

[CONTRIBUTION FROM THE LABORATORY OF THE CHILDREN'S CANCER RESEARCH FOUNDATION AND THE HARVARD MEDICAL SCHOOL, BOSTON, MASS.]

## The Helical Sense of Poly- $\beta$ -benzyl-L-aspartate. Synthesis and Rotatory Dispersion of Copolymers of $\beta$ -Benzyl-L and D-Aspartate with $\gamma$ -Benzyl-L-glutamate<sup>1</sup>

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The synthesis of poly- $\beta$ -benzyl-L-aspartate and poly- $\beta$ -benzyl-D-aspartate with weight average molecular weights of about 50,000 is described. The synthesis of twelve different copolypeptides of  $\gamma$ -benzyl-L-glutamate and  $\beta$ -benzyl-L and D-aspartate has also been effected. It has been concluded that poly- $\beta$ -benzyl-L-aspartate and poly- $\gamma$ -benzyl-L-glutamate exist as helices with the opposite sense of twist on the basis of the following observations. The helix  $\rightarrow$  random transformation of poly- $\beta$ -benzyl-L-aspartate in chloroform:dichloroacetic acid mixtures shows a much larger change of rotation and in the opposite direction to that observed with poly- $\gamma$ -benzyl-L-glutamate. The optical rotatory dispersion data of poly- $\beta$ -benzyl-L-aspartate from 360 to 600  $m\mu$  fit the two term equation proposed by Moffitt for helical polypeptides with  $b_0 \cong +630$ ; in this same region poly- $\gamma$ -benzyl-L-glutamate shows  $b_0 \cong -670$ . The copolymers of benzyl-D-aspartate with benzyl-L-glutamate show a regular variation of optical rotatory properties with composition, whereas the copolymers of benzyl-L-aspartate with benzyl-L-glutamate do not. Finally the ultraviolet (240 to 360  $m\mu$ ) rotatory dispersions of the L-aspartate and L-glutamate polypeptides show marked differences which strengthen the conclusion that the poly- $\beta$ -benzyl-L-aspartate helix has a sense of twist opposite to that of the poly- $\gamma$ -benzyl-L-glutamate helix.

### Introduction

In preliminary communications we reported<sup>3a,b</sup> that poly- $\beta$ -benzyl-L-aspartate showed distinctly different rotatory properties from the homologous synthetic polypeptide, poly- $\gamma$ -benzyl-L-glutamate, and in particular the rotatory properties of poly- $\beta$ -benzyl-L-aspartate (L-PBA) were more similar to those of poly- $\gamma$ -benzyl-D-glutamate (D-PBG) than to those of its L-isomer (L-PBG). One of the two explanations offered for these differences in properties was that the sense of the helix in L-PBA was similar to that of D-PBG. We now report extensive experimental evidence that leads to the conclusion that the helix sense of L-PBA is opposite from that of L-PBG and the same as that of D-PBG and further that D-PBA has the same sense of helix as L-PBG.

In this paper we describe (1) a new synthesis of  $\beta$ -benzyl-L-aspartate N-carboxyanhydride (NCA) and the synthesis of  $\beta$ -benzyl-D-aspartate NCA, (2) the synthesis of a series of copolymers derived from  $\gamma$ -benzyl-L-glutamate and  $\beta$ -benzyl-L-aspartate and a second series of copolymers derived from  $\gamma$ -benzyl-L-glutamate and  $\beta$ -benzyl-D-aspartate, (3) the characterization of the benzyl aspartate homopolymers and copolymers by viscosity and optical rotatory dispersion and finally (4) an interpretation of the optical rotatory dispersion data which leads to definite conclusions as to the relative sense of the helix in L-PBA compared with L-PBG.

### Experimental

All melting points are corrected.

**N-Carbonyloxy-aspartic Acid.**—The N-carbonyloxy derivatives of L, D and DL-aspartic acid were prepared according to the method of Bergman and Zervas.<sup>4</sup> The following results were obtained:

(1) This is Polypeptides. XXVII. For the preceding paper in this series see G. D. Fasman and E. R. Blout, *THIS JOURNAL*, **82**, 2262 (1960). Alternate address for E. R. Blout: Chemical Research Laboratory, Polaroid Corporation, Cambridge 39, Massachusetts.

(2) Gillette Safety Razor Company, Research Laboratories, So. Boston 6, Massachusetts.

(3) (a) E. R. Blout and R. H. Karlson, *THIS JOURNAL*, **80**, 1259 (1958). (b) E. R. Blout, "Proc. of the 1Vth International Congress of Biochemistry, Vienna, 1958," Vol. IX, Pergamon Press, London, 1959, p. 37.

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

*Anal.* *D-isomer*, m.p. 114–115°. Calcd. for  $C_{12}H_{12}NO_6$ : C, 54.0; H, 4.88; N, 5.24. Found: C, 54.6; H, 5.1; N, 5.1. *L-isomer*, m.p. 116–117°, reported 116°. *DL-isomer*, m.p. 76–77°. Found: C, 50.5; H, 5.1.

**Dibenzyl-N-carbobenzoyloxy-aspartate.**—The dibenzyl derivatives of L, D and DL-N-carbobenzoyloxy-aspartic acid were prepared according to the method of Berger and Katchalski.<sup>5</sup> The following results were obtained:

*Anal.* *D-isomer*, m.p. 64.0–64.8°. Calcd. for  $C_{26}H_{26}O_6N$ : C, 69.8; H, 5.6; N, 3.1. Found: C, 69.9; H, 5.7; N, 3.1.  $[\alpha]^{20}_D = -3.90$  ( $c = 10\%$ , benzene). *L-isomer*, m.p. 63.8–64.5°, reported 66.5°.  $[\alpha]^{20}_D = +4.14$  ( $c = 10\%$ , benzene) reported,  $[\alpha]^{20}_D = +3.5$  ( $c = 10\%$ , benzene). *DL-isomer*, m.p. 50.5–51.5°. Found: C, 69.9; H, 5.5; N, 3.1.

**Dibenzyl-aspartate-HBr.**—The dibenzyl-HBr derivatives of L, D and DL aspartic acid were prepared from the corresponding dibenzyl-N-carbobenzoyloxy-aspartate as follows: 51 g. of dibenzyl-N-carbobenzoyloxy-aspartate was covered with 100 ml. of 5.5 N HBr in glacial acetic acid<sup>6</sup> and allowed to stand at room temperature until  $CO_2$  evolution had ceased. The solution was diluted with anhydrous ether to almost the cloud point and placed at 0°. In 24 hr. the product had crystallized. The solid was filtered off, washed with ethyl ether and vacuum dried at 50°. Yield: 36 g. 79%. A sample was recrystallized from methanol-ethyl ether for analysis. The following results were obtained.

*Anal.* *D-isomer*, m.p. 128.0–128.4° (decomp.). Calcd. for:  $C_{18}H_{19}O_4N \cdot HBr$ : C, 54.8; H, 5.1; Br, 20.3. Found: C, 54.9; H, 5.1; Br, 20.0. *L-isomer*, m.p. 128.2–129.8° (decomp.). Found: C, 54.9; H, 5.1; Br, 20.3. *DL-isomer*, m.p. 104.0–106.0° (decomp.). Found: C, 54.0; H, 4.8; Br, 20.2.

