[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF ROCHESTER]

Products of Acidic Hydrolysis of S-Methyl-L-cysteine Sulfoxide; the Isolation of Methyl Methanethiolsulfonate, and Mechanism of the Hydrolysis[1]

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A quantitative study of the hydrolysis of S-methyl-L-cysteine sulfoxide by boiling 1 *N* hydrochloric acid shows that most of the material is converted to pyruvic acid, ammonia, dimethyl disulfide and methyl methanethiolsulfonate. The sulfur-containing products are probably formed from methyl methanethiolsulfinate, which has been shown to disproportionate to them under the conditions of the experiment. The thiolsulfinate may be formed from methanesulfenic acid. Alanine, S-methylcysteine and a carbonyl compound are formed in small amounts. Hydrolysis of the sulfoxide in neutral solution follows the same course as in acid solution. Two quantitative procedures for the determination of methyl methanethiolsulfonate have been developed.

S-Methylcysteine sulfoxide (I) has been isolated from cabbage[2,3] and from turnips[4]; the analogous S-allylcysteine sulfoxide (allicin, II) was isolated earlier from garlic.[5] The enzymatic hydrolysis of these and similar sulfoxides leads to the correspond-

$CH_3SCH_2CH(NH_2)COOH$
↓
O I

II, CH_3 is $CH_2{=}CHCH_2{-}$

ing thiolsulfinate III,[5,6] the diallyl compound being the antibacterial agent allicin.[7]

CH_3SSCH_3
↓
O
III

Acid hydrolysis of S-methylcysteine sulfoxide (I) has been reported to yield considerable quantities of S-methylcysteine,[2,4] dimethyl disulfide,[3] pyruvic acid[3] and traces of alanine.[4] In order to provide some information about the mechanism of the hydrolysis, to get a good material balance and to investigate the elusive but interesting sulfenic acid,[8,9] CH_3SOH, as a possible intermediate, we have carried out a quantitative study of the hydrolysis of S-methyl-L-cysteine sulfoxide (I) in boiling 1 *N* hydrochloric acid. The results show that

$$4CH_3SCH_2CHCOOH + 2H_2O \xrightarrow{1\ N\ HCl}$$
↓ |
O NH_2 I
O
↑
$CH_3SSCH_3 + CH_3SSCH_3 + 4NH_3 + 4CH_3COCOOH$ (1)
↓
O IV V

equation 1 accounts for at least 90% of the reaction under our conditions.

The S-methylcysteine sulfoxide (I) has two asymmetric centers, the carbon carrying the amino group and the sulfur atom. The methylation of L-cysteine yields S-methyl-L-cysteine (VI), without racemization: oxidation of this to the sulfoxide I introduces asymmetry at the sulfur atom

$$CH_3SCH_2CH(NH_2)COOH \xrightarrow{H_2O_2}$$
VI
$$CH_3SCH_2CH(NH_2)COOH \quad (2)$$
↓
O I

and hence the product is a mixture of diastereoisomers, containing both possible configurations of the sulfur but only one configuration around carbon.[10] Our experiments were carried out with a mixture of the diastereoisomeric sulfoxides, prepared from the optically active S-methylcysteine (VI) by oxidation with peroxide.[4] Inspection of models showed little difference between the diastereoisomers with respect to preferred conformations or steric effects on elimination.

Preliminary runs, in which the disappearance of S-methylcysteine sulfoxide was followed by paper chromatography, showed that in refluxing 1 *N* hydrochloric acid 18–24 hr. was necessary for complete disappearance of I. Pyruvic acid, determined as the dinitrophenylhydrazone, was formed in 83 ± 2% yield (average of six determinations) and ammonia was formed in 90 ± 3% yield. Paper chromatography showed the presence of a few per cent. of alanine and a smaller amount of S-methylcysteine (VI) at the end of the reaction.

The high yield of pyruvic acid and ammonia made it appear that the first stage in the hydrolysis was elimination of the sulfoxide group to give methanesulfenic acid (VII) and aminoacrylic acid (VIII). The latter then hydrolyzed to pyruvic acid and ammonia.[11]

The fate of the sulfur was more difficult to determine. Dimethyl disulfide (V) was formed in 46 ± 5% yield in six determinations[12]; it was collected

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(2) (a) R. L. M. Synge and J. L. Wood, *Biochem. J.*, **60**, XV (1955); (b) **64**, 252 (1956); we are indebted to a referee for pointing out ref. 2b to us.

