of hydrogen chloride, and the resulting tripeptide active ester hydrochloride was polymerized in dimethylformamide (50% solution) in the presence of triethylamine to poly-(γ-*t*-butyl-L-glutamyl-L-alanyl-γ-*t*-butyl-L-glutamic acid) (VI) in 42% yield. The *t*-butyl ester groups from polymer VI were removed by 90% trifluoracetic acid and the water-soluble poly-α-L-glutamyl-L-alanyl-L-glutamic acid (VII) was obtained in 94% yield. Further purification was achieved by dialysis[15] for the removal of low molecular weight polypeptides and possible cyclic peptides. *Anal.* Calcd. for $(C_{13}H_{19}N_3O_7.0.5H_2O)_\infty$: C, 46.1; H, 5.94; N, 12.4; equiv. wt., 169. Found: C, 46.34; H, 6.41; N, 12.10; equiv. wt., 173.5; intrinsic viscosity in dichloroacetic acid, 0.12 dl./g. Molecular weight as determined by DNP method was 20,000. Retention of optical purity was 98 ± 2%.[16]

Similarly N-carbobenzoxyglycylglycyl-L-phenylalanine pentachlorophenyl ester was polymerized to poly-(glycylglycyl-L-phenylalanine) in 58% yield.

Polypeptides with known repeating sequence of amino acids containing other than α-peptide bonds, *e.g.*, γ-glutamyl residues, are also important.[17] Preparation of poly-(γ-L-glutamyl-γ-aminobutyric acid) (VIII) from N-carbobenzoxy-α-*t*-butyl-L-glutamyl-γ-aminobutyric acid pentachlorophenyl ester (IX), m.p. 175–176°, $[\alpha]^{26}D$ −3° (*c* 2.0, chloroform), was achieved in 49% over-all yield after dialysis; intrinsic viscosity 0.135 dl./g. *Anal.* Calcd. for $(C_9H_{14}N_2O_4)_\infty$: C, 50.51; H, 6.55; N, 13.10. Found: C, 51.03; H, 7.08; N, 12.6.

Other polypeptides containing different amino acid residues were also prepared by this general procedure.

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(15) During the extensive dialysis the loss for various batches was between 38 and 62%.

(16) The retention of the optical purity was established by determining the specific rotation of the total hydrolysate of the polypeptides and comparing that with the specific rotation of the corresponding amino acids treated under the same conditions.

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Poly-β-L-aspartic Acid. Synthesis through Pentachlorophenyl Active Ester and Conformational Studies

Sir:

Utilization of the pentachlorophenyl active ester method for the synthesis of poly-β-L-aspartic acid has resulted in optically pure high molecular weight polypeptide, which was needed for biological and physical chemical investigations. The importance of optically pure poly-β-aspartic acids for immunochemical studies was emphasized in our earlier paper,[1] where a synthesis through *p*-nitrophenyl ester was also reported. Search for better polymerization methods led to the use of trichlorophenyl and later of pentachlorophenyl active esters.[2]

