LXIII. THE SYNTHESIS OF AMINO-ACIDS.

II. SULPHIDES AS REDUCING AND HYDROLYTIC AGENTS IN THE HYDANTOIN SYNTHESIS OF AMINO-ACIDS.

By WILLIAM JOHN BOYD AND WILLIAM ROBSON.

From the Department of Physiology, University of London King's College.

(Received February 1st, 1935.)

In the preceding paper of this series [1935] the authors have shown how the first, *i.e.* the condensation, stage of the Wheeler-Hoffman [1911] process of synthesising aromatic α -amino-acids may be modified, not only with regard to the catalyst but also the solvent employed, so that the yield and, in certain cases, the purity of the product may be improved.

The second and third stages of the synthesis consist respectively of the reduction of the unsaturated hydantoin (I), formed in the condensation stage referred to above, and of the hydrolysis of the resulting saturated hydantoin (II)

to the amino-acid (III). These two stages of reduction and hydrolysis, as shown by Wheeler and Hoffman, can be effected by one and the same reagent, *viz.* hydriodic acid and red phosphorus. By such means they obtained phenylalanine from benzalhydantoin and tyrosine from *p*-methoxybenzalhydantoin, whilst, more recently, Harington [1926] following the same procedure, prepared thyronine from 4-(4'-methoxyphenoxy)benzalhydantoin. When, however, the former workers attempted, by shortening the duration of treatment with hydriodic acid, to limit the process to one of reduction only, they found that, whilst the main product was the saturated hydantoin, it was invariably accompanied by amino-acid; in other words a certain degree of hydrolysis was inevitable. This difficulty can be readily overcome by the method adopted by Lamb and Robson [1931] who used a strictly limited quantity of hydriodic acid (dissolved in glacial acetic acid) and red phosphorus for the reduction of α -benzamidocinnamic acids to the corresponding α -benzamidophenylpropionic acids.

The method as described by Wheeler and Hoffman gives products in good yield and of excellent purity and, apart from the high cost of the hydriodic acid employed, has only one drawback as a general method. In the synthesis of certain aromatic α -amino-acids, *e.g.* tryptophan, the use of acid reducing agents such as hydriodic acid must be avoided. In such circumstances recourse is had to sodium amalgam. But the use of this reagent, in turn, has its disadvantages. Firstly, a high yield of the reduction product is only obtainable by the use of

a very large excess of amalgam. Secondly, some hydrolysis always occurs during the process and the recovery of the resulting amino-acid from the solution, which contains a large quantity of sodium salts, is exceedingly difficult, if not impossible.

It was such practical difficulties as these, encountered during attempts to synthesise derivatives of tryptophan, which led us to investigate whether it was possible to carry out the reduction stage of the Wheeler-Hoffman synthesis by reagents other than those named above.

Amongst the many possibilities which suggested themselves to us was ammonium sulphide. In this connection we had, so far as we knew, no precedent for supposing that hydrogen sulphide would reduce a compound of type I to one of type II, but we were encouraged to attempt the experiment by the observation of Harries and Weiss [1903] that ordinary hydantoin can be hydrolysed to glycine by heating it with ammonium sulphide at 150° for 2 hours. On heating benzalhydantoin in a closed vessel with 16 % ammonium sulphide solution for 72 hours at 100° we obtained a mixture consisting of phenylalanine (50–60 % of theory) and benzylhydantoin (30–40 % of theory). A similar yield of amino-acid was obtained after only 3 hours by carrying out the reaction at 180°. Under similar conditions, benzalthiohydantoin gave partly α -thiocarbamido- β -phenylpropionic acid and partly the corresponding thiohydantoin, the former of which on being boiled with hydrochloric acid is reconverted into the thiohydantoin, whilst the latter compound, as shown by Johnson and Nicolet [1913], can be desulphurised to give benzylhydantoin by treatment with boiling chloroacetic acid.