(3) G. P. Dateo, R. C. Clapp, D. A. M. MacKay, E. J. Hewitt and T. Hasselstrom, *Food Research*, **22**, 440 (1957).

(4) C. J. Morris and J. F. Thompson, *Chemistry & Industry*, 951 (1955); THIS JOURNAL, **78**, 1605 (1956).

(5) A. Stoll and E. Seebeck, *Helv. Chim. Acta*, **31**, 189 (1948).

(6) R. Gmelin, G. Hasenmaier and G. Strauss, *Z. Naturforsch.*, **12b**, 687 (1957).

(7) C. J. Cavallito, J. S. Buck and C. M. Suter, THIS JOURNAL, **66**, 1952 (1944).

(8) N. Kharasch, S. J. Potempa and H. L. Wehrmeister, *Chem. Rev.*, **39**, 269 (1946).

(9) J. A. Barltrop, P. M. Hayes and M. Calvin, THIS JOURNAL, **76**, 4359 (1954).

(10) The two compounds of structure I derived from L-cysteine are *not* a racemic pair, although they have been called one. The fact that the two diastereoisomers have approximately equal and opposite rotations[4] is purely coincidence. The separation of the two diastereoisomers by chromatography on an acidic ion exchange resin has been described.[2b]

(11) A. Stoll and E. Seebeck, *Helv. Chim. Acta*, **32**, 197 (1949).

(12) A 100% yield of disulfide would mean that two moles of the sulfoxide I yielded one mole of disulfide, *i.e.*, that all of the sulfur appeared as disulfide.

$$CH_3SCH_2CHCOOH \xrightarrow[HCl]{H_2O} [CH_3SOH] + [CH_2{=}CCOOH] \quad (3)$$

(O, NH_2, I; VII; VIII, NH_2)

$$[CH_2{=}CCOOH] \xrightarrow{H_2O} CH_3COCOOH + NH_3$$

in acetic acid wash bottles and titrated with bromate–bromide.[13] The bromination product (CH_3SO_2Br) was hydrolyzed to methanesulfonic acid identified by the infrared spectrum of the sodium salt; V was also identified as the mercuric mercaptide, $(CH_3S)_2Hg$, after reduction to CH_3SH.

No methyl mercaptan or hydrogen sulfide was formed in the hydrolysis, shown by failure to obtain a precipitate in a mercuric cyanide wash bottle.[3,14] The missing sulfur was not present as the corresponding sulfinic or sulfonic acids, CH_3SO_2H or CH_3SO_3H, in the reaction mixture, because a careful search using ion exchange resins failed to disclose more than a trace of these acids.

It was found eventually that the missing sulfur was present as methyl methanethiolsulfonate (IV); this was isolated from the reaction mixture after hydrolysis by continuous extraction with chloroform, and was identified by a comparison of its infrared spectrum with that of an authentic sample.[15a] It was determined quantitatively by bromate–bromide titration in 80% acetic acid at 100°, yielding a value of 45%.

It was also determined by titration of the sulfinic acid liberated on treatment with neutralized cysteine hydrochloride[16]

$$\underset{IV}{CH_3SO_2SCH_3} + HSCH_2CH(NH_2)COOH \longrightarrow CH_3SO_2H + CH_3SSCH_2(NH_2)COOH \quad (4)$$

This titration showed a yield of 37% of the thiosulfonate IV; the bromination determination result was probably too high because of the presence of a ketonic compound (see below).

The thiolsulfonate IV and the disulfide V probably arise by disproportionation of the thiolsulfinate III,[17] a reaction known to occur.[15b]

$$\underset{O}{\overset{III}{2CH_3SSCH_3}} \longrightarrow \underset{IV}{CH_3SO_2SCH_3} + \underset{V}{CH_3SSCH_3} \quad (5)$$

This possibility was supported in the present work by demonstration that an authentic sample of III[17] did yield the thiolsulfonate IV and the disulfide V when it was refluxed with hydrochloric acid under the conditions of the hydrolysis of the sulfoxide I.[18]

(13) S. Siggia, "Quantitative Organic Analysis *via* Functional Groups," John Wiley and Sons, Inc., New York, N. Y., 1949, p. 95.

