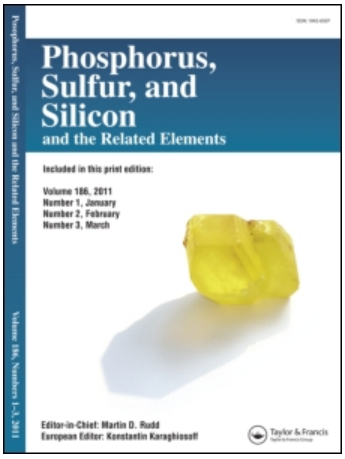

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THIONO COMPOUNDS, 7. OXIDATION OF THIOAMIDES IN RELATION TO ADVERSE BIOLOGICAL EFFECTS[1]

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THIONO COMPOUNDS. 7. OXIDATION OF THIOAMIDES IN RELATION TO ADVERSE BIOLOGICAL EFFECTS[1]

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Thioacetamide, thioacetamide *S*-oxide (**1**), and thiopivalamide *S*-oxide (**8**) were oxidized with H_2O_2 in $H_2^{18}O$ to generate the corresponding amides with at least 50% ^{18}O incorporation; hydrolysis of the *S*-oxides to the amides or ^{18}O exchange with the amides occurs much more slowly. When **1** and trifluorothioacetamide (**6**) were oxidized with three and four equivalents of H_2O_2, respectively, in the presence of benzylamine, *N*-acetylbenzylamine (**5**) and *N*-benzyltrifluoroacetamide (**7**) were isolated in respective yields of 17% and 37%. These results are interpreted as evidence for an oxidative desulfurization mechanism involving nucleophilic attack at the carbon atom of an *S*,*S*-dioxide or trioxide intermediate as the major pathway; a minor pathway may involve the intermediacy of an oxathiirane *S*-oxide (**3**) or dioxide (**4**) species. Understanding is added to the behavior of *S*-oxides in aqueous solution, as well as to thermal stability in deuterochloroform. Also reported are studies of the preparation and properties of some *N*,*N*-dialkyl derivatives of **8**, of reduction of **1** with NADH or NADPH, and of generation and trapping of species related to sulfoxylate ion.

INTRODUCTION

The question of why thioamides exert several adverse biological effects, such as hepatotoxicity, led us to initiate a study using chemical model systems for the oxidative desulfurization of thioamides. There is now substantial evidence linking inactivation of enzymes and tissue damage with reactive metabolites generated during the biotransformation of thioamides to amides.[2,3] The biological oxidation of thioamides, which may be accomplished principally by FAD-containing monooxygenase (MFMO),[3,4] seems most likely to proceed at first by generation of the corresponding thioamide *S*-oxide.[3] Several thioamide *S*-oxides have been prepared chemically, and apparently their ease of preparation as well as stability may be heavily influenced by substituents.[5]

Dyroff and Neal, in their study of the mechanism of oxidation of thioacetamide *S*-oxide (**1**) by rat liver microsomes, proposed a mechanism for the formation of *N*-ε-acetyllysine that involved nucleophilic attack of the ε-amino group of lysine upon thioacetamide *S*,*S*-dioxide (**2**), as outlined in Scheme 1.[6] This proposed mechanism involved expulsion of sulfoxylate ion (HSO_2^-), a species which has not been isolated as the free acid or salt. In an independent study, Walter and Bauer also proposed the elimination of sulfoxylic acid (HOSOH) in the oxidation of thioamides

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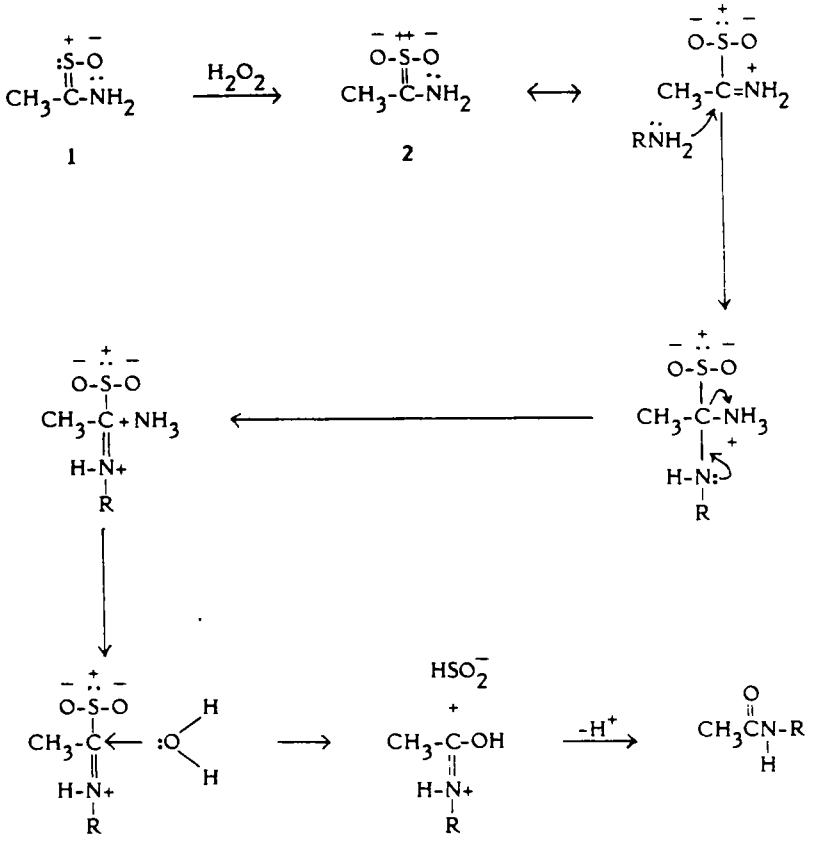
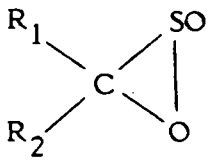


SCHEME 1

to imidates in an alcoholic medium.[7] The possibility exists, however, that the trioxide corresponding to the dioxide **2** may play a key role.

A major objective of the present study was to establish whether the mechanism of the chemical oxidation of thioamides (and *S*-oxides) paralleled that in biological systems. This objective is especially relevant since intermediates such as oxathiirane *S*-oxides (**3**) or oxathiirane *S*,*S*-dioxides (**4**) often have been proposed as reactive intermediates.[8] These reactive species could generate amides by extrusion of sulfur monoxide or sulfur dioxide, respectively.