From such results it was clear that we had found an excellent reducing agent, the use of which would eliminate many of the difficulties attached to the use of hydriodic acid and sodium amalgam. Since however the action of aqueous ammonium sulphide on the unsaturated hydantoin yields a mixture of products, it appeared essential to determine the conditions whereby maximum yields of the reduced hydantoin and of the amino-acid could be obtained.

The problem of obtaining a maximum yield of the reduced hydantoin was solved in the following way. Since certain unsaturated hydantoins were found to be extremely insoluble in aqueous ammonium sulphide even at 100°, an attempt was made to overcome this difficulty by carrying out the reaction at a higher temperature and by adding to the reaction mixture alcohol, pyridine or some such solvent miscible with water. Nevertheless, owing to the solubility factor, the amount of unsaturated hydantoin which could be treated by a given volume of ammonium sulphide solution was much less than the equivalent of the reducing power of the solution. In order therefore to make the method more convenient for the reduction of larger quantities of the more insoluble unsaturated hydantoins the possibility of effecting their reduction by hydrogen sulphide in anhydrous solvents, *e.g.* ethyl alcohol, pyridine *etc.*, was explored. Briefly it may be stated that the maximum yields of reduced hydantoin were obtained by heating the unsaturated hydantoin

(1) at 100° for 48–72 hours in pyridine saturated with hydrogen sulphide at 0°;

(2) at 58° in a mixture of aqueous ammonium sulphide and alcohol for a longer period;

(3) at 100° for 30–35 hours in an alcoholic solution of sodium or barium sulphide, the solution being saturated with hydrogen sulphide at 0° at the beginning of the experiment and again at that temperature after 16 hours.

The yields obtained by such methods was approximately the theoretical.

The optimum conditions for effecting the reduction of the unsaturated hydantoin having been determined, attention was next directed to the problem of converting the reduced hydantoin (II) into the amino-acid (III).

Reduced hydantoin can be hydrolysed by a variety of reagents both acid and alkaline. Lippich [1908] obtained a 92 % yield of leucine by heating its hydantoin for 24 hours with "fuming" hydrochloric acid. Dakin [1920] found that a 70 % yield of hydroxyproline was obtained when its hydantoin was boiled for 12 hours with a large excess of 10 % barium hydroxide solution. From experiments carried out in this laboratory, it appears that the respective times for complete hydrolysis of benzylhydantoin by 50 times its weight of constant boiling hydrochloric, hydrobromic and hydriodic acids are 20-30, 6-7 and 1-2 hours. Whilst these reagents are suitable from many points of view, they cannot, for reasons already given, be generally employed.

As has been mentioned, Harries and Weiss obtained glycine from hydantoin by heating the latter with commercial ammonium sulphide for 2 hours at 150°. These authors however omit to state the yield of amino-acid obtained. Since the use of ammonium hydroxide or ammonium sulphide (the sulphide being present presumably to protect the amino-acid in alkaline solution from atmospheric oxidation) has obvious advantages in the ready removal of the hydrolytic reagent and the isolation of the amino-acid, experiments were undertaken to determine the most suitable conditions for the hydrolysis of hydantoin by such means. From the results obtained, it appears that a suitable strength of reagent is about 6 % ammonium sulphide or 3 % ammonium hydroxide, and that whilst ammonium sulphide gives a practically theoretical yield, ammonium hydroxide under the same conditions gives only a 75-85 % yield of the amino-acid. Another very interesting fact was gleaned from these experiments. During the later stages of the hydrolysis of benzylhydantoin by 6 % ammonium sulphide solution, some colourless crystalline material separated. From its melting-point and other characteristics, this compound appeared to be phenylalanine anhydride. So far as we are aware the formation of this compound under such conditions has not been described before and it is being further investigated.

For purposes of presentation, the experimental work has been divided into the following sections:

(1) The action of aqueous ammonium sulphide at 100° on unsaturated hydantoin.

(2) The action of aqueous ammonium sulphide at 58° on unsaturated hydantoin.

(3) The action of sodium hydrogen sulphide at 100° on unsaturated hydantoin in (a) aqueous solution and (b) alcoholic solution.