**$\beta$ -Benzyl-aspartate-N-carboxyanhydride. Method A.**—The  $\beta$ -benzyl-aspartate-N-carboxyanhydride was prepared from the corresponding D and L dibenzyl-aspartate-HBr as follows: 75 g. of dibenzyl-aspartate-HBr was suspended in 200 ml. of dioxane<sup>7</sup> and phosgene bubbled in for 2 hr. while the temperature was maintained at 65°. The dioxane was removed in vacuo at 65°, the resultant oil diluted with 100 ml.  $CHCl_3$ , and *n*-hexane added to the cloud point whereupon the product crystallized. After standing for 12 hr. at 0°, the product was isolated and recrystallized three times from ethyl acetate-*n*-hexane. Yield, 16 g. The results obtained were:

*Anal.* *D-isomer*, m.p. 126.0–127.2°. Calcd. for:  $C_{12}H_{11}O_5N$ : C, 57.8; H, 4.5; N, 5.6. Found: C, 57.8; H, 4.4; N, 5.6.  $[\alpha]^{20}_D = +30.0$  ( $c = 5\%$ , ethyl acetate). *L-isomer*, m.p. 126.5–127.5°, reported 121°. Found: C, 58.0; H, 4.3; N, 5.6.  $[\alpha]^{20}_D = -30.0$  ( $c = 5\%$ , ethyl acetate).

(5) A. Berger and E. Katchalski, *THIS JOURNAL*, **73**, 4084 (1951).

(6) D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952).

(7) L. F. Fieser, "Experiments in Organic Chemistry," 2nd ed., D. C. Heath and Co., Boston, Massachusetts, 1941, p. 361.

**Method B.**—30 g.  $\beta$ -benzyl-L-aspartate<sup>8</sup> was suspended in 600 ml. of dioxane<sup>7</sup> with stirring, phosgene bubbled in, keeping the temperature at 50°, until all the solid had dissolved (approximately one hour). Nitrogen was bubbled through the solution for 4 hr. at room temperature to remove excess phosgene. The dioxane was removed at 40° at reduced pressure under nitrogen. The resulting oil was dissolved in 300 ml. of ethyl acetate, petroleum ether added to opalescence, the solution filtered through celite, 100 ml. petroleum ether added to the solution and it was placed at -30° overnight. The crystals were filtered yielding 25 g. of product. The solid was redissolved in 250 ml. ethyl acetate by warming to 40°, 25 ml. *n*-hexane added, the solution filtered through celite, 50 ml. more hexane added and placed at -30°. The next day 170 ml. of *n*-hexane was added to the crystallizing solution. After standing 2 days the crystals were filtered, yielding 22.4 g., m.p. 127–128°. Mixed melting point with anhydride from method A, 127–128°.

The solvent of the supernatant of the first crop was evaporated to dryness at reduced pressure, temperature below 40°, yielding 11 g. of oil. This was redissolved in 100 ml. of ethyl acetate by warming, filtered through celite, 130 ml. of *n*-hexane added and the solution placed at -30°. After standing overnight the crystals were filtered off, yielding 8.0 g.

**Method C.**—The procedure of Berger and Katchalski<sup>8</sup> using  $\beta$ -benzyl-N-carbobenzoyloxy-L-aspartate and  $\text{PCl}_5$  yielded the same anhydride, m.p. 127–128°. Mixed melting points with NCA from methods A and B showed no depressions.

**N-Chloroformyl-dibenzyl-aspartate.**—The N-chloroformyl derivatives of D, L and DL dibenzyl aspartate-HBr were prepared as follows: 35 g. of dibenzyl aspartate-HBr was treated with excess saturated sodium bicarbonate solution and the mixture extracted three times with ethyl ether. The combined extracts were dried over  $\text{MgSO}_4$ , and the solution concentrated to a volume of 100 ml. *n*-Hexane was added until the cloud point was approached, and phosgene was passed in at room temperature. An immediate formation of crystals occurred. After 20 minutes of phosgenation the product was filtered off and washed with *n*-hexane. It was recrystallized from chloroform-ethyl acetate, then from chloroform-*n*-hexane. Yield 25 g. The following results were obtained:

*Anal.* *D-isomer*, m.p. 126.0–127.0°. Calcd. for  $\text{C}_{15}\text{H}_{13}\text{O}_5\text{NCl}$ : mol. wt. 375.8, % benzyl, 50.0. Found: neutral equivalent ( $\text{NaOCH}_3$  titrated) 375, neutral equivalent (Cl titration) 374, benzyl (ultraviolet), 51.2%. *L-isomer*, m.p. 128.0–129.0°. Neutral equivalent ( $\text{NaOCH}_3$  titration), 360, benzyl, 50.4%. *DL-isomer*, m.p. 114.0–115°.

**Poly- $\beta$ -benzyl-L-aspartate.**—A typical polymerization was run as follows: To 25 ml. of  $\text{CH}_2\text{Cl}_2$  (freshly distilled from KOH) containing 27.8 ml. of 0.359 *N*  $\text{NaOCH}_3$  (25% methanol-75% benzene), [anhydride]/[initiator] (*A/I*) = 200, was added 0.5 g.  $\beta$ -benzyl-L-aspartate-N-carboxyanhydride and the clear solution allowed to stand for 24 hr., after which time  $\text{NaOCH}_3$  titration indicated the reaction was at least 95% complete. The solution was poured with stirring in to 100 ml. *n*-hexane, the polymer filtered off and air-dried:  $\eta_{sp}/c$  = 0.17 (0.2% in dichloroacetic acid).

*Anal.* Calcd. for:  $(\text{C}_{11}\text{H}_{11}\text{NO}_3)_n$ : C, 64.4; H, 5.4. Found: C, 64.3; H, 5.6.

**Copoly- $\gamma$ -Benzyl-L-glutamate: $\beta$ -benzyl-Aspartates.**—A series of copolymers of  $\gamma$ -benzyl-L-glutamate with D and L- $\beta$ -benzyl-aspartate were prepared from their respective anhydrides. A typical polymerization follows: 0.70 g. of  $\gamma$ -benzyl-L-glutamate-N-carboxyanhydride and 0.64 g. of  $\beta$ -benzyl-L-aspartate-N-carbobenzoyloxy-anhydride (1:1 mole ratio) were dissolved in 65 ml. of  $\text{CH}_2\text{Cl}_2$  (freshly distilled from KOH) and 74.2 cmm. of 0.375 *N*  $\text{NaOCH}_3$  (*A/I* = 200) was added. The polymerization was allowed to stand 24 hr., the solution was poured into 300 ml. of diisopropyl-ether causing precipitation of the copolymer, which was dried at 100° *in vacuo* (10<sup>-2</sup> mm. Hg). Yield = 0.96 g., 95% of theoretical.  $\eta_{sp}/c$  = 1.00 (0.2% in dichloroacetic acid).

**Increasing the Molecular Weight of Poly- $\beta$ -benzyl-L-aspartate.**—0.1 g. poly- $\beta$ -benzyl-L-aspartate,  $\eta_{sp}/c$  = 0.17 (*c* = 0.2%, dichloroacetic acid) stored at 3° after isolation was dissolved in 10 ml.  $\text{CH}_2\text{Cl}_2$  and 50 mg. of dicyclohexylcarbodi-

imide added. The reaction mixture was allowed to stand at room temperature for 3 days. The solution, which had become more viscous, was poured into *n*-hexane (30 ml.) and a fibrous precipitate formed. The polymer was isolated by centrifugation, washed two times with 30 ml. of ether and dried *in vacuo*. Yield = 0.1 g,  $\eta_{sp}/c$  = 0.40 (*c* = 0.2% in dichloroacetic acid).

