Oxidation and Other Reactions of Thiobenzamide Derivatives of Relevance to Their Hepatotoxicity

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The H_2O_2 oxidation of thiobenzamide (**3**) and 12 of its derivatives and the chemical reactivity of the oxidation products (*S*-oxides and *S,S*-dioxides) have been investigated to provide a basis for understanding the cytotoxic responses which occur following oxidative biotransformation of thioamides in vivo. Para-, 2,6-di-, and 4,*N*-disubstituted thiobenzamides undergo mono-S-oxidation with H_2O_2 smoothly at pH 7.4 and 25 °C with second-order rate constants ranging from 0.15 to 0.001 M^{-1} s^{-1} and Hammett ρ values of −0.4, −0.9, and −2.0, respectively. The oxidation of **3** shows no buffer catalysis and is indpenendent of pH from pH 3 to pH 9.1. This reaction undoubtedly proceeds via electrophilic attack by H_2O_2 on the thioamide sulfur. When treated with base, thiobenzamide *S*-oxide (**15**) undergoes clean elimination of the sulfur moiety, forming PhCN in high yield; the process is first order in HO^-. In contrast, 2.9 M HCl, Lewis acids, and $(CF_3CO)_2O$ convert **15** very efficiently to 3,5-diphenyl-1,2,4-thiadiazole (**28**), whereas 2,6-disubstituted *S*-oxides form only the corresponding nitrile under acidic or basic conditions. Thiobenzamide *S*-oxides are reduced to the parent thioamides by various thiols including *N*-acetylcysteine at pH 7.4 and 25 °C. The reactions are first order in both *S*-oxide and thiol and proceed at rates which could be significant under in vivo conditions ($k_r = 10^{-2}$–10^{-3} M^{-1} s^{-1}). Second-order rate constants for H_2O_2 oxidation of 4-substituted thiobenzamide *S*-oxides are much lower than those for the first oxidation (ca. 5×10^{-4} M^{-1} s^{-1}). For this reaction $\rho = 0$, but the rate increases markedly with pH above pH 9. Since no deprotonation of **15** was detectable spectrally from pH 3 to pH 10.3, the increase in oxidation rate at higher pH was attributed to nucleophilic attack by HOO^- on an electrophilic *S*-oxide sulfur atom. At pH 8–10 oxidation of **15** produced only PhCN, but at lower pH increasing amounts of $PhCONH_2$ formed; $PhCONH_2$ was the only product at pH 4. PhCN was not an intermediate in $PhCONH_2$ formation, and if ethanol was present, ethyl benzimidate was formed, showing that the oxidation of **15** generates an acylating agent (presumably imino sulfinic **29**). In contrast, oxidation of ortho-substituted thiobenzamides led only to the corresponding nitriles even at low pH where **15** yields exclusively amide or imidate products. The possible relevance of these observations to the hepatotoxicity of thiobenzamide derivatives and their metabolites is discussed.

A wide range of toxic thiocarbonyl compounds including thiourea derivatives,[1,2] thiobenzamides,[3,4] and thioacetamide[5,6] are thought to require metabolic activation, probably via one or more enzymatic S-oxidations, for expression of their toxicity.[7] Previous work in our laboratory has demonstrated that within a family of ring-substituted thiobenzamide derivatives, relative potency to hepatotoxicity is strictly correlated with the Hammett σ value of the para[3] or meta[8] substituent. Consistent with the postulated requirement for S-oxidation as a bioactivation step, the ρ values for various indices of toxicity range from −1.5 to −4.0, indicating that in each case toxicity is increased greatly by electron-donating substituents. We have also shown that thiobenzamide and its para-substituted derivatives undergo rapid S-oxidation by the flavin-containing monooxygenase (MFMO; EC 1.14.13.8) present in rat liver microsomes.[9] In contrast, 2,6-disubstituted thiobenzamides are not hepatotoxic[3] despite the fact that they too are rapidly S-oxidized in vitro and presumably in vivo. Although thiobenzamide *S*-oxides have been known by synthesis for some 20 years,[10] relatively little is known concerning their chemical reactivity. The present study was undertaken to obtain such base-line information in the hope that it would be helpful in understanding the metabolic activation and subsequent toxicity of thiobenzamides and related compounds.

Table I. Properties of Substituted Thiobenzamides and Their *S*-Oxides

compd (substituent)	mp,[a] °C	R_f,[b] solvent	λ_{max} (ϵ)[c]
	Thiobenzamides		
1 (4-CH_3O)	149	0.33, A	296 (11 640)
2 (4-CH_3)	168–169	0.60, B	293 (8310)
3 (4-H)	116–118	0.41, A	286 (8180)
4 (4-Cl)	128–129	0.56, B	302 (7810)
5 (4-CF_3)	135	0.49, A	305 (7470)
6 (4-NO_2)	160	0.29, A	255 (9600)
7 (2,6-(CH_3)$_2$)	153–154	0.71, A	268 (9610)
8 (2-CH_3, 6-Cl)	123–125	0.48, A	271 (8990)
9 (2,6-Cl_2)	151–152	0.50, A	271 (8210)
10 (*N*-CH_3)	114–115	0.82, A	271 (14 100)
11 (4-CH_3O, *N*-CH_3)	78–79	0.52, A	245 (9550)
12 (4-Cl, *N*-CH_3	153–154	0.59, A	252 (12 800)
13 (*N,N*-(CH_3)$_2$)	68–69	0.67, A	270 (10 200)
26 (2-Cl)	64–65	0.47, B	269 (8840)
	Thiobenzamide *S*-Oxides		
14 (4-CH_3)	92–93	0.07, B	331 (6730)
15 (4-H)	127–128	0.08, A	328 (8300)
16 (4-Cl)	170	0.05, B	335 (7500)
17 (4-CF_3)	168 dec	0.06, B	338 (6400)
18 (4-NO_2)	170	0.09, B	371 (6160)
19 (2,6-(CH_3)$_2$)	162–163	0.06, B	302 (7470)
20 (2-CH_3, 6-Cl)	164–165	0.05, B	292 (5800)
21 (2,6-Cl_2)	137–138	0.15, B	307 (7040)

