488. 2-*Mercaptoglyoxalines. Part I. The Synthesis of Ergothioneine.*

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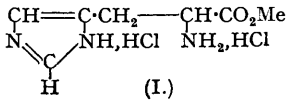
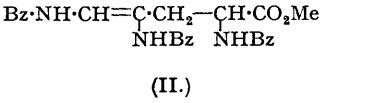
The synthesis of ergothioneine, the only 2-mercaptoglyoxaline known to occur naturally, is described. Histidine was converted by an improved procedure (cf. Tesar and Rittenberg, *J. Biol. Chem.*, 1947, **170**, 35) into 2-mercaptohistidine, the thiol group of which was protected by conversion into the carbethoxythio-derivative. Methylation of this, to give the betaine, followed by hydrolysis and decarboxylation, yielded ergothioneine.

Ergothioneine was isolated from ergot by Tanret (*J. Pharm. Chim.*, 1909, **30**, 145; *Compt. rend.*, 1909, **149**, 222) and from blood by Newton *et al.* (*J. Biol. Chem.*, 1926, **67**, 267; 1927, **72**, 367; see also Hunter and Eagles, *ibid.*, 1925, **65**, 623; 1927, **72**, 123). It was shown to be (V; R = H) by Barger and Ewins (*J.*, 1911, **99**, 2336) and Akabori (*Ber.*, 1933, **66**, 151). Attempts to synthesize it have been made by Ashley and Harington (*J.*, 1930, 2586), Harington and Overhoff (*Biochem. J.*, 1933, **27**, 338), and Jackson and Marvel (*J. Biol. Chem.*, 1933, **103**, 191).

2-Mercaptohistidine was prepared by a modification of the procedure of Ashley and Harington (*loc. cit.*), which was based on the observation of Kossel and Edlbacher (*Z. physiol. Chem.*, 1914—15, **93**, 396) that benzoylation of histidine methyl ester dihydrochloride (I) results in fission of the glyoxaline ring to yield methyl 2 : 4 : 5-tribenzamidopent-4-enoate * (II). This on treatment with methanolic hydrogen chloride yields methyl 2 : 5-dibenzamido-4-keto-pentanoate which after hydrolysis and treatment with potassium thiocyanate cyclises to give 2-mercaptohistidine (IV; R = H). Ashley and Harington obtained a 25% yield of the tribenzamido-compound (II), but by employing a two-phase benzoylation Tesar and Rittenberg (*J. Biol. Chem.*, 1947, **170**, 35) increased the yield to 60%. The method described below has given 80% yields.



(I.)



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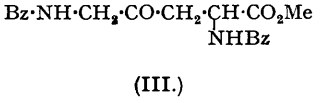
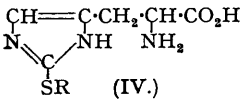
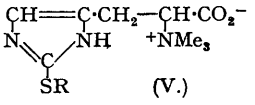


(III.)



(IV.)



(V.)

Attempts by previous workers to obtain ergothioneine by methylation of 2-mercapto-histidine were unsuccessful. The two difficulties to be overcome were (*a*) histidine betaine is unstable, readily losing trimethylamine to yield urocanic acid and (*b*) 2-mercaptoglyoxalines react readily with methylating agents to yield methylthioglyoxalines.

To prevent sulphur-methylation, 2-carbethoxythiohistidine was prepared by the action of ethyl chloroformate on an ethanolic suspension of 2-mercaptohistidine. Carbethoxylation of 2-mercaptohistidine in sodium hydroxide solution or aqueous pyridine did not yield the desired product as reaction occurred with the α-amino-group. Evidence for the structure of the carbethoxythio-derivative was (i) it gave a ninhydrin reaction indicating that the side-chain α-amino-group was intact, (ii) analysis proved it to be a dihydrochloride (the free mercapto-glyoxaline nucleus is not sufficiently basic to form salts), and (iii) the ultra-violet absorption spectrum failed to show the absorption maximum at *ca.* 2580 A. characteristic of the thiol group in marcaptoglyoxaline derivatives.

