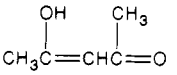
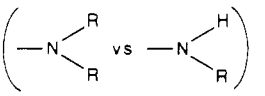
values) in the order primary < secondary < tertiary, probably as a result of varying inductive effects of the alkyl groups. 1,3-Diiodopropane is a noticeably stronger donor than iodoethane and 1-iodopropane, a fact which probably reflects the statistical advantage of two donor sites in the molecule. The cycloalkanones and 2,5-hexanedione are sufficiently strong donors to undergo 2:1 complex formation with *p*-chloranil. This diketone is a substantially better donor than the (mono)carbonyl compounds, again probably in reflection of a statistical factor. Unlike the hexanedione, 2,4-pentanedione is not a very strong donor, one which like acetone and butyraldehyde shows no significant inclination to form a termolecular complex. This no doubt reflects the fact that in nonpolar media 2,4-pentanedione exists largely in the enol form,[12] The ace-



tophenone–*p*-chloranil complex, in which the aromatic ring is the presumed donor site, is slightly weaker than the corresponding toluene complex[8,13] in reflection of the differences in inductive effects of methyl and acetyl groups. As a group the amides and the lactams used in this investigation are all relatively strong donors and demonstrate a disposition to form termolecular complexes. The lactones as donors are weaker than the *N,N*-dialkyl amides and the lactams, and, in fact, γ-butyrolactone is weak enough not to show evidence of 2:1 complex formation. Donor strengths of the lactones in their interactions with *p*-chloranil increase with increasing donor ring size for reasons which are not apparent. This is not the case for the two lactams included in the study. The *p*-chloranil complex of *N*-methylacetamide is weaker than the corresponding *N,N*-dialkyl amide complexes, presumably because of differences in the inductive effects of hydrogen atoms and alkyl groups In complexing with *p*-chloranil



vinyl acetate is a rather weak donor as compared to the lactones. Conjugation of the ester function with a carbon–carbon double bond is probably the underlying factor. Isopropenyl acetate is a better donor, though not a very strong one, possibly because of the favorable inductive effect of the methyl group attached to its carbon–carbon double bond.

With the exception of 2-iodopropane the various kinds of donors which have been investigated form markedly stronger complexes with *p*-fluoranil than with *p*-chloranil, as might be expected. The superiority in acceptor strength of *p*-fluoranil over *p*-chloranil was also apparent in the study of their interactions with ethers.

In summary, it can be concluded that the tendency of the tetrahalo-*p*-benzoquinones, (acceptors which offer two potential coordination sites, one on each face of the planar molecules) to form termolecular complexes is by no means restricted to aromatic π donor systems.[14] It appears to be a rather general phenomenon which is observed with relatively strong donors (those with relatively high K_c values) of varied structural type and in donor–acceptor solutions of relatively high donor concentration.

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Reactivity of Superoxide Ion with Thioamides in Dimethyl Sulfoxide

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The first step in the reaction of $O_2^{\bullet-}$ with thioamides (thiobenzamide, thionicotinamide, thioisonicotinamide, 2-ethyl-4-pyridinethiocarboxamide [ethionamide], thioacetamide, and thioacetanilide) in dimethyl sulfoxide is nucleophilic addition. Subsequent reaction of the initially formed peroxythiolate anion with a second $O_2^{\bullet-}$ yields O_2 and the peroxythiolate dianion; the latter undergoes nucleophilic addition with a second thioamide to give, after cleavage, 2RC(S$^-$) = NH + HOOH. The overall stoichiometry is one $O_2^{\bullet-}$ per thioamide. The formed thioamide anion and O_2 slowly react to form the corresponding nitrile (RC≡N), polysulfides, and a second HOOH. The rates of reaction for the primary step have been evaluated via rotated ring-disk voltammetry under pseudo-first-order conditions; the apparent second-order rate constants range from 115 ± 15 M^{-1} s^{-1} for thioacetamide to (6 ± 1) × 10^3 M^{-1} s^{-1} for thioacetanilide in dimethyl sulfoxide at 25 °C.