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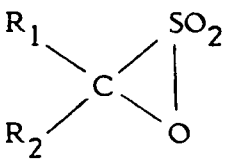

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RESULTS AND DISCUSSION

Studies Involving Sulfoxylate Ion

We first attempted to find a convenient precursor of sulfoxylate ion (HSO_2^-) and then to develop more efficient trapping agents for it. Kerber and Starnick suggested that the decomposition of Rongalit® (sodium hydroxymethanesulfinate) in aqueous acid may involve formation of sulfoxylate ion, which they trapped with acrylic acid derivatives to give sulfones (eq. 1; X = CN, CO_2H, etc).[9]

$$HOCH_2SO_2^-Na^+ \xrightarrow[\substack{pH\ 4\\ -CH_2O}]{H_3^+O} [HOSO^-Na^+] \xrightarrow{2CH_2=CH-X} (XCH_2CH_2)_2SO_2 \quad (1)$$

Accordingly, we began our study of thioamide oxidations by investigating the decomposition of Rongalit® to determine whether it was a possible source of sulfoxylate ion. In a search for optimum traps for sulfoxylate ion, Rongalit® was allowed to react at ca. pH 4 with more heavily functionalized olefins than were used by Kerber and Starnick.[9] The results are given in Table I. In each case, the only product observed was that from reduction of the olefin, and no sulfones were isolated (such use of Rongalit as a synthetic tool for related reductions appears to deserve further attention, although we have no such plans).

The decomposition of Rongalit with aqueous HCl in the presence of excess 2,4-dinitrophenylhydrazine was studied to determine whether formaldehyde (and hence sulfoxylate ion) was, in fact, liberated according to eq. 1. This reaction led only to the hydrazone of formaldehyde. Despite repeated attempts, the yield of the purified material never could be made to exceed 35%. These results clearly do not indicate a simple direct decomposition of Rongalit into formaldehyde and sulfoxylate ion but do seem consistent with homolytic cleavage of Rongalit to the radical ion (cf. ref. 10).

Two reported preparations of sulfoxylate salts were unsuccessful. Eq. 2 shows the first of these,[11] and eq. 3 shows the second,[12] which later was termed improbable.[13] A final attempt to prepare sodium sulfoxylate by reducing

$$Zn + SO_2Cl_2 \xrightarrow[0\text{–}5°C]{ether} ZnCl_2 + ZnSO_2 \quad (2)$$

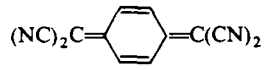
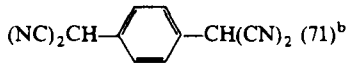
$$S + SO_3 \rightarrow S_2O_3 \xrightarrow[2)\ H_2O]{1)\ NaOEt} Na_2SO_2 \quad (3)$$

butadiene sulfone with sodium naphthalene gave only naphthalene; butadiene was not evolved, nor was sodium sulfoxylate isolated.

Our focus then turned toward the more direct investigation of the oxidation of thioacetamide (and its *S*-oxide **1**), together with other thioamides chosen to increase understanding of the effect of electronic and steric influences on the nature of the reactions and products. We now are endeavoring to learn whether there will be a correlation between these effects and biological studies in progress that will be of predictive value.

TABLE I

Reaction of sodium hydroxymethanesulfinate with substituted olefins in water at pH 4

Olefin	Product (Yield, %)
$NCCH{=}CHCN$	$NCCH_2CH_2CN$ (49)[a]
	
$HO_2CCH{=}CHCO_2H$	$HO_2CCH_2CH_2CO_2H$[c]

[a] Yield after purification; identified by mp and spectra.
[b] Crude yield; identified by the mp of recrystallized material and by NMR.
[c] Not isolated; identified by NMR spectroscopy. See Experimental for details.

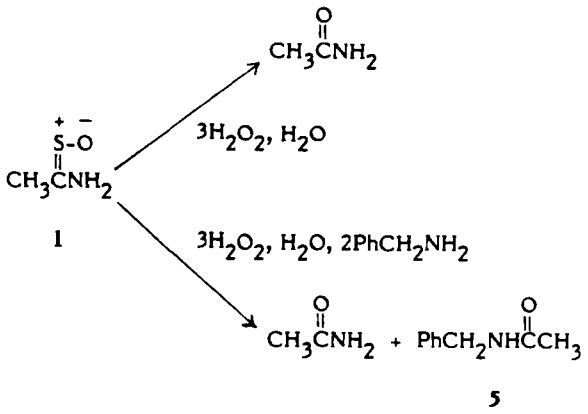
Oxidation of Thioacetamide S-oxide (**1**) *with Hydrogen Peroxide in Water*

In the absence of benzylamine. Thioacetamide *S*-oxide (**1**) was treated with three equivalents of hydrogen peroxide in water at ca. 25°C and pH 4–7 during six hours. The yield of acetamide obtained in this manner never exceeded 50%. Oxidation of thioacetamide with four equivalents of hydrogen peroxide under similar conditions gave the same results as oxidation of **1** with three equivalents.

In the presence of benzylamine. Thioacetamide *S*-oxide (**1**) was treated with three equivalents of hydrogen peroxide in the presence of approximately 2 equivalents of benzylamine under conditions identical with those above. *N*-Acetylbenzylamine (**5**) was obtained in 17% yield (purified). Acetamide and a small amount (ca. 10%) of unreacted thioacetamide *S*-oxide (**1**) also were identified. The results of these reactions are depicted in Scheme 2. Control experiments demonstrated that *N*-acetylbenzylamine (**5**) is not generated by reaction of benzylamine with acetamide under either neutral or acidic conditions.

Although the yield of (**5**) was not large (attributed primarily to difficulties with the solubility of benzylamine under the reaction conditions), the formation of this adduct agrees with previous results obtained enzymatically *in vitro* by Dyroff and Neal.[6] Specifically, the formation of this adduct supports the mechanism shown in Scheme 1. Efforts to trap sulfoxylate ion by oxidizing thioacetamide with four equivalents of hydrogen peroxide in the presence of divinyl sulfone led only to acetamide and recovery of the sulfone, however (similar experiments with thiobenzamide and acrylic acid gave only a polymer that contained negligible sulfur); presumably sulfoxylate ion, if present, is oxidized before it can add.

Oxidation of trifluorothioacetamide (**6**) *with hydrogen peroxide and benzylamine.* Trifluorothioacetamide (**6**) was studied next in order to afford a contrast to thioacetamide in permitting assessment of the effect of strong electron withdrawal. It was treated with four equivalents of hydrogen peroxide in the presence of two equivalents of benzylamine in water at ca. 25°C. After 90 min, *N*-benzyltrifluoroacetamide (**7**) was obtained in 37% yield (purified). The yield of the benzylamine adduct in this oxidative desulfurization, twice that obtained in the reaction of **1**



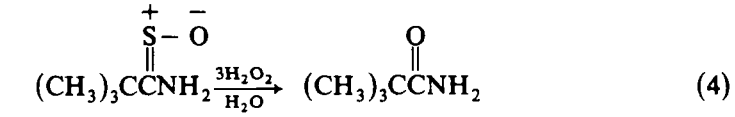
SCHEME 2

under similar conditions, indicates that an electron-withdrawing group attached to the thiono carbon increases the selectivity of this electrophilic center for benzylamine vs. water, relative to that of **1**.

