Synthesis and conformational studies of polymers and copolymers of *O,O'*-dimethyl-L-β-3,4-dihydroxyphenyl-α-alanine with γ-benzyl-L-glutamate

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Eight different copolymers of *O,O'*-dimethyl-L-β-3,4-dihydroxyphenyl-α-alanine (*O,O'*-dimethyl-L-Dopa) and γ-benzyl-L-glutamate with degrees of polymerization of 320—80 have been synthesized by the *N*-carboxyanhydride method. From the results obtained by a study of the optical rotatory dispersion and circular dichroism for the copolymer series, the conformation of poly(*O,O'*-dimethyl-L-Dopa) is a right-handed helix in helicogenic solvents such as chloroform, dioxane or 2-chloroethanol. Copolymers containing less than 50 mol % glutamate show a circular dichroism peak at 285 nm suggesting stacking of aromatic groups in the side chain. Poly(*O,O'*-dimethyl-L-Dopa) is in the random coil structure in trifluoroacetic acid or hexafluoroacetone. The results were compared with those of poly(*O,O'*-dicarbobenzoxy-L-Dopa) and poly(L-Dopa).

INTRODUCTION

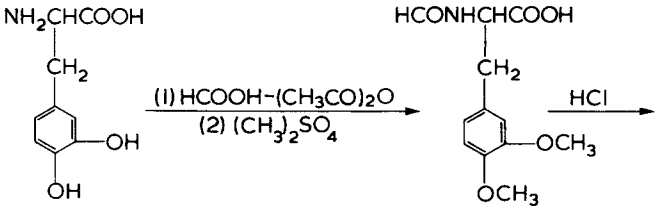
In the previous papers in this series[1-4] we have reported the synthesis and conformational studies of poly(L-β-3,4-dihydroxyphenyl-α-alanine) [poly(L-Dopa)] and poly(L-Dopa) derivative. The conformation of poly(*O,O'*-dicarbobenzoxy-L-Dopa) is anomalous and is solvent dependent. To study further the poly(L-Dopa) derivative, we widened the experiments on the polypeptide to include the aliphatic *O,O'*-dimethyl protecting group. In this paper, we report the synthesis and conformational studies of homopolymers and a series of random copolymers of *O,O'*-dimethyl-L-Dopa with γ-benzyl-L-glutamate. The conformation of the homo- and copolypeptides has been studied by circular dichroism (c.d.) and optical rotatory dispersion (o.r.d.) measurements.

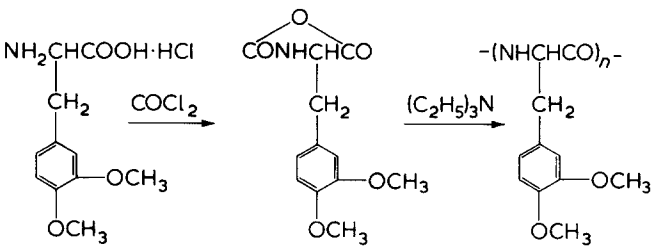
Since boron halides haye been used for the cleavage of methyl ethers[5], poly(*O,O'*-dimethyl-L-Dopa) is a good precursor for the preparation of high molecular weight poly(L-Dopa) and its copolymers[6].

EXPERIMENTAL

Polypeptides

L-Dopa was purchased from Tokyo Chemical Industry Co., Ltd. *O,O'*-Dimethyl-L-Dopa *N*-carboxyanhydride (NCA) was synthesized according to the following scheme:





N-formyl-O,O'-dimethyl-L-Dopa. *N*-formyl-*O,O'*-dimethyl-L-Dopa was prepared from L-Dopa (80 g), 90% formic acid—acetic anhydride and dimethyl sulphate under nitrogen as described by Schrecker and Hartwell[7], except that ethyl acetate was used to extract the product. The product was recrystallized twice from ethyl acetate; yield, 56.0 g (55%); m.p. 127°C; $[\alpha]_D^{24} = 70.2°$ (c = 2.0, ethanol). Calculated for $C_{12}H_{15}O_5N$; C, 56.91; H, 5.97; N, 5.53%. Found: C, 57.02; H, 5.78; N, 5.50%.

