XLVIII. A NEW SYNTHESIS OF 2-THIOLHISTIDINE TOGETHER WITH EXPERIMENTS TOWARDS THE SYNTHESIS OF ERGOTHIONEINE.

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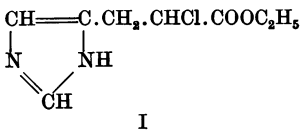
DURING the past year or two the problem of the synthesis of ergothioneine has received considerable attention in this laboratory. One mode of attack, which, whilst it failed in its ultimate object, led incidentally to the synthesis of 2-thiolhistidine has already been described [Ashley and Harington, 1930]. The work of these authors led immediately to an attempt to obtain ergothioneine by an analogous synthesis, namely by the benzoylation of ethyl α-chloro-β-glyoxaline-4 (or 5)-propionate (I) followed by conversion of the product (II) into ethyl α-chloro-γ-keto-δ-benzamidovalerate (III); it was hoped that the latter might be hydrolysed to the aminoketone (IV) and this converted into α-chloro-β-2-thiolglyoxaline-4 (or 5)-propionic acid (V) which, with trimethylamine, might be expected to yield ergothioneine (VI).



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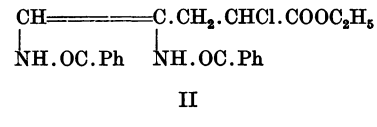

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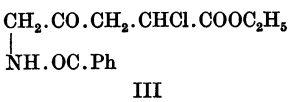

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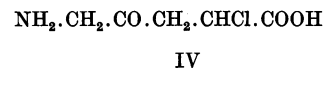

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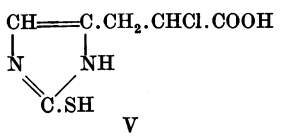

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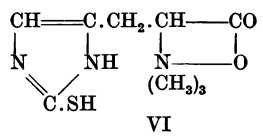

VI

All attempts to benzoylate ethyl α-chloro-β-glyoxaline-4 (or 5)-propionate have however proved fruitless. The conditions of the Bamberger fission of substituted glyoxalines are not very clearly established; according to Windaus, Dörries and Jensen [1921] the reaction does not occur with glyoxaline derivatives which contain a free carboxyl group in the side-chain, but succeeds with the esters of such derivatives; in no case however can the glyoxaline ring be ruptured by benzoylation if a carbonyl group be directly attached to it in the 4 (or 5) position, as for instance in glyoxaline-4 (or 5)-formaldehyde or ethyl glyoxaline-4 (or 5)-carboxylate. It appears that the presence of the negative halogen atom

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in the side-chain of ethyl α-chloro-β-glyoxaline-4 (or 5)-propionate brings this compound into the class of glyoxaline derivatives which are resistant to fission by benzoylation.

From time to time various other lines of attack have been tried and these are briefly outlined here in order to avoid unnecessary duplication by other workers.

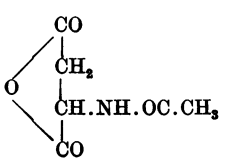
(*a*) One of the chief difficulties in working with 2-thiolglyoxalines consists in the lability of the sulphur atom which is removed with the greatest ease by oxidising agents. It was thought that this difficulty might be avoided by working with the corresponding disulphides and attempts were therefore made to oxidise 2-thiol-4 (or 5)-aminomethylglyoxaline [*cf.* Pyman, 1911] with iodine, the object being to substitute the amino-group of the disulphide which should result successively by hydroxyl and halogen and then to condense the product with ethyl sodiochloromalonate. The action of iodine on 2-thiol-4 (or 5)-aminomethylglyoxaline did not proceed smoothly however; a complex mixture of products was obtained which could not be readily purified, and this line of experiment was not pursued further.

