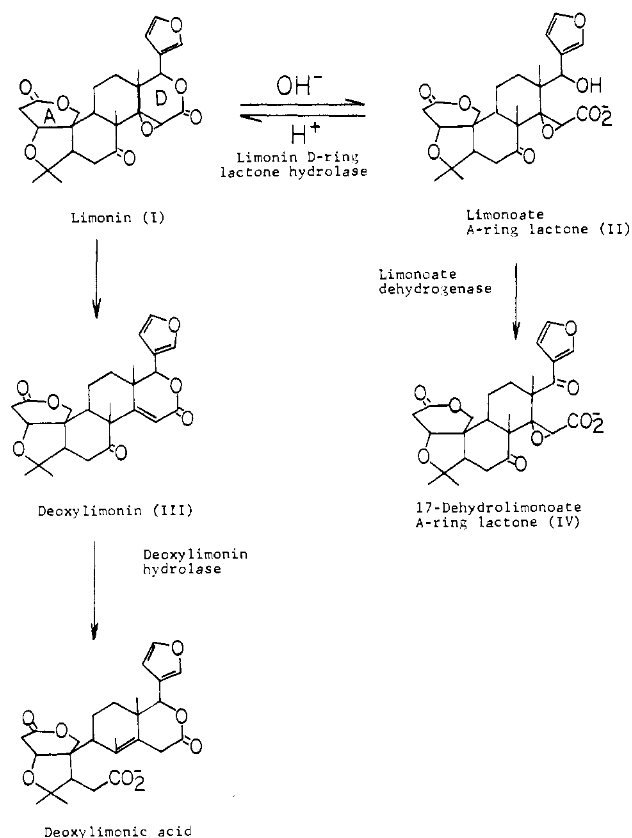

Figure 2. Metabolic pathways of limonoids in *Pseudomonas* sp. 321-18.

globiformis produced 17-dehydrolimonoate A-ring lactone as the major metabolite (Hasegawa et al., 1972b). Seemingly, the hydrolase was also present in *Arthrobacter globiformis* and catalyzed the conversion of limonin to limonoate A-ring lactone. The latter would then have been dehydrogenated by limonoate dehydrogenase, which has specificity for the open D-ring of limonoids (Hasegawa et al., 1972b).

Maier et al. (1969) questioned the biological functions of limonin D-ring lactone hydrolase, specifically whether it is involved in the synthesis of limonoids, their catabolism, or both. Our findings clearly show that the hydrolase was involved in the degradation of limonoids. Whether the enzyme also plays a role in their biosynthesis remains to be determined.

LITERATURE CITED

Arigoni, D., Barton, D. H. R., Corey, E. J., Jeger, O., et al., *Experientia* **16**, 41 (1960).
Barton, D. H. R., Pradhan, S. K., Sternhell, S., Templeton, J. F., *J. Chem. Soc.*, 255 (1961).
Chandler, B. V., *J. Sci. Food Agric.* **22**, 634 (1971).
Dreyer, D. L., *J. Org. Chem.* **30**, 749 (1965).
Flavian, S., Levi, A. J., *Food Technol.* **5**, 193 (1970).
Hasegawa, S., Bennett, R. D., Maier, V. P., *J. Agric. Food Chem.* **20**, 435 (1972a).
Hasegawa, S., Bennett, R. D., Maier, V. P., King, A. D., Jr., *J. Agric. Food Chem.* **20**, 1031 (1972b).
Hasegawa, S., Maier, V. P., Bennett, R. D., *Phytochemistry* **13**, 103 (1974a).
Hasegawa, S., Maier, V. P., Border, S. N., Bennett, R. D., *J. Agric. Food Chem.* **22**, 1093 (1974b).
Hasegawa, S., Maier, V. P., King, A. D., Jr., *J. Agric. Food Chem.* **22**, 523 (1974c).
Hsu, A. C., Hasegawa, S., Maier, V. P., Bennett, R. D., *Phytochemistry* **12**, 563 (1973).
Maier, V. P., Hasegawa, S., Hara, S., *Phytochemistry* **8**, 405 (1969).
Nomura, D., *Yamaguchi Daigaku Nogakubu Gakujutsu Hokoku* **17**, 903 (1966).

