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PHOTOLYSIS OF SULFIRAM: A MECHANISM FOR ITS DISULFIRAM-LIKE REACTION

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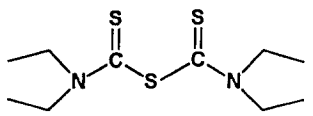
Abstract—Sulfiram, a drug applied topically to treat scabies, produces effects similar to those of disulfiram after subsequent ingestion of ethanol. Disulfiram, used in aversion therapy in the treatment of alcoholism, inhibits hepatic aldehyde dehydrogenase (ALDH) causing an accumulation of acetaldehyde after ethanol ingestion. The increased tissue levels of acetaldehyde cause a spectrum of undesirable side-effects including flushing, nausea, vomiting, and tachycardia, which are referred to as the disulfiram reaction. Previous studies have shown that *in vitro* sulfiram is a very weak inhibitor of ALDH, but solutions of sulfiram markedly increase in potency with time. In the present study, fresh solutions of sulfiram were exposed to fluorescent room light under ambient conditions and analyzed at timed intervals by HPLC. At least eight products, including disulfiram, were formed in the light-exposed sulfiram solutions, but not in solutions kept in the dark. Structural characterization of two of the photolysis products was obtained by on-line microbore HPLC-mass spectrometry (μLC-MS) and on-line microbore HPLC-tandem mass spectrometry (μLC-MS/MS) using continuous flow-liquid secondary ion mass spectrometry (CF-LSIMS) as the primary ionization method. Sulfiram was converted to disulfiram at an initial rate of 0.7%/hr, and the formation of disulfiram correlated with the increase in ALDH inhibition *in vitro*. The results of this investigation show that while sulfiram is a weak inhibitor of ALDH *in vitro*, it is readily photoconverted to disulfiram, a very potent inhibitor of ALDH, which may explain the adverse reaction to ethanol after sulfiram therapy.

Key words: disulfiram; disulfiram reaction; disulfiram–ethanol reaction; sulfiram; monosulfiram; photochemical reaction; free radical; aldehyde dehydrogenase; enzyme inhibition; tandem mass spectrometry

Sulfiram (**1**) (tetraethylthiuram monosulfide; monosulfiram) is applied topically to treat scabies. There have been reports of nausea, vomiting, tachycardia, and flushing in some individuals ingesting ethanol subsequent to sulfiram treatment [1–4]. These side-effects of sulfiram are similar to those of the "disulfiram reaction" observed with ethanol ingestion after treatment with disulfiram (**2**) (tetraethylthiuram disulfide).

Disulfiram, used in aversion therapy in the treatment of alcoholism, inhibits hepatic ALDH‖, causing an accumulation of acetaldehyde after ethanol ingestion [5, 6]. Disulfiram inhibits ALDH *in vitro* by the formation of mixed disulfide bonds with essential sulfhydryl groups in the protein [7]. An entirely different mechanism has been proposed for the inhibition of ALDH by disulfiram in intact animals because it is believed that disulfiram is too short-lived *in vivo* to directly inhibit the enzyme [8, 9]. The exact mechanism by which disulfiram inhibits aldehyde dehydrogenase *in vivo* is currently under investigation in several laboratories [10–14]. While most investigators are in agreement that disulfiram undergoes hepatic biotransformation to a reactive metabolite that inhibits ALDH, the identity of the ultimate inhibitor *in vivo* is still uncertain.



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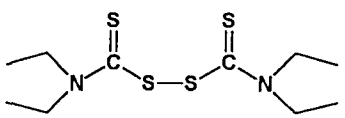

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Previous studies in our laboratory have shown that in freshly prepared solutions sulfiram is a very weak inhibitor of ALDH *in vitro*, but solutions of sulfiram markedly increase in potency within minutes

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‖ Abbreviations: ALDH, aldehyde dehydrogenase; CF-LSIMS, continuous flow-liquid secondary ion mass spectrometry; CID, collision-induced dissociation; μLC-MS, microbore HPLC-mass spectrometry; μLC-MS/MS, on-line HPLC-continuous flow-liquid secondary ion tandem mass spectrometry; MeCN, acetonitrile; Q_1 rf-only octapole collision cell; and Q_2 mass filter quadrupole.

[15]. In the present study, we determined that in solution sulfiram is photochemically converted to disulfiram in relatively high yield. We therefore propose that the disulfiram-like reaction observed with sulfiram treatment may be caused by disulfiram formed by photolysis of sulfiram prior to or after topical application. Photolysis products of sulfiram were analyzed using a combination of μLC-MS employing CF-LSIMS as the primary ionization method and μLC-MS/MS [16, 17].

