procedure described above, after the reaction mixture was allowed to warm to room temperature and stirred over night. Thianthrene-5,10-dioxide (0.11 mmol) was detected with phenyl sulfone as internal standard.

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