A light buff powder was obtained that began to darken at 210° and charred at 295°. J. von Braun[10] states that this salt does not melt below 240°. The yield was 1.2 g. or 72%.

Anal. Calcd. for $C_{17}H_{22}O_2N_2Cl_2$: Cl, 19.85. Found: Cl, 19.51.

The Solvates of the *p*-Nitrobenzoate Ester of *p*-(β-Di-*n*-butylaminoethyl)-phenol Hydrochloride with Benzene and Toluene.—The hydrochloride of *p*-(β-di-*n*-butylaminoethyl)-phenyl *p*-nitrobenzoate exhibits a rather high solubility in hot benzene and if such a solution was cooled slowly overnight pale yellow transparent prismatic crystals 3–4 mm. long were formed. A sample of 0.7801 g. of these crystals lost 0.1080 g. or 15.11% of their weight upon drying for 14 hours at 45°. This corresponds closely to a benzene solvation compound with a molecular ratio of 1:1, which should theoretically have a benzene content of 15.21%. The crystals slowly lose their transparency and the molecule of benzene of solvation at room temperature.

This compound forms a similar addition product when crystallized from hot toluene. The resulting solvation compound crystallizes as pale yellow needles that are 10–15 mm. long. A sample of 0.3016 g. lost 0.0447 g. or 14.82% of its weight after drying at about 75° for 12 hours and 0.0522 g. or 17.32% for 18 hours. The last figure corresponds closely to a toluene solvation compound with a molecular ratio of 1:1 which should have a toluene content of 17.45%. These crystals lost their transparency and the molecule of solvation more slowly at room temperature than the corresponding benzene solvate.

The Attempted Preparation of the *p*-Nitrobenzoate Ester of *p*-(β-Diethylaminoethyl)-phenol Hydrochloride by Reaction of Diethylamine with *p*-Nitrobenzoate Ester of *p*-(β-Iodoethyl)-phenol.—A mixture of 4 g. (0.01 mole) of the *p*-nitrobenzoate ester of *p*-(β-iodoethyl)-phenol, 2.3 ml. (0.022 mole) of diethylamine and 12 ml. of benzene was heated in a sealed tube for five hours at 100°. After filtration the benzene was removed by evaporation and the residue was distilled at 4 mm. Extensive decomposition took place and only a few drops of distillate boiling in the range 135–178° were obtained. The hydrochloride of the distillate melted at 169–172° and gave no depression when mixed with *p*-(β-diethylaminoethyl)-phenol hydrochloride (m.p. 172–173°).

Attempts were also made to isolate the desired product without distillation by using ethyl and methyl alcohol as solvents. Ethyl and methyl *p*-nitrobenzoate, respectively, were obtained indicating that transesterification had taken place.

Similar results were obtained when attempts to prepare the corresponding derivative of *p*-(β-di-*n*-butylaminoethyl)-phenol were made by this method.

Acknowledgment.—We want to express our appreciation to Mr. K. C. Li, Chairman of Board, Wah Chang Corporation, New York, and Dr. Ralph Montonna, former director of the Syracuse University Institute of Industrial Research, whose support made much of this study possible.

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[CONTRIBUTION FROM THE WEIZMANN INSTITUTE OF SCIENCE]

Poly-L-aspartic Acid

BY ARIEH BERGER AND EPHRAIM KATCHALSKI

The preparation of optically pure poly-L-aspartic acid is described. The synthesized poly-α-amino acid yields quantitatively on acid hydrolysis L-aspartic acid. The potentiometric titration curve and the dependence of viscosity on degree of ionization are employed to demonstrate the behavior of poly-L-aspartic acid as a polyelectrolyte. The infrared absorption spectrum of poly-L-aspartic acid is compared with that of synthetic poly-L-glutamic acid.

The synthesis of poly-aspartic acid from L-aspartic acid has been reported by Frankel and Berger.[1] A polymer obtained by the published procedure yielded on acid hydrolysis an extensively racemized aspartic acid. Since an optically pure poly-L-aspartic acid was required in the course of our enzymatic and biological studies of water-soluble polyamino acids,[2] the synthesis was reinvestigated and a procedure for the preparation of an optically pure poly-L-aspartic acid worked out.

The course of synthesis of poly-L-aspartic acid is represented in the scheme.