MATERIALS AND METHODS

Chemicals. Sulfiram (tetraethylthiuram monosulfide; [95-05-6]; $C_{10}H_{20}N_2S_3$; mol.wt 264.49) was a gift from Mr. D. E. Riley of ICI Pharmaceuticals (Macclesfield, U.K.). This reagent sulfiram was determined to be greater than 99.5% pure by HPLC and contained 0.2% (w/w) disulfiram. Tetmosol solution [pharmaceutical grade containing 25% (w/w) sulfiram in industrial methylated spirit equivalent to 70% (v/v) of absolute ethanol], was a gift from Dr. Kevin O'Hare (Imperial College of Science, Technology and Medicine, London, U.K.). Disulfiram (tetraethylthiuram disulfide; [97-77-8]; $C_{10}H_{20}N_2S_4$; mol.wt 296.54) from Sigma (St. Louis, MO) was recrystallized twice in ethanol before use (m.p. 71–72°). Aldehyde dehydrogenase (aldehyde: NAD(P) oxidoreductase, EC 1.2.1.5; sp. act. 51 U/mg enzyme protein) isolated from mitochondria of *Saccharomyces cervisiae*, NAD (grade 1 free acid, 100%), Tris–HCl, and Tris base were from Boehringer Mannheim (Mannheim, Germany). Acetaldehyde and KCl were obtained from Aldrich (St. Louis, MO). Acetonitrile (MeCN, Burdick & Jackson UV grade) and ethanol (redistilled before use) were obtained from Baxter (McGaw Park, IL).

HPLC analysis of photolysis products. Photolysis products were analyzed using a Pharmacia-LKB HPLC system (Uppsala, Sweden) consisting of a model 2157 autoinjector, two model 2150 pumps with a dynamic mixer, a 2152 LC controller, and a model 2140 photodiode array detector. Samples (5 or 10 μL in ethanol or MeCN) were injected directly into the HPLC, and analytes were separated on a Microsorb 4.6 × 100 mm C18 column (Cat. No. 80-200, pore size 100 Å, particle size 3 μm; Rainin, Worburn, MA). The mobile phase for the isocratic chromatography was H_2O/MeCN (40/60) at 0.5 mL/min. Absorbance at 225 nm was monitored continuously with a Hitachi model D-2500 integrator. UV spectra (190 to 370 nm) of the analytes were obtained and analyzed using the Pharmacia-LKB Wavescan program. Sulfiram and disulfiram eluted at 6.8 and 10.4 min, respectively, in the isocratic system.

Photolysis of sulfiram. Solutions of sulfiram in ethanol or MeCN were prepared fresh immediately before use by dissolving reagent sulfiram or diluting the Tetmosol solution in subdued laboratory light or under a red light in a darkroom. Dark control solutions of sulfiram in glass vials with teflon-lined screw caps were wrapped in foil and stored at room temperature in the dark between uses. If these solutions were protected from light, no additional degradation of sulfiram could be detected by HPLC 2 weeks after their preparation.

Solutions of sulfiram (0.44, 0.87, 1.74, 4.36, 8.72 and 17.4 mM), prepared from Tetmosol solution in 4-mL borosilicate glass vials with teflon-lined screw caps, were exposed to ordinary room light (fluorescent ceiling fixtures with 40 W cool white bulbs; Philips Lighting, Somerset, NJ). The intensity of light on the surface of the lab bench was approximately 1.7 $\mu W/cm^2$. The vials were in an upright position during exposure. In additional experiments, solutions containing sulfiram were irradiated at 350 nm at −25° in a Rayonet photochemical reactor (model RMR-600, Southern New England Ultraviolet, Branford, CT). Ninety percent of the energy output was between 320 and 380 nm. The light intensity at the center of the photochemical reactor was 9200 $\mu W/cm^2$.

Assay of ALDH activity. The activity of yeast ALDH was assayed with a microplate reader (Molecular Devices, Menlo Park, CA) at 22° by following the formation of NADH spectrophotometrically at 340 nm [18, 19]. All reagents were prepared in 0.1 M Tris/0.2 M KCl buffer, pH 8.0. The assay mixture, in a final volume of 225 μL, contained the following: Tris–KCl buffer (pH 8.0); 1.5 mM NAD; 0.03 U of yeast ALDH; 2.4 mM acetaldehyde; and disulfiram, sulfiram, or sulfiram photolysis products at varying concentrations in 1.75 μL of ethanol [0.88% (v/v) final concentration of ethanol]. An equal volume of ethanol was added to control assays without inhibitor. The order of addition of reagents was: NAD, Tris–KCl buffer, inhibitor, and yeast ALDH. All manipulations of solutions and incubation mixtures containing sulfiram were done in very subdued light. The solutions were mixed by gently tapping the microtiter plate and then equilibrated for 30 min at 22–24° in the dark. Following equilibration, the substrate acetaldehyde (2.4 mM final concentration) was added to start the reaction. The solutions were again mixed, and the initial rates obtained by following the absorbance at 340 nm for 3 min at 22°. Assays were performed in triplicate at each concentration of inhibitor.

μLC-MS. HPLC separations were performed on an Ultrafast Microprotein Analyzer (UMA) system (Michrome Bioresources, Inc., Pleasanton, CA) utilizing a Michrome UMA microbore column (1 × 150 mm) with Reliasil C-18 (100 Å, 3 μm) packing material. Separations were achieved using a mobile phase of H_2O and MeCN (80/20) with a 15-min linear gradient to 90% MeCN at a flow rate of 50 μL/min. A post-column split of 1:25 allowed ~2 μL/min of effluent to enter into the mass spectrometer system. The samples (1 or 2 μL) were introduced by direct injection and monitored at a wavelength of 225 nm.

