

Spontaneous Formation of Thiuram Disulfides in Solutions of Iron(III) Dithiocarbamates

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Dithiocarbamates are used as pesticides and rubber additives. Dithiocarbamates are the reduced forms of thiuram disulfides and both of these groups of substances induce allergic contact dermatitis. The allergic cross-reactivity pattern between dithiocarbamates and thiurams is unclear. The aim of this study was to investigate why these cross-reactions occur sometimes but not always. HPLC-analysis of buffer solutions of iron(III) dithiocarbamates demonstrated that thiuram disulfides were formed spontaneously and rapidly in high yield. No such oxidation was observed in solutions of copper(II), zinc(II), or sodium dithiocarbamates. However, sodium diethyldithiocarbamate and zinc diethyldithiocarbamate were oxidized in buffer solution when ferric salt was added. The influence of different metal ions on the oxidation reaction is probably an explanation for the cross-reactivity patterns seen between dithiocarbamates and thiurams. These findings also show that careful handling is necessary in analytical and biological studies with solutions of iron(III) dithiocarbamates. Oxidation of dithiocarbamates in aqueous buffer at physiological pH has not been shown before.

KEYWORDS: Contact allergy; cross-reactivity; dermatitis; disulfiram; ferbam; ferric compounds; HPLC; oxidation; pesticides; rubber chemicals; thiocarbamates; thiram

INTRODUCTION

Dithiocarbamates are substances with a multitude of interesting and useful properties. Their use as accelerators in rubber production and as fungicides makes them commercially important (1). Diethyldithiocarbamic acid is a thiol and the reduced form of tetraethylthiuram disulfide (disulfiram, TETD), which is employed therapeutically against alcoholism as the drug Antabuse (2). This effect is explained by an irreversible inhibition of aldehyde dehydrogenase. Several other biological effects have been attributed to dithiocarbamates. They inhibit copper–zinc superoxide dismutase (3) and the transcription factor NF- κ B (4). Burkitt et al. have shown that dithiocarbamates inhibit apoptosis, an effect that has been explained in terms of redox reactions between dithiocarbamates and thiuram disulfides (5). They report that the activity of dithiocarbamates is due to the oxidation to thiuram disulfides by copper ions without release of reactive oxygen species. The interactions with metal ions very often seem to be the explanation for the biological effects of dithiocarbamates.

As a consequence of the interest in these compounds in research and industry there is a need for sensitive analytical methods suitable for studies of dithiocarbamates, and some reports on this matter have recently been published (6–8).

A well-known adverse effect of dithiocarbamates and thiuram di- and monosulfides is their ability to induce allergic contact dermatitis (9), mainly from contact with rubber products such

as gloves (10). An increase in allergic contact dermatitis to thiurams has been noted in recent decades, probably because of an increase in the usage of rubber gloves. Both thiurams and dithiocarbamates are found in rubber products, and positive patch tests for thiurams are often, but not always, reported with a concomitant positive test for dithiocarbamates. We are at present studying these so-called cross-reactions, aiming to understand why they sometimes occur but not always and to clarify whether there is a simple chemical explanation for these test reactions. Both thiurams and dithiocarbamates have previously been components in the European standard patch test series used in the investigation of patients with suspected allergic contact dermatitis. Some years ago dithiocarbamates were excluded because of the frequently observed cross-reactions with thiurams.

During our studies of the oxidation of dithiocarbamates we noticed a fast reaction in the presence of ferric ions giving high yields of thiuram disulfides. Similar reactions in organic solvents have been reported earlier (11, 12), but not in buffered aqueous solutions at physiological pH. To investigate these reactions we followed the formation of thiuram disulfides from dithiocarbamates with HPLC. In earlier studies on rubber allergens, we have shown that test preparations of thiuram disulfides (13) or mercaptobenzothiazole compounds (14) are not stable.