The optical purity of the various intermediates was checked by their conversion into aspartic acid (by reduction or acid hydrolysis) and determination of the specific rotation of the latter. The use of benzyl esters (II, III, IV and V), originally suggested by Frankel and Berger,[1] was found advantageous, as the regeneration of free carboxyl groups by reduction with phosphonium iodide,[3] was found to cause no change in steric configuration. N-Carbobenzoxy-L-aspartic acid[4] (I) was converted to the dibenzyl ester II, with benzyl alcohol in toluene, using *p*-toluenesulfonic acid as catalyst. This procedure was found more convenient and gave higher yields than the esterification by the reaction of the disilver salt of I with benzyl iodide.[1] The removal of catalyst with magnesium oxide permitted isolation of the optically active compound, whereas aqueous potassium carbonate caused partial racemization. II yielded on treatment with an equimolar amount of sodium hydroxide in aqueous dioxane a monobenzyl ester to which formula III was attributed. The constitution of the monoester was proved by its conversion into N-carbobenzoxy-L-asparagine (VII) by means of liquid ammonia. Admixture of the latter with an authentic specimen[4] showed no depression in melting point. The monoester differed from α-benzyl N-carbobenzoxy-L-aspartate[5] in its melting point and dissociation constant. The lower pK value of the β-benzyl ester may be explained by the proximity of the carbobenzoxy group to the free carboxyl group in this compound. The preferential hydrolysis of the α-ester group of II is in accord with the findings of Pauly and Weir[6] for the hydrolysis of dimethyl N-benzoylaspartate. The alkaline hydrolysis of II in benzyl alcohol[1] leads to considerable racemization.

β-Benzyl N-carbobenzoxy-L-aspartate (III)

(1) M. Frankel and A. Berger, *Nature*, **163**, 213 (1949).

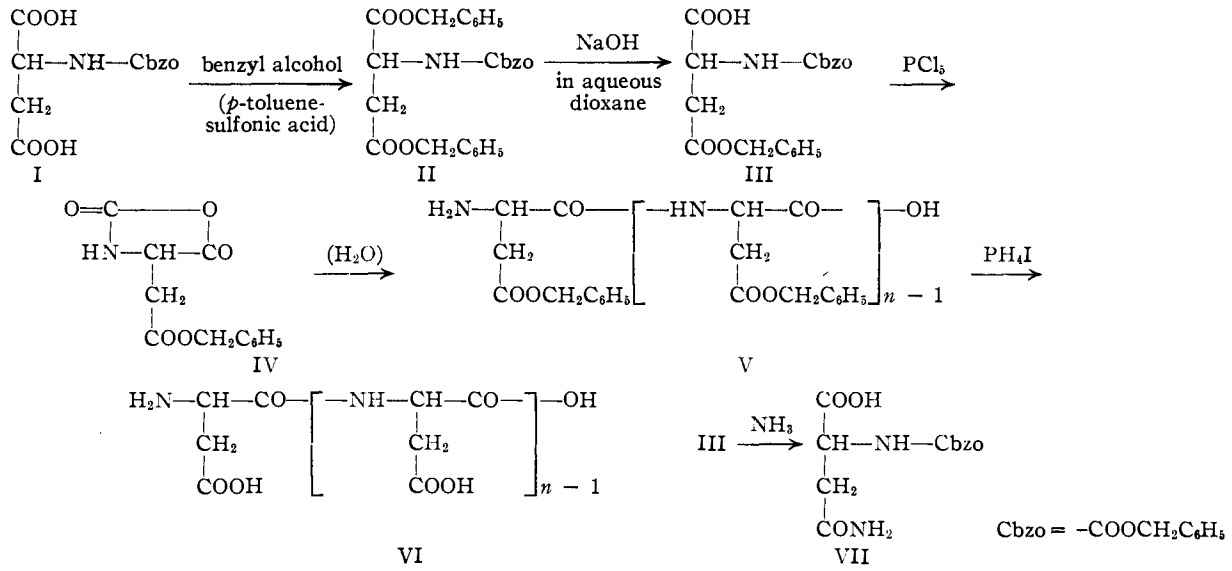
(2) E. Katchalski, *Advances in Protein Chem.*, **6**, in press; A. de Vries, A. Schwager and E. Katchalski, *Biochem. J.*, in press.

(3) C. R. Harington and T. H. Mead, *ibid.*, **29**, 1603 (1935).

(4) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

(5) M. Bergmann, L. Zervas and L. Salzmann, *ibid.*, **66**, 1288 (1933).

(6) H. Pauly and J. Weir, *ibid.*, **43**, 661 (1910).



I II III IV V VI VII

yielded with phosphorus pentachloride, β-benzyl-N-carboxy-L-aspartate anhydride (IV). This reaction is known to cause no change in steric configuration.[7] IV polymerized upon heating to poly-(β-benzyl-L-aspartate) (V) which was reduced with phosphonium iodide to poly-L-aspartic acid (VI), $[\alpha]^{25}D$ −11.2° (*c*, 5 in water containing one equivalent of sodium hydroxide). From VI, L-aspartic acid was obtained in quantitative yield upon acid hydrolysis.