(4) The action of hydrogen sulphide at 100° on unsaturated hydantoin in pyridine solution.

(5) The hydrolysis of reduced hydantoin by aqueous solutions of (a) ammonium sulphide and (b) ammonium hydroxide.

The successful use of hydrogen sulphide as a reducing agent for the purpose above described is novel and the possibility of the application of the reaction to other unsaturated substances of somewhat similar constitution suggests itself. It has already been found, for example, that hydrogen sulphide will reduce α -acetamidocinnamic acid and benzalcreatinine to phenylalanine and benzylcreatinine respectively. These and other extensions of the method will be discussed in a subsequent paper. Finally, the further possibility of the replacement of hydrogen sulphide by organic sulphides, *e.g.* thiolacetic acid or cysteine in such reactions is being investigated.

EXPERIMENTAL.

1. *The action of aqueous ammonium sulphide at 100° on unsaturated hydantoins.*

Benzylhydantoin and phenylalanine from benzalhydantoin. Benzalhydantoin (6.5 g.) was heated at 100° in a closed vessel for 64 hours with 80 ml. of commercial ammonium sulphide (16 %). The resulting solution was evaporated to dryness and treated with 200 ml. of boiling water and filtered. The filtrate was again evaporated to dryness, taken up in 200 ml. of hot water and re-filtered. The combined insoluble material consisting mainly of sulphur was extracted repeatedly with hot water and filtered. The united filtrates were concentrated until crystals of phenylalanine began to form on the surface. 100 ml. of 95 % alcohol were added, and the phenylalanine was allowed to crystallise out overnight. The amino-acid was collected and recrystallised from dilute alcohol. A second crop was obtained by concentrating the mother-liquor and adding more alcohol. This was collected in its turn and recrystallised. The total yield of amino-acid was 3.1 g. It melted at 263°, and contained 8.40 % N. Erlenmeyer [1893] gives m.p. 263°. $C_9H_{11}O_2N$ requires N 8.48 %.

The mother-liquors from which the phenylalanine separated were concentrated, acidified with hydrochloric acid and boiled for a few minutes. A white crystalline substance separated and this on recrystallisation from dilute alcohol melted at 188°; this was identified by m.p. and mixed m.p. with benzylhydantoin made from phenylalanine and potassium cyanate. On treating the two preparations separately with cold fuming nitric acid and adding cold water 4-benzyl-3-nitrohydantoin was obtained in each case. Both nitrohydantoins melted at 208° after recrystallisation from alcohol, and a mixture of the two melted at the same temperature. The benzylhydantoin obtained amounted to 2.2 g. and contained 14.67 % N. $C_{10}H_{10}O_2N_2$ requires N 14.74 %. These results showed that the reduction of the benzalhydantoin was practically quantitative.

p-Methoxyphenylalanine and p-methoxybenzylhydantoin from p-methoxybenzalhydantoin. In a similar manner to that described above, *p*-methoxybenzalhydantoin (1.0 g.) gave *p*-methoxyphenylalanine (0.5 g.; 50 %) melting at 235° and containing 7.25 % N. $C_{10}H_{13}O_3N$ requires N 7.18 %.

On acidification of the mother-liquor with hydrochloric acid and boiling, the corresponding hydantoin separated on cooling. On recrystallisation it melted at 182.5° and contained 12.64 % N. $C_{11}H_{12}O_3N_2$ requires N 12.73 %. The hydantoin prepared from *p*-methoxyphenylalanine and potassium cyanate melted at 181°, whilst a mixture of the two preparations melted at the same temperature. The yield of the hydantoin was 0.28 g.