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Specific Oxidation of Methionine to Methionine Sulfoxide by Dimethyl Sulfoxide

Samuel H. Lipton* and C. E. Bodwell

In the presence of hydrochloric acid or other hydrogen halides, the dipolar aprotic solvent and selective oxidant dimethyl sulfoxide (Me_2SO) oxidizes methionine to methionine sulfoxide and is concomitantly reduced to the hydrophobic and low-boiling dimethyl sulfide (Me_2S). The reaction is reversed (i.e., methionine sulfoxide is reduced to methionine) by an excess of Me_2S in a sealed tube. This oxygen exchange reaction occurs under relatively mild conditions, without interference from or oxidation of other common amino acids (except for oxidation of cysteine, for which a correction can be made). The reaction has been investigated as the basis of a methionine assay by measurement of the evolved Me_2S in a gas chromatograph equipped with a highly sensitive sulfur-specific flame photometric detector. This approach has potential for measuring methionine and/or nutritionally available methionine by a rapid chemical method which does not require conventional protein hydrolysis.

Dimethyl sulfoxide (Me_2SO) was used (Searles and Hays, 1958) for selective oxidation of organic sulfides to sulfoxides without formation of sulfones, although relatively severe conditions were required (8–12 hr at 160–175°C). More recently Me_2SO was used (Spencer and Wold, 1969) for the purpose of oxidizing cysteine and cystine to cysteic acid during the hydrolysis of proteins by HCl. The sulfur amino acids, methionine and cystine, as well as the amino acid tyrosine were reported (Lipton and Bodwell, 1973) to be oxidized by the presence of traces of Me_2SO under these same severe conditions. During efforts to develop a chemical method for estimation of nutritionally available methionine (Lipton et al., 1974), the

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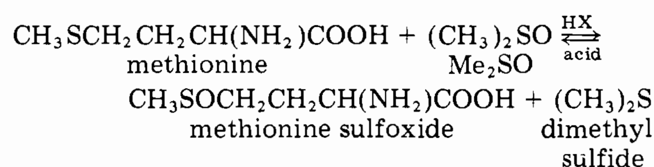
possibility of using Me_2SO as a specific oxidant of methionine under relatively mild conditions was investigated. Amino acid analyzer and gas chromatographic studies of the following reaction are thus the main subject of this paper.



EXPERIMENTAL SECTION

Materials. Amino acids and other chemicals were purchased from commercial sources. Reagent grade Me_2SO was from Eastman Kodak; glycyl-L-methionylglycine and the C-terminal octapeptide of glucagon were from Schwarz-Mann; type I amino acid calibration mixture, containing 18 amino acids at a concentration of 2.5 μmol/ml, was from Beckman Instruments. (Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.) *meso*-Lanthionine was a sample isolated by Dr. Millard J. Horn (Horn et al., 1941).

Amino Acid Analyzer Studies for Measurement of Oxidation by Me_2SO. Following the initial observation (Lipton and Bodwell, 1973) of the oxidation of amino acids by Me_2SO, experiments were designed to determine the relative susceptibility of the various amino acids to oxidation by Me_2SO. A Phoenix Biolyzer, Model 3000, was used to obtain quantitative amino acid analyses by procedures which have been previously referenced (Lipton and Bodwell, 1973). For use in the experiments on the relative susceptibility of amino acids to oxidation by Me_2SO, the amino acid calibration mixture was evaporated to dryness in vacuo at room temperature, and the residue was redissolved to its original concentration in 6 *N* HCl containing 0.1% Me_2SO. Of the amino acids present in the ordinary calibration mixture, it was obvious that methionine was the most easily oxidized. This was apparent from an analysis on the amino acid analyzer of the calibration mixture which had been heated in an evacuated vessel for only 3 hr at 100°C in 6 *N* HCl containing 0.1% Me_2SO. Before any of the other amino acids had been measurably affected, methionine had already been largely oxidized to methionine sulfoxide (MetSO).

In order to establish the requirements for the oxidation by Me_2SO, experiments were then conducted on the oxidation of methionine alone. These were done on 5-mg samples of L-methionine, treated with mixtures of 0.5 ml of Me_2SO and 0.5 ml of various acid reagents. Temperatures of either 24 or 100°C and various times of reaction were used. (For the room temperature experiments on the oxidation of methionine, the mixture of Me_2SO and the acid component was cooled before use, since the heat evolved by mixing equal volumes of Me_2SO and 6 *N* HCl caused an elevation of the temperature to about 65°C.) The results of these experiments were determined by immediately placing a 10-μl sample of the crude reaction mixture on the 60-cm column of the amino acid analyzer. The MetSO product of the oxidation occupied a position in the ion-exchange chromatogram just ahead of that normally occupied by aspartic acid, and it coincided with that of an authentic sample of MetSO. The MetSO peak had an asymmetric and double-headed appearance, since the chromatographic procedure gave only partial separation of the L-methionine sulfoxide isomers which had been formed. The results of these experiments are summarized in Table I, in which the yields of MetSO were calculated from the ratio of the MetSO peak area to the total area of all peaks, with the assumption that all components had equal peak areas per mole.