A certain discrepancy was observed between the mean residue weight (118) and the equivalent weight (125) of the poly-aspartic acid synthesized, as calculated from its total-N content and potentiometric titration, respectively. This discrepancy is not due to incomplete reduction of V,[8] since no characteristic absorption bands in the ultraviolet region (2550–2700 Å.) of the aromatic benzyl residues could be detected in an aqueous solution of sodium poly-aspartate (at a concentration of 6 mg. per ml.); moreover, the analyses of the copper and ammonium salts of poly-L-aspartic acid were in agreement with formula VI attributed to the reduced polymer. It may, therefore, be assumed that the inflexion point in the potentiometric titration curve (*cf.* Fig. 1) occurs before complete neutralization of all the free carboxylic groups.[9]

It is of interest that in all our poly-L-aspartic acid preparations, the carboxyl-N[10] values are close to the amino-N[11] values. As we found that isoasparagine[4] gives one mole of carbon dioxide on heating with ninhydrin, it seems plausible that the carbon dioxide evolved during the carboxyl-N determination of VI originated from the terminal aspartic acid residues bearing free α-amino and its adjacent β-carboxyl groups.

The behavior of poly-aspartic acid as a polyelectrolyte was demonstrated by potentiometric titration as well as by viscosity measurements at various degrees of ionization in aqueous solution. The potentiometric titration is represented in Fig. 1.

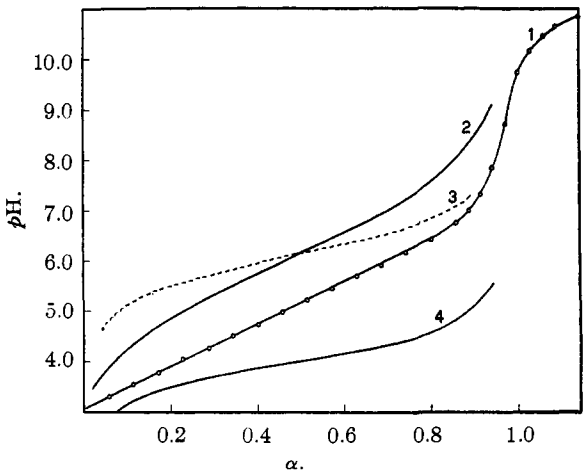


Fig. 1.—Potentiometric titration curves of: (1) poly-L-aspartic acid; (2) poly-acrylic acid[12]; (3) monobasic acid with dissociation constant of poly-acrylic acid (calculated)[12]; (4) natural poly-glutamic acid[13] in water. α = degree of ionization.

A comparison of the curve obtained, with the titration curves of monobasic acids reveals that the behavior of poly-aspartic acid is of the polymeric acid type, resembling closely titrations of poly-acrylic and polymethacrylic acids.[12] If the *p*H values be plotted against the logarithm of $(1 - \alpha)/(\alpha)$ (α being the degree of ionization), a straight line is obtained with a slope of 2, practically equal to that of the polymeric acids (Fig. 2). It is of interest that the potentiometric titration of natural polyglutamic acid[13] gives a slope much closer to 1, *i.e.*, the behavior of this poly-acid is closer to that of monobasic acids. The polymeric behavior of poly-aspartic acid is further emphasized by the strong depend-

(7) M. Bergmann, L. Zervas and W. F. Ross, *J. Biol. Chem.*, **111**, 245 (1935).

(8) W. E. Hanby, S. G. Waley and J. Watson, *J. Chem. Soc.*, 3239 (1950).

(9) Similar results were obtained in this Laboratory for polymethacrylic acid by Dr. A. Katchalsky (private communication).

(10) D. D. Van Slyke, R. T. Dillon, D. A. MacFadyen and P. Hamilton, *J. Biol. Chem.*, **141**, 627 (1941).

(11) D. D. Van Slyke, *ibid.*, **83**, 425 (1929).

(12) A. Katchalsky and P. Spitnik, *J. Polymer Sci.*, **2**, 432 (1947); A. Katchalsky and J. Gillis, *Rec. trav. chim.*, **68**, 879 (1949).

(13) W. E. Hanby and H. N. Rydon, *Biochem. J.*, **40**, 297 (1946).

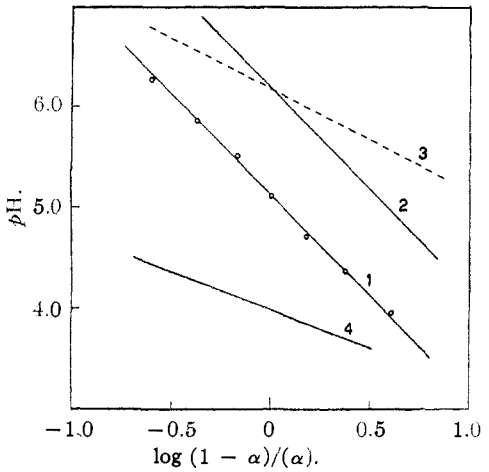


Fig. 2.—pH *versus* log $(1 - \alpha)/(\alpha)$ (α = degree of ionization) calculated from the potentiometric titration curves given in Fig. 1: (1) poly-L-aspartic acid; (2) poly-acrylic acid; (3) monobasic acid with dissociation constant of poly-acrylic acid[12]; (4) natural poly-glutamic acid.[13]

ence of the pH of its solution on the salt content: the addition of neutral salt to a partially neutralized poly-aspartic acid causes a very marked decrease in pH (Fig. 3), similar to that observed in other polymeric acids.[12] A fuller theoretical discussion of these results will be published in due course.