(14) F. Challenger and A. A. Rawlings, *J. Chem. Soc.*, 868 (1937).

(15) (a) H. J. Backer, *Rec. trav. chim.*, **67**, 902 (1948); (b) H. J. Backer and H. Kloosterziel, *ibid.*, **73**, 129 (1954).

(16) (a) S. Smiles and D. T. Gibson, *J. Chem. Soc.*, **125**, 176 (1924); (b) T. F. Lavine, *J. Biol. Chem.*, **113**, 593 (1936); (c) L. D. Small, J. H. Bailey and C. J. Cavallito, THIS JOURNAL, **71**, 3565 (1949).

(17) L. D. Small, J. H. Bailey and C. J. Cavallito, *ibid.*, **69**, 1710 (1947).

(18) Enzymatic hydrolysis[5,6] of the S-alkylcysteine sulfoxides stops at the thiolsulfinate stage, the mild conditions not leading to the subsequent disproportionation.

The thiolsulfinate III is presumably formed from two molecules of methanesulfenic acid VII, or its equivalent state of oxidation.[5] The summation of all of these stages

$$\underset{VII}{2[CH_3SOH]} \longrightarrow \underset{\underset{O}{\downarrow}\ III}{CH_3SSCH_3} + H_2O \quad (6)$$

leads to the over-all eq. 1; Table I summarizes the quantitative data discussed so far, which support this formulation.

TABLE I

Compound	Moles of compound per mole of (I) Calcd. from eq. 1	Found
Pyruvic acid	1.00	0.83
Ammonia	1.00	.90
IV	0.50	.37
V	.50	.46
Methyl mercaptan[a]	.00	.00

[a] Dateo, *et al.*,[3] also found no mercaptan or hydrogen sulfide.

The alanine formed in small amount during the acid hydrolysis may be due to transamination between the pyruvic acid and the starting material[19]; it may also be the result of a reductive cleavage of the $CH_3S \rightarrow O$ group from the starting material. The S-methylcysteine (VI), which in our experiments was formed only in very small amounts,[20] must result from a reductive removal of the sulfoxide oxygen by the sulfenic acid VII or the thiolsulfinate III.

In the chloroform extract of the hydrolysis mixture, which contained the thiolsulfonate IV, was present a small amount of a neutral carbonyl compound, showing absorption at 1725 $cm.^{-1}$, and forming a dinitrophenylhydrazone which melted at 136–156°. This product might have been formed by some aldol condensation involving pyruvic acid[21] and acetaldehyde. A product obtained by addition of acetaldehyde[22] to pyruvic acid in boiling 1 *N* hydrochloric acid showed the same infrared spectrum as the by-product of the hydrolysis of I. It formed a dinitrophenylhydrazone (m.p. 157–159°), whose infrared spectrum was identical with the one obtained from the dinitrophenylhydrazone (m.p. 136–156°) mentioned above. The nature of this product was not investigated further.

Hydrolysis of S-methylcysteine sulfoxide in neutral and in basic solution apparently followed the same general course as in acid solution, although it was not possible to get as good a material balance. It required between 24 and 48 hr. to bring about complete disappearance of the sulfoxide I when it

(19) *Cf.* R. M. Herbst and L. L. Engel, *J. Biol. Chem.*, **107**, 505 (1934); R. M. Herbst, THIS JOURNAL, **58**, 2239 (1936).

(20) Earlier workers[2,4] reported considerable amounts of S-methylcysteine; using the synthetic mixture of diastereoisomeric sulfoxides similar to ours, Dateo, *et al.*,[3] with 6 *N* hydrochloric acid obtained S-methylcysteine in unspecified amount, but in neutral solution obtained none. Morris and Thompson[4] hydrolyzed the sulfoxide I of unspecified stereochemistry with 1 *N* acid and obtained 38% of S-methylcysteine determined by quantitative paper chromatography.

