Studies of Methionine Sulfoxide. I. The Acidic Degradation of Methionine Sulfoxide

By Kensaku MORIHARA

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Although biochemical attention[1-3] has recently been focussed on methionine sulfoxide (MSO), the chemical properties of this amino acid have not been sufficiently investigated. It is well-known that MSO is converted to methionine by the action of hydrochloric acid under the usual conditions for the acidic hydrolysis of proteins with hydrochloric acid, and it is also known that on storage of a hydrochloric acid solution of methionine (Met), the formation of MSO by oxidation occurs in air.

This action of hydrochloric acid upon sulfoxide is unusual and has been reported in only a few cases. In the case of MSO, the reaction does not seem to be a simple reduction, as Reiner et al. observed in the course of their studies of the agene factor of wheat flour. They observed that MSO yielded two additional ninhydrin positive spots besides Met on a paper chromatogram after acidic hydrolysis.[4]

In the present work, in order to investigate the mechanism of the degradation and chemical properties of this amino acid through the action of hydrochloric acid upon it, the degradation products will be isolated.

On treating MSO with 12 N hydrochloric acid at 105°C in a sealed tube for 20 hr., seven ninhydrin positive spots were observed on two-dimensional chromatograms. The hydrolyzate was submitted to column chromatography, and four of the seven products were isolated and identified as methionine, homoserine, homocystine and homolanthionine.

Results and Discussion

Two-Dimensional Paper Chromatography.— The reaction mixture obtained by the hydrolysis of 31.7 mg. of DL-MSO with 2 ml. of 12 N hydrochloric acid in a sealed tube was treated with the cation exchange resin, Amberlite IR-120 (H^+) in the usual manner in order to remove hydrogen chloride. The resulting mixture of reaction products was submitted to two dimensional paper chromatography. As may be seen in Figs. 1 and 2, seven ninhydrin-positive spots were observed on the chromatograms and the main product, spot A, appeared to be Met judging from its R_f values. Spot B seemed rather unstable, and its location and size varied according to the reaction condition. The size of this spot B decreased with the reaction period, whereas the sizes of spots C, D and E increased with the reaction time. This must indicate that the compound giving spot B was a precursor of the substances in spots C, D and E. Spot B seemed to be too unstable and spots F and G were too small to attempt their identification. Therefore, the isolation of only the substances corresponding to spots C, D and E was carried out.

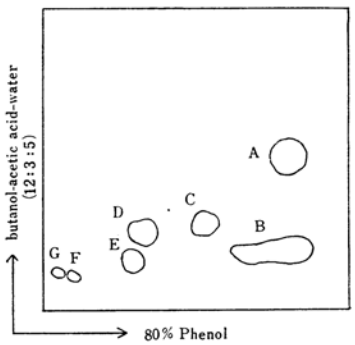

Fig. 1. Two-dimensional chromatogram I.

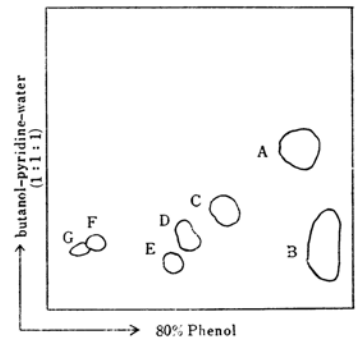

Fig. 2. Two-dimensional chromatogram II.

1) S. Black, E. M. Harte, B. Hudson and L. Wartofsky, *J. Biol. Chem.*, **235**, 2910 (1960).
2) J. F. Thompson, C. J. Morris and R. M. Zacharius, *Nature*, **178**, 593 (1956).
3) T. Sugawa, H. Akedo and M. Suda, *J. Biochem. (Japan)*, **47**, 131 (1960).
4) L. Reiner, F. Misani, T. W. Fair, P. Weiss and M. G. Cordasco, *J. Am. Chem. Soc.*, **72**, 2297 (1950).

