to predict the curves shown in Figure 4 with fair accuracy. The departure from linearity is obvious for ratios of α-DPND:β-DPND from 80:20 to 20:80. These data will be presented in detail elsewhere.

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Synthesis, Characterization, and Racemization of Poly-L-serine[*]

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Poly-*O*-benzyl-L-serine (III) with an average molecular weight of 52,000 was obtained by the polymerization of *O*-benzyl-*N*-carboxy-L-serine anhydride (II) in bromobenzene with sodium methoxide used as initiator. Debenzylation of III with anhydrous hydrogen bromide in dioxane yielded optically pure poly-L-serine (IV). Poly-L-serine is insoluble in water and the common organic solvents; it dissolves, however, in 7.0 to 8.5 M aqueous lithium bromide. Racemization of poly-L-serine was found to occur in alkaline media. The kinetics of racemization in concentrated aqueous lithium bromide solutions was investigated. The data obtained suggest that the racemization is base-catalyzed and that each of the seryl residues of the polymer undergoes optical inversion independently by a similar first-order reaction. The x-ray and infrared data indicate that the optically pure poly-L-serine (IV) has a β-conformation in the solid state. Optical rotatory dispersion data suggest that IV does not attain an α-helix conformation in aqueous lithium bromide or in aqueous lithium bromide–2-chloroethanol mixtures.

Poly-L-serine may serve as a suitable high-molecular-weight model compound in the elucidation of the physicochemical and biological properties of proteins with a high serine content, such as silk fibroin (Lucas *et al.*, 1958) and phosvitin (Mecham and Olcott, 1949; Williams and Sanger, 1959). The synthesis of a low-molecular-weight, optically inactive, water-soluble poly-DL-serine was described by Frankel *et al.* (1953). *O*-Acetyl-*N*-carboxy-DL-serine anhydride (Bamford *et al.*, 1956; Katchalski and Sela, 1958) was used as the starting monomer, and the resulting poly-*O*-acetyl-DL-serine was deacetylated with aqueous ammonia. A different procedure for the preparation of poly-DL-serine was described by Okawa and Tani (1954). *O*-Benzyl-*N*-carboxy-DL-serine anhydride was the monomer used, and the poly-*O*-benzyl-DL-serine obtained was debenzylated in dioxane with anhydrous hydrogen bromide. The synthesis of a water-soluble optically active polypeptide of serine reported to be poly-L-serine was recently described by Fasman and Blout (1960). *O*-Acetyl-*N*-carboxy-L-serine anhydride was polymerized in dioxane and the resulting poly-*O*-acetyl-L-serine was deacetylated with sodium methoxide in nitrobenzene. The marked solubility in water of the polypeptide obtained by Fasman and Blout seemed somewhat surprising in view of the insolubility in water of silk fibroin and of the fiber produced by *Chrysopa flava*, which is known to contain an exceptionally high percentage ($\sim$ 40%) of L-serine (Parker and Rudall, 1957; Lucas *et al.*, 1957). Furthermore, phosvitin, in which approximately half of the amino acid residues are L-serine, yields a water-insoluble product after exhaustive enzymatic dephosphorylation (Rabinowitz and Lipman, 1960). Since the deacetylation of poly-*O*-acetyl-L-serine was carried out by Fasman and Blout (1960) in an alkaline medium, the partial racemization of seryl residues had to be considered. Such a racemization has in fact been found to occur when proteins are treated with strong base (Daft and Coghill, 1931; Groh and Nyilasi, 1952; Nyilasi and Kovats, 1952; Nyilasi, 1956–57; Neuberger, 1948). Preliminary results, obtained in this laboratory, have shown that D-serine can be detected in acid hydrolysates of "poly-L-serine" samples obtained according to Fasman and Blout (1960). The synthesis of optically pure poly-L-serine was therefore undertaken.

In the following section we describe the synthesis of

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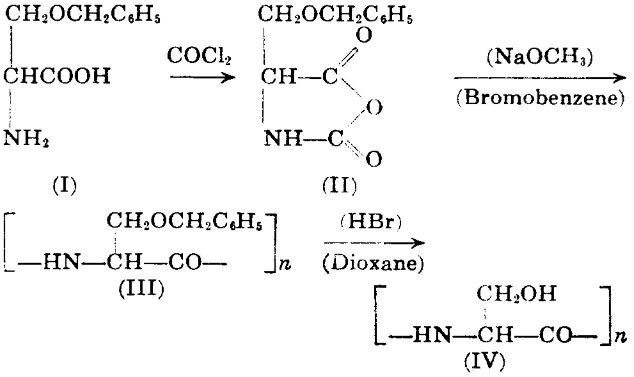
poly-L-serine by a procedure in which the use of basic conditions in the unmasking of the parent polyamino acid has been avoided. The polypeptide obtained is water insoluble, has a β-conformation in the solid state, and resembles proteins with a high serine content in its optical rotatory properties in 8.5 M lithium bromide solution. Poly-L-serine readily racemizes in alkaline media.

RESULTS AND DISCUSSION

Synthesis of Poly-L-serine.—Poly-L-serine was synthesized according to scheme 1. Optically pure O-benzyl-L-serine (I) was prepared by the enzymatic resolution of the corresponding racemic serine derivative (Okawa, 1956, 1957). On treatment with phosgene it yielded O-benzyl-N-carboxy-L-serine anhydride (II), which was purified by repeated crystallization from ether-petroleum ether and ethyl acetate–petroleum ether until free of chloride (chloride content less than 0.01 mole %). The N-carboxyanhydride II was polymerized in bromobenzene at room temperature with sodium methoxide as initiator. The poly-O-benzyl-L-serine (III) obtained had an intrinsic viscosity, [η], of 0.31 dl/g in dichloroacetic acid and a weight average molecular weight of 52,000 as determined by light scattering. The polymer was soluble in dichloroacetic acid and trifluoroacetic acid but insoluble in water, dioxane, chloroform, and bromobenzene. Debenzylation of poly-O-benzyl-L-serine with anhydrous hydrogen bromide in dioxane yielded poly-L-serine (IV). The polypeptide obtained was insoluble in water and dioxane. It dissolved in hot dichloroacetic acid and trifluoroacetic acid. Dissolution could also be effected at room temperature in 7.0 to 8.5 M aqueous lithium bromide. However, when the concentration of lithium bromide was increased above 8.5 M or decreased below 7.0 M the polymer separated out.



Scheme 1

The poly-L-serine synthesized yielded on acid hydrolysis a total amount of serine, as assayed by the periodate method (Friesell and Mackenzie, 1958), corresponding to approximately 88% of theory (see Table I). Pyruvic acid, determined as the dinitrophenylhydrazone (Friedemann and Haugen, 1943), was the main product of degradation. It accounted for about 9% of the seryl residues of the polypeptide. Similar results were obtained with poly-DL-serine, poly-O-benzyl-L-serine, and poly-O-acetyl-L-serine (Table I). Serine is known to be partly destroyed on heating with mineral acids, pyruvic acid being the main product of degradation (Rees, 1946). It is, therefore, recovered from peptides and proteins, under the standard conditions of acid hydrolysis, only in an 85 to 95% yield (Neuberger, 1948; Smith and Stockell, 1954).

No D-serine could be detected in acid hydrolysates of our poly-L-serine preparations (Table I) when assayed by means of D-amino acid oxidase (Greenstein and Winitz, 1961a). When acid hydrolysates of mixtures composed of a relatively small amount of poly-DL-serine and a large excess of poly-L-serine were subjected to the D-amino acid oxidase assay, the amount of D-serine obtained corresponded to about 80% of the amount to be expected (Table II). The experiments recorded in Tables I and II thus prove the optical purity of the poly-L-serine preparations derived from poly-O-benzyl-L-serine by the procedure described above.