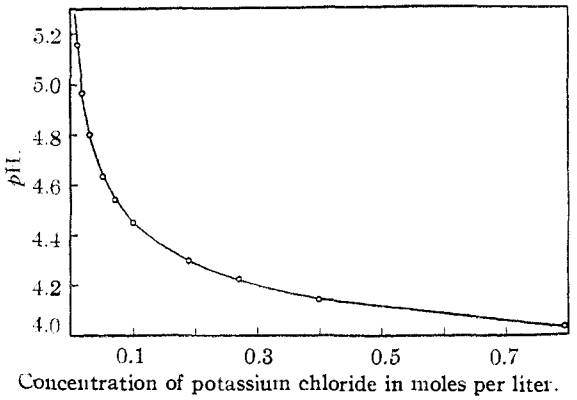


Fig. 3.—Change of pH of a half-neutralized 0.01 N aqueous solution of poly-L-aspartic acid on addition of potassium chloride.

The viscometric behavior of poly-aspartic acid having a molecular weight of about 7,000 (as determined by amino-N end-group analysis) is represented in Fig. 4. The abscissas are the degrees of ionization and the ordinates are the ratios of specific viscosity to the concentration (in basic moles per liter). Here, again, the typical behavior as a polyelectrolyte is observed—the viscosity starts with low values (α = 0.1 to 0.4), increases rapidly with further increase in ionization and approaches a limiting value (at α = 0.9 to 1.0). The increase is much more pronounced than that observed in proteins,[14] and seems to indicate an opening up of the molecule in a manner similar to the poly-acrylic acids.[15] It must, however, be noted that while the specific viscosity of poly-methacrylic acid starts to increase already at low degrees of ionization, the specific viscosity of poly-aspartic acid remains low up to about 40% of ionization—this, presumably, means that the opening up of the coiled molecule is prevented by the strong hydrogen bonding of the peptide links, and only at sufficiently high ionizations do the molecules stretch and increase the specific viscosity.

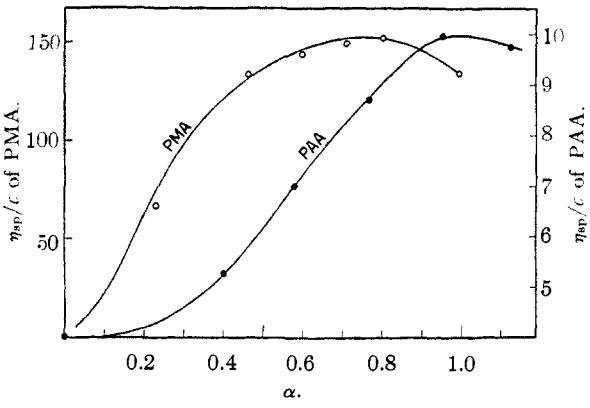


Fig. 4.—Variation of η_{sp}/c (η_{sp} = specific viscosity; c = concentration in basic moles per liter) with α (α = degree of ionization) for poly-L-aspartic acid (PAA; number average molecular weight 7000) and for poly-methacrylic acid[16] (PMA).

The infrared absorption spectrum (from 100 to 1800 cm.$^{-1}$) of a film of poly-L-aspartic acid cast from dimethyl formamide is given in Fig. 5. The absorption curve resembles that of poly-glutamic acid.[16] The spectral region of 1000 to 1500 cm.$^{-1}$ is considered to give absorption bands which are characteristic of the polypeptide side chains.[17] The absorption spectrum in this region differs considerably from that of poly-glutamic acid; it shows a strong band at 1380 cm.$^{-1}$ and two weaker bands at 1220 and 1160 cm.$^{-1}$.

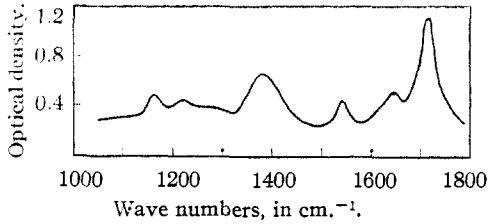


Fig. 5.—Infrared absorption spectrum of a film of poly-L-aspartic acid (determined with a Perkin-Elmer infrared spectrometer, model 12C).

In the region of 1500–1800 cm.$^{-1}$, three distinct bands appear at 1645, 1535 and 1715 cm.$^{-1}$. These bands appear, with rather different relative intensities, also in poly-glutamic acid and were ascribed[16] to the stretching mode of the C=O group in the –CONH– peptide linkage, to the deformation mode of the NH group of this linkage and to the stretching mode of the carboxyl –C=O groups of the side-chains, respectively.