Mass spectrometry. All mass spectrometry experiments were performed on a Finnigan MAT95Q mass spectrometer (Bremen, Germany) of BEQ_1Q_2 configuration, where B is the magnet, E is the electrostatic analyzer, Q_1 is an rf-only octapole collision cell, and Q_2 is a mass filter quadrupole. All samples were ionized by CF-LSIMS in positive mode using cesium as a source of ions at an ion voltage of 20 kV (Cs^+ ions). The CF-LSIMS probe consisted

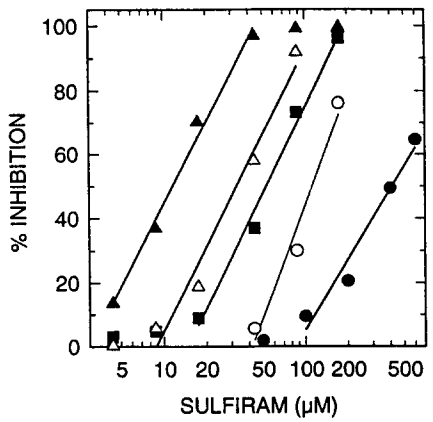


Fig. 1. Effect of light on sulfiram concentration–response curves for inhibition of aldehyde dehydrogenase (ALDH). Tetmosol solution [pharmaceutical grade containing 25% (w/w) sulfiram] was diluted in ethanol and exposed to room light for 11 hr. At timed intervals, a portion of each Tetmosol solution [Tetmosol 0 hr (○); 1 hr (■); 2 hr (△); and 11 hr (▲)] was added to the incubation mixture containing ALDH and NAD in 0.1 M Tris–0.2 M KCl (pH 8.0). Triplicate aliquots of each incubation mixure were transferred to a microtiter plate and incubated in the dark for 30 min at 22–24°. Immediately prior to assay, acetaldehyde (2.4 mM) was added to start the reaction, and the increase in absorbance at 340 nm was monitored for 3 min at 22° in a microplate reader. For comparison, solutions were prepared freshly from reagent sulfiram and assayed in the same way for inhibition of ALDH without exposure to light [sulfiram 0 hr (●)]. Each point is the average of three determinations. ALDH activity in control incubations was 28.7 ± 1.2 nmol NADH/min (N = 15). The lines were obtained by linear regression analysis of the percent inhibition of ALDH activity versus the log concentration of sulfiram at 3 or 4 data points on the linear portion of the curve.

Table 1. Inhibition of aldehyde dehydrogenase by sulfiram exposed to light

Time (hr)	Apparent IC_{50} (μM)		
	Tetmosol*		Sulfiram†
	Dark	Light	Dark
0	106	112	413
1	101	53	‡
2		32	
11	106	12	

Tetmosol solutions were diluted in subdued light with ethyl alcohol as described in Materials and Methods. Approximately equal portions of each solution were transferred into borosilicate glass vials; one set of solutions was placed in the dark, while the other was exposed to room light. At timed intervals, portions of each solution (light-exposed and the corresponding dark control) were assayed for inhibition of ALDH as described in Fig. 1. ALDH activity in control incubations was 28.7 ± 1.2 nmol NADH/min (N = 15). The IC_{50} values were calculated from the plot of the percent inhibition of ALDH activity versus log sulfiram concentration along the linear portion of each curve.

* Solutions were prepared by diluting pharmaceutical grade Tetmosol solution containing 25% (w/w) sulfiram.

† Solutions were freshly prepared from reagent sulfiram containing 0.2% (w/w) disulfiram as an impurity.

‡ Time points without data were not determined.

of a silica capillary connected directly to the HPLC surrounded by a tube with a larger i.d. in which an LSIMS matrix of 2% glycerol in 2-propanol was introduced post-column using a Harvard syringe pump at a rate of 1–2 μL/min. The ion source was maintained at 60° with an operating resolution of ~1200.

Direct infusion-mass spectrometry. Direct infusion experiments utilized a Harvard Apparatus 22 syringe pump (South Natick, MA), which delivered 1–2 μL/min for introduction of sample mixtures and standards directly into the mass spectrometer to obtain MS and MS/MS results.

Tandem mass spectrometry. Molecular ions (also referred to as precursor ions) of standards and photolysis products of sulfiram were selected with a resolution of ~1000 using BE (MS_1) and subjected to CID in the octapole collision cell (Q_1), using argon as the collision gas. Collision energies of ~20–30 eV with an argon gas pressure of ~6×10^{-6} mbar predominantly affording single precursor ion collisions were utilized to fragment the precursor ions. The product (fragment) ions were mass analyzed in Q_2 (MS_2) and product ion spectra acquired by scanning Q_2 over the mass range of 40 to 350 with 10–20 scans acquired and averaged to afford a composite product ion spectrum.

RESULTS

ALDH inhibition by sulfiram exposed to light. Figure 1 shows the inhibition of ALDH by sulfiram solutions after exposure to light for up to 11 hr. The apparent IC_{50} of sulfiram (Tetmosol diluted in ethanol) decreased from an initial value of 112 μM sulfiram in freshly prepared solutions protected from light to 12 μM sulfiram in solutions exposed to light for 11 hr (Table 1). In contrast, the apparent IC_{50} of sulfiram remained at 106 μM in solutions kept in the dark for 11 hr. Solutions freshly prepared from reagent sulfiram and kept in the dark were much less potent (IC_{50} 413 μM) than fresh solutions of Tetmosol. This difference in potency can be attributed to the lower initial concentration of disulfiram in the solutions prepared from reagent sulfiram, which contained 0.2% disulfiram (w/w). In contrast, pharmaceutical grade Tetmosol contained 0.8% (w/w) disulfiram with respect to its sulfiram content. Results of our previous study indicated that the increase in potency of dilute solutions of Tetmosol was independent of pH over the range of pH 4.5 to 8.5 [15].