TABLE I

COMPOSITION OF ACID HYDROLYSATES OF SOME SERINE POLYMERS[a]

Polymer	Total Serine (L + D) (moles per 100 moles polypeptide-N)	Pyruvic Acid (moles per 100 moles polypeptide-N)	D-Serine (% of total serine recovered)
Poly-O-benzyl-L-serine	88.0	9.4	<0.2
Poly-L-serine (A)[b]	88.2	8.8	<0.2
Poly-O-acetyl-L-serine[c]	87.9	6.1	<0.2
"Poly-L-serine" (B)[d]	89.8	9.8	4.7
Poly-L-serine (C)[e]	88.2	—	<0.2
Poly-DL-serine[f]	87.4	9.3	50.1

[a] Hydrolysis was carried out in 5.5 N hydrochloric acid at 105° for 20 hours, and the hydrolysates were assayed for total serine, D-serine, and pyruvic acid as described in the Experimental section. [b] Obtained by debenzylation of poly-O-benzyl-L-serine with anhydrous HBr in dioxane. [c] Prepared according to Fasman and Blout (1960). [d] Obtained by the deacetylation of poly-O-acetyl-L-serine according to the procedure of Fasman and Blout (1960), with one mole $NaOCH_3$ used per mole amino acid residues. [e] Derived from poly-O-acetyl-L-serine on treatment with hydrazine hydrate (99–100%). For details see Experimental section. [f] Obtained by debenzylation of poly-O-benzyl-DL-serine (Okawa and Tani, 1954) with anhydrous HBr in dioxane.

TABLE II

D-SERINE IN HYDROLYSATES OF MIXTURES OF POLY-L-SERINE AND POLY-DL-SERINE

Substrate Used for Hydrolysis[a]	D-Seryl Residues in Original Substrate Mixture (μmoles per 100 μmoles total (D + L)-seryl residues)	D-Serine in Hydrolysate[b] (μmoles per 100 μmoles total (D + L)-serine)
Mixture of poly-L-serine[c] and poly-DL-serine[d]	0.98	0.77
	0.50	0.40
	0.20	0.20
DL-Serine added to hydrolysate of poly-L-serine[c]	1.9	1.54
	0.37	0.28

[a] The amounts of substrate and the hydrolytic procedure used are described in the Experimental section. [b] D-Serine and total (D + L)-serine were determined by the D-amino acid oxidase assay and the periodate assay, respectively (see Experimental section). The amount of D-serine found was 78 to 79% of the amount to be expected from substrate composition. The amount of total serine found was 88 to 90% of the amount to be expected. [c] Obtained by debenzylation of poly-O-benzyl-L-serine with anhydrous HBr in dioxane. [d] Obtained by debenzylation of poly-O-benzyl-DL-serine (Okawa and Tani, 1954) with anhydrous HBr in dioxane.

Serine residues have been shown to undergo N → O acyl shift when proteins are treated with strong mineral acids (Elliott, 1953; Thompson, 1960). Poly-DL-serine has been shown to undergo such a shift when dissolved in sulfuric acid (Fasman, 1960). The possibility of an N to O shift occurring on the debenzylation of III with hydrogen bromide was therefore investigated. The N to O shift in polyserine should lead to the formation of ester bonds and to the appearance of an equivalent amount of free α-amino groups. No ester bonds could be detected by the hydroxamic acid test (Hestrin, 1949) in the polyserine synthesized, and the infrared absorption spectrum of the polymer did not show the characteristic absorption of the ester link at 1750 cm^{-1} (Bellamy, 1958a). The total amount of free amino groups as determined by Van Slyke's method did not exceed the amount of terminal amino groups as determined by oxidation with periodate (see below). It is thus obvious that no N → O acyl shift occurred during the debenzylation of poly-*O*-benzyl-L-serine, and that the poly-L-serine synthesized contains a normal polypeptide backbone.

Finally, it is worth mentioning that solutions of IV in 1 N sodium hydroxide did not show any significant absorption in the ultraviolet down to 235 mμ. This confirms the quantitative removal of benzyl ether groups and proves the absence of dehydroalanyl residues (Greenstein and Winitz, 1961b) formed by a β-elimination reaction (Hine, 1956).

The molecular weight of poly-L-serine could not be determined directly by the usual physicochemical methods, as the polymer is insoluble in water and in organic solvents which do not interact chemically with the polymer. An indirect estimate of the average degree of polymerization, $\overline{\text{DP}}$, of poly-L-serine could, however, be obtained from the $\overline{\text{DP}}$ of the parent poly-*O*-benzyl-L-serine ($\overline{\text{DP}}$ = 290, see Table III). The comparison of the average chain length of the water-soluble poly-DL-serine ($\overline{\text{DP}}$ = 200) with that of the parent poly-*O*-benzyl-DL-serine ($\overline{\text{DP}}$ = 320) (Table III) reveals that the debenzylation procedure adopted caused only a slight degradation of the polypeptide backbone. Assuming a similar extent of degradation during the debenzylation of the poly-*O*-benzyl-L-serine ($\overline{\text{DP}}$ = 290), an average degree of polymerization of 150 to 200 is obtained for the resulting optically pure poly-L-serine. An evaluation of the number average degree of polymerization of IV by amino end-group analysis (Van Slyke) or by an estimation of the *N*-terminal serine (oxidation with periodate) (Friesell and Mackenzie, 1958) was attempted. The determinations carried out on suspensions of poly-L-serine yielded irreproducible results from which average degrees of polymerization between 50 and 200 could be calculated.

In order to test the optical purity of the poly-L-serine described by Fasman and Blout (1960), the polymer was prepared by their procedure and hydrolyzed in acid, and the hydrolysate was assayed for D-serine. Whereas no D-serine could be detected by means of D-amino acid oxidase in the acid hydrolysate of the intermediate poly-*O*-acetyl-L-serine, the deacetylated "poly-L-serine" contained about 5% of the total serine in the D-form (see Table I). This shows that racemization occurs during the deacetylation with sodium methoxide. In this connection it is pertinent to note that exhaustive deacetylation of poly-*O*-acetyl-L-serine with hydrazine did not cause racemization and yielded a water-insoluble poly-L-serine. The treatment of poly-*O*-acetyl-L-serine with hydrazine leads, however, to partial hydrazinolysis of the peptide backbone.

