

Studies on the synthesis of Protein Analogus (PartV)

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Studies on the Synthesis of Protein Analogus (Part V)

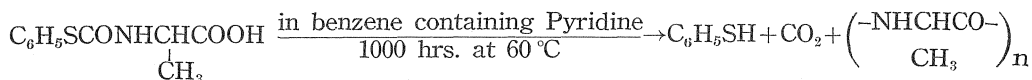
Polymerisation of DL-alanine*

By

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Successively to the preceeding method, we tested the polymerisation of DL-alanine by the method of N-carbothiophenyl-amino acid. Polyalanine having high molecular weight, about 22,400, was prepared.



N-carbothiophenyl-DL-alanine (m. p. 138°C) was prepared as the starting material by the Lindenmann's method.¹⁾ In this polymerisation, the solution polymerisation gave the better results than the block polymerisation. It was carried out at the heating of 60°C for 1000 hours in a sealed tube, in which the benzene solution contained about ten moles pyridine to the sample as in the case of polymerisation of glycine.²⁾ With the progress of the polymerisation, protein-like precipitation began to appear gradually. It was shaken now and again to make it uniform. After the reaction, the polymer was isolated with centrifuge from the mother liquor and then washed with alcohol and ether, and dried. The polymerisation rate was 70.8% of the theoretical. The analysis corresponded to polyalanine and the clear Biuret's reaction was observed.

It is insoluble in water, alcohol, benzene and acetic acid, and sparingly soluble in conc. alkali, and soluble in conc. hydrochloric acid, the mixture of acetic acid and monochloroacetic acid (1 : 1) and dichloroacetic acid.

The molecular weight by osmotic method and the viscosity with the Ostwald's viscometer were measured in the dichloroacetic acid solution, and the polymerisation degree and the intrinsic viscosity were determined, the molecular weight M. W. = 22,400, the polymerisation degree $n=316$, the intrinsic viscosity $[\eta]=0.292$.

The pyrolysis of N-carbothiophenyl-DL-alanine by thermobalance showed that the decomposition³⁾ occurred at about 140°C gradually, at about 150°C swiftly and at 160—170°C suddenly and at last the residue came close to the poly-DL-alanine (N=19.72%) in its nitrogen content but it was difficult to take a value in accordance with the theoretical, and the polymer gave always a little lower value.

N-carbothiophenyl-DL-alanine was kept at $100 \pm 1^\circ\text{C}$ for 2.5 hours under the reduced

* It was published in the Jan. of the Chem. Soc. of Japan **75**, 643—645 (1954) (in Japanese).

1) A. Lindenmann, N. H. Khan, K. Hofmann, J. Am. Chem. Soc. **74**, 476 (1952)

2) J. Noguchi, M. Asai, S. Ishino, T. Hayakawa, Jan. of the Chem. Soc. of Japan **75**, 642 (1954).

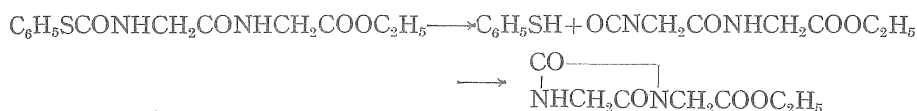
3) J. Noguchi, Jan. of the Chem. Soc. of Japan **74**, 962 (1953) Fig. 1.

pressure of 9 mm Hg below, and after the constant weight was obtained, the sample was taken out. It was hygroscopic powder and N=18.35%. When it was heated again at $25.0^\circ \pm 1^\circ\text{C}$ for 1.5 hours, it was N=19.54% and came close to poly-DL-alanine in its nitrogen content. When N-carbothiophenyl-DL-alanine was heated at $200 \pm 1^\circ\text{C}$ for about 4 hours, a part of white plate crystal sublimed and the residue was brown powder of the polymer. The white plate crystal did not show Biuret's reaction and was found to correspond to alanine diketo-piperazine, to judge from its nitrogen analysis and its picrate. The brown powdered polymer was hygroscopic and N=18.36%.

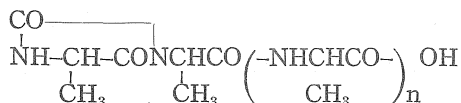
The monomer was heated at $150 \pm 1^\circ\text{C}$ for 4 hours in toluene with a sealed tube. It was dissolved in toluene and then almost gelatinized. After filtration, washing with toluene and ether, the polymer was dried. It was N=16.48%. When heated at $200 \pm 1^\circ\text{C}$ for 3 hours in xylene, it was N=17.81%. When heated at 180°C for 8 hours in toluene and then at 250°C for 2 hours, it was N=18.14%.

These polymers were hardly soluble in alcohol, a little soluble in water and showed clear Biuret's reaction.

