SINGLET OXYGEN OXIDATION OF SUBSTITUTED THIOBENZAMIDES†

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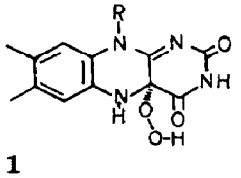
Summary

The relative rates of oxidation by singlet oxygen of a series of *p*-substituted thiobenzamides follow a Hammett $\rho\sigma$ correlation with $\rho = -0.39$ indicating that the oxidation proceeds via an electrophilic process. Initial product determinations and comparison with other examples of oxidation of thiocarbonyl compounds by singlet oxygen suggest that the reaction proceeds by attack of singlet oxygen at the sulfur atom.

1. Introduction

Thiobenzamides are hepatotoxins which are believed to require metabolic activation in order to express their toxicity [1]. Hepatotoxicity *in vivo* has been found to correlate with Hammett σ values when either plasma glutamic pyruvic transaminase ($\rho = -3.4$) or plasma bilirubin ($\rho = -1.4$) is used as an estimate of toxicity [2]. Hanzlik *et al.* [2] concluded that the activation involves an electrophilic oxidation step at the sulfur atom. Cashman and Hanzlik [3] have also shown that the oxidation is catalyzed by microsomal flavin-containing monooxygenase and not by cytochromes P-450. Using H_2O_2 as a model oxidant these same researchers have shown that *p*-substituted thiobenzamides are oxidized in a manner which correlates with Hammett σ values giving $\rho = -0.4$ and indicating the electrophilic nature of the oxidation [4].

The flavin-assisted monooxygenase produces a 4α-hydroperoxyflavin **1** during the course of the oxidation [5, 6] (**R** ≡ enzyme):



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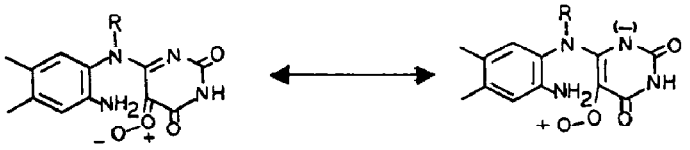
Hamilton [7] has suggested that this hydroperoxy compound is further transformed to a carbonyl oxide 2



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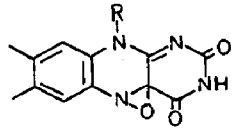
which because of favorable resonance stabilization is likely to transfer a positively charged terminal oxygen atom to the substrate in an electrophilic manner. In contrast, Orf and Dolphin [8] have suggested that the oxygen-atom-transferring species is the oxaziridine 3



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also arising from the precursor hydroperoxyflavin **1**. While **3** has apparently not yet been synthesized Davis *et al.* [9] have shown that suitably substituted oxaziridines are capable of oxygen atom transfer.

During the course of our work on the role of carbonyl oxides in ozone chemistry and, in particular, on the possible ability of these intermediates to transfer oxygen atoms we became interested in Hamilton's suggestion that carbonyl oxides could serve as models for the flavin-assisted monooxygenases. We [10 - 15] as well as others [16 - 26] have described a number of oxygen atom transfer reactions of the carbonyl oxides. Using the Hammett $\rho\sigma$ treatment we have shown that diaryl carbonyl oxides transfer oxygen atoms to naphthalene (ρ = +0.93) [14] and to diphenyl sulfide (ρ = +0.90) [27] in an electrophilic manner. Since oxidation at the sulfur atom in thiobenzamides has been shown to be catalyzed by microsomal flavin-containing monooxygenase [3] we planned to study the oxidation of substituted thiobenzamides using a carbonyl oxide as a model for the enzyme. The method of generation of carbonyl oxides in our recent studies involves singlet oxygen oxidation of diazo compounds [28]. As we began our studies on the thiobenzamides we discovered that these substrates are readily oxidized by singlet oxygen both under sensitized and, to some extent, under self-sensitized conditions. Carrying out the intended carbonyl oxide studies under these conditions would lead to results which would be difficult to interpret so we have proceeded instead to study the singlet oxygen oxidation of the substituted thiobenzamides.