TABLE III

VISCOSITY AND MOLECULAR WEIGHT OF SOME SERINE POLYPEPTIDES

Polypeptide	[η] (dl/g)	Average Molecular Weight	Average Degree of Polymerization
Poly-*O*-benzyl-L-serine	0.31[a]	52,000[b]	290
Poly-*O*-benzyl-DL-serine[c]	0.38[a]	58,000[b]	320
Poly-DL-serine[d]	0.15[e]	25,000[f]	290
		18,000[g]	200
		14,000[h]	160

[a] Obtained by a linear extrapolation of the reduced viscosity, measured in dichloroacetic acid, and the concentration range of 0.1 to 0.5 g/dl, at 30°. [b] The molecular weight was derived from light-scattering measurements. The Rayleigh ratio, R_{90}, for 546 mμ (Hg line) was determined in dichloroacetic acid with a Brice Phoenix-1000 instrument, at a concentration range of 0.3 to 1.0 g/dl, at 20°. The molecular weight was calculated from the value of KC/R_{90} extrapolated to $C = 0$. In the estimation of the characteristic light-scattering constant, K, a refractive index increment of 0.124 ml/g, given by Breitenbach and Koref (1955) for poly-DL-phenylalanine in dichloroacetic acid, was used. [c] Prepared from *O*-benzyl-*N*-carboxy-DL-serine anhydride by a procedure similar to that given for the preparation of poly-*O*-benzyl-L-serine (see Experimental section). [d] Obtained by the debenzylation of the poly-*O*-benzyl-DL-serine sample described (average molecular weight 58,000) according to the procedure adopted for the preparation of poly-L-serine (see Experimental section). [e] Obtained by a linear extrapolation of the reduced viscosity, measured in 0.1 M KCl, in the concentration range of 0.1 to 0.7 g/dl, at 30°. [f] Derived from light-scattering measurements in a manner similar to that given in *b*. 0.1 M KCl was used as solvent and the measurements carried out at 20°. For the refractive index increment at 546 mμ a value of 0.184 ml/g was assumed (Doty and Edsall, 1951). [g] Calculated from osmotic pressure measurements in 0.1 M KCl at 30°; a modified Zimm-Myerson osmometer (Riesel and Berger, 1959) with a cellophane membrane (Extra Dialyzer No. 300) obtained from Fisher Scientific Company was used. [h] Calculated from the sedimentation and diffusion coefficients ($s_{20,w} = 1.75\ S$; $D_{20} = 8.9 \times 10^{-7}\ \text{cm}^2\ \text{sec.}^{-1}$ in 0.1 M KCl, at a concentration of 0.35 g/dl, assuming a partial specific volume of 0.63. The latter value was determined picnometrically. The sedimentation experiments were carried out in a Spinco Model E ultracentrifuge at 59,780 rpm. The diffusion measurements were carried out in a synthetic boundary cell at 9,341 rpm.

Conformation of Poly-L-serine in the Solid State.—The infrared absorption spectrum of poly-L-serine in powder form in KBr discs has been described previously (Bohak and Ellenbogen, 1960). The spectrum shows a broad absorption band with a maximum at 3300 cm^{-1}. This band is due to the NH and OH stretching frequencies of the polymer and most likely includes the absorption of the adsorbed water. The absence of an absorption maximum at about 3500 cm^{-1}, where the hydroxyl of L-serine is known to absorb (Brockman and Muss, 1956), is an indication of hydrogen bonding (Bellamy, 1958b) in the solid state causing the OH frequency to approach that of the NH at 3300 cm^{-1}. A similar observation has been made with the serine-rich fiber of *Chrysopa flava* (Parker and Rudall, 1957).

In the absorption region of amide I, poly-L-serine shows a broad band with a sharp peak at 1634 cm^{-1} and a shoulder at 1650 cm^{-1}. In the region of amide II, a sharp and intense peak appears at 1530 cm^{-1}, accompanied by a less-intense peak at 1550 cm^{-1}. The absorption of poly-L-serine in these regions is identical with that of *Bombyx mori* fibroin, where dichroism studies have proved that the peaks at 1634 cm^{-1} and 1530 cm^{-1} are characteristic of the β crystalline structure, whereas the shoulders at about 1650 cm^{-1} and 1550 cm^{-1} result from the presence of some amorphous material (Elliott *et al.*, 1958; Lucas *et al.*, 1958). The broadening of the band around 1630 cm^{-1} observed in the case of poly-L-serine is most likely due to the presence of small amounts of water of hydration known to absorb in this region (Randal *et al.*, 1949).

The x-ray powder diagram of poly-L-serine shows four rings, corresponding to spacings of 5.7 A, 4.5 ± 0.2 A (very strong), 3.7 ± 0.1 A, and 2.9 ± 0.1 A. The characteristic feature of polypeptides in the β conformation is a very strong reflection at 4.70 A and a less strong one at 4.25 A (Marsch *et al.*, 1955). Hence it seems likely that the presence of the reflection at 4.5 A in poly-L-serine results from its being in the β conformation. The reflections corresponding to 3.7 ± 0.1 A and 2.9 ± 0.1 A are also found in powder diagrams of silk fibroin (Lucas *et al.*, 1958). Of special interest is the appearance of the reflection at 5.66 A, which has not been observed in silk. Parker and Rudall (1957) observed in the case of the fiber of *Chrysopa flava* a similar unusual reflection at 5.42 A and have shown that it is a *c* axis repeat. The *c* axis repeat in the β structure is determined by the side-chains which protrude into the space between the sheets (Marsch *et al.*, 1955) and increases from a value of 4.7 A in the *Bombyx mori* fibroin, rich in glycine and relatively poor in serine (~ 12%), to a value of 5.42 A in the fiber of *Chrysopa flava*, known to contain 40% serine. Hence it seems likely that the spacing of 5.7 in poly-L-serine is due to the same structural feature.

The infrared and x-ray data discussed above seem to indicate that poly-L-serine exists in the solid state in the β-conformation. The presence of a small amount of material in a random-coil formation cannot, however, be excluded.

*Racemization of Poly-*L*-serine in Alkaline Media.*—The susceptibility of poly-L-serine to racemization by alkali which was encountered in the preparative studies led us to investigate this phenomenon further.

Suspensions of poly-L-serine (50 mg) in 0.6 N and 1.0 N sodium hydroxide (1 ml) were incubated at 30° for varying time intervals and the extent of racemization followed by determining the amount of D-serine liberated on acid hydrolysis of the whole reaction mixture with 5.5 N hydrochloric acid. The data given in Table IV show that the racemization increased with alkali concentration and with the time of incubation. The seryl residues of poly-L-serine thus resemble the seryl residues of proteins which have also been shown to racemize in alkaline media (Daft and Coghill, 1931; Neuberger, 1948; Groh and Nyilasi, 1952; Nyilasi and Kovats, 1952; Nyilasi, 1956–57). They differ, however, from free serine, which retains full optical activity even after prolonged exposure to mineral base (Crawhall and Elliott, 1951; Weygand and Rinno, 1959).

TABLE IV

RACEMIZATION OF POLY-L-SERINE ON TREATMENT WITH AQUEOUS SODIUM HYDROXIDE[a]

	% D-Serine in Total Serine Recovered			
	Time of Incubation (min.)			
Medium	20	40	60	120
0.6 N NaOH	8.0	7.4	17.7	22.3
1.0 N NaOH	14.6	24.0	28.5	36.0

[a] Poly-L-serine (50 mg) was suspended in 0.6 N or 1.0 N NaOH (1 ml) and the mixture incubated at 30° with occasional shaking. At the times specified concentrated hydrochloric acid was added to a final concentration of 5.5 N. Hydrolysis was carried out at 105° for 20 hours and the hydrolysates assayed for total and D-serine as described in the experimental section.

Since the racemization reaction is accompanied by the solubilization of the polymer, it was of interest to examine whether the incubation mixtures contained soluble high-molecular-weight peptides with D-seryl residues. A suspension of poly-L-serine (200 mg) in 1 N sodium hydroxide (10 ml) was incubated for 20 minutes at 30°, the reaction mixture was neutralized, and the remaining insoluble material (28 mg) was removed by centrifugation. The supernatant was dialyzed exhaustively against water and lyophilized. The product (160 mg) yielded on acid hydrolysis 13% D-serine of the total amount of serine recovered. The solubility in water of the partially racemized poly-L-serine is most likely due to the disruption of the steric regularity and intermolecular hydrogen bonding of the water-insoluble β-conformation of the optically pure poly-L-serine as a result of the appearance of D-seryl residues along the polypeptide chain.

