INTRODUCTION.

In a paper dealing with the optical activity of cysteine Andrews (1) reported that the method of preparation of cysteine described by Harris (2) failed to give a completely reduced product. This method consisted in precipitating cystine with mercuric sulfate in sulfuric acid solution with subsequent treatment of the precipitate with hydrogen sulfide. The specific rotation of the product indicated approximately 85 per cent reduction. It seemed plausible that the function of the hydrogen sulfide was not only to precipitate the mercury but to reduce the newly liberated cystine to cysteine according to the reaction:

\[ 2H^+ + S^- + RSSR^{++} \rightarrow 2RSH^+ + S^0 \]

Thus sulfur would be precipitated and the cysteine formed by a fairly simple reaction and, although hydrogen sulfide is without action when directly applied to cystine, the simultaneous formation of mercuric sulfide and liberation of the cystine might be regarded as catalyzing the reduction of the latter. However, the above explanation was weakened by the fact that repeated attempts to indentify free sulfur in the precipitate from the action of hydrogen sulfide always failed.

It appeared desirable, therefore, to investigate the composition and properties of the precipitate of cystine with mercuric sulfate and the present paper records the results of such an investigation. Since these results were obtained, however, a paper by Vickery and Leavenworth (3) on the preparation of cysteine through the formation of a silver compound has recently appeared. In it these authors describe a reaction by which the original precipitate
formed by silver sulfate is not a compound of cystine but of cysteine, the latter having probably been formed by a mutual oxidation-reduction reaction which produces a small proportion of cysteic acid as well. Thus, that portion of the cystine which has been converted to silver cysteine is capable of direct regeneration to the free amino acid by either hydrogen chloride or hydrogen sulfide. The mechanism which Vickery and Leavenworth advance for the formation of the silver compound is supported by several observations which the present authors have made on the mercury compound. These observations are discussed in detail below.

EXPERIMENTAL.

The samples of the cystine-mercuric sulfate precipitate were prepared by dissolving 10 gm. of l-cystine in 2500 cc. of 5 per cent $\text{H}_2\text{SO}_4$ and adding an excess of the $\text{HgSO}_4$ reagent (10 per cent $\text{HgSO}_4$ in 5 per cent $\text{H}_2\text{SO}_4$). The flocculent white precipitate produced by an excess of the reagent was always preceded by the formation of a gray compound when smaller proportions of mercuric sulfate were used. This gray compound, while apparently stable in the cold, quickly gave place to the white flocculent precipitate when more of the reagent was added.

Two preparations made with excess mercuric sulfate were used for the analyses described below. Both preparations were carefully washed, but washing was difficult due to the cohesive, amorphous properties of the precipitate. Therefore, the precipitate was transferred from the filter to a large beaker and there carefully washed with distilled water. The suspension was then filtered in successive portions, each portion being again washed when on the filter. When the entire suspension had been so treated, the precipitate was again transferred to the beaker and the process was repeated. This method of washing was continued until the test for sulfate was almost negative, large quantities of water being used. The washings no longer gave a positive test for $\text{Hg}^{++}$ after 2.5 liters of distilled water had been used, and after the first 15 or 20 liters the amount of $\text{SO}_4^{=} \text{present in the filtrate showed a marked decrease, but thereafter it appeared roughly constant and the washings were always faintly acid. The samples were dried in a vacuum desiccator over $\text{P}_2\text{O}_5$.}
The hygroscopicity of the compound (see below) made drying a slow process, while the use of higher temperatures for drying was precluded by the instability of the compound. At temperatures of 250–300° the white compound blackens instantly, with formation of metallic mercury. This decomposition is initiated at a temperature of 120° and even below.

Methods of Analysis.—Total nitrogen was determined by Kjeldahl analysis, 10 per cent sodium sulfide solution being used to precipitate the mercury after digestion was complete. Blanks were run on all reagents used.

Mercury was determined as follows: A 0.5 gm. sample was subjected to Kjeldahl digestion; the solution was cooled and diluted to 200 cc. It was then heated to boiling and hydrogen sulfide passed in for about 1 hour, with the solution gently boiling. It was then allowed to stand 48 hours, filtered through a Gooch filter, and washed free from sulfates. Very little free sulfur was present and this, after drying, was extracted with carbon disulfide. The precipitate was dried to constant weight at 110°.