(14) H. Bull, *Trans. Faraday Soc.*, **36**, 80 (1940).

(15) A. Katchalsky, O. Kunzle and W. Kuhn, *J. Polymer Sci.*, **5**, 283 (1950); H. Markowitz and G. E. Kimball, *J. Colloid Sci.*, **5**, 115 (1950).

(16) E. J. Ambrose, *J. Chem. Soc.*, 3246 (1950).

(17) W. T. Astbury, C. E. Dalgliesh, S. E. Darmon and C. B. B. M. Sutherland, *Nature*, **162**, 596 (1948).

Experimental[18]

Dibenzyl N-Carbobenzoxy-L-aspartate (II).—N-Carbobenzoxy-L-aspartic acid (9.0 g.)[4] and benzyl alcohol (40 ml.) were added to a boiling solution of *p*-toluenesulfonic acid (0.5 g.) in toluene (50 ml.). Refluxing was continued for 2.5 hours, during which time water was removed azeotropically (2.5 ml.). The solution was cooled to room temperature, shaken for ten minutes with magnesium oxide (1.0 g.) and filtered. The toluene was removed at the water-pump and the excess of benzyl alcohol in high vacuum (90°, 10^{-2} mm.). The viscous residue crystallized on trituration with petroleum ether. Dibenzyl N-carbobenzoxy-L-aspartate (11.0 g.) was obtained as a white powder (m.p. 58°) after repeated washing with petroleum ether. The crude product was crystallized from boiling petroleum ether and recrystallized from dibutyl ether before analysis; m.p. 66.5°; $[\alpha]^{25}D$ −2.5° (*c* 10 in glacial acetic acid); $[\alpha]^{25}D$ +3.5° (*c* 10 in benzene).

Anal. Calcd. for $C_{26}H_{25}O_6N$: C, 69.8; H, 5.6; N, 3.1. Found: C, 70.1; H, 5.6; N, 3.1.

Dibenzyl N-carbobenzoxy-L-aspartate (II) readily dissolves in the usual organic solvents, is sparingly soluble in petroleum ether and insoluble in water.

The optical purity of II was checked by determining the specific rotation of the aspartic acid derived from it by reduction as follows:

Reduction of II with Phosphonium Iodide.—Phosphonium iodide (5 g.) was added to a solution of dibenzyl N-carbobenzoxy-L-aspartate (1000 mg.) in glacial acetic acid (15 ml.). A stream of dry hydrogen was passed for 5 hours through the mixture kept at 55°. The solution was concentrated at reduced pressure (5 mm., bath temperature 30–40°), water (15 ml.) was added and the solution again brought to dryness *in vacuo* (5 mm.). Two repetitions of this procedure caused total removal of benzyl iodide. The final residue was made up to 10 ml. with 6 *N* hydrochloric acid. A 1-ml. aliquot was withdrawn, neutralized, and diluted to 25 ml. The amino-N[11] and carboxyl-N[10] content of the diluted solution proved that the reduction of II yielded aspartic acid quantitatively.

Anal. Calcd. for 297.5 mg. aspartic acid derived from 1000 mg. II: amino-N, 31.3 mg.; carboxyl-N, 62.6 mg. Found: amino-N, 31.4 mg.; carboxyl-N, 62.2 mg.

From the optical rotation of the 6 *N* hydrochloric acid solution the specific rotation of the aspartic acid formed was calculated, $[\alpha]^{25}D$ 23.2° (*c* 2.975, in 6 *N* hydrochloric acid).[19]

When L-aspartic acid, $[\alpha]^{25}D$ 24.5° (*c*, 10 in 6 *N* hydrochloric acid), was treated with phosphonium iodide as above, it was recovered quantitatively with no change in specific rotation.

When employing potassium carbonate to remove *p*-toluenesulfonic acid from the esterification mixture of carbobenzoxy-L-aspartic acid (*cf.* preceding paragraph), a dibenzyl ester II with $[\alpha]^{25}D$ −1.25° (*c* 10, in glacial acetic acid) was obtained. On reduction with phosphonium iodide II yielded aspartic acid with $[\alpha]^{25}D$ 12.3° (*c* 10, in 6 *N* hydrochloric acid).

β-Benzyl N-Carbobenzoxy-L-aspartate (III).—A mixture of 2 *N* sodium hydroxide (5 ml.), water (24 ml.) and dioxane (60 ml.) was added to a solution of dibenzyl N-carbobenzoxy-L-aspartate (4.56 g.) in dioxane (50 ml.) and water (20 ml.). These amounts of solvents permitted the formation of a single phase. After standing at room temperature for 24 hours, the *p*H of the solution was adjusted to 5.5 with hydrochloric acid and the solvents were evaporated *in vacuo*. The residue was treated with 1 *N* aqueous potassium bicarbonate (10 ml.) and the mixture extracted with ether (20 ml.) to remove unreacted dibenzyl ester. β-Benzyl N-carbobenzoxy-L-aspartate (2.2 g.) separated out as a colorless crystalline precipitate (m.p. 102°) on acidification of the aqueous layer with 6 *N* hydrochloric acid (2 ml.). It was filtered, washed with cold water and dried over sulfuric acid. Recrystallized from benzene, m.p. 108°; $[\alpha]^{25}D$ 12.1° (*c* 10, in glacial acetic acid).