Tyrosine from p-hydroxybenzalhydantoin. *p*-Hydroxybenzalhydantoin (2.65 g.) [cf. Boyd and Robson, 1935] was heated with 30 ml. of ammonium sulphide at 100° for 72 hours. On cooling, the mixture became semisolid owing to separation of the tyrosine. The crystals of amino-acid were collected and the remainder of the amino-acid was isolated as in the previous experiments. After recrystallisation from water and drying, the tyrosine weighed 0.75 g., contained 7.69 % N, and melted at 309° when heated rapidly, 300° when heated slowly. Wheeler and Hoffman [1911] state that the melting-point of synthetic tyrosine is "indefinite" and varies for the same specimen from 295 to 340° according to the rate of heating. $C_9H_{11}O_3N$ requires N 7.74 %. The yield was 32 % of the theoretical.

Benzylthiohydantoin and benzylhydantoin from benzalthiohydantoin. Benzalthiohydantoin (2.4 g.) was heated with a mixture of 50 ml. of alcohol and 100 ml. of commercial ammonium sulphide at 100° in a closed vessel for 72 hours. The

liquid was evaporated to dryness and the residue was taken up in dilute sodium hydroxide and filtered. The solution was made strongly acid by the addition of dilute hydrochloric acid and boiled for some minutes. The crystalline precipitate which separated on the solution cooling was recrystallised from hot water. It melted at 180°; after a further recrystallisation from water, at 185°. Johnson and Nicolet [1913] quote 185°. It contained 13.86 % N. $C_{10}H_{10}ON_2S$ requires N 13.60 %. It weighed 1.66 g., equivalent to 68 % of the theoretical.

Benzylthiohydantoin (1.0 g.) was boiled for one hour with 10 ml. of 50 % chloroacetic acid. The solution was then diluted with water and cooled. The hydantoin was collected and recrystallised from hot water. It melted at 189° and contained 14.60 % N. The yield was 0.67 g. or 73 % of theory.

p-Methoxybenzylthiohydantoin and *p*-methoxybenzylhydantoin from *p*-methoxybenzalthiohydantoin. *p*-Methoxybenzalthiohydantoin (2.0 g.) was heated with 50 ml. of 16 % ammonium sulphide for 72 hours at 100°. The reduced hydantoin was isolated as in the case of benzylthiohydantoin. It weighed 1.24 g., equivalent to 62 % of the theoretical. It melted at 215° and contained 11.73 % N. $C_{11}H_{12}O_2N_2S$ requires N 11.86 %. The preparation of this substance has not been recorded hitherto.

p-Methoxybenzylthiohydantoin (0.6 g.) was boiled with 10 ml. of 50 % chloroacetic acid for 1 hour and the *p*-methoxybenzylhydantoin was isolated as in the case of the benzylhydantoin. It weighed 0.41 g., melted at 182.5° and contained 12.84 % N.

2. The action of aqueous ammonium sulphide at 58° on unsaturated hydantoins.

Benzylhydantoin from benzalhydantoin. Benzalhydantoin (2.0 g.) was heated with a mixture of 50 ml. of 16 % ammonium sulphide solution and 50 ml. of alcohol for 240 hours at 58° in a closed bottle. The solution was evaporated to dryness, the residue boiled with 200 ml. of water and the mixture filtered. The insoluble sulphur remaining on the filter was extracted twice with hot water. The combined filtrates were acidified with hydrochloric acid, boiled with a little charcoal and filtered. The filtrate was concentrated and the benzylhydantoin allowed to crystallise out for 3 days. It was collected and washed with a little cold water. The dry hydantoin weighed 1.88 g. The volume of mother-liquor was about 25 ml. and since the solubility of benzylhydantoin is of the order of 1 g. per 1000 ml., it may be assumed that at least 0.03 g. remained in solution. The total yield was therefore 1.91 g. equivalent to 95 % of the theoretical value. The product melted at 187° and contained 14.63 % N. It is probable that a shorter period of heating the benzalhydantoin with the ammonium sulphide would suffice.