[a] Melting points were taken in a Hoover apparatus and are uncorrected. [b] R_f values were measured on 0.25-mm silica plates; solvent A is $CHCl_3$/MeOH (97.5:2.5) and solvent B is EtOAc/hexane (40:60). [c] UV-vis spectra were recorded in 0.1 M sodium phosphate buffer at pH 7.4 containing 2.5% acetonitrile; λ values are in units of nm, and ϵ values are in units of M^{-1} cm^{-1}.

Experimental Section

Chemicals. All chemicals used were reagent grade or better, and all compounds listed in Table I were fully characterized by

(1) Boyd, M. R.; Neal, R. A. *Drug Metab. Dispos.* **1976**, *4*, 314.
(2) Hollinger, M. A.; Giri, S. N.; Hwang, F. *Drug Metab. Dispos.* **1976**, *4*, 119.
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(4) Chieli, E.; Malvaldi, G.; Tongiani, R. *Toxicology* **1979**, *13*, 101.
(5) Hunter, A. L.; Holscher, M. A.; Neal, R. A. *J. Pharmacol. Exp. Ther.* **1977**, *200*, 439.
(6) DeFerreyra, E. C.; DeFenos, O. M.; Castro, J. A. *Toxicology* **1980**, *16*, 205.
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(8) Parikh, K.; Cashman, J. R., unpublished results.
(9) Cashman, J. R.; Hanzlik, R. P. *Biochem. Biophys. Res. Commun.* **1981**, *98*, 147.
(10) Walter, W.; Curts, J. *Chem. Ber.* **1960**, *93*, 1511.

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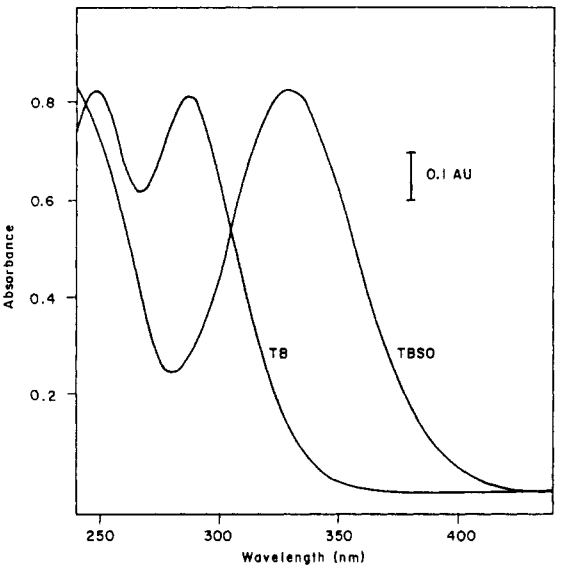

Figure 1. UV–visible spectra of thiobenzamide (TB, 0.1 mM) and thiobenzamide *S*-oxide (TBSO, 0.1 mM) in 0.1 M sodium phosphate buffer (pH 7.4).

elemental analysis (C, H, N) and IR, UV–Vis, ^{1}H NMR, and in most cases mass spectrometry as well. Primary thiobenzamides were synthesized by using the method of Fairful et al.[11] [3,5-^{3}H]Thiobenzamide was prepared similarly from [3,5-^{3}H]benzonitrile.[12] Other thiobenzamides were prepared from the corresponding benzamide by using P_2S_5 in pyridine.[13] Thiobenzamide *S*-oxides were prepared as described by Walter and Curts[10] and were recrystallized from MeCN.

Measurement of Rate and Equilibrium Constants. All measurements were made spectrophotometrically at 25.0 ● 0.2 °C by using a Cary 118C spectrophotometer. The buffers employed were HCl (pH <3), acetate (pH 3–6), phosphate (pH 6–8 or 10–13), bicine (pH 8–10), and hydroxide (pH >13); the ionic strength was maintained at 0.26 with NaCl. Metal-free conditions were ensured by extracting the buffers with dithizone in chloroform or by the addition of 1 mM EDTA. Hydrogen peroxide solutions were standardized by iodometric titration. Stock solutions of thiobenzamides and their *S*-oxides were prepared in dry acetonitrile, and aliquots were diluted into the aqueous reaction solutions such that the final concentration of acetonitrile never exceeded 2.5%.