Attempts to prepare the *S*-oxide of **6** using hydrogen peroxide in acetonitrile were unsuccessful. The only products isolated from these reactions were ammonium sulfate (ca. 30% yield, identified by elemental analysis) and sulfur (40% yield, identified by mp).

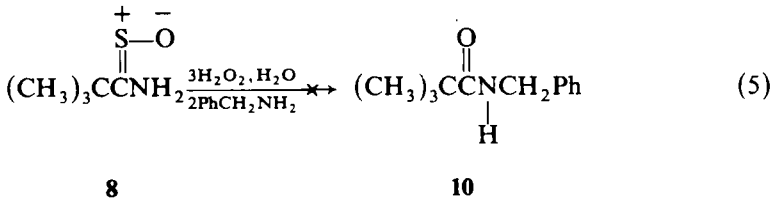
Oxidation of thiopivalamide S-oxide (**8**) *with hydrogen peroxide in H_2O.* The reactive species responsible for hepatotoxicity probably is an *S,S*-dioxide[2a] (or trioxide) species, each successive oxidation at sulfur rendering the thiono carbon more electrophilic and more subject to nucleophilic attack. It would seem reasonable that steric hindrance towards nucleophilic attack at this site might result in decreased observed hepatotoxicity, even though the *S,S*-dioxide or trioxide may be formed rapidly from the *S*-oxide. The effect of a bulky group attached to the thiono carbon atom of a thioamide *S*-oxide upon the nature of oxidative desulfurization (with and without added benzylamine) therefore was investigated. For this study, thiopivalamide *S*-oxide (**8**) was prepared (58% yield). Unlike the *S*-oxide of **6**, **8** was quite stable (halflife in $CDCl_3$ at 55°C ca. 21 days); inferences from the first-order decomposition observed for **8** will be discussed presently.

In the absence of benzylamine. Thiopivalamide *S*-oxide (**8**) was treated with three equivalents of hydrogen peroxide in water at ca. 25°C over 21 hours. The pH then was adjusted to 7, and the solution was left for a further 27 hours. After workup, pivalamide (**9**) was isolated in 75% crude yield (eq. 4).



8 **9** (4)

In the presence of benzylamine. Thiopivalamide *S*-oxide (**8**) was treated with three equivalents of hydrogen peroxide and two equivalents of benzylamine in water at ca. 25°C under the conditions described above (eq. 5). After workup there was no indication (3% or less by ^{1}H NMR spectroscopy) of the presence of *N*-benzylpivalamide (**10**), by comparison with the ^{1}H NMR spectrum obtained from authentic **10**. The implications of this and the preceding experiment will be discussed below.



$$(CH_3)_3CC(=S^+-O^-)NH_2 \xrightarrow[2PhCH_2NH_2]{3H_2O_2,\,H_2O} (CH_3)_3CC(=O)NHCH_2Ph \qquad (5)$$

8 **10**

Oxidations with Hydrogen Peroxide in $H_2{}^{18}O$

We next sought to learn if Scheme 1 best explained the oxidation (in contrast to whether oxathiiranes **3** and **4** might be involved), as well as to determine whether the mechanism of oxidative desulfurization would change when the substrate was changed from **1** to the hindered *S*-oxide **8**. A series of ^{18}O labeling experiments therefore were performed using $H_2^{18}O$ and hydrogen peroxide (prepared *in situ* from anhydrous sodium peroxide and sulfuric acid) with thioacetamide, thioacetamide *S*-oxide (**1**) and thiopivalamide *S*-oxide (**8**).

Thioacetamide, thioacetamide *S*-oxide (**1**) and thiopivalamide *S*-oxide (**8**) were treated with hydrogen peroxide in the presence of a large excess of $H_2{}^{18}O$ (98 atom % ^{18}O) at ambient temperature. The hydrogen peroxide was prepared *in situ* according to the method of Anbar,[14a] who also demonstrated that hydrogen peroxide prepared in this manner did not exchange its oxygen atoms with water at a rate significant for our purposes (cf. also ref. 14b). After the reaction was complete, the acidic solutions were adjusted to pH 7 with 0.1 N sodium hydroxide solution, and the water was evaporated under reduced pressure at or below ambient temperature. The crude product was extracted with organic solvent, and the solvent was evaporated to give the amide. The residue obtained in this manner was analyzed by mass spectrometry for the extent of ^{18}O incorporation in the amide product. It has been established previously that amides do not undergo oxygen exchange under acidic conditions, although exchange does occur slowly in aqueous base.[15-17] The results of these labeling experiments are given in Table II. It should be added that there was considerable difficulty in obtaining amide product from these oxidations in reproducible fashion. Often the reactions turned green and were alkaline after 6–7 hours, and no amide was isolated (the pH always was acidic and usually was ca. 1 in successful reactions). The overall success rate for these oxidation reactions was judged to be about 50%, but fortunately unsuccessful oxidations were not misleading since negligible labeled or unlabeled amide could be isolated.

TABLE II

Oxidation of thioamides and *S*-oxides with hydrogen peroxide in the presence of $H_2{}^{18}O$

Substrate	Product	% Excess ^{18}O in Amide[a]
$CH_3\overset{\overset{S}{\|}}{C}NH_2$	$CH_3\overset{\overset{O}{\|}}{C}NH_2$	60.2
$CH_3\overset{\overset{\overset{+}{S}-\overset{-}{O}}{\|}}{C}NH_2$	$CH_3\overset{\overset{O}{\|}}{C}NH_2$	53.3, 67.9[b]
$(CH_3)_3C\overset{\overset{\overset{+}{S}-\overset{-}{O}}{\|}}{C}NH_2$	$(CH_3)_3C\overset{\overset{O}{\|}}{C}NH_2$	73.5, 80.5

[a] m = parent peak intensity; $m + 2$ = parent peak + 2; % excess $^{18}O = (m + 2)(100)/[m + (m + 2)]$, where the $m + 2$ has been corrected for $m + 2$ intensity owing to the presence of the natural-abundance amide.

[b] An average of two scans at 70 eV (67.0%) and 14 eV (68.8%).

There appears to be no significant difference in the extent of ^{18}O incorporation in the amide product as the thiono substrate was changed. Although the oxidative desulfurization of **8** with H_2O_2 is distinctly slower than of **1** (as determined by model experiments), the extent of ^{18}O incorporation in the respective amides was not significantly different. Therefore, within experimental uncertainty all three substrates behaved alike under the reaction conditions. These results suggest that a large fraction of the substrates are oxidatively desulfurized via a mechanism such as that depicted by the last three steps of Scheme 1.