HPLC analysis of sulfiram photolysis products. In dilute solutions of Tetmosol exposed to room light for 11 hr, the concentration of sulfiram decreased by 24%, and several new compounds appeared (Fig. 2). The material eluting at 10.40 min from the HPLC in the isocratic system had a retention time and UV

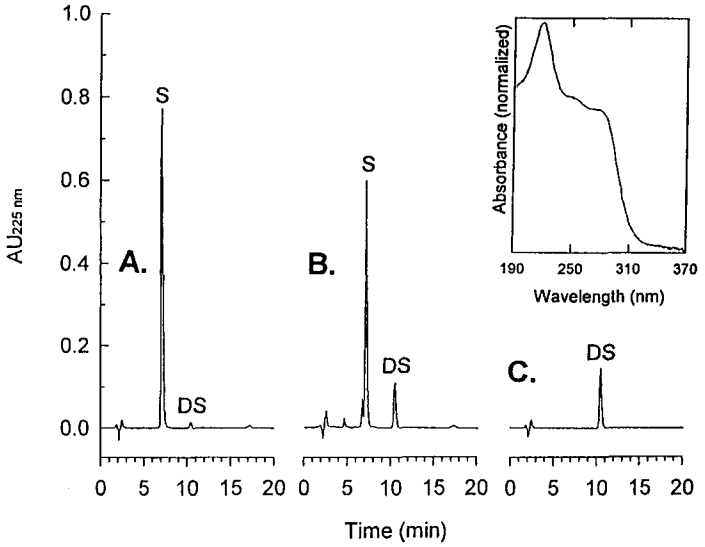


Fig. 2. HPLC chromatograms of light-exposed solutions of sulfiram. Tetmosol solution [25% (w/w) sulfiram, pharmaceutical grade] was diluted to a final concentration of 4.36 mM sulfiram in subdued light, and 5 μL was injected directly into the HPLC immediately after preparation (A) and after exposure to room light for 11 hr (B). Five microliters of a standard solution of 0.5 mM disulfiram in ethanol was analyzed under the same conditions (C). The inset shows the superimposition of the UV spectra of the peaks eluting at 10.40 min in the chromatograms of the light-exposed Tetmosol and the standard disulfiram. Abbreviations: S, sulfiram; DS disulfiram.

spectrum that were identical to those of authentic disulfiram. The photochemical product of sulfiram eluting at 10.40 min was later conclusively identified as disulfiram by μLC-MS and μLC-MS/MS (see MS results).

The decline in sulfiram concentration in dilute solutions of Tetmosol exposed to room light was accompanied by a concomitant increase in disulfiram concentration (Fig. 3). The HPLC patterns of photolysis products of sulfiram solutions made from reagent sulfiram or from pharmaceutical grade Tetmosol were essentially the same. In Tetmosol solutions diluted in ethanol and exposed to room light, the yield for disulfiram typically was about 36% of the loss of sulfiram. Irradiation of 5 mM reagent sulfiram in ethanol at 350 nm for 5 min in the RMR-600 photochemical reactor resulted in a 74% loss of sulfiram with a 31% yield of disulfiram. Irradiation of sulfiram with room light or in the photochemical reactor at 350 nm produced similar patterns of photolysis products.

Contribution of disulfiram to ALDH inhibition by photolyzed sulfiram. The potency of diluted Tetmosol solutions in the inhibition of ALDH was greater than expected based on their concentration of disulfiram. The mean $IC_{50} \pm SD$ (N = 4) of Tetmosol solutions was 1.03 ± 0.09 μM with respect to their concentration of disulfiram compared with 1.68 ± 0.07 μM for standard disulfiram (Fig. 4; significantly different by Student's *t*-test, $P < 0.0001$). The intrinsic activity of sulfiram is too low to account for this difference [$IC_{50} > 600$ μM when corrected for inhibition due to 0.2% (w/w) contamination with disulfiram]. The possibility that other photolysis

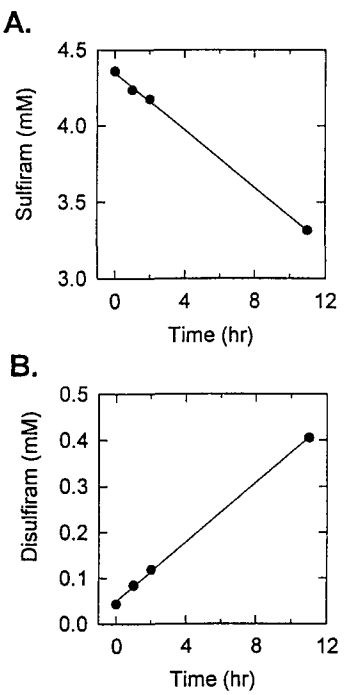


Fig. 3. Time course of sulfiram photolysis in solutions of sulfiram. A freshly prepared dilute solution of Tetmosol (4.36 mM sulfiram) was analyzed by HPLC after exposure to light for 0, 1, 2, and 11 hr. The concentrations of sulfiram (A) and disulfiram (B) in the Tetmosol solution were determined by comparison of peak areas with those of standard solutions.