A similar experiment in which 50 ml. of ammonium sulphide without alcohol were used gave a yield of 51 % of the theoretical figure after 1 week at 58°. The insolubility of the benzalhydantoin in ammonium sulphide at 58° however was such that it was not completely dissolved until the sixth day. The reduction was therefore retarded by the insolubility of the unreduced substance.

p-Hydroxybenzylhydantoin from *p*-hydroxybenzalhydantoin. *p*-Hydroxybenzylhydantoin was prepared in a similar manner to that described for benzylhydantoin, the period of heating being extended to 2 weeks. The product melted at 254° and contained 13.69 % N. The yield was 90 % of the theoretical. $C_{10}H_{10}O_3N_2$ requires N 13.59 %.

p-Hydroxybenzylthiohydantoin from *p*-hydroxybenzalthiohydantoin. *p*-Hydroxybenzylthiohydantoin prepared from *p*-hydroxybenzalthiohydantoin as

described for benzylhydantoin melted at 209–210°, and contained 12.78 % N. $C_{10}H_{10}O_2N_2S$ requires N 12.62 %. Johnson and Nicolet [1913] quote 211° as the m.p. of a specimen of *p*-hydroxybenzylthiohydantoin made by the action of ammonium thiocyanate and acetic anhydride on tyrosine.

3. *The action of sodium hydrogen sulphide at 100° on unsaturated hydantoins.*

(a) *In aqueous solution.*

Benzylhydantoin from benzalhydantoin. Benzalhydantoin (2.0 g.) was treated with the equivalent amount (0.4 g.) of sodium hydroxide in 30 ml. of water. The bottle containing the solution was immersed in ice and the solution saturated with hydrogen sulphide, the hydantoin being precipitated thereby. The bottle was sealed and heated at 100° for 15 hours. It was then cooled, again saturated with hydrogen sulphide and finally re-heated at 100° for a further 16 hours. The solution was then evaporated to dryness *in vacuo*. The residue was treated with hot water and filtered from the insoluble sulphur. The filtrate, made strongly acid by the addition of hydrochloric acid, was boiled for 10 minutes. After cooling and keeping overnight 1.0 g. of pure benzylhydantoin separated. It melted at 188° and contained 14.66 % N. The mother-liquor was concentrated, made neutral to litmus by the addition of sodium hydroxide and boiled with 0.5 g. of potassium cyanate for 30 minutes to re-convert the amino-acid, produced by hydrolysis, into carbamido-acid. The liquid was then made strongly acid with hydrochloric acid and boiled for 10 minutes. A further crop of 0.59 g. of benzylhydantoin separated when the liquid cooled. It melted at 189° and contained 14.60 % N. If allowance be made for the solubility of benzylhydantoin in the 250 ml. of mother-liquor on the assumption that one part dissolves in 1000 ml. of water, the total yield of benzylhydantoin was 1.84 g. or 92 % of the theoretical value. Part of this (32 %) however consisted of amino-acid reconverted into hydantoin. The reduction was therefore practically quantitative.

A second experiment was carried out in which the heating period was only 16 hours. Examination of the resulting product showed that only partial reduction had been effected.

(b) *In alcoholic solution.*

Benzylhydantoin from benzalhydantoin. Benzalhydantoin (2.0 g.), sodium hydroxide (5 ml. of 10 % aqueous solution), water (25 ml.) and alcohol (60 ml.) were mixed and saturated with hydrogen sulphide at 0–5°. The mixture was heated in a closed vessel at 100° for 16 hours, re-saturated with hydrogen sulphide at 0–5° and again heated for 44 hours longer. The resulting solution was evaporated to dryness *in vacuo* and the residue taken up in 100 ml. of hot water. The hot solution was filtered and the insoluble sulphur repeatedly extracted with boiling water and filtered. The combined filtrates were acidified strongly with hydrochloric acid, boiled with a little charcoal and filtered hot. The filter was washed twice with hot water. The combined filtrates were concentrated to 150 ml. *in vacuo* and allowed to cool overnight. 1.32 g. of benzylhydantoin, m.p. 188°, were obtained. After addition of 0.15 g. as a correction for the amount of material remaining in solution, the total yield amounted to 1.47 g. or 73 % of the theoretical.

A similar experiment carried out in 90 % alcohol instead of 60 % alcohol gave a total yield, on the same basis, of 1.60 g. of benzylhydantoin, or 78 % of the theoretical.

