

Reaction of Disulfides with Mercuric Ions*

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ABSTRACT: Mercuric ions have been found to react with disulfide compounds. Both the nitrate and chloride salts reacted with each of the cyclic and linear disulfides studied. Solid reaction products were isolated and characterized when the coenzyme, α -lipoic acid, and di-*n*-butyl disulfide were used as substrates. The isolation of a compound of apparent formula $n\text{-C}_4\text{H}_9\text{SHgNO}_3$

from $n\text{-C}_4\text{H}_9\text{SSC}_4\text{H}_9$ and $\text{Hg}(\text{NO}_3)_2$ shows clearly that chemical reaction, probably involving cleavage of the disulfide link, has taken place. The solubility of the compound formed by the reaction of $\text{Hg}(\text{NO}_3)_2$ with α -lipoic acid was found to be dependent on the solvent and the pH of the solution as well as the nature of the anion present.

In the past, it has been generally assumed that mercurials do not react with disulfides. Based on this premise, mercuric compounds have been used in biochemistry to determine SH content, to determine if enzyme function or metabolic reactions depend on SH groups, to estimate the number and reactivity of SH groups in proteins, and to localize SH groups in tissues or cells (Webb, 1966). It was found in our work on the reactions of α -lipoic acid with metal ions (Brown, 1968) that α -lipoic acid (1,2-dithiolane-3-valeric acid) reacted readily with mercuric ions in acid solution, wherein a white solid containing mercury and a lipoic acid residue was formed. In the past, it was thought that reduction of the disulfide bond was a prerequisite to reaction of mercuric ion with disulfides, since it is well known that mercuric ions react with sulfhydryl groups to form products of the types RSHgX or RSHgSR . From our observations on the reaction of α -lipoic acid with mercuric nitrate (Brown, 1968), however, it appeared that covalent change in the disulfide compound might have occurred in the formation of the solid complex. Therefore, the present work was undertaken in order to find out whether mercuric ions react with other disulfides, what effect the nature of the anion has on the reaction, what effect other conditions (pH, solvent, etc.) have on the reaction, and whether there was any evidence for cleavage of the disulfide link.

Experimental Section

In all the reactions, the substrate was dissolved in the minimum amount of 95% ethanol (EtOH). The mercuric salt was added to water and acid was added dropwise until all the salt was dissolved. A 1:1 ratio of ligand to

TABLE I

	Salt	Acid
1	$\text{Hg}(\text{NO}_3)_2$	HNO_3
2	HgCl_2	HCl
3	$\text{Hg}(\text{NO}_3)_2$	HCl
4	HgCl_2	HNO_3

Hg^{2+} was used and, in each case, the solution of disulfide was added to the solution of mercuric salt. The infrared spectra were determined as KBr pellets on a Perkin-Elmer 337 grating infrared spectrophotometer. The elemental analyses were done by the Schwartzkopf Microanalytical Laboratory. The α -lipoic acid and the di-*n*-butyl disulfide were obtained from the Aldrich Chemical Co., the mercuric nitrate ($\text{Hg}(\text{NO}_3)_2$) from Mallinckrodt, and the mercuric chloride (HgCl_2) from Fisher Scientific Co. All solvents and reagents were reagent grade.

1. *Reaction of α -Lipoic Acid with Mercuric Salts.* α -Lipoic acid (0.10 g) was dissolved in 2 ml of 95% EtOH. The mercuric salt (0.16 g) was dissolved in 12 ml of H_2O plus the necessary acid. The reaction was carried out four times, varying the anion and acid (Table I). The solid product isolated from 1 was washed with H_2O , EtOH, and Et_2O . The product was dried under vacuum at ambient temperatures.

Anal. for $\text{Hg-}\alpha$ -lipoic acid product prepared from $\text{Hg}(\text{NO}_3)_2$ in dilute HNO_3 . Found (sample I-51): C, 21.49; H, 3.22; Hg, 45.62. Found (sample J-17): C, 19.86; H, 3.73; Hg, 43.77.

Although the analytical data do not give indication of the exact nature, the percentages for mercury and carbon show that the product has one mercury for each lipoic acid residue.

2. *Reaction of Di-*n*-butyl Disulfide with Mercuric Salts.* The same procedure was followed using 0.16 g of di-*n*-butyl disulfide and 0.18 g of mercuric salt.

Anal. for $\text{Hg-di-}n$ -butyl disulfide product prepared from $\text{Hg}(\text{NO}_3)_2$ in dilute HNO_3 . Calcd for $\text{C}_4\text{H}_9\text{SHgNO}_3$:

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TABLE II: Effect of Anion and Acid on Reaction of α -Lipoic Acid (a) and Di-*n*-butyl Disulfide (b) with Mercuric Ions.