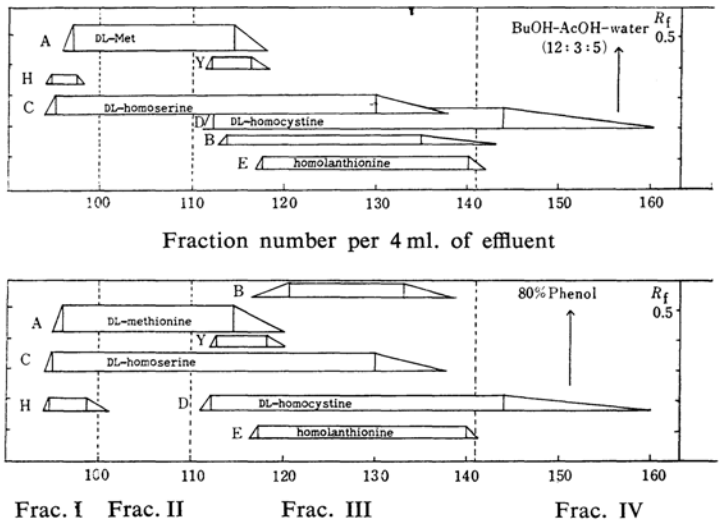

Fig. 3. Displacement chromatography of the hydrolyzate.

Elution Chromatographic Separation. — To isolate the reaction products from the hydrolyzate of 5 g. of DL-MSO, fractionation was carried out by displacement chromatography on a column of the Amberlite CG-120 type II with 0.5 N ammonia and then by gradient chromatography with 4 N hydrochloric acid, following the procedure of Hirs et al.[5] with some modifications. Aliquots of each fraction were analyzed by one dimensional paper chlomatography. The results are summarized in Figs. 3, 4 and 5. The five spots of Fig. 1, spots A, B, C, D and E, correspond to five zones of Fig. 3, zones A, B, C, D and E respectively. However, the zones corresponding to spots F and G were not obtained, and, on the other hand, a new zone H appeared which did not correspond to any spot in Fig. 1. These products were too small in quantity to be identified. Moreover, unknown ninhydrin-negative yellow zone was observed, as is shown in Fig. 3.

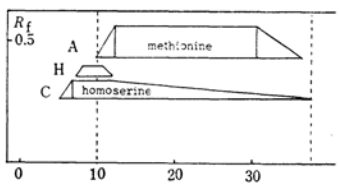

Fig. 4. Rechromatography of fraction II.

Products Isolated and Identified. — DL-Met, corresponding to spot A, was isolated as crystals from fractions II-[2] and III-[4]; DL-homoserine, corresponding to spot C, from fraction III-[2]; DL-homocystine, corresponding to spot D, from fraction IV, and homolanthionine, corresponding to spot E, from fraction III-[6].

From the other fractions, though they contained ninhydrin-positive materials, no pure product was isolated.

The Mechanism of Degradation.—From the results obtained, the route of the degradation of MSO seems to be complex; the most plausible one is drawn in Fig. 6. Route A is the ordinary route of the reduction of MSO to Met.

The initial products in route B may be supposed to be methane sulfinic and α-amino-β,γ-dehydro butyric acid if an elimination reaction takes place, or methane sulfinic acid and homoserine, if hydrolytic splitting occurs.

However, according to Ostemayer et al.[6], *S*-methylcysteine sulfoxide is split into methane sulfinic acid and dehydroalanine, the latter being further decomposed to ammonia and pyruvic acid in hot dilute sulfuric acid. Therefore, we suggest that an analogous reaction series occurs in the case of MSO and that it is reasonable to assume that α-amino-β,γ-dehydro butyric acid is an intermediate. Route C is the rearrangement of the sulfoxide to yield homocysteine and formaldehyde, and the resulting homocysteine can be easily converted to homocystine by air oxidation.

The formation of homolanthionine is explained by the addition of homocysteine to α-amino-β,γ-dehydro butyric acid.

When MSO was treated with 6 N hydrochloric acid for 12 hr., only one ninhydrin-positive

5) H. W. Hirs, S. Moore and M. W. Stein, *J. Am. Chem. Soc.*, **76**, 6063 (1954).