O,O'-dimethyl-L-Dopa hydrochloride. A solution of 3.3 g of *N*-formyl-*O,O'*-dimethyl-L-Dopa in 48 ml of 0.5 N hydrochloric acid was refluxed for 3 h[8] and evaporated to dryness under reduced pressure. The residue was treated with acetone. The precipitate, *O,O'*-dimethyl-L-Dopa hydrochloride, was filtered and recrystallized from ethanol and ether; yield, 2.9 g (85%); m.p., 220°C; $[\alpha]_D^{25} = -4.4°$ (c = 1.04, N hydrochloric acid). Calculated for $C_{11}H_{16}O_4NCl$: C, 50.48; H, 6.16; N, 5.35%. Found: C, 50.65; H, 5.94; N, 5.32%.

O,O'-dimethyl-L-Dopa NCA. The NCA compound was prepared from 3.0 g of *O,O'*-dimethyl-L-Dopa hydrochloride using phosgene for 1 h at 45°C in ten-fold dry dioxane and recrystallized from ethyl acetate and n-hexane; yield, 2.7 g (94%); m.p., 120°C; infra-red (i.r.) bands at 1785 and 1847 cm^{-1}. Calculated for $C_{12}H_{13}O_5N$: C, 57.37; H, 5.21; N, 5.58%. Found: C, 57.39; H, 5.32; N, 5.60%.

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Table 1 Molecular weight for copoly(*O,O'*-dimethyl-L-Dopa, γ-benzyl-L-glutamate)

L-Glutamate content (mol %)	*O,O'*-Dimethyl-L-Dopa NCA		γ-Benzyl-L-glutamate NCA		Yield (%)	$[\eta]_{DCA}^{25}$	Degree of polymerization	
	mg	mmol	mg	mmol				
0	3900	15.5	–	–	92	0.49	320[a]	
5	1969	9.5	132	0.5	90	0.27	130	
10	1865	9.0	263	1.0	88	0.25	115	
20	1658	8.0	526	2.0	87	0.20	80	
33	1388	6.7	723	3.3	87	0.20	80	
50	2288	11.0	2420	11.0	95	0.21	90	135[b]
75	518	2.5	1644	7.5	90	0.20		125
100	–	–	12900	49.0	96	0.64		470

[a] Calculated from $[\eta] = 3.2 \times 10^{-2} M_w^{0.66}$; [b] calculated from $[\eta] = 2.78 \times 10^{-5} M_w^{0.87}$

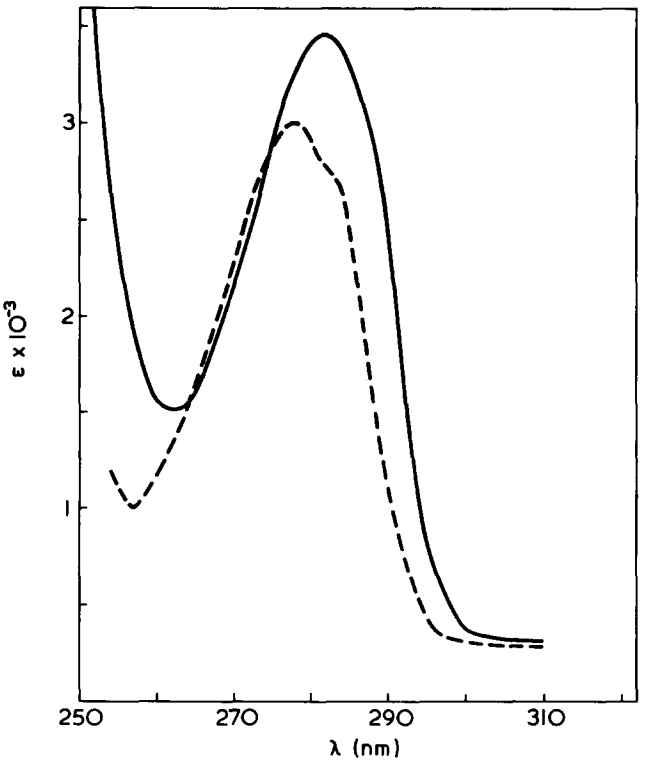

Figure 1 U.v. spectra of poly(*O,O'*-dimethyl-L-Dopa) at 25°C: ——, in chloroform–1% TFA; – – – –, in TFA

γ-Benzyl-L-glutamate NCA. The NCA compound was prepared from γ-benzyl-L-glutamate by the usual procedure[9].