	Anion	Acid	Reaction
a.	NO_3^-	HNO_3	Heavy, white precipitate
	NO_3^-	HCl	White suspension $\xrightarrow{\text{Standing}}$ white precipitate
	Cl^-	HNO_3	White suspension $\xrightarrow{\text{Standing}}$ white precipitate
	Cl^-	HCl	White precipitate
b.	NO_3^-	HNO_3	White suspension $\xrightarrow{\text{Standing}}$ white precipitate
	NO_3^-	HCl	Gray suspension $\xrightarrow{\text{Standing}}$ gray precipitate
	Cl^-	HNO_3	Gray suspension $\xrightarrow{\text{Standing}}$ gray precipitate
	Cl^-	HCl	Gray suspension $\xrightarrow{\text{Standing}}$ gray precipitate

C, 13.65; H, 2.57; Hg, 57.02. Found: C, 14.08; H, 2.66; Hg, 56.83.

3. *Reaction of $\text{Hg}(\text{NO}_3)_2$ with Diphenyl Disulfide and Bis(*p*-nitrophenyl) Disulfide in Dilute Nitric Acid.* The procedure for the reaction of diphenyl disulfide ($\text{C}_6\text{H}_5\text{S}$)₂ with $\text{Hg}(\text{NO}_3)_2$ was the same as that followed with α -lipoic acid. The bis(*p*-nitrophenyl) disulfide, however, did not dissolve in EtOH. Therefore, it was dissolved in a minimum amount of $\text{CH}_3\text{C}(=\text{O})\text{CH}_3$ and the EtOH was added to the dissolved disulfide. This solution was added to the $\text{Hg}(\text{NO}_3)_2$ in dilute HNO_3 . Solid products were obtained but not further identified.

4. *Reaction of α -Lipoic Acid with $\text{Hg}(\text{NO}_3)_2$ in Varying Amounts of EtOH.* The procedure for the reaction of α -lipoic acid with HgCl_2 was the same as described in 1 of Table I; however, no acid was added to the HgCl_2 . The solvents in which the HgCl_2 was dissolved were: H_2O , 50% EtOH, 95% EtOH, and 100% EtOH.

5. *Effect of pH on Formation of Solid Product in Reaction of α -Lipoic Acid with HgCl_2 .* Three HgCl_2 solutions were prepared. No acid was added to any of them. After the α -lipoic acid was mixed with the HgCl_2 , one was made acid with HCl to pH 2.0, one was made basic with dilute NaOH to pH 7.5, and neither base nor acid was added to the third (pH 5.0–6.0).

6. *Preparation of Reference Compounds.* The same procedure was followed as described in 1 of Table I using substrates *n*-butylmercaptan, dihydrolipoic acid, and octanoic acid. The Hg–dihydrolipoic acid and Hg–*n*-butylmercaptan were sent out for elemental analysis.

Anal. for Hg–dihydrolipoic acid. Calcd for $(\text{C}_8\text{H}_{14}\text{S}_2)_2\text{Hg}$: C, 23.61; H, 3.44; Hg, 49.30. Found: C, 24.16; H, 3.71; Hg, 49.05.

Anal. for Hg–*n*-butylmercaptan. Calcd for $(\text{C}_4\text{H}_9\text{S})_2\text{Hg}$: C, 25.35; H, 4.80; Hg, 52.93; N, 0.0. Calcd for $\text{C}_4\text{H}_9\text{S}-\text{HgNO}_3$: C, 13.65; H, 2.57; Hg, 57.02; N, 3.96. Found: C, 21.65; H, 4.50; Hg, 53.28; N, 1.14.

The results of the analysis of Hg–*n*-butylmercaptan suggest that the product formed by the reaction of $\text{Hg}(\text{NO}_3)_2$ and $\text{C}_4\text{H}_9\text{SH}$ has a mixture of products of the formulas $\text{C}_4\text{H}_9\text{SHgNO}_3$ and $\text{C}_4\text{H}_9\text{SHgSC}_4\text{H}_9$.

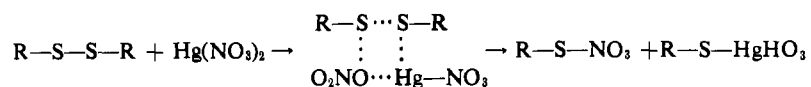
Results

In the reactions of α -lipoic acid and reference compounds, dihydrolipoic acid and *n*-butylmercaptan, with

$\text{Hg}(\text{NO}_3)_2$ in dilute HNO_3 , heavy, white precipitates were formed. A white precipitate was formed with octanoic acid and white suspensions with the linear disulfides, di-*n*-butyl disulfide, $\text{C}_6\text{H}_5\text{SSC}_6\text{H}_5$, and 5,5'-dithiobis(2-benzoic acid). Mercuric compounds of octanoic acid, dihydrolipoic acid, and *n*-butylmercaptan were prepared so that the infrared spectra of these products could be compared with those from the disulfide reactions.