Equilibrium acidity constants were determined from plots of the shift in λ_{max} of the compound vs. pH. Rate constants (k_{obsd} were measured under pseudo-first-order conditions by monitoring the formation or disappearance of the *S*-oxide (usually at 370 nm; see Figure 1) for at least 5 half-lives, and second-order rate constants were obtained from plots of k_{obsd} vs. the concentration of the reactant present in large excess (usually H_2O_2 or thiol). Buffer catalysis could not be detected in any of the reactions studied. Reaction products were isolated from kinetic runs (2 mL/cuvette) or larger scale reactions (same concentrations of reactants) by ether extraction followed by preparative thin-layer chromatography on silica gel; for the studies with [^{3}H]TB, Whatman LK5DF TLC plates with a preadsorbent loading zone were used. In these cases 1–2 ppm of diphenylamine added to the solvent system effectively stopped the spurious formation of oxidation products during development of the plates.

Results

Reaction of Thiobenzamide with Hydrogen Peroxide. Both the reaction of thiobenzamide (**3**) with excess hydrogen peroxide and the reaction of hydrogen peroxide with excess **3** were observed to follow pseudo-first-order kinetics for 3–5 half-lives. Thiobenzamide *S*-oxide (**15**) was the only detectable product, although at long reaction times it too decomposed in the presence of H_2O_2 (see below). In practice the second-order rate constants (eq 1)

$$d[15]/dt = k_1[3][H_2O_2] \quad (1)$$

were determined from the slopes of plots of k_{obsd} vs. $[H_2O_2]$. Such plots had zero intercepts and were linear throughout the range of H_2O_2 concentration of 0–120 mM.

To investigate the effect of pH on the reaction of **3** and H_2O_2, we first established that the UV–vis spectrum of **3** did not vary from pH 3 to pH 10, which suggested that no ionizations were occurring. Various concentrations of acetate (pH 4.0), phosphate (pH 7.4), and bicine (pH 9.1) buffers were employed to determine whether buffer catalysis was occurring for this reaction, but buffer catalysis was not detected.

The second-order rate constant for the reaction of **3** and H_2O_2 was constant over the range of pH 3 to pH 9.1. (Figure 2), and at each pH studied UV–vis scans at the end of the kinetic run showed that **15** was the only product formed.

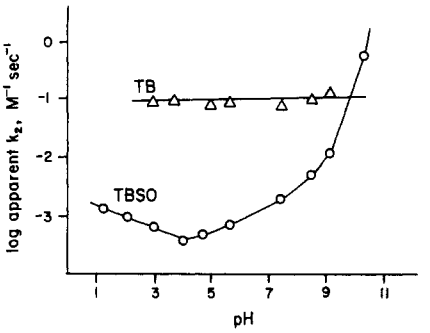

Figure 2. Plot of apparent second-order rate constants vs. pH for the H_2O_2–oxidation of thiobenzamide (△) and thiobenzamide *S*-oxide (○).

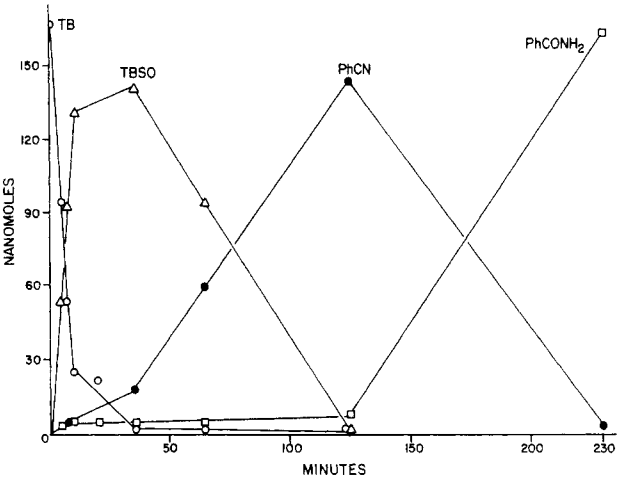

Figure 3. Plot of product composition vs. time for the reaction of [^{3}H]thiobenzamide with excess H_2O_2 at pH 7.4: thiobenzamide, ○; thiobenzamide *S*-oxide, △; benzonitrile, ●; benzamide, □.

Reaction of [^{3}H]Thiobenzamide with H_2O_2. In order to investigate the complete multistep H_2O_2 oxidation of **3**, we prepared tritiated **3** and subjected it to oxidation with excess H_2O_2 at pH 7.4. The reaction conditions chosen were essentially identical with those above. However, a TLC assay system was used in order to monitor multiple reaction products simultaneously and to observe products which are hidden spectrally by the end absorption of H_2O_2. Figure 3 shows the product composition vs. time profile for the oxidation of **3** with excess H_2O_2. It is apparent that

(11) Fairful, A. E. S.; Lowe, J. L.; Peak, D. A. *J. Chem. Soc.* **1952**, 742.
(12) Hanzlik, R. P.; Wiley, R. A.; Gillesse, T. J. *J. Labelled Compd.* **1979**, *16*, 523, 531.
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3 is rapidly oxidized to 15 which is then converted successively to benzonitrile (22) and benzamide (23).