(*b*) Phenoxyacetyl chloride gives, with diazomethane, a good yield of phenoxychloroacetone; the latter can be converted into phenoxyiodoacetone and this in turn, by condensation with hexamethylenetetramine and subsequent hydrolysis according to the method of Delépine [1895], yields phenoxyaminoacetone hydrochloride. Treatment of the latter with one molecular equivalent of sodium thiocyanate gives 2-thiol-4 (or 5)-phenoxyglyoxaline. This compound cannot however be satisfactorily hydrolysed to 2-thiol-4 (or 5)-hydroxymethylglyoxaline nor can the phenoxy-group be directly substituted by bromine with concentrated hydrobromic acid.

(*c*) A theoretically possible method of building up the type of compound necessary for the synthesis of an α-halogeno-β-glyoxaline-4 (or 5)-propionic acid by Gabriel's reaction consists in the condensation of an acylaminohalogenoacetone with ethyl sodiochloromalonate followed by liberation of the amino-group, formation of the thiolglyoxaline ring and hydrolysis and decarboxylation of the product. Very numerous attempts were made to condense phthalimidohalogenoacetones with ethyl sodiochloromalonate and indeed with ethyl sodiomalonate itself under various conditions, but all were in vain. The halogenoacetones reacted exothermically with the sodiomalonates with rapid elimination of the sodium halide, but normal condensation did not occur, the products consisting for the most part of highly pigmented resins. As a matter of fact the work to be described in the present paper shows that even if such a condensation had been successfully achieved the synthesis on these lines would have failed at a later stage.

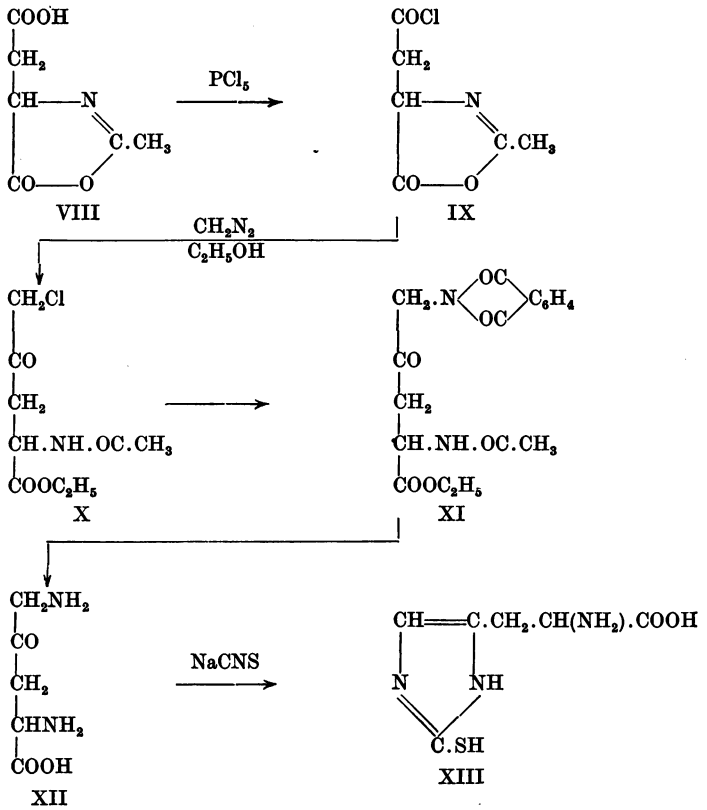
The object of the present paper is to describe an entirely different mode of attack. Although this has again failed to yield ergothioneine it has led to an independent synthesis of 2-thiolhistidine and is thought to be of sufficient chemical interest to be worthy of description.

Bergmann *et al.* [1926] described an anhydride resulting from the action of acetic anhydride on aspartic acid to which they ascribed the formula VII.