6) F. Ostermayer and D. S. Tarbell, *J. Am. Chem. Soc.*, **82**, 3752 (1960).

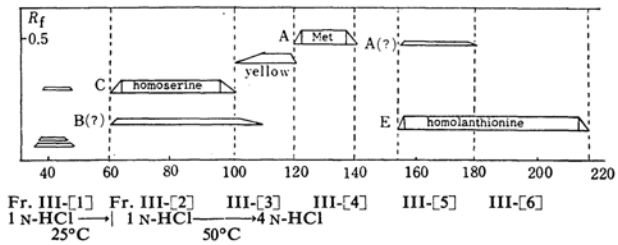

Fig. 5. Gradient chromatography of fraction III.

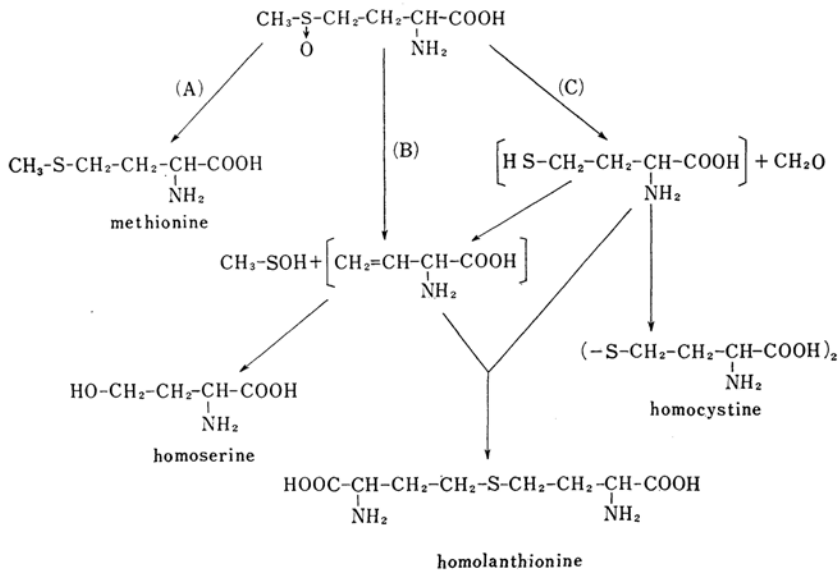

Fig. 6

spot of Met was detected on the paper chromatogram, showing that only the reduction of route A took place, and that only when it is treated with 12 N hydrochloric acid the complex reactions of routes B and C take place, thus giving rise to the above-mentioned products. Therefore, the fact that these products have not been detected in the usual hydrolyzate of protein with 6 N hydrochloric acid does not completely exclude the possibility of the presence of a MSO residue in protein molecules.

Experimental

Materials.—DL-Methionine sulfoxide was prepared by the method of Toenis et al.[7] with the slight modification of using more methanol and less water. M. p. 186°C (decomp.).

Found: C, 36.35; H, 6.89; N, 8.56. Calcd. for $C_5H_{11}O_3NS$: C, 36.35; H, 6.71; N, 8.48%.

Concentrated hydrochloric acid prepared by the saturation of distilled water with hydrogen chloride gas was used first, but later concentrated hydrochloric acid, special grade, purchased from Wakō Pure Chemical Industries, Ltd., was used, as it was proved to give the same result.

Hydrolysis and Chromatographic Separation.—Five grams of DL-methionine sulfoxide were dissolved in 30 ml. of concentrated hydrochloric acid, and the mixture was heated in a sealed tube at 105°C for 20 hr. The hydrolyzate was then brought to dryness and transferred to a column of the Amberlite CG-120 type II (H^+ form), 1.5 cm.×25 cm. Distilled water was passed through the column until the effluent became neutral. Then the absorbed hydrolytic products were eluted with 0.15 N ammonium hydroxide at 60°C, and the effluent was collected in 4 ml. fractions. An aliquot of each fraction was submitted to one dimensional chromatography (BuOH : AcOH : Water—12 : 3 : 5) in order to analyze the components. Fraction II was rechromatographed in the same way.