A relatively minor alternative to Scheme 1 may involve formation of an oxathiirane *S*-oxide (**3**) or dioxide (**4**), however, which could lose SO or SO_2 respectively and lead thereby to the amide. Amide thus produced would account for the natural-abundance amide which was found in the product (averaging ca. 33% for all values in Table II). Also consistent with involvement of an oxathiirane or oxo derivative are: (a) decomposition of thiopivalamide-*S* oxide (**8**) at 55°C to pivalamide (trimethylacetamide, **9**; 21%) and thiopivalamide (79%; presumably produced by disproportionation of **8** to give the oxathiirane oxide or dioxide); and (b), the fact mentioned above that the decomposition of **8** appeared to be kinetically first-order.

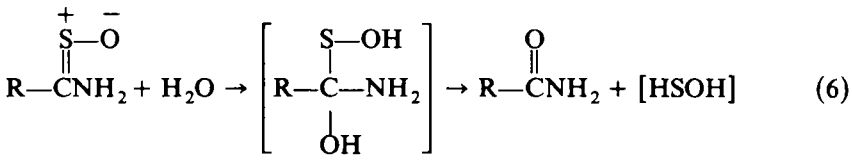
The large amount of ^{18}O incorporation into pivalamide (**9**) along with the lack of formation of *N*-benzylpivalamide (**10**) when thiopivalamide *S*-oxide (**8**) was treated with hydrogen peroxide in the presence of benzylamine deserves comment. These two results, although apparently inconsistent, probably reflect the limited accessibility of benzylamine towards the modestly sterically hindered system stemming from the three methyl groups. In contrast, with thioacetamide *S*-oxide (**1**) there is little restriction to attack at thiono carbon by suitable nucleophiles, so that benzylamine as well as water can easily react with intermediates derived from **1**.

Hydrolysis of Thioacetamide S-Oxide (**1**) *and Thiopivalamide S-Oxide* (**8**) *in Deuterium Oxide at 37°C*

In order to establish that the incorporation of ^{18}O did not arise simply from hydrolysis of the *S*-oxides **1** and **8**, a series of ^1H NMR experiments were done. Thioacetamide *S*-oxide (**1**) and thiopivalamide *S*-oxide (**8**) were dissolved in D_2O and allowed to stand at 37°C. The reactions were followed by ^1H NMR spectroscopy, and the products were identified by comparison with ^1H NMR spectra obtained from authentic specimens. The results are summarized in Table III. Relative proportions were determined by ^1H NMR integration.

Thioacetamide *S*-oxide (**1**) appeared to be half consumed only after approximately 100 hours at 37°C whereas thiopivalamide *S*-oxide (**8**) was half consumed only after approximately 30 days. The respective times for half-hydrolysis (100 hours, 30 days) thus contrast markedly with those for the complete oxidations (5–7 and 20 hours).

The uncatalyzed hydrolysis probably proceeds by addition of water to the thiono carbon atom (by analogy with the hydrolysis of sulfines),[18] as shown in eq. 6. If the rate-determining step is formation of the tetrahedral intermediate



(6)

and is the same for both *S*-oxides, this difference in the rate of hydrolysis may reflect the difference in accessibility of the thiono carbon to attack by water. This conclusion thus reinforces the suggestion made above that benzylamine might not add readily to the pivaloyl thiono-carbon atom. If true, it implies that hindered thioamides and their *S*-oxides potentially could be less damaging biologically than unhindered thioamides, since covalent binding to proteins may require an easily accessible functional group.

Reduction of thioacetamide S-oxide (**1**) *with NADH and NADPH in deuterium oxide at 37°C.* Thioacetamide *S*-oxide (**1**) is mutagenic in the Ames assay without metabolic activation by microsomal oxidative enzyme additives, but it is not mutagenic in the presence of such additives.[19] Since this result indicates that the mutagenicity of **1** can be destroyed by a biological oxidation, it is important also to establish the effect of biological reducing agents. Cashman and Hanzlik have considered the possibility that thiols may effect detoxication of toxic thioamide *S*-oxides to nontoxic thioamides.[20] Moreover, it has been observed previously *in vitro* that high levels of glutathione will convert the *S*-oxide **1** to thioacetamide, thereby reducing the amount of **1** available for metabolism to the species responsible for covalent binding to proteins.[6]

TABLE III

Hydrolysis of thioamide *S*-oxides in deuterium oxide (D_2O) at 37°C

S-Oxide	Products	Estimated time of reaction, one-half (complete)[a]	Relative proportion (%)
$CH_3\overset{\overset{+}{S}-\overset{-}{O}}{\overset{\|}{C}}NH_2$	$CH_3\overset{O}{\overset{\|}{C}}NH_2$	100 h (75 days)	65[b]
	CH_3CN		15
	$CH_3\overset{S}{\overset{\|}{C}}NH_2$		15
	Unidentified (cf. Experimental)		5
$(CH_3)_3C\overset{\overset{+}{S}-\overset{-}{O}}{\overset{\|}{C}}NH_2$	$(CH_3)_3C\overset{O}{\overset{\|}{C}}NH_2$	30 days (ca. 111 days)	85[b]
	$(CH_3)_3C\overset{S}{\overset{\|}{C}}NH_2$		15

[a] Estimated by NMR (see Experimental).
[b] Conceivably a mixture of amide and acid.

Two particularly ubiquitous biological reducing agents are the reduced forms of nicotinamide-adenine dinucleotide (NADH) and the 2′-dihydrogen phosphate (NADPH). To determine whether these could reduce *S*-oxide **1** to thioacetamide, equivalent proportions of **1** and either NADH or NADPH were combined in D_2O in an NMR tube. The solutions were maintained at 37°C, while ^1H NMR spectra were obtained at regular intervals.

Under these conditions, thioacetamide *S*-oxide (**1**) was half consumed after approximately 1 day, both NADH and NADPH being about equally effective in this reduction. The reaction products (identified by spiking with authentic materials) and relative proportions (by integration) were determined after 4–6 days at 37°C, and are given in Table IV.

A control experiment demonstrated that the reaction products were stable under the conditions employed in the NMR study. Small portions of the observed acetamide and acetonitrile in Table IV of course derive from the hydrolysis of the *S*-oxide concurrent with the reduction, as reflected in Table III. These results appear to support the view that *S*-oxides are quite reactive chemically and not only can be easily oxidized to metastable species but also can be reduced readily, principally to thioamides. It thus seems reasonable to conclude that while *S*-oxides may be mutagenic, they are easily converted by oxidation or reduction to species that are not [19] mutagenic.

TABLE IV

Reaction of thioacetamide *S*-oxide (**1**) with NADH and NADPH in deuterium oxide at 37°C[a]

Reagent	Products	Estimated time of reaction, one-half (complete)	Relative proportion (%) at completion
NADH	$CH_3\overset{\overset{S}{\|}}{C}NH_2$	1 day (6 days)	55
	CH_3CN		39
	$CH_3\overset{\overset{O}{\|}}{C}NH_2$		6
NADPH	$CH_3\overset{\overset{S}{\|}}{C}NH_2$	1 day (4 days)	53
	CH_3CN		40
	$CH_3\overset{\overset{O}{\|}}{C}NH_2$		7

[a] Relative times for half and complete reactions, as well as relative proportions of products at completion, were estimated from NMR integrals. The identity of all products was established by spiking the solutions with authentic materials.