Reversibility of Oxidation by Me_2SO. The reduction of MetSO by Me_2S was demonstrated in reactions which were run in test tubes sealed with ground glass stoppers which were held in place by metal clamps. The experiments summarized in Table II were based on the reaction of 5-mg samples of MetSO dissolved in 0.9-ml volumes of various concentrations of HCl. Immediately after the addition of 0.1 ml of liquid Me_2S, the tubes were sealed for the 3- or 5-min reaction at either 24 or 100°C. Results were determined by then immediately placing a 10-μl sample on the 60-cm column of the amino acid analyzer.

Oxidation of Glycyl-L-methionylglycine by Me_2SO. The suitability of Me_2SO for the oxidation of methionyl residues of peptides was also first demonstrated on the amino acid analyzer. A 5-mg sample of glycyl-L-methionylglycine was dissolved in 0.5 ml of 6 *N* HCl, to which was added 0.5 ml of Me_2SO with no effort to control the temperature. After standing for 30 min, a 10-μl sample was analyzed on the 60-cm column of the amino acid analyzer. Hydrolysis of the main portion of the oxidized peptide was carried out by first evaporating in vacuo at room temperature to dryness and then heating for 12 hr at 110°C in 1 ml of 15% NaOH. After acidifying the hydrolysate with HCl, a 10-μl sample was analyzed as above on the amino acid analyzer.

Identification of Me_2S as the Reduction Product of Me_2SO. The formation of Me_2S was apparent from its strong characteristic odor which was noted during the reaction of Me_2SO and methionine. The identification was confirmed by confining a reaction in a sealed serum bottle and by injecting a sample of the headspace gas into a mass spectrometer. The observed spectrum corresponded with the spectrum obtained on an authentic sample of Me_2S. Spectra were run on a Hitachi RMU-6E spectrometer at an ionizing voltage of 25 V and displayed major peaks at m/e 62 (M^+), 47 (M − 15), and 35.

Gas Chromatography. A Tracor Model 550 gas chromatograph was used with a flame photometric detector and a 394-nm filter. The column was a 6 ft × 0.25 in. glass tube packed with 3% OV-1 on 80–100 mesh Chromosorb W. Rates of gas flow to the detector were adjusted for maximum response to Me_2S at the following values: H_2, 125 ml/min; air, 90 ml/min; O_2, 9 ml/min. Using nitrogen carrier gas at a flow rate of 80 ml/min and a column temperature of 50°C, the retention time for Me_2S was about 30 sec. The choice of the OV-1 column was deliberate so as to achieve a very rapid analysis. In the analysis of the Me_2S headspace samples with the sulfur-specific detector the only appreciable peak observed was the Me_2S peak. Since the Me_2S peak was a very sharp spike, quantitation was by measurement of peak height. We did not consider it necessary to employ other columns which provided longer Me_2S retention time and high resolutions. For reactions with simple amino acids and peptides, it might actually be possible to operate with an empty column, as long as the sulfur-specific detectors were used. However, during analysis of food samples, levels of interfering sulfur compounds might be encountered which would necessitate the use of another column.

Measurement of Me_2S Evolved during the Oxidation of Amino Acids by Me_2SO. Reactions of amino acids with Me_2SO (Table III) were run in 125-ml sealed Wheaton serum bottles which had a total internal volume

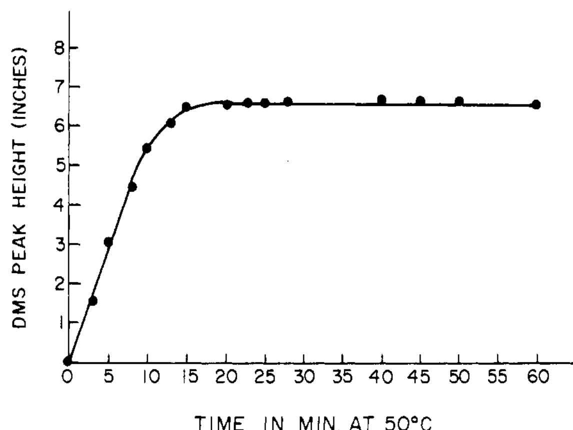


Figure 1. Dimethyl sulfide (Me_2S) formation at 50° C. Experimental conditions are described in the text.

