THE DECOMPOSITION OF METHIONINE IN SULFURIC ACID*

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The classical procedure for the preparation of homocystine consists of boiling a solution of methionine in 50 per cent sulfuric acid followed by neutralization (1). Although the yields of homocystine account for only 25 to 45 per cent of the methionine, the nature of the reaction has received little attention since originally published by Butz and du Vigneaud (1). These authors noted the formation of dimethyl disulfide and also observed that the complete removal of sulfate by barium hydroxide resulted in a solution of considerable alkalinity.

The present study provides experimental evidence for the following mechanism for the reaction (R and Me signify \(-\text{CH}_2\text{CH}_2\text{CH(}\text{NH}_2\text{)}\text{COOH}\) and \(-\text{CH}_3\), respectively):

\[
\begin{align*}
2\text{RSMe} + 2\text{H}_2\text{SO}_4 \rightarrow 2\text{RSH} + 2\text{MeSO}_4\text{H} & \quad (1) \\
2\text{RSMe} + 2\text{MeSO}_4\text{H} \rightarrow 2\text{R(Me)}_2\text{S}^+\text{SO}_4\text{H}^- & \quad (2) \\
\text{RSH} + \text{H}_2\text{SO}_4 \rightarrow \text{RSSO}_3\text{H} + \text{H}_2\text{O} & \quad (3) \\
\text{RSH} + \text{RSSO}_3\text{H} \rightarrow \text{RSSR} + \text{H}_2\text{SO}_4 (\text{H}_2\text{O} + \text{SO}_2) & \quad (4)
\end{align*}
\]

or

\[
4\text{RSMe} + 3\text{H}_2\text{SO}_4 \rightarrow \text{RSSR} + 2\text{R(Me)}_2\text{S}^+\text{SO}_4\text{H}^- + 2\text{H}_2\text{O} + \text{SO}_2 & \quad (5)
\]

Reaction 1 could not be directly demonstrated, but it has its counterpart in the Baernstein demethylation procedure (2) in which the action of hydriodic acid on methionine gives rise to the volatile methyl iodide and homocysteine thiolactone. Alternatively, sulfuric acid may react with methionine as follows:

\[
\text{RSMe} + \text{H}_2\text{SO}_4 \rightarrow \text{MeSH} + \text{RSO}_4\text{H} \quad (1, a)
\]

and would thus account for the small amount of dimethyl disulfide that is formed.

The alkylating action of dimethyl sulfate in sulfonium salt formation is

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well documented\textsuperscript{1} and was found to be especially facile in sulfuric acid. The reaction between equivalent amounts of methionine and dimethyl sulfate in 16 N H\textsubscript{2}SO\textsubscript{4} is essentially complete in 15 minutes at 135°. From this it may be inferred that Reaction 2 is rapid under these conditions. Additional data pertaining to Reaction 2 is shown in Fig. 1. The reaction of dimethyl sulfate consists of an initially very rapid phase which is followed by a rate corresponding to that of the acid sulfate.

The oxidation of mercaptans, including homocysteine (5), to disulfides by sulfuric acid is also well known and presumably occurs by way of Re-

![Graph](http://www.jbc.org/)

**Fig. 1.** The formation of methionine methylsulfonium hydrogen sulfate in 16 N H\textsubscript{2}SO\textsubscript{4}. Original concentration of methionine 0.860 M, of methyl derivative 0.046 N.

actions 3 and 4. Reaction 3 bears a formal analogy to the formation of an alkyl hydrogen sulfate. Sulfur dioxide was estimated by absorption in alkali and iodometric titration and also by the loss in weight of the mixture during the reaction.

A similar mechanism has been advanced by Haas and Dougherty (6) to account for the decomposition of benzyl sulfide in sulfuric acid.

**EXPERIMENTAL**

The reaction mixtures were prepared by weight and resulted in final concentrations of about 0.87 M methionine and 16 N sulfuric acid. For

\textsuperscript{1} Ray and Farmer (3) review sulfonium sulfates and Atkinson and Poppelsdorf (4) describe the preparation of methionine methylsulfonium methosulfate.
analysis, 1 ml. aliquots of the reaction mixture were weighed and diluted to 10 ml.