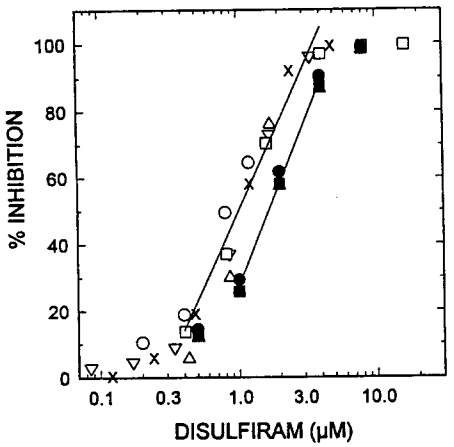


Fig. 4. Inhibition of ALDH by light-exposed solutions of sulfiram as a function of disulfiram concentration. Freshly prepared dilutions of Tetmosol solution were exposed to room light as described in Materials and Methods and Figs. 1 and 2. At timed intervals, portions of each Tetmosol solution [0 hr (△); 1 hr (▽); 2 hr (×); 11 hr (□)] were assayed for inhibition of ALDH and analyzed by HPLC for disulfiram concentration. Solutions of sulfiram, freshly prepared from reagent sulfiram and protected from light [sulfiram 0 hr (○)] and standard solutions of disulfiram immediately after preparation [DS 0 hr (▲)] and after exposure to room light for 2 and 69 hr [DS 2 hr (●); and DS 69 hr (■); respectively] were also assayed. ALDH activity in control incubations was 28.2 ± 1.6 nmol NADH/min (N = 21). Percent inhibition of ALDH was plotted against the log concentration of disulfiram in each solution. Each point is the average of three determinations. The lines were obtained separately for the solutions containing sulfiram and standard disulfiram by linear regression analysis of the data on the linear portion of the inhibition curves.

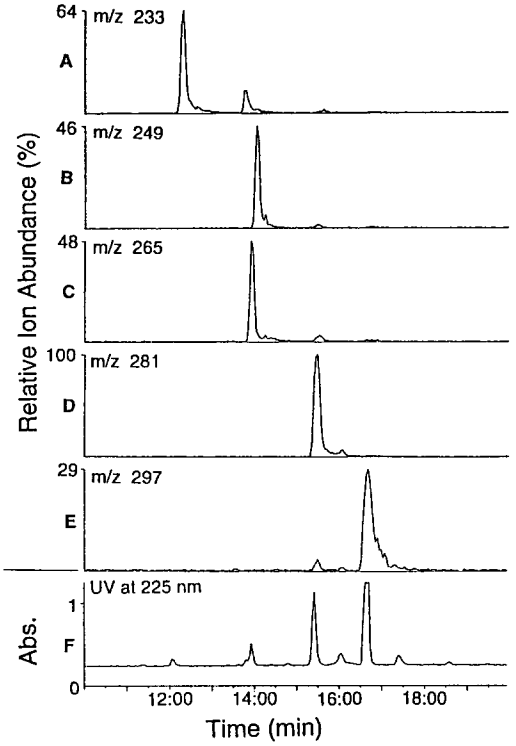


Fig. 5. μLC-MS ion chromatograms of the photolysis products of sulfiram. A 5 mM solution of sulfiram in a quartz 5 mm o.d. NMR tube was irradiated at 350 nm in a Rayonet RMR-600 photochemical reactor maintained at −25°. After irradiation for 18 min, 1 μL of the photolyzed solution was injected into the microbore HPLC system and analyzed on a Finnigan MAT95Q mass spectrometer as described in Materials and Methods. (A) MH^+ = 233; (B) MH^+ = 249; (C) MH^+ = 265 corresponding to unreacted sulfiram (15.4 min) and unknown products; (D) MH^+ = 281 corresponding to **10**; (E) MH^+ = 297 corresponding to disulfiram (**2**); and (F) UV absorbance at 225 nm of the HPLC effluent prior to introduction into the mass spectrometer.

products may be inhibiting ALDH activity prompted an effort to identify these compounds by μLC-MS.

μLC-MS analysis of the photolysis compounds derived from sulfiram. On line μLC-MS analysis using CF-LSIMS as the primary ionization method revealed molecular ions at *m/z* 265 (HPLC retention time 15.4 min) corresponding to unreacted sulfiram (**1**) and at *m/z* 297 (r.t. = 16.6 min), which was tentatively identified as disulfiram (**2**) by comparison of its retention time and molecular weight with standard disulfiram. A major product was observed at *m/z* 281 (r.t. = 15.3 min), as well as four other products at *m/z* 265 (r.t. = 13.8 min), 249 (r.t. = 13.9 min), 233 (r.t. = 12.3 min), and 233 (r.t. = 13.7 min). These results are shown in Fig. 5, which contains μLC-MS ion chromatograms and the UV absorbance at 225 nm derived from the HPLC analysis of the photolysis mixture.