MATERIALS AND METHODS

Chemicals. Iron(III) dimethyldithiocarbamate (97%), iron(III) diethyldithiocarbamate (95%), copper(II) dimethyldithiocarbamate (98%), copper(II) diethyldithiocarbamate (97%), and zinc(II) diethyldithiocar-

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Table 1. Abbreviations of Dithiocarbamates and Thiuram Disulfides Studied.

abbreviation	names and synonyms	CAS registry number
Fe(DMTC) ₃	iron(III) dimethyldithiocarbamate, ferbam	14484-64-1
Fe(DETC) ₃	iron(III) diethyldithiocarbamate	13963-59-2
Cu(DMTC) ₂	copper(II) dimethyldithiocarbamate	137-29-1
Cu(DETC) ₂	copper(II) diethyldithiocarbamate	13681-87-3
Zn(DETC) ₂	zinc(II) diethyldithiocarbamate	14324-55-1
NaDETC	sodium diethyldithiocarbamate, sodium dithiocarb	148-18-5
TMTD	tetramethylthiuram disulfide, thiram	137-26-8
TETD	tetraethylthiuram disulfide, disulfiram, Antabuse	97-77-8

bamate (99%) were obtained from TCI, Tokyo, Japan. Sodium diethyldithiocarbamate trihydrate (99%) was obtained from Aldrich, Steinheim, Germany. Tetramethylthiuram disulfide (95%) and tetraethylthiuram disulfide (97%) were obtained from Lancaster, Morecambe, England. A list of the compounds studied is presented in **Table 1**. Acetonitrile and dichloromethane (both HPLC grade) was obtained from Labscan Ltd., Dublin, Ireland. Iron(III) chloride hexahydrate (p.a.) was obtained from Merck, Darmstadt, Germany.

The low solubility of some of the dithiocarbamates and thiuram disulfides in aqueous solutions made it necessary to modify the reaction medium with acetonitrile. Stock solutions (1.0 mM) of dithiocarbamates were made in acetonitrile immediately before use. The reaction media in experiments with buffered solutions were mixtures of acetonitrile and 0.05 M sodium phosphate buffer in a proportion that gave a final solution containing equal amounts of acetonitrile and buffer after the addition of dithiocarbamate.

HPLC. Chromatographic analyses were performed by HPLC on a reversed-phase column (Genesis C₁₈, 150 × 4.6 mm, 4 μm, Jones Chromatography Limited, Mid Glamorgan, UK), eluted with acetonitrile/water (40:60) with 0.1% trifluoroacetic acid for analysis of TMTD, and acetonitrile/water (68:32) with 0.1% trifluoroacetic acid for analysis of TETD. Eluent was pumped with a Waters 600 pump (Waters Chromatography Division, Milford, MA) at a flow rate of 1 mL/min and monitored at 280 nm with a 1100 Series diode array detector (Hewlett-Packard Co., Palo Alto, CA). Peak area was used to determine the concentration, and the thiuramdisulfides were identified by comparison of retention times and UV spectra recorded by the diode array detector.

Spontaneous Oxidation of Dithiocarbamates at Physiological pH.

One of the stock solutions (2 mL) of Fe(DMTC)₃, Fe(DETC)₃, Cu(DMTC)₂, or Cu(DETC)₂ was added to 8 mL of a mixture of acetonitrile and buffer at pH 5.5 or 7.5 in a test tube with a screw cap. The concentration of dithiocarbamates at start was 200 μM in 50% acetonitrile in buffer. The reaction was run at room temperature, and after mixing the test tube was placed on a shaker. Samples were taken for analysis on HPLC of the amount of thiuram disulfide formed at different times.

Spontaneous Oxidation of Dithiocarbamates in Acetonitrile.