(VII)

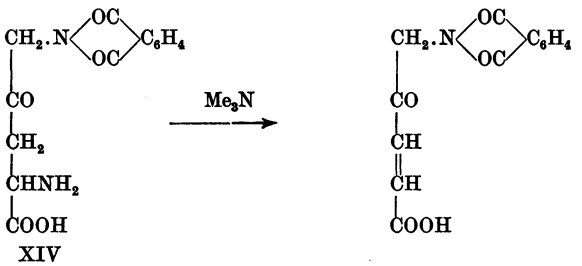
The experimental results upon which this formula was based were not conclusive however and could be equally well explained by making the more likely assumption that the compound was an azlactone (VIII); as will be seen from the experiments about to be described the main product of the action of acetic anhydride on aspartic acid or acetylaspartic acid is in fact this azlactone.

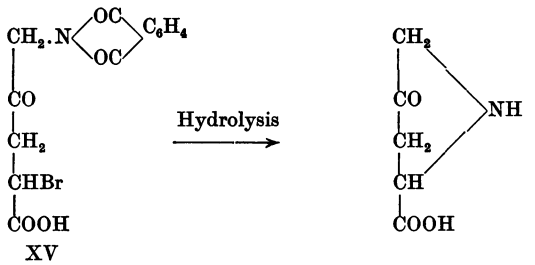


When the acetylation product (VIII) is treated with phosphorus pentachloride in acetyl chloride an acid chloride (IX) is produced; the action of diazomethane in dry ethereal solution on IX gives a product which, on dissolution in alcohol, yields ethyl α-acetamido-γ-keto-δ-chlorovalerate (X). The latter can be readily converted into the phthlamido-derivative (XI) which is hydrolysed by boiling with 20 % hydrochloric acid to give the hydrochloride of αδ-diamino-γ-ketovaleric acid (XII). The action of one molecular equivalent of sodium thiocyanate on XII yields the hydrochloride of 2-thiolhistidine from which the free amino-acid (XIII) is readily obtained.

In the attempt to synthesise ergothioneine the above series of reactions was modified as follows. Owing to the relative firmness with which the phthalimido-residue is held it is possible to effect a partial hydrolysis of compound XI with liberation of the α-amino- and carboxyl-groups to give α-amino-γ-keto-δ-phthalimidovaleric acid (XIV), and we hoped, by methylation of this compound, followed by hydrolysis of the phthalimido-group and synthesis of the thiol-glyoxaline ring, to obtain ergothioneine.

In this hope we were disappointed however since all attempts to methylate XIV led only to liberation of trimethylamine and production of an unsaturated acid. It is evident that, as in the case of aspartic acid, the presence of a carbonyl group in the β-position relative to the carbon atom to which the nitrogen is attached renders the betaine highly unstable.





As a last resort the amino-group of compound (XIV) was interchanged for bromine by the action of nitrosyl bromide, the object being to hydrolyse the resulting α-bromo-γ-keto-δ-phthalimidovaleric acid (XV) and use the product for the preparation of α-bromo-β-2-thiolglyoxaline-4 (or 5)-propionic acid, which should finally be treated with trimethylamine to give ergothioneine. In this instance we were defeated by the fact that hydrolysis of the bromo-acid (XV) even under the mildest conditions consistent with removal of the phthalimido-residue was accompanied by elimination of hydrogen bromide and ring-closure.

It appears to us therefore that the only reasonable hope of synthesising ergothioneine rests in the action of trimethylamine on an α-halogeno-β-2-thiol-glyoxaline-4 (or 5)-propionic acid and that such an acid will have itself to be obtained by synthesis from an appropriate thiolglyoxaline; that is to say the formation of the thiolglyoxaline ring cannot be postponed until the last stage. In view of the difficulties referred to above of working with thiolglyoxalines the prospects for a synthesis of this kind are not encouraging.

The 2-thiolhistidine obtained in the course of the present work differs from that prepared by Ashley and Harington [1930] in being optically inactive since racemisation of the aspartic acid occurs during the treatment with acetic anhydride; it closely resembles the *l*-acid however in its physical and chemical properties.

Experimental.