Methylation of the 2-carbethoxythiohistidine was first attempted with methyl sulphate and alkali according to Carter and Melville's method (*J. Biol. Chem.*, 1940, **133**, 109). The crystalline product obtained after hydrolysis of the methylated material (to remove the carbethoxy-group) was not, however, easily purified and, though apparently a betaine with a free thiol group, it did not give the deep magenta colour characteristic of ergothioneine in the Hunter diazo-test (*Biochem. J.*, 1928, **22**, 4). Methylation was achieved under neutral con-

* Geneva numbering, CO_2H = 1.

ditions with silver oxide and methyl iodide. The reaction mixture thus obtained, though boiled with hydrochloric acid, still contained silver, which was removed only by hydrogen sulphide. Such formation of a silver complex was to be expected as ergothioneine hydrochloride on treatment with silver nitrate forms the complex $(AgCl)_2(C_9H_{15}N_3SO_2)_2Ag_2O$ (Tanret, *loc. cit.*). The ergothioneine isolated was identical in all respects, except optical activity, with the natural material obtained from ergot. The optical activity of the 2-mercaptohistidine ($[\alpha]_D$ $-10°$) was the same as that observed by Ashley and Harington and by Tesar and Rittenberg, who proved by reconversion into L-histidine that racemization did not take place during the synthesis of 2-mercaptohistidine. Partial racemization of the ergothioneine ($[\alpha]_D$ $+47°$ instead of $+110°$) must have occurred during the formation of the betaine.

Experimental.

All m. p.s are uncorrected. Analyses are by Drs. Weiler and Strauss.

Methyl 2 : 4 : 5-*Tribenzamidopent*-4-*enoate*.—Histidine methyl ester dihydrochloride (24·1 g., 0·1 mol.) was dissolved in water (600 ml.) and cooled in an ice-salt bath. Redistilled benzoyl chloride (110 ml., 0·9 mol.) and benzene (400 ml.) were added with mechanical stirring. Sodium carbonate decahydrate (286 g., 1·0 mol.) was added in small portions during 2 hours, the cold mixture being stirred continuously for a further 5 hours. Water (350 ml.) was then added and, after filtration, the aqueous layer was extracted with benzene. The bulked benzene extracts, after being washed with water and dried (Na_2SO_4), were concentrated under reduced pressure to remove most of the benzene. Ether (2 l.) was added, and the precipitated resinous tribenzamido-compound set aside at $-10°$ overnight. The ether was decanted and the residue washed with ether. The crude product was heated in ethanol (150 ml.); it first dissolved, but rapidly crystallised when the solution was boiled; ether (300 ml.) was added and after being kept overnight at $-10°$ the crystals of methyl 2 : 4 : 5-tribenzamidopent-4-enoate (m. p. 219°; 37·9 g., 80·5%) were filtered off and washed with ether. A small quantity could be obtained from the mother-liquor by concentration under reduced pressure and addition of ether. The product thus obtained was pure enough for the next stage in the synthesis.

Methyl 2 : 5-*Dibenzamido*-4-*ketopentanoate*.—Methyl 2 : 4 : 5-tribenzamidopent-4-enoate (34 g.) was refluxed on a boiling water-bath with methanol containing 10% (w/v) of hydrogen chloride (475 ml.) for 0·5 hour. The solution was cooled and distilled under vacuum until crystals appeared. Ether (100 ml.), then iced water (400 ml.), were added. A white precipitate of methyl 2 : 5-dibenzamido-4-ketopentanoate, m. p. 158° (22·75 g., 85·7%), was formed, and on shaking complete precipitation took place. After being kept at 4° the precipitate of keto-ester was filtered off and washed with water and ether.

2 : 5-*Diamino*-4-*ketopentanoic Acid Dihydrochloride*.—The foregoing ester (65 g.) was boiled under reflux with water (300 ml.) and concentrated hydrochloric acid (350 ml.) for 7 hours. After cooling, the benzoic acid was removed by filtration. The filtrate and washings were distilled nearly to dryness under reduced pressure, dissolved in water, and extracted with ether to remove the last traces of benzoic acid. Owing to the instability of the acid dihydrochloride this concentrated solution was used directly.

2-*Mercaptohistidine*.—The above solution of 2 : 5-diamino-4-ketopentanoic acid dihydrochloride was heated on a boiling water-bath with the addition of potassium thiocyanate (10 g. every 0·5 hour, until a total of 40 g. had been added). After 3 hours' heating the solution was boiled with decolorizing carbon, filtered, and concentrated under reduced pressure to about 75 ml. On adjustment to pH 5 with sodium carbonate, crystallization rapidly set in, and after storage at 4° the almost colourless 2-mercaptohistidine (19·3 g., 58%) was filtered off, washed with water, and dried. It decomposed at 300° without melting. The mother-liquor darkened considerably on storage, even at 4°, and although this did not affect the crystals of 2-mercaptohistidine it was not possible to isolate any further quantity by means of mercuric chloride solution.