Product ion spectra of standards of sulfiram and disulfiram by direct infusion MS/MS. The molecular ion of sulfiram (MH^+ = 265) was subjected to CID conditions as described in Materials and Methods and product ions (with appropriate relative abundance values) at *m/z* 148 (10), 116 (100), 88 (10), 72 (<5) and 44 (<5) were readily detectable. The molecular ion of disulfiram (MH^+ = 297) was also subjected to CID and product ions were detected at the following masses: *m/z* 224 (<5), 180 (<5), 148 (25), 116 (100), 88 (10), 72 (<5), and 44 (<5).

μLC-MS/MS product ion spectra of photolysis products derived from sulfiram. By subjecting individual molecular ions detected in the μLC-MS analysis of the photolysis mixture to CID with the inert gas argon in the octapole collision cell (Q_1), product ion spectra were acquired on all compounds detected. The product ion spectrum of *m/z* = 265 (r.t. = 15.4 min), corresponding to unreacted sulfiram, was very similar to the product ion spectrum obtained on authentic standard, as can be seen in Fig. 6A. Product ions at *m/z* 88 and 116 formed by homolytic cleavage of the —C(S)—S— bond are indicative of the presence of the thiocarbonyl group (>C=S).

The product ion spectrum of the molecular ion at *m/z* 297, tentatively identified as disulfiram (r.t. = 16.6 min), revealed ions at *m/z* 224, 180, 148, 116, 88, 72, 60, and 44, which are identical to those ions

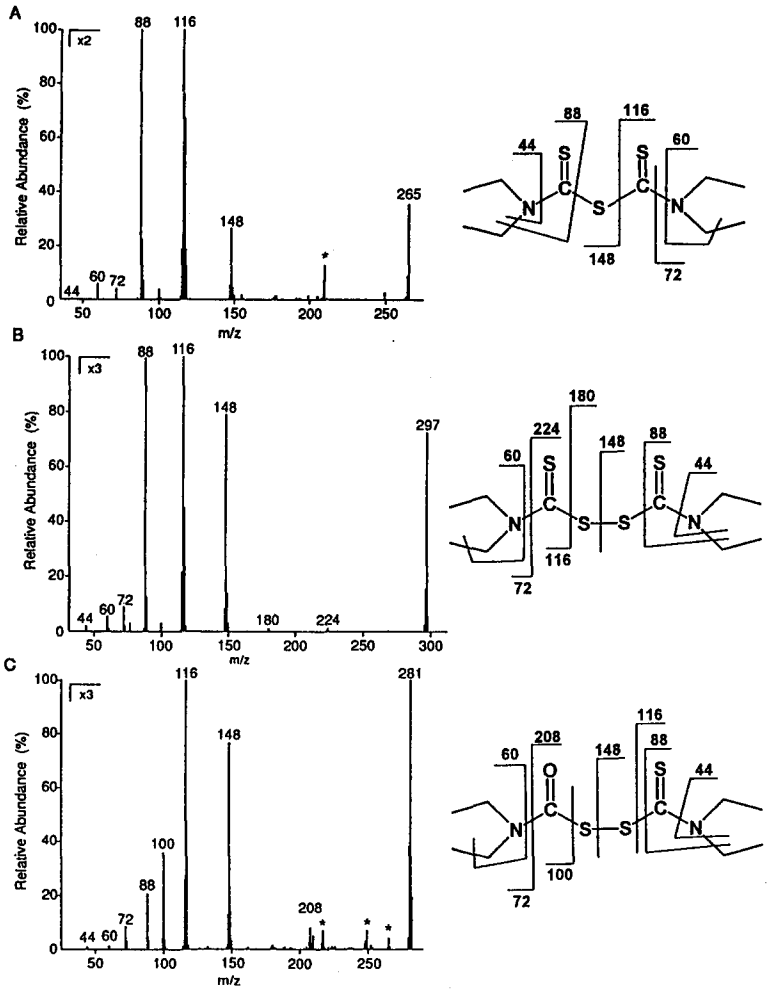
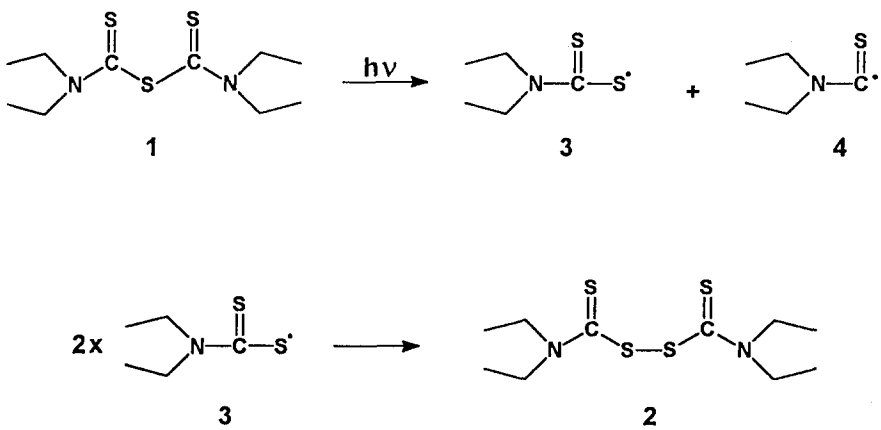
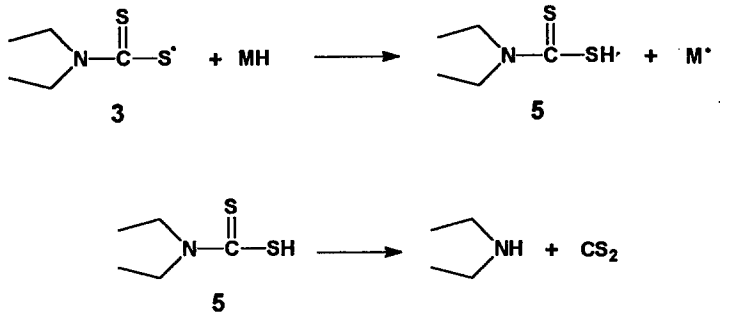