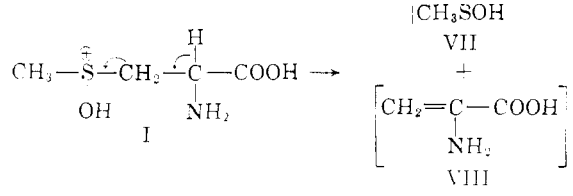
(21) It is probably not the self condensation product of pyruvic acid (L. Wolff, *Ann.*, **317**, 1 (1901)), or α-keto-γ-methyl-γ-butyrolactone (A. Rossi and H. Schinz, *Helv. Chim. Acta*, **31**, 473 (1948)), which are both enolic; the latter should be formed by condensation of pyruvic acid with acetaldehyde.

(22) Acetaldehyde might be present in the reaction mixture as a product of decarboxylation of pyruvic acid, perhaps during transamination reactions.[19]

was refluxed in neutral aqueous solution; pyruvic acid (58–60%), ammonia (84–90%) and dimethyl disulfide (41–43%) were determined as before. The presence of the thiolsulfonate IV was shown by its infrared spectrum.

In refluxing 1 *N* sodium hydroxide, the sulfoxide had disappeared completely after 3 hr. Pyruvic acid (36%), and dimethyl disulfide (45%) were formed, but it was not possible to establish the presence of either the thiolsulfonate or the thiolsulfinate; the latter is known[17] to form the disulfide and sulfur dioxide in basic solution.

The hydrolysis of S-methylcysteine sulfoxide is best regarded as a β-elimination reaction; in acid solution, the protonated sulfoxide I undergoes elimination of the sulfenic acid VII



In basic solution, when the reaction takes place 5–10 times faster than in acidic solution, the base may attack the hydrogen α to the carboxyl group to induce a concerted 1,2-elimination.

In neutral solution (slowest reaction conditions), a water molecule may initiate the elimination by hydrogen bonding on the sulfoxide oxygen.

The presence of the more basic sulfoxide group (as compared to the sulfide group in S-methylcysteine) explains[23] why S-methylcysteine sulfoxide I undergoes elimination under conditions when S-methylcysteine VI itself is stable.[24]

Similar cases are found in O-derivatives of serine.[25,26]

Experimental[27]

S-Methylcysteine (VI) was prepared[28] from commercial L-cysteine and methyl iodide in 60–70% yield, m.p. 180–200° dec. By recrystallization from concentrated solution in hot water, it was obtained chromatographically pure, m.p. 217–221° dec., $[\alpha]^{25}D$ −29.4° (*c* 1, water). The reported[29] values are m.p. 248° dec., $[\alpha]^{23}D$ −31.2°.

S-Methyl-L-cysteine sulfoxide (I) was prepared by the published procedure[4]; when cystine-free S-methyl-L-cysteine was used, the product (80–90% yield, m.p. 152–153° dec., $[\alpha]^{30}D$ −13.0° (*c* 1, water)) was chromatographically homogeneous and was used without further purification. The pure diastereoisomers are reported[4] to melt at 174–175° dec., $[\alpha]^{25}D$ +124° and −127°.

Methyl methanethiolsulfinate (III) was prepared from dimethyl disulfide and peracetic acid,[17] and had b.p. 44–45° (1.5 mm.), $n^{25}D$ 1.5540, and the following ultraviolet absorption in 95% ethanol: λ_{max} 245–248 mμ (ε 1880), λ_{min} 225 mμ (ε 880). The reported values are b.p. 64° (0.5 mm.), $n^{25}D$ 1.5481[17]; λ_{max} 248 mμ (ε 2090), λ_{min} 224 mμ (ε 1020).[15b]

Methyl methanethiolsulfonate (IV), prepared from dimethyl disulfide and hydrogen peroxide,[15a] showed $n^{25}D$ 1.5104; the reported value[15b] is $n^{17}D$ 1.5137.

Methanesulfinic acid was synthesized for comparison purposes from methylmagnesium bromide and sulfur dioxide.[30] The main difficulty arose from extraction of hydrogen bromide as well as the sulfinic acid by ether. After neutralization with sodium hydroxide, the resulting mixture of sodium methanesulfinate and sodium bromide had to be recrystallized repeatedly from absolute alcohol to obtain the pure sulfinate salt. The use of methylmagnesium chloride should facilitate the separation because of the lower solubility of sodium chloride in absolute alcohol.