Previous studies[1-5] have demonstrated that superoxide ion ($O_2^{\bullet-}$) in aprotic media reacts with carbonyl carbons via nucleophilic addition. With esters the alkoxide is a good leaving group and there is net hydrolysis via subsequent reduction of the percarboxylate radical by a second

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$O_2^{\bullet-}$. Enolizable α-dicarbonyl compounds undergo a rapid reaction with $O_2^{\bullet-}$ via proton abstraction from the enol.[1] Nonenolizable α-diketones are oxygenated to carboxylate ions [e.g., $PhC(O)C(O)Ph + 2O_2^{\bullet-} \rightarrow 2PhC(O)O^- + O_2$]. An analogous reaction is observed with dehydroascorbic acid to give threonate and oxalate.[6] Because of the absence of adequate leaving groups there is not a net reaction for $O_2^{\bullet-}$ with benzaldehyde or aliphatic amides.[5]

Early investigations[7,8] of the enzymatic S-oxidation of thioamides [2-ethyl-4-thioisonicotinamide (ethionamide) and thioisonicotinamide] have implicated $O_2^{\bullet-}$ as the oxidant. This has been based on (a) the requirement for oxygen in the S-oxidative process and (b) the inhibition of the process by superoxide dismutase. Several other thioamides (thiobenzamides, thioacetamide, and thioacetanilide) that are used as drugs or pesticides[9-11] are converted to their *S*-oxides in biological matrices, but $O_2^{\bullet-}$ has not been implicated. A recent study[12] used 18-crown-6 ether to solubilize KO_2 in dimethylformamide for the desulfurization of thioamides. A continuing interest in the nucleophilic character of $O_2^{\bullet-}$ has prompted the present investigation of its addition to the thiocarbonyl function of several thioamides.

Experimental Section

Materials. Dimethyl sulfoxide (Me_2SO) from Burdick and Jackson (H_2O, 0.01%) and the supporting electrolyte, tetraethylammonium perchlorate (TEAP, G.F. Smith Chemical Co.), were used as received. The thioamides (Aldrich), ethionamide (Sigma), and all other materials and gases were reagent grade and used as received. The nitrile derivative of 2-ethyl-4-thioisonicotinamide was prepared by the same procedure that converts thiobenzamide to benzonitrile.[13,14] This procedure also was used to convert of thioisonicotinamide to its nitrile; the product was identical with an authentic sample of 4-cyanopyridine on the basis of their cyclic voltammetry in Me_2SO (0.1 M TEAP) and their UV spectra in hexane.

Instrumentation. The cyclic voltammetric and electrolysis measurements were accomplished with a Bioanalytical Systems (BAS) Model CV-27 potentiostat, and the voltammograms were recorded with a Houston Instruments Omni-graphic *X–Y* recorder. The working electrodes were a BAS glassy-carbon disk (3-mm diameter) for the voltammetric measurements and a glassy-carbon flag (0.5 cm × 1 cm × 4 cm) for the electrolysis measurements. All potentials were measured versus a BAS Ag/AgCl (3 M NaCl) reference electrode (–0.04 V vs SCE). The auxiliary electrodes were platinum wires; the electrolysis experiments used a platinum-wire coil in a solvent-filled compartment that was isolated from the bulk solution by a medium-porosity glass frit. The reference electrode was isolated from the bulk solution in a glass tube with a porous Vycor tip.

The kinetic measurements for the $O_2^{\bullet-}$/thioamide reactions made use of a Pine Instruments Co. system that included a Model AFMNT 29 glassy-carbon–glassy-carbon ring-disk electrode, a Model RDE 4 dual potentiostat, and a Model AFMSR rotator. The spectroscopic measurements were made with a Perkin-Elmer Lambda 3A UV–vis spectrophotometer.

Methods. The electrochemical studies used Me_2SO (0.1 M TEAP) for the solvent/supporting electrolyte and were made at 25 °C. Superoxide ion (1–4 mM) was generated by controlled potential electrolysis at –1.1 V vs Ag/AgCl of O_2 (1 or 0.21 atm, bubbled through the solution); after electrolysis excess O_2 was removed by purging with nitrogen. Aliquots of thioamide (1–4 mM) in Me_2SO were then added to these deaerated solutions. The O_2 released on reaction of thioamides with $O_2^{\bullet-}$ was determined by cyclic voltammetry with a glassy-carbon electrode in a calibrated, sealed electrochemical cell without head space. Superoxide ion concentrations were determined by measurement of the anodic voltammetric current peak at –0.6 V vs Ag/AgCl (the electrode system was calibrated with standard solutions of $O_2^{\bullet-}$).