Further experiments were carried out using in each case 2.0 g. of benzalhydantoin dissolved in 100 ml. of absolute alcohol, to which was added (1) 0.6 g. of sodium hydrogen sulphide (1 mol.) or (2) 0.3 g. of sodium hydrogen sulphide respectively. The cold solution was saturated with hydrogen sulphide, heated for 16 hours at 100°, cooled, re-saturated with hydrogen sulphide and again heated for 16 hours at 100°. The experiment with 1 mol. of sodium hydrogen sulphide yielded 1.5 g. of reduced product, or 75 % of the theoretical. That with 0.5 mol. of sodium hydrogen sulphide yielded 1.62 g. of the required product or 80 % of the theoretical figure.

Finally, an experiment was carried out using 1.8 g. of benzalhydantoin, 2 ml. of 20 % sodium hydroxide solution and 100 ml. of absolute alcohol. The other conditions were the same as in the two preceding experiments. The yield of pure reduced product was here 1.59 g. or 87 % of theory.

It may be noted that solutions of benzylhydantoin which have been saturated at a higher temperature and then kept at room temperature do not attain equilibrium for several days. The solubility figure used above refers to solutions in pure water. The actual yields were probably a little higher than the values given.

p-Hydroxybenzylhydantoin from p-hydroxybenzalhydantoin. *p*-Hydroxybenzalhydantoin (2.0 g.) was dissolved in sodium hydroxide (2.0 ml. of 20 %) and alcohol (100 ml.). The solution was saturated at 0° with hydrogen sulphide, heated at 100°, cooled, re-saturated with hydrogen sulphide and again heated at 100° for 16 hours. After being cooled, the solution deposited 0.80 g. of pure *p*-hydroxybenzylhydantoin which melted at 256°. Wheeler and Hoffman [1911] quote 257–258°. A further 0.60 g. was obtained in the usual way after removal of sulphur from the solution. The yield was thus 70 % of the theoretical. The material contained 13.62 % N. $C_{10}H_{10}O_3N_2$ requires N 13.59 %.

Experiments carried out with larger proportions of sodium hydrogen sulphide or sodium hydroxide yielded an oily by-product.

4. *The action of hydrogen sulphide at 100° on unsaturated hydantoins in pyridine solution.*

p-Methoxybenzylhydantoin from p-methoxybenzalhydantoin. *p*-Methoxybenzalhydantoin (2.0 g.) was dissolved in pyridine (20 ml.) to which concentrated ammonia (1 ml.) had been added. The mixture was saturated at 0–5° with hydrogen sulphide and then heated at 110° in a closed vessel for 48 hours. The solution was cooled to 5°, re-saturated with hydrogen sulphide and again heated under the same conditions for a further 18 hours. The solution was evaporated to dryness *in vacuo*, the residue taken up in boiling water and the whole filtered to remove the insoluble sulphur. The latter was boiled out repeatedly with fresh quantities of hot water, the suspension being filtered each time. The combined filtrates were concentrated to 250 ml. and cooled. 1.62 g. of *p*-methoxybenzylhydantoin, melting at 182° and containing 12.80 % N, was thus obtained. On concentrating the mother-liquors a further 0.3 g. of the required material was obtained. The yield amounted to 95 % of the theoretical figure.

Benzylhydantoin from hydantoin and benzaldehyde without intermediate isolation of benzalhydantoin. Benzaldehyde (5.5 g.), hydantoin (4.5 g.), pyridine (10 ml.) and diethylamine (10 drops) were heated to 98° for 42 hours in a closed bottle. The mixture was then cooled, more pyridine (20 ml.) added, and the solution was saturated with hydrogen sulphide at –5°. It was thereafter heated for 66 hours at 98°. The benzylhydantoin was isolated as in the preceding experiments. It weighed 4.1 g. or 48 % of the theoretical.