Methionine was estimated by its reversible iodine consumption (7). Both homocystine and, to a lesser degree, the sulfonium salt interfere by also liberating iodine. The interference by homocystine was eliminated with mercuric sulfate as follows: To 5 ml. of the diluted aliquot, in a graduated centrifuge tube, are added 2.5 ml. of water and 1 ml. of 0.5 M HgSO₄ in 1.8 N H₂SO₄. When no further immediate precipitation occurs on drop-wise addition of the HgSO₄ reagent to the supernatant solution, the mixture is diluted to 10 ml. and allowed to stand for 2 hours. After centrifugation methionine is determined on an aliquot of the supernatant liquid.

The relatively small contribution of sulfonium salts to the methionine value may be evaluated by a "blank" in which methionine is oxidized to the sulfoxide prior to the iodometric procedure (7).

Iodate Oxidation—The total reducing power of solutions was estimated by oxidation with excess iodate (at least 100 per cent) in N hydrochloric acid (i.e. 2 ml. of the diluted aliquot, 5 ml. of 5 N HCl, and 15 ml. of 0.1 N KI₂O₃ are diluted to 25 ml.). Aliquots were titrated at intervals (usually 10 minutes, 1 and 2 hours) until a constant value was obtained, which was calculated in terms of moles of iodine or atoms of oxygen and correlated with methionine (1I₂ per mole) or homocystine (5I₂ per mole).

Homocystine was estimated by colorimetric determination with phospho-18-tungstic acid (8) and indirectly by iodate oxidation. At the end of Reaction 5 the oxidation value showed good agreement (within 2 per cent) with the colorimetric figures.

Sulfur Dioxide—The odor of sulfur dioxide is readily apparent during the reaction of methionine with hot sulfuric acid. Quantitative estimation was made by passing N₂ through the boiling reaction mixture into N NaOH for a period of 8 hours. Iodometric titration after acidification indicated the formation of 101.5 per cent of the amount of sulfite required by Reaction 5. When formaldehyde was added at pH 9, the iodine consumption was reduced to 3.5 per cent, thus indicating the virtual absence of mercaptan. Examination by the mass spectrograph of the gas evolved on acidification showed only sulfur dioxide to be present. The loss in weight of a solution of 500 mM of DL-methionine in 500 ml. of 18 N H₂SO₄ upon refluxing at 135° for 3 hours (the time required for complete reaction) amounted to 8 gm., in agreement with the expected amount of sulfur dioxide.

Dimethyl Disulfide—The formation of dimethyl disulfide was detected by passing N₂ through the boiling reaction mixture for 8 hours into, first, 2.5 N NaOH and, secondly, 3 per cent HgCl₂. The HgCl₂ trap yielded 0.2 gm. of precipitate (from 50 mM of methionine) which sintered at 142°; m.p.,

2 The authors wish to thank the Houdry Process Corporation, Marcus Hook, for this determination.
147–148° with decomposition. On treatment with 5 \( \text{n} \) HCl at 60° the precipitate evolved a gas which gave a precipitate with Hg(CN)\(_2\). This behavior characterizes dimethyl disulfide (9, 10). Control experiments established that dimethyl disulfide passes through the alkaline sulfite solution. No evidence was obtained for the presence of dimethyl sulfide. On the basis of the iodate oxidation values and the colorimetric estimation of homocystine, only 1 to 2 per cent of the methionine was converted to dimethyl disulfide.

**Rate of Reaction**—Table I shows the results obtained on boiling a solution of methionine in 50 per cent \( \text{H}_2\text{SO}_4 \). The methionine disappeared in about 3 hours. The original iodate oxidation value corresponds to methionine; the final value, 133, corresponds to 1.6 mm of dimethyl disulfide and 25 mm of homocystine. Both the sulfonium salt and homocystine appear to be stable against sulfuric acid under these conditions.

**Isolation Procedures**—A solution of 500 mm (75 gm.) of \( \text{L-} \text{methionine} \) and 500 ml. (749 gm.) of 18.5 \( \text{n} \) \( \text{H}_2\text{SO}_4 \) was refluxed at 135° for 3 hours; density of solution, 1.453. Analysis in terms of the theory for Reaction 5 was as follows: methionine, absent; homocystine (colorimetric), 100.4 per cent; homocystine (iodate oxidation), 102.5 per cent; methionine methylsulfonium phosphotungstate, 95 per cent; mercaptan (nitroprusside test with cysteine standards), 5.6 mm or 1.1 per cent of original methionine. This solution was used for the following isolation procedures. Methionine