Anal. Calcd. for $C_{19}H_{19}O_6N$: C, 63.8; H, 5.4; N, 3.9; equiv. wt., 365. Found: C, 63.3; H, 5.3; N, 4.0; equiv. wt., 357 (determined by titration in alcohol).

The monoester III is soluble in the usual organic solvents; practically insoluble in water and petroleum ether.

The optical purity of III was checked by determining the specific rotation of the aspartic acid derived from it, as in the case of II; $[\alpha]^{25}D$ 24.0° (*c* 5.17, in 6 *N* hydrochloric acid).[19]

The hydrolysis of II ($[\alpha]^{25}D$ −2.5° in glacial acetic acid) with potassium hydroxide in benzyl alcohol[1] gave III with $[\alpha]^{25}D$ 4.5° (*c* 10, in glacial acetic acid). This partially racemized monoester yielded on reduction aspartic acid with $[\alpha]^{25}D$ 11.5° (*c* 10, in 6 *N* hydrochloric acid).

A potentiometric titration of a 0.05 *N* solution of a twice-recrystallized sample of β-benzyl *N*-carbobenzoxy-L-aspartate in a water–dioxane mixture (1:2 by volume) with 0.05 *N* sodium hydroxide in the same solvent mixture, was carried out at 25° in a nitrogen atmosphere, using a Beckman *p*H-meter with a glass electrode. A *pK* of 6.05 was calculated from the titration curve.

The dissociation constant and the melting point of the synthesized monoester were compared with those of α-benzyl N-carbobenzoxy-L-aspartate prepared according to Bergmann, *et al.*[5]; found for α-benzyl N-carbobenzoxy-L-aspartate: *pK* 6.55 in water–dioxane (1:2 by volume) (calculated from a titration carried out under conditions similar to those given for the β-benzyl ester); m.p. 84°; on admixture with the corrresponding β-benzyl ester (m.p. 108°) m.p. 64–66°.

Conversion of III to N-Carbobenzoxy-L-asparagine (VII).—A solution of III (1.0 g.) in liquid ammonia (5 ml.) was kept in a sealed glass tube at room temperature for 24 hours. The residue left after removal of ammonia at room temperature was dissolved in water (5 ml.) and the aqueous solution extracted with ether (5 ml.). On acidification of the aqueous layer with concentrated hydrochloric acid, a colorless crystalline precipitate was obtained; m.p. 163° after two recrystallizations from boiling water. No depression of m.p. was observed on admixture with an authentic sample of N-carbobenzoxy-L-asparagine (m.p. 164°) prepared according to Bergmann and Zervas.[4] Admixture with N-carbobenzoxyisoasparagine[4] (m.p. 164°) caused a depression in m.p. to 148–150°.

β-Benzyl-N-carboxy-L-aspartate Anhydride (IV).—Phosphorus pentachloride (11.5 g.) was added to an ice-cold solution of III (17.0 g.) in anhydrous benzene (150 ml.). On shaking the mixture for 10 minutes at 0°, practically all the phosphorus pentachloride went into solution. The filtered solution was left at room temperature for 4 hours. The crystalline mass which had formed was filtered and thoroughly washed with petroleum ether (50 ml.). It was dried *in vacuo* over sulfuric acid and soda lime; yield 8 g.; recrystallized from acetone–petroleum ether; m.p. 121° (with carbon dioxide evolution); soluble in acetone and ethyl acetate, sparingly soluble in benzene; insoluble in petroleum ether.

Anal. Calcd. for $C_{12}H_{11}O_5N$: C, 57.8; H, 4.5; N, 5.6. Found: C, 57.5; H, 4.5; N, 5.6.

Another crop of crude β-benzyl-N-carboxy-L-aspartate anhydride (2.5 g., m.p. 70°) was obtained from the benzene mother liquor by precipitation with two volumes of petroleum ether.

Poly-(β-benzyl-L-aspartate) (V) (*n* average = 80).—The polymerization of IV was carried out at 120° in high vacuum (10^{-3} mm.) under conditions similar to those given by Frankel and Berger[1] and Katchalski, *et al.*[20] The polymer obtained was dissolved in a small amount of hot dimethylformamide and precipitated by pouring the clear solution into cold water, slightly acidified with hydrochloric acid. The supernatant was decanted and the precipitate washed several times with water; dried *in vacuo* over sulfuric acid; yield quantitative. Poly-(β-benzyl-L-aspartate) is soluble in acetone, hot dimethylformamide and hot glacial acetic acid; is slightly soluble in hot methanol. It is easily drawn into fibers above its softening point (160°).

