CXXXVII. THE ERLENMEYER SYNTHESIS OF AMINO-ACIDS.

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OF the many methods available for the synthesis of α -amino-acids, that originated by Erlenmeyer [1893] has been most generally adopted by biochemists working in this field. For example, in more recent years, Ellinger and Flamand [1907], by its use, synthesised tryptophan, Pyman [1916] histidine, Barger and Ewins [1917] *Pr*-2-methyltryptophan, and Harington and Barger [1927], with a modified procedure, 3:5-diiodothyronine, the immediate precursor in the synthesis of thyroxine.

The main reasons for this choice are, firstly, that the starting materials are more or less readily available, and, secondly, that the benzoyl derivative of the amino-acid, obtainable at one stage of the synthesis, lends itself, as Fischer [1900] showed, to resolution into its optically active isomerides.

Nevertheless, the scheme, as Erlenmeyer himself pointed out, has certain weaknesses and these have been encountered repeatedly by later workers, *e.g.* Ellinger and Flamand, Pyman, Barger and Ewins.

The method as elaborated by Erlenmeyer consisted of four stages, *viz.*: (i) the formation of an oxazolone by the condensation of an aldehyde and hippuric acid; (ii) the hydrolysis of the oxazolone to an α -benzoylamino-acrylic acid; (iii) the reduction of the latter compound to the α -benzoylamino-propionic acid, and finally (iv) the hydrolysis of this compound to the α -amino-acid. Of these, stages (i) and (ii) proceed smoothly and with satisfactory yields. The same, however, cannot be said of stages (iii) and (iv).

The reduction as carried out by Erlenmeyer with the calculated quantity of sodium amalgam yielded only some 10–15 % of the theoretical amount of the saturated acid. This procedure was modified by Fischer who, using much larger quantities of sodium amalgam, obtained 80 % and 65 % yields respectively of benzoylphenylalanine and *N*-benzoyltyrosine. In other cases, however, the yields were persistently poor and moreover, the method has a further disadvantage. When the reduction has been completed, the unreduced acid remaining must invariably first be destroyed before the reduced compound can be isolated in a pure condition.

With regard to stage (iv), the hydrolysis, as carried out by these workers, takes a considerable time to accomplish and the yield of amino-acid is often

unsatisfactory. Thus Erlenmeyer found that benzoylphenylalanine, even after treatment in a sealed tube with concentrated hydrochloric acid for 16 hours at 140°, did not yield phenylalanine quantitatively, while Pyman, using Fischer's modification, reported that benzoylhistidine boiled with 60 times its weight of 20 % hydrochloric acid for 4 hours gave 70 % of the theoretical yield of histidine.

Harington and Barger [1927], in their synthesis of thyroxine on the lines of Erlenmeyer's method, were beset with yet another difficulty. To avoid removal of iodine by the alkaline reducing agent, they substituted for Erlenmeyer's sodium amalgam constant-boiling hydriodic acid and red phosphorus. On subjecting their benzoylaminoacrylic acid to this reducing reagent, they obtained, not the benzoylaminopropionic acid, but the α -amino-acid itself. Later Harington and McCartney [1927], modifying slightly this procedure by the introduction of acetic anhydride as a solvent, synthesised phenylalanine and tyrosine from the corresponding α -benzoylaminoacrylic acids or ethyl esters, whichever proved the more suitable.

Following the publication of these results of Harington and McCartney, Hoffmann-La Roche [1928, 1929] took out patents for the direct synthesis of thyroxine from the oxazolone, the reagent used being essentially that of Harington and McCartney. Apparently the oxazolone, like the benzoylaminoacrylic acid gives satisfactory yields of the amino-acid. Thus Erlenmeyer's original synthesis of an optically inactive α -amino-acid from the aldehyde had been reduced to a three-stage process [Harington and Barger, 1927], and to a two-stage process [Hoffmann-La Roche, 1928, 1929].

Nevertheless it will be seen that both these modifications, otherwise highly successful, suffer from one grave disadvantage. Reference has already been made to Fischer's use of the α -benzoylamino-acid for the purpose of resolution. Yet this is the very stage which is eliminated by the use of the acetic anhydride-hydriodic acid reagent, and thus we find Harington, in order to resolve his synthetic thyroxine (stage iv), under the necessity of doubling back to stage (iii) (he made formyldiiodothyronine), resolving and then hydrolysing the optically active isomerides into the respective amino-acids. It is apparent, therefore, that if the method of Harington and Barger could be so modified that the reduction took place without the simultaneous hydrolysis of the reduced compound, such a procedure would be a distinct advance.

Requiring certain amino-acid derivatives recently for metabolism experiments, and obtaining unsatisfactory yields with the sodium amalgam method of reduction, we turned our attention to the acetic anhydride-hydriodic acid method of Harington and McCartney. It occurred to us, in studying the method as described by these workers, that it might be modified in at least two directions, *e.g.*

- (i) by variation of the amount of the hydriodic acid used, and
- (ii) by the substitution of glacial acetic acid for acetic anhydride as the solvent.