Fig. 6. Tandem mass spectra of components of the sulfiram photolysis mixture. The product ion spectra were acquired either by on-line μLC-MS/MS or direct infusion MS/MS. The product (or fragment) ions produced are assigned to: (A) unreacted sulfiram ($MH^+ = 265$); (B) disulfiram ($MH^+ = 297$); and (C) product **10** ($MH^+ = 281$). * Product ions observed in control MS/MS experiments for the same parent ion.



Scheme 1. Proposed pathway for photochemical formation of disulfiram.



Scheme 2. Proposed pathway for formation of diethylamine and carbon disulfide.

obtained on an authentic standard of synthetic disulfiram. The product ion spectrum of the disulfiram produced in the photolysis reaction of sulfiram is shown in Fig. 6B, along with the appropriate product ion assignments derived from fragmentation of the disulfiram molecular ion ($MH^+ = 297$).

The product ion spectrum of a major photolysis product of sulfiram (r.t. 15.3 min at m/z 281) revealed ions at m/z 208, 148, 116, 100, 88, 72, 60, and 44, as shown in Fig. 6C. Several product ions observed are similar to those observed in the disulfiram spectrum. In particular, the ion at $m/z = 116$ corresponding to $[(CH_3CH_2)_2N{=}C{=}S]^+$ clearly indicates the presence of the thiocarbonyl group. However, by analogy an ion at m/z 100 (not present in disulfiram) corresponds to the functional group $[CH_2CH_2)_2N{=}C{=}0]^+$ and is indicative of a carbonyl group in the product. This can only be accommodated by the structure shown in Fig. 6C. The product ion spectra and structural assignments of the other compounds detected in the photolysis mixture will be discussed in detail elsewhere*.

DISCUSSION

Previous studies in our laboratory have shown that, while sulfiram itself is a very weak inhibitor of ALDH, solutions of the compound become more potent over time [15]. Based on those initial studies, we proposed that inhibition of ALDH may explain the adverse reaction seen with sulfiram. Results of the present investigation demonstrated that conversion of sulfiram to a more potent inhibitor of ALDH is catalyzed by ordinary room light (Fig. 1, Table 1). The potency of sulfiram solutions stored in the dark did not change. HPLC analysis of the light-exposed solutions of sulfiram revealed that several products were formed by photolysis of sulfiram (Fig. 2). The major photolysis product of sulfiram (**1**) was identified as disulfiram (**2**) by HPLC retention time, UV spectrum (Fig. 2), and μLC-MS with CF-LSIMS (Figs 5 and 6). Disulfiram, which is structurally similar to sulfiram, is a potent inhibitor of ALDH [6] and thus accounts for most, but probably not all, of the increase in activity of sulfiram solutions exposed to light. When the activities of the sulfiram solutions exposed to light were plotted against their disulfiram concentrations, there was inhibition of ALDH that cannot be explained by disulfiram alone (Fig. 4). The additional activity in the light-exposed Tetmosol solutions may be due to other photochemical products (Fig. 5). Kitson [20] reported that bis(diethylcarbamyl) disulfide, which is structurally similar to the photochemical product **10** (diethylthiocarbamyl diethylcarbamyl disulfide), is a potent inhibitor of ALDH.

In the treatment of scabies mites (*Sarcoptes scabiei*), one part Tetmosol solution [25% (w/w) sulfiram] is diluted with 2–3 parts water. The resulting fine suspension is applied over the entire body except for the face and scalp, rubbed in well, and allowed to dry naturally (about 10 min) before the patient dresses. This procedure may be repeated daily for several days. Using this treatment regimen, there are ample opportunities for a potentially significant exposure of the patient to disulfiram. Our analysis has shown that undiluted Tetmosol solution (analyzed 14 months prior to the expiration date) stored at room temperature in the original packaging (dark amber bottle protected from light) contained about 0.2% (w/w) disulfiram. Furthermore, exposure of sulfiram to light could occur during and after dilution of Tetmosol, particularly if the dilute solution is stored and reused. During topical application, the sulfiram is distributed over almost the entire body in a thin film providing a large surface area for photolysis. Once formed, disulfiram, a lipophilic drug, is likely to be adsorbed through the skin. Two percent photoconversion of sulfiram to disulfiram, which occurred in about 2 hr in our chemical studies (Fig. 3), would correspond to a topical dose of about 250 mg of disulfiram, an amount slightly greater than the daily oral maintenance dose of 200 mg [21]. Depending on the extent of percutaneous absorption of disulfiram, some individuals, particularly those who are deficient in the active form of ALDH [22], may be at an increased risk to a disulfiram-like reaction from treatment with sulfiram.