2. Experimental details

2.1. Materials and methods

Thiobenzamide was purchased from Aldrich Chemical Company and was recrystallized from toluene prior to use. Substituted benzonitriles (*p*-chloro, *p*-methyl, *p*-nitro, *p*-methoxy and *p*-trifluoromethyl) were purchased from Aldrich Chemical Company. Pyridine (Gold Label), triethylamine and thiolacetic acid were purchased from Aldrich Chemical Company. H_2S was purchased from Matheson (NJ) and HCl was purchased from Air Products Company (PA). Spectra grade CH_2Cl_2 was purchased from Fisher Scientific Company. Ⓟ Rose Bengal [29] was purchased from Hydron Laboratories Inc. Tetramethylethylene (TME) (99%; Gold Label), purchased from Aldrich Chemical Company, was redistilled from calcium hydride prior to use. Authentic 3-hydroperoxy-2,3-dimethyl-1-butene was prepared following Schaap's procedure [30]. 1,1-dimethoxyethane, purchased from Eastman Organic Chemicals, was used in the nuclear magnetic resonance (NMR) analysis for the quantitative determination of the hydroperoxide product.

The substituted thiobenzamides (*p*-methoxy, *p*-chloro, *p*-methyl and *p*-trifluoromethyl) were synthesized by passing H_2S into a solution of the corresponding benzonitrile in pyridine and triethylamine as described by Fairful *et al.* [30]. *p*-nitrothiobenzamide was synthesized by reacting *p*-nitrobenzonitrile with thiolacetic acid in absolute ether, which had been saturated with HCl [31].

Thin layer chromatography (TLC) was performed using Analtech precoated silica gel 1000 μm glass plates 20 cm × 20 cm. All gas phase chromatography (GPC) work was performed on a Perkin–Elmer model Sigma 2000 gas chromatograph using a Supelco fused silica capillary column (15 m × 0.25 mm (inside diameter)). The liquid phase was Supelco SPB-5 with a film thickness of 0.25 μm. A flame ionization detector was used with a split ratio of 50:1. All the quantitative GPC work was done using a Hewlett–Packard model 3390A reporting integrator. The GPC conditions used were as follows: A, column temperature of 130 °C for 2 min then programmed at 5 °C min^{-1} to 180 °C and held for 5 min, injector temperature of 230 °C, detector temperature of 230 °C; B, column temperature of 110 °C (isothermal), injector temperature of 200 °C, detector temperature of 230 °C.

2.2. Sensitized photo-oxidation of thiobenzamide

The photo-oxidations were carried out at 5 °C in a Pyrex vessel in 125 ml of CH_2Cl_2 and using 200 mg of Ⓟ Rose Bengal as sensitizer with a General Electric Company DWY 650 W lamp. In all cases 5 mmol (0.685 g) of thiobenzamide were used. After photo-oxidation for different time periods (*vide infra*) the sensitizer was filtered off and the CH_2Cl_2 removed at reduced pressure. Unreacted thiobenzamide was separated from the reaction residue by preparative TLC using aliquots of the crude residue and several plates and using chloroform:methanol (98:2) as the developing solvent (R_f = 0.4). The

extent of oxidation for various reaction times was as follows (time (in minutes):percentage): 5:18.1; 10:27.3; 15:70.7; 30:98.5; 35:100.

2.3. Unsensitized photo-oxidation of thiobenzamide

The photo-oxidation was carried out as described above except that no Ⓟ Rose Bengal was used. The reaction was continued for 35 min and unreacted thiobenzamide isolated as before. This reaction led to 34.5% oxidation of the thiobenzamide. A similar reaction carried out for 45 min led to 38% oxidation of the thiobenzamide.