In order to investigate the kinetics of the racemization of poly-L-serine in a homogeneous system, the polypeptide was dissolved in concentrated aqueous lithium bromide and the decrease in the optical rotation with time was followed polarimetrically at room temperature after the addition of lithium hydroxide. The results obtained are given in Figure 1 and Table V. Measurements were carried out at 405 mμ, since the optical rotations at this wave length are considerably higher than those at 589 mμ (see below). In all the experiments recorded, the rotation dropped to zero within 10 to 12 hours. The data presented in Figure 1 show that the change in optical rotation with time follows first-order kinetics. It may thus be described by equation (1), where α denotes the optical rotation

$$\frac{d\alpha}{dt} = -k\alpha \qquad (1)$$

of the polyserine at any given instant, and k is the specific rate constant of the reaction. Doubling the concentration of LiOH (from 0.17 to 0.34 M) caused a twofold increase in the rate of racemization (Table V). It is thus obvious that the reaction is base catalyzed. Equation (1) suggests that each of the seryl residues of the polymer undergoes optical inversion independently by a similar first-order reaction. The rate of change in the concentration of L-seryl residues, in such a system, should be given by equation (2), where (L) and (D)

$$\frac{d(\text{L})}{dt} = -k'[(\text{L}) - (\text{D})] = -k'[2(\text{L}) - C_0] \qquad (2)$$

denote the respective concentrations of L- and D-seryl residues, and C_0 gives their total concentration. As a first approximation, the optical rotation of the system at any given moment can be described by equation (3),

$$\alpha = [\alpha_{\text{L}}][(\text{L}) - (\text{D})] = [\alpha_{\text{L}}][2(\text{L}) - C_0] \qquad (3)$$

TABLE V

SPECIFIC FIRST-ORDER RATE CONSTANT DESCRIBING THE CHANGE IN OPTICAL ROTATION OF POLY-L-SERINE IN 7.1 M AQUEOUS LiBr ON INCUBATION WITH BASE AT 20°

Conc. of LiOH in Reaction Mixture (moles/liter)	$10^2 \times k$ (min.$^{-1}$)	$10^2 \times k/[\text{OH}^-]$ (mole^{-1} liter min.$^{-1}$)
0.34	2.9	8.5
0.26	1.9	7.5
0.17	1.4	8.3

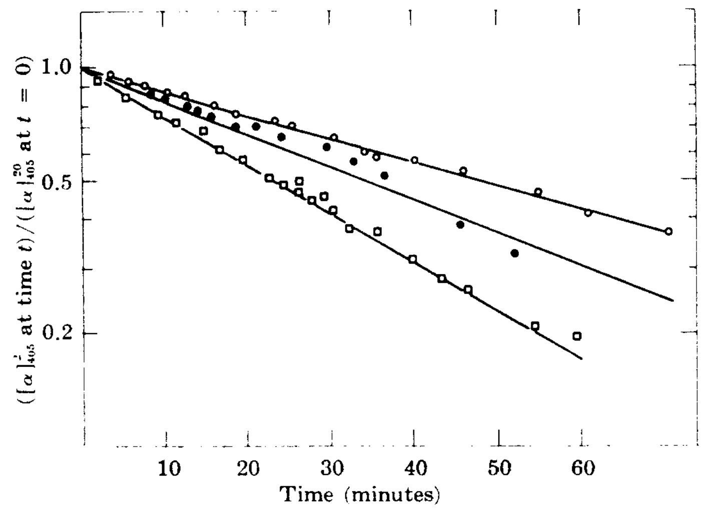


FIG. 1.—Kinetics of racemization of poly-L-serine dissolved in 7.1 M aqueous LiBr in the presence of LiOH, at 20°. Concentration of polypeptide 1.0 g/dl. Optical rotation measured at 405 mμ. ○, 0.17 M LiOH; ●, 0.26 M LiOH; □, 0.34 M LiOH.

$[\alpha_L]$ denoting the specific optical rotation of an L-seryl residue and $-[\alpha_L]$ that of a D-seryl residue. From (2) and (3) one obtains (4).

$$\frac{d\alpha}{dt} = -2k'\alpha \qquad (4)$$

Comparison of equation (4) with (1) shows that the values of the specific rate constants for the change in optical rotation are twice as large as the corresponding constants for the inversion of the L- or D-seryl residues. The data given in Table V thus show that the base-catalyzed racemization of the L and D-serine residues is characterized under the experimental conditions used by the specific rate constant $k' \cong 4 \times 10^{-2}$ [OH$^-$] min.$^{-1}$.

To compare the rate of racemization of poly-L-serine in concentrated aqueous lithium bromide with that in an aqueous solution devoid of mineral salt, the kinetics of racemization in aqueous alkali of a partially racemized water-soluble polypeptide was investigated. The polymer used was the one described above, which yielded on acid hydrolysis 13% D-serine of the total amount of serine recovered. The rate of racemization in 0.1 M and 0.3 M aqueous NaOH, at 24°, was determined polarimetrically (λ = 405 mμ). The change in optical rotation with time followed first-order kinetics, and the specific rate of racemization was proportional to the concentration of base. An average value of k/[OH$^-$] = 9.1 × 10^{-2} mole^{-1} liter min.$^{-1}$ was obtained under the experimental conditions used. The racemization of poly-L-serine in water at 24° seems, therefore, to proceed somewhat faster than that in 7.1 M aqueous lithium bromide at 20° (k/[OH$^-$] ≅ 8.1 × 10^{-2} mole^{-1} liter min.$^{-1}$, see Table V). It is pertinent to note that the base-catalyzed racemization of *N*-acetyl-L-serine amide (see Experimental section; measured polarimetrically at 325 mμ) in 0.2 M and 0.4 M NaOH, at 24°, could also be described by a first-order reaction with an average specific rate constant (k/[OH$^-$] ≃ 9.3 × 10^{-2} mole^{-1} liter min.$^{-1}$; k′ ≃ 4.65 × 10^{-2} [OH$^-$] min.$^{-1}$) close to that recorded for the racemization of the seryl residues of polyserine, under similar conditions.

The base-catalyzed racemization of the seryl residues in poly-L-serine is most likely due to the ionization of the hydrogen of the asymmetric α-carbon atom and the formation of a symmetric carbanion (Neuberger, 1948). A similar mechanism has recently been established for the base-catalyzed racemization of compounds containing an amide, ester, or nitrile group adjacent to an optically active carbon atom (Cram *et al.*, 1961). The ease of racemization of the seryl residues as compared with that of other amino acid residues suggests that the electrophilic β-hydroxyl group markedly enhances the ionization of the hydrogen attached to the α-carbon (Neuberger, 1948).

Optical Rotatory Dispersion of Poly-L-serine in Aqueous LiBr Solution.—The optical rotatory dispersion of poly-L-serine in aqueous 8.5 M LiBr was measured at different temperatures (13° to 65°) in the range of 365 mμ to 579 mμ. Straight lines were obtained when the specific rotation $[\alpha]_\lambda$ at each temperature was plotted against the corresponding $[\alpha]_\lambda\lambda^2$ (Fig. 2). The optical rotatory dispersion at each of the temperatures studied can thus be described by the well-known one-term Drude equation $[\alpha]_\lambda = K/(\lambda^2 - \lambda_c^2)$. The numerical values obtained for the characteristic constants K and the dispersion constants λ_c are given in Table VI. The data given in the Table show that λ_c remains practically constant ($\lambda_c \cong 233$ mμ) within the temperature range of 16° to 65°. The somewhat higher value obtained for λ_c (λ_c = 249 mμ) at 13° may be due to error in the measurement of the low optical rotations at this temperature. The numerical value of the negative specific rotation at 546 mμ increases when the temperature is increased from 13° to 45°. Further increase in temperature to 65° does not cause, however, any significant change in the optical rotation.