Experimentation on these lines soon indicated that drastic reduction of the amount of hydriodic acid used led to the reduction of the unsaturated compound taking place without the loss of the benzoyl radical from the amino-group. For example, α -benzoylamino-cinnamic acid gave benzoylphenylalanine, the product being obtained in excellent yield and in an easily purified form. The reaction was carried on for one hour, although it is probably complete in a shorter period of time. Treated with similar quantities of hydriodic acid, the oxazolone also yields the benzoylamino-acid, although, from results obtained, it would appear that the yield in this case is not quite so satisfactory as that obtained by the reduction of the acrylic acid.

From this point we were led to try the effect of increasing the amount of hydriodic acid, while still retaining acetic acid as the general solvent. It was now found that, should the hydriodic acid be present in sufficient quantity, the reduction was also accompanied by the removal of the benzoyl group, with the consequent production of the α -amino-acid. Thus α -benzoylamino-cinnamic acid under these conditions yields phenylalanine. Here again the yields are good and the product is easily purified. The reaction is complete in from 60–90 minutes, a period which, however, must be considerably lengthened if the hydriodic acid is required to effect the removal of other groups, *e.g.* methyl, as in the synthesis of tyrosine. With similar quantities of hydriodic acid, the oxazolone can be made to yield the α -amino-acid, whilst it also follows that the same method will remove the benzoyl group from the benzoylamino-acid.

It will thus be seen that the reaction can be so controlled that the one weakness of Harington and McCartney's procedure is eliminated. Moreover, the reagents involved are such that the synthesis can be carried out at a considerably reduced cost.

The results obtained may be summarised thus:

Reactions 1 and 2—by the use of minimum quantities of hydriodic acid.

Reactions 3, 4 and 5—by the use of much larger quantities of hydriodic acid.

EXPERIMENTAL.

Phenylalanine.

Benzoylphenylalanine. (a) From α -benzoylamino-cinnamic acid. 10 g. of α -benzoylamino-cinnamic acid, prepared according to the directions of Erlenmeyer [1893], were dissolved in 40 cc. of glacial acetic acid A.R., 1.6 cc. of hydriodic acid (sp. gr. 1.7) and 3 g. red phosphorus were added to the solution, and the resulting mixture was boiled under a reflux condenser for one hour. The hot solution was filtered through a glass filter, the separated phosphorus being afterwards washed with hot glacial acetic acid. The combined filtrate and washings were then evaporated to dryness *in vacuo*. Water was added and the evaporation to dryness repeated. The residue was taken up in the minimum quantity of hot alcohol, the solution boiled with a little charcoal and filtered. To the filtrate hot water was added until the solution became cloudy when it was set aside overnight. Practically colourless benzoylphenylalanine separated. A further crystallisation produced a product which melted at 184–5° (Fischer gives M.P. 186°). The yield was 82 % of the theory. (Found: N, 5.2. Calc.: N, 5.2 %.)

An alternative procedure, which, however, does not give quite such a good yield, is to pour the hot reaction mixture, after the removal of the red phosphorus, into a large excess of water. The crude benzoylphenylalanine which separates is purified as described above.

(b) From 4-benzylidene-2-phenyl-5-oxazolone. 10 g. of this oxazolone [Erlenmeyer, 1893] were boiled under a reflux condenser with a mixture of 70 cc. glacial acetic acid A.R., 1.6 cc. constant-boiling hydriodic acid and 3 g. red phosphorus for one hour. The reaction mixture was filtered hot and worked up in the manner already detailed. The benzoylphenylalanine, obtained in the first crystallisation from dilute alcohol, melted at 181°. The yield did not equal that obtained from the cinnamic acid, being only 72 % of the theoretical.

Phenylalanine. (a) From benzoylphenylalanine. 10 g. of benzoylphenylalanine, prepared by either of the above methods, were dissolved in 40 cc. glacial acetic acid A.R., and to the solution were added 40 cc. of hydriodic acid (sp. gr. 1.7) and a little red phosphorus, the resulting mixture being refluxed for 90 minutes. At the end of this time the hot mixture was filtered, the separated phosphorus washed with hot acetic acid, and the combined filtrate and washings were evaporated to dryness *in vacuo*. Water was added to the residue and the evaporation repeated. The residue was then dissolved in the minimum quantity of boiling water, the solution cooled under the tap, and extracted several times with ether to remove the benzoic acid and any unchanged benzoylphenylalanine. The aqueous solution was then concentrated to about 50 cc. On making it neutral to litmus by the addition of ammonia, the solution began to deposit almost colourless phenylalanine. After standing overnight, the phenylalanine was separated and recrystallised from boiling water. It

was thus obtained in the form of colourless plates. The yield of the analytically pure amino-acid was 5.3 g. or 80 % of theory.