Superoxide ion also was produced in the presence of thioamide substrates via O_2 reduction by cyclic voltammetry or controlled potential electrolysis. Ratios of electron equivalents for O_2 reduction relative to mols of reacted thioamide were obtained from the thioamide peak heights before and after coulometric reduction of O_2 in the presence of excess thioamide. Reaction stoichiometries were determined by titration of a known concentration of $O_2^{\bullet-}$ in Me_2SO with aliquots of thioamide; the residual $O_2^{\bullet-}$ was assayed by anodic voltammetry. The acid/base stoichiometries were obtained via syringe injection of 1.0 M tetrabutylammonium hydroxide in slight excess of thioamide, followed by a back-titration with 0.10 M $HClO_4$; the thioamide concentration was monitored by cyclic voltammetry.

The kinetics for the $O_2^{\bullet-}$/thioamide reactions were measured with a 15-fold excess of thioamide relative to the $O_2^{\bullet-}$ generated at the disk electrode of a rotated glassy-carbon–glassy-carbon ring-disk assembly. Unreacted $O_2^{\bullet-}$ was monitored at the ring electrode by oxidation to O_2. The ratio of ring current-to-disk current decreased when a thioamide was present, and this change was used to determine the pseudo-first-order rate constants with an analytical function.[15] Effective second-order rate constants were obtained by dividing the first-order constants by twice the thioamide concentration (the reaction consumes two $O_2^{\bullet-}$ per thioamide). The rate of reaction of $O_2^{\bullet-}$ with thioisonicotinamide was determined from the decay of the $O_2^{\bullet-}$ concentration (monitored by cyclic voltammetry) in a solution to which a substoichiometric amount of amide had been added. Product nitriles were identified by comparison with the cyclic voltammograms of authentic nitrile samples in Me_2SO (0.1 M TEAP) and were assayed by standared addition to the product solutions. The nitrile products also were identified by comparison of their UV spectra (hexane extractions from 1:1 Me_2SO/H_2O mixtures) with those of authentic nitrile samples. Polysulfides were identified by comparison of the UV spectrum for the light blue solutions that result from reaction of elemental sulfur and hydroxide ion in

Table I. Voltammetric Peak Potentials for the Reduction of the Thioamides and for the Oxidation and Reduction of the Product Species from their Reaction with $O_2^{\bullet-}$ in Me_2SO (0.1 M Tetramethylammonium Perchlorate)

	V vs Ag/AgCl			
			product species	
thioamide [$RC(S)NH_2$]	$(E_{p,c})_1$[a]	$(E_{p,c})_2$[a]	$E_{p,a}$[b]	$E_{p,c}$[c]
thioacetamide [$MeC(S)NH_2$]	–2.45		+0.09	
thioacetanilide [MeC(S)NHPh]	–2.13		+0.39	
thiobenzamide [$PhC(S)NH_2$]	–1.82	–2.5	+0.10	–2.24
thionicotinamide [3-[$C(S)NH_2$]py]	–1.66	–2.4	+0.13	–1.86
thioisonicotinamide [4-[$C(S)NH_2$]py]	–1.39	–2.3	+0.20	–1.65
ethionamide [2-Et-4-[$C(S)NH_2$]py]	–1.44	–2.3	+0.18	–1.75

[a] First and second reduction peaks for thioamide under a nitrogen atmosphere. [b] Anodic peak potential for major product [$RC(S^-)=NR'$] from the reverse scan after reduction of O_2 in the presence of thioamide via cyclic voltammetry. [c] Reduction peak for the secondary product from the slow decay ($k \sim 10^{-3}$ s^{-1}) of the primary product of the $RC(S)NH_2/O_2^{\bullet-}$ reaction. Authentic nitriles ($RC\equiv N$) have identical reduction potentials.

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Table II. Stoichiometries and Kinetics for the Reaction of $O_2^{\bullet-}$ with Thioamides in Me_2SO (0.1 M Tetraethylammonium Perchlorate) at 25 °C

thioamide (S)	e^- per (O_2 + S)	$O_2^{\bullet-}$ per S	O_2 released per S[a]	$k/2[S]$, M^{-1} s^{-1} [b]
thioacetamide	1.3 ± 0.2	1.0 ± 0.1	0.5 ± 0.2	(0.11 ± 0.02) × 10^3
thioacetanilide	1.0 ± 0.2	1.0 ± 0.2	0.5 ± 0.2	(6.0 ± 1.0) × 10^3
thiobenzamide	1.1 ± 0.2	1.0 ± 0.2	0.5 ± 0.2	(1.2 × 0.2) × 10^3
thionicotinamide	1.2 ± 0.3	1.0 ± 0.1	0.5 ± 0.2	(3.6 ± 0.5) × 10^3
thioisonicotinamide	1.0 ± 0.2	1.0 ± 0.1	0.5 ± 0.2	(4.9 ± 1.0) × 10^3
ethionamide	1.0 ± 0.2	1.0 ± 0.1	0.5 ± 0.2	(3.8 ± 0.5) × 10^3