Poly (O,O'-dimethyl-L-Dopa). High molecular weight poly(*O,O'*-dimethyl-L-Dopa) was prepared by polymerizing *O,O'*-dimethyl-L-Dopa, NCA (2.7 g) in ten-fold dioxane for 3 days at room temperature using triethyl amine as an initiator (A/I = 100). The polymer was precipitated with water, filtered and dried: yield, 2.1 g (94%). Calculated for $(C_{11}H_{13}O_3N)_n$: C, 63.76; H, 6.32; N, 6.76%. Found: C, 63.55; H, 6.10; N, 6.55%. The polypeptide showed i.r. absorptions at 3270, 3030, 2940, 2830, 1670, 1550 and 1030 cm^{-1}.

Poly(*O,O'*-dimethyl-L-Dopa) is soluble in chloroform, methylene dichloride, 2-chloroethanol, trimethyl phosphate (TMP), pyridine, dichloroacetic acid (DCA), trifluoroacetic acid (TFA), hexafluoroacetone sesquihydrate (HFA), 2,2,2-trifluoroethanol or 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), slightly soluble in 1,2-dichloroethane or 1,2-dichloropropane, and insoluble in dioxane, tetrahydrofuran and carbon tetrachloride.

Copolymerization

High molecular weight copolymers were prepared by copolymerizing the NCA compounds in ten-fold dioxane for 3 days at room temperature using triethylamine as an initiator (A/I = 100). The copolymers were precipitated with water, filtered and dried. The results are shown in *Table 1.*

Methods

Ultra-violet (u.v.), i.r., o.r.d. and c.d. spectra were measured on UVIDEC-1, IR DS-301, ORD/UV 5 and CD J-40A instruments, respectively, all made by the Japan Spectroscopic Co., Ltd. For rotation measurements, cells with path lengths of 0.1–10 mm were used under nitrogen flush. The concentrations of the samples were in the 0.4–1.0% (o.r.d.) and 0.03–0.3% (c.d.) range. The experimental data were expressed in terms of specific rotation $[\alpha]$, mean residue ellipticity $[\theta]$ (degree cm^2/dmol) for c.d. or molar extinction coefficient ϵ for u.v. The parameters a_0 and b_0, derived from the Moffitt–Yang equation were calculated from o.r.d. curves using λ_0 = 212 nm as in the case of chemically related aromatic polypeptides[2–4,10].

The intrinsic viscosities were measured in DCA at 25°C using an Ubbelohde viscometer and are listed in *Table 1.* The molecular weights were estimated from empirical equations $[\eta] = 3.2 \times 10^{-2} M_w^{0.66}$ for poly(*O*-carbobenzoxy-L-tyrosine)[11] and $[\eta] = 2.78 \times 10^{-5} M_w^{0.87}$ for poly(γ-benzyl-L-glutamate)[12] both measured in DCA at 25°C.

RESULTS

Conformation of poly(O,O'-dimethyl-L-Dopa)

U.v. spectra. Poly(*O,O'*-dimethyl-L-Dopa) does not dissolve completely in chloroform since a partial gelation takes place, however, it can be solubilized by the added 1% TFA. The u.v. absorption spectra of poly(*O,O'*-dimethyl-L-Dopa) in chloroform–1% TFA or TFA are shown in *Figure 1.* The peak at 282 nm in chloroform shifted to 278 nm and decreased the coefficient ϵ from 3480 to 3020.

C.d. spectra. The c.d. spectra of poly(*O,O'*-dimethyl-L-Dopa) are shown in *Figure 2.* In chloroform or 2-chloroethanol, the polypeptide showed a peak at 285 nm, a small peak at 240–242 nm, a trough at 231–233 nm and a strong peak at 205 nm (data not shown; $[\theta]_{205}$ = 90 000 in 2-chloroethanol, TMP or HFIP). In TFA, the polypeptide showed a trough at 285 nm and a small peak at 240 nm. The molar ellipticity values at 285 nm are listed in *Table 2.* In a helix-promoting solvent such as chloroform, methylene

dichloride, 1,2-dichloroethane, 2-chloroethanol or TMP, or HFIP, the $[\theta]_{285}$ values were 5100–7900 and in a coil-promoting solvent such as TFA or HFA, the values were −1600 to −500. The positive $[\theta]_{285}$ value suggests the helical conformation (see *Figure 3*).

