THE ACTION OF MERCURIC SULFATE AND CHLORIDE
ON CYSTEINE, CYSTINE, CYSTEINE SULFINIC ACID
(R–SO₂H), AND CYSTEIC ACID WITH REFERENCE
TO THE DISMUTATION OF CYSTINE

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Preceding studies (1) show that one of the products of the dismu-
tation of cystine disulfoxide (R–(SO)₂–R) is the sulfinic acid
(R–SO₂H), a compound which possesses greater stability in
solution than any other intermediate oxidation product of cystine
obtained thus far. It was interesting, therefore, to determine
whether the sulfinic acid results from the dismutation of cystine in
the presence of heavy metal salts, especially since published data
can be so interpreted.

It becomes increasingly evident that many reactions of cystine

\[ R-S-S-R + H_2O \rightarrow R-SH + R-SOH \]  

(1)

In the presence of heavy metal salts, cysteine is eliminated by
precipitation, thus leaving the sulfenic acid free to dismute

\[ 2R-SOH \rightarrow R-SH + R-SO_2H \]  

(2)

If, as postulated, the dismutation stops with the sulfinic acid—
and as yet there is no evidence of dismutation of the sulfinic acid—
then the dismutation of cystine can be expressed by the following
stoichiometrical equation

\[ 2R-S-S-R + 2H_2O \rightarrow 2R-SH + R-SO_2H \]  

(3)

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tute, Philadelphia.

1 There is some evidence that cysteic acid is produced by the dismutation
of cystine disulfoxide in acid solution (1).
with 75 per cent of the cystine being converted to cysteine and 25 to the sulfinic acid. Since the sulfinic acid is an intermediate oxidation product which can, under suitable conditions, be either reduced to cystine or oxidized to cysteic acid, there are various means which can be employed to determine whether or not the dismutation proceeds according to Reaction 3.

The dismutation was effected in the present studies by mixing sulfuric acid solutions of cystine and mercuric sulfate. The white precipitate which formed was filtered off and resuspended in water, the mercury removed as HgS, and the excess HgS expelled from the solution by CO₂ or N₂. The colorimetric determination of cysteine in the solution, by Shinohara’s modification of the phospho-18-tungstic acid procedure (2), a test which is not affected by the sulfinic acid, showed that 75 per cent of the original cystine had been converted to cysteine.

Direct confirmation of this result by iodometric oxidation of cysteine to cystine in KI-HCl solution (3) is not possible, since the sulfinic acid is here reduced to cystine with the liberation of I₂. Indeed, a solution containing cysteine and sulfinic acid in a molar ratio of 3:1, such as would be produced by Reaction 3, will neither consume nor liberate I₂, even in the presence of excess I₂, when it is made 2 M in KI-HCl, as shown by Reactions 4 and 5.

\[ R-\text{SO}_2\text{H} + 3\text{HI} \rightarrow \frac{3}{2}R-\text{S}-\text{S}-R + 3\text{I}_2 + 2\text{H}_2\text{O} \]  
\[ 3R-\text{SH} + 1\frac{1}{2}\text{I}_2 \rightarrow 1\frac{1}{2}R-\text{S}-\text{S}-R + 3\text{HI} \]

This fact which was confirmed with the above solution of the dismutation products of cystine may be accepted as indicating the presence of an intermediate oxidation product.

The summation of Reactions 4 and 5 results in Reaction 6

\[ 3R-\text{SH} + R-\text{SO}_2\text{H} \rightarrow 2R-\text{S}-\text{S}-R + 2\text{H}_2\text{O} \]

which is seen to be the reverse of the original dismutation Reaction 3, and which is free to proceed after the heavy metal has been removed. Accordingly, owing to the formation of cystine, the optical rotation of such solutions becomes increasingly negative (1) and, as might be expected from Reactions 4 and 5, the rate of Reaction 6 is accelerated by iodide ions.

After oxidation of cysteine to cystine by aeration, the sulfinic
acid present was determined by the amount of I₂ liberated in 2 M KI-HCl solution (since cystine in contrast to cysteine does not consume I₂ under these conditions). The sulfenic acid can apparently withstand aeration and also evaporation on a water bath, since in some cases the theoretical amount of I₂ was liberated. The acidity of the solution also suggests the formation of the sulfenic acid. The attempts to isolate both a barium salt and the free acid resulted in impure products which were not positively identified.