[a] Moles of O_2 evolved per mol of $RC(S)NH_2$ added to excess $O_2^{\bullet-}$. [b] Pseudo-first-order rate constant normalized to twice the substrate concentration (a stoichiometric factor of 2). Obtained via rotated ring-disk voltammetry at glassy carbon electrodes, with 0.4 mM O_2 reduced to $O_2^{\bullet-}$ at the disk and oxidized to O_2 at the ring (all in the presence of 6 mM thioamide and at a rotation speed of 2500 rpm). An increase in thioamide concentration from 6 to 12 mM caused a 20% decrease in the value for the apparent second-order rate constant, and a change of rotation speed from 2500 to 1600 rpm resulted in a 25% decrease in the value for the rate constant.

Me_2SO (0.1 M TEAP) with those solutions that developed blue-green colors after the reaction of $O_2^{\bullet-}$ with thioamides. Other sulfur systems give blue colors, but within the context of these experiments polysulfides are the likely form of the sulfur from the $O_2^{\bullet-}$/thioamide reactions.

Results

Electrochemistry. The reduction potentials for thioamides in dimethyl sulfoxide, which are summarized in Table I, are much more negative than that for the $O_2/O_2^{\bullet-}$ couple. Aromatic thioamides are reduced at less negative potentials than thioacetamide and exhibit two reduction peaks that are separated by about 0.7 V. The first reduction for all of the thioamides appears to be a one-electron processes on the basis of controlled potential coulometry. The product solutions exhibit oxidation peaks between 0.0 and +0.4 V. Each of the thioamides is oxidized at potentials more positive than +1.0 V vs Ag/AgCl.

Cyclic voltammograms for the $O_2/O_2^{\bullet-}$ couple alone and in the presence of excess ethionamide are shown in Figure 1. For an initial negative voltage scan O_2 is reduced to $O_2^{\bullet-}$; reversal of the voltage scan causes the $O_2^{\bullet-}$ to be oxidized back to O_2 with the peak height for the oxidation proportional to the amount of $O_2^{\bullet-}$ formed at the electrode surface. In the presence of ethionamide the amount of $O_2^{\bullet-}$ is diminished (shown by a smaller anodic peak current), which indicates that a reaction has occurred. The enhanced cathodic current with the presence of ethionamide is due to the production of O_2 from the primary reaction products; the anodic peak at +0.18 V for the reverse scan is due to the oxidation of a reaction product. The presence of the other thioamides in an O_2 solution has an effect on the oxygen electrochemistry that is essentially identical with that illustrated in Figure 1. Each of the thioamides reacts with $O_2^{\bullet-}$ to yield a product that exhibits an oxidation peak between +0.1 and +0.4 V on the reverse scan of its cyclic voltammogram (Table I).

The same anodic peaks of the reverse scans for the cyclic voltammetry of O_2/thioamide systems also are observed for the major product from bulk $O_2^{\bullet-}$/thioamide reactions. On the basis of incremental titrations by thioamide solutions of deaerated $O_2^{\bullet-}$ solutions the reaction stoichiometries are 1:1 for each thioamide. The reactions produce 0.5 mol of O_2 per mol of thioamide that has reacted. Cyclic voltammograms for these solutions also exhibit reduction peaks for minor products at potentials about 0.4 V more negative than those for the parent thioamide. Addition of $HClO_4$ eliminates the oxidation peak for the major product and regenerates 90% of the parent thioamide peak.