**Optical Rotation.**—In the spectral range 350 to 600  $m\mu$  a G.E. AH-4 mercury arc and a Sylvania K-100 zirconium arc were used as light sources. The spectral lines were isolated with a Beckmann D.U. monochromator and, in some cases, interference filters as described previously.<sup>9</sup> Optical rotation was measured with a Rudolph photoelectric polarimeter model 200. Solutions were contained in one or two decimeter standard polarimeter tubes. Precautions were taken against evaporation of volatile solvents.

Below 350  $m\mu$  the light source was a Westinghouse short arc mercury lamp, SAH-250A, whose outer glass bulb had been removed to pass ultraviolet light. Since operation of the lamp unprotected from air oxidation of the external electrodes resulted in short lamp life, it was finally mounted in an evacuable metal container having a quartz window; a pressure of ca. 2–4 mm. Hg was maintained while operating.<sup>10</sup> Measurements were made during periods of stable intensity. Fluctuations were presumably a consequence of arc shifting and increased with the age of the lamp. The radiation source was cooled by circulating air.

Solutions measured in the ultraviolet were contained in 1 and 0.1 cm. fused quartz cells. The 0.1 cm. cell was found to possess insignificant birefringence down to 237  $m\mu$ . However, the windows of the 1 cm. cell were slightly birefringent and therefore care was taken in positioning the cells between reading the blank and the solution. The 1 cm. cell was used at wave lengths above 265  $m\mu$  and the 0.1 cm. cell below 265  $m\mu$ . Errors in measurement were small above 280  $m\mu$  since the observed rotations were large and the slits narrow. Below this wave length it was necessary to use wide slits and short path lengths.

**Calculation of Rotatory Data.**—The dispersion constant,  $\lambda_0$ , was obtained from the square root of the slope of  $[\alpha]\lambda^2$  vs.  $[\alpha]$  (*i.e.*, modified Lowry plot proposed by Yang and Doty.<sup>11</sup>)

In the spectral region 365 to 578  $m\mu$  the coefficient,  $b_0$ , of the second term in the Moffitt equation<sup>12</sup>

$$[m']_{\lambda} = \frac{a_0\lambda_0^2}{\lambda^2 - \lambda_0^2} + \frac{b_0\lambda_0^4}{(\lambda^2 - \lambda_0^2)^2} \quad (1)$$

was derived from the slope of the plot  $[m'](\lambda^2 - \lambda_0^2)$  vs.  $[\lambda^2 - \lambda_0^2]^{-1}$  as suggested by Moffitt and Yang<sup>13</sup> with the exception that the specific rotation  $[\alpha]$  rather than the mean residue rotation  $[m']$  was used

$$[\alpha]_{\lambda} = [m']_{\lambda} \frac{100 n^2 + 2}{M} \quad (2)$$

Thus  $b_0$  is the slope of the linear plot divided by  $\lambda_0^4(100/M)(n^2 + 2/3)$ . In this form of the Moffitt plot the dispersion of the refractive index is neglected; the Lorentz term in eq. 2 was assumed constant and the value of solvent refractive index used was that at the sodium D line. Optical rotatory dispersions of poly- $\gamma$ -benzyl-L-glutamate in  $\text{CHCl}_3$  when plotted in this manner, with  $\lambda_0 = 212 m\mu$  as found by Moffitt and Yang,<sup>13</sup> showed no significant deviation from linearity in the range 365 to 578  $m\mu$ ,  $b_0 = -670$ . Unless noted otherwise, all values of  $b_0$  reported herein refer to measurements calculated without the dispersion of refractive index correction in the range 365 to 578  $m\mu$ . In some instances Moffitt plots of optical rotatory dispersions from 240 to 578  $m\mu$  (Fig. 7) were also made using the correction for the dispersion of solvent refractive index. The refractive index was estimated from Sellmeir's approximation formula

$$n^2 = 1 + \frac{a\lambda^2}{\lambda^2 - \lambda_v^2} \quad (3)$$

(9) E. R. Blout and G. D. Fasman, "Recent Advances in Gelatin and Glue Research," Pergamon Press, London, 1958, p. 122.

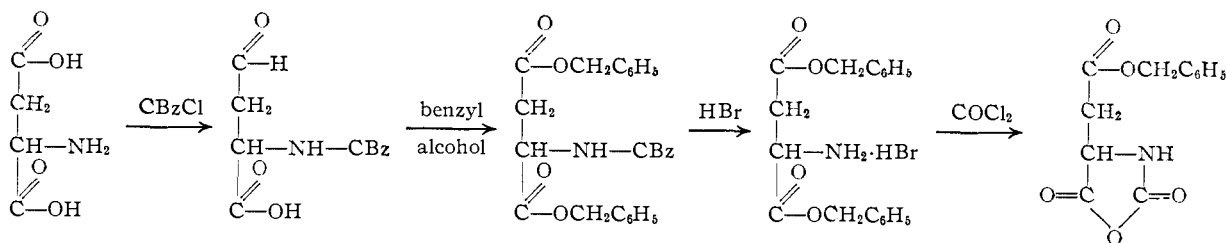
(10) We wish to acknowledge the advice and assistance of V. Eloranta and J. King of Polaroid Corporation in the design and construction of this device.

(11) J. T. Yang and P. Doty, *THIS JOURNAL*, **79**, 761 (1957).

(12) W. Moffitt, *J. Chem. Phys.*, **25**, 467 (1956).

(13) W. Moffitt and J. T. Yang, *Proc. Natl. Acad. Sci. U. S.*, **42**, 596 (1956).

(8) J. Noguchi, J. Kurtz and E. Katchalski. We thank these authors for this procedure before publication.



the constants  $a$  and  $\tau_v$  being determined from measurements of the refractive index of  $\text{CHCl}_3$  at 434 and 658  $\mu$ .

### Results and Discussion of Syntheses

The synthesis of poly- $\beta$ -benzyl-L-aspartate was first reported by Frankel and Berger<sup>14</sup> in 1949 and an improved method by Berger and Katchalski<sup>5</sup> appeared in 1951. The  $\beta$ -benzyl-L-aspartate-N-carboxyanhydride used in the polymerizations reported in this paper was prepared by three independent methods to confirm that the NCA obtained by these methods yielded the  $\alpha$ -amino-N-carboxyanhydride and not the  $\beta$ -amino-N-carboxyanhydride. This was thought to be necessary when the results obtained from optical rotatory studies on polymer made by synthetic method A (see above), were found to be distinctly different than those previously obtained with poly- $\gamma$ -benzyl-L-glutamate.

The first synthetic procedure (A) shown below, is a new method for the synthesis of an amino acid N-carboxyanhydride (NCA).

N-carbobenzoxy aspartate was prepared according to the Bergman and Zervas<sup>4</sup> method. This was then converted to the dibenzyl-N-carbobenzoxy aspartate following the Berger and Katchalski procedure.<sup>5</sup> The carbobenzyloxy group was removed by HBr in glacial acetic acid<sup>6</sup> yielding dibenzyl aspartate. The NCA was obtained by treatment of the dibenzyl aspartate with phosgene. The crystalline NCA obtained in this manner from L-aspartic acid had a melting point of 126.5–127.5°, whereas that previously reported was 121°. The Berger and Katchalski synthesis<sup>5</sup> (B) was then repeated and the NCA so obtained had a melting point of 127° which gave an undepressed mixed melting point with the NCA from the first procedure. If the conditions of phosgene treatment were altered, in method A, it was found to be possible to isolate the N-chloroformyl dibenzyl aspartate instead of the NCA. The third procedure (C) involved the synthesis of  $\beta$ -benzyl-L-aspartate<sup>5</sup> and then phosgene treatment to yield the NCA. This material again gave an undepressed melting point with the NCAs obtained from the other methods. As Berger and Katchalski<sup>5</sup> had unambiguously proved the structure of N-carbobenzoxy  $\beta$ -benzyl-L-aspartate the NCAs made from the above three procedures can definitely be assigned to the  $\alpha$ -amino-N-carboxyanhydride. Further evidence was provided by removing the N-carbobenzoxy group, by means of HBr in glacial acetic acid<sup>6</sup> from  $\beta$ -benzyl-N-carbobenzoxy-L-aspartate to yield  $\beta$ -benzyl-L-aspartate. This compound on treatment with phosgene again yielded  $\beta$ -benzyl-L-aspartate NCA with identical properties to those mentioned above.