The estimation of total cysteine and sulfenic acid (or after air oxidation, total cystine and sulfenic acid) by means of the I₂ required for oxidation of the two compounds to cysteic acid (4) showed approximately 94 per cent of the theoretical. The optical rotation of cystine solutions obtained by adding KI to the original dismutation mixture (Reactions 4 and 5) and subsequently removing Hg by extraction with ether (as HgI₂·HI) indicated recovery of only 90 per cent of the original cystine; iodometrically, 92 to 98 per cent recovery was indicated.

Cysteine, cysteic acid, and the sulfenic acid were also precipitated individually by mercuric sulfate from 2 N H₂SO₄ solution. The behavior of the above compounds as well as cystine with mercuric chloride was also investigated.

EXPERIMENTAL

Cysteine and Mercuric Sulfate—The addition of a solution containing 8.05 mM of HgSO₄ in 11 cc. of 1.9 N H₂SO₄ to a solution of 4 mM of cysteine (5) in 6 cc. of 1.9 N H₂SO₄ resulted in the formation of a white precipitate which after standing 2 hours was filtered off by suction, washed with water, alcohol, and ether, and dried in vacuo over H₂SO₄ at room temperature; yield 2.276 gm. The completeness with which cysteine is precipitated was shown by the fact that only 0.003 mM remained in the filtrate, which also contained 0.973 milli-atom of Hg++. The precipitate was analyzed as follows: 0.500 gm. was suspended in H₂O and Hg++ removed and weighed as HgS (total of 7.18 milli-atoms of Hg++); after H₂S was expelled from the filtrate by a current of N₂, aliquots were taken and cysteine determined both colorimetrically (4.05 mM of R—SH) and by I₂ oxidation in 1 M KI-HCl (3.96 mM of R—SH). The H₂SO₄ in the remainder of the filtrate was titrated.
with NaOH with methyl red as indicator (3.76 mM of H$_2$SO$_4$) and was subsequently precipitated and weighed as BaSO$_4$ (3.84 mM of BaSO$_4$). The analysis indicated the following molecular proportions $1R$—$S^-$ : $1.8Hg^{++}$ : $0.96SO_4^{2-}$, corresponding to a total weight equal to 101 per cent of that of the precipitate. No evidence of the formation of $Hg^+$ was obtained; i.e., no blackening with KI or NH$_4$OH and no $HgCl$ formation when dissolved in HCl.

The optical rotation, $\alpha^2$$_{Hg}$, of a solution of 0.5 gm. of the precipitate (approximately 0.105 gm. of cysteine) in 10 cc. of m HCl was $+0.79^\circ$ per dm. On standing, this solution deposited dense tufts of crystals (cf. $HgCl_2$ below). After the addition of 0.5 gm. of the original precipitate to 5 cc. of 2 m HCl, followed by the addition of 6.32 mM of KI (4 times the amount of $Hg^{++}$ present) the mixture was diluted to 10 cc. and the mercury removed by extraction with ether (as $HgI_2$·HI). The resulting aqueous solution now possessed a rotation, $\alpha^2$$_{Hg}$, of $+0.088^\circ$ per dm. in agreement with that of cysteine.

Cysteic Acid and Mercuric Sulfate—Cysteic acid was prepared by the oxidation of cystine with Br$_2$; the equivalent weight of this material determined by titration with NaOH, with methyl red as indicator, amounted to 174 gm. instead of the theoretical 169 gm. Mixture of a solution of 4.06 mM of $R$—SO$_3$H (0.708 gm.) in 5 cc. of 1.9 N H$_2$SO$_4$ with a solution of 8 mM of $HgSO_4$ in 12 cc. of 1.9 N H$_2$SO$_4$ caused a heavy white precipitate to form. After standing 1$\frac{1}{2}$ hours, the mixture was filtered by suction, washed once with 2 N H$_2$SO$_4$, 50 per cent alcohol and water, alcohol, and finally ether, and dried in vacuo over H$_2$SO$_4$ at room temperature. A small additional amount (0.122 gm.) precipitated from the filtrate on standing; total weight of the precipitate, 1.312 gm. This precipitate is semicrystalline, appearing as small blades, and dissolves quite easily in chloride solutions, viz. NH$_4$Cl, BaCl$_2$, and HCl. Analysis, similar to that of the cysteine precipitate, showed 3.22 milli-atoms of $Hg^{++}$, 4.11 milli-equivalents of acid, and 0.17 mM of $SO_4^{2-}$ to be present. Deduction of the $SO_4^{2-}$ (which probably represents contamination) from the total acid leaves 3.77 mM of $R$—SO$_3$H as occurring in the precipitate or about 94 per cent the amount used. The molecular ratio of $1Hg^{++}$ : $1.17R$—$SO_3$ : $0.053SO_4^{2-}$ which was obtained indicates 100.2 per cent of the
weight of the precipitate. The rotation, $\alpha_{\text{He}}^{25}$, of 0.19 gm. of the precipitate (containing 0.095 gm. of cysteic acid according to the analytical evidence) dissolved in 10 cc. of $\text{m HCl}$ was $+0.118^\circ$ per dm., in agreement with the figure $+0.116^\circ$ obtained with a solution of 0.1 gm. of cysteic acid in 10 cc. of $\text{m HCl}$.

