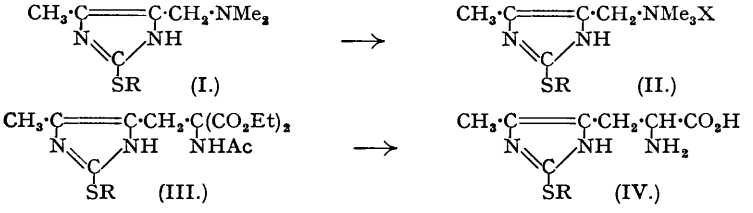
490. 2-*Mercaptoglyoxalines. Part III.* Synthesis of* 2-*Mercapto*-4(5)-*methylhistidine and* 4-*Methylergothioneine.*

By H. Heath, Alexander Lawson, and C. Rimington.

5(4)-Dimethylaminomethyl-2-mercapto-4(5)-methylglyoxaline, after methylation with methyl sulphate, can be condensed with ethyl sodioacetamidomalonate to yield, after hydrolysis and decarboxylation 4(5)-methyl-2-methylthiohistidine. This can then be converted into 2-metcapto-4(5)-methylhistidine by treatment with sodium in liquid ammonia, and into 4-methylergothioneine by the method described in Part I.

The projected method for the synthesis of 2-mercapto-4(5)-methylhistidine was to methylate 5(4)-dimethylaminomethyl-2-mercapto-4(5)-methylglyoxaline (I; R = H), to the quaternary ammonium salt (II; R = H) and condense the latter with ethyl sodioacetamidomalonate, in accordance with the procedure used by Rydon (*J.*, 1948, 705) for the methyltryptophans. By hydrolysis and decarboxylation of this condensation product (III; R = H), 2-mercapto-4(5)-methylhistidine (IV; R = H) should result.



This synthesis was at first unsuccessful because it was not found possible to form the quaternary ammonium salt without methylating the thiol group: the dipicrate of 5(4)-dimethylaminomethyl-4(5)-methyl-2-methylthioglyoxaline (II; R = Me) was isolated. It was therefore thought advisable to protect the sulphur atom by either a methyl or preferably (on account of the ease of removal at a later stage) a benzyl group. As reported in Part II the Mannich condensation proceeds more easily with the free mercapto-compound than with the *S*-benzyl derivative. Benzylation of the Mannich base (I; R = H), however, brought about decomposition, probably owing to the instability of the dimethylaminomethyl group under alkaline conditions (cf. Mannich and Dannehl, *Arch. Pharm.*, 1938, **276**, 206).

The alternative procedure of starting with the benzylated mercaptoglyoxaline for the Mannich reaction also yielded a resinous material, which, after methylation to the quaternary ammonium compound (II; R = CH_2Ph), was condensed with ethyl sodioacetamidomalonate.

* Part II, preceding paper.

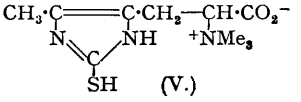
This yielded a fraction which analysis showed to be $C_{31}H_{42}O_{10}N_4S$, corresponding to the introduction of two ethyl acetamidomethylmalonate groups into the molecule.

Protection of the sulphur atom by benzylation having thus proved impracticable, the Mannich base (I; R = H) was converted into the quaternary methyl derivative with simultaneous methylation of the thiol group. The malonic ester condensation product (III; R = Me) could not be crystallised; it was hydrolysed and decarboxylated to yield 4(5)-methyl-2-methylthiohistidine (IV; R = Me). From this the pure amino-acid hydrochloride was obtained after esterification and subsequent hydrolysis.

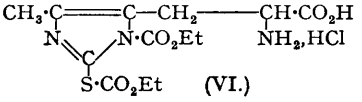
Attempts to remove the methyl group from the sulphur of the amino-acid methyl ester with sodium in liquid ammonia did not yield the expected product. The reaction was obviously not a simple demethylation; the end-point was not sharp and much more than the theoretical amount of sodium was taken up before the solution remained blue. A crystalline basic product isolated from the reaction gave the sulphur dioxide test and the ultra-violet absorption maximum characteristic of the mercaptoglyoxaline ring. The ninhydrin reaction was, however, negative, even after hydrolysis. It is possible that, in addition to the removal of the protecting group from the sulphur atom, an amide group formed from the ester by the action of the liquid ammonia was reduced to the corresponding amino-alcohol.