Fe(DMTC)₃ (16.6 mg) was dissolved in 20 mL of acetonitrile. A 1-mL aliquot of this solution was added to 9 mL of acetonitrile in a test tube with screw cap, giving a concentration of dithiocarbamates at start of 200 μM. The reaction was run at room temperature, and after dissolution the test tube was placed on a shaker. Samples were taken for HPLC analysis of the amount of TMTD formed at different times. In a separate experiment, a solution of 20 mg of Fe(DMTC)₃, dissolved in 20 mL of dichloromethane, was washed three times with 20 mL water each time in a separatory funnel to remove free iron ions. The organic phase was dried over sodium sulfate and evaporated under reduced pressure. The dry residue was dissolved in acetonitrile and diluted to a concentration of dithiocarbamates of 200 μM. The reaction was run at room temperature, and after dissolution the test tube was placed on a shaker. Samples were taken for HPLC analysis of the amount of TMTD formed at different times.

Oxidation with Ferric Chloride. Stock solutions of Zn(DETC)₂ or NaDETC (2 mL) were added to 8 mL of a mixture of acetonitrile and buffer at pH 5.5 or 7.5 containing iron(III) chloride in a test tube

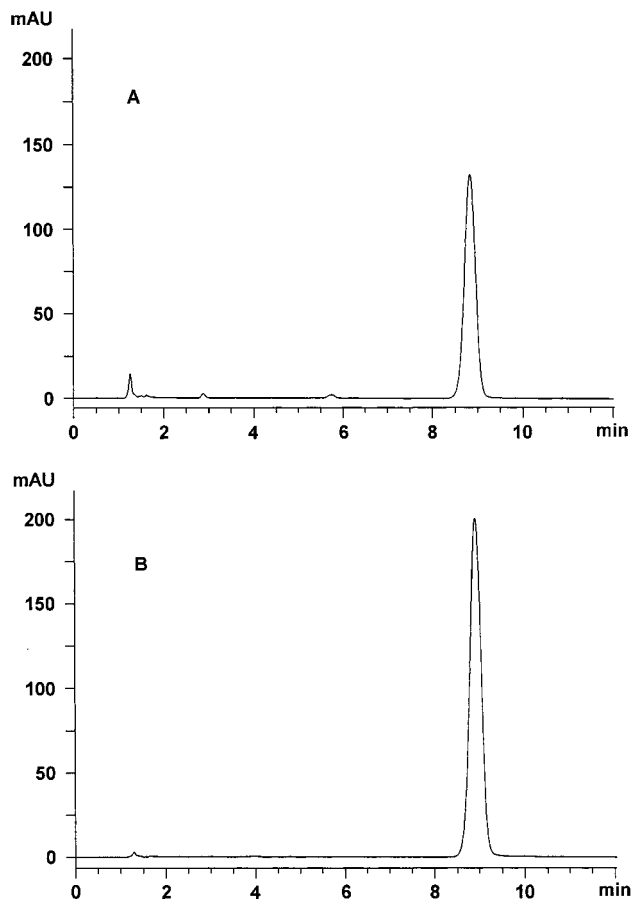


Figure 1. Spontaneous oxidation of Fe(DMTC)₃. (A) HPLC chromatogram of 200 μM Fe(DMTC)₃ after standing in buffer solution at pH 5.5 for 4 h. (B) HPLC chromatogram of a reference solution containing 200 μM TMTD.

with screw cap. The concentration of dithiocarbamates at start was 200 μM in 50% acetonitrile in buffer and the concentration of iron(III) chloride was 200, 400, 600, or 2000 μM. The reaction was run at room temperature, and after mixing the test tube was placed on a shaker. Samples were taken for HPLC analysis of the amount of thiuram disulfide formed at different times.

RESULTS

Spontaneous Oxidation of Dithiocarbamates at Physiological pH. Chromatograms of standard solutions of thiuram disulfides are shown in **Figures 1B** and **2B**. The retention time for TMTD was 8.8 min and the retention time for TETD was 6.4 min, with the chromatographic conditions specified in Materials and Methods. The UV-spectra recorded with the diode-array detector for the thiuram peaks in all experiments showed that the peaks were pure and identical with those of reference material.