No thiobenzamide oxidation was observed when similar reactions were attempted (a) with sensitizer and no irradiation, (b) without sensitizer and no irradiation or (c) with N_2 used instead of O_2.

2.4. The effect of quenchers on the photo-oxidation of thiobenzamide

Sensitized photo-oxidations similar to those described above were run for the purpose of determining the effect of various quenchers on the oxidation. When the photo-oxidation (35 min run) was carried out with 2,6-di-*tert*-butylphenol present in either a 5:1 or a 1:5 ratio (thiobenzamide:phenol) the reaction was found to proceed to 100% conversion. When 1,4-diazabicyclo-[2.2.2]octane (DABCO) is used as quencher at a ratio of thiobenzamide: DABCO of 1:1 then the percentage conversion falls to 74.3. When the thiobenzamide:DABCO ratio is 1:5 then the conversion falls to 19.1%.

Photo-oxidations were also carried out to determine the influence of a filter solution (K_2CrO_4, 1.5 g l^{-1}). When a photo-oxidation was carried out without sensitizer but with the filter solution the conversion observed after 35 min was 5% (compared with 34.5% without filter). When the photo-oxidation was carried out with Ⓟ Rose Bengal sensitizer and the K_2CrO_4 filter solution the conversion after 35 min was 64.6%. In all these cases the percentage conversion was determined using TLC analysis.

2.5. Determination of relative rates of thiobenzamide photo-oxidations

The competitive photo-oxidations were carried out by using 1 mmol each of thiobenzamide and one of the series of substituted thiobenzamides. The reactions were performed in 100 ml of CH_2Cl_2 at 0 °C and using a K_2CrO_4 filter solution. The reactions were stopped after 5 min of irradiation. The Ⓟ Rose Bengal was filtered off and the solutions were concentrated. The concentrated reaction mixtures were then brought to 100 ml using CH_2Cl_2 and volumetric flasks. The yields of unreacted thiobenzamides were determined by GPC using response curves based on standardized injections of the thiobenzamides. The GPC conditions used were A except for substituent trifluoromethyl where condition B was used. Each conversion determination was carried out three times. The results of these competitive photo-oxidations are given in Table 1.

A plot of the logarithm of the relative rate A/A_0 (where A and A_0 are the percentage conversions of substituted and unsubstituted thiobenzamides respectively) *versus* the Hammett σ values was constructed and found to give

TABLE 1

Substituent	*Relative rate*	*σ value* [32]
$-OCH_3$	1.06	−0.268
$-CH_3$	1.04	−0.170
−Cl	0.80	0.226
$-CF_3$	0.52	0.551
$-NO_2$	0.45	0.778
−H	(1.0)	—

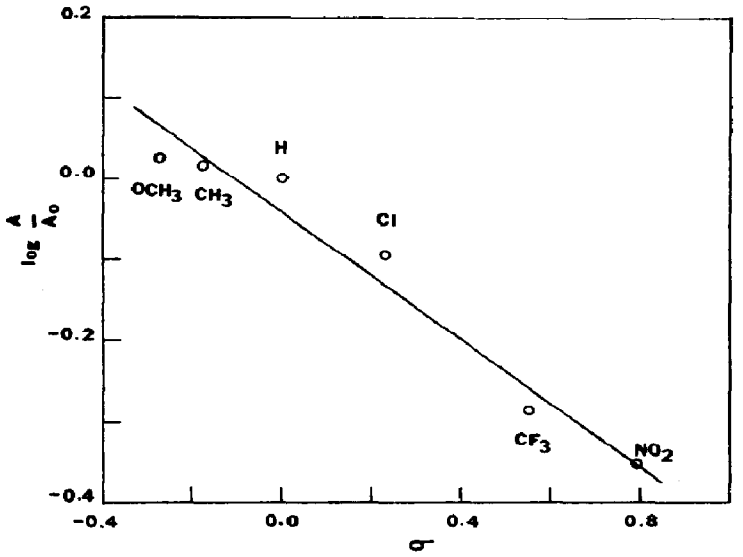


Fig. 1. Hammett plot for the oxidation of *p*-substituted thiobenzamides by singlet oxygen ($\rho = -0.39$).

a straight line with $\rho = -0.39$ (correlation coefficient, 0.981) (Fig. 1). When a similar set of data was collected for a run time of 2.5 min and the data were analyzed in the same way then $\rho = -0.42$ (correlation coefficient, 0.98).