Steric effects: Preparation and properties of N,N-diethyl- and N,N-dimethylthiopivalamide S-oxide. Efforts were made to prepare two thiopivalamide *S*-oxides having *N,N*-dialkyl substitution, so that the effects of still greater steric hindrance could be assessed on ^{18}O incorporation and on the kinetics of decomposition. Of interest first as a more sterically hindered *S*-oxide than thiopivalamide *S*-oxide (**8**) was *N,N*-diethylthiopivalamide *S*-oxide (**11**). Attempts to prepare **11** by reaction of *N,N*-diethylthiopivalamide with aqueous H_2O_2, H_2O_2 in aqueous acetonitrile, or aqueous peroxytrifluoroacetic acid were unsuccessful, yielding only sulfur and *N,N*-diethylpivalamide. However, when the oxidation was performed with hydrogen peroxide in acetic acid buffered with sodium acetate over 20 hours, a yellow oil was obtained in 22% yield that had a ^1H NMR spectrum and thermal decomposition products consistent with the structure of *N,N*-diethylthiopivalamide *S*-oxide (**11**). This product

$$(CH_3)_3C\overset{\overset{+\ \ -}{S\text{—}O}}{\overset{\|}{C}}N(CH_2CH_3)_2$$

11

when maintained at 37°C overnight in $CDCl_3$ was completely converted to a 1:2 mixture of *N,N*-diethylthiopivalamide and *N,N*-diethylpivalamide. This *S*-oxide (**11**) was not studied further, since it seemed too fragile for purification and elemental analysis. Only preliminary experiments were done on the *N,N*-dimethyl homologue when it proved to have no advantages over the diethyl. The facile decomposition of the *N,N*-dialkylpivalamide *S*-oxides underscores the possible involvement of oxathiirane oxides in the decomposition, alluded to above for the parent *S*-oxide.

CONCLUSIONS

The results presented above appear to support (at least in the chemical oxidation model) the mechanism proposed by Dyroff and Neal,[6] or a similar one, as the principal route through which thioamides are oxidatively desulfurized to amides. The relative importance of a minor pathway involving an oxathiirane *S*-oxide (**3**) (or *S,S*-dioxide **4**) intermediate has yet to be established, although the decomposition and isotopic experiments suggest that such intermediates account for perhaps 25–35% of the amide product in the reaction of **1** and **8** with H_2O_2; somewhat similar possibilities have been suggested for related systems by Walter and Bauer.[7]

The yields of *S*-oxide decreased in the sequence of **8** (58%) > **1** (30%) > $F_3CC{=}S(O)NH_2$ (0%). This sequence contrasts with the yields of *N*-benzylamide from the thioamides in the reversed sequence of **7** (37%) > **5** (17%) > **10** (0%). Interpretation of this reversal of yields should await more data, but it may be informative to learn which sequence will apply in biological testing.

S-Oxides are quite reactive chemically, being easily oxidized or reduced to species which have been shown to be not mutagenic, whereas thioacetamide *S*-oxide (**1**) itself is mutagenic. It would be of interest to extend this conclusion to other adverse biological effects.

EXPERIMENTAL

Melting points were determined by using a Thomas-Hoover stirred-liquid apparatus and are corrected. NMR spectra were recorded in $CDCl_3$ with Me_4Si as internal standard [or in D_2O with $Me_3Si(CH_2)_3SO_3Na$] using either a JEOL JNM-MH-100 or a JEOL FX-90Q spectrometer. IR spectra were obtained with a Perkin-Elmer Model 727 spectrometer. Electron-impact mass spectra were obtained using an LKB 9000 GC-MS instrument at 70 eV (except where otherwise specified) with an unheated direct-inlet probe. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn. Moist extracts were dried using anhydrous $MgSO_4$, and solvents then were removed under reduced pressure using a rotary-flask evaporator. TLC was performed on Eastman Chromagram silica gel plates (No. 13181) with visualization by UV. Preparative TLC was done on 1000-μm Whatman PK6F silica gel plates. Thioacetamide *S*-oxide (**1**) was prepared in DMF from thioacetamide in a yield of 30% by the method of Breau *et al.*:[19] mp 125–126°C; lit.[19] mp 125–126°C (others have reported a different crystalline form with mp in the range 134–137°C; cf. ref. 19). $H_2{}^{18}O$ (98 atom % ^{18}O) was obtained from Cambridge Isotope Laboratories, Woburn, Mass. "Hexane" refers to Fisher "Hexanes," bp 65–69°C. Rongalit® (sodium hydroxymethanesulfinate, provided and used as the dihydrate) was obtained from the Fluka Chemical Corp. Hydrogen peroxide ("H_2O_2") was a 30% solution (Fisher). All other materials were commercial unless otherwise stated.

Reactions of Rongalit

7,7,8,8-Tetracyanoquinodimethane. A solution of Rongalit (200 mg, 1.30 mmol) in H_2O (30 mL) was added to 7,7,8,8-tetracyanoquinodimethane (500 mg, 2.45 mmol) in dioxane (30 mL). The solution was adjusted to ca. pH 3–4 with 4 *N* HCl. After 4 h, extraction with CH_2Cl_2 gave 360 mg of solid (71%, based on the quinone), which after recrystallization (MeOH) had mp 240–242°C (decomp); undepressed by authentic *p*-phenylenedimalononitrile prepared using thiophenol;[21] lit.[21] mp 244–245°C; ^1H NMR congruent with *p*-phenylenedimalononitrile.

Fumarodinitrile. Rongalit (4.00 g, 26.0 mmol) was added to fumarodinitrile (2.00 g, 25.6 mmol) in H_2O (100 mL). The solution was adjusted to pH 3–4 and heated under reflux for 3 h. Extraction (CH_2Cl_2) gave 1.50 g, mp 49.5–53.5°C. Distillation at ca. 760 torr afforded succinodinitrile as a colorless solid (1.0 g, 49%): mp 52.5–54°C, lit.[22] mp 54.5°C; IR and ^1H NMR ($CDCl_3$) spectra were identical with those of authentic succinodinitrile.[22]

Fumaric acid. Rongalit (4.32 g, 28.1 mmol) was added in two portions over 48 h to fumaric acid (1.00 g, 8.62 mmol) in H_2O (50 mL). The solution was maintained at pH 3–4 and heated under reflux for 6 days. Extraction (Et_2O) gave ca. 380 mg (38%); mp > 200°C. The ^1H NMR spectrum (CD_3OD) was consistent with a 9:1 mixture of succinic acid and fumaric acid, as estimated by comparison with spectra obtained from authentic specimens.