The formation of disulfiram from sulfiram may proceed via a free radical mechanism. The photolysis of organic disulfides to form free radicals was first reported by Lyons in 1948 [23]. It was discovered

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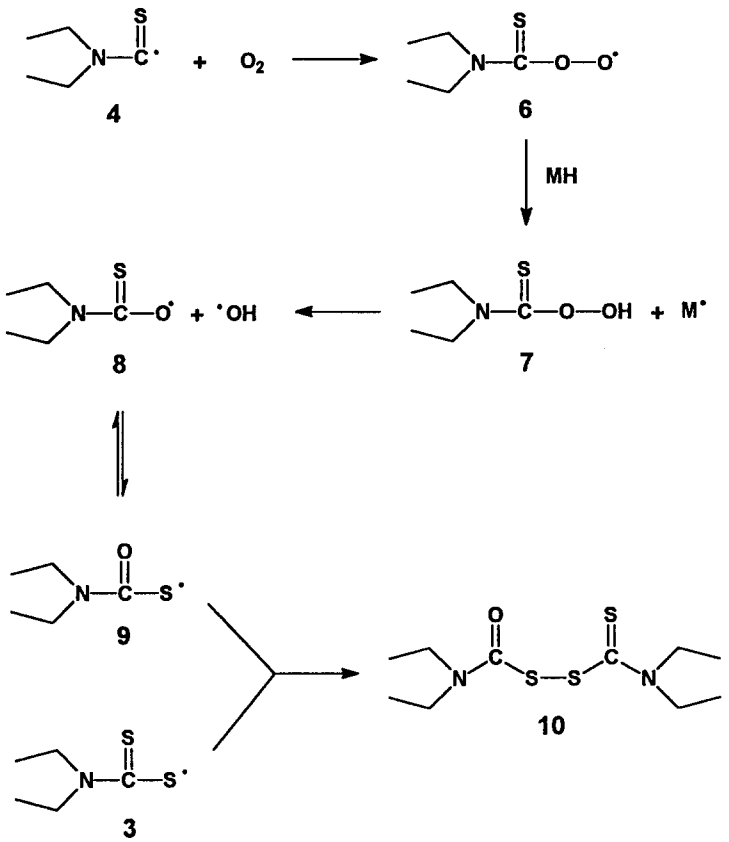

Scheme 3. Proposed pathway for formation of product **10**.

soon thereafter that free radical polymerization could be achieved by thermal activation or photoactivation of the related compounds tetramethylthiuram monosulfide and tetramethylthiuram disulfide [24, 25]. Tetramethylthiuram monosulfide was unusual in that a large amount of homolytic cleavage of the C–S bond was observed with visible light [24]. This phenomenon can be attributed to a strong absorption maximum for tetramethylthiuram monosulfide at 277 nm, which extends well into the visible region and gives the compound its characteristic yellow color. The disulfide, in contrast, is essentially transparent in the visible region of the spectrum and stable in visible light [26].

Analogous to the previously suggested photolytic pathways for tetramethylthiuram monosulfide, we propose that the first step in the photochemical reaction of sulfiram (**1**) is homolytic cleavage of the C–S bond to yield diethyldithiocarbamoyl (**3**) and diethylthiocarbamoyl (**4**) free radicals (Scheme 1). Subsequent combination of two diethyldithiocarbamoyl free radicals (**3**) would yield disulfiram (**2**).

Combination of two of the free radicals (**3**) has been proposed previously to explain the formation of disulfiram by the action of lipid peroxides on diethyldithiocarbamate anion, which was oxidized to **3** by lipid peroxy radicals [27]. Diethyldithiocarbamoyl free radical (**3**) can also abstract a hydrogen atom from a suitable donor (**MH**) to form diethyldithiocarbamic acid (**5**), which can decompose to the weak ALDH inhibitors, diethylamine and carbon disulfide (Scheme 2).

Alternatively, CS_2 may be eliminated directly from **3** to form diethylamine free radical. CS_2 was identified as a product in the photolysis mixture by comparison of its HPLC retention time (8.3 min) and UV spectrum (absorbance maximum at 204 nm) with that of authentic CS_2. Proposed pathways for the formation of additional radicals are shown in Scheme 3.

The carbon-centered free radical (**4**) has been observed with photolysis of metal complexes of dialkyl dithiocarbamates and, as expected, is reactive with oxygen readily forming the corresponding peroxy free radical (**6**) [28]. Abstraction of hydrogen by **6** results in the hydroperoxy radical (**7**), which can undergo homolytic scission of the peroxide bond. The free radical (**8**) and its resonance equivalent (**9**) provide the precursors for additional products. For example, simple radical combination of **3** and **9** could produce **10**, the most abundant product after disulfiram. Analogous reactions could account for other products observed by mass spectrometry. It is also possible that products such as **10** could undergo additional reactions, similar to those of sulfiram, either by direct photoactivation or indirect activation via a photosensitizer as has been described for

dithiocarbamic anhydrides and related compounds [29–31].

In summary, these studies have shown that sulfiram is photoconverted to disulfiram, a potent inhibitor of ALDH. This photochemical reaction may explain the adverse, disulfiram-like reaction observed in patients ingesting ethanol after topical treatment with sulfiram. Additional photochemical products may also contribute to the toxicity of sulfiram. The reaction mechanism likely involves formation of free radical intermediates.

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