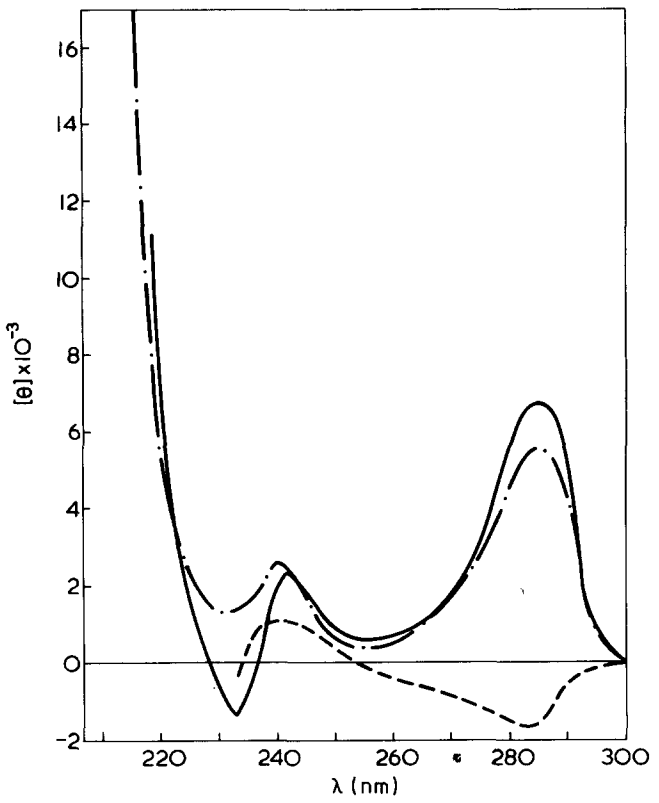

Figure 2 C.d. spectra of poly(*O,O'*-dimethyl-L-Dopa) at 25°C: ——, in chloroform; — · — · —, in 2-chloroethanol; — — — —, in TFA

O.r.d. spectra. When a few percent of TFA were added to the chloroform solution, the specific rotation increased from $[\alpha]_{546} = 260°$ to 285°. 5% TFA decreased the specific rotation and 6% TFA inverted the rotation from dextro to laevo. On the other hand, in dioxane–TFA mixed solvents the rotation of the polypeptide changed from dextro to laevo at about 70% TFA. The parameters, a_0 and b_0, were calculated from the o.r.d. curves. *Figure 3* shows the specific rotation, a_0 and b_0 and $[\theta]_{285}$ values of poly(*O,O'*-dimethyl-L-Dopa) in chloroform–TFA or dioxane–TFA mixed solvents. The b_0 behaviour in chloroform–TFA mixed solvents coincided with the results of the specific rotation. Thus, the sharp change in $[\alpha]_{546}$ and b_0 corresponds to the helix–coil transition and the transition of poly(*O,O'*-dimethyl-L-Dopa) occurred at 5% TFA in chloroform–TFA and at 70% TFA in dioxane–TFA mixed solvents. It is also

Table 2 Residue ellipticity of c.d. extrema of poly(*O,O'*-dimethyl-L-Dopa) in organic solvents

Solvent	Ellipticity (degree cm²/dmol)	Extrema position (nm)
Chloroform	6800	285
Methylene dichloride	7200	285
1,2-Dichloroethane	5100	285
2-Chloroethanol	5700	285
Dioxane[a]	5100	285
TMP	7900	285
HFIP	3300	280
TFA	−1600	285
HFA	− 500	280

[a] Values of copoly(95% *O,O'*-dimethyl-L-Dopa, 5% γ-benzyl-L-glutamate)