When a slight excess of thioamide is combined with $O_2^{\bullet-}$ and the solution is allowed to remain closed to the atmosphere for periods of 30 min or more, a subsequent voltammogram shows a significant increase in the height for

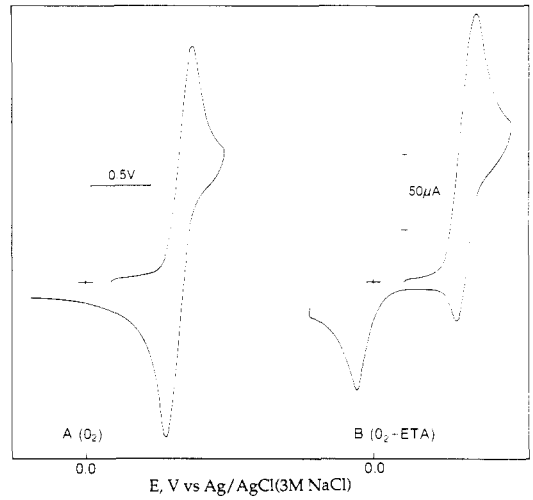


Figure 1. Cyclic voltammograms for 2.1 mM O_2 in the absence (A) and presence (B) of 4 mM 2-ethyl-4-pyridinecarboxamide (ethionamide, ETA) in Me_2SO (0.1 M TEAP) solutions: scan rate; 0.1 V s^{-1} at a glassy-carbon electrode (3-mm diameter).

the cathodic peak of the secondary product (Table I). However, the amount of secondary product is never more than 25% of the initial thioamide concentration. Light green-blue colors develop with time for the thiobenzamide/$O_2^{\bullet-}$ and thioacetamide/$O_2^{\bullet-}$ solutions. The reactions of the pyridine-ring thioamides (thioisonicotinamide, thionicotinamide, and ethionamide) do not produce significant color changes, but the cyclic voltammograms for the product solutions are similar (the sulfur from these thioamides may be elemental rather than polysulfides). The reduction peak potentials for the secondary products are the same as those for the nitrile derivatives of the parent carboxylic acids, which indicates that the secondary products from thiobenzamide, thionicotinamide, thioisonicotinamide and ethionamide are benzonitrile, 3-cyanopyridine, 4-cyanopyridine, and 2-ethyl-4-cyanopyridine, respectively. The UV spectra of hexane extractions from 1:1 mixtures of the product solutions with water are identical with those of authentic samples of the nitriles. (Benzonitrile is readily identified by its UV spectrum in hexane with characteristic absorptions at 275, 268, and 261 nm.)

Similar results are observed when O_2 is electrochemically reduced in the presence of thioamide substrates. Cyclic voltammograms that are recorded after electrolysis reveal a decrease of the thioamide peak and a new reduction peak at more negative potentials. The electron stoichiometry relative to the decrease in peak height for the thioamides is close to 1:1 (Table II). Cyclic voltammograms of deaerated thioamide solutions that contain $(Bu_4N)OH$ are similar to those obtained by reaction with $O_2^{\bullet-}$. The ad-

dition of ^-OH eliminates the thioamide peak and gives rise to the same peaks as those from the products in the reactions with $O_2^{\bullet-}$. The addition of acid regenerates all of the thioamide peak, and the acid/base stoichiometry is one-to-one. For the thioamide/^-OH systems the secondary nitrile product only is formed in the presence of oxygen and excess hydroxide ion. The rate of production of nitrile appears to be the same as or slower than the decomposition rate of hydroxide ion in Me_2SO (0.1 M TEAP), which precludes quantitative measurements. Solutions that contain 5 mM thiobenzamide and 10 mM hydroxide ion (saturated with O_2) yield 1 mM benzonitrile after 45 min.

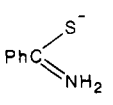
The reactivity of thioamides with hydrogen peroxide increases markedly when hydroxide ion is present; the reaction products in basic solution are the same as those obtained with $O_2^{\bullet-}$. Millimolar quantities of thiobenzamide react with H_2O_2 and tetrabutylammonium hydroxide to yield a green-blue solution whose UV–vis spectrum is the same as that from the $O_2^{\bullet-}$/thiobenzamide reaction. These green-blue colors appear to be due to the formation of polysulfides on the basis of UV–vis spectrophotometry.[16]

Electrochemical oxidation of an O_2-free solution that contains equimolar amounts of thioamide and ^-OH yields a product that has the same reduction potential as the corresponding nitrile.

Reaction Rates. The apparent pseudo-first-order rate constants for the $O_2^{\bullet-}$/thioamide reactions have been measured by rotated ring-disk voltammetry and are summarized in Table II. Variation of substrate concentration confirms that the process is first order in thioamide and first order in $O_2^{\bullet-}$. The apparent second-order rate constant for isonicotinamide is 3 ± 1 M^{-1} s^{-1}, which is dramatically slower than the rate for the $O_2^{\bullet-}$/thioisonicotinamide (k_{bi}, 4900 M^{-1} s^{-1}).