The free methanesulfinic acid was prepared from its sodium salt by ion exchange over Dowex-50. Since this acid has been poorly characterized, the following derivatives were made.

The S-benzylisothiouronium methanesulfinate was prepared from equivalent amounts of sodium methanesulfinate and S-benzylisothiouronium chloride in water, followed by evaporation to dryness and extraction of the derivative with absolute alcohol; recrystallization from the same solvent yielded material of m.p. 150–152°, which was analyzed, since the reported[31] m.p. was 136–137°.

Anal. Calcd. for $C_9H_{14}N_2O_2S_2$: C, 43.88; H, 5.73; N, 11.37; S, 26.03. Found: C, 43.70; H, 5.73; N, 11.52; S, 26.37.

The phenylhydrazine salt was prepared from the unstable free acid in ether, and melted, after recrystallization from absolute alcohol–ether, at 107.5–108.5°.

Anal. Calcd. for $C_7H_{12}N_2O_2S_2$: C, 44.66; H, 6.43; N, 14.88; S, 17.03. Found: C, 43.78; H, 6.51; N, 14.74; S, 16.53.

Methods of Quantitative Determination.—Dimethyl disulfide V was determined by bromate–bromide titration.[13] Methyl methanethiolsulfinate (III) was determined by the same procedure

$$4/3\ HBrO_3 + 20/3\ HBr \longrightarrow 4\ Br_2 + 4\ H_2O$$

$$CH_3SO\text{–}SCH_3 + 4\ Br_2 + 3\ H_2O \longrightarrow 2\ CH_3SO_2Br + 6\ HBr$$

Methyl methanethiolsulfonate (IV) could also be determined by this method, provided the bromate–bromide titration was carried out at 100°; below 90° the bromine uptake was too slow to give a reliable end-point (persistence of the yellow color for 2 min.)

$$HBrO_3 + 5\ HBr \longrightarrow 3\ Br_2 + 3\ H_2O$$

$$CH_3SO_2\text{—}SCH_3 + 3\ Br_2 + 2\ H_2O \longrightarrow 2\ CH_3SO_2Br + 4\ HBr$$

All of these three compounds gave satisfactory results on determination of knowns.

Methyl methanethiolsulfonate (IV) with cysteine was quantitatively determined[16]: The thiolsulfonate (0.5–1 mole) in 5 ml. of water was neutralized, if necessary, to methyl red, and treated with a neutralized (methyl red) solution of 2–3 equiv. of cysteine hydrochloride in water (*cf.* eq. 4); the solution immediately turned strongly acidic. After standing 5–10 min., the liberated methanesulfinic acid was titrated with standard base. Thiosulfinates react with cysteine, but do not liberate titratable acid.[16]

Hydrolysis of S-Methylcysteine Sulfoxide (I) in 1 *N* Hydrochloric Acid. A. Paper Chromatography.—The sulfoxide I (300 mg.) was refluxed in 20 ml. of 1 *N* hydrochloric acid, and 5-μl. samples were withdrawn at intervals, chromatographed on paper strips, dried, developed by dipping into a 0.25% ninhydrin solution in acetone,[32] and heated 5–10 min. in an oven at 80–90°. The spot at R_f 0.09–0.11, due to the starting material, decreased as the reaction progressed, and had disappeared after the hydrolysis had proceeded for 18 hr. The 2-hr. sample showed a weak spot of R_f 0.25, which increased in intensity as the hydrolysis proceeded for longer periods. Descending chromatography on longer sheets (25–35 cm.) showed separation of the latter spot into two spots. The more intense spot was

(23) R. P. Linstead, L. N. Owen and R. F. Webb, *J. Chem. Soc.*, 1211 (1953).

(24) F. Challenger and H. D. Hollingworth, *ibid.*, 64 (1959).

(25) G. Riley, J. H. Turnbull and W. Wilson, *Chemistry & Industry*, 1181 (1953).

(26) F. Micheel and W. Busse, *Chem. Ber.*, **91**, 985 (1958); there may be more examples of this type.