It has been shown by du Vigneaud and Behrens (*J. Biol. Chem.*, 1937, **117**, 27) that histidine can easily be obtained by the reductive debenzylation of N^{α}-benzylhistidine. When 4(5)-methyl-2-methylthiohistidine (IV; R = Me) was used in place of its ester, reductive fission smoothly yielded 2-mercapto-4(5)-methylhistidine (IV; R = H).

Attempts to prepare 4-methylergothioneine (V) from 2-mercapto-4(5)-methylhistidine (IV; R = H) by the method described in Part I of this series for the synthesis of ergothioneine showed again that the methyl group in the 4(5)-position has a marked influence on the behaviour of the glyoxaline ring. The preparation of the carbethoxythio-derivative gave a deliquescent material of unexpectedly low melting point, the analysis for which indicated a dicarbethoxy-derivative (VI). Methylation and decarboxylation of this compound yielded 4-methylergothioneine (V).



(V.)



(VI.)

4-Methylergothioneine responds to all the usual colour tests for ergothioneine except the Hunter diazo-reaction (*Biochem. J.*, 1928, **22**, 4). This was expected from the work of Lawson, Morley, and Woolf (*Nature*, 1951, **167**, 82) since the 4(5)-position is blocked. However, when the pH of the coupling solution in the Hunter test was lowered to about 7 by using 2% sodium acetate instead of the usual buffer, a very pale yellow primary colour resulted. On addition of the sodium hydroxide, trimethylamine was liberated and an indigo colour (absorption maximum at 5900 A.) developed. The sensitivity of this reaction is about one-tenth of the Hunter ergothioneine test.

Experimental.

Analyses are by Drs. Weiler and Strauss.

4(5)-*Methyl-2-methylthiohistidine Methyl Ester Dihydrochloride.*—This condensation must be carried out under strictly anhydrous conditions. 5(4)-Dimethylaminomethyl-2-mercapto-4(5)-methylglyoxaline (17·7 g.) and ethyl acetamidomalonate (25·4 g.) were suspended in ethanol (dried over magnesium–iodine; 150 ml.). A solution of sodium (5·4 g.) in ethanol (150 ml.) was added and to the resulting solution methyl sulphate (28 ml.) was added, the temperature being allowed to rise as the reaction proceeded. After the solution had been kept overnight at room temperature, water (200 ml.) was added and the solution was distilled under reduced pressure to a low volume. 25% (w/v) Sulphuric acid (250 ml.) was added and the solution boiled for 5 hours, cooled, and diluted to 500 ml. The solution was brought to pH 4 with sodium hydroxide and treated with a hot solution of mercuric chloride (100 g.) in water (500 ml.). After adjustment of the pH to 7·4 with sodium carbonate and storage overnight, the precipitate of the mercury complex of 4(5)-methyl-2-methylthiohistidine was centrifuged off and washed with water until free from sulphate. The mercury derivative was decomposed with hydrogen sulphide, and the mercury-free filtrate evaporated to dryness under reduced pressure. The dry residue was then refluxed with methanol (400 ml.), saturated with hydrogen chloride, for 1 hour whereupon crystals began to separate. After being kept at −10°, the colourless crystals of 4(5)-*methyl-2-methylthiohistidine methyl ester dihydrochloride*, m. p. 224° (16·8 g., 65%) (Found: C, 36·1; H, 5·6; N, 14·2; S, 10·0. $C_9H_{17}O_2N_3SCl_2$ requires C, 35·8; H, 5·7; N, 13·9; S, 10·6%), were collected. They recrystallised from methanol in long, thin rectangular prisms. The absorption maximum was at 2550 A., ε = 7800. The compound is very soluble in water, sparingly so in methanol, and insoluble in ether.

2-Mercapto-4(5)-methylhistidine.—4(5)-Methyl-2-methylthiohistidine methyl ester dihydrochloride (10 g.) was refluxed for 1 hour with 10% hydrochloric acid (100 ml.) and then distilled to dryness under reduced pressure. The dried white crystalline residue was dissolved in liquid ammonia (400 ml.), and sodium added in small pieces until a permanent blue colour developed. Ammonium chloride (2 g.) was then added and the ammonia allowed to evaporate spontaneously. The white solid residue was dissolved in dilute sodium hydroxide solution (15 ml.). On adjustment to pH 4 with acetic acid the *2-mercapto-4(5)-methylhistidine* crystallised (3·8 g., 57%) (Found: C, 42·3; H, 5·6; N, 20·4; S, 15·7. $C_7H_{11}O_2N_3S$ requires C, 41·8; H, 5·5; N, 20·9; S, 16·0%), soluble in hot water, sparingly so in cold water, and insoluble in ethanol. The absorption maximum in water was at 2600 A., $\varepsilon = 15{,}300$. The compound crystallises from water in thin microscopic prisms which do not melt, but decompose above 300°. It gives positve reactions with ninhydrin, sulphur dioxide, mercuric chloride, and the Folin–Marenzi reagent. The Hunter diazo-test is negative.