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### Table 1

**Decomposition of 0.89 mm Methionine in 16.4 n Sulfuric Acid at 135°**

<table>
<thead>
<tr>
<th>Time (hrs.)</th>
<th>Methionine (mm)</th>
<th>Homocystine (mm)</th>
<th>Iodate Oxidation (millimols O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100.2 (100)†</td>
<td>0 (0)†</td>
<td>99.8 (100)†</td>
</tr>
<tr>
<td>1</td>
<td>12</td>
<td>25.0</td>
<td>134.5</td>
</tr>
<tr>
<td>2</td>
<td>4.4</td>
<td>24.9</td>
<td>135.5</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>24.9</td>
<td>134.8</td>
</tr>
<tr>
<td>8‡</td>
<td>0</td>
<td>25.2</td>
<td>133.2</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>25.5</td>
<td>132.6</td>
</tr>
<tr>
<td>16</td>
<td>0 (0)†</td>
<td>25.3 (25)†</td>
<td>133.2 (125)†</td>
</tr>
</tbody>
</table>

* 100 mm of methionine and 100 ml. of 18.5 n \( \text{H}_2\text{SO}_4 \).
† The figures in parentheses represent theoretical values.
‡ At the end of 8 hours the solution was allowed to stand at room temperature for 15 hours; the heating was then resumed for an additional 8 hours.

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5 ml. of the reaction mixture were diluted with 85 ml. of water (i.e. 0.9 n \( \text{H}_2\text{SO}_4 \)) and 10.5 gm. of phosphotungstic acid in 20 ml. of 1 n \( \text{H}_2\text{SO}_4 \) added. The mixture was allowed to stand for an hour and then filtered and the precipitate washed twice successively with water, methanol, and ether; yield, 4.60 gm.
methylsulfonium sulfate, because of its hygroscopic nature, was unsatisfactory for isolation and the bromide (11) was utilized for this purpose. Its identity and purity were verified routinely by the Volhard and formol titrations (equivalent weight, 244.2). Drying was carried out at room temperature in vacuo over P₂O₅. While the bromide is relatively stable in the dry state, the odor of dimethyl sulfide is always detectable and on long storage the substance may become yellow, particularly if exposed to light. The gradual decomposition renders the melting point of limited value and dependent on the rate of heating, although it is sharply defined by much foaming (i.e. evolution of dimethyl sulfide). The value 139° (in agreement with Toennies and Kolb (11)) was obtained by heating at a rate of 2° per minute in an open tube in an electrically heated apparatus of the Hershberg' type. Recrystallization was carried out in aqueous alcohol in which methionine methylsulfonium bromide possesses the following solubilities at 25°, expressed as gm. per 100 ml.: methanol, 0.439; 90 per cent methanol, 2.685; ethanol, 0.012; 90 per cent ethanol, 0.41; it is insoluble in acetone and ether.

Phospho-12-tungstic acid forms with methionine methylsulfonium salts in dilute acid solution (1 to 2 n) a characteristic, voluminous microcrystalline precipitate which is insoluble in water, dilute acid, alcohol, and ether and soluble in acetone and hot acidified aqueous alcohol. Recrystallization of the sulfonium phosphotungstate (0.644 and 0.648 per cent nitrogen) was carried out in hot 50 per cent aqueous ethanol containing 1 n HCl (4 gm. of sulfonium phosphotungstate per 100 ml.) with 93.5 per cent recovery (0.643 and 0.651 per cent nitrogen). (The determination of nitrogen by the Kjeldahl procedure with peroxide is technically somewhat difficult, owing to the insolubility of the phosphotungstate.) In terms of the N remaining in solution, the solubility of the sulfonium phosphotungstate at 25° is \(1 \times 10^{-5} \text{ M}\) in acidic 50 per cent ethanol and \(6 \times 10^{-5} \text{ M}\) in aqueous 1 n H₂SO₄.

The solubility of the phosphotungstate in water-acetone mixtures is given in Table II. The rate of solution in absolute acetone was relatively slow and suggests reaction of the components. This was also indicated by the finding of a continuous increase in nitrogen content of the phosphotungstate after attempts at recrystallization from aqueous acetone.