Rongalit with 2,4-dinitrophenylhydrazine. A solution of Rongalit (0.250 g, 1.62 mmol) in 50% aqueous EtOH (10 mL) was acidified with 2 drops of conc. HCl and immediately was combined with a solution of 2,4-dinitrophenylhydrazine (1.00 g, 5.05 mmol) in a mixture of 95% EtOH (10 mL), H_2O (5 mL) and conc. HCl (5 mL). After 1 h, a yellow precipitate was isolated (0.185 g, mp 153–160°C). Evaporation of several extracts with hot hexane gave a yellow solid (0.175 g, 51% crude yield, mp 159–163°C). Recrystallization (EtOH) gave yellow needles (0.100 g, 29%): mp 165–166°C, undepressed by the authentic 2,4-DNPH of formaldehyde; lit.[23] mp 167°C. A second crop was obtained (10 mg, mp 162–164°C).

Preparation of thioamides. Thioamides were prepared in general by heating the corresponding amide with Lawesson's reagent [**12**; 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide; Aldrich Chemical Co.] in tetrahydrofuran (THF) under reflux. The molar ratio of the amide to **12** was 2:1 unless otherwise specified. Times of reaction were determined by disappearance of the amide by TLC. The crude product obtained after evaporation of solvent was purified by flash chromatography with Baker 7024-2 silica gel; EtOAc/hexane mixtures were used for elution. Particulars were as follows:

Trifluorothioacetamide (6). Trifluoroacetamide (10.00 g) and **12** (18.00 g) after 6 h of reflux in THF (100 mL), evaporation of solvent, and chromatography (10% EtOAc/hexane) gave a yellow oil. Distillation at ca. 20 torr from 80–90°C afforded a yellow oil (6.0 g, 53%), which crystallized on being cooled: mp 40–41°C; lit.[24] mp 42–43°C.

Thiopivalamide. Pivalamide (**9**, 15.0 g, 148 mmol) and **12** (25.5 g, 63.0 mmol) when heated for 4 h in THF (200 mL) gave after evaporation of the solvent and chromatography (20% EtOAc/hexane) a colorless solid (9.80 g, 66%): mp 108–112°C; recrystallization from H_2O gave the thioamide as colorless needles: mp 115–117°C; lit.[25] mp 116–119°C; ^1H NMR ($CDCl_3$) δ 1.36 (s).

N,N-Dimethylthiopivalamide. N,N-Dimethylpivalamide (14.0 g, prepared from pivaloyl chloride with dimethylamine) and **12** (22.0 g) were heated for 16 h in THF (150 mL). The solution then was poured into H_2O (150 mL), and the mixture was extracted with Et_2O(3 × 100 mL). The Et_2O extracts were combined, dried, filtered, and evaporated to give a brown oil (11.8 g). Chromatography (15% EtOAc/hexane) gave a yellow oil (4.0 g, 25%) which crystallized on standing: mp 37–38.5°C, lit.[26,27] mp 40°C; ^1H NMR ($CDCl_3$) δ 1.44 (s, 9 H), 3.48 (s, 6 H); lit.[26] ^1H NMR ($CDCl_3$) δ 1.4 (s, 9 H), 3.5 (s, 6 H).

N,N-Diethylthiopivalamide. N,N-Diethylpivalamide (15.00 g, 95.5 mmol, prepared from pivaloyl chloride with diethylamine) and **12** (30.0 g, 74.2 mmol) were heated in THF (200 mL) for 10 days. The solvent was evaporated, and the residue was chromatographed (10% EtOAc/hexane). The material thus obtained was distilled at ca. 0.1 torr from 65–67°C to give a pale yellow oil (5.60 g, 34%); lit.[27] bp 74–75°C at 0.3 torr; ^1H NMR ($CDCl_3$) δ 1.28 (t, 6 H), 1.44 (s, 9 H), 3.88 (q, 4 H); lit.[27] ^1H NMR ($CDCl_3$) δ 1.29 (t, 6 H), 1.43 (s, 9 H), 3.95 (q, 4 H).

Oxidation of thioacetamide S-Oxide (**1**) *with* H_2O_2

In the absence of benzylamine. H_2O_2 (0.35 mL, 3.40 mmol) was added with stirring to a solution of thioacetamide *S*-oxide (**1**, 0.100 g, 1.10 mmol) in H_2O (10 mL) at ca. 25°C, while the pH was maintained at ca. 4–7 by means of 1.0 *N* NaOH. After 6 h (the pH then was steady at 7), the solvent was evaporated to give a colorless solid. This material was extracted repeatedly with MeOH. The extracts were combined, filtered, and evaporated to yield a colorless hygroscopic solid (0.031 g, 48%). The ^1H NMR (D_2O) spectrum of this material was identical with that of authentic acetamide.

In the presence of benzylamine. H_2O_2 (1.2 mL, 12 mmol) was added dropwise with stirring to a solution of thioacetamide *S*-oxide (**1**, 0.350 g, 3.85 mmol) in water (15 mL) at ca. 25°C. After ca. 2–3 min, benzylamine (1.00 mL, 9.16 mmol) was added, and the resulting mixture was stirred for 24 h while the pH was kept at ca. 6–7 by periodic addition of 1.0 *N* NaOH. The mixture then was diluted with H_2O (20 mL) and extracted with CH_2Cl_2 (3 × 25 mL). The organic extracts were combined, dried, filtered, and evaporated to give a brown oil (0.380 g). TLC (CH_2Cl_2) indicated at least 4 spots. The oil in EtOAc was chromatographed on a short column of silica gel, and the eluate was treated with decolorizing carbon. Evaporation of the solvent and recrystallization of the yellow solid residue (hexane) gave colorless plates (0.095 g, 17%); mp 60.5–62.0°C, undepressed by authentic *N*-acetylbenzylamine (**5**, prepared from AcCl with benzylamine): lit.[28] mp 61–62°C); ^1H NMR ($CDCl_3$) identical with that of authentic **5**. NMR spectra of the evaporated aqueous layer showed the presence only of acetamide (δ 1.96) and thioacetamide *S*-oxide (**1**, δ 2.20) in ca. 4:1 ratio, along with benzylamine (δ 4.18 and ca. 7.50).

In one control experiment, acetamide (0.0200 g, 0.339 mmol) and benzylamine (0.035 mL, 0.32 mmol) were combined in D_2O (ca. 1.0 mL). The mixture was maintained at 55°C for 19 h, then extracted with $CDCl_3$ (ca. 0.5 mL). The ^1H NMR spectrum of this extract had no absorptions corresponding to *N*-acetylbenzylamine (**5**), by comparison with the ^1H NMR spectrum obtained from authentic **5**. A second experiment identical with the first except for adjustment to pH 3 (dilute H_2SO_4) and for a heating period of 4 h also showed no formation of **5**.