N-Carbobenzoxy-α-benzyl-L-aspartic acid pentachlorophenyl ester (I), m.p. 151–152°, $[\alpha]^{26}D$ 20.0° (*c* 2.36, chloroform), was obtained in 95% yield from N-carbobenzoxy-α-benzyl-L-aspartate and pentachlorophenol using the DCCI method.[3] *Anal.* Calcd. for $C_{25}H_{18}O_6NCl_5$: C, 49.57; H, 3.00; N, 2.31. Found: C, 49.57; H, 3.24; N, 2.35. Hydrogen bromide cleavage of I [10 g. of I in 140 ml. of 8.5% solution of hydrogen bromide in acetic acid–dichloroacetic acid (1:6)] gave after two recrystallizations from absolute alcohol 84% α-benzyl-β-pentachlorophenyl-L-aspartate hydrobromide (II), m.p. 201–202°, $[\alpha]^{26}D$ 7.12° (*c* 3.38, dimethylformamide). *Anal.* Calcd. for $C_{17}H_{13}O_4NCl_5Br$: C, 36.96; H, 2.37; N, 2.54. Found: C, 37.04; H, 2.46; N, 2.87. Active ester II polymerized readily in dimethylformamide in the presence of a tertiary amine to poly-β-(α-benzyl)-L-aspartic acid (III) in 95% yield of which 62% was a polypeptide with an average molecular weight of 10,000, as determined by end-group analysis.[4] (During the polymerization of II, the higher molecular weight polypeptide precipitated from the dimethylformamide solution (62%) and the lower molecular weight fraction was precipitated by ether.) The intrinsic viscosity of III in dichloroacetic acid is 0.09 dl./g. *Anal.* Calcd. for $(C_{11}H_{11}O_3N)_\infty$: C, 64.38; H, 5.40; N, 6.88. Found: C, 63.90; H, 5.41; N, 6.76. Catalytic hydrogenation of III gave optically pure water-soluble poly-β-L-aspartic acid (IV) in 99% yield. After dialysis polypeptide IV was obtained in 50% yield; intrinsic viscosity in dichloroacetic acid 0.11 dl./g.; molecular weight, as determined by the DNP method, 19,000; equivalent weight 125.1, calcd. 124. *Anal.* Calcd. for $(C_4H_5O_3N.0.5\ H_2O)_\infty$; C, 38.79; H, 4.80; N, 11.21. Found: C, 39.01; H, 4.55; N, 11.42.

The optical purity of the dialyzed sample was established by determining the optical purity of the aspartic acid after total hydrolysis; 58.6 mg. of sample was refluxed for 24 hr. in 3 ml. of 12 *N* hydrochloric acid, the solution was evaporated to dryness, the residue was dried for 24 hr. over sodium hydroxide and phosphorus pentoxide and then dissolved in 5 ml. of water, and the specific rotation was determined with a Rudolph Model 200AS-80Q spectropolarimeter; $[\alpha]^{26}D$ 19.65° (*c* 1.25, water). As a control, 68.3 mg. of aspartic acid was treated in the same manner; $[\alpha]^{26}D$ 20.03° (*c* 1.35, water), which indicated at least 98.1% optical purity. The exclusive presence of β-peptide linkages was determined by Hofmann degradation.[5]

Poly-β-L-aspartic acid was also prepared through the α-*t*-butyl ester derivative. N-Carbobenzoxy-α-*t*-butyl-L-aspartic acid pentachlorophenyl ester (V), m.p. 121–123°, $[\alpha]^{26}D$ 37.5° (*c* 2, chloroform) [*Anal.* Calcd. for $C_{22}H_{20}O_6NCl_5$: C, 46.20; H, 3.54; N, 2.45. Found:

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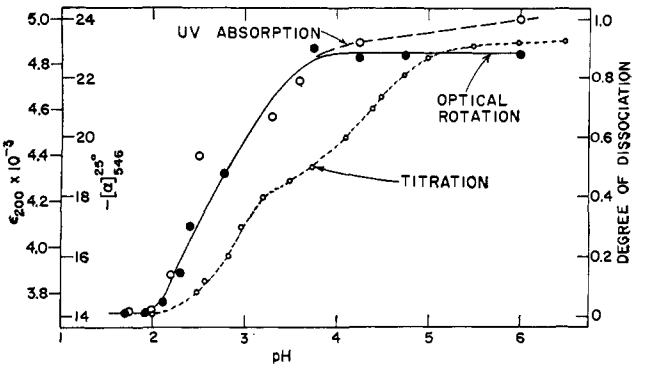

Figure 1. Optical rotation, ultraviolet absorption, and titration of poly-β-L-aspartic acid, mol. wt. 19,000, in 0.2 *M* NaCl: (○) residue-molar extinction coefficient at 200 mμ, concentration 0.034 g./dl.; (●) specific rotation at 546 mμ, concentration 0.62 g./dl. Titration at concentration = 0.0276 g./dl. Ordinate scales are adjusted to make the ranges of the various measurements approximately coincide.