The insolubility of the sulfonium phosphotungstate in cold aqueous alcohol separates it from homocystine phosphotungstate, while its solubility in acetone distinguishes it from ammonium phosphotungstate. The latter fact was utilized for conversion of the phosphotungstate to the bromide by adding a slight excess of tetraethylammonium bromide to a solution of the sulfonium phosphotungstate in 80 to 90 per cent acetone. (Quaternary ammonium phosphotungstates form larger crystals than the simple ammonium compound and are easier to handle.) The filtrate contains methi-
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Methionine methylsulfonium bromide hydrobromide, indicating that the sulfonium and aminium groups each bind an acid radical of phosphotungstic acid. This and the nitrogen values suggest the following composition for the phosphotungstate: \( (\text{C}_6\text{H}_{14}\text{O}_2\text{NS})_3(\text{H}_3\text{PO}_4\cdot 12\text{WO}_3\cdot 7\text{H}_2\text{O})_2 \) equivalent weight per N, calculated 2169; found 2170. Alternatively, phosphotungstic acid may be removed from acid solutions of the sulfonium salt by extraction with amyl alcohol-ether mixtures.

Unless otherwise stated, the following yields of homocystine and methionine methylsulfonium salt refer to per cent of the theory according to Reaction 5. For maximal yields of homocystine, precipitation was allowed to occur at low temperature until the homocystine content of the supernatant liquid reached a constant value.

**TABLE II**

_Solubility of Methionine Methylsulfonium Phosphotungstate in Acetone-Water Mixtures_

<table>
<thead>
<tr>
<th>Acetone, %</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfonium phosphotungstate, gm. per ml.</td>
<td>0.02</td>
<td>0.04</td>
<td>0.12</td>
<td>0.45</td>
<td>0.64</td>
<td>0.67</td>
<td>1.40</td>
<td>1.51</td>
<td>1.88</td>
</tr>
</tbody>
</table>

_NH₃-Methanol Procedure_—The reaction mixture (100 ml.) was diluted with ice (300 gm.) and neutralized with 15 N NH₃ to pH 6.5 to 7 to precipitate homocystine; yield, 90 per cent. Additional precipitation during the subsequent isolation of the sulfonium salt increased the yield to 99.2 per cent. Estimation by iodate oxidation indicated 98.1 per cent purity. The filtrate from homocystine was divided in two equal portions, each representing 50 ml. of the original reaction mixture.

Sulfate was precipitated as \((\text{NH}_4)_2\text{SO}_4\) from one portion by evaporating in vacuo and treating the residue with 15 N NH₃ (22 ml.) and methanol (200 ml.). After standing in the refrigerator overnight, the mixture was filtered, the precipitate washed with methanol, and the filtrate evaporated to semidryness in vacuo. The residue was dissolved in water and found by analysis to consist largely of the thetin, \((\text{CH}_3)_2\text{SCH}_2\text{CH}_2\text{CH(NH}_3\text{)}\text{COO}^-\).

² The thetin is the inner salt derived from the sulfonium hydroxide. It reacts alkaline and accounts for the earlier observation of the alkaline solution resulting after removal of sulfate by barium hydroxide (1). If the ratio of alcohol to 15 N NH₃ is decreased to 2:1 in the precipitation of \((\text{NH}_4)_2\text{SO}_4\), the sulfonium salt is recovered as the neutral sulfate \((\text{CH}_3)_2\text{SCH}_2\text{CH}_2\text{CH(NH}_4\text{)}\text{COO}^-\)\text{SO}_4\).
and to contain 101 per cent of the amino N calculated for the sulfonium salt (22.3 mM) and the remaining homocystine (1.1 mM). The solution was neutralized to pH 6 with HBr, sulfate was removed by barium bromide (4.4 mM), and the filtrate was taken to semidryness in vacuo. Precipitation with 2 volumes of acetone from 80 per cent methanol solution yielded a crude product (5.45 gm.) which was decolorized in aqueous solution at 50° with Norit. On evaporation, a small amount of homocystine separated (see above). Methionine methylsulfonium bromide crystallized from the filtrate (20 ml.) on the addition of 10 volumes of ethanol; yield, 70 per cent. Analysis of different preparations: C 29.6, 29.7; H 5.95, 5.96; N 5.67, 5.58; S 12.9, 12.9; NH₂-N (formol titration) 5.75, 5.79; Br (Volhard titration) 32.9, 33.1 per cent. Theory for C₆H₁₄O₂NSBr, C 29.5, H 5.75, N 5.74, S 13.1, Br 32.7 per cent.