**Sulfinic Acid and Mercuric Sulfate; Some Observations on the Sulfinic Acid**—The procedure previously described for the preparation of the sulfinic acid by ammoniacal decomposition of cystine disulfoxide (1) apparently gives rise to several hydrates. When the solution containing the sulfinic acid was evaporated to small volume, crystals were deposited in the form of small, crossed, elongated hexagons. These crystals, after drying on a porous plate or between filter papers in a desiccator over CaCl$_2$, yielded the following equivalent weights (cf. (1)): 188.5 by titration with alkali; 180.7 by reduction in 2$\text{m KI-HCl}$; 183.1 by I$_2$ oxidation; m.p. 146°. Although the substance becomes discolored on drying in vacuo at 100°, there was no loss in weight. The above figures suggest the dihydrate, R—SO$_2$H$\cdot$2H$_2$O; molecular weight 189.

In the previously reported preparation (1) crystals of the non-hydrated acid (R—SO$_2$H) were deposited when a hot 50 per cent alcohol solution was cooled. However, this treatment usually produces two liquid layers, of which the lower can be solidified by stirring to loose, slightly yellow crystals. This material after being filtered off, washed with alcohol and ether, and dried in vacuo over H$_2$SO$_4$ at room temperature yielded the following data: molecular weight 171.5 by titration with alkali, 161.3 by KI-HCl reduction, 144.2 by I$_2$ oxidation; m.p. 143° (with decomposition). The specific rotation, $[\alpha]_{\text{He}}^{25}$, of a solution containing 1 gm. in 100 cc. of $\text{n HCl}$ was $+29.3^\circ$; if the substance is assumed to be the monohydrate R—SO$_2$H$\cdot$H$_2$O, the value is $+32.7^\circ$, in fair agreement with the value $+33.4^\circ$ previously obtained for the sulfinic acid R—SO$_2$H(1). Although the analytical data are not very satisfactory, since the acid titration indicates a monohydrate R—SO$_2$H$\cdot$H$_2$O (mol. wt. 171) and the other data R—SO$_2$H (mol. wt. 153), nevertheless the material was used as the best available, especially since reprecipitation by alcohol did not improve the results. Mention should be made of the fact that the sulfinic acid consumes I$_2$ relatively rapidly, which may be a consideration in iodometric determinations, when the sulfinic acid
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might be present. Thus, although the acid cannot be titrated directly with I₂, in the presence of a 4-fold excess of I₂ back titration of the excess with Na₂S₂O₃ yielded the following molecular weights: 152.5 after 20 minutes (i.e. theoretical for R—SO₃H), 146 after 1 hour, and 144.2 after 48 hours. The difference between the last two figures may be attributed to the 0.5 per cent cystine which was present and which is more slowly oxidized to cysteic acid.

The addition of 0.855 gm. of the above sulfinic acid (5 mM, with a molecular weight of 171) dissolved in 8 cc. of 1.9 N H₂SO₄ to a solution of 3.0 gm. of HgSO₄ (10 mM) in 12 cc. of 1.9 N H₂SO₄ caused the immediate formation of a thick, white paste. After standing overnight, the mixture was filtered by suction, washed once with 1.9 N H₂SO₄, with 50 per cent alcohol, with alcohol, and with ether, and dried in vacuo; yield 2.36 gm. The addition of NH₄Cl to the filtrate precipitated a white flocculent precipitate (0.0928 gm.) which, when titrated with I₂ containing KI, consumed only 0.292 milli-equivalent of I₂ instead of the theoretical 0.39 milli-equivalent required by the precipitate if it were HgCl. After the Hg⁺⁺ remaining in the filtrate (3.30 mM) was removed with H₂S, it was necessary to bubble N₂ through the solution for 10 hours before a negative test with lead acetate paper could be obtained. 0.365 mM of sulfinic acid remained in the filtrate, indicating precipitation of about 93 per cent.