There was a significant difference in the behavior of ferric dithiocarbamates compared to that of cupric dithiocarbamates in buffered solutions (**Table 2**). Both Fe(DMTC)₃ and Fe(DETC)₃ were oxidized to TMTD and TETD, respectively. Cu(DMTC)₂ or Cu(DETC)₂, on the other hand, did not produce thiurams spontaneously in solution. Instead, the small amount of thiuram disulfide present at the start of the experiment with the copper compounds decreased during the 6 h the solutions were examined from about 2 μM to about 1 μM. In the buffer solution at pH 5.5 of Fe(DMTC)₃, 43% of the dithiocarbamate was oxidized to TMTD within 2 h, based on the concentration of formed thiuram disulfide. The reaction reached a plateau and no further reaction was seen within the next hours (**Figure 1**).

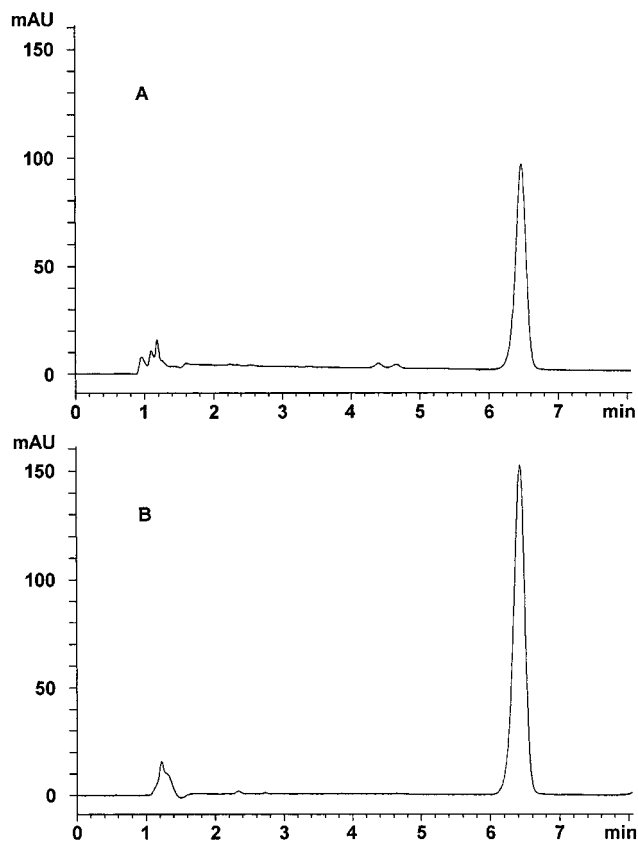


Figure 2. Oxidation of NaDETC by ferric ions. (A) HPLC chromatogram of 200 μM NaDETC after standing in buffer solution at pH 7.5 containing 2 mM iron(III) chloride for 1 h. (B) HPLC chromatogram of a reference solution containing 100 μM TETD.

Table 2. Yields of Thiuram Disulfides after Dissolution of Different Dithiocarbamates in Buffer at pH 7.5 and 5.5 (reaction time was 4 hours)

compound	pH	product	yield
$\text{Fe}(\text{DMTC})_3$	7.5	TMTD	21%
	5.5	TMTD	43%
$\text{Fe}(\text{DETC})_3$	7.5	TETD	24%
	5.5	TETD	20%
$\text{Cu}(\text{DMTC})_2$	7.5	TMTD	0%
	5.5	TMTD	0%
$\text{Cu}(\text{DETC})_2$	7.5	TETD	0%
	5.5	TETD	0%

At pH 7.5, the same dithiocarbamate was oxidized at a much slower rate. In the solutions of $\text{Fe}(\text{DETC})_3$ the reaction stopped at yields of thiuram between 20 and 25% after a couple of hours at both pH 5.5 and 7.5 (Figure 3).

