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Studies of Poly-β-benzyl-L-aspartate Helix. I. The Synthesis and Rotatory Dispersion of Copolymers of β-*p*-Methyl, Chloro, Cyano, or Nitrobenzyl-L-aspartate with β-Benzyl-L-aspartate

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β-*p*-Methyl, chloro, cyano and nitrobenzyl-L-aspartates were synthesized, and the respective homopolymers and copolymers of these β-(*p*-substituted benzyl)-L-aspartates with β-benzyl-L-aspartate were prepared. The introduction of a methyl, chloro, or cyano group into the para position of the aromatic ring in the side chain of poly-β-benzyl-L-aspartate (PBLA) also causes a reversal from the left- to the right-handed in the helical sense of the polypeptide in a chloroform solution, as in the case of the nitro group. The residue of the copolymers where the transition takes place contained: nitro, 20—30; cyano, 40—50; chloro, ca. 50; and methyl group, ca. 70 mol.-%. These values may indicate the relative stabilities between the PBLA helix and poly-β-(*p*-substituted benzyl)-L-aspartate helices. These series of copolymers had no absorption band in the longer wavelength region except for a nitrobenzyl series, which had an absorption band at around 330 mμ. The absence of an absorption band resulted in a normal b_0 value. The results suggest that the factors which determine the helical sense of the PBLA helix are not very simple and that the left-handed helix of PBLA is much less stable.

It has been shown that poly-β-benzyl-L-aspartate (PBLA) forms the left-handed α-helix in a chloroform solution.[1,2] Recently Goodman and his co-workers have reported that the introduction of a nitro group in the para position of the aromatic ring in the side chain of poly-β-benzyl-L-aspartate causes a reversal from the left- to the right-handed in the helical sense of the polypeptide in a chloroform solution.[3,4] It has also been suggested that the right-handed helix is stabilized by the nitro-aromatic groups in the side chain, which form an electronically-coupled side chain helix rigidly arrayed about the main chain helix.

These findings stimulated our interest in studying the helical sense of poly-β-benzyl-L-aspartate homologues which have other side chains. In this paper, we shall describe the synthesis of poly-β-(*p*-methylbenzyl)-L-aspartate (PMeBLA), poly-β-(*p*-chlorobenzyl)-L-aspartate (PClBLA), poly-β-(*p*-cyanobenzyl)-L-aspartate (PCNBLA), and also poly-β-(*p*-nitrobenzyl)-L-aspartate (PNBLA), together with the copolymers derived from these β-(*p*-substituted benzyl)-L-aspartates and β-benzyl-L-aspartate. It will be described as well how optical rotatory dispersion data show that the introduction of a methyl, chloro, or cyano group in the aromatic side chain also causes a reversal in helical sense of the polypeptide in a chloroform solution, as in the case of a nitro group.

Results and Discussion

Syntheses.—β-Benzyl-L-aspartate (BLA) has been prepared by the partial hydrolysis of α, β-dibenzyl-*N*-carbobenzoxy-L-aspartate[5] and by selective β-esterification using concentrated hydrochloric acid[6] or aqueous sulfuric acid[7]. Goodman et al.[4] have prepared β-*p*-nitrobenzyl-L-aspartate by the nitration of β-benzyl-L-aspartate with nitronium fluoroborate.

In the present study, β-(*p*-substituted benzyl)-L-aspartates were prepared by the direct β-esterification of L-aspartic acid with *p*-substituted benzyl alcohol. β-*p*-Methylbenzyl-L-aspartate (MeBLA) was obtained in only a trace amount by using concentrated hydrochloric acid, but it was prepared in a 10% yield by using *p*-toluenesulfonic acid as the catalyst. β-*p*-Chlorobenzyl-L-aspartate (ClBLA) and β-*p*-cyanobenzyl-L-aspartate (CNBLA) were obtained in better yields by using *p*-toluenesulfonic acid than by using concentrated hydrochloric acid as the catalyst. The esterification of L-aspartic

1) R. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, *J. Am. Chem. Soc.*, **82**, 2268 (1960).
2) E. M. Bradbury, A. R. Downie, A. Elliott and W. E. Hanby, *Proc. Roy. Soc. (London)*, **A259**, 110 (1960).
3) M. Goodman, C. M. Deber and A. M. Felix, *J. Am. Chem. Soc.*, **84**, 3773 (1962).
4) M. Goodman, A. M. Felix, C. M. Deber, A. R. Brause and G. Schwartz, *Biopolymers*, **1**, 371 (1963).
5) A. Berger and E. Katchalski, *J. Am. Chem. Soc.*, **73**, 4084 (1951).
6) N. Izumiya, Z. Uchio and T. Yamashita, *J. Chem. Soc. Japan, Pure Chem. Sect. (Nippon Kagaku Zassi)*, **79**, 420 (1958).
7) T. Hayakawa, J. Noguchi, H. Nishi, S. Ikeda, T. Yamashita and T. Isemura, ibid., **82**, 601 (1961).