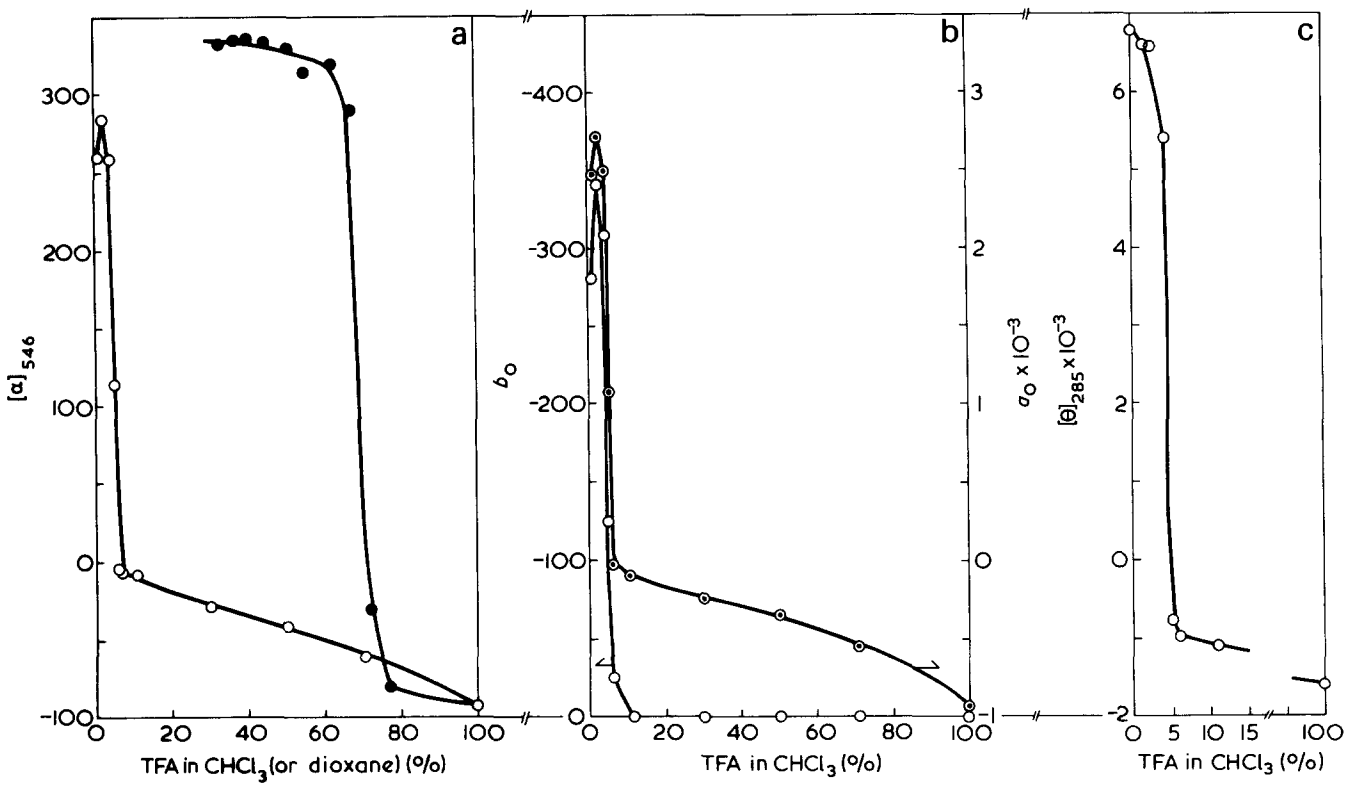

Figure 3 (a) Specific rotation: (b) b_0, a_0 and (c) $[\theta]_{285}$ values of poly(*O,O'*-dimethyl-L-Dopa) with the change of solvent composition at 25°C: ○ and ⊙, in chloroform–TFA; ●, in dioxane–TFA