Reaction of Thiobenzamide *S*-Oxide with Hydrogen Peroxide. The reaction of excess hydrogen peroxide with 15 is a pseudo-first-order process and at neutral pH leads to benzonitrile as the major (≥94%) organic product. The reaction of H_2O_2 with excess 15 is also pseudo first order. The second-order rate constant for oxidation of 15 (eq 2) was determined from a plot of k_{obsd} vs. H_2O_2 concentration (0–480 mM). In the neutral pH range such plots were linear and had zero intercepts. However, in the alkaline pH range (pH ≥10.0) a second kinetic term was apparent from the markedly nonzero intercept of k_{obsd} vs. H_2O_2 concentration plots. This kinetic contribution was apparently not associated with ionization of 15 since careful rapid UV–vis scans of solutions of 15 did not show any spectral changes from pH 3.0 to pH 13.8, although a pK_a of ~0.8 was determined spectrophotometrically by titrating 15 below pH 3.0. In 2.9 M HCl the UV–vis spectrum of 15 shows maxima at 281 (ϵ 8690 M^{-1} cm^{-1}) and 255 nm (ϵ 10 500 M^{-1} cm^{-1}).

$$-d[15]/dt = k_2^{app}[15][H_2O_2]_T \quad (2)$$

The above observations prompted us to investigate the effect of pH on the oxidation of 15. By employing acetate (pH 4.0), phosphate (pH 7.4), and bicine (pH 9.1) buffers, buffer dilution studies established that buffer catalysis is also absent from the H_2O_2-mediated decomposition of 15. Apparent second-order rate constants for the pH–rate profile were determined from the slopes of k_{obsd} vs. H_2O_2 concentration at various pH values. The results of these studies are also shown in Figure 2. It is apparent that alkaline pH substantially increases the rate of TBSO oxidation. At neutral or alkaline pH, the major initial organic product from oxidation of 15, as judged by UV–vis scans, is benzonitrile (22). In contrast, the major product (97%) from oxidation of 15 in acidic solution (pH ≤4) is benzamide (23). Although kinetic studies of this reaction were not undertaken, it was clear that the presence of excess H_2O_2 both greatly accelerated the decomposition of 15 and caused the formation of a product different from that formed in alkaline peroxide or in acid alone (see below). Oxidation of 15 at intermediate pH values led to the formation of various mixtures of 22 and 23, the ratio of which varied with pH. When these oxidations were repeated in buffered 50 mol % ethanol solutions, a new product, ethyl benzimidate, (24), was formed. Both 22 and 24 were hydrolytically stable under the conditions of these experiments. Thus the amide and imidate which formed must have arisen directly from reaction of an oxidation product of 15 with water or ethanol, respectively.

Effect of Substituents on Oxidation of Thiobenzamide and Thiobenzamide *S*-Oxide with H_2O_2. We determined the effect of substituents on the oxidation of three classes of thiobenzamide derivatives (Table I). In all cases, excellent pseudo-first-order plots were obtained, from which second-order rate constants were determined as described above. Figure 4 shows the results of this study in a typical Hammett fashion. The second-order rate constant for oxidation of *N,N*-dimethylthiobenzamide is also included in Figure 4 for comparison. For all three classes of compounds linear correlations are apparent. Furthermore, as the rates of oxidation decrease from one class of compounds to another, the ρ values become progressively larger and more negative. Thus the ρ values for the oxidation of para-substituted thiobenzamides, 2,6-disubstituted thiobenzamides, and para-substituted *N*-methylthiobenzamides are –0.4, –0.9, and –2.0, respectively. In contrast to these results the Hammett plot for the oxidation of para-substituted thiobenzamides *S*-oxides with H_2O_2 (Figure 5) is markedly different; the ρ value is essentially zero despite the very low oxidation rates. This may imply a different mechanism of reaction for this group of substrates.

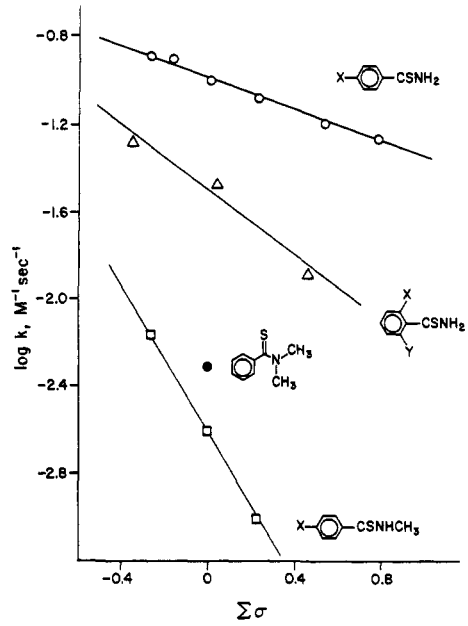


Figure 4. Hammett plot for the oxidation of substituted thiobenzamides with H_2O_2 at pH 7.4. $\Sigma\sigma$ is the sum of the Hammett para-substituent constants for X and Y.

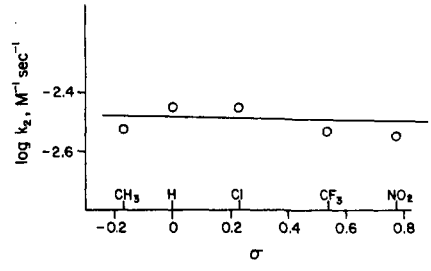


Figure 5. Hammett plot for the reaction of para-substituted thiobenzamide *S*-oxides with H_2O_2 at pH 7.4.

The oxidation of substituted thiobenzamide *S*-oxides sometimes took a different overall course from the oxidation of 15. For example, whereas the oxidation of 15 with excess H_2O_2 at low pH led cleanly to amide 23 (or imidate 24 if ethanol was present), similar oxidations of 20 led only to 2-chloro-6-methylbenzonitrile (25) under all conditions of pH or added ethanol. Surprisingly, oxidation of 2-chlorothiobenzamide (26) with excess H_2O_2 also led only to 2-chlorobenzonitrile (27) at all pH values studied (pH 4–10.5).

