SHORT COMMUNICATIONS

Polycondensation of Tripeptide with Bis-o-phenylene Pyrophosphite

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Many attempts to prepare high molecular polypeptides, in which amino acid residues were arranged in a regular order, have been made thus far[2-10]. These methods will be divided into two types. The one is the use of peptide derivative, e. g. peptide ester, *N*-carbothiophenylpeptide, as a monomer[2-7,9], and the other is the use of free peptide in the presence of the reagents which form peptide linkage, such as dicyclohexyl carbodiimide[8], tetraethyl pyrophosphite[9], or phosphorus pentoxide[10]. Among them, the carbothiophenyl method[7] and the tetraethyl pyrophosphite method[9] gave rather favorable results.

The present authors found a very handy procedure for the polycondensation of oligopeptides by the aid of bis-*o*-phenylene pyrophosphite. The present reagent was used previously for the peptide bond formation between acylamino acids and amino acid esters by Crofts et al.[11] In the present communication, polycondensation of L-leucylglycylglycine is described. Bis-*o*-phenylene pyrophosphite[12] resembles tetraethyl pyrophosphite[13] in the chemical nature, but the synthesis of the former reagent was much easier than that of the latter.

L-Leucylglycylglycine (m. p. 215~216°C with decomp. Found: C, 49.08; H, 7.96; N, 17.06. Calcd. for $C_{10}H_{19}O_4N_3$: C, 48.96; H, 7.81; N, 17.13%.) was synthesized from carbobenzoxy-L-leucine and glycylglycine ethyl ester by the use of isobutyl chloroformate, subsequent saponification and catalytic hydrogenation. Bis-*o*-phenylene pyrophosphite was synthesized according to the method of Crofts et al.[12]

A mixture of 1 g. of L-leucylglycylglycine, 1.2 g. of bis-*o*-phenylene pyrophosphite (an equimolar ratio) and 6 ml. of dry pyridine was heated at 100°C for 6 hr. in a sealed tube. During the reaction, the solution gradually gelled and became pale yellow. After the reaction completed, the product was poured into a flask containing 30 ml. of water. Then pyridine and water were removed in vacuo. The residues were dissolved in a small amount of ethanol, and an equal volume of water was added to the solution to decompose the terminal activated groups and the solvents were removed in vacuo. These procedures were repeated. An oily material remained was suspended in ethanol. Dry ether was added to the suspension and the syrupy material resulted was centrifuged off, washed several times with ethanol and ether*1, and finally dried. The weight of the dried material was 770 mg. The crude polypeptide was partly dissolved in hot dimethylformamide and precipitated by the addition of ether. In order to decompose the activated end groups completely, the precipitate was dissolved in 80% aqueous phenol*2 (5 ml.) and 1% aqueous ammonia (15 ml.) was added to the solution. The above solution was dialyzed against deionized water for 50 hr. in a cellophane tube. The precipitate obtained during dialysis was centrifuged off (fraction 1, which amounted to approximately 9% for L-leucylglycylglycine in weight) and the supernatant was lyophilized (fraction 2, which amounted to about 8%).

The polypeptide (fraction 1) was soluble in 80% aqueous phenol and 85% aqueous formic acid, and insoluble in glacial acetic acid,

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*1 By these procedures the syrupy material changed gradually into powder.
*2 Sometimes the precipitate can dissolve in hot dimethylformamide.

pyridine, dimethylformamide, hot ethanol, water, benzene, chloroform and ethyl acetate. Found: C, 49.30; H, 7.41; N, 16.56. Calcd. for $(C_{13}H_{17}O_3N_3 \cdot H_2O)_n$: C, 48.96; H, 7.81; N, 17.13%*3. The phosphorus content of this material was determined by the method of Allen[14], and the value of 0.99% was obtained*4.

The average molecular weight of the polypeptides, fraction 1 and fraction 2 of poly-(L-leucylglycylglycine), was determined by titrating the end carboxyl groups with sodium methoxide in dimethylformamide[15], and the values of 3220 and 1730 were obtained for the fractions 1 and 2 respectively.

The infrared absoption bands which were characteristic for polypeptides could be observed at 3290, 1660, 1540 and 1250 cm^{-1}, especially in the fraction 1.

Further investigations on this condensation reaction are now in progress and details will be presented elsewhere.

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*3 On the elementary analysis, the polypeptide gave 3.78% of the incombustible material.

*4 In turn, the phosphorus content of the fraction 2 was determined in a fashion analogous to the fraction 1, and the value of 18.0% was obtained. It appeared to be probable that the phosphorus might affect the elementary analysis of C, H and N considerably. Found: C, 39.38; H, 6.88; N, 12.81%. On the analysis, the incombustible material amounted to 7.67% in weight.

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