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Studies on the Synthesis of Protein Analogus (Part VI)
Polymerisation of L-leucine, Co-polymerisation of L-leucine
and DL-phenylalanine, and Co-polymerisation
of Glycine and DL-alanine.

By

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By using the Noguchi's method¹⁾ by which N-carbothiophenyl-amino acids can be polymerized in polypeptide, poly-L-leucine, copoly L-leucine and DL-phenylalanine, and copoly glycine and DL-alanine, each with high polymerisation degree, were prepared.

The N-carbothiophenyl-L-leucine (syrup) and N-carbothiophenyl-DL-phenylalanine (m. p. 56 °C) which were employed in polymerisations were prepared as described in the Part I.²⁾

In these polymerisations, the solution polymerisation also gave better results than the block polymerisation. Benzene was used as solvent in the polymerisation of L-leucine and in the co-polymerisation of L-leucine and DL-phenylalanine, and dioxane was used in the copolymerisation of glycine and DL-alanine. Adding a small quantity of pyridine to the solutions, the polymerisation reaction were accelerated. In poly-L-leucine, the monomer, which was dissolved in benzene or in benzene containing ten moles pyridine to the sample, was heated at 80 °C for long hours in a sealed tube. The viscosity of solution increased with the progress of polymerisation, and protein-like polymer precipitated in swollen clear gel. Now and again, it was agitated to become uniform. The gel polymer was precipitated from the solvent with the progress of polymerisation.

It was insoluble in water, alcohol, benzene and acetic acid, and soluble in dichloroacetic acid. Biuret's reaction was not observed. The analysis corresponded to poly-L-leucine.

The molecular weight and the polymerisation degree were measured by osmotic method in dichloroacetic acid solution and the viscosity was determined with Ostwald's viscosimeter.

molecular weight $M=26,689$, polymerisation degree $n=236$, intrinsic viscosity $[\eta]=0.312$

In the copolymerisation of L-leucine and DL-phenylalanine, N-carbothiophenyl-L-

* It was published in the Jan. of the Chem. Soc. of Japan **75**, 645—647 (1954) (in Japanese).

1) J. Noguchi, *ibid.* **74**, 963 (1953) ; *ibid.* **75**, 639—645 (1954) (in Japanese).

2) J. Noguchi, K. Miyamori, S. Ishino, Jan. of the Chem. Soc. of Japan **75**, 641 (1954)

leucine and N-carbothiophenyl-DL-phenylalanine were used in equivalent moles and polymerised in benzene at 60 °C for 1000 hours.

This polymer did not show the Biuret's reaction as poly-DL-phenylalanine.³⁾ It was insoluble in conc. alkali, conc. hydrochloric acid, alcohol and ether, soluble in benzene, and easily soluble in dichloroacetic acid. When the benzene solution was evaporated, it formed film. It is clear from the analysis that the copolymer which was copolymerized at the rate of equivalent moles of DL-phenylalanine and L-leucine has the components of almost the same ratio. Each single polymer of DL-phenylalanine and L-leucine is both hardly soluble in benzene, but the co-polymer is soluble in benzene. Its solubility is similar to that of the co-polymer of DL-phenylalanine and L-leucine which was prepared by Woodward *etc.*³⁾ by the method of N-carboxylic anhydride. From these facts, it is clear the polymer is not mixed-polymer but copolymer. The molecular weight by Osmotic method is $M=161,000$ and the intrinsic viscosity is $[\eta]=2.42$.

In the copolymerisation of glycine and DL-alanine, N-carbothiophenylglycine and N-carbothiophenyl-DL-alanine were used in equivalent moles and polymerized in dioxane having pyridine at 60 °C for 1000 hours.

The copolymer was precipitated from the dioxane in opaque light deposit of protein like. It was separated, treated with alcohol, washed with ether and dried. The rate of polymerisation was about 36% of the theoretical. It is white powder and shows clear Biuret's reaction. It is easily soluble in water, hardly soluble in alcohol, and insoluble in ether. The molecular weight by osmotic method in water solution is $M=11,457$, and polymerisation degree $n=90$ and intrinsic viscosity $[\eta]=0.093$.