Measurements of the optical rotatory dispersion of poly-L-serine were also carried out in aqueous 8.5 M LiBr–2-chloroethanol (1:1 v/v). The dispersion, at each of the temperatures studied, could be described by a one-term Drude equation, the characteristics of which are given in Table VI. Of particular interest is the finding that the dispersion constant, λ_c, increases markedly with an increase in the temperature from 30° to 50°. The observed direction of change of the dispersion constant, λ_c, with temperature is opposite to that encountered in most proteins, where it has been shown that denaturation is accompanied by a decrease in the value of λ_c from 250–270 mμ to 200–220 mμ (Linderstrøm-Lang and Schellman, 1954). An abnormal increase in λ_c as a result of denaturation has been

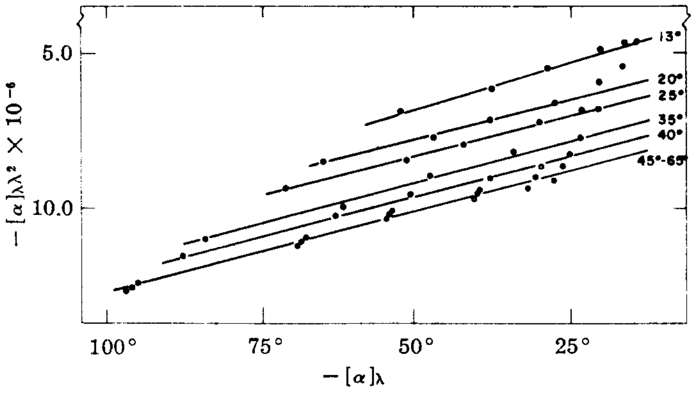


FIG. 2.—Optical rotatory dispersion of poly-L-serine in aqueous 8.5 M LiBr at different temperatures.

recorded for proteins possessing a relatively high content of serine, such as human γ-globulin, Bence-Jones protein, and pepsin (Jirgensons, 1958, 1959). Fasman and Blout (1960) reported for their partially racemized poly-L-serine λ_c values of 250 mμ, 212 mμ, and 206 mμ in 8 M urea, water, and dioxane-water (70:30 v/v), respectively. It thus seems that also in this case the λ_c value obtained for a medium (dioxane-water) known to favor a helical conformation in proteins and polypeptides (Yang and Doty, 1957) is considerably lower than the corresponding value obtained for a medium favoring a random-coil structure (8 M urea) (Schellman, 1958; Schellman and Schellman, 1958). A value of $b_0 = -20°$ to $-50°$ was obtained when the optical rotatory dispersion of poly-L-serine in aqueous lithium bromide or in aqueous lithium bromide-2-chloroethanol was described by the well-known Moffitt equation (Moffitt, 1956; Doty, 1959), assuming $\lambda_0 = 212$ mμ. A value of $b_0 \cong 0$ was reported for films of water-soluble *Bombyx mori* fibroin (Elliott *et al.*, 1958), where the absence of an α-helix conformation has also been corroborated by infrared measurements. In view of the above observations, it might be concluded that poly-L-serine does not attain an α-helix conformation in the solvent systems investigated. It should be noted, however, that Liquier-Millward (1930), Darmois (1930), and Bigelow and Geschwind (1961) have shown that the presence of salts at high concentration can significantly alter the optical rotation of compounds in which no helix formation is possible. It is, therefore, questionable, at our present state of knowledge, whether changes in optical rotation accompanying changes of solvent can be interpreted in terms of helix formation or breakdown.

TABLE VI
OPTICAL ROTATORY DISPERSION OF POLY-L-SERINE AT DIFFERENT TEMPERATURES[a]

	Solvent					
Temperature (°C)	Aqueous 8.5 M LiBr			Aqueous 8.5 M LiBr–2-Chloroethanol (1:1, v/v)		
	$[\alpha]_{546}$	$10^{-6} \times K$	λ_c (mμ)	$\alpha[_{546}]$	$10^{-6} \times K$	λ_c (mμ)
13	−15.4	−3.7	249	—	—	—
15	—	—	—	−19.8	−5.3	197
16	−19.4	−4.6	233	—	—	—
20	−19.9	−5.1	233	−20.3	−5.3	197
25	−22.5	−5.6	233	−20.9	−5.3	197
30	−26.1	−6.2	232	−21.9	−5.8	196
35	−29.5	−6.4	236	—	—	—
40	−28.8	−7.0	233	−21.5	−5.0	243
45	−31.4	−7.4	232	−27.4	−6.3	265
50	−28.0	−7.4	232	−28.7	−6.4	260
60	−30.3	−7.3	232	—	—	—
63	—	—	—	−27.8	−6.3	265
65	−29.5	−7.4	232	—	—	—

[a] The values of K and λ_c appearing in the one-term Drude equation $[\alpha]_\lambda = K/(\lambda^2 - \lambda_c^2)$ were calculated from the linear plots of $[\alpha]_\lambda\lambda^2$ *versus* $[\alpha]_\lambda$.

Optical Rotatory Dispersion of Poly-O-benzyl-L-serine in Solution.—The change in the specific optical rotation at 546 mμ of poly-*O*-benzyl-L-serine with solvent composition in the systems ethylene dichloride–dichloroacetic acid and ethylene dichloride–trifluoroacetic acid is presented in Figure 3. In the ethylene dichloride–dichloroacetic acid system a significant increase in the specific rotation occurred when the volume % of dichloroacetic acid was decreased below 10. The polymer is insoluble in ethylene dichloride, and therefore no measurements could be carried out in this solvent. The optical rotatory dispersion in all the solvent systems tested could be described by a one-term Drude equation with $\lambda_c = 200 \pm 15$ mμ. It is of interest to note that the optical rotation of poly-*O*-acetyl-L-serine varies with solvent composition, in ethylene dichloride–dichloroacetic acid and $CHCl_3$–dichloroacetic acid mixtures, in a manner similar to that described for poly-*O*-benzyl-L-serine in the ethylene dichloride–dichloroacetic acid system. Thus the optical rotation at 546 mμ of poly-*O*-acetyl-L-serine increases from ~ + 40° to ~ + 120° when the volume % of dichloroacetic acid is decreased from 50 to 40% (Fasman and Blout, 1960).

The infrared absorption spectrum of poly-*O*-benzyl-L-serine in powder form, in a KBr disc, reveals two characteristic amide absorption bands, the first at 1640 cm^{-1} (amide I) and the second at 1500–1505 cm^{-1} (amide II). The x-ray powder diagram showed three rings corresponding to spacings of 4.6 ± 0.2 A (very strong) and to 3.8 A and 2.9 A, suggesting a β-conformation.

The optical rotatory dispersion data described above suggest that poly-*O*-benzyl-L-serine exists as a random coil in the ethylene dichloride–dichloroacetic acid solvent system at dichloroacetic acid concentrations exceeding 10% by volume. At lower dichloroacetic acid concentrations aggregation sets in and the increase in the optical rotation is most likely due to the appearance of a β-conformation. (For the optical rotatory behavior of the β-structure of poly-γ-benzyl-L-glutamate in ethylene dichloride, see Wada *et al.*, 1961.) A similar explanation was proposed by Fasman and Blout (1960) to account for the optical rotatory behavior of poly-*O*-acetyl-L-serine in the $CHCl_3$–dichloroacetic acid system.