The precipitate, which is hygroscopic, contained 6.29 milliatoms of Hg⁺⁺, 4.69 mM of R—SO₃H, 11.93 milli-equivalents of acid titrated by NaOH, and 3.75 mM of sulfate; deduction of the sulfate from the total acid indicates 4.43 mM of R—SO₂H to be present. The analysis indicates the following molecular proportions, 1R—SO₂⁻:1.34 Hg⁺⁺:0.80 SO₄⁻. The precipitate dissolves in solutions of NH₄Cl, HCl, and BaCl₂ (precipitation of BaSO₄ occurs in the latter case). 0.4015 gm. dissolved in 10 cc. of m HCl and filtered from 0.0038 gm. of a white precipitate possessed an optical rotation, α₅₀H₂O, amounting to +0.39° per dm. When +0.29° is used as the rotation per dm. of 0.1 gm., the above figure indicates 0.781 gm. of sulfinic acid to be present in the precipitate or 91.5 per cent of that used.

The extraction of Hg with ether after addition of KI did not yield a solution whose rotation could be taken, because of con-
continued liberation of $I_2$. No $Hg^+$ was indicated upon addition of KI.

Cystine and $HgSO_4$; Determination of Cysteine Colorimetrically and Iodometrically, and Estimation of Cysteine Plus Intermediates

$0.300 \text{ gm. (2.5 milli-equivalents) of cystine dissolved in 5 cc. of 1.9 N H}_2\text{SO}_4$ was added to 1.50 gm. of $HgSO_4$ (5.0 mm) in 8.2 cc. of 1.9 N $H_2SO_4$ contained in a 50 cc. volumetric flask. The mixture containing the white precipitate was diluted to the mark with water, $H_2S$ passed in, $HgS$ removed by filtration with suction (no washing), and the excess $H_2S$ removed from the filtrate by a current of $N_2$. The amount of cysteine present, determined colorimetrically with Shinohara's modification (2) of the phospho-18-tungstic acid test, was 1.950 mm or 78 per cent of the cystine used. However, when 5 cc. of 0.1 N $I_2$ was added to a 10 cc. aliquot containing 5 cc. of 10 N HCl and 10 cc. of 5 N KI (total volume 50 cc.), there was no $I_2$ consumed after 2 hours (compared with a corresponding blank). In view of the cysteine present this can only mean that the amount of $I_2$ necessary for oxidation of cysteine to cystine was liberated by reduction of some intermediate oxidation product to cystine.

The total $I_2$ consumed in oxidation to cysteic acid by an excess of $I_2$ in weakly acid solution (3) amounted to 5.90 mm or 94.5 per cent of the theoretical value for the calculated amounts of $R-SH$ and $R-SO_2H$, i.e. 6.25 mm.

Recovery of Cystine Polarimetrically and Reaction of Cysteine with Sulfinic Acid—2.5 milli-equivalents of cystine were dissolved in 4 cc. of 2.2 N $H_2SO_4$, contained in a 25 cc. volumetric flask, and 5.0 mm of $HgSO_4$ in 8 cc. of 2.2 N $H_2SO_4$ were added. The mixture was shaken for 5 minutes, 4 cc. of 5 M KI (20 mm) were added, the mixture was diluted to 25 cc., and the mercury extracted with two 25 cc. portions of ether. The rotation, $\alpha_{Hg}^{25}$, of the

Because of the time required for reduction of the sulfinic acid, it is necessary to wait 1 or 2 hours in this test before determining the $I_2$ consumed or liberated (1); thus in the above instance 0.236 milli-equivalent of $I_2$ was consumed immediately after mixing, indicating a total of 1.18 mm of cysteine.

Titration of the ether extracts with NaOH to the turning point of methyl red indicated that an amount of acid equivalent to the mercury was extracted from the aqueous solution; viz., 4.6 milli-equivalents from the dismutation solution, 4.9 milli-equivalents from the cysteine blank, and 5.0 milli-equivalents from the cysteine solution ($HgI_2\cdot HI$).
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clear solution immediately after extraction amounted to $-2.24^\circ$ per dm., which changed to $-2.56^\circ$ on standing overnight and remained constant 24 hours later. A blank of 2.5 milli-equivalents of cystine, 5 mM of HgI$_2$, and 10 mM of KI treated in the same way yielded $-2.85^\circ$ per dm. The rotation therefore indicates 90 per cent recovery of cystine (90 and 92 per cent in other experiments).