acid with *p*-nitrobenzyl alcohol, using *p*-toluenesulfonic acid as the catalyst, afforded a mixture of β-*p*-nitrobenzyl and α-*p*-nitrobenzyl-L-aspartates, with the former predominant. However, hydrochloric acid also gave a mixture, with the former predominant. It was more favorable to obtain the β-isomer of *p*-nitrobenzyl-L-aspartate by the reaction using concentrated hydrochloric acid than to use *p*-toluenesulfonic acid, since the repeated recrystallizations needed to separate the β-isomer lowered the yield.

In order to confirm that these synthesized esters were β-esters and L-isomers, these esters were treated with aqueous ammonia to convert them to asparagine, which was identified as L-asparagine monohydrate. The *N*-carboxyanhydrides (NCA) of these esters were prepared by a method similar to that of Karlson et al.[1] (method B). The polymerizations of NCA's were carried out in glass ampules, in which the air was replaced with dry nitrogen, using triethylamine[8] as an initiator ($A/I=100$).

Optical Rotatory Dispersion in a Chloroform Solution.—*1) The Poly-β-(p-methylbenzyl)-L-aspartate and Copolymers of β-p-Methylbenzyl-L-aspartate with β-Benzyl-L-aspartate.*—The values of b_0 derived from the Moffitt equation[9] have been empirically correlated with the sense of helix; the b_0 values of −630 obtained for poly-γ-benzyl-L-glutamate have been associated with the right-handed helix, and that of +600 for poly-β-benzyl-L-aspartate have been correlated with the left-handed one.[10]

The optical rotatory measurement of PMeBLA in a chloroform solution gave b_0 values ($\lambda_0=212$ mμ) of −680, which seem to indicate that PMeBLA exists in a right-handed helix. However, Fasman[11] pointed out that the sign is not always an indication of the helical sense. If PMeBLA exists in a right-handed helix, the copolymer of β-*p*-methylbenzyl-L-aspartate with β-benzyl-L-aspartate, which have been demonstrated to exist in a left-handed helix in a chloroform solution,[1,2] must exhibit a drastic change in b_0 values when the copolymer composition varies. Truly, b_0 values displayed a sharp change when they were plotted against copolymer composition (Fig. 1a). The values of λ_c and $[\alpha]_{546}$ also showed drastic changes (Table I). PMeBLA showed values of 256 for λ_c which are empirically correlated with a right-handed helix.[12] These typical drastic and abrupt changes in b_0, λ_c, and $[\alpha]_{546}$ indicate that a transition from the left-handed helix of PBLA to the right-handed helix of PMeBLA occurs at ca. a 70 mol.-% methyl residue content.

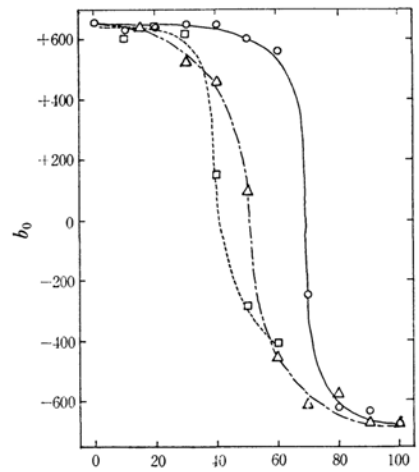

Fig. 1. Values of b_0 against copolymer composition; a) ○—○, copolymers of BLA and MeBLA, b) △-·-△, copolymers of BLA and ClBLA, c) □---□, copolymers of BLA and CNBLA.