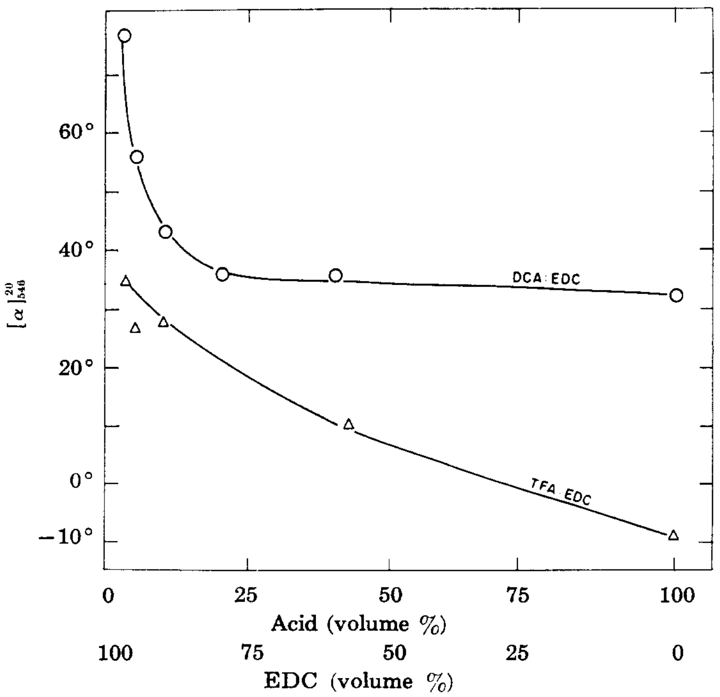


FIG. 3.—Specific optical rotation of poly-*O*-benzyl-L-serine at 546 mμ in dichloroacetic acid (DCA)–ethylene dichloride (EDC) and trifluoroacetic acid (TFA)–ethylene dichloride (EDC).

Experimental

Solvents.—Tetrahydrofuran was kept for 4 days over potassium hydroxide pellets (100 g per liter), filtered, and refluxed for 12 hours over metallic sodium. The solid was filtered off and the solvent distilled from a sodium-potassium alloy and kept in a brown glass bottle over sodium wire. The tetrahydrofuran used in the preparation of the *N*-carboxyserine anhydride derivatives was distilled into the reaction vessel from a sodium-potassium alloy.

Bromobenzene was refluxed for 2 days over barium hydroxide and distilled. It was kept over a mixture of aluminium oxide and magnesium sulfate. The bromobenzene used in the polymerization experiments of *O*-benzyl-*N*-carboxyserine anhydride was distilled over a small amount of a sodium wire into the reaction vessel in a closed system at 0.05 mm Hg.

The chloroform used in the chloride determination was purified according to Vogel (1948) and kept in a brown bottle over anhydrous potassium carbonate.

Enzymes.—The D-amino acid oxidase used was prepared from hog kidney (250 g) by following the procedure of Massey *et al.* (1961) up to the end of stage 2. The material obtained by precipitation with ammonium sulfate to 0.2 saturation was dissolved in 0.05 M phosphate buffer, *p*H 6.3 (50 ml) and dialyzed overnight against the same buffer. The dialyzed solution was mixed with a suspension of calcium phosphate gel (50 mg) and cellulose powder (350 mg) in 0.05 M phosphate buffer, *p*H 6.3 (5 ml) and centrifuged at 0° for 20 minutes at 20,000 × *g* (Yagi *et al.*, 1960). The clear yellow supernatant was collected and lyophilized. The preparation thus obtained (350 mg), when assayed at 30° with DL-alanine under standard conditions (Burton, 1955), showed a specific activity of 34 units/mg protein. (One unit is defined as that amount of enzyme that will cause the uptake of 1 μl O_2/minute under these conditions.)

Takadiastase was obtained from Parke-Davis and was specified (undiluted) to assay 2000–2500%.

A commercial grade catalase from Worthington was used.

Determination of Chloride in N-Carboxy-α-amino Acid Anhydride Preparations.—A modified spectrophotometric silver-dithizonate method (Kirsten, 1955) was used. The carboxyanhydride (10 mg) was weighed into a Beckman DU tube (10-mm light path), silver-dithizonate reagent was added (3 ml), and the optical density was read at 600 mμ against pure reagent. The reagent was prepared by mixing 1% phosphoric acid in ethyl acetate (15 ml) with 0.01% silver-dithizonate (Pariaud and Archinard, 1952) in anhydrous, phosgene-free chloroform (3 ml). Freshly mixed reagent was used in each run. Calibration was carried out with 0.001 N hydrochloric acid in dioxane added from an Agla buret (0.0 to 0.03 ml). In this concentration range a linear correlation was obtained between the optical density and the concentration of chloride, one μg of chloride corresponding to an optical density of 0.252.

Determination of Serine

(*a*) TOTAL AMOUNT OF SERINE.—The total amount of L- and D-serine was determined by the periodate method (Friesell and Mackenzie, 1958).

(*b*) D-SERINE.—The amount of D-serine in the polyserine hydrolysates was determined by means of D-amino acid oxidase (Greenstein and Winitz, 1961). Samples containing 50 to 100 μmoles of total serine were taken for each assay in Warburg flasks. Each flask contained 40 to 50 units of the D-amino acid oxidase and an excess of catalase in a total volume of 2 ml of 0.02 M pyrophosphate buffer of *p*H 8.3. Five μmoles of D-serine were completely oxidized within 100 minutes and could be estimated within an error of ± 5%.

Pyruvate Determination.—Pyruvate was determined as 2,4-dinitrophenylhydrazone (Friedemann and Haugen, 1943).

Determination of Ester Groups.—The hydroxamic acid method (Hestrin, 1949), as adapted by Dr. Y. Lapidoth of this laboratory, for the determination of ester derivatives of serine was used. The solution or suspension to be tested (1 ml) was incubated for 20 minutes at room temperature with 2 ml of basic hydroxylamine solution, obtained by mixing 2 M hydroxylamine hydrochloride (1 ml) and 3.5 N sodium hydroxide (1 ml). The reaction mixture was acidified with 4 N HCl (1 ml), and exactly 1 minute later 1 ml of 0.37 M $FeCl_3$ in 0.1 N HCl was added and the optical density read in a Klett-Summerson photoelectric colorimeter with filter No. 54.

Optical Rotatory Dispersion.—These measurements were carried out with the aid of a home-made instrument constructed from a Schmidt and Haensch polarimeter and a Beckman DU spectrophotometer. The polarimeter (eyepiece and half shadow prism removed) was inserted between the monochromator and the photomultiplier of the Beckman spectrophotometer, and the "optical density" readings were taken at ± 3° by the method of Gallop (1957). A mercury lamp (GE AH3) was used as light source. Calibration against sucrose yielded the following values for the constant K (in degrees) of the instrument (see Gallop, 1957): 1.93 (365 mμ); 1.91 (405 mμ); 1.89 (436 mμ); 1.86 (492 mμ); 1.83 (546 mμ); and 1.82 (579 mμ). It should be noted that the variation of K with wave length is considerably smaller than that recorded for the Keston Polarimeter attachment in which polaroids are used (Poulsen, 1960; Nebbia, 1960).