(27) All m.p.'s were taken on a Kofler block and were corrected. Analyses by Microtech Laboratories, Skokie, Ill., and by W. Manser, ETH, Zürich, Switzerland. Paper chromatograms were carried out with Whatman No. 1 paper and 1-butanol–acetic acid–water (4:1:1 by volume).

(28) Following the procedure of A. Stoll and E. Seebeck, *Helv. Chim. Acta*, **32**, 866 (1949), for S-isopropylcysteine.

(29) V. du Vigneaud, H. S. Loring and H. A. Craft, *J. Biol. Chem.*, **105**, 481 (1934).

(30) H. G. Houlton and H. V. Tartar, THIS JOURNAL, **60**, 544 (1938); P. Allen, Jr., *J. Org. Chem.*, **7**, 23 (1942).

(31) F. Kurzer and J. R. Powell, *J. Chem. Soc.*, 3728 (1952).

(32) G. Toennies and J. J. Kolb, *Anal. Chem.*, **23**, 823 (1951).

due to alanine, R_f 0.21–0.25 (reddish), the violet one showed in parallel chromatograms the same R_f-value (0.25–0.30) as S-methylcysteine.

The identity of the alanine spot was confirmed by use of a ninhydrin–cupric nitrate reagent,[33] which gives a purple spot with alanine, and a bluish-gray spot with S-methylcysteine. This reagent appears to be less sensitive, and gave no color with the small amount of S-methylcysteine present from the hydrolysis.

B. Quantitative Determination of Dimethyl Disulfide (V), Pyruvic Acid, Ammonia and Methyl Methanethiolsulfonate (IV).—In general, runs were carried out by refluxing *ca.* 500 mg. of the sulfoxide in 30 ml. of 1 *N* hydrochloric acid, with a nitrogen stream going through the solution and leading into several wash bottles. In one set of runs, the wash bottles contained 3% mercuric cyanide solution and 3% mercuric chloride solution. There was no precipitate in the first wash bottle, showing the absence of hydrogen sulfide and methyl mercaptan; a white precipitate formed in the mercuric chloride bottle and increased up to 22 hr. This precipitate was due to dimethyl disulfide.

In other runs, the two wash bottles contained 80% acetic acid, cooled in ice, and glacial acetic acid at room temperature. The contents of the first bottle were refluxed with 25 g. of zinc, and the methyl mercaptan generated was passed into 0.05 *M* mercuric cyanide solution. The precipitated $(CH_3S)_2Hg$ obtained melted, after recrystallization from ethanol, at 177–187° dec.,[34] undepressed by mixture with an authentic sample.

The dimethyl disulfide (III), formed by refluxing 604 mg. of I in 40 ml. of 1 *N* hydrochloric acid 18–24 hr., was determined quantitatively by absorption in acetic acid (see above) and titration with 0.1 *M* bromate–bromide solution yielding 46% of III. In a known run, in which the disulfide III was refluxed with 1 *N* acid, and collected and titrated as above, 95% of the initial amount of III was found.

The aqueous hydrolysis solution was made up to 50.0 ml. and 5.0 ml. of this solution was treated with a hot solution of 0.15 g. of dinitrophenylhydrazine in 3 ml. of 2 *N* hydrochloric acid. The derivative obtained (93 mg., corresponding to 86% of pyruvic acid) melted at 214–216° (some softening from 205°). A mixed m.p. with authentic material showed no depression (reported 218°,[35] 215–216°[6]).

The ammonia content was determined on another 5.0-ml. portion of the above solution, by making basic and distilling into standard acid; 93% of ammonia was found. S-Methylcysteine was shown not to evolve any ammonia under these conditions.

The remaining 40 ml. of the hydrolysis solution was continuously extracted with chloroform for 22 hr., the extract was washed with saturated potassium bicarbonate solution, with water, dried and the solvent removed through a short column at atmospheric pressure. The infrared spectrum of the residue (1% in chloroform) showed the four strong bands[36] of the methyl methanethiolsulfonate (IV) spectrum, as determined from an authentic sample,[15a] at 1325, 1302, 1127 and 950 cm.$^{-1}$, in addition to bands at 1724 and 1010 cm.$^{-1}$, apparently due to some carbonyl impurity.