Condensation of Mannich Base of 2-Benzylthio-4(5)-methylglyoxaline with Sodioacetamidomalonic Ester.—The viscous solution of the Mannich base prepared from 2-benzylthio-4(5)-methylglyoxaline (20·4 g.) (cf. Part II) was dissolved in ethanol (100 ml.). Ethyl sodioacetamidomalonate, prepared from ethyl acetamidomalonate (21·7 g.) by addition of ethanol (200 ml.) containing sodium (2·3 g.), was mixed with the Mannich base, and methyl sulphate (19 ml.) added. Trimethylamine was evolved and, after storage and removal of the solvent under reduced pressure, the solution was made alkaline with sodium carbonate and extracted with ether, which on evaporation left a pale yellow non-crystalline residue. This crystallised after 2 months from amyl acetate or carbon tetrachloride. The analytical figures, though not entirely satisfactory, were obtained on using material once recrystallised from amyl acetate and twice from carbon tetrachloride without change of m. p. (115°) (Found: C, 56·2; H, 6·4; N, 9·0; S, 5·3. $C_{31}H_{42}O_{10}N_4S$ requires C, 56·2; H, 6·4; N, 8·5; S, 4·8%). This *compound* is therefore a condensation product between the Mannich di-base of 2-benzylthio-4(5)-methylglyoxaline and two molecules of ethyl acetamidomalonate.

5(4)-Dimethylaminomethyl-4(5)-methyl-2-methylthioglyoxaline Dipicrate.—5(4)-Dimethylaminomethyl-2-mercapto-4(5)-methylglyoxaline (5 g.) was dissolved in ethanol (100 ml.), and methyl iodide (4·2 g.) in ethanol (10 ml.) added. After being kept at room temperature, the alcohol was removed under reduced pressure and excess of hot aqueous picric acid solution added. On cooling, short yellow needles, m. p. 201°, of *5(4)-dimethylaminomethyl-4(5)-methyl-2-methylthioglyoxaline dipicrate* separated (Found: C, 37·5; H, 3·2; N, 19·7; S, 4·7. $C_{20}H_{21}O_{14}N_9S$ requires C, 37·3; H, 3·3; N, 19·6; S, 5·0%). The formation of a dipicrate can best be accounted for on the basis of the thiol group having been methylated, thus rendering the glyoxaline ring basic.

1-Carbethoxy-2-carbethoxythio-4(5)-methylhistidine Hydrochloride.—2-Mercapto-4(5)-methylhistidine (3·8 g.), suspended in ethanol (100 ml.), was refluxed with ethyl chloroformate (4 ml.) till dissolution was effected. After cooling and addition of dry ether (150 ml.), the oil which separated crystallised rapidly when scratched. Additional material was obtained from the mother-liquor (total yield, 4·76 g., 60%). The *hydrochloride* was deliquescent and had m. p. 60° (Found: C, 36·2; H, 5·5; S, 7·5; Cl, 8·3. $C_{13}H_{20}O_6N_3SCl,2H_2O$ requires C, 37·3; H, 5·8; S, 7·7; Cl, 8·5%).

4-Methylergothioneine.—The dicarbethoxy-derivative obtained as above was treated as described in the preparation of ergothioneine (Part I). The *4-methylergothioneine* crystallised from aqueous alcohol and had m. p. 265° (decomp.) (Found: C, 48·7; H, 7·1; N, 17·2; S, 13·5. $C_{10}H_{17}O_2N_3S$ requires C, 49·4; H, 7·1; N, 17·3; S, 13·2%). This compound, like ergothioneine, was soluble in water, sparingly so in ethanol. The tests with sulphur dioxide, gold chloride, mercuric chloride, iodobismuthous acid, and the Folin–Marenzi reagent were positive. The absorption maximum in water was at 2600 A., $\varepsilon = 15{,}600$.

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DEPARTMENT OF CHEMICAL PATHOLOGY,
UNIVERSITY COLLEGE HOSPITAL MEDICAL SCHOOL, UNIVERSITY STREET, LONDON, W.C.1.
DEPARTMENT OF CHEMISTRY, ROYAL FREE HOSPITAL SCHOOL OF MEDICINE,
8 HUNTER STREET, LONDON, W.C.1. [*Received, March 27th,* 1951.]