Trifluorothioacetamide (**6**) *with* H_2O_2 *in the presence of benzylamine.* H_2O_2 (1.20 mL, 12 mmol) was added rapidly dropwise with stirring to a solution of trifluorothioacetamide (**6**, 0.350 g, 2.71 mmol) in H_2O (10 mL) at ca. 25°C, benzylamine (0.60 mL, 5.50 mmol) was immediately added, and the mixture was stirred for 90 min (the pH remained at ca. 8–9 without addition of NaOH). The mixture was diluted with H_2O (15 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The organic extracts were combined, dried, filtered, and evaporated to give a yellow solid and oil (0.67 g). This material was left under reduced pressure (ca. 1 torr) overnight, and the solid that resulted was recrystallized twice from hexane (10 mL) to give off-white needles (0.170 g, 31%): mp 72.5–74°C, undepressed by authentic *N*-benzyltrifluoroacetamide (**7**, from benzylamine and trifluoroacetic anhydride); lit.[29] mp 73–74°C; ^1H NMR identical with that of authentic **7**. A second crop of **7** was obtained (0.036 g, mp 70.5–72.5°C, overall yield 37%).

Preparation and stability of thiopivalamide S-oxide (**8**). H_2O_2 (0.27 mL, 2.62 mmol) was added to a suspension of thiopivalamide (0.320 g, 2.74 mmol) in MeCN (3 mL) at ca. 0–5°C. A solution immediately resulted, and colorless needles appeared after 1 h. After 16 h at ca. 5°C, colorless needles were isolated. Evaporation of the mother liquor at ca. 20 torr and recrystallization from CH_3CN gave additional **8**; the combined yield of **8** was 0.210 g (58%): mp 141–142°C; ^1H NMR (D_2O) δ 1.28 (s); ^1H NMR ($CDCl_3$) δ 1.30 (s); ^{13}C NMR ($CDCl_3$) δ 22.7, 36.3, 205.8; IR (Nujol) 3250, 1630, 1360, 1220, 970, 930, 880, 795 cm^{-1}; Anal. Calcd. for $C_5H_{11}NOS$: C, 45.08; H, 8.32; N, 10.51; S, 24.07. Found: C, 44.96; H, 8.36; N, 10.47; S, 24.23.

For the determination of its stability, thiopivalamide *S*-oxide (**8**, 30.0 mg, 0.226 mmol) in $CDCl_3$ (ca. 0.5 mL) was sealed in an NMR tube (final *S*-oxide conc'n., 0.45 M) and maintained at 55°C. ^1H NMR spectra were obtained at regular intervals. The ratio of unchanged *S*-oxide (**8**) to reaction products was determined by NMR integration. A first-order plot of ln[*S*-oxide (**8**)] versus time was linear to at least 3 half lives, with the half life ca. 21 days (since $k = 0.693/t_{1/2}$, $k = 1.4 \times 10^{-3}$ hr^{-1}). The reaction products were thiopivalamide (79%) and pivalamide (**9**, 21%, identified by spiking with authentic specimens). A small amount of an off-white precipitate was observed in the NMR tube after several weeks. This material was not identified but was presumed to be sulfur.

Oxidation of thiopivalamide S-oxide (**8**) *with* H_2O_2

In the absence of benzylamine. H_2O_2 (0.25 mL, 2.4 mmol) was added to a solution of **8** (0.100 g, 0.752 mmol) in H_2O (10 mL) at ca. 25°C. The solution became turbid after 2 h, and after 21 h the pH was adjusted to 7 with 0.1 *N* NaOH. After 48 h the neutral solution was evaporated to give a colorless solid,

which was extracted repeatedly with hot EtOAc. These extracts were combined, filtered through glass wool, and evaporated to give colorless needles of pivalamide (**9**; 0.066 g, 75%), mp 148–150.5°C. Recrystallization from EtOAc gave 0.040 g of **9** (45%): mp 151–153°C; lit. mp 152–153°C,[30] 155–157°C.[31]

In the presence of benzylamine. H_2O_2 (0.25 mL, 2.4 mmol) was added with stirring to a solution of thiopivalamide *S*-oxide (**8**, 0.100 g, 0.752 mmol) in H_2O (15 mL) at ca. 25°C. After ca. 2–3 min, benzylamine (0.160 mL, 1.47 mmol) was added. The mixture was stirred for 48 h (the pH was adjusted to 7 with 1.0 *N* NaOH after 24 h) and then was extracted with CH_2Cl_2 (3 × 50 mL). The extracts were combined, dried, filtered, and evaporated to give a yellow solid (0.075 g). The ^1H NMR spectrum ($CDCl_3$) of this material indicated that it consisted of **8** and **9**, with benzylamine, and that less than 3% was *N*-benzylpivalamide (**10**), by comparison with the ^1H NMR spectrum of authentic **10** (prepared by reaction of benzylamine with pivaloyl chloride,[32] mp 77.5–80°C; lit.[32] mp 82°C).

Oxidation of thioacetamide and thioamide S-oxides with H_2O_2 in $H_2{}^{18}O$

Thioacetamide. Sodium peroxide (Na_2O_2, Aldrich Chemical Co., 95–98%; 21.0 mg, 0.270 mmol) was added to a solution of H_2SO_4 (95% min., 14 μL, 0.25 mmol) in $H_2{}^{18}O$ (98 atom % ^{18}O, 0.450 mL; at least 22 mmol) at ca. 25°C. After ca. 2–3 min, thioacetamide (5.0 mg, 0.067 mmol) was added, and the solution was allowed to stand for 5 h (complete reaction, based on model experiments). The pH (ca. 1) then was adjusted to ca. 7 with 0.1 *N* NaOH, and the water was evaporated under reduced pressure at or below 25°C to give a colorless solid. This material was extracted repeatedly with MeOH. The extract was filtered, and evaporated at ca. 25°C and ca. 760 torr. The solid residue (1.7 mg, 44%) thus obtained was subjected to mass spectral analysis to determine the extent of ^{18}O incorporation in the acetamide produced (*m/z* 59). The excess ^{18}O% (Table II) was determined as: [*m* + 2, the relative peak intensity at *m/z* 61 (after correction for the natural-abundance *m* + 2 intensity)](100)/[(*m*, the relative peak intensity at *m/z* 59) + (the corrected relative peak intensity at *m/z* 61)].

Thioacetamide S-oxide (**1**). The reaction, workup, analytical procedures and calculation used were those described above with the following quantities of reagents: Na_2O_2 16 mg, 0.205 mmol; H_2SO_4, 11 μL, 0.20 mmol; $H_2{}^{18}O$ 0.450 mL; and *S*-oxide (**1**), 6.0 mg, 0.066 mmol. The reaction times for the two results of Table II were 5h and 7 h, respectively.