Reaction of Thiobenzamide *S*-Oxides with Acid, Base, and Thiols. The effect of thiols on the decomposition of thiobenzamide *S*-oxides (50 μM) at neutral pH (phosphate buffer, pH 7.4) was determined spectrophotometrically. By varying the initial concentrations of the reactants, the reactions of 15 and 2-chloro-6-methylthiobenzamide *S*-oxide (20) with *N*-acetylcysteine, dithiothreitol, or *o*-mercaptobenzoic acid were found to be pseudo first order in both thiol and *S*-oxide. In all cases the *S*-oxides were cleanly reduced to the corresponding thioamides, which were identified by TLC and their UV–vis spectrum. The second-order rate constants (eq 3) given

$$-d[S\text{-oxide}]/dt = k_r[S\text{-oxide}][RSH] \quad (3)$$

in Table II were determined by a linear least-squares fit-

Table II. Rate Constants for Reduction of Thioamide S-Oxides by Thiols[a]

S-oxide	thiol	k_r, M^{-1} s^{-1}
TBSO	o-$C_6H_4(SH)CO_2H$	1.0×10^{-2}
2-Cl-6-MeTBSO		3.8×10^{-2}
TBSO	dithiothreitol	8.2×10^{-3}
2-Cl-6-MeTBSO		5.8×10^{-2}
TBSO	N-acetylcysteine	6.6×10^{-4}
2-Cl-6-MeTBSO		1.6×10^{-2}

[a] Reaction conditions: pH 7.4, 25 °C, μ = 0.26 (NaCl).

ting of k_{obsd} vs. thiol concentration (0–12.0 mM) for each thioamide S-oxide.

For the decomposition of **15** in NaOH solution (0.01 M), repetitive scans of the UV–vis spectrum of the reaction mixture revealed a pseudo-first-order loss of S-oxide ($t_{1/2}$ = 44.4 min) with no evidence for the occurrence of spectrally detectable intermediates. Since this reaction was also found to be first order in hydroxide, the rate law is as give in eq 4, with the value of k_B being 0.316 M^{-1} s^{-1}.

$$-d[15]/dt = k_B[15][HO^-] = k_{obsd}[15] \quad (4)$$

The major product (ca. 94%, estimated spectrophotometrically) was benzonitrile. Similarly, the base-catalyzed decomposition of **20** was pseudo first order with a half-life of 37.8 min in 0.01 M NaOH. The major product (ca. 97%) of this reaction was nitrile **25**, as judged by TLC, HPLC, mass spectrometry, and comparison of the UV–vis spectrum at the end of the reaction with a spectrum of authentic nitrile.

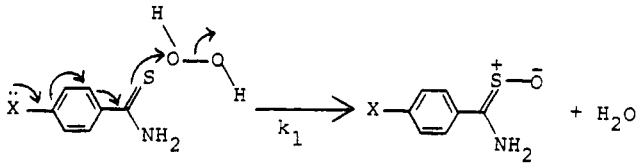
When thiobenzamide S-oxide was placed in 2.9 M HCl, repetitive scans of the UV–vis spectrum revealed that a first-order loss of **15** ($t_{1/2}$ = 75.5 h) coincided with the emergence of one major new product. A preparative-scale reaction (30 mg) was performed, and at completion the mixture was extracted with ether. Preparative thin-layer chromatography of the extract on silica gel with hexanes as an elution solvent separated elemental sulfur (R_f 0.75) from the remaining material. The latter was rechromatographed with ethyl acetate/hexanes (40:60) to yield 3,5-diphenyl-1,2,4-thiadiazole[14] (**28**; R_f = 0.72; 7 mg, uncorrected for recovery efficiency) and traces of **3** and **22**. The structure of this compound was confirmed by its melting point and IR, UV, and mass spectral comparison with an authentic sample.[14] The same thiadiazole plus elemental sulfur also formed in high yield upon treatment of **15** in acetonitrile with $(CF_3CO)_2O$, $BF_3{\cdot}Et_2O$, or anhydrous $CaCl_2$ (eq 5) or upon treatment of **3** in acetonitrile with I_2.

(5)

28

In 2.9 M HCl **20** decomposed with a half-life of 13.2 min. At the end of the reaction UV–vis scans and TLC examination of the reaction mixture clearly showed that by far the major product was **25**. Treatment of **20** with $(CF_3CO)_2O$ in MeCN again gave **25** as essentially the sole organic product. The rapid elimination of **20** to form nitrile **25** contrasts sharply with the very slow dimerization of **15** to thiadiazole **28** under comparable conditions. Since the methyl and chloro substituents in **20** have nearly equal and opposite inductive effects, differences between **15** and **20** are more likely attributable to the steric effects of the two ortho substituents which force the thioamide S-oxide moiety into a plane perpendicular to the aromatic ring and thus out of conjugation with it. The fact that compounds **7–9** and **19–21** are colorless or nearly so while compounds **1–6** are yellow and **14–18** are vividly yellow is also indicative of a sterically imposed inhibition of conjugation in the 2,6-disubstituted compounds.