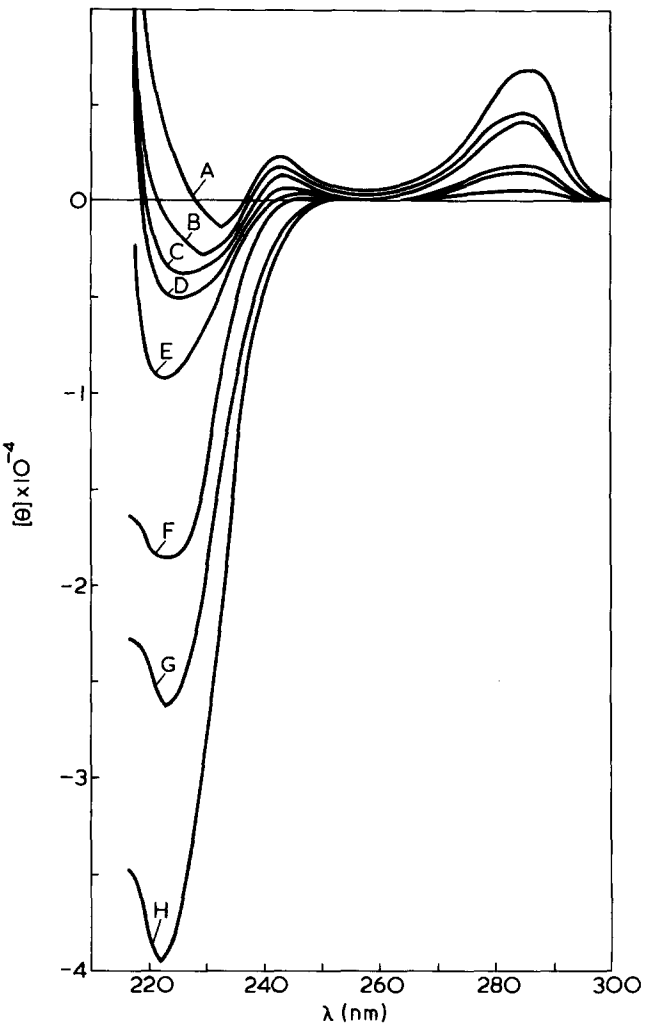

Figure 4 C.d. spectra of copoly(*O,O'*-dimethyl-L-Dopa, γ-benzyl-L-glutamate) in chloroform at 25°C. Mol % of γ-benzyl-L-glutamate in copolymers: A, 0%; B, 5%; C, 10%; D, 20%; E, 33%; F, 50%; G, 75%; H, 100%

clear that the sharp drop of the $[\theta]_{285}$ value is paralleled by the conformational transition as pointed out by Horie and Toda[13].

Unfortunately, full rotation data of poly(*O,O'*-dimethyl-L-Dopa) in dioxane–TFA mixed solvents are not available since the polypeptide precipitates in this mixed solvent.

Conformation of copoly (O,O'-dimethyl-L-Dopa, γ-benzyl-L-glutamate)

C.d. spectra. The c.d. spectra of copoly(*O,O'*-dimethyl-L-Dopa, γ-benzyl-L-glutamate) in chloroform are shown in *Figure 4*. Approximately, the same series of c.d. spectra in the range 300 to 190–200 nm were obtained in 2-chloroethanol, dioxane (copolymers containing more than 5 mol % glutamate), TMP or HFIP. The positions and magnitudes of the multiple Cotton effects of poly(*O,O'*-dimethyl-L-Dopa) changed with increasing γ-benzyl-L-glutamate content; that is, a peak with $[\theta]_{285} = 6800$ decreased, a peak with $[\theta]_{242} = 2300$ disappeared and a trough with $[\theta]_{233} = -1400$ shifted to 222 nm and increased the magnitude to −40 000 (*Figure 4*). Copolymers containing more than 50 mol % glutamate showed the typical spectra of a right-handed α-helix.

Figures 5 and *6* show the dependence of $[\theta]_{285}$, $[\theta]_{222}$ and $[\theta]_{208}$ on the composition of copoly(*O,O'*-dimethyl-L-Dopa, γ-benzyl-L-glutamate). The $[\theta]_{285}$ values of the copolymers in chloroform, dioxane or 2-chloroethanol decreased gradually and, at 50 mol % glutamate, the copolymer showed a very small peak at 285 nm. This implies the stacking of poly(*O,O'*-dimethyl-L-Dopa) and is discussed below. The $[\theta]_{222}$ and $[\theta]_{208}$ ellipticity values of the copolymers in chloroform, dioxane or 2-chloroethanol were linear. The linear relationship between the $[\theta]_{222}$ and $[\theta]_{208}$ values and composition indicates that poly(*O,O'*-dimethyl-L-Dopa) has the same helical sense to that of poly(γ-benzyl-L-glutamate).

O.r.d. spectra. The o.r.d. curves of this series of copolymers were measured in chloroform–1% TFA mixed solvents over the wavelength range 320–600 nm. *Figure 7* shows the dependence of b_0 on the composition of copoly(*O,O'*-dimethyl-L-Dopa, γ-benzyl-L-glutamate). The b_0 value for poly(*O,O'*-dimethyl-L-Dopa) was found to be −280. The