Lindenmann etc.¹⁾ showed the following reaction in the water solution of N-carbothiophenyl-dipeptide ester :



In consideration of the above reaction and of the results which did not show any trace of thiophenol in the polymer, the formula of these polymers would be as follows :



The formula corresponds to the experimental results as the following shows.

n	1	2	3	4	5	6	7	∞
Calc. N%	16.35	17.1	17.52	17.86	18.12	18.30	18.47	19.72
anal. N%	16.48	—	—	17.81	18.14	18.35	—	19.54

It is natural that some analytical values take the intermediate of the theoretical, because each polymer analysed was not a pure substance having the same polymerisation degree. But they can be classified as above.

After all, in the preparation of polyalanine by N-carbothiophenyl-DL-alanine, the solution polymerisation at low temperature gave good result as in other cases.

Experimental

Poly-DL-alanine—N-carbothiophenyl-DL-alanine 2.25 g was dissolved in benzene 70 cc containing pyridine 7.9 g and polymerized at 60°C for 1000 hours in a sealed tube. After several days some opaque fibrous precipitate separated out and after

several hundred hours a large amount of protein-like precipitate was obtained. It was centrifuged after 1000 hours, washed with alcohol and ether, and dried. It was white powder. Yield 0.503 g (70.8% of the theoretical). Clear Biuret's reaction was observed.

N analysis found N=19.91%

for $(C_3H_5ON)_n$ calc. N=19.72%

Molecular weight : It was measured by the osmotic method using Meshizuka's apparatus.⁴⁾

The polymer 0.0521 g was dissolved in dichlor-acetic acid and the total volume diluted to 10 cc and measured at $30 \pm 0.1^\circ C$.

Concentration $C=5.21g/L$, Specific gravity $\rho=1.485$

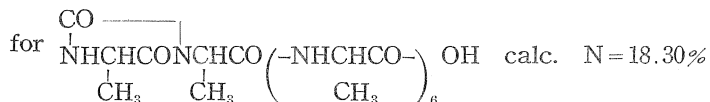
Osmotic press. $\pi=3.89 \text{ cm}=5.75 \times 10^{-3} \text{ atm}$.

Molecular weight M. W.=22,400, Polymerisation degree $n=316$

Polymerisation of N-carbothiophenyl-DL-alanine at high temperature — (1)

N-carbothiophenyl-DL-alanine 1 g was taken in a test tube and heated at $150 \pm 1^\circ C$ for 2.5 hours in 9 mm Hg vacuum. Hygroscopic powder 0.316 g was obtained.

N analysis found N=18.35%



This sample was heated more at $250 \pm 1^\circ C$ for 1.5 hours. It was somewhat hygroscopic.

N analysis found N=19.54%

for $(C_3H_5ON)_\infty$ calc. N=19.72%

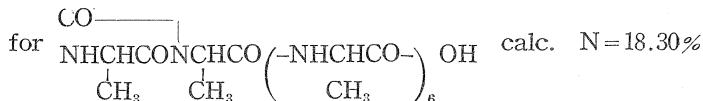
(2) N-carbothiophenyl-DL-alanine 1g was taken in a test tube and heated at $200 \pm 1^\circ C$ for 40 minutes in 2 mm Hg vacuum. Some white crystal sublimed on the wall and brown polymer was obtained. When the brown polymer was washed with water, alcohol and ether, it became brown powder. Yield 0.2 g.

Analysis of white crystal found N=19.74%

for $(C_3H_5ON)_2$ calc. N=19.72%

It was dissolved in water, had ammonium picrate solution added to it. It gave yellow precipitate. Biuret's reaction was not observed. It must be diketopiperazine.

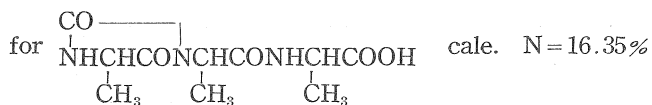
Analysis of brown polymer found N=18.36%



(3) N-carbothiophenyl-DL-alanine 0.3 g was dissolved in toluene 10 cc and heated at $150 \pm 1^\circ C$ for 4 hours in a sealed tube. It was almost solidified in about one hour of the first reaction. It was washed with toluene and ether, and dried.

N analysis found N=16.48%

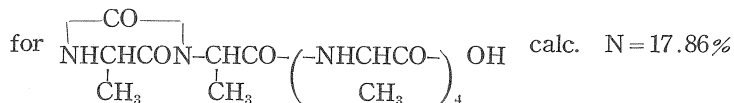
4) G. Meshizuka, The Chemistry of Highpolymer 6, 305 (1949)



(4) N-carbothiophenyl-DL-alanine 1 g was dissolved in xylene 10 cc and heated at $200 \pm 1^\circ\text{C}$ for 3 hours in a sealed tube. It was treated with alcohol.

N analysis

found $N = 17.81\%$



(5) N-carbothiophenyl-DL-alanine 2 g was dissolved in toluene 20 cc and heated at 180°C for 8 hours and then at 250°C for 2 hours. When it was washed with alcohol, it became white powder.

N analysis

found $N = 18.14\%$