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

The aspartic acid derivatives necessary for the synthesis of the NCA via method A, were prepared for the L, D and DL-aspartic acid, namely N-carbobenzoxy aspartic acid, dibenzyl-N-carbobenzoxy aspartate and dibenzyl aspartate·HBr. The analyses and physical constants are listed in the experimental section. The NCAs of D and L- $\beta$ -benzyl aspartic acid were also prepared. (See above.) Of the three methods employed the third, via  $\beta$ -benzyl-L-aspartate, was found to be the simplest and gave the best yields.

The polymerization of the NCAs was investigated in chloroform, dioxane, ethylene dichloride, methylene dichloride, pyridine and acetonitrile using both sodium methoxide and triethylamine as initiators, at various anhydride-initiator ( $A/I$ ) ratios. Chloroform, methylene chloride and ethylene dichloride were found to be the most suitable solvents, and sodium methoxide was the preferred initiator at  $A/I = 100$ , to yield the highest molecular weights. The highest molecular weight polymer obtained had a reduced specific viscosity of 0.33 ( $c = 0.2\%$  in dichloroacetic acid (DCA)). Polymer of this viscosity has an estimated weight average molecular weight of 50,000 using the calibration of viscosity and light scattering for poly- $\gamma$ -benzyl-L-glutamate.<sup>15</sup>

It should be noted that the solubility of the polymers in weakly hydrogen-bonding solvents varied according to the polymerization solvent. Polymers obtained in ethylene dichloride were not soluble in chloroform and only partially redissolved in ethylene dichloride. Polymers prepared in reagent grade chloroform were found to readily redissolve in this solvent. As reagent grade chloroform usually contains 0.5% ethyl alcohol, the postulate can be advanced that the polymerization termination step involves the alcohol and as such the end group may be different from that obtained in a polymerization carried out in ethylene dichloride. This difference in the end group may account for the observed differences in solubility of the polymers.

As the molecular weights obtained on polymerization of  $\beta$ -benzyl aspartate NCA (Table I) were considerably lower at the same  $A/I$  than those obtained for poly- $\gamma$ -benzyl-L-glutamate<sup>16</sup> attempts were made to increase the molecular weight using the carbodiimide method<sup>17</sup> as adapted by Blout and DesRoches.<sup>18</sup> Increases in molecular weight were obtained in both dioxane and ethylene dichloride, starting with a polymer having a reduced specific viscosity of 0.17 ( $c = 0.2\%$ , DCA), made

(15) P. Doty, J. H. Bradbury and A. M. Holtzer, *THIS JOURNAL*, **78**, 947 (1956).

(16) R. R. Blout and R. H. Karlson, *ibid.*, **78**, 941 (1956).

(17) J. C. Sheehan and G. P. Hess, *ibid.*, **77**, 1067 (1955).

(18) E. R. Blout and M. E. DesRoches, *ibid.*, **81**, 370 (1959).

in ethylene dichloride initiated at  $A/I$  400 with sodium methoxide. The carbodiimide treatment in dioxane yielded a higher molecular weight polymer with a reduced specific viscosity of 0.30 ( $c = 0.2\%$ , DCA). Using the same starting polymer in ethylene dichloride solution, carbodiimide treatment yielded a polymer with a reduced specific viscosity of 0.40 (0.2%, DCA). Only freshly prepared polymer, or that stored for short periods at 3° yielded increased molecular weights.

To ascertain that no racemization took place during the synthesis of the D and L poly- $\beta$ -benzyl aspartates, the polymers were hydrolyzed in 6  $N$  HCl to their respective amino acids. Poly- $\beta$ -benzyl-L-aspartate on hydrolysis gave  $[\alpha]_{578}^{22} = +23.9$  in 6  $N$  HCl; reported for L-aspartic acid  $[\alpha]_{25D} = +24.6$ ; poly- $\beta$ -benzyl-D-aspartate on hydrolysis gave  $[\alpha]_{578}^{22} = -23.0$ .

A series of copolymers was synthesized with  $\gamma$ -benzyl-L-glutamate with both D and L-benzyl-aspartate in varying compositions. Table I lists

TABLE I  
COPOLYMERS OF  $\beta$ -BENZYL-L-ASPARTATE AND  $\gamma$ -BENZYL-L-GLUTAMATE<sup>a</sup>

Mole ratio in polymerization mixture Aspartate NCA    Glutamate NCA	Polymer no.	( $\eta_{sp}/c$ ) <sup>b</sup>	Chromat. anal., <sup>c</sup> mole % glutamate	
0	10	RK1333	1.17	
1	9	RK1323	1.0	90
3	7	RK1324	0.77	71
5	5	RK1325	.76	51
7	3	RK1326	.69	30
9	1	RK1327	.37	13
10	0	RK1145	.26	
10	0	GF-5-64	.33 <sup>d</sup>	

<sup>a</sup> Polymerized in  $CH_2Cl_2$ , 2% NCA. Initiated with  $NaOCH_3$ ,  $A/I = 200$ . <sup>b</sup>  $c = 0.2\%$ , DCA. <sup>c</sup> Determined by hydrolysis of the polymer in 6  $N$  HCl (20 mg./3 ml.) at 120° for 24 hr. C. W. Hirs, W. H. Stein and S. Moore, *J. Biol. Chem.*, 211, 941(1954) and then by paper chromatography according to R. E. Kay, D. C. Harris and C. Entennan, *Arch. Biochem. Biophys.*, 63, 14 (1956). The error in these determinations is estimated to be  $\pm 2.5\%$ . <sup>d</sup> Polymerized in  $CHCl_3$ , 1.5% NCA. Initiated with  $NaOCH_3$ ,  $A/I = 100$ .

the copolymers of  $\beta$ -benzyl-L-aspartate and  $\gamma$ -benzyl-L-glutamate and their specific viscosities in dichloroacetic acid. The mole ratio of NCAs used in the polymerization reaction are listed as well as the composition analysis of the copolymers as determined by hydrolysis and chromatography. As can be seen in this table the found ratios all fall within a few per cent. of the calculated ratios. In subsequent data the calculated values are used. Table II lists the copolymers of  $\beta$ -benzyl-D-aspartate and  $\gamma$ -benzyl-L-glutamate and their accompanying data.