(b) From α -benzoylamino-cinnamic acid. 10 g. of α -benzoylamino-cinnamic acid, 40 cc. of glacial acetic acid A.R., 40 cc. of constant-boiling hydriodic acid and 3 g. of red phosphorus were boiled together under a reflux condenser for 75 minutes. The coloured mixture which resulted was worked up in a precisely similar manner to that obtained in the preparation of phenylalanine from its benzoyl derivative. The yield was 82 % of the theoretical amount, and the product melted at $263-5^{\circ}$ (Erlenmeyer gives 263°).

(c) From 4-benzylidene-2-phenyl-5-oxazolone. 10 g. of the oxazolone dissolved in 70 cc. of glacial acetic acid A.R. were boiled together with 50 cc. of constant-boiling hydriodic acid and 3 g. red phosphorus for 90 minutes. The subsequent treatment of the reaction mixture followed closely that already described. The yield of phenylalanine obtained amounted to some 68 % of that demanded by theory.

Tyrosine. 4-(p-Methoxybenzylidene)-2-phenyl-5-oxazolone was synthesised from anisaldehyde and hippuric acid in the usual way. 24 cc. of anisaldehyde, 60 g. of acetic anhydride, 40 g. of hippuric acid and 8 g. of freshly fused sodium acetate were well mixed in a conical flask and heated on the boiling water-bath for half an hour. The solid gradually went into solution and after 20 minutes the oxazolone began to separate; finally the whole became solid. The crude oxazolone was rubbed up with some 300 cc. of hot water and rapidly filtered, a process which was repeated twice. It was then dried in the steam-oven and finally crystallised from benzene, from which it separated in long needles. The product so obtained melted at $161-2^{\circ}$. It weighed 35 g. (62 % of theory). (Found: C, 72.7; H, 4.63; N, 4.8 %. Calc. for $C_{17}H_{13}O_3N$; C, 73.1; H, 4.67; N, 5.04 %.)

α -Benzoylamino-p-methoxycinnamic acid. The product of the last experiment was dissolved in the minimum quantity of hot alcohol, and to the solution was added $1\frac{1}{2}$ times the theoretical equivalent of sodium hydroxide in the form of a 2 % aqueous solution. The mixture was kept hot and stirred on the boiling water-bath for 15 minutes. It was then cooled and neutralised (Congo paper) with dilute sulphuric acid. The almost colourless solid which separated was sucked as dry as possible on the Büchner funnel, washed several times with distilled water and finally crystallised from dilute alcohol. The yield was quantitative. The melting-point of the product was 230° . (Found: N, 4.6. Calc.: N, 4.7 %.)

Benzoyl-p-methoxyphenylalanine. (a) From α -benzoylamino-p-methoxycinnamic acid. A mixture of 10 g. of this acid, dissolved in 35 cc. of glacial acetic acid A.R., 1.4 cc. constant-boiling hydriodic acid and 2.5 g. of red phosphorus was boiled for one hour. The reaction mixture was filtered, and the separated phosphorus washed with hot acetic acid. The combined filtrate and washings were evaporated to dryness *in vacuo*. Water was added and the evaporation repeated. The residue was extracted several times with small

volumes of 10–15 % acetic acid, in which the tar present in the residue was insoluble. The filtrates were combined, boiled with a little charcoal, and filtered. Concentration *in vacuo* of the colourless solution so obtained led to the separation of the desired product. The yield was 65 % of the theory. The melting-point of the product was 173°, and this could not be raised by further recrystallisation from dilute acetic acid or alcohol. (Found: C, 68.1; H, 5.5; N, 4.62 %. Calc. for $C_{17}H_{17}O_4N$; C, 68.2; H, 5.7; N, 4.62 %.)

(b) From 4-(*p*-methoxybenzylidene)-2-phenyl-5-oxazolone. The method of preparation of the benzoyl-*p*-methoxyphenylalanine from the oxazolone was exactly similar to that in which the starting-point is the cinnamic acid, with the exception that the amount of glacial acetic acid employed was increased from 35 to 60 cc. The yield in this case was 57 % of the theory, while the product, after the first crystallisation, melted at 172°.

Tyrosine. From (a) benzoyl-*p*-methoxyphenylalanine; (b) α -benzoyl-amino-*p*-methoxycinnamic acid; (c) 4-(*p*-methoxybenzylidene)-2-phenyl-5-oxazolone.

The preparation of tyrosine from any one of these compounds followed closely that already described for the preparation of phenylalanine from its corresponding derivative. In order to effect the hydrolysis of the methoxy-group the time of reaction was increased from 1½ hours to 3½ hours. It was found best to treat the starting material with 10 times its weight of a mixture of equal volumes of glacial acetic acid A.R. and hydriodic acid (sp. gr. 1.7) together with 2–3 g. of red phosphorus. The yields obtained were (a) 75 %, (b) 63 % and (c) 55 % of those demanded by theory. The specimen of tyrosine prepared from benzoyl-*p*-methoxyphenylalanine darkened at 300° and melted in the neighbourhood of 316°. (Found: N, 7.7. Calc.: N, 7.75 %.)

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