Discussion and Conclusions

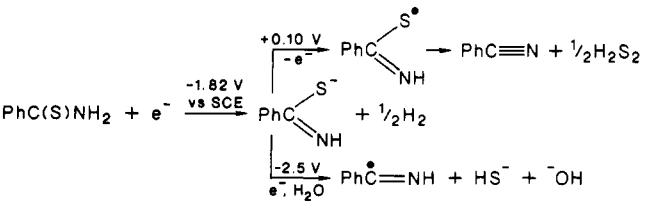
The negative values of the reduction potentials for these thioamides (Table I) indicate that proton transfer from NH_2 to $O_2^{\bullet-}$ is not a major reaction.[17-19] Consideration of the results in Table II and Figure 1 indicate that the reaction pattern for the $O_2^{\bullet-}$/thioamide system is similar to that for the reaction of $O_2^{\bullet-}$ with esters via nucleophilic addition.[2-5] Scheme I outlines the reaction patterns for the electrochemistry of thiobenzamide and for its $O_2^{\bullet-}$ adduct. As in the case of the ester adduct, a second $O_2^{\bullet-}$ reacts to reduce the peroxy radical and give O_2. The reversibility of the $O_2^{\bullet-}$/thioamide reactions upon addition of protons indicates that the adduct from nucleophilic $O_2^{\bullet-}$ attack does not undergo elimination because of the poor leaving group (e.g., loss of ^-OR in the case of related reactions with esters). However, the peroxide adduct apparently attacks a second thiobenzamide via nucleophilic addition in a second rapid step to give the major product,



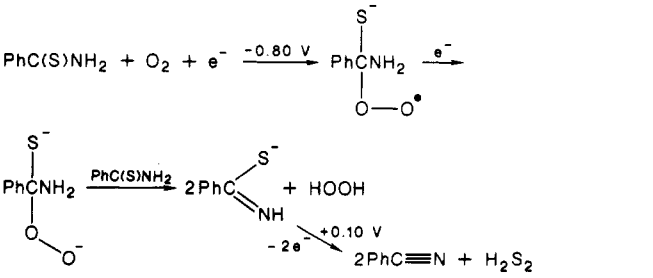
The other thioamides of Table I appear to have analogous reaction pathways (Scheme I), but the primary products for thioacetanilide cannot be converted to a nitrile.

Scheme I

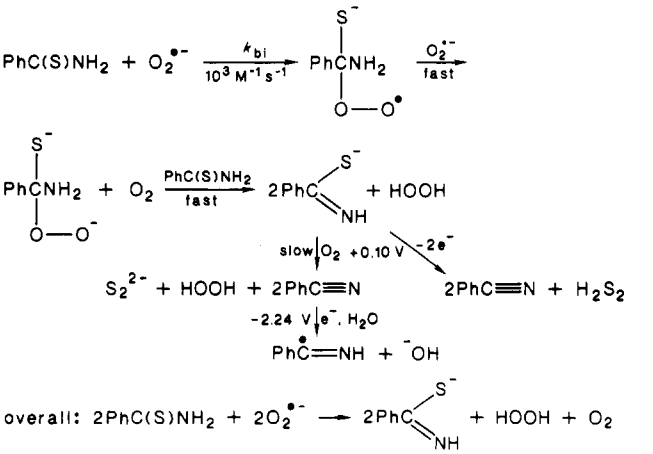
a. electrochemistry



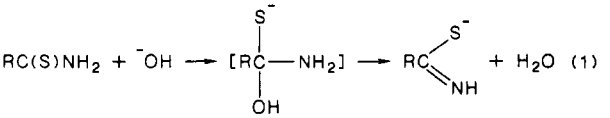
b. electrochemistry with O_2



c. reaction with $O_2^{\bullet-}$



The reaction of ^-OH with thioamides in the absence of O_2 yields the same product as the $O_2^{\bullet-}$ reaction of Scheme I,



which is consistent with their known acidic properties.[20] Addition of protons reverses the process to give the parent thioamide. Whereas the ^-OH reaction is a deprotonation process, the addition of $O_2^{\bullet-}$ to a thioamide is followed by rapid electron transfer from a second $O_2^{\bullet-}$. Electrochemical oxidation of this product species (Table I and Scheme I) yields the corresponding nitrile and H_2S_2. The same oxidation occurs by O_2, but the process is much slower. Previous studies have detected nitriles as oxidation products of thioamides.[20]