To determine that we were dealing with truly random copolymers of  $\beta$ -benzyl aspartate and  $\gamma$ -benzyl glutamate the optical properties,  $[\alpha]_{546}$  and  $b_0$ , were determined for several mixtures of poly- $\beta$ -benzyl-L-aspartate and poly- $\gamma$ -benzyl-L-glutamate in chloroform solution and compared with the optical properties of the copolymers having the same ratio of aspartate to glutamate. The mixtures of the homopolymers gave the optical properties expected and calculated from those of

TABLE II  
COPOLYMERS OF  $\beta$ -BENZYL-D-ASPARTATE AND  $\gamma$ -BENZYL-L-GLUTAMATE<sup>a</sup>

Mole ratio in polymerization mixture Aspartate NCA    Glutamate NCA	Polymer no.	( $\eta_{sp}/c$ ) <sup>b</sup>	Chromat. anal., <sup>c</sup> mole % glutamate	
0	10	RK1333	1.17	
1	9	RK1328	0.64	86
2	8	RK1195	.80	
3	7	RK1329	.24	67
5	5	RK1330	.17	53
7	3	RK1331	.14	36
9	1	RK1332	.13	11
10	0	RK1355	.30	

<sup>a</sup> Polymerized in  $CH_2Cl_2$ , 2% NCA. Initiated with  $NaOCH_3$ ,  $A/I = 200$ . <sup>b</sup>  $c = 0.2\%$ , DCA. <sup>c</sup> Determined by hydrolysis of the polymer as in Table I, footnote <sup>a</sup>.

TABLE III  
SOME OPTICAL PROPERTIES OF POLYPEPTIDE HOMOPOLYMERS, OF MIXTURES OF HOMOPOLYMERS AND OF COPOLYMERS

	$[\alpha]_{546}$	$b_0$
Poly- $\gamma$ -benzyl-L-glutamate (L-PBG)	+ 14	-670
Poly- $\beta$ -benzyl-L-aspartate (L-PBA)	-172	+630
Calcd. for 5:5 mixture of L-PBA:L-PBG	- 79	~ 0
Found for 5:5 mixture of L-PBA:L-PBG	- 73	~ 0
Found for 5:5 copolymer L-BA:L-BG	- 8	-615
Calcd. for 9:1 mixture of L-PBA:L-PBG	-153	+500
Found for 9:1 mixture of L-PBA:L-PBG	-153	+453
Found for 9:1 copolymer L-BA:L-BG	- 73	-355

the respective homopolymers, while the copolymers showed different properties (Table III). It can be concluded therefore that the copolymers described herein are random copolymers and not mixtures of homopolymers or block copolymers.

### Results and Discussion of Rotatory Dispersion Measurements

**Poly- $\beta$ -benzyl Aspartate.**—The optical rotation,  $[\alpha]_{546}$ , of poly- $\gamma$ -benzyl-L-glutamate (L-PBG) and poly- $\beta$ -benzyl-L-aspartate (L-PBA) as a function of solvent composition is shown in Fig. 1. The change in optical rotation observed for L-PBG is toward *increased* levorotation and occurs at *ca.* 70% dichloroacetic acid (DCA) whereas L-PBA exhibited *decreased* levorotation at *ca.* 8% DCA. Furthermore the magnitude of the change is much greater for L-PBA (*ca.* 150°) than for L-PBG (*ca.* 40°). Since the residue weight of L-PBA (205 g./residue mole) differs only slightly from that of L-PBG (219 g./residue mole) calculation of the rotation changes on a molar residue basis will not alter the relative magnitude of the rotations appreciably. The direction of the change in optical rotation observed for L-PBA is opposite to that normally encountered in proteins and some polypeptides when the constituent amino acids are of the L-configuration and the conformational transition is from a presumed right-handed helix to random chain.

The rotatory dispersion of L-PBA in  $CHCl_3$  in the region 360 to 600  $m\mu$  fits eq. 1 with a  $b_0$  similar in magnitude but opposite in sign to that observed for L-PBG.<sup>3</sup> The fact that the data for L-PBA fit the Moffitt eq., 1, suggests that L-PBA is helical

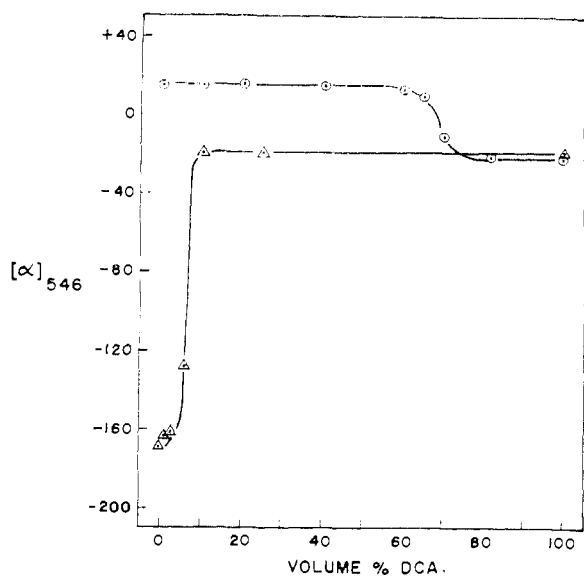


Fig. 1.—The specific rotation,  $[\alpha]_{546}$ , of poly- $\gamma$ -benzyl-L-glutamate,  $\circ-\circ-\circ$ , and poly- $\beta$ -benzyl-L-aspartate,  $\triangle-\triangle-\triangle$ , in chloroform-dichloroacetic acid (DCA) solutions. The volume % DCA is shown on the abscissa.

in this solvent. This suggestion is supported by infrared measurements in solution and in the solid state.<sup>19,20</sup> Further evidence for the existence of L-PBA and D-PBA in the helical conformation is offered by X-ray diffraction results.<sup>19,21</sup> Therefore the change in optical rotation observed in Fig. 1 can be interpreted as reflecting a helix  $\rightarrow$  random chain transition similar to that found for L-PBG in  $\text{CHCl}_3$ :DCA.<sup>22</sup> Since the transformation occurs below *ca.* 8% DCA the L-PBA helix appears to be thermodynamically less stable in this solvent system than L-PBG which retains the helical conformation up to 68% DCA. A similar conclusion regarding the stability of the L-PBA helix has been reached by Bradbury, *et al.*, on the basis of deuterium exchange studies.<sup>19</sup>

The direction of the change in the specific rotation  $[\alpha]_{546}$  supports the assignment of a left-handed helical structure to L-PBA as previously suggested,<sup>3a</sup> but it should be emphasized that the magnitude of the change is larger than expected. This is more apparent when the change in  $[m']_{546}$  is considered (eq. 2). The contribution of an  $\alpha$ -helix to  $[m']_{546}$  has been estimated to be *ca.*  $100^{\circ 23,24,25}$ ; the benzyl

(19) E. M. Bradbury, L. Brown, A. R. Downie, A. Elliott, W. E. Hanby and T. R. R. McDonald, *Nature*, **183**, 1736 (1959).

(20) K. S. Noiland, G. D. Fasman and E. R. Blout, to be published.

(21) C. Johnson, Ph.D. Thesis, Massachusetts Institute of Technology, 1959.

(22) E. R. Blout, P. Doty and J. T. Yang, *THIS JOURNAL*, **79**, 749 (1957).

(23) P. Doty and R. D. Lundberg, *Proc. Natl. Acad. Sci. U. S.*, **43**, 213 (1957).

(24) A. R. Downie, A. Elliott, W. E. Hanby and B. R. Malcolm, *Proc. Roy. Soc. (London)*, **A242**, 325 (1957).

(25) A simple rotatory dispersion is one which fits a one term Drude

equation,  $(\alpha)_\lambda = \frac{K}{\lambda^2 - \lambda_0^2}$ ; a complex rotatory dispersion is one which does not fit a one term Drude equation. An anomalous rotatory dispersion is defined (T. M. Lowry, "Optical Rotatory Power," Longmans, Green and Co., London, 1935) as one in which either (a)  $(\alpha)_\lambda$  changes sign or (b) the first derivative of  $(\alpha)_\lambda$  with respect to wave length is zero or (c) the second derivative is zero. An anomalous dispersion is necessarily complex.