Acetylaspartic acid. (*a*) Aspartic acid (10 g.) was dissolved in 2*N* sodium hydroxide (82·5 cc.) and the solution was treated with acetic anhydride (15·3 cc.) and 2*N* sodium hydroxide (165 cc.); the acetic anhydride and sodium hydroxide were added alternately in small portions, the mixture being vigorously shaken and cooled under the tap after each addition. At the end of the reaction 6*N*

sulphuric acid (82·5 cc.) was added and the solution evaporated under diminished pressure. The residue was extracted several times with boiling alcohol and the combined alcoholic extracts, after addition of a little water to inhibit esterification, were evaporated *in vacuo*. The syrupy residue set to a crystalline mass after keeping in an evacuated desiccator for several days; yield 9·5 g.

(*b*) Aspartic acid was dissolved in 2*N* sodium hydroxide (2 equivalents) and ketene was distilled into the solution until the latter was acid to litmus; 5*N* sulphuric acid (2 equivalents) was then added and the solution was evaporated under diminished pressure. The further working up was as described above.

The acetylaspartic acid obtained by either of these methods was difficult to recrystallise satisfactorily, being exceedingly soluble in water, alcohol and acetic acid and very sparingly so in other organic solvents. It was obtained in micro-crystalline condition on cooling a very concentrated solution in hot water and melted at 142–143°. (Found: C, 40·7; H, 5·3; N, 7·8 %. $C_6H_9O_5N$ requires: C, 41·2; H, 5·1; N, 8·0 %.) The compound had $[\alpha]_{5461}^{20°}$ + 5·9° (*c* = 2·552 in water).

Azlactone (VIII). (*a*) Aspartic acid (10 g.) was suspended in freshly distilled acetic anhydride (50 cc.) and the mixture was boiled for 35 minutes. The solution was then cooled, filtered from a trace of undissolved material and concentrated as rapidly as possible in a good vacuum, the bath temperature not exceeding 60°. The partly crystalline residue was dissolved in hot ethyl acetate, and the solution, on cooling, deposited 5·5 g. (47 %) of crystalline material. After recrystallisation from ethyl acetate or acetic acid it formed hard prisms melting at 145–146°.

(*b*) Acetylaspartic acid (5 g.) was suspended in acetic anhydride (10 cc.) and the mixture was boiled; a clear solution was rapidly obtained and boiling was continued for 2 minutes after this had occurred. The subsequent working up was as described above and the yield and properties of the product were similar. (Found: C, 45·7; H, 4·6; N, 8·7 %. $C_6H_7O_4N$ requires: C, 45·8; H, 4·5; N, 8·9 %.)

If the acetylaspartic acid employed for the reaction were not perfectly dry and if the reaction were carried out at 95° in an oil-bath instead of at the boiling temperature, an *anhydride* was obtained in variable yield which was isomeric with the above-described azlactone; this second anhydride was much less soluble than the azlactone and crystallised from ethyl acetate in prisms melting at 175°. It seems probable that the higher-melting anhydride possesses the constitution VII. (Found: C, 46·1; H, 4·5; N, 8·9 %. $C_6H_7O_4N$ requires: C, 45·8; H, 4·5; N, 8·9 %.)

Acid chloride (IX). Perfectly dry and very finely powdered azlactone (5 g.) was suspended in acetyl chloride (30 cc.) and the mixture was treated with powdered phosphorus pentachloride (7 g., 5 % excess). On shaking for a few minutes practically everything passed into solution with slight evolution of heat, after which crystallisation of the product set in rapidly and was soon complete. The mixture was cooled in ice and salt and diluted with an equal volume of anhydrous ether, after which the precipitate was filtered off, with exclusion of atmospheric moisture, and washed with light petroleum. The yield of dry white excessively hygroscopic crystalline powder was 94 % of the theoretical. (Found: Cl, 20·1 %. $C_6H_6O_3NCl$ requires: Cl, 20·2 %.)