TABLE I. COPOLYMERS OF β-*p*-METHYLBENZYL-L-ASPARTATE WITH β-BENZYL-L-ASPARTATE

Polymer No.	Mol-% of methyl-benzyl residues*1	b_0*2	λ_c*2	$[\alpha]_{546}$*2	η_{sp}/C*3
C0014	0	+659	152	−178	0.38
C3011	9.9	+631	154	−178	0.34
C3021	19.8	+645	139	−175	0.41
C3031	29.9	+651	146	−172	0.29
C3041	39.9	+653	142	−166	0.30
C3051	49.9	+602	148	−159	0.36
C3061	59.9	+564	148	−158	0.27
C3071	70.0	−248	230	−128	0.17
C3081	80.0	−623	254	−114	0.25
C3091	90.1	−631	257	−110	0.20
C3101	100.0	−680	256	−120	0.31

*1 Mol.-% of residues in polymerization mixture of NCA's.
*2 At 30.0±0.5°C.
*3 $C=0.5\%$, dichloroacetic acid.

2) The Poly-β-(p-chlorobenzyl)-L-aspartate and Copolymers of β-p-Chlorobenzyl-L-aspartate with β-Benzyl-L-aspartate.—Drastic changes in b_0, λ_c, and $[\alpha]_{546}$ similar to those shown above were observed for this series of copolymers (Fig. 1b, Table II). These results reveal that a reversal in the helical sense from the left-handed helix of PBLA to the right-handed helix of PClBLA takes place at a nearly equimolar composition of these residues.

3) The Copolymers of β-Cyanobenzyl-L-aspartate with β-Benzyl-L-aspartate.—Data are shown in Fig. 1c and Table III. Unfortunately, copolymers with cyano-residue contents greater than 60 mol.-%

8) E. R. Blout and R. H. Karlson, *J. Am. Chem. Soc.*, **78**, 941 (1956).
9) W. Moffitt, *J. Chem. Phys.*, **25**, 467 (1956).
10) E. R. Blout and R. H. Karlson, *J. Am. Chem. Soc.*, **80**, 1259 (1958).
11) G. D. Fasman, *Nature*, **193**, 681 (1962).
12) J. A. Schellman and C. G. Schellman, *J. Polymer Sci.*, **49**, 129 (1961).

Table II. Copolymers of β-*p*-chlorobenzyl-L-aspartate with β-benzyl-L-aspartate

Polymer No.	Mol.% of chlorobenzyl residues*1	b_0*2	λ_c*2	$[\alpha]_{546}$*2	η_{sp}/C*3
C1021	15.1	+644	149	−168	0.40
C1031	30.0	+522	156	−146	0.33
C1041	40.0	+458	164	−139	0.41
C1051	49.9	− 93	218	−115	0.39
C1061	60.0	−454	254	−105	0.32
C1071	70.1	−612	263	−99	0.26
C1081	79.9	−578	265	−92	0.30
C1091	90.0	−677	267	−97	0.23
C1101	100.0	−686	269	−94	0.21

*1 Mol.% of residues in polymerization mixture of NCA's.
*2 At 27.5±0.5°C.
*3 C=0.5%, dichloroacetic acid.

Table III. Copolymers of β-*p*-cyanobenzyl-L-aspartate with β-benzyl-L-aspartate

Polymer No.	Mol.% of cyanobenzyl residues*1	b_0*2	λ_c*2	$[\alpha]_{546}$*2	η_{sp}/C*3
C4011	10.0	+613	138	−165	0.73
C4021	20.0	+615	142	−174	0.69
C4031	30.0	+626	147	−167	0.69
C4041	39.9	+156	193	−135	0.64
C4051	50.0	−280	234	−98.6	0.54
C4061	60.0	−401	259	−83.5	0.30

*1 Mol.% of residues in polymerization mixture of NCA's.
*2 At 30.0±0.5°C.
*3 C=0.5%, dichloroacetic acid.

were insoluble in chloroform. In this series a transition from the left-handed to the right-handed helix occurs at a 40—50 mol.-% cyano-residue content.

4) The Copolymers of β-Nitrobenzyl-L-aspartate with β-Benzyl-L-aspartate.—Goodman et al.[4] have reported that a transition from the left-handed helical sense of PBLA to the right-handed helical sense of PNBLA occurs at a 26—32 mol.-% nitro-residue content, and that copolymers with more than a 32% nitro-residue content have large negative b_0 values. In our series a transition takes place at a 20—30 mol.-% nitro residue, Moffitt's plots for copolymers containing 30 and 40 mol.-% nitro residues are non-linear, and those with nitro-residue contents greater than 40 mol.-% are insoluble in chloroform (Table IV).