For the further fractionation of fraction III, the chromatographic system described by Hirs et al.[5] was employed with some modifications. A shorter column than that described by Hirs et al. (40 cm. ×2 cm.) was used, and Amberlite CG-120 type II was employed instead of Dowex 50×4. The column was pretreated with 10 volumes of 1 N hydrochloric acid.

7) G. Toenis and J. J. Kolb, *J. Biol. Chem.*, **128**, 399 (1939).

Fraction III was brought to dryness and dissolved in a few milliliters of 1 N hydrochloric acid, and the solution was transferred to the column. The chromatography was carried out by elution with 1 N hydrochloric acid at a room temperature of 25°C, and the effluent was collected in 4 ml. fractions. When 324 ml. of effluent had been eluted, the concentration of hydrochloric acid was gradually increased from 1 to 4 N using a mixing chamber of 200 ml., and the temperature of the column was raised and kept at 50°C. Each fraction was dried, and the components were analyzed by one-dimensional paper chromatography. As may be seen in Fig. 5, homocystine was not eluted under these conditions.

DL-Methionine.—Fraction II-[2] was decolorized with active charcoal and concentrated under reduced pressure until it was almost dry. The ethanol was added, and the resulted solution was allowed to stand. The precipitate formed was collected by filtration and recrystallized from water and ethanol. The recrystallization was repeated until the contaminating homoserine and another ninhydrin-positive material had been removed. The crystals formed were identical in their infrared spectrum with authentic methionine. From fraction III-[4], pure methionine was also isolated without decolorization after the fraction had been treated with ion exchange resin to remove the hydrogen chloride.

DL-Homoserine. — Fraction III-[2] was concentrated under reduced pressure and treated with cation exchange resin to remove the hydrogen chloride in the usual manner. The ammoniacal effluent was concentrated, ethanol was added to this solution until the cloudy point was obtained, and then the solution was allowed to stand. The resulting precipitate was collected and recrystallized from water and ethanol.

Found: C, 39.68; H, 7.53; N, 11.04. Calcd. for $C_4H_9O_3N$: C, 40.33; H, 7.62; N, 11.76%.

The infrared spectrum of this material and its R_f values in two solvent systems, *n*-butanol - acetic acid - water (12 : 3 : 5) and 80% aqueous phenol, were identical with those of authentic L-homoserine obtained from the Kyowa Hakko Kogyo Co., Ltd.

DL-Homocystine. — Fraction IV was treated in virtually the same way as methionine, and an amino acid was isolated. M. p. 258°C.

Found: C, 35.43; H, 6.60; N, 9.50. Calcd. for $C_8H_{16}O_4N_2S_2$: C, 35.82; H, 6.01; N, 10.45%.

Though the value of the nitrogen content was less for homocystine, the chromatographic behavior and infrared spectrum of this material were identical with those of authentic DL-homocystine perchased from Wakō Pure Chemical Industries Co., Ltd.

Homolanthionine. — An amino acid was isolated from fraction III-[6] in virtually the same way as homoserine. In this case, decolorization was necessary as the ammoniacal effluent was acompanied with an unknown red violet material. M. p. 270°C (decomp.).

Found: C, 40.25; H, 6.97; N, 11.92. Calcd. for $C_8H_{16}O_6N_2S$: C, 40.67; H, 6.83; N, 11.86%.

The authentic homolanthionine prepared from DL-menthionine and DL-homoserine according to the procedure described by Stekol[8] was found to contain three components on paper chromatograms, DL-homolanthionine, meso-homolanthionine and a trace of unchanged DL-methionine. However, the infrared spectrum of the isolated amino acid was identical with that of a mixture of DL and meso-homolanthionine, and its R_f values in two solvent systems, *n*-butanol - acetic acid - water (12 : 3 : 5) and 80% aqueous phenol, were identical with those of one component of the mixture. Therefore, the amino acid isolated must be an isomer of homolanthionine.

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Division of Organic Chemistry
Institute for Protein Research
Osaka University
Kita-ku, Osaka

8) J. A. Stekol, *J. Biol. Chem.*, **173**, 153 (1948).