The I$_2$ consumed by cystine in aliquots of the two solutions on

![Fig. 1. Illustrating the reaction R-SO$_2$H + 3R-SH $\rightarrow$ 2R-S-S-R. Solution 1, 0.15 M R-SH, 0.05 M R-SO$_2$H; Solution 2, 0.15 M R-SNa, 0.05 M R-SO$_2$Na; Solution 3, 0.15 M R-SH, 0.05 M R-SO$_2$H, 1 M HCl; Solution 4, 0.15-M R-SH, 0.05 M R-SO$_2$H, 1 M HCl, 0.01 M KI; Solution 5, filtrate from 0.5 gm. of dismutation precipitate in 10 cc. of M HCl after removal of Hg by H$_2$S. $\alpha_{Hg}$ = $-5.95^\circ$ per dm. for 0.1 M R-S-S-R in M HCl. Cystine precipitated from Solutions 1 and 2 after 48 hours. Oxidation to cysteic acid by an excess of I$_2$ indicated 0.0110 gm. of cystine per cc. of the dismutation solution and 0.0120 gm. of cystine per cc. of the blank or 91.8 and 100 per cent recovery, respectively; in another case 98 per cent recovery was indicated by the I$_2$ oxidation.

Although these figures lack quantitative precision, nevertheless they are in such striking contrast to those obtained from similarly treated cysteine solutions that something other than iodide is clearly responsible for the formation of cystine. Thus 2.5 mM of
cysteine treated as above with 5 mm of HgSO₄ and 20 mm of KI yielded after extraction with ether a solution with an optical rotation, \( \alpha_{Hg}^{24} \), equal to +0.13° per dm., which remained practically unchanged for 48 hours (± 0.12°).

**Table I**

*Precipitation of Cystine by HgSO₄*

40 milli-equivalents of R–S–S–R in 60 cc. of 2.2 N H₂SO₄ and 80 mm of HgSO₄ in 90 cc. of 2.2 N H₂SO₄ were mixed.

In Preparation 1, HgSO₄ was added to R–S–S–R and the mixture allowed to stand for 3 days; in Preparation 2, the mixture was filtered 2 hours after mixing; in Preparation 3, cystine was added to HgSO₄ and the mixture filtered after 2 days.

<table>
<thead>
<tr>
<th>Preparation No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Theoretical</th>
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<td><strong>Filtrate</strong></td>
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<tr>
<td>Hg(^+), milli-atoms</td>
<td>3.75</td>
<td>3.72</td>
<td>3.97</td>
<td></td>
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<td>Hg(^++) “</td>
<td>10.2</td>
<td>10.7</td>
<td>6.9</td>
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<tr>
<td>R–SH, mm.</td>
<td>0.085</td>
<td>0.026</td>
<td>0.024</td>
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<tr>
<td>R–SO₂H “</td>
<td>0.65</td>
<td>0.27</td>
<td>0.53</td>
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<th><strong>Precipitate</strong></th>
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<tr>
<td>Weight, gm.</td>
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<td>21.8</td>
<td>22.7</td>
<td></td>
</tr>
<tr>
<td>Hg(^+), milli-atoms</td>
<td>8.7</td>
<td>11.8*</td>
<td>18.2†</td>
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</tr>
<tr>
<td>Hg(^++) “</td>
<td>55.5</td>
<td>53.4</td>
<td>51.6</td>
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<tr>
<td>Total Hg “</td>
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<td>79.6</td>
<td>80.7</td>
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<td>R–SH, colorimetric, mm.</td>
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<td>29.7</td>
<td>27.4</td>
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<td>“ I₂ + HI “</td>
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<td>5.7</td>
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<td>0</td>
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<td>R–SO₂H, calculated from</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>R–SH + I₂, mm.</td>
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<td>8.2</td>
<td>10.6</td>
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<tr>
<td>R–SO₂H (HI reduction), mm</td>
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<td>9.8</td>
<td>9.5</td>
<td>10.0</td>
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<tr>
<td>Total acid-H₂SO₄, m.-eq</td>
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<td>Sulfate (H₂SO₄) “</td>
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<td>79.5</td>
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<td>( \alpha_{Hg} ) per dm.,°, degrees</td>
<td>+0.65</td>
<td>+0.59</td>
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* 12.5 mm of HgCl by titration with I₂ + KI.
† 18.4 mm of HgCl by titration with I₂ + KI.
‡ 0.5 gm. of the precipitate dissolved in 10 cc. of m HCl.