Table IV. Copolymers of β-*p*-nitrobenzyl-L-aspartate with β-benzyl-L-aspartate

Polymer No.	Mol.% of nitrobenzyl residues*1	b_0*2	$[\alpha]_{546}$*2	η_{sp}/C*3
C2011	10.2	+613	−168	0.31
C2021	20.1	−7.6	−155	0.24
C2031	30.2	N. L.	−157	0.31
C2041	40.0	N. L.	−157	0.24
C2101	100.0	—	—	0.13

*1 Mol.% of residues in polymerization mixture of NCA's.
*2 At 27.5±0.5°C, N. L.; Moffitt's plot was non linear, —; insoluble in chloroform.
*3 C=0.5%, dichloroacetic acid.

In the present study, as has been described above, the optical rotatory dispersion studies with series of copolymers of β-benzyl-L-aspartate and *p*-substituted esters are carried out. The introduction not only of a nitro group but also of a methyl, chloro, or cyano group in the para position of the aromatic ring in the side chain of poly-β-benzyl-L-aspartate causes a reversal from the left- to the right-handed in the helical sense of the polypeptide in chloroform.

Goodman and his co-workers[4] have proposed an explanation based on competitive hydrogen bonding between the carbonyls of the side-chain esters and the main-chain amides. They have suggested that if PBLA were in a right-handed helical conformation, normal intramolecular hydrogen bonding could be disrupted through the interaction of main-chain NH groups with suitably-placed side-chain ester groups. The stabilization of the right-handed conformation in the poly-β-*p*-nitrobenzyl-L-aspartate helix has been explained by assuming that nitroaromatic groups form an electronically-coupled side-chain helix; they have also suggested that when the nitro group is not present, certain π–π^* transitions are absent, or that there is insufficient π-electron cloud density to allow side chains to overlap.

In our studies, PMeBLA, PClBLA, and PCNBLA have neither an asymmetric absorption band in the long wavelength region nor the enhanced b_0 values which have been reported on PNBLA.[4] Ultraviolet absorption spectra[13] show that these three have small molar extinction coefficients compared with that of PNBLA. Especially, PMeBLA and PClBLA have molar extinction coefficients associated with the aromatic groups which do not differ much from that of PBLA. Nevertheless, these polymers are in right-handed helices. It is remarkable that the helical sense is reversed even by a methyl group which is weakly electron-releasing in contrast with the strongly electron-withdrawing nitro group. Therefore, it is unlikely that stabilization forces of an electronically-coupled aromatic group in the side chain are the primary forces which bring about a reversal

13) Molar extinction coefficients are as follows: PBLA, $\lambda_{max}^{CHCl_3}$ 259 mμ (ε, 210); PMeBLA, $\lambda_{max}^{CHCl_3}$ 265 mμ (ε, 281); PClBLA, $\lambda_{max}^{CHCl_3}$ 268 mμ (ε, 254); PCNBLA, λ_{max}^{DMF} 274 mμ (ε, 825); PNBLA, λ_{max}^{DMF} 275 mμ (ε, 7770).

into a right-handed helix, just as Goodman et al. have proposed for PNBLA. The helix reversals found in the present study also can not be interpreted by the dipole properties of the *p*-substituted benzene rings, since the effects of the introduction of a methyl group on the dipole properties of the aromatic ring should be opposite to those of the other three groups. (The dipole moments of mono-substituted benzenes, Ph-X, are as follows[14]: CH_3, $-0.4(\times 10^{-18})$; Cl, 1.58; C≡N, 4.0; NO_2, 3.98, where the dipole moment of $\overrightarrow{Ph-X}$ is taken to be positive.)

However, in the series of nitro, cyano, chloro, and methyl groups, the transitions occur at 20—30, 40—50, ca. 50, and ca. 70 mol.-%. These values may indicate relative stabilities between PBLA helix and poly-β-(*p*-substituted benzyl)-L-aspartate helices. From these values, it is evident that the helix of PNBLA is the most stable and that that of PMeBLA is the most unstable among the homopolypeptides in the present study. However, the stabilities relative to the PBLA helix differ much less between PCNBLA and PClBLA, while the difference is significant between PMeBLA and PClBLA. These results can be interpreted neither by the electronic nor by the dipole properties of *p*-substituted benzene rings.

These results suggest that the factors which determine the helical sense of PBLA helix are not simple, and that the left-handed helix of PBLA is far less stable.

Experimental

Samples and Apparatus.—The optical rotatory dispersion was measured with a Rudolph photoelectric polarimeter, model 200-S, using a mercury lamp as the light source. Measurements were made at five wavelengths from 5780 to 3650 Å; the concentration was 0.5% w/v, and the path length was 2 dm. The chloroform used was refluxed over and distilled from phosphorus pentoxide.