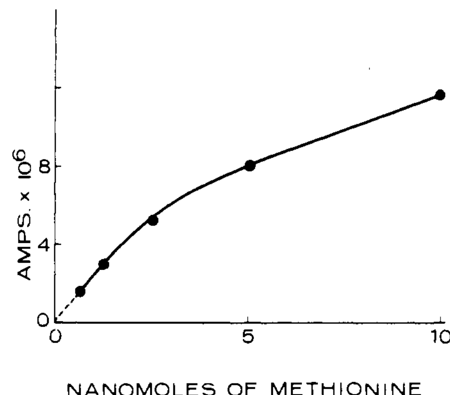


Figure 2. Response of flame photometric detector to headspace dimethyl sulfide at various levels of methionine reacted with dimethyl sulfoxide. Experimental conditions are described in the text.

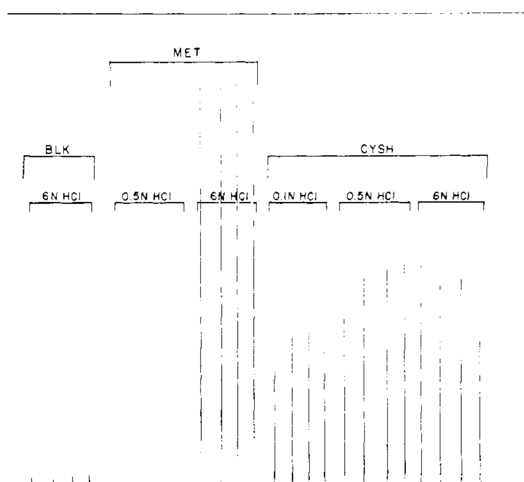


Figure 3. Photograph of recorder chart showing the flame photometric detector response to headspace injections following dimethyl sulfoxide oxidations of blank (BLK), methionine (MET), and cysteine (CYSH). Duplicate 100-μl injections were made from two replicates of each sample. Oxidations were done in 160-ml Wheaton serum bottles for 30 min at 50° C, as described in the text. The electrometer setting was $10^4 \times 64$ for the blanks and $10^4 \times 512$ for the other samples.

of 160 ml. The large bottle volume, as compared with the small 100-μl volume of headspace gas sample used for injection into the gas chromatograph, permitted good replication of successive samples withdrawn from the same bottle. This large bottle volume, combined with a low volume of liquid reactants, also favored complete escape of the hydrophobic Me_2S into the confined headspace gas. Pressure-Lok Series A gas syringes (Precision Sampling Co., Baton Rouge, La.) were used.

A typical procedure for the reaction was to pipet a 0.10-ml volume of the amino acid sample (dissolved in 6 *N* HCl) into a tilted serum bottle, which was then cooled in a dry ice bath in order to freeze the sample on one side of the bottle. A 0.10-ml volume of Me_2SO was then added (without contacting the sample) and frozen on the opposite side of the bottle. Subsequently, while the bottle was still in the ice bath, it was flushed free of air with a nitrogen stream and sealed by means of a special crimping tool, which tightened an aluminum collar around a rubber septum in the neck of the bottle. Incubation was in a water bath, with occasional rotation of the bottle, in order to thoroughly mix the contents. Various times and temperatures of incubation were used to determine the most suitable conditions for oxidation of methionine (Figure 1).

The relationship between detector response of the gas chromatograph and the level of methionine was studied in experiments in which the level of methionine added to a reaction bottle was varied from 1 up to 16 μmol. Since in each case the volume of headspace gas injected into the chromatograph was 100 μl, the sample corresponded to $^1/_{1600}$ of the methionine reacted in the 160-ml serum bottle. The response curve (Figure 2) thus corresponded to methionine levels of 0.625 to 10 nmol.

A comparison was made between the responses to dilutions made from weighed quantities of Eastman liquid Me_2S and response to headspace samples from weighed samples of methionine. The responses to the Me_2S standards were about 10% less than to the headspace samples of corresponding molar quantities of methionine. (The exact purity of the Me_2S was not determined and there may have been inaccuracy in the technique for diluting the Me_2S standard.) Weighed quantities of methionine were used as standards.

Specificity of Me_2SO for Oxidation of Methionine. Solutions of individual common amino acids (Table III) were made up in 6 *N* HCl at a concentration of 40 m*M*. Volumes of 0.10 ml (equivalent to 4 μmol) were allowed to react with 0.10-ml volumes of Me_2SO in 160-ml serum bottles in the manner which was described above. Since interference might be expected from common reducing sugars, the effectiveness of several of these and related compounds as reductants of Me_2SO was tested (Table IV).