A quantitative determination of the thiolsulfonate showed 37% yield by the cysteine method and 45% by bromination (*cf.* Methods of Quantitative Determinations).

When the chloroform extract, which had been treated with cysteine, was again continuously extracted with chloroform, 10 mg. of a compound was isolated whose infrared spectrum in carbon tetrachloride showed major peaks at 2870, 1725, 1297 and 1134 cm.$^{-1}$ as well as a weak absorption at 1017 cm.$^{-1}$.

In another experiment the chloroform extract, containing IV and the unidentified carbonyl compound, gave negative tests with Tollens and Schiff reagents. With dinitrophenylhydrazine a yellow crystalline precipitate was obtained, which melted at 136–156° after two recrystallizations from ethanol.

Disproportionation of Methyl Methanethiolsufinate (III) in 1 *N* Hydrochloric Acid.—In a set-up as described for the quantitative determination of dimethyl disulfide in the hydrolysis of I, 0.42 g. of the thiolsulfinate III was refluxed in 40 ml. of 1 *N* hydrochloric acid. After 24 hr. the amount of dimethyl disulfide in the acetic acid traps was determined by bromate–bromide titration (yield 60% of the calcd. value (eq. 5)).

The aqueous solution was continuously extracted with chloroform, yielding 76% of the calcd. amount of the thiolsulfonate IV (identified by its infrared spectrum and determined by bromination).

Hydrolysis of S-Methylcysteine Sulfoxide (I) in Neutral Solution.—Paper chromatography showed that more than 24 hr. was necessary to hydrolyze all the starting material. After 48 hr. all the sulfoxide I had disappeared and more alanine than S-methylcysteine was found.

Quantitative experiments, carried out as described in the acid hydrolysis, yielded values of 58–60% of pyruvic acid as dinitrophenylhydrazone, m.p. 208–213°. A recrystallized sample (ethanol) melted undepressed by admixture of authentic material at 215–216°.

Ammonia (84–90%) and 41–43% of dimethyl disulfide were found.

The reaction mixture remained neutral all the time, indicating that strong acids were not formed. At the end of the reaction the aqueous solution was continuously extracted overnight with chloroform. The isolated oil showed the infrared absorption of the thiolsulfonate IV.

Hydrolysis of S-Methylcysteine Sulfoxide (I) in 1 *N* Sodium Hydroxide.—Refluxing the sulfoxide I in a 10-fold molar excess of 1 *N* sodium hydroxide for 3 hr. caused destruction of essentially all ninhydrin-active material. Dimethyl disulfide was determined quantitatively by bromination (90% of the calcd. value, eq. 1); 36% of pyruvic acid was found as its dinitrophenylhydrazone, m.p. 214–217° (ethanol), undepressed by mixing with an authentic sample.

Small amounts of bases (2 mole per cent. of pyridine, 5 mole per cent. of sodium hydroxide or 1 molar equivalent of sodium hydroxide) did not influence the rate of reaction appreciably.

Reaction of Pyruvic Acid with Acetaldehyde in 1 *N* Hydrochloric Acid.—A solution of 0.44 g. of acetaldehyde in 50 ml. of ice-water was added slowly to 4.4 g. of pyruvic acid in 200 ml. of boiling 1 *N* hydrochloric acid. The refluxing was continued for 21 hr.

A neutral carbonyl compound (0.30 g.) was isolated which absorbed in the infrared at 1725, 1353, 1297, 1130 and 1020 cm.$^{-1}$. It formed a dinitrophenylhydrazone (m.p. 157–159° after several recrystallizations from ethanol) whose infrared spectrum was identical with the one obtained from the derivative of the neutral carbonyl compound isolated from the hydrolysis mixture of I.

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(33) E. D. Moffat and R. I. Lytle, *Anal. Chem.*, **31**, 926 (1959).

(34) F. Challenger, "Aspects of the Organic Chemistry of Sulphur," Academic Press, Inc., New York, N. Y., 1959, p. 3.

(35) H. H. Strain, THIS JOURNAL, **57**, 760 (1935).

(36) J. Cymerman and J. B. Willis, *J. Chem. Soc.*, 1332 (1951).