With all the disulfide substrates, reactions of Hg^{2+} occurred readily. With the five-membered cyclic disulfide and the reference compounds, the solid products could be filtered immediately. With the di-*n*-butyl disulfide, a white solid settled out after standing several hours; with the diphenyl disulfide, white crystalline material settled out after standing overnight. Table II shows the behavior when chloride anion is used rather than nitrate ion, and compares the effect of HNO_3 and HCl when the same anion, substrate, and solvent were used. In all these reactions, the pH of the solution was ~ 2 . When 100 or 95% EtOH was used as the solvent for HgCl_2 , no reaction was observed on adding the α -lipoic acid. A slight turbidity was noted when 50% EtOH was the solvent and a white suspension was formed in H_2O . In these reactions, no acid was present. The results demonstrate the effect of solvent on the reaction and possibly explain the difference in the reaction of the linear disulfide, which required 10 ml of 95% EtOH for complete solution, and the α -lipoic acid, which dissolved completely in only 2 ml. The effect of pH was observed by varying with dilute HCl and NaOH the pH of a solution of α -lipoic acid and HgCl_2 . When the pH ~ 2 , a white precipitate was formed; when it was 5–6, a white suspension. At a pH greater than 7, the precipitate dissolved and the solution turned yellow.

In the product formed by the reaction of octanoic acid with $\text{Hg}(\text{NO}_3)_2$, there was no band at 1705 cm^{-1} (protonated carboxyl) in the infrared spectrum; there was, however, a strong band at 1580 cm^{-1} (carboxylate ion). This showed that there was no protonated carboxyl in Hg–octanoic acid; all the carboxyl was present as carboxylate ions. In the products formed with mercuric ions and α -lipoic acid and dihydrolipoic acid, the protonated carboxyl band showed up strongly (at 1705 cm^{-1}) and there was no band in the $1600\text{--}1550\text{ cm}^{-1}$ region. This indicates that the carboxyl group is not involved



in the metal complexing in either product. The spectra of the Hg-dihydrolipoic acid and Hg- α -lipoic acid, however, were significantly different, both in the region of 900–600 cm^{-1} and in the ratio of absorbances of bands at 1700 cm^{-1} to those in the 1475–1300- cm^{-1} region. This indicates that different mercuric compounds were formed with dihydrolipoic acid and α -lipoic acid. In Hg- α -lipoic acid, the spectrum had a band at 1038 cm^{-1} , suggesting the presence of sulfoxide. There is no evidence for the presence of SH groups in any of the compounds as indicated by the absence of absorption $\sim 2600\text{--}2550\text{ cm}^{-1}$ in the SH stretching region. The weak band at 1525 cm^{-1} in Hg- α -lipoic acid suggests the presence of nitrate. The spectra of Hg-*n*-butylmercaptan and Hg-di-*n*-butyl disulfide were very similar but showed minor differences in the region of 900–600 cm^{-1} .

Discussion

It is seen that mercuric compounds react readily with cyclic and linear aryl and alkyl disulfides. The solubility of the resultant product is dependent upon the solvent, pH of the solution, kind of acid present, and the nature of the anions. In reactions with α -lipoic acid, the ratio of ligand to mercury was 1:1, although the carbon and mercury analyses were both lower in Hg- α -lipoic acid than in Hg-dihydrolipoic acid. This suggests the presence of an anion in Hg- α -lipoic acid. With the linear disulfide, di-*n*-butyl disulfide, cleavage of the disulfide bond appears to have taken place. From the elemental analysis of the product isolated, a compound of

the type RSHgNO_2 is probable; the nature of the other half of the cleaved disulfide, however, is unknown. It is possible that there is a concomitant electrophilic-nucleophilic attack of mercuric nitrate on the disulfide. This type of mechanism has been proposed by Kice (1968) for the reaction of disulfides with other reagents. This reaction, however, could also be stepwise and a kinetic study is necessary to differentiate the two possibilities. There are alternative possibilities that can be considered for the structure of this reaction product. Although these alternatives are less likely, all involve significant covalent change.

Since disulfide bonds are an integral part of enzymes and proteins, it is suggested that the results of biochemical determinations of SH groups by mercurials be examined in the light of the evidence presented here and that allowance be made for the possible reaction of the mercuric ion with disulfide. Although our results have been obtained on rather concentrated solutions which are unlikely in the conditions normally employed in studying proteinaceous material with mercurials, there can be no doubt that chemical attack at a disulfide link by mercury(II) compounds can occur.

References

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