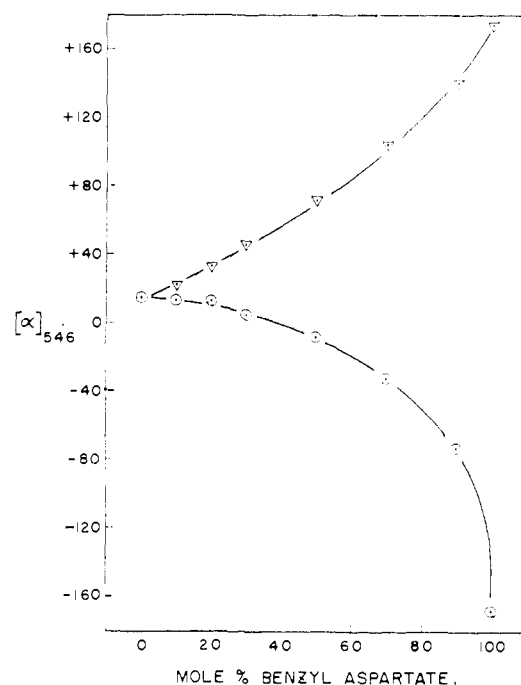


Fig. 2.—The specific rotation,  $[\alpha]_{546}$ , of copolymers of  $\beta$ -benzyl-L-aspartate: $\gamma$ -benzyl-L-glutamate,  $\circ-\circ-\circ$ , and of copolymers of  $\beta$ -benzyl-D-aspartate: $\gamma$ -benzyl-L-glutamate,  $\triangle-\triangle-\triangle$ , in chloroform solutions.

aspartate helix contributes about twice this amount (*ca.*  $-250^{\circ}$  as determined from Fig. 1). This increased levorotation is not compatible with solvent effects previously observed in polypeptide and protein solutions.<sup>11</sup>

**Copolymers of  $\beta$ -Benzyl Aspartate.**—The two series of copolymers of  $\gamma$ -benzyl-L-glutamate (L-BG) with  $\beta$ -benzyl-L-aspartate (L-BA) and with  $\beta$ -benzyl-D-aspartate (D-BA) have been shown to exist in helical conformations in the solid state and chloroform solution by X-ray diffraction and infrared spectroscopy.<sup>20,21</sup> The optical rotations of these copolymers are shown in Fig. 2 ( $\text{CHCl}_3$  solution) and in Fig. 3 (DCA solution). In chloro-

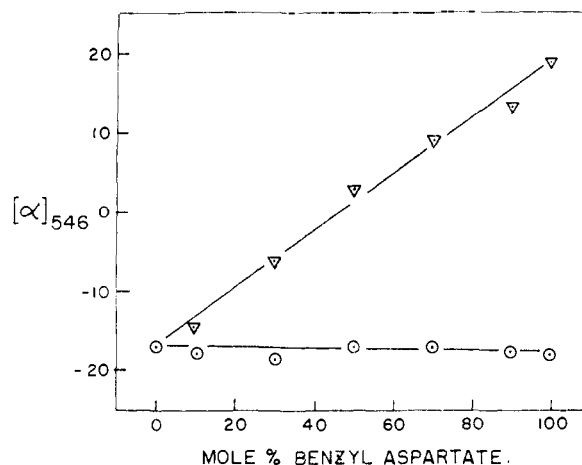


Fig. 3.—The specific rotation,  $[\alpha]_{546}$ , of copolymers of  $\beta$ -benzyl-L-aspartate: $\gamma$ -benzyl-L-glutamate,  $\circ-\circ-\circ$ , and of copolymers of  $\beta$ -benzyl-D-aspartate: $\gamma$ -benzyl-L-glutamate,  $\triangle-\triangle-\triangle$ , in dichloroacetic acid solutions.

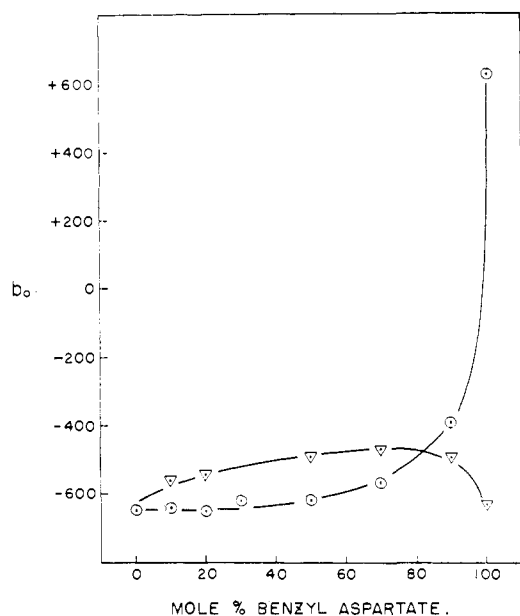


Fig. 4.—The  $b_0$  values for the wave length range 365 to 578  $m\mu$  (from eq. 1 assuming  $\lambda_0 = 212 m\mu$ ) of copolymers of  $\beta$ -benzyl-L-aspartate: $\gamma$ -benzyl-L-glutamate,  $\circ$ — $\circ$ — $\circ$ , and copolymers of  $\beta$ -benzyl-D-aspartate: $\gamma$ -benzyl-L-glutamate,  $\Delta$ — $\Delta$ — $\Delta$ , in chloroform solutions.

form (Fig. 2) the optical rotation, in the series L-BA:L-BG, is non-linear and markedly dependent, particularly above 70% L-BA, upon the mole % benzyl aspartate in the copolymer. On the other hand, in the series D-BA:L-BG, the optical rotation varies in a regular manner with the mole % benzyl aspartate. In dichloroacetic acid solution both the D and L series of benzyl aspartate: benzyl-L-glutamate copolymers show a linear variation of  $[\alpha]_{546}$  with mole % benzyl aspartate (Fig. 3).

When the optical rotatory dispersion of the copolymers in chloroform solution is fitted to eq. 1, the coefficient of the second term,  $b_0$ , is found to vary in the manner shown by the optical rotation, *i.e.*, marked dependence upon mole % benzyl aspartate in the L-BA:L-BG series above 70 mole % and a relatively linear dependence in the D-BA:L-BG series (Fig. 4). Similarly,  $b_0$ s determined in dichloroacetic acid solution parallel the behavior of the optical rotation in this solvent; the dependence of  $b_0$  upon mole % benzyl aspartate was approximately linear for both series (Fig. 5).