\textit{NH₄Br-Methanol Procedure}—The second portion of the filtrate from homocystine was evaporated in vacuo and (NH₄)₂SO₄ salted-out by the addition of NH₄Br (44 mM) and 80 per cent methanol (70 ml.). Sulfate was removed from the filtrate by barium bromide, and, after evaporation in vacuo, methionine methylsulfonium bromide was precipitated by acetone from methanol solution. Yield, 75 per cent; recrystallization from 80 per cent methanol resulted in 86 per cent recovery.

\textit{Ba(OH)₂ Procedure}—50 ml. of the original reaction mixture were neutralized to pH 5.5 to 6 with Ba(OH)₂ and the sulfate remaining was quantitatively removed with barium bromide (11 mM); theory for methionine methylsulfonium sulfate, 11.15 mM. The filtrate and washings from the barium sulfate were evaporated in vacuo to semidryness. The residue was extracted with 80 per cent methanol and filtered from homocystine. The filtrate was evaporated, the residue dissolved in methanol, and methionine methylsulfonium bromide precipitated by ethanol; yield, 65.5 per cent. The total recovery of homocystine, including extraction of the barium sulfate with dilute acid, was 69.8 per cent.

\textit{Phosphotungstic Acid Procedure}—A solution of 100 ml. of the reaction mixture, 300 ml. of water, and 200 ml. of 95 per cent ethanol was heated nearly to boiling and 100 gm. of phosphotungstic acid in 100 ml. of hot water were added. The mixture was allowed to cool protected from light. When well settled, the precipitate was removed by filtration and washed successively with 50 per cent ethanol, absolute ethanol, and ether; yield, 88.3 gm. or 91.3 per cent of the theory.

Homocystine was precipitated from the filtrate after removal of phosphotungstic acid by extraction with ether-amyl alcohol; yield, 83 per cent.

For conversion of methionine methylsulfonium phosphotungstate to the bromide, 30 gm. of the phosphotungstate were dissolved in 75 ml. of 90

\textsuperscript{4} C, H, N, and S analyses by J. F. Alicino, Metuchen, New Jersey.
per cent acetone and 35 ml. of 1 M tetraethylammonium bromide added. After the addition of an equal volume of water to complete the precipitation, tetraethylammonium phosphotungstate was removed by filtration and washed with water. The estimation of amino N (14.3 mM) and neutralization equivalent (14.0 mM) indicated complete recovery as methionine methylsulfonyl bromide hydrobromide. The solution was concentrated in vacuo to remove acetone (at this point, filter, if necessary, from any tetraethylammonium phosphotungstate) and then neutralized with the calculated amount of ammonia (14 mM) and evaporation was continued to about 5 ml. The residue was dissolved in methanol and methionine methylsulfonyl bromide precipitated by ethanol; yield, 88 per cent of the theory; m.p., 136-136.5° with decomposition.

Reaction of Methionine with Dimethyl Sulfate in 18 H₂SO₄—10 ml. (105 mM) of dimethyl sulfate were added to a solution of 29.8 gm. (200 mM) of L- or D-methionine in 200 ml. of 18 H₂SO₄. After the dimethyl sulfate had dissolved (slightly exothermic), the solution was refluxed at 137° for 1 hour. Methionine determinations indicated that 43, 99, and 100 per cent of the methionine had reacted after solution of the components and after heating for 15 minutes and 1 hour, respectively. Removal of sulfate by the Ba(OH)₂ procedure resulted in a 70 per cent yield of methionine methylsulfonyl bromide, an additional 23 per cent being recovered by working up the mother liquors. The NH₃-ethanol procedure resulted in a 74 per cent yield. Analysis: C 29.5, H 5.80, N 5.74, S 3.0 per cent.

SUMMARY

Evidence was presented to show that in the decomposition of methionine by hot 50 per cent sulfuric acid essentially one-half of the methionine is converted to homocystine and the other half to methionine methylsulfonium hydrogen sulfate. Intermediates in the reaction are homocysteine and methylsulfuric acid which arise by cleavage of the thio ether linkage by sulfuric acid. The homocysteine is subsequently oxidized to the disulfide by sulfuric acid which is reduced to sulfur dioxide. The methylsulfuric acid combines stoichiometrically with undecomposed methionine to yield the corresponding methylsulfonium salt. The latter reaction is very rapid in hot sulfuric acid and it was utilized for preparative purposes. Details are given of procedures for the isolation of the sulfonium salt as the bromide and phosphotungstate. Methionine methylsulfonium phosphotungstate is described for the first time.

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