Sulfur was determined by precipitation as barium sulfate, oxidation being accomplished with fuming nitric acid. The results of a number of analyses of both samples averaged as follows:

Sample I. N 2.87, Hg 61.86, S 7.55.

" II. " 3.04, " 60.34, " 7.22.

The individual figures used in making these averages varied by ± 0.03 per cent for N, ± 0.05 per cent for Hg, and ± 0.10 per cent for S.

Difficulties in drying have already been noted. The hygroscopic properties of the dried compound were also evidenced by a rapid gain in weight during the weighing of samples. To determine the hygroscopicity of the compound a sample was placed on a weighed watch-glass in the balance case and weighed at intervals. The gain in weight was at first fairly rapid; 5 hours exposure to open air caused an increase in weight of over 0.5 per cent. On further exposure the sample gained in weight more slowly but quite steadily, particularly on damp days.

Optical Activity.—To determine the completeness of precipitation of the cystine-mercuric sulfate precipitate the original filtrate was tested for optical activity. A rotation of ±0.15° was ob-
served, while the initial washings gave a rotation of +0.13°. Similar results were obtained on other such filtrates; for example, +0.11°, +0.05°, +0.10°, and +0.07°. It will be noted that in view of the possible formation of cysteic acid such rotations would be expected.

**Potentiometric Titration.**—For comparison with the nitrogen to mercury ratio obtained analytically (see below) several potentiometric titrations were made. 0.5 gm. of l-cystine were dissolved in 50 cc. of 5 per cent H₂SO₄ and the 10 per cent HgSO₄ reagent was added from a burette. The voltage was measured by a type K potentiometer, with a standard Weston cell and calomel half-cell. Both bright and platinized platinum electrodes were used, but the most satisfactory results were obtained with the latter. An agar-KCl bridge between the calomel cell and the cystine solution was necessitated by the fact that the presence of halides greatly increased the solubility of the mercury compound and gave very erratic curves. Further experiments with bromide and iodide gave parallel results; the compound is easily soluble in alkali halides leaving only, in the case of the iodide, a slight precipitate of mercuric iodide.

In the absence of any halide, a smooth and reproducible curve was obtained with a definite minimum at about 7 cc. of 10 per cent HgSO₄ reagent, with no indication of any other maximum or minimum. This would indicate slightly more than a 1:1 ratio of cystine to mercuric sulfate (0.5 gm. of cystine reacting with 0.62...
gm. of HgSO₄), with no indication of a compound with the proportions shown by the analysis (see "Discussion"). The first addition of the reagent caused the formation of some precipitate, which appeared to come down at all times without delay. Table I shows the data corresponding to a typical curve of this sort. In all cases the minimum of the curve was very close to the 7 cc. point.

**Gray Compound.**—Mention has been made above of a gray compound obtained with smaller proportions of mercuric sulfate. Samples of this precipitate showed, under the microscope, a mass of long needle-like crystals somewhat enlarged at one end, in sharp contrast to the amorphous character of the white precipitate to which the gray changed on addition of more mercuric sulfate. These needle-like crystals were very fragile and were easily broken into irregular fragments. No further information concerning this intermediate compound has been obtained. It may, of course, represent the first step in the reaction which we postulate below: a simple mercuric dicysteine.

**DISCUSSION.**

The compositions recorded for Samples I and II, when calculated in terms of atomic ratios of nitrogen to mercury, yield the following:

<table>
<thead>
<tr>
<th></th>
<th>Sample I</th>
<th>Sample II</th>
</tr>
</thead>
<tbody>
<tr>
<td>N.</td>
<td>2.87</td>
<td>3.04</td>
</tr>
<tr>
<td>14.01</td>
<td></td>
<td>14.01</td>
</tr>
<tr>
<td>Hg.</td>
<td>61.86</td>
<td>60.34</td>
</tr>
<tr>
<td>200.6</td>
<td></td>
<td>200.6</td>
</tr>
</tbody>
</table>

Thus in Sample I a very definite ratio of 2 atoms of nitrogen to 3 of mercury is indicated, while in Sample II the proportion of mercury is about 8 per cent below a 2:3 ratio. If we accept this proportion, there is indicated a compound of 3 mols of mercury combined with either 1 cystine or 2 cysteine molecules. In such a compound, the mercury may replace the hydrogen of carboxyl or (in cysteine) sulfhydryl groups or a mercury-containing group may be attached to one or more amino groups. If we assume a mercury compound analogous to the silver cysteine described by Vickery and Leavenworth we may expect to have 1 mercury atom substituted in the two sulfhydryl groups of 2 cysteine molecules.
Mercury Derivatives of Cysteine

with two more mercury-containing groups attached as indicated above. These latter groups may be either HgSO₄ or a hydrolysis product (HgO or Hg(OH)₂). Consideration of the various possible compounds of this type shows that the percentage composition recorded above is probably best explained by the assumption that we are dealing with a mixture of two or more of the following compounds.