Reactions were also run to determine the effectiveness of the K_2CrO_4 solution filter. Using thiobenzamide as sensitizer and no filter the 5 min reaction led to 23.4% oxidation while with filter the oxidation was reduced to 10.6% conversion. The corresponding Ⓟ-Rose-Bengal-sensitized reaction gave 30.6% conversion at 5 min. The corresponding results using *p*-methoxythiobenzamide as sensitizer were 27.8%, 12.1% and 36.7% conversion respectively.

2.6. Photo-oxygenations of tetramethylethylene sensitized with thiobenzamides

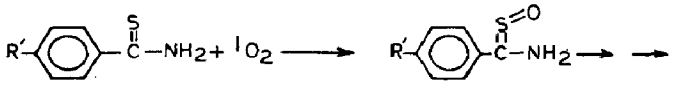
A solution of 8.4 g (100 mmol) of TME in 100 ml CH_2Cl_2 was photo-oxygenated using thiobenzamide (0.137 g, 1 mmol) as sensitizer and the lamp described above. The reactions were carried out at 5 °C with aliquots

(0.5 ml) being removed for analysis every 30 min. The extent of hydroperoxide formation (3-hydroperoxy-2,3-dimethyl-1-butene) was determined by NMR. After 2 h of reaction the yellow color of the thiobenzamide had disappeared. The solvent was removed from the reaction mixture by distillation under argon at atmospheric pressure using a Vigreaux column (25 cm × 8 mm) filled with glass helices. The bath temperature was maintained at 60 °C. The residue (about 18 ml) was mixed with 100 mg (1.1 mmol) of 1,1-dimethoxyethane as an internal standard for NMR. The resulting mixture was brought to 25 ml with CH_2Cl_2 in a volumetric flask. NMR analysis of this solution revealed that the hydroperoxide (identified by comparison with an authentic sample) was formed in 3.19% yield (3.19 mmol).

A similar experiment was carried out only using *p*-methoxythiobenzamide as sensitizer. In this case the hydroperoxide was formed in 3.29% yield.

3. Results and discussion

The relative rates of singlet oxygen oxidation of a series of *p*-substituted thiobenzamides were determined by co-oxidizing each member of the series with an equimolar amount of unsubstituted thiobenzamide. The amount of thiobenzamide reacted after a short reaction time (5 min) was determined by GPC. Under these reaction conditions the relative conversions are taken as indicative of the relative rates. The data indicate that the rate of oxidation is increased by electron-donating substituents and hindered by electron-withdrawing substituents. A plot of the logarithms of the relative rates *versus* the Hammett substituent constants σ [32] gives a straight line (Fig. 1) with slope $\rho = -0.39$, *i.e.* the oxidation is electrophilic in character:



where $R' \equiv H, OCH_3, CH_3, Cl, CF_3, NO_2$.