Ethyl α-acetamido-γ-keto-δ-chlorovalerate (X). The acid chloride freshly prepared as described above from 5 g. of azlactone was suspended in anhydrous ether (250 cc.). The suspension was cooled in ice and diazomethane from 9 cc. of nitrosomethylurethane was passed into it, the mixture being continually agitated. The addition of the diazomethane was accompanied by vigorous evolution of nitrogen and dissolution of most of the solid. 15 minutes after the last of the diazomethane had been added the pale yellow solution was decanted

from a small amount of undissolved material, chilled in ice and salt and saturated with hydrogen chloride. As soon as the yellow colour due to the excess of diazomethane was discharged a voluminous white precipitate (presumably α-acetamido-γ-keto-δ-chlorovaleryl chloride) began to separate. Addition of hydrogen chloride was interrupted when this precipitate no longer increased in amount. Alcohol (80 cc.) was then added which caused immediate dissolution of the precipitate; if the operation had been properly conducted the solution was colourless at this stage. Solvents were evaporated under diminished pressure, overheating being carefully avoided and the last traces of alcohol being removed in a vacuum desiccator. The partly crystalline residue so obtained was rubbed up with cold aqueous sodium carbonate to neutralise traces of hydrochloric acid and the almost colourless crystalline precipitate was collected.

The product could be crystallised from hot water, in which it was easily soluble, or from toluene or xylene; it formed colourless leaflets melting at 128°. The yield was 2·25 g. or 32 % calculated on the azlactone employed. (Found: C, 46·0; H, 6·0; N, 6·0; Cl, 15·0 %. $C_9H_{14}O_4NCl$ requires: C, 45·8; H, 6·0; N, 6·0; Cl, 15·1 %.)

Ethyl α-acetamido-γ-keto-δ-phthalimidovalerate (XI). The chloroketo ester (X) (4·5 g.) was dissolved in anhydrous xylene (9 cc.) and to the solution, heated in an oil-bath at 135°, there was added freshly prepared potassium phthalimide (3·45 g.); heating was continued for 45 minutes. On cooling, the contents of the tube set to a crystalline mass; this was pulverised, freed from xylene and thoroughly washed with cold water.

The product was recrystallised from boiling 80 % alcohol from which it separated, on cooling, in fine white needles melting at 175°. The compound was readily soluble in alcohol; in water it was fairly soluble at the boiling temperature but very sparingly so in the cold.

The yield was 5 g. or 76 % of the theoretical. (Found: C, 59·0; H, 5·1; N, 8·1 %. $C_{17}H_{18}O_6N_2$ requires: C, 59·0; H, 5·2; N, 8·1 %.)

αδ-Diamino-γ-ketovaleric acid monohydrochloride (XII). Ethyl α-acetamido-γ-keto-δ-phthalimidovalerate (5 g.) was boiled under reflux for 2·5 hours with 55 cc. of 20 % hydrochloric acid. The solution was cooled and the phthalic acid which had separated was filtered off, the last traces being removed by extraction with ether. The solution was then concentrated to a low volume under diminished pressure and transferred to a vacuum desiccator over potassium hydroxide; removal of the last traces of hydrochloric acid in this way left a crystalline residue of the hydrochloride.

The compound was recrystallised from 60 % alcohol from which it separated in colourless non-hygroscopic prisms. It was extremely soluble in water and sparingly so in alcohol. On heating it turned yellow at 175° and blackened without melting properly at 210°.

The yield was 3 g. or 95 % of the theoretical. (Found: C, 33·0; H, 6·1; N, 14·8; Cl, 19·7 %. $C_5H_{11}O_3N_2Cl$ requires: C, 32·9; H, 6·0; N, 15·3; Cl, 19·5 %.)