The thiol amino acid cysteine was the subject of special attention. Although the oxidized form cystine did not appreciably reduce Me_2SO, cysteine was oxidized by Me_2SO under the conditions used for oxidation of methionine. (The ordinary amino acid calibration mixture contains no cysteine but only cystine.) Since food proteins might contain cysteine, lower HCl concentrations were investigated for distinguishing between the reaction of Me_2SO and thiols as compared with its reaction with methionine (Figure 3).

Reduction of Me_2SO by Methionyl Peptides. Glycyl-L-methionylglycine and the C-terminal octapeptide from glucagon were allowed to react with Me_2SO under the same conditions described for the amino acids, except that 0.20 ml each of 6 *N* HCl and Me_2SO was used for the oxidation of the octapeptide (Table VI). In each case 100-μl samples of headspace gas were injected into the gas chromatograph.

Oxidation of Other Sulfides by Me_2SO. The susceptibility of selected organic sulfides to oxidation by Me_2SO was tested (Table V) by running reactions of 4-μmol quantities of sulfide in 0.1 ml of 6 *N* HCl with 0.1

Table I. Requirement of Acid for Oxidation of Methionine by Dimethyl Sulfoxide (Me_2SO)

Acid component[a]	Time	Temp, °C	Yield of MetSO, %
None (H_2O)	5 min	100	0
1 *N* HCl	5 min	100	7
3 *N* HCl	5 min	100	89
6 *N* HCl	5 min	100	97
6 *N* HCl	2 hr	24	50
6 *N* HCl	5 hr	24	73
6 *N* HCl	21 hr	24	98
12 *N* HCl	5 min	24	95
48% HBr	5 min	24	90
48% HBr	5 min	100	96
85% H_3PO_4	5 min	100	0
6 *N* H_2SO_4	5 min	100	0.4
3 *N* *p*-toluenesulfonic	5 min	100	0
3 *N* HCl + 3 *N* H_2SO_4	5 min	100	95
100% acetic	5 min	100	0
88% formic	5 min	100	0

[a] The acid component (0.5 ml) was mixed with Me_2SO (0.5 ml) for oxidation of 5 mg of L-methionine. During the reaction the concentration of acid was thus one-half of the above stated values.

ml of Me2SO, by using the standard conditions of incubation described for the amino acids. Samples of butyl methyl sulfide, ethyl sulfide, biotin, tetrahydrothiophene, pentamethylene sulfide, homocystine, *S*-carboxymethylcysteine, and lanthionine were tested. With the conditions used, the butyl methyl sulfide and the diethyl sulfide were easily separated from the Me2S, so that the volatility of these samples did not confuse the interpretation of the results.

RESULTS AND DISCUSSION

Acid Requirements for Oxidation of Methionine by Me2SO. The data presented in Table I show that oxidation of methionine by Me2SO depends upon the presence of either HCl or HBr. Oxidation increased with an increase in the HCl concentration. (Oxidation also occurred in the presence of HI; HF was not tested.) Oxidation did not occur either in the absence of acid, or after substitution of the haloacid by a mineral acid such as sulfuric or phosphoric acid, or an organic acid, such as acetic or formic acids. The nonoxidizing strong acid *p*-toluenesulfonic acid was ineffective. Oxidation of methionine in the presence of 3 *N* HCl was not inhibited by sulfuric acid.

Although an explanation of the role of haloacid in the oxidation of methionine by Me2SO will not be attempted in this paper, its essentiality must be emphasized. As mentioned previously, organic sulfides could be oxidized by Me2SO in the absence of acid only under rather severe conditions (Searles and Hays, 1958). Oxidation of methionine by Me2SO in the presence of HCl occurred rapidly and was almost complete within 5 min at 100°C (Table I). The reaction was quite selective for forming the sulfoxide, since under the mild conditions used, only traces of sulfone were obtained and homocysteic acid could not be detected.

Reversal of the Reaction. The experiments summarized in Table II clearly demonstrated that reduction of MetSO to methionine occurred readily when MetSO was confined in a sealed tube with an excess of Me2S in the presence of HCl. This reduction of MetSO by Me2S was promoted by increasing the HCl concentration; it did not occur in the absence of a haloacid. The reversibility of the reaction supports the view that the reaction is a simple oxygen exchange which is catalyzed by hydrogen halides.