Spontaneous Oxidation of Dithiocarbamates in Acetonitrile. To determine the effects of potential free Fe^{3+} and Fe^{2+} , a sample of $\text{Fe}(\text{DMTC})_3$ in dichloromethane was extracted with water to wash out the metal ions. Both washed and unwashed samples oxidized spontaneously in solution, with the difference that oxidation unexpectedly occurred faster in the solution of washed dithiocarbamate. Washed $\text{Fe}(\text{DMTC})_3$ reached a plateau after 4 h at about 200 μM of TMTD, but the solution of unwashed dithiocarbamate contained only about half that concentration of thiuram disulfide at the same time, although the concentration was still increasing.

Oxidation with Ferric Chloride. TETD was formed in 63% yield after 1 h in the reaction of 200 μM NaDETC with 2mM FeCl_3 in buffer solution at pH 7.5 (Table 3, Figure 2). At the

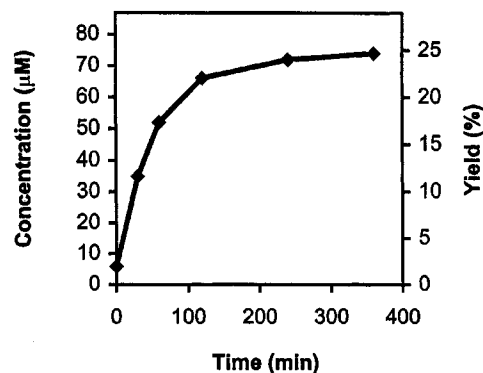


Figure 3. Spontaneous oxidation of 200 μM $\text{Fe}(\text{DETC})_3$ at pH 7.5; yield of the oxidation product TETD and its concentration at different reaction times.

Table 3. Yields of TETD in the Reactions of 200 μM $\text{Zn}(\text{DETC})_2$ and NaDETC with 600 μM and 2 mM FeCl_3 in Buffer at pH 7.5 and 5.5 (reaction time was 1 hour)

compound	pH	concentration of FeCl_3	yield
$\text{Zn}(\text{DETC})_2$	7.5	600 μM	7%
	7.5	2 mM	17%
	5.5	600 μM	4%
NaDETC	5.5	2 mM	22%
	7.5	600 μM	9%
	7.5	2 mM	63%
	5.5	600 μM	6%
	5.5	2 mM	6%

oxidations with 600 μM FeCl_3 , the yields were significantly lower. The low yield in the reaction at pH 5.5 is probably due to the fast degradation of the dithiocarbamate to carbon disulfide and diethylamine at low pH, as shown earlier (15). The more stable $\text{Zn}(\text{DETC})_2$ is probably less reactive toward both oxidation and acid-catalyzed degradation compared to NaDETC. This stability may explain the lower yield at pH 7.5. Reaction times longer than 1 h did not produce more thiuram disulfide.

DISCUSSION

The redox behavior of thiurams and dithiocarbamates is often discussed nowadays, but the studies are seldom accompanied by analytical data. Direct analysis of dithiocarbamates by HPLC is laborious and requires a precolumn-derivatization procedure (16) that makes studies of fast reactions difficult to perform. Thiuram disulfides, on the other hand, are easily studied by chromatographic techniques, and the formation of thiuram disulfides from dithiocarbamates in simple models can be demonstrated by analytical methods, as shown in this investigation.

It is important in studies of the biological effects of iron(III) dithiocarbamates to be aware of the chemical stability of the solutions used in laboratory experiments. Besides the spontaneous oxidation to thiuram disulfides, the fast degradation of dithiocarbamates at low pH to amine and carbon disulfide has to be considered when interpreting results from biological and chemical investigations of dithiocarbamates (17). When handling reactive organic substances in solution, it is also necessary to choose an appropriate solvent. We have previously shown how mercaptobenzothiazole compounds react spontaneously with acetone in solution, yielding new compounds (18), and other investigators have identified adducts between TMTD and acetone formed in solutions of the thiuram disulfide in hot solvent (19).

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