We have observed that the thiobenzamides are able to self-sensitize similar oxidations by singlet oxygen. While the competitive oxidations described above were carried out using (P) Rose Bengal sensitization, it is conceivable that the thiobenzamides could have different sensitization efficiencies and that such different efficiencies could interfere with our procedure for determining relative rates. Several precautions were taken to minimize any such contribution to the data. A concentrated solution of K_2CrO_4 was used as a filter by circulating it through the cooling jacket of the photo-oxidation apparatus. Control experiments indicated that the use of such a filter solution leads to appreciable reductions in contributions from self-sensitized reactions, *i.e.* a 58% reduction for unsubstituted thiobenzamide and a 57% reduction for the *p*-methoxy compound. Secondly reactions were run in an attempt to determine any differences in the self-

sensitization efficiency directly. In these reactions singlet oxygen, produced by thiobenzamide sensitization, was used to oxidize a reactive acceptor, namely TME. In order to minimize the reaction with the sensitizer the reactions were run with a large excess of TME (100:1). Reactions were run with thiobenzamide and *p*-methoxythiobenzamide. In both cases small but nearly equal amounts of the singlet oxygen product, 3-hydroperoxy-2,3-dimethyl-1-butene, were produced, consistent with an equal or nearly equal sensitization efficiency of the thiobenzamides tested. While a small contribution from unequal self-sensitization efficiencies to the data cannot be completely excluded, we believe that the observed differences in thiobenzamide reaction rate are, as far as we can determine, due to inherent differences in substrate reactivity. We have also shown that the free-radical inhibitor, 2,6-di-*tert*-butylphenol, has no effect on the extent of oxidation, while the known singlet oxygen quencher, DABCO, causes a significant reduction in the observed oxidation. The experimental data are thus most consistent with a singlet oxygen oxidation of the thiobenzamides. The reaction is electrophilic in nature consistent with the known [33] electrophilicity of singlet oxygen. The ρ value obtained here (—0.39) is comparable with that (—0.4) when a similar series of thiobenzamides is oxidized with H_2O_2 [4] or when a series of *p,p'*-substituted thiobenzophenones is oxidized by peroxybenzoic acid (ρ = —0.88) [34].

We have determined that the initially produced oxidation product in each case is the *S*-oxide corresponding to the thiobenzamide. These first products are transformed to a series of other products under longer reaction times. Details of the product analysis studies will be published elsewhere. The *S*-oxides are also the first-formed products when thiobenzamides are oxidized by H_2O_2. There have been a number of reports of the oxidation of thiocarbonyl compounds by singlet oxygen [35 - 37]. In at least one case [35] the *S*-oxide has been identified as a product of these oxidations. Hanzlik *et al.* [2] have shown that thiobenzamide *S*-oxide is significantly more hepatotoxic than thiobenzamide itself. Furthermore these researchers have shown that, when plasma glutamic pyruvic transaminase is used as a response measure, then a series of *p*-substituted thiobenzamide *S*-oxides gives a Hammett correlation with a ρ value which is about the same as that obtained from the corresponding series of unoxidized thiobenzamides. Thus hepatotoxicity requires metabolic activation and evidence suggests that oxidation at the sulfur atom, by an electrophilic oxidation process, is an essential element in the observed toxicity.

We have shown that benzamide itself is not oxidized under our reaction conditions. It is interesting that the deleterious effects of a number of thiocarbonyl compounds are associated with the presence of the sulfur functionality. Thus thioacetamide, thiourea, methylthiouracil and propylthiouracil have all been found [1] to inhibit the P-450-containing monooxygenases while their oxygen analogs do not cause a similar inhibition. Likewise, thiourea, thioacetamide, thiouracil and a number of other thiocarbonyl compounds have been shown to produce cancer in experimental

animals while their oxygen analogs have not been found to be carcinogenic in similar tests [1].

While our method of generation of carbonyl oxides for use in monooxygenase modeling studies could not be applied to the thiobenzamide substrates, the use of singlet oxygen instead has proven instructive. While the mechanism of the singlet oxygen oxidation of the thiobenzamides has not been determined, its electrophilic character suggests an attack initiated by an electron-deficient oxygen atom similar to that observed for the carbonyl oxide system [14, 27] and expected for the Hamilton [7] model for the flavin-assisted monooxygenase system. To the best of our knowledge the electronic requirements for oxygen atom transfer by oxaziridine 3 as suggested in the Orf–Dolphin model [8] have not been determined.

Acknowledgment

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