Oxidation of Methionyl Residue of a Peptide. With a view toward the possible use of Me2SO as an analytical reagent for measurement of methionine without protein hydrolysis, the oxidation of the simple tripeptide glycyl-L-methionylglycine was carried out with examination of the products on the amino acid analyzer. The oxidized tripeptide was eluted as a single new peak at 132 min on the 60-cm column, about 7 min after the elution position of methionine. This peak was apparently the tripeptide sulfoxide; there was no contamination by free glycine or methionine or by other peptides. By analysis of the alkaline hydrolysate of this tripeptide sulfoxide on the amino acid analyzer, glycine and MetSO peaks were observed at a molar ratio of approximately 3 to 1 instead of the theoretical 2 to 1. Partial destruction of MetSO was thus indicated to have occurred during alkaline hydrolysis. Although MetSO has been considered to be stable to alkaline hydrolysis (Neumann, 1972), this stability to alkali has been questioned (Lunder, 1972).

Table II. Reduction of Methionine Sulfoxide (MetSO) to Methionine (Met) by Dimethyl Sulfide (Me_2S)[a]

Acid	Conditions	Final composition, %: MetSO	Met
1 *N* HCl	5 min at 100°C	95	5
3 *N* HCl	5 min at 100°C	40	60
6 *N* HCl	3 min at 100°C	7	93
12 *N* HCl	5 min at 24°C	1	99

[a] Reactions were run in sealed tubes by using 5 mg of MetSO dissolved in 0.9 ml of acid solution to which 0.1 ml of liquid Me_2S was added. A 10-μl sample was assayed on the amino acid analyzer.

Gas Chromatographic Experiments. Although Me2SO has found wide use as a selective oxidant (see Durst, 1969; Epstein and Sweat, 1967), we have found no previous attempt to follow its reduction through the measurement of Me2S formation. Despite the technical difficulties associated with analysis of headspace gases, our interest in applying this approach for measurement of methionine was derived from recognition of the drastic differences in chemical properties between the high-boiling (189°C), hydrophilic solvent Me2SO and its low-boiling (38°C), hydrophobic Me2S reduction product. The solvent properties of Me2SO appeared to be ideal for reaction with samples in aqueous medium while the physical character of the Me2S reduction product would favor its quick expulsion from aqueous medium into the headspace gas.

The 50:50 mixture of Me2SO and 6 *N* HCl was adapted from the experiments with the amino acid analyzer for use in the gas chromatographic experiments. The high levels of Me2SO were intended to drive the oxidation reaction to completion, although in the initial observation of methionine oxidation only traces of Me2SO had been used (Lipton and Bodwell, 1973). As mentioned in the Experimental Section, the deliberate use of small volumes of liquid reactants in large bottles was also intended to favor evolution of the Me2S into the gas phase.

Me2S Formation Curve and Relationship of Detector Response and Me2S Level. Data obtained by periodic sampling (during an oxidation of methionine by a 50:50 mixture of Me2SO and 6 *N* HCl at 50°C) are plotted as the formation curve (Figure 1). The Me2S release into the headspace gas was rapid and reached a maximal level at about 15 min. This maximal level was maintained for the 1-hr duration of the experiment. This experiment was the basis for the selection of the standard conditions of incubation at 50°C for 30 min. Under these conditions, there was no appreciable increase during 1 hr in the Me2S formed in the blank. Although Me2S formation was more rapid at the higher temperatures, use of

Table III. Specificity of Dimethyl Sulfoxide (Me_2SO) as an Amino Acid Oxidant

Sample[a]	Dimethyl sulfide peak height, A × 10^8
Blank	1.47
L-Serine	0.96
L-Tyrosine	1.22
L-Cystine	1.28
L-Methionine	449
Blank	0.96
L-Arginine	1.09
L-Tryptophan	1.98
L-Histidine	1.22
L-Methionine	425

[a] In each case, oxidation of 4 μmol of amino acid was done for 30 min at 50° C. The amino acid dissolved in 0.1 ml of 6 *N* HCl was mixed with 0.1 ml of Me_2SO in a 160-ml Wheaton serum bottle, in a nitrogen atmosphere. A 100-μl sample of headspace gas was injected into the gas chromatograph.

higher temperatures resulted in appreciable increases in the reagent blanks.

Figure 2 shows the detector response plotted vs. level of methionine, over the range of 0.625 to 10 nmol. The detector response is not linear, although in the range from 0.625 to 2.5 it does not decline greatly. (The characteristics of the flame photometric detector response to various sulfur compounds have been reported by Mizany, 1970.) The detector response at the 2.5-nmol level was about 4.5 × 10^{-6} A; since the maximum sensitivity of the electrometer was 10^{-12} A, it is obvious that much lower levels are detectable. (For analyses of samples of very limited supply, one could also use smaller serum bottles and smaller volumes of headspace gas.)