Because of $O_2^{\bullet-}$/thioamide reaction produces a product that is more easily oxidized (by more than 1 V) than the parent thioamide, this may account for the requirement of O_2 in the enzymatic transformation of ethionamide to its *S*-oxide and for its inhibition by superoxide dismu-

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tase.[7,8] Thus, $O_2^{\bullet-}$ may act as an activating agent for the S-oxygenation of ethionamide in vivo. Replacement of the carbonyl oxygen of amides by a sulfur atom enhances the reactivity of $O_2^{\bullet-}$ by at least a factor of 1000.

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Some New Phytotoxic Ophiobolins Produced by *Drechslera oryzae*

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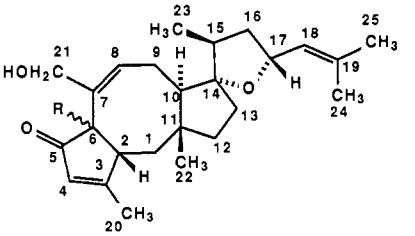
Several previously unreported phytotoxic compounds were isolated from the fungus *Drechslera oryzae*, a plant pathogen of rice. The compounds were characterized as 6-epiophiobolin I (**1**), ophiobolin J (**3**), 8-deoxyophiobolin J (**4**) by spectroscopic analyses and comparisons with recently determined ophiobolin I (**2**).

A plant pathogenic fungus, *Drechslera oryzae* (*Helminthosporium oryzae*) causes brown leaf spot disease on rice.[1] The first work on the phytotoxins of this organism was reported in the mid 1960's by Nozoe and co-workers,[2,3] who elucidated the structures of ophiobolin A and B. As a consequence of our recent findings of new ophiobolins from *D. maydis* and *D. sorghicola*,[4] we undertook a more detailed investigation of the phytotoxic metabolites of *D. oryzae* because the sexual stages of all of these fungi are genetically compatible and they are likely to produce the same or related compounds. Six ophiobolins (A, 6-epi A, B, I, 25-hydroxy I, and 3-anhydro-6-epi A)[5] were characterized from culture broth of *D. oryzae* and identified with 400-MHz ^{1}H NMR and high-resolution mass spectra (HRMS). However, three previously unreported ophiobolins were isolated from this fungus and their structures were characterized with spectroscopic methods, which is the subject of this report.

Our earlier work had defined the structure of ophiobolin I (**2**) by single-crystal X-ray diffraction methods,[4] and it serves as a convenient starting point for the characterization of the new ophiobolins. The chromatographic behavior of **1** was very similar to that of **2**. Its molecular formula was determined by HRMS as $C_{25}H_{36}O_4$ and its fragmentation pattern closely resembled that of **2**. The ^{1}H NMR showed a doublet at δ 3.55 (J = 6.5 Hz) assigned for H6 and a multiplet at δ 3.17 for H2 and these were consistent with cis ring fusion, whereas a coupling constant



1: R = Hα

2: R = Hβ

of J = 2.6 Hz exists in the case of **2** which is trans ring fused. A doublet shifted to low field at δ 6.04 (J = 8.1 Hz) for H8 and a doublet of H6 indicated the position of a double bond on C7. These combined with the other signals led to the assignment of **1** as the 6-epimer of **2**. An exposure of **1** in methanol solution with 1 N HCl[6] afforded a single product which was identified as **2** by chromatographic analyses (HPLC and TLC), ^{1}H NMR, and HRMS. These results implied the stereochemical structure of **1** to be the same as to that of **2** on C2, C10, C11, C14, C15, and C17.

Ophiobolin J **1** has a molecular formula of $C_{25}H_{36}O_4$ as determined by HRMS. The 400-MHz ^{1}H NMR of **3** was similar to that of **2**, but there were differences primarily in the A–B ring fusion region. The olefinic proton H8 at δ 5.78 and the bridgehead proton H6 at δ 3.67 of **2** were replaced by a signal at δ 4.68 in **3**. The bridgehead proton at C2 of **3** appeared at δ 4.04 (broad doublet, J = 11.2 Hz) rather than a multiplet at δ 2.53 as in **2**. These changes suggest that the double bond moved to C6 from C7 also

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