XLIII. HYDROLYSIS OF PROTEINS WITH AN ALCOHOLIC SOLUTION OF HYDROGEN CHLORIDE.

PART I.

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In the usual method of isolating the mono-amino-acids resulting from protein hydrolysis, aqueous hydrochloric acid is used as the hydrolysing agent, and subsequently a saturated alcoholic solution of hydrogen chloride is used for the purpose of esterification. An attempt was made to shorten this double process by using a saturated alcoholic solution of hydrogen chloride from the beginning to serve both as a hydrolysing and an esterifying agent.

Further, a saturated alcoholic solution of hydrogen chloride is undoubtedly a milder reagent than a saturated aqueous solution of the same, first, because the solubility of hydrogen chloride is smaller in alcohol than in water, and secondly, because it cannot be heated to as high a temperature as the aqueous solution; so it was expected that the process of hydrolysis would not be quite complete, but might stop at an earlier stage than that of the amino-acids.

While the research was in progress, Pribram [1911] and Abderhalden and Hanslian [1912] published their investigations, in which the action of this reagent on proteins was studied. But their object being quite different from that of the present investigation, they have not pursued the subject further.

In the present investigation, experiments have so far been made on caseinogen from cow's milk, and on silk-fibroin. In each case it was found that the protein was only partially attacked. The corresponding mono-amino-acids were isolated by the usual methods, and converted into copper salts, which were analysed. It must be admitted that the evidence of these analyses is not absolutely conclusive; but the authors believe that it is sufficient to show the identity of the various substances, which are known to be present in the different fractions by the more thorough investigations made by using the ordinary method.

Although the proportion of the protein attacked could not always be exactly determined, as the reaction liquid could not be filtered free from the solid floating in it, it appeared certain that a very large proportion had been attacked. The yield of the amino-acids, however, was comparatively poor. This makes it extremely probable that a large proportion of products, more complex than the simple amino-acids, had been formed. The isolation of individual substances from this complex mixture is an extremely difficult task; and experiments are still being carried on for that purpose.

EXPERIMENTAL.

Caseinogen.

200 grams of caseinogen were hydrolysed by boiling for several hours with 2 litres of alcohol, saturated with hydrogen chloride. There was some unchanged solid which could not be filtered. The mixture was, therefore, evaporated in vacuo, and the residue decomposed with sodium hydroxide, and extracted with ether in the usual way. A very strong smell of ammonia was evident throughout this process.

The ethereal extract was then fractionated under 14 mm. pressure, and the following fractions collected. The temperatures are those of the bath.

I -60° , II $60-100^{\circ}$, III $100-140^{\circ}$, IV $140-170^{\circ}$.

They were further worked up in the usual way.

Fraction I: This gave 0.4 gram of alanine, which was confirmed by analysing the copper salt.

0.0500 grm. gave 0.0162 grm. CuO. Cu = 25.92 per cent.

Calculated for alanine, Cu = 26.51 per cent.

Fraction II: This gave 3.5 grams of a mixture of leucine (also isoleucine), valine, and proline. They were isolated in the usual way, and confirmed by analysing the copper salts.

Leucine (and isoleucine):

0.1225 grm. gave 0.0293 grm. CuO. Cu = 19.13 per cent.

Calculated for leucine, Cu = 19.63 per cent.

Valine:

0.1098 grm. gave 0.0300 grm. CuO. Cu = 21.85 per cent.

Calculated for valine, Cu = 21.48 per cent.

Fraction III: This gave about 1.5 gram of a mixture of leucine, etc., and a little glutamic acid. The latter was converted into the copper salt, which was analysed.

0.0724 gm. gave 0.0280 gm. CuO. $\text{Cu} = 30.93$ per cent.

Calculated for glutamic acid, $\text{Cu} = 30.45$ per cent.

Proline: About 1 gram of this was obtained from the last two fractions. It was confirmed by analysing the copper salt, which was first made anhydrous.

0.0572 gm. gave 0.0154 gm. CuO. $\text{Cu} = 21.53$ per cent.

Calculated for proline, $\text{Cu} = 21.77$ per cent.

Fraction IV: Phenylalanine was isolated from this in the usual way, and confirmed by estimating the chlorine in the hydrochloride, of which about 0.4 gram was obtained.

0.1105 gm. gave 0.0778 gm. AgCl. $\text{Cl} = 17.38$ per cent.

Calculated for phenylalanine hydrochloride, $\text{Cl} = 17.61$ per cent.

The rest of the fraction gave 0.5 gram of a mixture of solids.

The dark brown residue in the distilling flask was extracted with hot water. On evaporating the aqueous solution, about 10 grams of a yellowish white powder were obtained, which was most probably a mixture of diketopiperazines.

The residue in the extraction flask was not worked up as usual for re-esterification, but is being worked up with the object of isolating the more complex products of hydrolysis.

Silk-fibroin.

50 grams of silk-fibroin were boiled for several hours with about a litre of alcohol, saturated with hydrogen chloride. The fibre was entirely broken down, but a very fine solid was still floating in the brown liquid, which, in this case, could be easily filtered off. The fine solid weighed about 14 grams on drying.

The liquid was further dealt with exactly as in the last case; here, too, the smell of ammonia was very evident during the process of extraction with ether. The ethereal extract was distilled in the usual way, the temperature of the bath being only taken up to 100° . About 5.5 grams of a mixture of glycine and alanine were obtained. They were not separated quantitatively; but the picrate of glycine was prepared according to Levene's method, and melted at 190° .

No crystalline solid could be obtained from the residue in the distilling flask, which, however, gave very beautifully Denigès' test for tyrosine, as modified by Mörner [1902].

The residue from the extraction is being worked up for the isolation of complex hydrolytic products, as in the last case.

Another interesting experiment was made with silk-fibroin. About a gram of it was heated with about 10 c.c. of absolute alcohol saturated with hydrogen chloride, in a sealed tube for about seven hours at 110–120°. The whole of the fibroin was found to have gone into solution without any residue.

REFERENCES.

- Abderhalden and Hanslian (1912), *Zeitsch. physiol. Chem.* **77**, 285.
Mörner (1902), *Zeitsch. physiol. Chem.* **37**, 86.
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