Specificity of Me_2SO for the Amino Acid Methionine. The treatment of individual amino acids with Me_2SO and HCl and the measurement of the detector response to evolved Me_2S indicated that Me_2SO was quite specific for methionine. In the experiments summarized in Table III, individual amino acids of the common calibration mixture which might be expected to be oxidized by Me_2SO were tested. These included serine and tyrosine, each of which contains a hydroxyl group, and histidine, tryptophan, and arginine, which undergo losses during some amino acid analysis procedures. The high specificity for methionine was confirmed, as these amino acids yielded less than 0.5% of the Me_2S peak height observed from an equal level of methionine. (Results with threonine closely resembled those obtained with serine.) Me_2S formation was in fact no higher than in a reagent blank. Cystine and tyrosine were not oxidized under these mild conditions of 50°C for 30 min in contrast with their previously reported (Lipton and Bodwell, 1973) oxidation under more severe conditions.

The results of reacting some carbohydrates and related compounds with Me_2SO and HCl are summarized in Table IV. The hexoses glucose and fructose did not have much reducing power toward Me_2SO, even when tested at 10 times the methionine concentration. The more powerful reducing compound fructose–glycine (Hagan et al., 1970) was also found to be ineffective as a Me_2SO reductant. However, ascorbic acid was found to reduce Me_2SO to a considerable extent. Although many protein foods do not have levels of ascorbic acid which would interfere, one would have to know the ascorbic acid contents before attempting to use Me_2SO as a methionine reagent for some foods. Alternatively, one might take advantage of the high lability of ascorbic acid to eliminate its interference by a preliminary mild alkaline heat treatment.

Table IV. Reduction of Dimethyl Sulfoxide to Dimethyl Sulfide (Me_2S) by Glucose, Fructose, Fructose-Glycine and Ascorbic Acid

Compound	μmol[a]	Me_2S peak height, A × 10^8
D-Glucose	40	5.12
D-Fructose	40	1.02
D-Fructose-glycine	40	0.67
Ascorbic acid	4	88.8
Ascorbic acid (no HCl)	4	0.014
Blank	0	0.83
L-Methionine	4	465

[a] Levels of compound in the 160-ml serum bottle, conditions were the same as for oxidation of the amino acids. A 100-μl volume of headspace gas was injected into the gas chromatograph.

Table V. Susceptibility of Selected Sulfides to Oxidation by Dimethyl Sulfoxide

Compounds oxidized[a]	Compounds not oxidized
Butyl methyl sulfide	Homocystine
Diethyl sulfide	*S*-Carboxymethylcysteine
Biotin	Lanthionine
Tetrahydrothiophene	
Pentamethylene sulfide	

[a] Oxidation conditions were 30 min at 50° C, as detailed in the text. Oxidation was measured by dimethyl sulfide formation.

Reduction of Me_2SO by Cysteine. Because mercaptoethanol has been used for the reduction of MetSO to methionine during the hydrolysis of proteins (Keutmann and Potts, 1969), we expected reduction of Me_2SO by the thiol amino acid cysteine. The experiments illustrated in Figure 3 demonstrated the reduction of Me_2SO by cysteine as well as the means to distinguish between the behavior of cysteine and methionine to Me_2SO. When the concentration of the HCl was reduced from 6 to 0.5 *N*, there was no discernible reduction of Me_2SO by methionine, while cysteine reduced Me_2SO as efficiently as at the higher acid concentration. Thus a simple correction could be made for cysteine content by running the reaction in 0.5 *N* HCl. (It should be noted that Me_2SO headspace analysis of Me_2S thus also offers the possibility of a rapid determination of cysteine by a gas chromatographic procedure.) Reduced glutathione was found to reduce Me_2SO in a way which was similar to reduction of Me_2SO by cysteine.

The data of Figure 3 also serve to illustrate the reproducibility of this technique of headspace analysis with detector response; there was little variation between successive injections or between replicate bottles.

Oxidation by Me_2SO of Other Sulfides. Although methionine and cystine are respectively the most abundant sulfide and disulfide constituents which are present in most foods, it was nevertheless of interest to see whether Me_2SO and HCl might oxidize other sulfides as well as methionine. As the reagent was quite selective in yielding the sulfoxide without forming sulfones or other oxidation products, Me_2SO and HCl might find wider use as a general reagent for preparation of organic sulfoxides. Interest in this possibility was derived from the previous use of Me_2SO as an oxidant (Searles and Hays, 1958) under rather severe conditions. A summary of some selected compounds tested, by observation of the formation of a Me_2S reduction product, is given in Table V. Most of the simple sulfides, whether open chain or cyclic, were oxidized by Me_2SO under the same conditions used for the oxidation of methionine. However, the sulfides lanthionine and *S*-