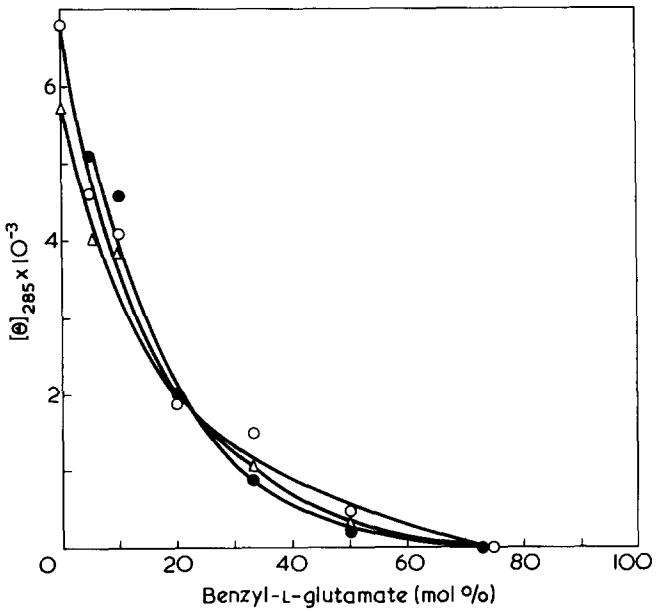

Figure 5 Dependence of $[\theta]_{285}$ on the composition of copoly(*O,O'*-dimethyl-L-Dopa, γ-benzyl-L-glutamate) at 25°C: ○, in chloroform; △, in 2-chloroethanol; ●, in dioxane

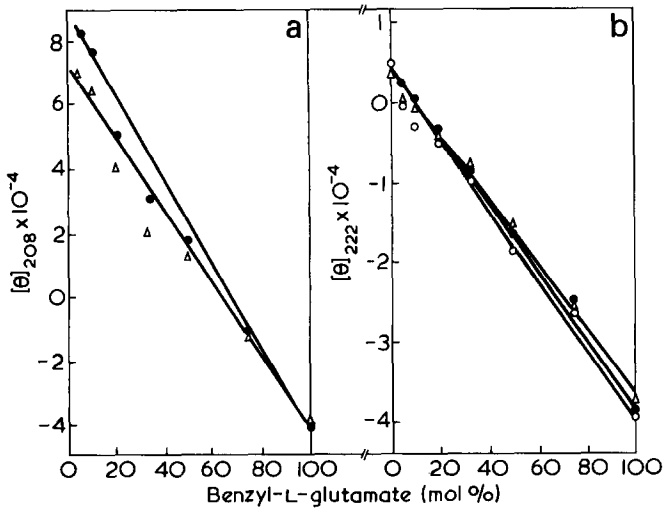

Figure 6 Dependence of (a) $[\theta]_{208}$ and (b) $[\theta]_{222}$ on the composition of copoly(*O,O'*-dimethyl-L-Dopa, γ-benzyl-L-glutamate) at 25°C; ○, in chloroform; △, in 2-chloroethanol; ●, in dioxane

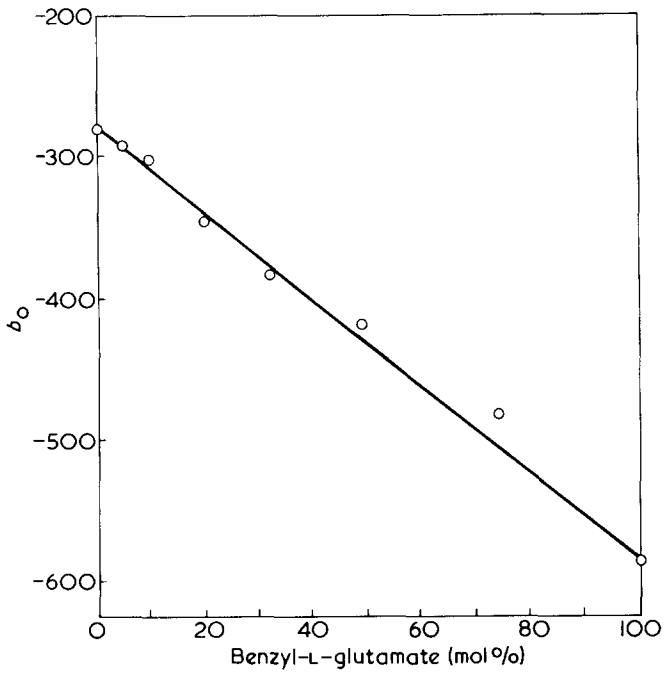

Figure 7 Dependence of b_0 on the composition of copoly(*O,O'*-dimethyl-L-Dopa, γ-benzyl-L-glutamate) in chloroform–1% TFA at 25°C

b_0 value for helical poly(γ-benzyl-L-glutamate) was found to be −590. The b_0 values varied linearly from −280 to −590 for this series of copolymers. This again indicates that the same helical sense is maintained in these copolymers, namely a right-handed helix.