A. HgC₆H₁₂O₄N₂S₂ (HgSO₄) (Hg(OH)₂).
   Mol. wt. 972.15, N 2.88, Hg 61.90, S 9.89.
B. HgC₆H₁₂O₆N₂S₂ (HgSO₄) (Hg(OH)₂)₂H₂O.
   Mol. wt. 990.17, N 2.83, Hg 60.78, S 9.71.
C. HgC₆H₁₂O₄N₂S₂(2Hg(OH)₂)(2H₂O).
   Mol. wt. 946.14, N 2.96, Hg 63.60, S 6.78.

The low percentage of sulfur found as compared with the calculated values for Compounds A and B suggests that a larger proportion of sulfate was probably hydrolyzed out. The persistence of sulfuric acid in the wash water supports this view. However, the above structures are advanced for the sole purpose of meeting the needs of the analytical data at hand.

In this connection it is interesting to note that a similar mercury-cysteine compound was prepared by Brenzinger (4) directly from cysteine and mercuric chloride. The analysis of this compound also showed a proportion of 2 nitrogen to 3 mercury atoms and indicated a compound analogous in all respects to our compound with mercuric sulfate. Brenzinger commented on the ease with which hydrochloric acid is lost by hydrolysis and assigned a constitution in which 1 mercury atom replaced the 2 hydrogen atoms of the sulfhydryl groups, while the other 2 were combined with the amino groups.

It is obvious that a number of rearrangements of each of these compounds are possible. In opposition to the analytical findings, the curve of electrode potential versus cc. of mercuric sulfate (Table I) shows such a pronounced break at a 1:1 ratio of cystine to mercury (or 2:1 cysteine to mercury) as to leave no doubt about definite compound formation at this ratio. If we assume a compound of cystine with mercuric sulfate, there appears to be no reason why the introduction of the 1st atom of mercury should produce any sharper change than the 2nd or the 3rd. But if we assume a compound of cysteine with mercury, it is clear that the introduction of the 1st atom of mercury has ac-
J. C. Andrews and P. D. Wyman

compromised the reduction of cystine to cysteine whereas the others have probably combined in a purely additive way. Thus we should expect a sharp break in the curve only from the first step: the reduction of cystine.

The dextrorotatory solutions which, as noted above, were obtained when the precipitate from the action of mercuric sulfate on l-cystine is filtered appear to substantiate the assumption of cysteic acid as a by-product of the reaction. The specific rotation of cysteic acid as reported by Friedmann (5) is about +8.25.

In some studies on the separation of cystine and tyrosine Plimmer (6) reported that, as determined by Kjeldahl, mercuric sulfate removed 81 per cent of the cystine and left 19 per cent unprecipitated. This figure agrees satisfactorily with that calculated from the equation postulated by Vickery and Leavenworth, 83.3 per cent.

In addition, the mechanism outlined provides a satisfactory explanation for the absence of free sulfur in the mercuric sulfide precipitate when the mercury compound is decomposed by hydrogen sulfide.

**SUMMARY AND CONCLUSION.**

The composition of the compound formed by the action of mercuric sulfate on cystine has been determined and a general structure has been assigned to it. This structure, corresponding to that assigned by Vickery and Leavenworth to the compound formed by silver sulfate on cystine, is probably that of a mercury di-cysteine containing 2 more atoms of mercury. This hypothesis is supported by other experimental observations, such as the absence of free sulfur from the precipitate obtained when the mercury compound is treated with hydrogen sulfide, the dextrorotatory solutions resulting from the precipitation of l-cystine with mercuric sulfate, and the form of the electrode potential curve given by the reaction.

**BIBLIOGRAPHY.**

MERCURY DERIVATIVES OF CYSTEINE
James C. Andrews and Pauline D. Wyman


Access the most updated version of this article at http://www.jbc.org/content/87/2/427.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/87/2/427.citation.full.html#ref-list-1