This difference in behavior is attributed to the previously noted (1) reaction between cysteine and the sulfinic acid (Reaction 6) which proceeds in the solution of the dismutation products of cystine after the mercury has been removed. This reaction is illustrated in Fig. 1 which shows the influence of acidity on the
reaction rate as well as the accelerating action of iodide (Reactions 4 and 5). The change in rotation of solutions from which the mercury was removed by \( \text{H}_2\text{S} \) was so slow that it was not utilized for quantitative purposes; one such solution from the precipitate Preparation 2 of Table I is shown in Fig. 1.

Table I contains additional data on the precipitation of cystine by \( \text{HgSO}_4 \). \( \text{Hg}^+ \) was determined in the filtrate by adding \( \text{NH}_4\text{Cl} \) and weighing the precipitated \( \text{HgCl} \); the \( \text{Hg}^+ \) in the precipitate from the dismutation was determined by dissolving 0.5 gm. in 10 cc. of \( \text{m HCl} \), the precipitated \( \text{HgCl} \) was filtered off, dried, and weighed, and the weight confirmed by titration with \( \text{I}_2 \) containing \( \text{KI} \). The polarimetric data were obtained on this filtrate. \( \text{Hg}^{++} \) was precipitated as \( \text{HgS} \) after removal of \( \text{Hg}^+ \); the total \( \text{Hg} \) re-covered was nearly quantitative. After the excess \( \text{HgS} \) had been expelled from the filtrate with \( \text{N}_2 \), the cysteine was determined colorimetrically as before. Titration of the cysteine in \( \text{m KI-HCl} \) solution with excess \( \text{I}_2 \) yielded very low results; on the assumption that \( 1\text{R-SO}_2\text{H} \) liberates \( \text{I}_2 \) equivalent to \( 3\text{R-SH} \), and using the \( \text{R-SH} \) content obtained colorimetrically, one can calculate the amount of \( \text{R-SO}_2\text{H} \) present, as shown in Table I. Neutralization of the solution to the turning point of methyl red yielded the total acid content (\( \text{R-SO}_2\text{H} + \text{H}_2\text{SO}_4 \)). Aeration at pH 7 to 8 converted \( \text{R-SH} \) to \( \text{R-S-S-R} \), after which the \( \text{R-SO}_2\text{H} \) was determined by reduction with 2 \( \text{m KI-HCl} \). Deduction of sulfate, determined as \( \text{BaSO}_4 \), from the total acid also gives information as to the amount of sulfinic acid that formed. Removal of \( \text{Hg} \) by extraction with ether (as \( \text{HgI}_2\cdot\text{HI} \)) resulted in solutions which possessed rotations similar to those of the preceding preliminary experiments.

Isolation of Sulfinic Acid—This was complicated not only by the difficulty of removing \( \text{Hg} \), sulfate, and cysteine from the precipitate of cystine dismutation products without loss of sulfinic acid but also by the solubility and difficulty of crystallizing the sulfinic acid. The precipitation of sulfate as \( \text{BaSO}_4 \) especially was found to cause losses because of the sulfinic acid carried down with the precipitate (cf. also Simonsen (6)) from which, however, it can be extracted with \( \text{HCl} \). The attempts to isolate both the \( \text{Ba} \) salt and the sulfinic acid are as follows.

\( \text{Hg} \) was removed by \( \text{H}_2\text{S} \) from 21.8 gm. of the precipitate; the
filtrate after \( \text{H}_2\text{S} \) had been expelled was made slightly alkaline to litmus with \( \text{Ba(OH)}_2 \), \( \text{BaSO}_4 \) was filtered off, and the filtrate aerated (0.1 cc. of 1 n \( \text{CuSO}_4 \) was added) until the nitroprusside test became negative. The solution was then evaporated, taken up in a small amount of \( \text{H}_2\text{O} \), filtered from cystine, and the \( \text{Ba} \) salt of sulfinic acid precipitated by 4 volumes of alcohol; yield 1.3 gm.

This material contained 29.3 per cent \( \text{Ba} \); 219 gm. consumed 1 mm of \( \text{I}_2 \) (equivalent to 1 gm. atom of \( \text{O} \)) on oxidation to cysteic acid; 222 gm. liberated 1 mm of \( \text{I}_2 \) on KI-HCl reduction. (\( \text{R-SO}_2\text{Ba} \), molecular weight 441.6, contains 31.1 per cent \( \text{Ba} \), consumes 1 mm of \( \text{I}_2 \) per 220.8 gm., and liberates 1 mm of \( \text{I}_2 \) per 147.2 gm.