DISCUSSION

The newly synthesized poly(*O,O'*-dimethyl-L-Dopa) showed an unexpectedly good solubility in many organic solvents in spite of the high degree of polymerization.

As is well known, the helical senses of poly(β-benzyl-L-aspartate)[14,15], poly(*O*-carbobenzoxy-L-tyrosine)[11], poly(L-tyrosine)[16], poly(L-tryptophan)[17] and poly(*O,O'*-dicarbobenzoxy-L-Dopa)[4] were solved by copolymer studies. If poly(*O,O'*-dimethyl-L-Dopa) and poly(γ-benzyl-L-glutamate) are helices of the opposite sense of twist, the b_0 value of their copolymers should change markedly over a relatively narrow range of copolymer composition, representing a region of transition from one helical sense to the other, as found for copolymers of β-benzyl-L-aspartate and γ-benzyl-L-glutamate[14,15]. However, the linear relationship between the $[\theta]_{222}$, $[\theta]_{208}$ and b_0 values and composition (*Figures 6* and *7*) indicates that poly(*O,O'*-dimethyl-L-Dopa) in helicogenic solvents has the same helical sense as that of poly(γ-benzyl-L-glutamate) which exists as a right-handed α-helix[18].

Poly(*O,O'*-dimethyl-L-Dopa) showed the three positive ellipticity bands at 285, 241 and 205 nm in helicogenic solvents in the u.v. region. In the case of the absence of chromophores other than the peptide group in this region the n–π* band at 222 nm and the π–π* band at 208 nm immediately related to the α-helix conformation and the helical sense and content can be assigned. But in poly(*O,O'*-dimethyl-L-Dopa) it is evident that the experimental results cannot allow any conformation to be attributed since the n–π* and π–π* transitions involve the contribution of the ^1L_a and ^1L_b transitions of the substituted benzenes in the Platt notation[19–21] and of the stacking of the side chain[13].

Goodman *et al.*[21] have given a discussion of the possibility of mixing the symmetry-forbidden π–π* transition of the benzene nucleus with the n–π* transition of the non-bonding orbitals of the oxygen atom as in the case of L-tyrosine. *O*-Methylation in L-tyrosine does not change this mixing, but *O*-acylation is expected to alter the nature of the transition substantially. The c.d. spectrum of helical poly(*O,O'*-dimethyl-L-Dopa) was very similar to that of helical poly(L-Dopa) which showed three positive peaks at 280, 230 and 204 nm in aqueous solution or TMP ($[\theta]_{280} = 2000$, $[\theta]_{230} = 3700$ and $[\theta]_{204} = 60\,000$)[3]. But the c.d. spectrum of the former polypeptide differed from that of *O*-acylated poly(*O,O'*-dicarbobenzoxy-L-Dopa) which showed no peak at around 280 nm and a trough at 225 nm with $[\theta]_{225} = -13\,600$ in chloroform[2]. The comparison of the three poly(L-Dopa) and poly(L-Dopa) derivatives is an experimental proof of the discussion described by Goodman[21].

Poly(*O,O'*-dimethyl-L-Dopa) and its copolymers with γ-benzyl-L-glutamate showed a peak at 285 nm in the c.d. spectra (*Figures 2* and *4*) and the peak is strong evidence of the stacking in the side chains. This c.d. peak at around 280 nm has been assigned to the arrangement of the induced dipole moments of the stacked side chain[13]. On the other hand, poly(*O,O'*-dicarbobenzoxy-L-Dopa) which has three aromatic groups in the side chain, showed no peak above 250 nm in the c.d. spectrum[2,4]. In an ordinary sense, the more aromatic groups a polymer has, the more stacking it causes. To avoid contradiction of the experimental results, we can give only one explanation which is that the three aromatic groups in one side chain are sterically too bulky to maintain the stacking. This speculation, of course, requires further experimental and theoretical evidence.

Though we feel that the results of poly(*O,O'*-dimethyl-L-Dopa) do not provide sufficient evidence to discuss why poly(L-Dopa) and its derivatives show anomalous rotation behaviour and how the polypeptide causes the stacking, side chain effects should be emphasized here. Finally, to study further the synthesis and conformational studies of poly(L-Dopa) derivatives, work is in progress to synthesize the aliphatic acyl (*O*-acetyl) and aromatic ether (*O*-benzyl) derivatives of L-Dopa[22] for comparison with the results we have reported.

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