ABSTRACTS FROM THE ORIGINAL PAPERS.

*On the Products of Partial Hydrolysis
of the Silkfibroin.*

By Hisashi ARIYAMA.

(Received June 14th, 1925)

In 1923 W. S. Ssodikow and N. D. Zelinsky (Biochem. Zeitschr., 136. 241; 143, 504; 147. 30) reported on a new method for hydrolysing the protein. Their method consists in the following two principal processes: (1) hydrolysis of the proteins by heating them with 1 % HCl in autoclave at high temperature for several hours (2) successive extraction of resulting products with organic solvents. By this method they obtained from goose feather and horse hair various kinds of anhydride of amino acids. The writer applied this method to the silkfibroin, slightly modifying the conditions, and isolated as the decomposition products four kinds of anhydride, in which glycine, alanine and tyrosine were the constituents respectively. Hydrolysis of the silkfibroin:—

1 kg silkfibroin was heated with 8 times its weight of 0.5% HCl in autoclave for 8.5 hours at 170°C. After filtered from the voluminous undissolved matter (300g. in dry state), the solution was neutralized with dilute NaOH and was concentrated to one-tenth of the original volume below 40°C.

Isolation of the anhydrides:—

Crystal A (2 tyrosine + 2 glycine + 1 alanine anhydride):

This separated as crystalline plates during the operation of concentrating the neutralized solution. Decolorized and recrystallized several times from hot water, it crystallized in heavy transparent plates, m. p. 255–258°C (incorr). It is almost insoluble in ordinary organic solvents, but is soluble in water and glacial acetic acid when warmed and gives Millon's—and picric acid reactions (anhydride reaction), but biuret—and ninhydrin reactions are absent.

Complete hydrolysis of this compound gave tyrosine and the mixture of glycine and alanine. From these results, as well as from the results of elementary analysis, Crystal A may be regarded as an anhydride consisted of 2 mol. tyrosine + 2 mol. glycine + 1 mol. alanine.

Crystal B (glycyl-l-tyrosine anhydride):

The concentrated solution, after being filtered from Crystal A, was extracted with acetic ether in Dakin's extraction apparatus for 35 hours. From this extract Crystal B was obtained as fine white needles, m. p. 267–275°C (incorr.), and $[\alpha]_D^{20} = +100.2^\circ$ in ammoniacal solution. It is insoluble in ether and CHCl_3 , soluble in warm alcohol and water and easily soluble in dilute NH_4OH and glacial acetic acid in the cold. As regards its color reactions, Crystal B behaves just analogously to Crystal A.

On evaporating the mother liquor of the recrystallization of crystal B, a further crop of white needles was obtained which may be called here Crystal C. From the results of elementary analysis and various properties, Crystal C may be regarded as almost identical with Crystal B, except that it shows a little difference in its melting point, i.e. 282–285°C (incorr).

Crystal D (glycyl-alanine anhydride):

When the filtrate of Crystal B was evaporated down in vacuo, yellowish solid substance separated out which was taken in warm acetic ether. On cooling the acetic ether solution, Crystal D separated out as transparent heavy crystals, m. p. 242–243°C (incorr) and $[\alpha]_D^{20} = +5.7^\circ$ in aqueous solution. It is soluble in water and glacial acetic acid, but rather difficultly in alcohol, methylalcohol and ether. It gives strong picric acid reaction while ninhydrin- and Millon's reactions, are absent. Cryoscopic measurement in Water gave the molecular weight of 131 (theory 128).

By the hydrolysis, Crystal D gave glycine and alanine.

Crystal E (glycyl-d-alanine anhydride): This was obtained from the filtrate of Crystal D and recrystallized from warm methyl alcohol in very fine white needles, m. p. 245–247°C (incorr.) and $[\alpha]_D^{20} = -3.8$ in aqueous solution. In the solubility and color reactions Crystal E is almost analogous to Crystal D and also the results of elementary analysis stand close to that of Crystal D. The complete hydrolysis of Crystal E gave glycine and alanine. Measurement of its molecular weight gave close value to that of Crystal D, i. e. $M=134$ (Crystal D. 131).

While on the various points, as above mentioned, these two crystals are almost

analogous, they differs as to the crystall form and rotatory power each other, and these differences may be due to the degree of racemization of these compounds during operations.

Of these four kinds of anhydride, Crystal B (C) (glycyl-l-tyrosine anhydride) and Crystal E (glycyl-d-alanine anhydride) are identical with those previously obtained from the silkfibroin by F. Fischer and E. Abderhalden while as to the isolation of the other two anhydrides, Crystal A (2 tyrosine + 2 glycine + 1 alanine anhydride) and Crystal D (dextro rotatory glycyl-alanine anhydride) it seems that nothing has been reported up to present.

Studies on Proteins. (II).

By Kinsuke KONDO.

(Contribution No. 3. from the Laboratory of Nutritional Chemistry, Department of Agriculture, Kyoto Imp. University) (Received July 1st, 1925)

A. It has been shown by surveying the beautiful works of S. P. L. Sørensen and J. Loeb that the Donnan theory has been of great importance in the development of the theoretical chemistry of proteins. Viewed in the light of the activity theory, the facts are in reality very little altered. The electric potential difference E_M is nearly determined here by

$$E_M = \frac{RT}{F} \ln \frac{a'}{a''} \dots\dots\dots (1)$$

where a' and a'' represent the activity in a state of equilibrium of one of the monovalent diffusible ions on the one side and on the other side of the membrane. And if we take sodium chloride as the only diffusible electrolyte, Donnan's simple equation

$$C'_{Na} \cdot C'_{Cl} = C''_{Na} \cdot C''_{Cl}$$

is altered accordingly to

$$a'_{Na} \cdot a'_{Cl} = a''_{Na} \cdot a''_{Cl} \dots\dots\dots (2)$$

B. The methods and apparatus serving for determining the membrane potential of casein chloride solution are described in detail. The measurement of the membrane potential is effected by means of the following element:

Hg, HgCl, 0.1n KCl | 3.5n KCl | Prot. sol. || Membrane || outer liquid | 3.5n KCl | 0.1n KCl, HgCl, Hg.
where the protein solution contains, in addition to the protein, also the diffusible