The substitution of D-isomers into a helix composed of L-residues has been used to estimate the contribution of a right-handed helix to the optical rotation of polypeptides.<sup>22, 23, 24</sup> As a result of these investigations it was concluded that a single sense of helix was maintained from  $L/(D + L) = 1.0$  (where  $L$  and  $D$  refer to the fraction of L and D isomer respectively) to  $L/(D + L) \cong 0.9-0.6$  depending on the particular poly- $\alpha$ -amino acid being studied. The fact that the optical rotation, a criterion of helix of a single sense was not linear over the complete range of  $L/(D + L)$  (from 1.0  $\rightarrow$  0.5) was ascribed to (a) the increasing thermodynamic stability of a mixture of right and left-handed helices as the fraction of D-isomer increased or (b) the greater

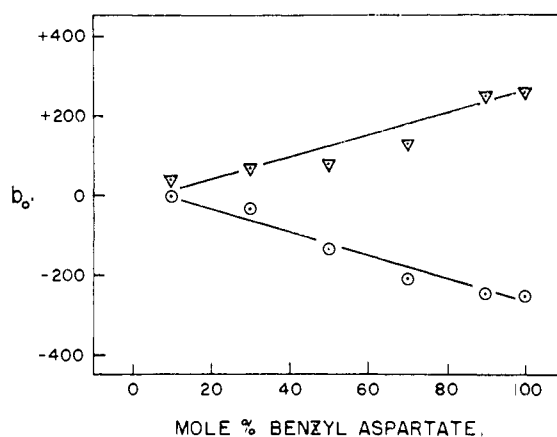


Fig. 5.—The  $b_0$  values for the wave length range 365 to 578  $m\mu$  (from eq. 1 assuming  $\lambda_0 = 212 m\mu$ ) of copolymers of  $\beta$ -benzyl-L-aspartate: $\gamma$ -benzyl-L-glutamate,  $\circ$ — $\circ$ — $\circ$ , and copolymers of  $\beta$ -benzyl-D-aspartate: $\gamma$ -benzyl-L-glutamate,  $\Delta$ — $\Delta$ — $\Delta$ , in dichloroacetic acid solutions.

conformational stability of the random chain for the mesopolyptide.<sup>24</sup> For PBG in  $\text{CHCl}_3$ :DCA the stability of the helix relative to the random chain was shown to be inversely proportional to the fraction of D-isomer down to  $L/(D + L) = 0.5$ .<sup>22</sup> While all these studies were performed with D,L-isomers of the same amino acid, the qualitative features would be expected to remain in a copolymer of two different amino acids, when the respective pure polypeptides are helical.

Thus it might be argued that if L-PBA and L-PBG are helices of the opposite sense of twist, the optical rotation and  $b_0$  of their copolymers should change markedly over a relatively narrow range of copolymer composition, representing a region of transition from one helix sense to the other. Furthermore, since it has been demonstrated that the L-benzyl aspartate helix is less stable than that of L-benzyl glutamate the transition should occur at less than 50% L-BG. On the other hand the optical rotation and  $b_0$  of D-BA:L-BG copolymer in the helical conformation should show a regular, almost linear, dependence on composition which would be evidence that no change of helix sense occurs in this series. This is indeed the case as seen in Fig. 2 and 4, and therefore we conclude that there is a change of helical sense in the series L-BA:L-BG while no change in sense of twist occurs in the D-BA:L-BG series.

It is noted that there is a departure from linearity in the optical properties in chloroform solution of the D-BA:L-BG series which may be correlated with the varying viscosities of the copolymers shown in Table II. In addition, the average environment of a D-BA or L-BG residue in a copolymer would differ from the environment in the pure polypeptide, influencing the mean residue rotation in a manner analogous to the solvent effects often found with optically active compounds.

In DCA solution, Fig. 3 and 5, both copolymer series exhibit the relatively linear dependence of optical rotation and  $b_0$  anticipated for random chain conformations. One further point should be noted about the  $b_0$  values found in strong hydrogen

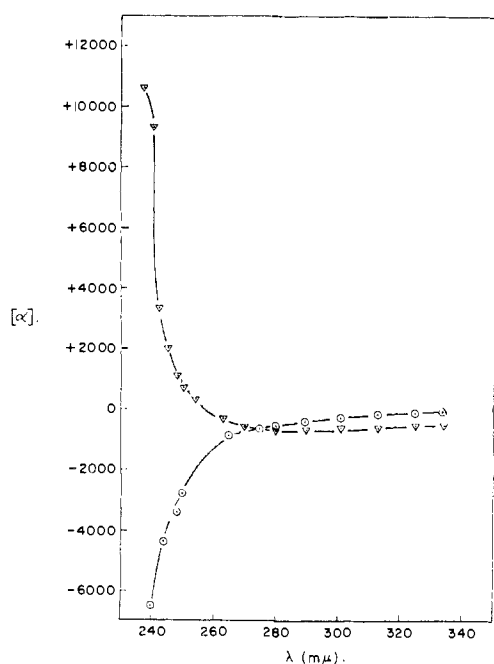


Fig. 6.—The ultraviolet rotatory dispersions of poly- $\beta$ -benzyl-L-aspartate,  $\Delta$ — $\Delta$ — $\Delta$ , and poly- $\gamma$ -benzyl-L-glutamate in chloroform solutions. The specific rotations,  $[\alpha]_{\lambda}$  are plotted as a function of wave length in  $m\mu$ .

bonding solvents. In DCA solution  $b_0 \cong -250$  for L-PBA,<sup>19</sup> in trifluoroacetic acid solution it differs only slightly ( $b_0 = -270$ ,  $[\alpha]_{546} \cong -5^\circ$ ). Since it is known that solvents of this type break down more stable polypeptide helices, *e.g.*, L-PBG, there is no doubt that the L-PBA helix is also transformed to a random chain in these solvents. Therefore the  $b_0$  values observed with PBA in these strong hydrogen bonding solvents must be characteristic of the residue and thus the  $b_0$  observed for L-PBA in weakly hydrogen bonding solvents should reflect both helical and residue contributions.

**Ultraviolet Rotatory Dispersions.**—The optical rotatory dispersions of L-PBG and L-PBA from *ca.* 240 to *ca.* 340  $m\mu$  are shown in Fig. 6. The dispersion of L-PBA is clearly anomalous.<sup>25</sup> In contrast to L-PBA, the dispersion of L-PBG is not anomalous in the region 240 to 360  $m\mu$  but is complex.<sup>25</sup>

Since it is known that the optical rotatory dispersion of L-PBG is anomalous in the visible and fits eq. 1, it was of interest to determine whether this relation held in the ultraviolet and in particular whether the anomalous dispersion found for L-PBA could be described by the Moffitt equation. It was found that the data for L-PBG could be fit to eq. 1 from 240 to 600  $m\mu$ ,  $b_0 = -660$ , Fig. 7. However the optical rotatory dispersion of L-PBA only fit eq. 1 down to 265  $m\mu$  ( $b_0 = +740$ ) and at shorter wavelengths deviated markedly, particularly below 250  $m\mu$ . (Fig. 7).

Returning now to a consideration of the data in Fig. 6, it should be emphasized that both L-PBA and L-PBG show anomalous rotatory dispersion; for L-PBA in the ultraviolet, for L-PBG in the visible region. The observed anomaly for L-PBG has been correlated with its existence as a pre-

sumably right-handed helix.<sup>26,27</sup> Since the anomaly observed with L-PBA is opposite in sign, it lends support to the argument that the sense of helix in L-PBA is the opposite of that in L-PBG. The displacement of the anomaly to the ultraviolet might be accounted for by the expected levorotation of a left-handed helix above *ca.* 285  $m\mu$ .<sup>28</sup> Qualitatively the change to positive rotation would then be a result of the dextrorotation of the left-handed  $\alpha$ -helix below *ca.* 285  $m\mu$ .