The mole-per-cent residue content for each of the copolymers is given in a molar ratio in the polymerization mixture of NCA's.[15] The measurements of viscosity were made on solutions in dichloroacetic acid at a concentration of 0.5% w/v using an Ostwald viscometer (35°C) for a qualitative comparison of the relative molecular weights of the various preparations.

Preparation.[16]—*β-Benzyl-L-aspartate.*—The preparation was carried out according to the procedure of Hayakawa, Noguchi, et al.[7] A 32% yield of white powder was obtained; m. p. 221°C (decomp.) $[\alpha]_D^{24}$ +24.4 (*c* 1, 0.1 N HCl).

β-p-Methylbenzyl-L-aspartate. — A mixture of well-pulverized L-aspartic acid (10.0 g.), *p*-toluenesulfonic acid (14.4 g.), and *p*-methylbenzyl alcohol (9.2 g.), which had been prepared by the reduction of *p*-methylbenzaldehyde with sodium borohydride in a methanol solution, was heated at 80°C for 2 hr. with stirring; during the reaction water was removed three or four times under reduced pressure from a reaction vessel. After water (150 ml.) had been added to the reaction mixture and it had been washed with ether (150 ml.), the aqueous layer was neutralized by adding sodium bicarbonate; after the mixture had then stood in a cold place, the precipitate was filtered and recrystallized from water (300 ml.). After decoloring with Norit, β-*p*-methylbenzyl-L-aspartate was obtained (1.70 g. (9.5%)) as white leaflets; m. p. 225—226°C (decomp.) $[\alpha]_D^{25}$ +14.3 (*c* 1, glacial acetic acid).

Found: C, 60.46; H, 6.44; N, 6.02. Calcd. for $C_{12}H_{15}O_4N$: C, 60.75; H, 6.37; N, 5.90%.

β-p-Chlorobenzyl-L-aspartate.—A mixture of L-aspartic acid (1.2 g.), *p*-toluenesulfonic acid (1.5 g.), and *p*-chlorobenzyl alcohol (2.5 g.), which had been prepared by the hydrolysis of *p*-chlorobenzyl chloride in an aqueous sodium carbonate solution, was treated according to the same procedure as was used for β-*p*-methylbenzyl-L-aspartate; recrystallization from boiling water furnished 1.3 g. (58.5%) of the product as white leaflets, m. p. 208°C (decomp.), $[\alpha]_D^{23}$ +25.6 (c 1, glacial acetic acid).

Found: C, 51.35; H, 4.58; N, 5.32; Cl, 13.94. Calcd. for $C_{11}H_{12}O_4NCl$: C, 51.27; H, 4.69; N, 5.44; Cl, 13.76%.

A similar procedure but using concentrated hydrochloric acid instead of *p*-toluenesulfonic acid afforded the product in about a 15% yield.

β-p-Cyanobenzyl-L-aspartate. — The procedure was exactly the same as was used for β-*p*-methylbenzyl-L-aspartate (yield 10—15%); m. p. 187—188°C (decomp.), $[\alpha]_D^{25.5}$ +11.2 (*c* 1, glacial acetic acid).

Found: C, 57.88; H, 5.04; N, 11.60. Calcd. for $C_{12}H_{12}O_4N_2$: C, 58.06; H, 4.87; N. 11.29%.

p-Cyanobenzyl alcohol was prepared by the chlorination of *p*-methylbenzonitrile, followed by hydrolysis with aqueous potassium carbonate.[17]

β-p-Nitrobenzyl-L-aspartate. — a) A mixture of *p*-nitrobenzyl alcohol (15 g.) and L-aspartic acid (7 g.) was stirred for 0.5 hr., with the temperature kept at 110°C; concentrated hydrochloric acid (3 ml.) was added. After the mixture had been stirred for 2 hr., concentrated hydrochloric acid (1 ml.) was added; then the mixture was stirred for another hour. During the reaction water was removed from the reaction vessel two or three times under reduced pressure. The reaction mixture was then diluted with water, neutralized with sodium bicarbonate, and filtered. Treatment with Norit and recrystallization from water afforded pale yellow crystals whose infrared spectra have two

14) C. P. Smyth, "Dielectric Behavior and Structure," McGraw-Hill, New York (1955), p. 253.