Anal. Calcd. for V (*n* average = 80): C, 64.4; H, 5.4; N, 6.8; amino-N, 0.085. Found: C, 64.2; H, 5.4; N, 6.8; amino-N, 0.085.[11]

Poly-L-aspartic Acid (VI) (*n* average = 80).—Phosphonium iodide (16 g.) was added in four portions to a solution of V (*n* average = 80) (4.0 g.) in glacial acetic acid (30 ml.)

(18) All melting points are uncorrected.

(19) M. S. Dunn and L. B. Rockland (*Advances in Protein Chem.*, **3**, 354 (1947)) report $[\alpha]^{25}D$ 24.62° (*c*, 2 in 6 *N* hydrochloric acid).

(20) E. Katchalski, I. Grossfeld and M. Frankel, THIS JOURNAL, **70**, 2094 (1948).

kept at 55°. Dry hydrogen was bubbled through the hot mixture during the period of reduction (5 hours). Ether (100 ml.) was added to the final reaction mixture, the precipitate filtered and repeatedly washed with ether (30 ml.). Further purification was effected by boiling the reduced polymer with 0.1 *N* hydrochloric acid for 10 minutes.

Anal. Calcd. for VI (*n* average = 80): C, 41.7; H, 4.3; N, 12.2; amino-N, 0.15. Found: C, 41.0; H, 4.3; N, 12.0; amino-N, 0.15[11]; carboxyl-N, 0.13.[10]

The equivalent weight of the polymer determined by dissolving in excess of 0.1 *N* sodium hydroxide and potentiometric back-titration with 0.1 *N* hydrochloric acid was 125; the value calculated for complete reduction is 115; $[\alpha]^{25}D$ −11.2° (*c* 5 in water in the presence of one equivalent of sodium hydroxide); $[\alpha]^{25}D$ −10.8° (*c* 5 in water in the presence of 0.67 equivalent of sodium hydroxide); $[\alpha]^{25}D$ −10.4° (*c* 5 in water in the presence of 0.33 equivalent of sodium hydroxide).

Two other poly-L-aspartic acid preparations gave the following analysis: Preparation (a) prepared under conditions similar to those described above): N, 12.0; amino-N, 0.20; carboxyl-N, 0.18 (*n* average calcd. = 61).

Preparation (b) (prepared from IV kept in a desiccator over sulfuric acid for 5 months): N, 12.2; amino-N, 0.61; carboxyl-N, 0.52 (*n* average calcd. = 20).

Total Hydrolysis of VI (*n* average = 80).—Poly-L-aspartic acid (50 mg.) was hydrolyzed by refluxing in 6 *N* hydrochloric acid (4 ml.) for 24 hours. The resulting clear hydrolysate was diluted to a volume of 25 ml. and the amino-N[11] and carboxyl-N[10] determined in 1-ml. aliquots after neutralization.

Anal. Calcd. for a hydrolysate of 100 mg. VI (*n* average = 80); amino-N, 12.2 mg.; carboxyl-N, 12.2 mg. Found: amino-N, 11.8 mg. carboxyl-N, 11.5 mg.

In order to determine the specific rotation of the aspartic acid derived from the polymer on acid hydrolysis, the following experiment was carried out: Poly-L-aspartic acid (50 mg.) was hydrolyzed, as above. The hydrolysate was brought to dryness, the residue dissolved in 6 *N* hydrochloric acid (0.5 ml.) and brought to a volume of 1 ml. with the same solvent. From the optical rotation of this solution the specific rotation of the monomeric aspartic acid (56.9 mg., *cf.* amino-N of previous experiment) was calculated: $[\alpha]^{25}D$ 24.9.[19] On isolation of the aspartic acid from the hydrolysate, it was found that the acid showed the normal specific rotation of L-aspartic acid.

The ammonium salt of poly-L-aspartic acid (*n* = 80) was obtained by dissolution of VI in an excess of 3 *N* ammonium hydroxide and evaporation to dryness on a water-bath. The salt was dried over sulfuric acid for two days before analysis.

Anal. Calcd. for $(C_4H_8O_3N_2)_n$: N, 21.2. Found: N, 20.7.

Sodium Salt of VI (*n* = 80).—VI was dissolved in a slight excess of 1 *N* sodium hydroxide and absolute ethanol (10 volumes) added. The flocculent precipitate was washed with ethanol, redissolved in a small amount of water and reprecipitated with absolute ethanol (10 volumes). The purified salt was collected, washed with absolute ethanol and anhydrous ether and dried *in vacuo* over sulfuric acid.

Anal. Calcd. for $(C_4H_4O_3NNa)_n$: Na, 16.8. Found: Na: 14.8.