In another case Hg was removed as \( \text{HgS} \) from 18.3 gm. of the precipitate; the filtrate (700 cc.) contained 8.5 mm of acid derivatives in addition to \( \text{H}_2\text{SO}_4 \), 6.5 mm of \( \text{R-SO}_2\text{H} \) (KI-HCl reduction value), and 23.6 mm of \( \text{R-SH} \). After the solution was neutralized to the turning point of litmus with \( \text{Ba(OH)}_2 \), the \( \text{BaSO}_4 \) was filtered off and the filtrate aerated and evaporated at approximately 30°, taken up in a small amount of \( \text{H}_2\text{O} \) (6 days after starting), and filtered from cystine. The yellow filtrate (50 cc.) contained 4.3 mm of \( \text{R-SO}_2\text{H} \) (an additional 0.63 mm was extracted from the \( \text{BaSO}_4 \) by 25 cc. of 2 n \( \text{HCl} \)). A slight excess of \( \text{H}_2\text{SO}_4 \) was added, the solution heated with charcoal, filtered from both \( \text{BaSO}_4 \) and charcoal, and evaporated. Since crystallization could not be induced, the syrup produced by evaporation was repeatedly heated with acetone and decanted until a solid was obtained; yield 0.97 gm. This material after correction for the 5.3 per cent cystine which was present possessed an equivalent weight of 171.5 based on titration with alkali (brom-thymol blue as indicator). This corresponds to both cysteic acid (mol. wt. 169) and \( \text{R-SO}_2\text{H-H}_2\text{O} \) (mol. wt. 171). The KI-HCl reduction indicated that the substance contained 50.3 per cent \( \text{R-SO}_2\text{H-H}_2\text{O} \), and the \( \text{I}_2 \) oxidation showed 77 per cent, the remainder in each case being presumably cysteic acid. It appears that cysteic acid formation does take place to a certain extent, possibly during aeration, although this has not been confirmed as yet.

A method which offers promise consists of triturating a thin suspension of the precipitate with \( \text{BaCl}_2 \). Because of differences
in solubility in chloride solutions, the sulfinic acid is thereby separated at once from cysteine. However, the details of obtaining a solid material by this procedure have yet to be worked out.

Behavior of Cystine Derivatives with \( \text{HgCl}_2 \)—Mercuric chloride

![Graph showing the change in rotation of solutions containing 1 mM of cysteine in 10 cc. of \( \text{M} \) HCl with the amount of \( \text{HgCl}_2 \) present. Several precipitates were isolated and suspended in \( \text{H}_2\text{O} \), Hg determined as \( \text{HgS} \), R—SH determined colorimetrically after \( \text{H}_2\text{S} \) had been expelled; \( \text{HCl} \) was titrated with \( \text{NaOH} \).]

<table>
<thead>
<tr>
<th>Ratio, R—SH: ( \text{HgCl}_2 ) in solution</th>
<th>Analysis of ppt.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{Hg}^{2+} )</td>
</tr>
<tr>
<td></td>
<td>( \mu )</td>
</tr>
<tr>
<td>1:0.5*</td>
<td>1.00</td>
</tr>
<tr>
<td>1.3</td>
<td>3.00</td>
</tr>
<tr>
<td>1:4†</td>
<td>1.98</td>
</tr>
</tbody>
</table>

* The precipitate loses crystalline structure on addition of \( \text{H}_2\text{O} \).
† This precipitate was washed with alcohol and ether.

because of its un-ionized nature, differs materially from the sulfate in its behavior with cystine and its derivatives. Although dismutation of cystine slowly takes place with higher ratios of \( \text{HgCl}_2 \): R—S—S—R, nevertheless the optical rotation of cystine in \( \text{M} \) HCl (1 gm. per 100 cc.) is unchanged by the addition of 2 \( \text{M} \)
equivalents of HgCl₂ (α₂₄⁵₆₇₈⁹₀₁₂ = −2.50° per dm.) and remains constant for at least 3 days.

Cysteic acid behaved similarly; α₃₄⁵₆₇₈⁹₀₁₂ of a solution of 1 gm. of R—SO₃H in 100 cc. of M HCl was +0.116° per dm., which remained unchanged with ratios of HgCl₂ to cysteic acid of 0.5:1 and 3:1 (+0.115° and +0.117° per dm. respectively).

The rotation, α₅₆₇₈⁹₀₁₂, of 1 gm. of R—SO₃H in 100 cc. of M HCl amounted to +0.293° per dm., which was unchanged by the addition of 0.5 M equivalents of HgCl₂; with a ratio of 4HgCl₂ to 1R—SO₃H, the rotation was +0.306° per dm.