5. (a) *The hydrolysis of reduced hydantoin by aqueous ammonium sulphide.*

Phenylalanine from benzylhydantoin. Benzylhydantoin (2.0 g.) was dissolved in a mixture of 10 ml. of concentrated ammonia (sp. gr. 0.880) and 90 ml. of water. The solution was saturated with hydrogen sulphide at 0°. The vessel containing the resulting mixture was then closed and heated for 5 days at 100°. At the end of this time the solution was clear except for a small quantity of white flocculent matter. It was filtered, the filtrate concentrated until the amino-acid began to separate and finally treated with 50 ml. of alcohol. Pure phenylalanine crystallised out in well-formed plates which were filtered off and washed with dilute alcohol. It weighed 1.4 g., melted at 262° and contained 8.39 % N. A second crop was obtained by concentrating the mother-liquor and treating it again with alcohol. This contained 8.35 % N and weighed 0.3 g. The total yield was, therefore, 1.7 g., which was practically theoretical.

The flocculent material removed from the solution by filtration before isolating the amino-acid melted at 292°, and was evidently phenylalanine anhydride. Phenylalanine anhydride was found by Erlenmeyer and Lipp [1883] to melt at 290–291°. The amount isolated in the above experiment weighed 0.03 g.

5. (b) *The hydrolysis of reduced hydantoin by aqueous ammonium hydroxide.*

An exactly similar experiment to the preceding was carried out but omitting the saturation of the solution with hydrogen sulphide. In this case the deposit found in the solution after the period of heating was dark brown in colour indicating that oxidation had taken place to a certain extent. The yield of amino-acid isolated was 1.41 g., equivalent to 80 % of the theoretical. It contained 8.37 % N.

SUMMARY.

Reduction of unsaturated hydantoin, obtained by condensing aromatic aldehydes with ordinary hydantoin and with 2-thio-3-acetylhydantoin respectively, is readily effected quantitatively by heating them at 100° with solutions of sulphides for 24–72 hours. For this purpose aqueous or alcoholic solutions of ammonium sulphide, sodium hydrogen sulphide or sulphides of organic bases may be employed. The accompanying hydrolysis which occurs at 100° in the presence of water may be avoided by using anhydrous or almost anhydrous solutions of ammonium sulphide or sodium hydrogen sulphide in alcohol, or of hydrogen sulphide in pyridine or by heating the unsaturated hydantoin with aqueous ammonium sulphide at a lower temperature (58°) for a longer period (7–14 days). Some alcohol is usually added to the aqueous ammonium sulphide to increase the solubility of the unsaturated hydantoin.

In general a cleaner product is obtained by this method than by those hitherto employed, and it is especially applicable in cases where the use of hydriodic acid or of sodium amalgam is precluded by the instability of other parts of the molecule.

Hydrolysis of the reduced product to the amino-acid may be carried out in conjunction with the reduction process or separately. Dilute solutions of ammonium sulphide give a quantitative yield of pure amino-acid from the reduced hydantoin in 120 hours at 100°. Dilute ammonium hydroxide solutions are almost equally satisfactory but the presence of the sulphide appears to inhibit the darkening (? oxidation) of the solution during the process of hydrolysis.

A small amount of material separates during the course of hydrolysis by ammonium sulphide. This seems to consist of amino-acid anhydride.

REFERENCES.

- Boyd and Robson (1935). *Biochem. J.* **29**, 542.
Dakin (1920). *J. Biol. Chem.* **44**, 518.
Erlenmeyer (1893). *Liebig's Ann.* **275**, 1.
—— and Lipp (1883). *Liebig's Ann.* **219**, 179.
Harrington (1926). *Biochem. J.* **20**, 300.
Harries and Weiss (1903). *Liebig's Ann.* **327**, 372.
Johnson and Nicolet (1913). *Amer. Chem. J.* **49**, 197.
Lamb and Robson (1931). *Biochem. J.* **25**, 1231.
Lippich (1908). *Ber. deutsch. chem. Ges.* **41**, 2957.
Wheeler and Hoffman (1911). *Amer. Chem. J.* **45**, 368.