Synthetic Procedures

O-Benzyl-DL-*serine*.—(The procedure given is based on that of Okawa and Tani, 1954, and of Grassmann *et al.*, 1958.) Ethyl dibromopropionate (Marvel *et al.*, 1940) (520 g, 2 moles), mercuric acetate (Bretschneider *et al.*, 1953) (16 g), and anhydrous ether (200 ml) were put into a three-necked reaction flask (3 liter) fitted with a strong mechanical stirrer, a dropping funnel (1 liter), and a thermometer. Moisture was excluded by means of $CaCl_2$ drying tubes. Sodium benzylate, obtained by reacting metallic sodium (50 g, 2.2 moles) with benzyl alcohol (600 ml), was mixed with anhydrous ether (1000 ml) and the mixture was added dropwise from the funnel over a 2-hour period. The reaction mixture was kept under vigorous stirring at −5° to 0° by cooling in an ice-salt bath. The cooling bath was then removed and the reaction mixture stirred for another hour at room temperature. Cooling was resumed and 5 N NaOH (440 ml) was added at 5°. Stirring was continued for 1 hour at room temperature, ether (3000 ml) was added, and the aqueous and organic layers were separated. The organic solution was washed twice with water (200-ml portions) and discarded. The aqueous solution was combined with the washings, cooled to 0°, and acidified to congo red with concentrated hydrochloric acid. The α-bromo-β-benzyloxypropionic acid was finally extracted with ether (an 800-ml portion followed by two 400-ml portions). The combined ethereal solution was dried over anhydrous sodium sulfate, and the solvent was evaporated by heating. The crude α-bromo-β-benzyloxy-propionic acid was left as a yellow oil (600 g) and was used in the synthesis of O-benzyl-DL-serine without further purification. Titration with sodium methoxide in ethanol, with thymol blue as indicator, as well as bromide analysis (Volhard), after alkaline hydrolysis, revealed that the crude material contains approximately 90% by weight of the required intermediate.

The crude α-bromo-β-benzyloxy-propionic acid (600 g) was aminated with aqueous 27% ammonia (8000 ml) in a stainless steel autoclave at 80° for 14 hours. The solvent was evaporated under reduced pressure at 45° until a heavy precipitate formed. The crystalline precipitate was filtered and the mother liquor concentrated to 250 ml and cooled, whereupon a further crop of crystals was obtained. The combined precipitate was washed with acetone and anhydrous ethanol and recrystallized from boiling water (1000 ml). Norite A was used for decolorization. Yield of O-benzyl-DL-serine 235 g.

Anal. Calcd. for $C_{10}H_{13}NO_3$: C, 61.5; H, 6.7; N, 7.2. Found: C, 61.2; H, 7.4; N, 7.2.

O-Benzyl-L-*serine*.—*O*-Benzyl-DL-serine was acetylated according to Okawa (1956, 1957) to yield *N*-acetyl-*O*-benzyl-DL-serine, which was used in the enzymatic resolution described below.

Takadiastase (5 g) was mixed with cold distilled water (25 ml) and the suspension centrifuged at 15,000 × *g* for 5 minutes at 0°. The residue was resuspended in cold water (25 ml) and the centrifugation repeated. The combined clear supernatants were added to a solution obtained by mixing *N*-acetyl-*O*-benzyl-DL-serine (72 g) with 3 N NaOH (100 ml), the *p*H of which was adjusted to 7.2. The solution was incubated under toluene at 37°. The crystals of *O*-benzyl-L-serine formed were collected on the third and sixth day, and the *p*H of the remaining solutions was readjusted to 7.1. After further incubation for 4 days the solution was brought to *p*H 5.0 with glacial acetic acid and refrigerated overnight, and the last batch of crystals was collected. Recrystallization of the combined crystalline material was effected from boiling water (150 ml) with Norite A used for the removal of protein contaminants. The purified *O*-benzyl-L-serine was obtained as colorless platelets in a yield of 22 g (72% of theory). $[\alpha]^{20}_{D} = +6.0 \pm 0.4°$ (*c*, 2 in 5 N HCl). A value of $[\alpha]^{20}_{D} = +5.86$ (*c*, 2 in 5 N HCl) has been given by Okawa (1956, 1957).

Anal. Calcd. for $C_{10}H_{13}NO_3$: C, 61.5; H, 6.7; N, 7.2; equivalent weight 195; $C_6H_5C \cdot H_2O$, 54.9. Found: C, 61.4; H, 7.3; N, 7.0; equivalent weight 197 as determined by the Sørensen formol titration (Sørensen, 1908). $C_6H_5C \cdot H_2O$, 55.4 (Patchornik and Ehrlich-Rogozinski, 1961).

The optical purity of the *O*-benzyl-L-serine preparation was tested by the procedure of Greenstein and Winitz (1961a) with use of D-amino acid oxidase. The material synthesized contained less than 0.3 μmoles of *O*-benzyl-D-serine per 1000 μmoles of *O*-benzyl-serine. The *O*-benzyl-L-serine (100 μg) gave a single spot with ninhydrin, $R_F = 0.95$, on a chromatogram developed with *n*-butanol–acetic acid–water (77:16:6 v/v). Chromatography of the acid hydrolysate of *O*-benzyl-L-serine (5.5 N hydrochloric acid for 20 hours at 105°) in the same solvent system gave a single ninhydrin-positive spot with $R_F = 0.22$, corresponding to that of serine.

O-Benzyl-N-carboxy-L-*serine Anhydride*.—*O*-Benzyl-L-serine (10 g) was powdered, dried *in vacuo* (0.05 mm Hg) at 60°, and suspended in an anhydrous tetrahydrofuran (250 ml). Dry phosgene was passed through the mixture for 1.5 hours at room temperature with rigorous exclusion of moisture. Excess phosgene was removed from the resulting clear solution by a stream of nitrogen and the solvent evaporated under reduced pressure. The remaining oil was kept for 15 minutes *in vacuo* (0.05 mm Hg) and triturated with petroleum ether (b.p. 40–60°). The crude crystalline *N*-carboxyanhydride obtained was recrystallized by dissolution in boiling ether (150 ml), filtration through a sintered glass filter covered with a 5-mm layer of kieselguhr, and addition of petroleum ether (450 ml).

Further purification was obtained by recrystallization from ethyl acetate–petroleum ether at −20°. Recrystallizations from ether–petroleum ether and ethyl acetate–petroleum ether were repeated until the colorless crystals contained 8 × 10^{-5} moles Cl^- per mole anhydride. Yield 5 g; m.p. 72° (decomp.).

Anal. Calcd. for $C_{11}H_{11}NO_4$: C, 59.7; H, 5.0; N, 6.3; equivalent weight 221. Found: C, 60.5; H, 5.0; N, 6.5; equivalent weight 224, as determined by titration in dioxane with 0.1 M sodium methoxide with thymol blue as indicator (Berger *et al.*, 1953).

Poly-O-benzyl-L-serine.—*O*-Benzyl-*N*-carboxy-L-serine anhydride (5.5 g) was dissolved in purified bromobenzene (110 ml) and 3.5 M sodium methoxide in methanol was added to give a molar monomer-to-initiator ratio of 200:1. The reaction mixture was kept in a dry box for 3 days at room temperature. The resulting gelatinous mass was poured into a mixture of ether (500 ml) and petroleum ether (500 ml), and the precipitate formed was centrifuged. It was washed with boiling methanol followed by boiling water and dried *in vacuo* (0.1 mm Hg) for 12 hours at 60°. Yield of poly-*O*-benzyl-L-serine, 3.2 g.