dl-*2-Thiolhistidine.* αδ-Diamino-γ-ketovaleric acid hydrochloride (2·8 g.) was dissolved in water (3 cc.) and heated in a boiling water-bath. Sodium thiocyanate (1·3 g.) was added in three portions at 20-minute intervals and heating was continued for 1 hour after the last addition. The solution was then treated with saturated aqueous sodium acetate until no longer acid to Congo red, whereupon the amino-acid began rapidly to crystallise; after keeping overnight in the ice-chest the precipitate was collected and amounted to 1·5 g. (62·5 % of the theoretical). When recrystallised from hot water (charcoal) it formed pale straw-coloured plates of irregular shape similar in appearance to the *l*-2-thiolhistidine

synthesised by Ashley and Harington [1930]. (Found: C, 38·5; H, 4·9; N, 21·8; S, 16·9 %. $C_6H_9O_2N_3S$ requires: C, 38·5; H, 4·8; N, 22·4; S, 17·1 %.)

The product gave a ninhydrin reaction; in aqueous solution it decolorised alkaline permanganate instantaneously in the cold. With diazobenzenesulphonic acid and sodium carbonate it gave an orange colour which deepened in intensity and acquired a purplish tinge on addition of concentrated aqueous sodium hydroxide.

On evaporation of a solution of the amino-acid in concentrated hydrochloric acid in a vacuum desiccator over potassium hydroxide there was obtained a *dihydrochloride* which formed large colourless prisms, melting at 204–206° (decomp.).

When the amino-acid was oxidised with ferric sulphate under conditions precisely similar to those described by Ashley and Harington [1930] for the oxidation of *l*-2-thiolhistidine it gave a satisfactory yield of *dl*-histidine. No doubt remains therefore as to the constitution of the synthetic amino-acid.

α-Amino-γ-keto-δ-phthalimidovaleric acid (XIV). Ethyl α-acetamido-γ-keto-δ-phthalimidovalerate (2·5 g.) was boiled under reflux for 3 hours with *N* hydrochloric acid (25 cc.). On cooling the solution a small amount of phthalic acid separated; this was filtered off and the mother-liquor was thoroughly extracted with ether; the total amount of phthalic acid recovered was 0·3 g. corresponding with 25 % of the starting material. The aqueous solution was then neutralised by the addition of 2*N* sodium carbonate (12·5 cc.) when the aminoketophthalimidovaleric acid separated.

The precipitate was collected and recrystallised from hot water from which it separated in colourless needles melting at 165–166° (decomp.). (Found: C, 56·3; H, 4·8; N, 9·7 %. $C_{13}H_{12}O_5N_2$ requires: C, 56·5; H, 4·35; N, 10·15 %.)

All attempts to methylate this compound whether in the ordinary way with methyl sulphate and potassium hydroxide or with methyl iodide and anhydrous sodium carbonate in acetone (a mild method which has been employed with success in the preparation of the betaine of phenylalanine) led only to liberation of trimethylamine and formation of an unsaturated acid.

α-Bromo-γ-keto-δ-phthalimidovaleric acid (XV). α-Amino-γ-keto-δ-phthalimidovaleric acid (1·38 g.) was dissolved in 3*N* sulphuric acid (8·3 cc.) and potassium bromide (2 g.) was added. The solution was cooled in ice and vigorously stirred with a mechanical stirrer whilst finely powdered sodium nitrite (0·525 g.) was added in small portions during 20 minutes. When about half the nitrite had been added the bromo-acid started to crystallise. At the end of the reaction the product was collected and recrystallised from water; it formed fine colourless needles, melting at 185–186° (decomp.). (Found: C, 46·3; H, 3·2, N, 4·3; Br, 22·9 %. $C_{13}H_{10}O_5NBr$ requires: C, 45·9; H, 2·9; N, 4·1; Br, 23·5 %.)

The hydrolysis of this compound proceeded with extreme ease, 1 hour's boiling with 2*N* hydrochloric acid sufficing to liberate all the phthalic acid; whether the hydrolysis were conducted under these mild conditions however, or more vigorously with the employment of concentrated acid, the solution, after removal of the phthalic acid and evaporation under diminished pressure, yielded an oily residue, not easily soluble in water and non-basic in character.

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