Scheme I



Discussion

Reactions. Initially the reaction of thiobenzamide (**3**) with excess H_2O_2 was investigated to identify the products formed. H_2O_2 was chosen as the oxidant because the MFMO enzyme which oxidizes **3** generates a 4(a)-hydroperoxyflavin as the enzymic oxidant.[15,16] As shown in Figure 3, **3** is rapidly oxidized to thiobenzamide S-oxide (**15**) which is then slowly converted to PhCN and in turn to $PhCONH_2$. The conversion of **15** to PhCN must involve an oxidative step, since in the absence of H_2O_2 this reaction is extremely slow; e.g., at pH 7.4 the value of k_{obsd} calculated by eq 4 is 7.93×10^{-8} s^{-1} whereas in the presence of 0.1 M H_2O_2 k_{obsd} is 2.8×10^{-4} s^{-1}. The ultimate formation of benzamide is attributed to the well-known[17] H_2O_2-catalyzed hydrolysis of nitriles and not to any direct reaction of **15**.

Kinetic studies showed that the reaction of **3** with H_2O_2 is first order in both reactants and is not subject to acid, base, or buffer catalysis in the range from pH 3 to pH 9. Studies with substituted thiobenzamides (Figure 4) indicated that those with electron-donating substituents were oxidized faster. The ρ values obtained for these oxidations range from −0.4 to −2.0, and may be compared to the ρ value of −0.88 reported for the oxidation of 4-substituted thiobenzophenones with perbenzoic acid,[18a] or the ρ values of −0.32 and −0.56 reported for the oxidation of substituted thiobenzanilides.[18b] The results in Figure 4 clearly show that the influence of ring substituents becomes greater as the overall reactivity of the particular group of thiobenzamides decreases, in accordance with the reactivity–selectivity principle.[19] While it is customary to exclude ortho-substituted compounds from correlations such as that in Figure 4 because of the intervention of steric effects, the steric effects are essentially constant among compounds **7–9**, and the electronic effect may clearly be seen. From these data we conclude that H_2O_2 behaves as a typical electrophilic oxidant toward the nucleophilic thiobenzamide sulfur atom (Scheme I).

Kinetic studies of the oxidation of **15** with H_2O_2 showed that while this reaction also is first order in both reactants, it differs from the analogous oxidation of **3** in several respects. For example, as seen in Figure 2 the apparent second-order rate constant increases rapidly above pH 8, and above pH 10.3 it is too fast to measure conveniently without stopped-flow equipment (e.g., $t_{1/2}$ < 23 s in 0.1 M H_2O_2). Since this reaction is not subject to buffer catalysis, the increase in rate with unit slope above pH 8 must be attributed to specific base catalysis. This suggests that

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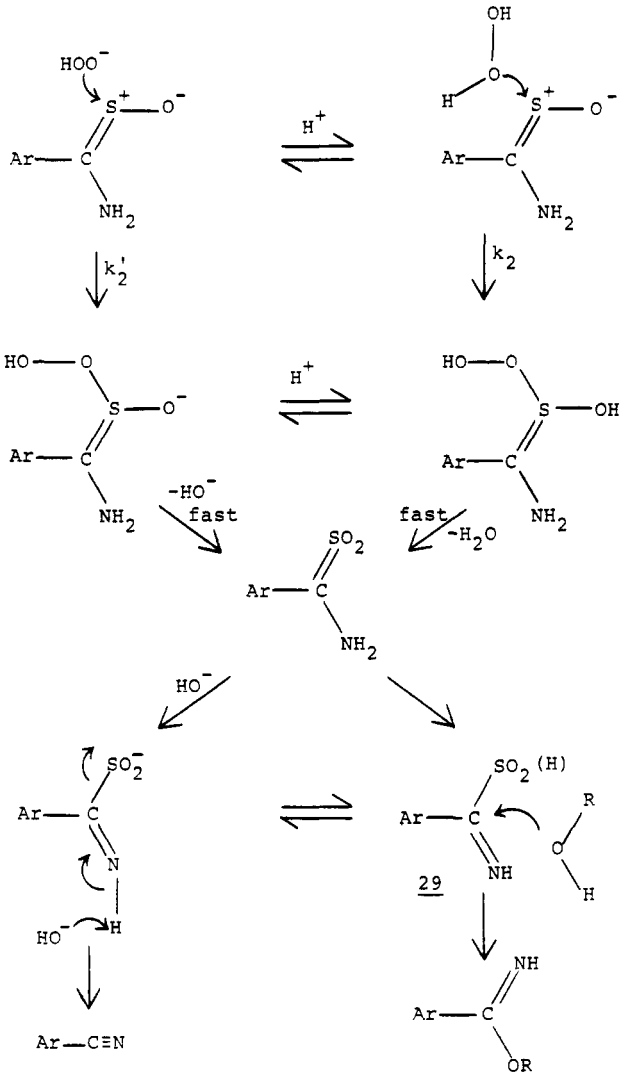
(19) Giese, B. *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 125.

Scheme II



one of the two reactants deprotonates, giving a more reactive conjugate base.

Treatment of **15** with base alone results in slow formation of PhCN in a reaction that is first order in hydroxide (eq 4). Since negligible benzamide is formed, hydroxide must be reacting as a base and *not* as a nucleophile, and since no intermediates were observed spectrally, the conjugate base of **15** must decompose as fast as it is formed. Finally, since there is no indication of deviation from eq 4 up to pH 13, the pK_a of **15** must be very high.

