Studies of Methionine Sulfoxide. II. The Transformation of Methionine Hydantoin Sulfoxide

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During our study[1] of the acidic degradation of methionine sulfoxide (MSO), the products homocystine, homolanthionine, homoserine and methionine (Met) were isolated. The formation of homocystine and homolanthionine indicated that the demethylation of MSO occurs much more easily than that of Met, for Met does not give the above-mentioned products under these conditions. Moreover, it may be assumed that this demethylation is due to the conversion of the sulfoxide group to give *S*-hydroxymethyl-homocysteine.

Smythe studied the action of some kinds of hydrogen chloride on dibenzyl sulfoxide in detail and suggested that there was a dynamic tautomerism between the sulfoxide and the corresponding α-oxy-sulfide during the reaction.[2] Later, Horner et al. demonstrated that several sulfoxides were converted to the corresponding α-acyloxy-sulfoxides in a good yield by the action of acid anhydride.[3] Therefore, it is reasonable to assume that such a rearrangement occurs to yield *S*-hydroxymethyl-homocysteine derivatives from MSO prior to demethylation, as has already been reported in the previous paper.[1] This transformation of sulfoxide to α-hydroxy-sulfide radicals is very interesting with regard to the biochemical role of sulfoxides, especially that of MSO.[4]

In this paper, it will be demonstrated that the quantitative transformation of methionine hydantoin sulfoxide (MHSO) occurs through the action of acetic anhydride, yielding the corresponding α-acetoxy-sulfide, the structure of which will be determined mainly from the fact that formaldehyde is quantitatively released from the product when it is hydrolyzed with acid or alkali.

Results and Discussion

The Transformation of MHSO. — The substrate of this reaction, MHSO, was prepared as white crystals by the oxidation of DL-methionine hydantoin (MH) with hydrogen peroxide. MHSO is a strongly polar substance, as it is freely soluble in water, slightly soluble in alcohol, and insoluble in other non-polar solvents, while MH is freely soluble in non-polar solvents but insoluble in water. MHSO was used instead of MSO in this study because when MSO was treated just as MHSO with acetic anhydride, the color of the reaction mixture turned red-brown, showing that an undesirable side reaction, mainly due to the formation of azlactone, took place. On the other hand, when a suspension of MHSO (1.74 g.) in acetic anhydride (3 ml.) was heated under reflux on an oil-bath, it was resolved in a few minutes, and the color of the reaction mixture turned only slightly yellow, showing that no side reaction occurred. The reaction was allowed to proceed for about an hour, and then excess water was added to decompose the unchanged acetic anhydride. The resulting mixture was concentrated under reduced pressure to remove the acetic acid and water. The residue was then extracted with ethyl acetate, an the extract washed with water and dried on sodium sulfate. When the ethyl acetate was removed under reduced pressure, a yellow syrup was obtained (about 2.0 g.). This material was quite different from MHSO; it was insoluble in water and easily soluble in ethyl acetate, showing, as expected, that the strong polarity of MHSO had been lost by the action of acetic anhydride to give the corresponding α-acetoxy-sulfides, as is shown in Fig. 1. Only when the reaction was stopped within a few minutes was the starting MHSO recovered from the aqueous layer.

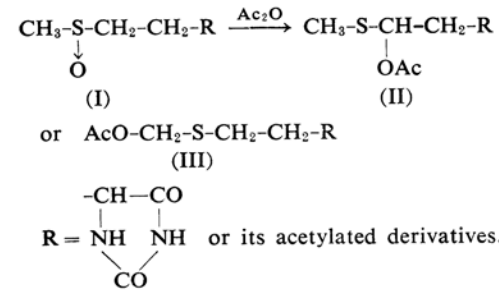

Fig. 1

1) K. Morihara, This Bulletin, **37**, 1781 (1964).
2) J. A. Smythe, *J. Chem. Soc.*, **95**, 349 (1909).
3) L. Horner and P. Kaiser, *Annal. D. Chem.*, **626**, 19 (1959).
4) S. Oae, T. Kitao, S. Kawamura and Y. Kitaoka, 35th General Meeting of the Japanese Biochemical Society, September, 1962.

The Structure of the Product.—Attempts to purify and crystallize the product in order to determine its structure were unsuccessful. However, from the results of Horner et al. it seems that the structure is either II or III.

Desulfurization with Raney nickel[5] was carried out to see whether the structure was II or III. If it was II, homoserine derivatives should be formed, while if it is III, α-amino-butyric acid derivatives may be expected by this treatment. A small portion of the syrup was treated with Raney nickel for about 3 hr. The catalyst was then filtered off, and the filtrate was hydrolyzed with 6 N hydrochloric acid. The hydrolyzate was evaporated to dryness and submitted to paper chromatography.