15) For almost these copolymers prepared, the results of their elementary analyses agreed, within ±0.35%, with the values calculated from the molar ratios of the NCA's in the polymerizations. The chloro-residue contents of the copolymers of ClBLA with BLA were determined by the ultraviolet spectra (utilizing the aromatic absorption bands) and by the infrared spectra (utilizing the out-of-plane deformation bands of the aromatic rings). These results agree, within 2 mol.%, with the mixing molar ratios of the NCA's in the polymerizations. For all the copolymers, their molar ratios may, therefore, not differ much from those of the NCA's in the polymerization mixtures.

16) All melting points are uncorrected.

17) J. N. Ashley, H. J. Barber, A. J. Ewins, G. Newberg and A. D. H. Self, *J. Chem. Soc.*, **1942**, 103.

ester-carbonyl stretching absorptions (1740 cm^{-1} and 1720 cm^{-1}). Repeated recrystallizations from hot water gave leaflets (2.7 g.); m. p. 190.5—191°C (decomp.), $[\alpha]_D^{19.5}$ +16.7 (*c* 2, glacial acetic acid), which have a single ester-carbonyl stretching absorption at 1720 cm^{-1} in the infrared spectrum; this was proved to be the β-ester by the conversion to L-asparagine to be described below.

Found: C, 49.17; H, 4.70; N, 10.31. Calcd. for $C_{11}H_{12}O_6N_2$: C, 49.26; H, 4.51; N, 10.44%.

On the other hand, the concentration of the mother liquor gave crystals which have an ester-carbonyl stretching absorption at 1740 cm^{-1}, with a weak shoulder at 1720 cm^{-1}. Repeated recrystallizations afforded α-*p*-nitrobenzyl-L-aspartate (m. p. 180—181.5°C (decomp.), ester νC=O 1740 cm^{-1}). The specimen was converted to L-isoasparagine monohydrate, which was identified by a comparison of its infrared spectrum with that of an authentic sample.[18]

b) The same procedure utilizing *p*-toluenesulfonic acid as was used for β-*p*-methylbenzyl-L-aspartate afforded a mixture which contained more α-esters than was obtained by the procedure of (a).

Conversions of p-Substituted Benzyl-L-aspartates to L-Asparagine.—To establish that these *p*-substituted benzyl-L-aspartates are β-esters and that no racemization takes place during the preparation of the products, these esters were converted as follows. β-*p*-Methylbenzyl-L-aspartate (0.517 g.) was treated with 28% aqueous ammonia at 100°C for 25 hr. in the sealed glass ampule. The solution was then heated to remove the ammonia and washed with ether, the aqueous layer was concentrated, and the residue was recrystallized from aqueous alcohol to give crystals (0.264 g.); m.p. 230°C (decomp.), $[\alpha]_D^{24.5}$ +31.0 (*c* 1, 6 N HCl); reported for L-asparagine monohydrate[2] $[\alpha]_D^{20}$ +31.2.

Found: C, 32.07; H, 7.00; N, 18.35. Calcd. for $C_4H_8N_2O_3 \cdot H_2O$: C, 32.00; H, 6.70; N, 18.66%.

Exactly the same treatments of β-(*p*-chloro, *p*-cyano and *p*-nitro)-benzyl-L-aspartates (0.49, 0.53, and 0.53 g.) as for β-*p*-methylbenzyl-L-aspartate were carried out to afford 0.18, 0.23 and 0.26 g. of L-asparagine monohydrate; their specific rotations were as follows: $[\alpha]_D^{24.5}$ +29.6, +33.7 and +30.7 (*c* 1, 6 N HCl) respectively.

β-Benzyl-L-aspartate-N-carboxyanhydride.—The procedure of Karlson et al.[1] (method B) utilizing the reaction of phosgene with β-benzyl-L-aspartate suspended in anhydrous dioxane[19] at 40°C and the recrystallization of the anhydride from ethyl acetate-*n*-hexane furnished the product as needles.

β-p-Methylbenzyl-L-aspartate-N-carboxyanhydride.—The same procedure as that described above for β-*p*-methylbenzyl-L-aspartate (10.0 g.) afforded the product (7.0 g.) as leaflets, m. p. 129—131°C, $[\alpha]_D^{27}$ −30.6 (*c* 2.6, ethyl acetate).

Found: C, 59.22; H, 5.09; N, 5.45. Calcd. for $C_{13}H_{13}NO_5$: C, 59.31; H, 4.98; N, 5.32%.