In contrast, the pK_a of H_2O_2 is 11.2, so it would appear more reasonable to attribute base catalysis in this reaction to *nucleophilic* attack by HOO^- on the *electrophilic sulfur atom* in **15** (see Scheme II). Although peroxides and similar oxidizing agents are usually thought of as electrophilic reagents, there is ample precedent for their reaction as nucleophiles. Examples include the oxidation of thiolsulfinates exclusively at the sulfinyl (SO) sulfur by periodate and related oxyanions,[20] the base catalysis of the oxidation of *p*-tolyl methyl sulfoxide by aromatic peracids,[21] the epoxidation of α,β-unsaturated systems (nitriles, sulfones, chalcones) by alkaline peroxide, and possibly the selective oxidation of sulfoxides to sulfones in the presence of olefins by buffered potassium hydrogen persulfate.[22]

The idea that further oxidation of the *S*-oxides involves nucleophilic attack by the oxidant on sulfur may help to explain the apparent insensitivity of this reaction, at least at pH 7.4, to the effects of para substituents (Figure 5). Because nucleophilic attack of HOOH or HOO^- on sulfur (k_2 or k_2'= in Scheme II) would involve empty d orbitals on the sulfur atom, one would not expect para substituents to exert much influence through either inductive or resonance effects. This argument would perhaps be more effective had the effect of para substituents on the oxidation of TBSO been studied at a higher pH where the k_2' pathway was clearly the dominant one. However, the only logical alternative to Scheme II would involve an electrophilic oxidation analogous to Scheme I. In this latter case, from the reactivity–selectivity principle and the low order of reactivity of the *S*-oxides vs. the thiobenzamides, one might have expected a large negative ρ value for the second oxidation.

The overall reaction of **15** at neutral or alkaline pH can thus be described by eq 6, in which each kinetic term

$$\frac{-d[\mathbf{15}]}{dt} = [\mathbf{15}]\left(\frac{k_B K_W}{[H^+]} + \frac{k_2[H_2O_2][H^+]}{K_a + [H^+]} + \frac{k_2'[H_2O_2]K_a}{K_a + [H^+]}\right) \quad (6)$$

represents a process which converts **15** to PhCN. The k_B term is equivalent to eq 4, and the terms k_2 and k_2' are as defined in Scheme II. In all three cases PhCN formation appears to be attributable to a simple *elimination* reaction from either an imino sulfenic acid tautomer of **15** or from an imino sulfinic acid such as **29**, the latter reaction being much faster than the former. The same comments probably also apply to analogous reactions of **20** with base or with neutral or alkaline peroxide; although the kinetics of these processes were not investigated in detail, nitrile **25** was essentially the only product formed under these conditions.

At acidic pH the oxidation of **15** with excess H_2O_2 takes a different course, leading directly to benzamide without the intermediacy of PhCN. This behavior can be rationalized (Scheme II) in terms of imino sulfonic acid **29** acting as an acylating agent (i.e., *substitution* rather than elimination of the sulfur leaving group). Acylation of water leads (after tautomerization) to benzamide, but if ethanol is present the stable imidate **24** is formed. Similar reactivity has been reported for the H_2O_2 oxidation of thiobenzanilide *S*-oxide in buffered aqueous ethanol,[23] although in this case nitrile formation is not possible. Furthermore, chemical oxidation (H_2O_2) of radiolabeled thioacetamide *S*-oxide in the presence of protein results in the covalent binding of radioactivity to the protein, and subsequent amino acid analysis revealed that the ϵ-amino side chains of lysine had been acetylated.[24] Evidently the reactivity of **29** as an acylating agent is effectively eliminated by the presence of even a single ortho substituent. In the 2,6-disubstituted cases it is clear that the approach of nucleophiles to the carbonyl carbon is completely blocked. In the 2-substituted case (e.g., the *S*,*S*-dioxide of **26**) nucleophiles can approach the carbonyl carbon from one side, but progress to a tetrahedral intermediate is greatly impeded by the steric crowding imposed by the ortho substituent. This view is reinforced by the observed failure of **20** to form a 1,2,4-thiadiazole dimerization

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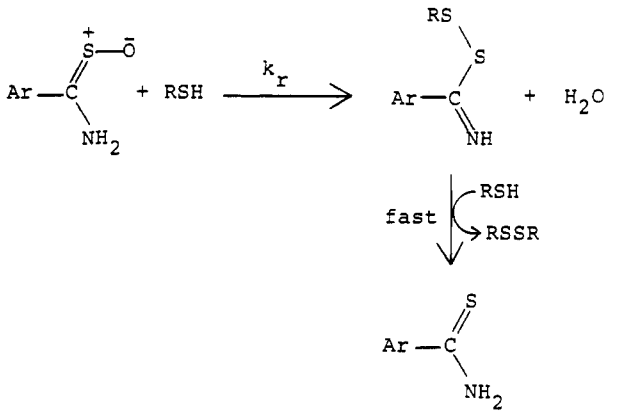
Scheme III



product when treated with Lewis or Brönsted acids, conditions which readily dimerize *S*-oxide **15** to **28**. Such dimers, however, are unlikely to play a significant role in the biotransformation of thiobenzamides in vivo.