The copper salt of VI (*n* = 80) was obtained as a green voluminous precipitate on adding an excess of aqueous copper sulfate to an aqueous solution of sodium poly-aspartate.

Anal. Calcd. for $(C_4H_4O_3N^1/_2Cu)_n$: Cu, 21.8; N, 9.6. Found: Cu, 22.0; N, 10.0.

The silver salt of VI (*n* = 80) was precipitated from an aqueous solution of sodium poly-aspartate on adding an excess of aqueous silver nitrate.

Anal. Calcd. for $(C_4H_4O_3NAg)_n$: Ag, 48.7. Found: Ag, 40.0.[21]

Carboxyl-N of L-Isoasparagine.—L-Isoasparagine hydrate was prepared according to Bergmann and Zervas[4] and analyzed for carboxyl-N.[10]

Anal. Calcd. for L-isoasparagine hydrate: carboxyl-N, 9.3. Found: carboxyl-N, 9.5.

Potentiometric Titration of VI (*n* average = 61).—The titration curve given in Fig. 1 was obtained by the back-titration of an aqueous solution of sodium poly-L-aspartate (*n* = 61; 30.0 mg. in 20 ml.) with 0.05 *N* hydrochloric acid. The titration was carried out at 25° in a nitrogen atmosphere, using the Beckman *p*H-meter with a glass electrode.

The influence of potassium chloride at various concentrations on the *p*H of an aqueous solution of a 50% neutralized poly-L-aspartic acid is given in Fig. 3.

Viscosity of Poly-L-aspartic Acid (*n* = 61) **Solutions.**—The viscosity of poly-L-aspartic acid (*n* = 61) in aqueous solution at various degrees of neutralization was measured at 30 ± 0.02° in a modified Ostwald-Fenske viscosimeter (A.S.T.M. D445) of efflux time 180.6 seconds for water. The results are summarized in Fig. 4. Concentrations are given in basic moles per liter (1 basic mole 115 g.).

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(21) The silver salt of poly-glutamic acid obtained by Hanby, Waley and Watson[8] contained silver in a percentage (40.7%) considerably less than the theoretical (45.8%).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF TEXAS]

Synthesis and Reaction with Morpholine of *cis*-1,2-Dibenzoyl-1-*p*-tolylethylene[1]

BY PHILIP S. BAILEY, ELIAS E. KAWAS AND LELAND L. SMITH[2]

cis-1,2-Dibenzoyl-1-*p*-tolylethylene, a vinylog of 1,2-dibenzoylpropene, has been synthesized and shown to react with morpholine by simple addition, in contrast to 1,2-dibenzoylpropene which undergoes a novel reaction with morpholine to give 1,2-dibenzoyl-3-(4-morpholinyl)-propane.

Earlier work has shown that 1,2-dibenzoylpropene and similar compounds will react with secondary amines to give 3-amino-1,2-dibenzoylalkanes.[3,4,5] In view of certain addition reactions of conjugated systems involving aromatic rings found in the literature[6] it seemed desirable to make 1,2-dibenzoyl-1-*p*-tolyl- and *o*-tolylethylenes in order to see if the amine reaction could be extended to vinylogs (through the benzene ring) of 1,2-dibenzoylpropene. Of these only the first one could be prepared.

1,2-Dibenzoyl-1-*p*-tolylethylene (I) was made by well known and general reactions[7] involving the

(1) This is the fifth in a series of papers concerning 1,3-shifts of hydrogen in the reactions of dibenzoylalkenes and related compounds. For the first four see (a) R. E. Lutz and P. S. Bailey, THIS JOURNAL, **67**, 2229 (1945); (b) P. S. Bailey and R. E. Lutz, *ibid.*, **67**, 2232 (1945); (c) P. S. Bailey and G. Nowlin, *ibid.*, **71**, 732 (1949); (d) P. S. Bailey and W. W. Hakki, *ibid.*, **71**, 2886 (1949).

(2) Taken in part from the M.A. Thesis of Leland L. Smith, May, 1948.

(3) R. E. Lutz and P. S. Bailey, THIS JOURNAL, **67**, 2229 (1945).

(4) P. S. Bailey and G. Nowlin, *ibid.*, **71**, 732 (1949).

(5) P. S. Bailey and W. W. Hakki, *ibid.*, **71**, 2886 (1949).

(6) (a) E. P. Kohler and E. M. Nygaard, *ibid.*, **52**, 4128 (1930); (b) M. Couper and R. E. Lutz, *J. Org. Chem.*, **7**, 79 (1942); (c) H. Gilman, J. E. Kirby and C. R. Kinney, THIS JOURNAL, **51**, 2252 (1929).

(7) (a) R. E. Lutz and W. R. Tyson, *ibid.*, **56**, 1341 (1934); (b) R. E. Lutz and C. J. Kibler, *ibid.*, **61**, 3007 (1939); (c) R. E. Lutz and F. N. Wilder, *ibid.*, **56**, 978 (1934).