Anal. Calcd. for $(C_{10}H_{11}NO_2)_n$: C, 67.8; H, 6.3; N, 7.9. Found: C, 67.9; H, 6.4; N, 7.7.

The polymer obtained had a reduced specific viscosity (η_{sp}/c) of 0.31 dl/g (*c*, 0.5 g per 100 ml dichloroacetic acid) at 30° and an approximate weight average molecular weight of 52,000 as calculated from light-scattering data in dichloroacetic acid.

Poly-*O*-benzyl-L-serine is insoluble in water, dioxane, chloroform, and bromobenzene. It is soluble in dichloroacetic acid and trifluoroacetic acid, and stays in solution on addition of an excess of dioxane, chloroform, bromobenzene, and ethylene dichloride. It may be precipitated from its solutions in dichloroacetic acid and trifluoroacetic acid by addition of water, dimethylformamide, glacial acetic acid, or formic acid.

Poly-L-serine.—Poly-*O*-benzyl-L-serine (3.0 g) was suspended in cold dioxane (60 ml) previously saturated with dry bromine-free hydrogen bromide, and the suspension was kept at 2–4° overnight. The mixture was poured into anhydrous ether (600 ml), and the precipitate was centrifuged, washed with methanol until free of benzyl bromide, and dried *in vacuo* over phosphorous pentoxide. Purification of the water-insoluble poly-L-serine obtained was accomplished by dissolution in concentrated lithium bromide solution and precipitation by dialysis against water. The dry polymer (1.3 g) was dissolved in 8.5 M LiBr (35 ml) and the solution clarified by centrifugation at 20,000 × *g*, suspended in water, and recentrifuged. This procedure was repeated until the washings were free of bromide. The purified gel was lyophilized and kept in a desiccator over phosphorous pentoxide. Yield 0.80 g. Complete debenzylation by the above procedure was proved by the absence of benzyl groups in the final product as determined by the method of Patchornik and Ehrlich-Rogozinski (1961).

Anal. Calcd. for $(C_3H_5O_2N{\cdot}H_2O)_n$: C, 34.3; H, 6.7; N, 13.3; amino N, 0. Found: C, 35.2; H, 6.7; N, 13.7; amino N, 0.2 (Van Slyke).

The elementary analysis indicates that the above poly-L-serine preparation contains approximately one mole of water per serine residue. The finding that the polymer loses weight on prolonged drying and that its nitrogen content increases to 14.7% after 2 days *in vacuo* (0.1 mm Hg) at 60° over phosphorous pentoxide corroborated this assumption. The anhydrous poly-L-serine is exceedingly hyrogscopic and yields the hydrated polymer on exposure to moisture.

The poly-L-serine prepared gave on hydrolysis with 5.5 N hydrochloric acid for 20 hours at 105° (see Table I) L-serine in a yield of 88.2% of theory. The hydrolysate contained also pyruvic acid in an 8.8% yield. No D-serine could be detected by oxidation with D-amino acid oxidase.

Poly-L-serine is insoluble in water, dioxane, ethanol, dimethylformamide, *m*-cresol, and 2-chloroethanol. It dissolved on heating for 5 minutes at 60° in dichloroacetic acid and trifluoroacetic acid. The dissolution in these cases is most likely accompanied by esterification (Narita, 1959; Rabinowitz, 1960). The polymer is insoluble in 7.5 M aqueous urea, 5.5 M guanidinium hydrochloride, 85% phosphoric acid, or 27% aqueous ammonia. It is soluble in 7.0 to 8.5 M aqueous lithium bromide and can be precipitated out by dilution with water or by increasing the concentration of lithium bromide above 8.5 M. Dissolution can be effected by stirring in 1 M sodium hydroxide. This procedure leads, however, to partial racemization (see Table IV).

Determination of D-Serine in Hydrolysates of Mixtures of Poly-L-serine and Poly-DL-serine.—To estimate the precision with which D-serine can be determined in the acid hydrolysates of the various polyserine preparations obtained, hydrolysates of known mixtures of poly-L-serine and poly-DL-serine were prepared and assayed for D-serine with D-amino acid oxidase (Greenstein and Winitz, 1961a). The results obtained are summarized in Table II. The hydrolysates were prepared and analyzed as follows: Poly-L-serine (in an amount corresponding to 100 to 200 μmole L-seryl residues, as calculated from total N) was mixed with poly-DL-serine (in an amount corresponding to 0.4 to 1.0 μmole D-seryl residues, as calculated from total N), and the mixture was hydrolyzed in 2.5 ml 5.5 N hydrochloric acid at 110° for 20 hours in an evacuated sealed tube. The hydrolysate was evaporated to dryness at 40° (20 mm Hg) and the residue dissolved in 1 ml water. The solvent was removed *in vacuo* and the residue redissolved in a mixture of 0.5 ml water and 0.4 ml 0.5 N NH_4OH. The solution was brought to dryness and the residue again dissolved in 1 ml water. The solution used for analysis was finally obtained by removal of water *in vacuo* and dissolution of the residue in 0.6 ml 0.05 M pyrophosphate buffer, *p*H 8.3. An aliquot of 0.5 ml was withdrawn for determination of D-serine by the D-amino acid oxidase assay, and an aliquot of 0.005 ml for determination of total (D + L)-serine by the periodate method (Friesell and Mackenzie, 1958). In two control experiments DL-serine was added to the acid hydrolysate of poly-L-serine and the amounts of D- and (D + L)-serine determined (Table II).

The data given in Table II show that the recovery of D-serine in the hydrolysates amounts to 78 to 79% of theory. The average recovery of total (D + L)-serine was 88 to 90%. The found molar ratios of D-serine to total (D + L)-serine in the hydrolysates were rather close to the expected values derived from the composition of the corresponding substrate mixtures hydrolyzed.

Deacetylation of Poly-O-acetyl-L-serine with Hydrazine.—A suspension of poly-*O*-acetyl-L-serine (Fasman and Blout, 1960) (500 mg) in 99–100% hydrazine hydrate (25 ml) was stirred at room temperature for 2 hours. The suspension was poured into anhydrous ethanol (500 ml) and the precipitate was collected by filtration, washed with anhydrous ethanol and ether, and dried at 60° *in vacuo* (0.05 mm Hg). The polymer (310 mg) was dissolved in 8.5 M lithium bromide (20 ml) and dialyzed for 3 days against distilled water, and the gel which formed in the dialysis bag was lyophilized, yielding a white powder (285 mg). The polypeptide was devoid of acetyl groups as determined by the hydroxamic acid test. On acid hydrolysis L-serine was obtained in 88% of theory, and no D-serine could be detected in the hydrolysate (Table I). Determination of acyl hydrazide groups (Seifter *et al.*, 1960) revealed that the polypeptide contained 1.7 acyl hydrazide groups per 100 seryl residues.

N-Acetyl-L-serine Amide.—*N*-Acetyl-L-serine amide was prepared from L-serine by the procedure described by Rothstein (1949) for the synthesis of the corresponding racemic compound. M.p. 141°. A m.p. of 138–139° was recorded by Rothstein for *N*-acetyl-DL-serine amide. The optically active compound in water (*c*, 0.4%) at 24° gives a specific rotation ($[\alpha]_\lambda^{24}$) of −135°, −26.4°, and −9.4° at λ = 300 mμ, 400 mμ, and 500 mμ respectively.

Anal. Calcd. for $C_5H_{10}N_2O_3$: C, 41.1; H, 6.9; N, 19.2. Found: C, 41.3; H, 6.75; N, 19.0.

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