C, 45.85; H, 3.48; N, 2.36] gave after catalytic hydrogenation and polymerization poly-β-(α-*t*-butyl)-L-aspartic acid (VI), which on treatment with 90% trifluoroacetic acid yielded polypeptide IV; intrinsic viscosity in dichloroacetic acid 0.125 dl./g.; molecular weight, as determined by the DNP method, 24,000.

The physical properties of poly-β-L-aspartic acid solutions have been examined for the purpose of learning about possible secondary structure in this material. Evidence for such secondary structure was reported previously[6] from a study of low molecular weight poly-β-L-aspartic acid prepared *via* the N-carboxyanhydride. The higher molecular weight samples prepared by the present method seemed more suitable for this purpose, since the stability conferred on an ordered structure as a result of cooperative effects increases with the molecular weight. In view of the current interest in these structures, a brief report of results is appropriate at this time.

In Figure 1 are shown the dependence upon pH of the specific rotation $[\alpha]^{25}_{546}$ and the residue-molar extinction coefficient ϵ_{200} at 200 mμ, which is within the amide absorption band centered at 190 mμ. The degree of dissociation of carboxyl groups shown was determined by potentiometric titration with 1 *M* NaOH. The changes in optical properties with pH are strikingly similar to those observed for poly-α-L-glutamic acid (PGA)[7-9] and ascribed in that case to a transition from helix to random coil as the molecule becomes charged. The change in rotation at 546 mμ and the hypochromic effect at 200 mμ are smaller for poly-β-L-aspartic acid than for PGA, but are in the same direction. Furthermore, neither effect can be simply a measure of the degree of dissociation of carboxyl groups, since most of the change occurs when the molecules are less than 50% dissociated. It is therefore concluded that the observed changes arise from an ordered secondary structure which is stable only at very low degrees of electostatic charge. The anomalous hump in the titration curve near pH 3 is most likely associated with this structural change, since similar anomalies have been observed for PGA.[10,11]

This conclusion is supported by two additional lines of evidence: (i) The rate of deuterium exchange with amide hydrogen in D_2O solution was followed by the infrared absorption method of Blout, *et al.*[12] The exchange at room temperature was complete within 10 min. at pD 7.5, but required 94 min. for half completion at pD 2.8. As in the case of PGA,[12] the decrease in exchange rate at low pD may result from the participation of amide groups in hydrogen bonds among each other. (ii) Heating a solution at pH 2.34 produced a gradual change in optical rotation in the negative direction, suggesting a gradual breaking up of a secondary structure whose stability is roughly comparable to that of PGA.[13]

The details of the secondary structure cannot be stated with certainty at present. However, a one-stranded helical structure is more appealing than an intermolecular aggregate, in view of the fact that the analogous structures in poly-α-L-lysine are distinguished by the appearance of hypochromism in the helix and hyperchromism in the aggregate.[14] A number of poly-β-amino acid helices have been constructed with Courtauld stereomodels.[15] One helix that appears to be particularly favorable on the basis of the criteria laid down by Pauling, Corey, and Branson[16] is formed by hydrogen bonding each carbonyl to the third amide group removed toward the N-terminus. This model has 3.4 residues/turn and an axial translation of 1.58 Å./residue. It is necessarily right-handed for β-amino acids with the L-configuration at the α-carbon (α-amino acid convention).

It is interesting to note that a homologous compound, poly-γ-D-glutamic acid, has also been found to show changes in optical rotation with degree of dissociation.[17,18] Edelhoch and Lippoldt[17] interpreted their data to mean that the un-ionized form is hypercoiled as a result of random intramolecular hydrogen bonds, while Rydon[18] has suggested that a helical structure would account for the observed effects.

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