β-p-Chlorobenzyl-L-aspartate-N-carboxyanhydride.—The same procedure as has been described above for β-*p*-chlorobenzyl-L-aspartate (15.0 g.) gave the product (5.8 g.) as leaflets, m. p. 162°C (decomp.), $[\alpha]_D^{24}$ −30.1 (*c* 1.5, ethyl acetate).

Found: C, 50.81; H, 3.65; N, 4.74; Cl, 21.86. Calcd. for $C_{12}H_{10}O_5NCl$: C, 50.81; H, 3.55; N, 4.94; Cl, 12.50%.

β-p-Cyanobenzyl-L-aspartate-N-carboxyanhydride.—The same procedure as has been described above for β-*p*-cyanobenzyl-L-aspartate (8.0 g.) and the recrystallization of the anhydride from dioxane-*n*-hexane furnished the product (6.5 g.) as leaflets, m. p. 153—154°C (decomp.), $[\alpha]_D^{27}$ −53.6 (*c* 2.4, dioxane).

Found: C, 57.06; H, 3.95; N, 10.12. Calcd. for $C_{13}H_{10}O_5N_5$: C, 56.94; H, 3.68; N, 10.22%.

β-p-Nitrobenzyl-L-aspartate-N-carboxyanhydride.—M. p. 174—176°C (decomp.), $[\alpha]_D^{25}$ −48.5 (*c* 1.5, dioxane). (Found: C, 48.91; H, 3.67; N, 9.22%.)

Poly-β-benzyl-L-aspartate and Poly-β-p-methyl chloro, cyano or nitrobenzyl-L-aspartate.—A typical polymerization was carreid out as follows: to 7 ml. of dioxane[17] containing 0.4 ml. of a solution of 100 mg. of triethylamine in 10 ml. of dioxane, 1.0 g. of β-benzyl-L-aspartate-*N*-carboxyanhydride was added; A/I=100. The clear solution in a glass ampule[20] (dried for 7 hr. at 140°C) was sealed under an atmosphere of dry

Table V

Polymer No.	MeBLA-NCA mg.	BLA-NCA mg.	Methylbenzyl content mol.%	Triethylamine* mg.	A/I**	Yield %
C3011	104	896	9.9	4.0	100	76
C3021	207	793	19.8	4.0	100	83
C3031	310	687	29.9	4.0	100	84
C3041	411	586	39.9	4.0	100	87
C3051	513	488	49.9	4.0	100	86
C3061	611	387	59.9	3.9	100	84
C3071	711	289	70.0	3.9	100	86
C3081	808	191	80.0	3.9	100	83
C3091	903	94	90.1	3.8	100	84
C3101	1000	0	100.0	3.8	100	85

* A solution of 0.10 g. in 10 ml. dioxane; the triethylamine was purified by refluxing over and distilling from sodium metal.

** Molar ratio of the total anhydride to initiator.

18) J. Kovacs, H. N. Kovacs and R. Ballina, *J. Am. Chem. Soc.*, **85**, 1839 (1963).

19) L. F. Fieser, "Experiments in Organic Chemistry," 2nd Ed., Heath, Boston, Mass. (1941), p. 361.

20) Sealed glass ampules were used for the polymerization to prevent the moisture.

TABLE VI

Polymer No.	ClBLA-NCA mg.	BLA-NCA mg.	Chlorobenzyl content mol.%	Triethylamine* mg.	A/I**	Yield %
C1015	152	750	15.1	3.6	100	73
C1031	295	606	30.0	3.5	100	60
C1041	389	513	40.0	3.5	100	73
C1051	479	423	49.9	3.4	100	67
C1061	568	332	60.0	3.4	100	77
C1071	652	244	70.1	3.3	100	78
C1081	737	163	79.9	3.3	100	75
C1091	821	80	90.0	3.2	100	76
C1101	1470	0	100.0	5.3	100	81

* A solution of 0.10 g. in 10 ml. dioxane; the triethylamine was purified by refluxing over and distilling from sodium metal.
** Molar ratio of the total anhydride to initiator.

TABLE VII

Polymer No.	CNBLA-NCA mg.	BLA-NCA mg.	Cyanobenzyl content mol.%	Triethylamine* mg.	A/I**	Yield %
C4011	109	889	10.0	4.0	100	81
C4021	216	785	20.0	4.0	100	84
C4031	321	680	30.0	3.9	100	82
C4041	423	578	39.9	3.9	100	82
C4051	524	477	50.0	3.9	100	84
C4061	623	377	60.0	3.8	100	86
C4075	720	200	76.6	3.8	100	26
C4081	816	185	80.0	3.8	100	27
C4091	909	93	89.9	3.7	100	19
C4101	1050	0	100.0	3.7	100	46

* A solution of 0.10 g. in 10 ml. dioxane; the triethylamine was purified by refluxing over and distilling from sodium metal.
** Molar ratio of the total anhydride to initiator.