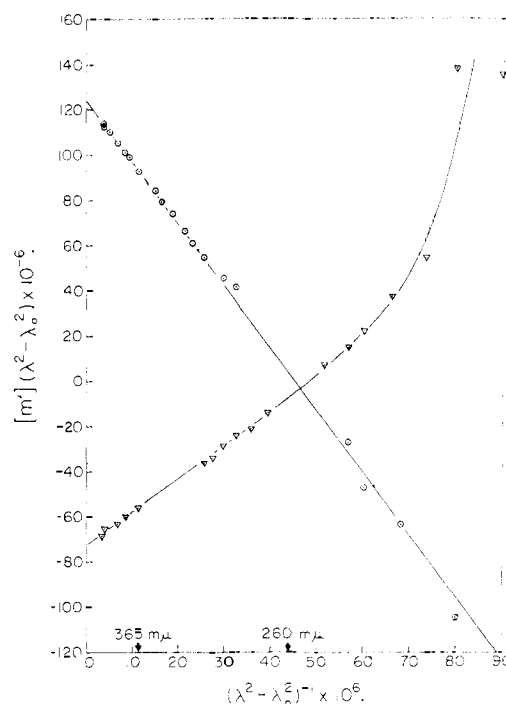


Fig. 7.—Moffitt plot (from eq. 1 using  $\lambda_0 = 212 m\mu$ ) of the optical rotatory dispersion of poly- $\beta$ -benzyl-L-aspartate,  $\Delta$ — $\Delta$ — $\Delta$ , and poly- $\gamma$ -benzyl-L-glutamate,  $\circ$ — $\circ$ — $\circ$ , in chloroform solutions over the wave length range 237 to 578  $m\mu$ .

However the dextrorotations measured for L-PBA at wave lengths below 245  $m\mu$  are in excess of those anticipated for a left-handed helix.<sup>29</sup> This excess dextrorotation may be a consequence of the onset of a positive Cotton effect arising from an interaction between the asymmetric  $\alpha$ -C and the

$\text{—C—O—}$  chromophore which absorbs around 220–230  $m\mu$ . The existence of a Cotton effect would imply

that the free rotation of the  $\text{—C—O—R}$  group is re-

(26) D. D. Fitts and J. G. Kirkwood, *Proc. Natl. Acad. Sci. U. S. A.*, **43**, 1046 (1957).

(27) P. Doty and J. T. Yang, *THIS JOURNAL*, **78**, 498 (1956).

(28) Using the values  $a_0 = +680$ ,  $b_0 = -500$ ,  $\lambda_0 = 212 m\mu$  found by Doty and Lundberg<sup>29</sup> for a presumably right-handed  $\alpha$ -helical optical rotatory dispersion, it is found that the contribution of a right-handed  $\alpha$ -helix is positive above *ca.* 285  $m\mu$  and becomes highly negative below. A left-handed  $\alpha$ -helix would be expected to mirror this behavior.

(29) From the constants given above (ref. 28) the optical rotation of a right-handed  $\alpha$ -helix at 240  $m\mu$  is estimated to be  $[m']_{\text{helix}, 240} \cong -3700^\circ$ . Then the optical rotation of a left-handed  $\alpha$ -helix would be *ca.*  $+3700^\circ$ , much less than the observed rotation for L-PBA at this wave length ( $[m']_{\text{L-PBA}, 240} \cong +17,000^\circ$ ).

stricted.<sup>30</sup> On the other hand a positive Cotton effect around 225  $m\mu$  is inconsistent with the excess levorotation observed in the visible for helical L-PBA unless it is assumed that there is a net large levorotatory contribution from absorption bands lying at shorter wave lengths. However, it should be noted at this point that *all* the optical effects observed for L-PBA cannot be explained simply by the presence of oriented chromophores in the side chain but almost necessarily involve a change of helical sense compared to L-PBG.

Finally, measurements have been made in the region 240 to 360  $m\mu$  on 9:1 copolymers of L-BA:L-BG and D-BA:L-BG. The results are shown in Fig. 8 plotted on a log scale. The anomaly observed and noted above for L-PBA is completely lost upon the incorporation of 10 mole % L-BG (Fig. 8). In contrast to this observation the incorporation of 10 mole % L-BG in D-PBA does not result in a loss of the anomaly observed with D-PBA. Thus the rotatory dispersions shown in Fig. 8 indicate that a structural change is involved when 10 mole % L-BG is incorporated in L-PBA, whereas no such change is found upon incorporation of L-BG in D-PBA. These data offer additional support for the conclusions reached above concerning the opposite sense of helix in L-PBA and L-PBG on the basis of optical rotatory dispersion measurements at wavelengths longer than 360  $m\mu$ .

### Conclusions

From the interpretation of the rotatory dispersions of the fifteen polymers and copolymers reported here it is concluded that the sense of helix in poly- $\beta$ -benzyl-L-aspartate is opposite to that observed in poly- $\gamma$ -benzyl-L-glutamate. Consideration has been given to possible effects of rotatory

contributions due to the  $\text{-}\overset{\text{O}}{\parallel}\text{C-O-}$  group and to the benzyl chromophore. It is suggested that the optical rotations observed with poly- $\beta$ -benzyl-L-

(30) We have considered the possibility that the benzyl groups in the side chains may be oriented. However since the absorption of this chromophore did not allow measurement around its absorption maximum ( $\lambda_{\text{max}} = 258 m\mu$ ) and since it would be expected that this grouping would make only a weak contribution, if any, to the optical rotatory dispersion (W. Kauzmann, "Quantum Chemistry," Academic Press, Inc., New York, 1957, p. 718) we can only conclude that the effect of this chromophore is small.

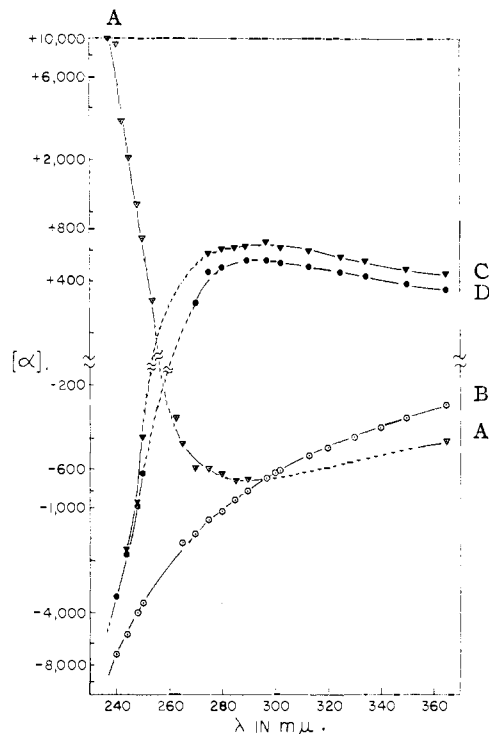


Fig. 8.—Ultraviolet rotatory dispersions in chloroform solutions: A,  $\Delta$ — $\Delta$ — $\Delta$ , poly- $\beta$ -benzyl-L-aspartate; B,  $\circ$ — $\circ$ — $\circ$ , 9:1 copolymer of  $\beta$ -benzyl-L-aspartate: $\gamma$ -benzyl-L-glutamate; C,  $\blacktriangle$ — $\blacktriangle$ — $\blacktriangle$ , poly- $\beta$ -benzyl-D-aspartate; D,  $\bullet$ — $\bullet$ — $\bullet$ , 9:1 copolymer of  $\beta$ -benzyl-D-aspartate: $\gamma$ -benzyl-L-glutamate.

aspartate are due in part to the  $\text{-}\overset{\text{O}}{\parallel}\text{C-O-}$  group in the  $\beta$  position. Optical rotatory dispersion measurements in the ultraviolet (240 to 360  $m\mu$ ) reveal effects not seen in previous rotatory work on polypeptides carried out in the more easily accessible region between 360 and 600  $m\mu$ .

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