2-*Carbethoxythiohistidine Dihydrochloride*.—2-Mercaptohistidine (4·6 g.) was suspended in ethanol (100 ml.), and ethyl chloroformate (5 ml.) slowly added. This suspension was refluxed on a boiling water-bath until dissolution was effected. After cooling, dry ether (150 ml.) was added, and on storage at $-10°$ crystallization occurred. The almost colourless crystals of 2-*carbethoxythiohistidine dihydrochloride* (5·8 g., 71%), m. p. 189° (decomp.), were filtered off and washed with dry ether (Found: C, 33·5; H, 4·9; N, 12·5; S, 9·6; Cl, 21·2. $C_9H_{15}O_4N_3SCl_2$ requires C, 33·5; H, 4·6; N, 12·6; S, 9·6; Cl, 21·3%). 2-Carbethoxythiohistidine dihydrochloride is very soluble in water, sparingly so in methanol and ethanol. It gives a yellow colour with sulphur dioxide in aqueous solution. In water it exhibits an ultra-violet absorption maximum at 2400 A., $\varepsilon = 9{,}400$.

Ergothioneine.—2-Carbethoxythiohistidine dihydrochloride (3·32 g., 0·01 mol.) was dissolved in water (20 ml.). Freshly prepared silver oxide (12 g.) was added as an aqueous suspension (50 ml.). Methyl iodide (1·9 ml., 0·03 mol.) was added with shaking and cooling. The mixture was then mechanically shaken. After 1 hour concentrated hydrochloric acid (50 ml.) was added and the suspension centrifuged. The precipitate was washed twice with 5N-hydrochloric acid (25 ml.). The bulked solution was boiled for 2 hours, evaporated to dryness under reduced pressure, and dissolved in water (50 ml.), and the silver removed with hydrogen sulphide. After boiling and centrifugation, the supernatant liquid was treated with saturated aqueous phosphotungstic acid until all the ergothioneine was precipitated. The precipitate of ergothioneine phosphotungstate was centrifuged off and washed with water, suspended in water, and made alkaline with saturated barium hydroxide solution. The barium phosphotungstate was centrifuged off and the supernatant solution was immediately acidified with 2N-sulphuric acid. The barium phosphotungstate was extracted twice with water, and the solution

adjusted to pH 7 with barium hydroxide solution. After centrifugation and decolorisation, the solution was concentrated under reduced pressure until crystallization of the ergothioneine occurred. Recrystallised from aqueous ethanol and dried *in vacuo* over phosphoric oxide at 105° the material had m. p. 290° and $[\alpha]_D$ +47° ($c = 1$, $l = 2$, in water) (0·8 g., 30%) (Found: C, 47·1; H, 6·7; N, 18·3; S, 14·0. Calc. for $C_9H_{15}O_2N_3S$: C, 47·1; H, 6·6; N, 18·3; S, 14·0%). The ultra-violet absorption spectrum showed a maximum at 2580 A., $\varepsilon = 16{,}000$.

The synthetic and the natural ergothioneine behaved identically in the following tests: formation of a stable dihydrate and a monohydrochloride, m. p. 250° (Found: C, 40·6; H, 6·1; N, 16·0; S, 12·0; Cl, 13·39. Calc. for $C_9H_{16}O_2N_3SCl$: C, 40·7; H, 6·1; N, 15·8; S, 12·1; Cl, 13·35%); paper chromatography (R_F 0·87 in phenol, 0·32 in collidine); Hunter diazo-, Folin–Marenzi, and Mayer's reactions. The synthetic material formed the requisite precipitates with mercuric chloride, silver oxide, potassium bismuth iodide, phosphotungstic acid, and iodine. Sulphur dioxide produced a yellow solution.

2-*Mercaptourocanic Acid.*—The synthetic ergothioneine (1 g.) was boiled with 40% sodium hydroxide solution (25 ml.) for 3 hours, cooled, filtered, and neutralised with hydrochloric acid. After storage at 4° the pale yellow crystals of 2-mercaptourocanic acid were collected (0·62 g.). They did not melt but decomposed at 300° (Found: C, 42·5; H, 3·9; N, 16·8; S, 18·8. Calc. for $C_6H_6O_2N_2S$: C, 42·4; H, 3·6; N, 16·5; S, 18·8%). They give the typical magenta colour with the Hunter test when the final solution is adjusted to pH 10; addition of an equal volume of 50% sodium hydroxide solution is not necessary.

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