Finally, several thiols were observed to reduce **15** and **20** to their parent thioamides. While this is formally a two-electron reduction which presumably consumes 2 equiv of RSH and forms 1 mol of RSSR, the rate law (eq 3) indicates only first-order dependence on [RSH]. In addition, dithiothreitol does not show significantly greater reactivity than the monothiols studied (Table II), and no intermediates were observed spectrally during the course of the reaction. These observations are consistent with the mechanism shown in Scheme III. The overall rates of this nonenzymatic reduction are such that similar reductions, e.g., with glutathione, might readily be expected to occur under in vivo conditions. Their possible significance in the context of thioamide toxicity is discussed below.

Metabolism and Toxicity of Thioamides. There is now general agreement that the toxicity of thioamides (including thiocarbamides) stems in most if not all cases from products of their oxidative biotransformation rather than from the parent compounds or the hydrolytic generation of H_2S as was once believed.[1-7] The rapid enzymatic oxidation of thioamides to thioamide *S*-oxides is well documented to occur both in vivo[4,25-27] and in vitro.[9,28-30] In the specific case of thiobenzamide derivatives the S-oxidation is catalyzed essentially exclusively, at least in vitro, by the MFMO enzyme system.[9,30]

The observation that the hepatotoxicity of meta- and para-substituted thiobenzamides is strictly correlated with the electron-donating ability of the substituents[3,8] is certainly consistent with the hypothesis that an S-oxidative bioactivation step is required prior to expression of hepatotoxicity. However, the Hammett ρ value for hydrogen peroxide oxidation of para-substituted thiobenzamides, −0.4, is very small compared to the ρ values for in vivo hepatotoxic reactions, which range from −1.5 to −4.0 for various indices of toxicity. Furthermore, because the substrate oxygenation step is not rate limiting in the turnover of the MFMO enzyme,[15,16] virtually all substrates are oxidized at the same rate and with very low apparent K_m values, and, indeed, no substituent effects were detected for the first S-oxidation of a series of thiobenzamide derivatives by rat liver microsomes.[30] Thus it is unlikely that the striking substituent effects on the toxicity of compounds **1–5** and related meta-substituted thiobenzamides arise because electron-donating substituents give enhanced rates of S-oxidation, at least for the *first* oxidation step.

The enzymic oxidation of thioacetamide and several thiourea derivatives leads, among other things, to metabolites having sufficient chemical reactivity to undergo spontaneous covalent binding to cellular nucleophiles. Furthermore, the occurrence and extent of such covalent binding often correlates closely with the extent of biological injury induced.[1,2,7,27,28] Evidence presented by Neal and co-workers indicates that with thioacetamide the expression of hepatotoxicity requires *two successive* S-oxidation steps, leading to an *S,S*-dioxide or sulfene metabolite as the chemically reactive cytotoxic species.[27,28] An analogous situation may obtain with thiobenzamide. In fact, *S*-oxide **15** is known to be oxidized slowly by the MFMO system.[9] Although we have not yet determined whether enzymic oxidation of **3** of **15** leads to covalent binding to macromolecules, the observation that nonenzymic oxidation of **8**, **20**, and **26** leads to reactive intermediates which undergo only elimination and do not react as acylating agents could be an important clue in understanding why ortho- and 2,6-disubstituted thiobenzamides have no significant hepatotoxic properties.[3,30]

Observations in man and several other mammalian species indicate that thioamides and their *S*-oxides are rapidly *interconvertible* in vivo in a "futile metabolic cycle".[25-27] Thus there must exist in vivo a rapid mechanism for the reduction of thioamide *S*-oxides back to the parent thioamides. Presumably, since the thioamides are not themselves toxic, this process constitutes a *detoxication* step. The mechanism of these in vivo reductions is not known, but our experiments have shown that the *nonenzymic* reduction of *S*-oxides **15** and **20** by various simple thiols proceeds at rates (Table II) which could be significant under physiological conditions in intact animals. Enzymic systems are also known which efficiently reduce sulfoxides to thioethers[32-35] but it is not known whether these systems will reduce thioamide *S*-oxides. However, it is not inconceivable that such chemical or enzymic reduction reaction(s) might show a substituent dependence *opposite* to that predicted and observed for the oxidation direction. Thus in the context of a futile metabolic cycle the steady-state concentration of *S*-oxide, and any toxicity stemming from it, would be decreased by electron-withdrawing substituents. Further work will obviously be required to substantiate this hypothesis.

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Registry No. **1**, 2362-64-3; **2**, 2362-62-1; **3**, 2227-79-4; **4**, 2521-24-6; **5**, 72505-21-6; **6**, 26060-30-0; **7**, 51643-31-3; **8**, 23822-01-7; **9**, 1918-13-4; **10**, 5310-14-5; **11**, 32872-35-8; **12**, 60253-29-4; **13**, 15482-60-7; **14**, 75554-00-6; **15**, 20199-04-6; **16**, 75554-01-7; **17**, 83312-58-7; **18**, 83312-59-8; **19**, 83312-61-2; **20**, 83312-60-1; **21**, 83312-62-3; **24**, 825-60-5; **26**, 15717-17-6; **28**, 4115-15-5; **29**, 83312-63-4; *o*-$C_6H_4(SH)CO_2H$, 147-93-3; H_2O_2, 7722-84-1; dithiothreitol, 3483-12-3; *N*-acetylcysteine, 616-91-1; water, 7732-18-5; ethanol, 64-17-5.

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