Only one ninhydrin-positive spot was detected, and this had the same R_f value as an authentic sample of α-amino-butyric acid, showing that only III was formed when MHSO was treated with acetic anhydride. Furthermore, it was expected that formaldehyde should be formed from III or methyl mercaptan from II when the syrup was hydrolyzed with acid or alkali. Small portions of the syrup was transferred to Kjehldahl distilling tubes, together with 6 N sulfuric acid, or to 3 N sodium hydroxide, and the samples were submitted to steam-distillation for 15 min. The distillate was neutralized with sodium bicarbonate when it had been treated with sulfuric acid. To the distillates was added a 0.4% aqueous solution of dimedone, and the mixtures were allowed to stand overnight. White needle-shaped crystals were obtained; they melted at 189°C and were identified as formaldimedone[6] by infrared spectroscopy. This also showed that III was the product, and, though a slight mercaptan-like odor was detected in the distillate, no aldehyde was detected with Schiff's reagent in the residual mixture. In the same way, when the reaction mixture of MHSO, treated with acetic anhydride and then by hydrolysis with sulfuric acid or sodium hydroxide, was submitted to steam distillation, formaldehyde was also caught directly as the dimedone derivative.

The Recovery of Formaldehyde.—To confirm the above results, the formaldehyde released from MHSO was determined quantitatively. Five samples of MHSO, each about 100 mg., were boiled with 2 ml. of acetic anhydride for 3, 10, 30, 60 and 180 min. respectively, while another sample of about 100 mg. of methionine hydantoin (MH), was treated in the same way, for 180 min., as a control. The reaction was stopped by then addition of 1 ml. of water to each reaction mixture. Then to each was added 5 ml. of 6 N sulfuric acid, and the solutions were distilled for 15 min. with steam as has been described above. The distillates were received in ice-cooled Erlenmeyer flasks containing sodium bicarbonate roughly equivalent to 2 ml. of acetic anhydride suspended in 10 ml. of water. The distillate was neutralized with sodium bicarbonate or acetic acid, and then the distilled formaldehyde was determined by iodometry by the direct bisulfite method.[7] The results are shown in Table I. The quantitative liberation of formaldehyde from MHSO was observed during the first 30 min. of the reaction period. This confirms that the product of the rearrangement is not II but III, and it also suggests that this rearrangement is essentially useful for the determination of MSO and its derivatives. When MSO was treated by the same procedure as MHSO, the reaction mixture turned red-brown; formaldehyde was also distilled off and trapped as the dimedone derivative, but its recovery was low and not quantitative, and, furthermore, this results was so reproducible as that with MHSO.

TABLE I. QUANTITATIVE LIBERATION OF FORMALDEHYDE FROM MHSO

Substrate mg. (μ mol.)	Reaction period	Formaldehyde found μ mol.	Recovery %
MHSO 99.5 (523)	3	112	21.2
100.2 (527)	10	417	92.2
101.7 (534)	30	533	100
101.2 (532)	60	525	99
100.8 (531)	180	539	101
MH 99.6 (573)	180	0	0

In conclusion, although this procedure is not suitable for the determination of MSO for the above-mentioned reason, it may be practically useful for the determination of MHSO itself, even if the iodometric titration by the direct bisulfite method is somewhat troublesome and time-consuming.

Experimental

The Preparation of DL-Methionine Hydantoin (MH) and Its Sulfoxide (MHSO).—DL-Methionine hydantoin (MH) was prepared by boiling DL-methionine with an aqueous urea solution in the usual manner.[8] M. p. 94°C.

Found: C, 41.43; H, 5.63; N, 16.25. Calcd. for $C_6H_{10}O_2N_2S$: C, 41.38; H, 5.79; N, 16.09%.

Seventeen and a half grams (0.1 mol.) of DL-methionine-hydantoin was dissolved in 200 ml. of methanol while being gently warmed. This solution was then cooled, and 15 ml. of a (30%) hydrogen

5) K. Vogler, *Helv. Chim. Acta*, **30**, 1766 (1947).
6) D. Vorländer, *Ber.*, **58B**, 2656 (1925).
7) L. H. Dounally, *Ind. Eng. Chem. Anal. Ed.*, **25**, 91 (1933).
8) F. Lippich, *Ber.*, **41**, 2974 (1908).

peroxide solution was added drop by drop over about a 30 min. period. The reaction temperature was kept below 20°C by cooling, and the solution was allowed to stand for a further 3 hr. Then the mixture was concentrated to 20~30 ml. under reduced pressure at below 40°C. One hundred milliliters of benzene was added, and the resulting azeotropic mixture was distilled off to remove any traces of water. This procedure was repeated twice, and on concentration the mixture became cloudy and the crystalline product precipitated. About 40 ml. of acetone was added in small portions to precipitate the crystalline product completely. White crystals were collected by filtration and washed first with acetone and then with a small amount of ether. The yield of the crude product was 19 g. Nineteen grams of the product was suspended in 19 ml of water, resolved by warming, and filtered. Then 250 ml. of acetone was added in small portions while the solution was being cooled, and the mixture was stored in a refrigerator overnight. The fine white crystals obtained were washed with a small amount of acetone and then with ether. The yield was 15.75 g.; M. p. 156~157°C (corr.)

Found: C, 37.46; H, 5.27; N, 14.42. Calcd. for $C_6H_{10}O_3N_2S$: C, 37.90; H, 5.30; N, 14.73%.

This substance is soluble in water and hot methanol. It is slightly soluble in hot ethanol but insoluble in methanol, ethanol, acetone, ethyl acetate and ether.

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