Thiopivalamide S-oxide (**8**). Na_2O_2 (14.0 mg, 0.180 mmol) was added to a solution of H_2SO_4 (10 μL, 0.18 mmol) in $H_2{}^{18}O$ (98 atom % ^{18}O, 0.450 mL; at least 22 mmol) at ca. 25°C. Thiopivalamide *S*-oxide (**8**, 8.0 mg, 0.060 mmol) was added, and the resulting solution was left for 20 h (complete reaction in model experiments). The pH (ca. 1) then was adjusted to 6–7 with 0.1 *N* NaOH, and the H_2O was evaporated at or below 25°C (ca. 0.5 torr). The residue thus obtained was extracted repeatedly with EtOAc, and the filtrate was evaporated at ca. 25°C and ca. 760 torr to give a colorless solid. For the results of Table II, this material was subjected to mass spectral analysis to determine the excess ^{18}O% present in the pivalamide produced (**9**, *m/z* 101). The excess ^{18}O% in **9** was determined as: [*m* + 2 peak, the relative peak intensity at *m/z* 103 (after correction for natural-abundance *m* + 2 intensity)](100)/[(*m*, the relative peak intensity at *m/z* 101) + (the corrected relative peak intensity at *m/z* 103)].

Hydrolysis of thioamide S-oxides at 37°C

Thioacetamide S-oxide(**1**). Thioacetamide *S*-oxide (**1**, 0.0100 g, 0.110 mmol) was dissolved in D_2O (ca. 0.5 mL) in an NMR tube and the solution was maintained at 37°C. The *S*-oxide (**1**) appeared by NMR integrations at δ 2.19 to be half consumed after ca. 100 h, with an off-white precipitate first being observed after 22 h (presumably sulfur). After 75 days, the reaction was judged to be complete (absence of the NMR peak at δ 2.19 for **1**). The reaction products and estimated relative proportions after completion were shown by NMR to be acetamide (65%, δ 1.96), acetonitrile (15%, δ 2.04), and thioacetamide (15%, δ 2.50), along with two unidentified singlets (5%) at δ 2.60, 2.80. The identity of the products named was established by spiking with authentic materials.

Thiopivalamide S-oxide (**8**). Thiopivalamide *S*-oxide (**8**, 10.0 mg, 0.0752 mmol) was dissolved in D_2O (ca. 0.5 mL) in an NMR tube and the solution was maintained at 37°C. ^1H NMR spectra were obtained at regular intervals as before. The *S*-oxide (**8**, δ 1.28) appeared to be half consumed after ca. 30 days. After 111 days, the reaction was at least 95% complete; final pH ca. 4–5. The reaction products (relative proportions by NMR integration) were pivalamide (**9**) and/or pivalic acid (85%, δ 1.21), and thiopivalamide (15%, δ 1.35), identified as before by spiking with authentic materials.

Other experiments

Reduction of thioacetamide S-oxide (1) with NADH in deuterium oxide at 37°C. Thioacetamide *S*-oxide (**1**, 11.0 mg, 0.12 mmol) and sodium NADH · $4H_2O$ (100 mg, 0.128 mmol; Sigma Chemical Co.) were dissolved in D_2O (ca. 0.50 mL), in an NMR tube and maintained at 37°C. ^1H NMR spectra were obtained at regular intervals. The *S*-oxide (**1**) was judged to be half-consumed after ca. 26 h. After 6 days no *S*-oxide (**1**) remained. Acetamide (6%) and thioacetamide (55%) were identified by spiking with authentic specimens and were estimated in amount from NMR integrals. The solution was extracted with $CDCl_3$ (ca. 0.5 mL). The ^1H NMR spectrum of this extract exhibited a singlet at δ 1.99, identified as MeCN (39%) by spiking with authentic MeCN.

In a control experiment for the above, a solution of thioacetamide (10 mg, 0.133 mmol), acetamide (80 mg, 0.136 mmol), acetonitrile (ca. 6 μL, 0.115 mmol), and NADH · $4H_2O$ (100 mg, 0.128 mmol) in D_2O (ca. 1.0 mL) was maintained at 37°C. ^1H NMR spectra were obtained at regular intervals over 5 days. At this time, there was no change in the relative proportions of the mixture, as judged by NMR integration.

Reduction of **1** *with NADPH.* As with NADH, a solution of **1** (10.0 mg, 0.110 mmol) and sodium NADPH (100 mg, 0.113 mmol; Sigma) in D_2O (ca. 0.50 mL) in an NMR tube was maintained at 37°C, and ^1H NMR spectra were obtained at regular intervals. The *S*-oxide (**1**) was judged to be half consumed after ca. 1 day and at least 95% consumed after ca. 4 days. The reaction products and relative proportions (from NMR integration), as shown in Table IV, were thioacetamide (53%), acetonitrile (40%), and acetamide (7%).

Studies in the preparation and properties of N,N-diethyl- and N,N-dimethylthiopivalamide S-oxide

Oxidation of N,N-diethylthiopivalamide. H_2O_2 (0.30 mL, 2.91 mmol) was added to a solution of *N,N*-diethylthiopivalamide (0.500 g, 2.89 mmol) in 10% NaOAc/glacial AcOH (10 mL), according to the method of Walter and Bauer.[5] The solution was allowed to stand at ca. 25°C for 20 h and then was poured into saturated $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 50 mL). The organic extracts were combined, dried, filtered, and evaporated to give a yellow oil containing a little solid (0.253 g). TLC (CH_2Cl_2) exhibited 3 spots. Preparative TLC (CH_2Cl_2) gave a major band at ca. R_f 0.1–0.2, which was extracted with EtOAc. Filtration and evaporation of the extract gave a yellow oil (**11**) (0.120 g, 22%); ^1H NMR ($CDCl_3$) δ 1.24 (t, 6 H), 1.40 (s, 9 H), 3.84 (q, 4 H). The following experiment indicated that **11** was too fragile for elemental analysis elsewhere.

For assessment of the thermal stability of **11**, a purified sample (50 mg, 0.265 mmol) in $CDCl_3$ (ca. 0.5 mL) was maintained at 37°C in an NMR tube. After 7 h an off-white precipitate (presumably sulfur) was observed, and the ^1H NMR spectrum indicated that **11** was about half consumed. After 24 h, no **11** remained; *N,N*-diethylthiopivalamide and *N,N*-diethylpivalamide were observed in a relative proportion of 1:2. The identity of the products was confirmed by spiking this mixture with authentic specimens.

Oxidation of N,N-dimethylthiopivalamide. By means of the general procedure described above, *N,N*-dimethylthiopivalamide was oxidized with 1 equivalent of H_2O_2 to give, after workup, a pale yellow oil. The ^1H NMR ($CDCl_3$) spectrum of the crude *S*-oxide exhibited absorptions at δ 1.40 (s, 3 H) and 3.42 (s, 2 H) along with other absorptions congruent with *N,N*-dimethylthiopivalamide and *N,N*-dimethylpivalamide. The *N,N*-dimethyl compound was not studied further because it proved even more difficult to handle than the *N,N*-diethyl homologue.

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