In spite of the fact that the simple polymer of glycine or DL-alanine is insoluble in water at the molecular weight of about over 10,000 order, the co-polymer is easily soluble in water and differs in the solubility from them. The analytical value corresponds also to the ratio of about 1 : 1 of glycine and DL-alanine. So it will be not the mixed-polymer of polyglycine and poly-DL-alanine, but the copolymer of their components.

Experimental

Poly-L-leucine—(A) N-carbothiophenyl-L-leucine 0.3 g (0.0011 moles) was dissolved in benzene 10 cc and heated at 80 °C for 670 hours in a sealed tube. The solution gelatinized completely. It was centrifuged with alcohol, washed with alcohol and ether, and dried. It was white powder. Yield 0.08 g (63% of the theoretical).

N analysis found N=12.51%
for (C₈H₁₀ON)_n calc. N=12.39%

(B) N-carbothiophenyl-L-leucine 0.4 g (0.0015 moles) was dissolved in the mixture of benzene 10 cc and pyridine 1.2 g (0.015 moles). It was heated at 80 °C for 1000 hours. Some gelatinous polymer deposited on the glass wall, and the clear solution

3) R. B. Woodward, C. H. Schramm, J. Am. Chem. Soc. **69**, 1551 (1947)

was somewhat viscous. It was clearer and in more soluble state than in pure benzene solvent (A). It was precipitated with a large amount of alcohol, centrifuged, washed with ether and petrol ether, and dried. White powder. Yield 49 mg. (29% of the theoretical).

The molecular weight and the viscosity were measured with the sample (A).

Molecular weight : It was measured at 30 °C by the Meshizuka's osmotic method⁴⁾ in dichlor-acetic acid

Concentration $C=4.78$ g/L, Osmotic press. $\pi=4.45 \times 10^{-3}$ atm.

Molecular weight $M=26,689$, polymerisation degree $n=236$

Viscosity : It was measured at 30 °C in dichlor-acetic acid.

intrinsic viscosity $[\eta]=[\eta]_{sp}/c]_{c \rightarrow 0}=0.312$

Copoly-(L-leucine and DL-phenylalanine)—N-carbothiophenyl-L-leucine 0.67 g and N-carbothiophenyl-DL-phenylalanine 0.75 g was dissolved in benzene 40 cc and kept at 60 °C for 1000 hours. The viscosity increased with the progress of polymerisation. It was precipitated with ligroin, centrifuged, washed with ether several times and dried. Yield 0.3 g (44.3% of the theoretical)

N analysis

found N=10.49%

for $(C_9H_9NO:C_6H_{11}NO=1:1)_n$ calc. N=10.76%

Molecular weight : Sample of about 40 mg was refluxed with benzene 12 cc for several hours, dissolved completely, and left overnight. The concentration was determined after evaporating the solution to dryness.

Concentration $C=3.30$ g/L, Osmotic press. $\pi=5.09 \times 10^{-4}$ atm.

Molecular weight $M=161,000$

Viscosity : It was measured at 30 ± 0.1 °C with Ostwald's viscosimeter.

intrinsic viscosity $[\eta]=[\eta]_{sp}/c]_{c \rightarrow 0}=2.42$

Copoly-(glycine and DL-alanine)—N-carbothiophenyl-glycine 2.11 g and N-carbothiophenyl-DL-alanine 2.25 g was dissolved in hot dioxane 50 cc containing pyridine 7.9 g and polymerized at 60 °C for 1000 hours. Protein-like polymer separated out in opaque gel. It was centrifuged, separated from the mother liquor, refluxed with absolute alcohol for scores of ten minutes, centrifuged, washed with ether and dried. Further, it was dried at 150 °C in vacuum for 3 hours. It was white powder and a little hygroscopic. Yield 0.46 g (35.93% of the theoretical).

N analysis

found N=20.60%

for $(C_2H_3ON:C_3H_5ON=1:1)_n$ calc. N=21.88%

It was dissolved in water and the molecular weight and the viscosity were measured at 30 ± 0.1 °C.

Concentration $C=3.38$ g/L, Osmotic press. $\pi=7.33 \times 10^{-3}$ atm.

Molecular weight $M=11,457$, polymerisation degree $n=89.5$, intrinsic viscosity $[\eta]=0.093$

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