With cysteine the rotation varied with the amount of HgCl₂ present through a maximum when the solution contained 1R—SH to 0.5HgCl₂ and reached a minimum value when 1R—SH:1HgCl₂ was present as shown by Fig. 2. Moreover, the compounds responsible for the changes in rotation evidently possess different solubilities, since precipitation in the form of loose needles starts with a ratio of 1R—SH:0.25HgCl₂ and continues through 1:0.7 (denser precipitate). No precipitation occurred with 1:1 and 1:2 ratios of cysteine to HgCl₂ but with a 1:3 ratio a densely granular precipitate is formed which comes out so quickly at 1:4 that the rotation could not be taken.

Evidently the anomalously high rotation of the cysteine-Ag complex first noted by Vickery and Leavenworth (7) is a general characteristic of all cysteine-metal compounds.

DISCUSSION

The foregoing evidence may be accepted as indicating that the products of the dismutation of cystine are cysteine and an intermediate oxidation product, the sulfinic acid, rather than cysteine and cysteic acid. Although more definite proof of the presence of the sulfinic acid is desirable, nevertheless the material presented warrants further consideration of the dismutation problem. Of the published data, Vickery and Leavenworth (7) report 72 to 84 per cent recovery of cystine (based on N determinations after removal of Ag), figures which may be high owing to the presence of the sulfinic acid; of the remainder of the cystine, 0.3 to 1.7 per cent was isolated as cysteic acid, while the balance was presumably in an unidentified syrup. Simonsen (8) consistently found that about 75 per cent of the cystine was converted to cysteine by
Hg dismutation and it should be pointed out that the analytical data (N, S, and acid value) reported by her for the isolated cysteic acid could also indicate a monohydrate of the sulfinic acid, R—SO₂H·H₂O, as isolated by Schubert (9). In regard to the quantitative results obtained by Preisler and Preisler (10) for the dismutation of dithiodihydraerlyc acid, it can only be said that, while this compound evidently dismutes to form a sulfonic acid, the analogy with cystine is unwarranted.

Because of the lack of success attending the attempts at isolation of the sulfinic acid it cannot be unequivocally asserted that the sulfinic acid is the intermediate oxidation product produced, although the indirect evidence is most pointed. One fact which should perhaps be considered is the difficulty experienced in removing H₂S after precipitating Hg from both sulfinic acid solutions and solutions of dismuted cystine, which suggests interaction of H₂S with a cystine derivative and its subsequent slow elimination. In fact the capabilities of H₂S for reacting with other sulfur compounds as well as its reducing properties should advise caution in its use. Similarly the use of heavy metal salts in studying the dismutation suffers from the disadvantage that they are capable of several valence states and may participate in the oxidation and reduction rather than acting merely as precipitating agents. Thus, the formation of Hg²⁺ (first noted by Preisler and Preisler (10)) and its part in the dismutation is still unexplained.

SUMMARY

Cysteine, cysteic acid, and the sulfinic acid of cysteine (R—SO₂H) are all precipitated from 2N H₂SO₄ solution by HgSO₄. Analytical evidence indicates that the precipitate obtained from cystine and HgSO₄ contains cysteine and sulfinic acid; consequently, the equation 2R—S—S—R + 2H₂O → 3R—SH + R—SO₂H governs the dismutation of cystine rather than the previously proposed equation, 3R—S—S—R + 3H₂O → 5R—SH + R—SO₂H. This conclusion is supported by the amount of cysteine produced and by the evidence for the sulfinic acid, which

4 Since sulfinic acids are known to react with free sulfur (11) (R—SO₂H + S → R—SO₂SH), the slow elimination of H₂S as well as cysteic acid formation could be explained by hydrolysis (R—SO₂·SH + H₂O → H₂S + R—SO₂H).
rests mainly on its power of liberating I\textsubscript{2} from KI-HCl solutions; the isolation of a pure sulfinic acid was not accomplished. Reversal of the dismutation, i.e. reformation of cystine, occurs when the mercury is removed from the precipitate.

While HgCl\textsubscript{2} does not affect the optical rotation of HCl solutions of cystine, sulfinic acid, or cysteic acid, the rotation of cysteine was found to depend on the amount of HgCl\textsubscript{2} present.

**BIBLIOGRAPHY**

THE ACTION OF MERCURIC SULFATE AND CHLORIDE ON CYSTEINE, CYSTINE, CYSTEINE SULFINIC ACID (R—SO₂H), AND CYSTEIC ACID WITH REFERENCE TO THE DISMUTATION OF CYSTINE
Theodore F. Lavine