Table VI. Reduction of Dimethyl Sulfoxide (Me_2SO) to Dimethyl Sulfide (Me_2S) by Methionyl Residues of Peptides

Sample	Me_2S peak height, A × 10^8
Blank[a]	1.4
L-Methionine[a]	435
Glycyl-L-methionylglycine[a]	428
Blank[b]	2.6
L-Methionine[b]	467
C-Terminal octapeptide from glucagon[b]	402

[a] Conditions and levels of amino acid or peptide were the same as in Table III. [b] Instead of 0.1-ml volumes of 6 *N* HCl and Me_2SO which were used above, 0.2-ml volumes were used for reactions containing 4 μmol of amino acid or peptide per bottle. The octapeptide was analyzed, assuming 1 methionyl residue per mole and a 100% purity on a weight basis. However, the sample was only about 80% pure (see text).

carboxymethylcysteine were not appreciably oxidized. The presence of carboxyl groups on both sides of the sulfur apparently protected it from oxidation by Me_2SO. The disulfide amino acid homocystine, like cystine, resisted oxidation as well. Since the oxidation of a disulfide without carboxyl groups was not attempted, it cannot be stated that all disulfides are resistant to oxidation by Me_2SO.

Me_2SO Reduction by Methionyl Residues of Peptides. The data of Table VI summarize observations made on the reduction of Me_2SO by a methionyl tripeptide and an octapeptide. Headspace Me_2S peak height observed for the tripeptide closely corresponded to that found for the same level of crystalline methionine. For the octapeptide sample (assumed to be 100% pure), the evolved Me_2S was somewhat less than from the methionine control. However, amino acid analysis of this sample indicated a purity of only 80% based on a content of 1 methionyl residue per mole of peptide. These results thus confirm the earlier amino acid analyzer study of the tripeptide, and demonstrate the reactivity of the methionyl residue with the Me_2SO reagent.

Potential Application of Me_2SO as a Reagent for Methionine. The ultimate objective of the current investigations is to develop a chemical assay for nutritionally available methionine which would be faster and more economical than the existing biological methods (see Shorrock and Ford, 1973, and citations therein). Interest in such a method for methionine is especially great since this sulfur amino acid limits the nutritional value of many common foods and feeds, other than cereal grains (Miller and Donoso, 1963; Swendseid and Wang, 1970). Even though an established quantitative method has not been developed, it is in response to interest in such a method that the novel approach of using Me_2SO as a methionine oxidant is reported. The results described offer encouragement for further study of this approach for the measurement of methionine. The accompanying paper (Lipton and Bodwell, 1976) suggests an alternative approach for methionine measurement which involves the formation and decomposition of a sulfonium derivative.

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LITERATURE CITED

Durst, T., *Adv. Org. Chem.* **6**, 643 (1969).
Epstein, W. W., Sweat, F. W., *Chem. Rev.* **6**, 247 (1967).
Hagan, S. N., Horn, M. J., Lipton, S. H., Womack, M., *J. Agric. Food Chem.* **18**, 273 (1970).
Horn, M. J., Jones, D. B., Ringel, R. J., *J. Biol. Chem.* **138**, 141 (1941).
Keutmann, H. T., Potts, J. T., Jr., *Anal. Biochem.* **29**, 175 (1969).
Lipton, S. H., Bodwell, C. E., *J. Agric. Food Chem.* **21**, 235 (1973).
Lipton, S. H., Bodwell, C. E., *J. Agric. Food Chem.*, following paper in this issue (1976).
Lipton, S. H., Bodwell, C. E., Dutky, R. C., 168th National Meeting of the American Chemical Society, Atlantic City, N.J., Sept 1974, Abstract AGFD-9.
Lunder, T. L., *Anal. Biochem.* **49**, 585 (1972).
Miller, D. S., Donoso, G., *J. Sci. Food Agric.* **14**, 345 (1963).
Mizany, A. I., *J. Chromatogr. Sci.* **8**, 151 (1970).
Neumann, N. P., *Methods Enzymol.* **25**, 393–400 (1972).
Searles, S., Hays, H. R., *J. Org. Chem.* **23**, 2028 (1958).
Shorrock, C., Ford, J. E., *Proteins Hum. Nutr. Proc. NATO Adv. Study Inst.*, 207–233 (1973).
Spencer, R. L., Wold, F., *Anal. Biochem.* **32**, 185 (1969).
Swendseid, M. E., Wang, M., in "Symposium: Sulfur in Nutrition", Muth, O. H., and Oldfield, J. E., Ed., Avi Publishing Co., Westport, Conn., 1970, pp 209–221.

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