TABLE VIII

Polymer No.	NBLA-NCA mg.	BLA-NCA mg.	Nitrobenzyl content mol.%	Triethylamine* mg.	A/I**	Yield %
C2011	119	884	10.2	4.0	100	74
C2021	230	772	20.1	3.9	100	52
C2031	340	666	30.2	3.8	100	76
C2041	440	560	40.0	3.8	100	71
C2101	1070	0	100.0	3.7	100	34

* A solution of 0.10 g. in 10 ml. dioxane; the triethylamine was purified by refluxing over and distilling from sodium metal.
** Molar ratio of the total anhydride to initiator.

nitrogen and allowed to stand for 3.5 days at 30°C. The solution was poured into 80 ml. of diethyl ether in order to precipitate the polymer, which was washed with ether and reprecipitated from a chloroform solution with ether and dried at 60°C in vacuo.

These β-*p*-methyl, chloro, cyano, and nitrobenzyl-L-aspartate-*N*-carboxyanhydrides were polymerized according to the same procedure as was used for β-benzyl-L-aspartate. The quantities used and the yields obtained for these polymers are summarized in Tables V—VIII. Poly-β-*p*-cyano and nitro benzyl-L-aspartates were reprecipitated from a dimethylformamide solution with ether.

PMeBLA; Found: C, 65.63; H, 6.11; N, 6.58. Calcd. for $C_{12}H_{13}O_3N$: C, 65.74; H, 5.98; N, 6.39%. PClBLA; Found: C, 55.08; H, 4.44; N, 5.77; X, 11.99. Calcd. for $C_{11}H_{10}C_3NCl$: C, 55.13; H, 4.21; N, 5.84; X, 14.79%. PCNBLA; Found: C, 62.73; H, 4.49; N, 11.99. Calcd. for $C_{12}H_{10}N_2O_3$: C, 62.20; H, 4.38; N, 12.17%. PNBLA; Found: C, 52.39; H, 4.26; N, 11.05. Calcd. for $C_{11}H_{10}O_5N_2$: C, 52.80; H, 4.03; N, 11.20%.

Copolymers of β-p-Methylbenzyl-L-aspartate with β-Benzyl-L-aspartate.—Tabel V summarizes the quantities used

and the yields obtained for these copolymers. A typical polymerization was as follows: 0.513 g. of β-*p*-methylbenzyl-L-aspartate-*N*-carboxyanhydride and 0.488 g. of β-benzyl-L-aspartate-*N*-carboxyanhydride (1 : 1 molar ratio) were dissolved in 7.0 ml. of dioxane. To a total of 14.3% w/v NCA's in a dioxane solution, 0.40 ml. of a solution of 100 mg. of triethylamine in 10 ml. of dioxane was added ($A/I=100$). The mixed solution in a glass ampule was sealed under an atmosphere of dry nitrogen and allowed to stand for 3.5 days at 30°C. The workup was the same as for poly-β-benzyl-L-aspartate described above.

Copolymers of β-p-Chlorobenzyl-L-aspartate with β-Benzyl-L-aspartate.—Table VI summarizes the yields obtained for these copolymers. Polymerizations were carried out in a manner similar to that employed for the copolymers of MeBLA with BLA. The NCA's were in a 5% w/v solution, and polymerizations were allowed to proceed for 7.5 days.

Copolymers of β-p-Cyanobenzyl-L-aspartate with β-Benzyl-L-aspartate.—Table VII summarizes the quantities used and the yields obtained for these copolymers. Polymerizations were carried out according to the procedure used for the copolymers of MeBLA with BLA. The NCA's were in a 10% solution for C4011—C4051 and in a 5% solution for C4061—C4091, and polymerizations were allowed to proceed for 5 days. The copolymers were dissolved in chloroform for C4011—C4061 and in dimethylformamide for C4075—C4091, and they were precipitated with ether.

Copolymers of β-p-Nitrobenzyl-L-aspartate with β-Benzyl-L-aspartate.—Table VIII summarizes the quantities used and the yields obtained for these polymers. Polymerizations were carried out according to the procedure used for the copolymers of MeBLA with BLA. The solutions of NCA's were 5% except C2101 (3%), and polymerizations were allowed to stand for 5 days.

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