of certain groups between soluble and crystalline proteins are expected, since a small number of these groups are likely to participate in intermolecular interactions responsible for molecular order in the crystal. Five to ten ionizable groups are involved in interactions of this sort in myoglobin, a sufficient number to explain the titration differences found for the larger hemoglobin molecule. Any structure comparison between crystal and solution which is based upon their reactivities must consider new intermolecular interactions as a source of differences, and only a difference unaccountable in these terms can be used to infer a structural change.

The titration data just quoted and those obtained in this work show that ionizing groups in the crystalline protein are accessible and behave normally. They participate with hydrogen ions in rapidly established equilibria, characterized by constants the same or nearly the same as for the soluble protein. Consequently, it is reasonable to expect that reactions such as those routinely used for protein study will yield data interpretable in terms of a comparison between the crystal and solution. Several such experiments have recently been reported. Banaszak *et al.* (1963) studied the reaction of bromoacetate with the histidines of myoglobin, and found that the same groups reacted in the crystal and solution. Doscher and Richards (1963) have shown that ribonuclease is enzymically active in the crystal, and that only small changes in X-ray diffraction pattern are caused by substrate or inhibitor binding to the crystalline protein. Praissman and Rupley (1964) have studied the tritium-hydrogen-exchange behavior of insulin in solution and in the crystal; differences were observed which require explanation in terms of a structural change. More experiments of this sort are needed, and one hopes they will be forthcoming.

ACKNOWLEDGMENT

The able technical assistance given by Mr. Peter Monheit is gratefully acknowledged.

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Conformational Aspects of Polypeptide Structure XIII. A Nonionic Helical Polypeptide in Aqueous Solution*

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Received June 18, 1964

Poly-δ-hydroxy-*O*-acetyl-L-α-aminovaleric acid was prepared from L-glutamic acid and was shown to be in a fully helical conformation in trifluoroethanol and dimethylformamide. In order to avoid the problem of racemization during deacetylation of poly-δ-hydroxy-*O*-acetyl-L-α-aminovaleric acid, we utilized a method involving amidolysis with 3-aminopropanol. Poly-δ-hydroxy-L-α-aminovaleric acid was isolated and found to be soluble in 0.75–9.8 M aqueous lithium bromide and in hexafluoroacetone trihydrate. The conformational studies are based primarily on optical rotatory dispersion measurements, and reveal a transition from the random coil at lithium bromide concentrations greater than 2.75 M to the helical conformation at about 2.0 M lithium bromide concentration. In 0.75 M lithium bromide and in hexafluoroacetone trihydrate poly-δ-hydroxy-L-α-aminovaleric acid is essentially completely helical. The polypeptide precipitates from solution at concentrations of lithium bromide below 0.75 M.

Since water forms the natural environment of proteins, poly-α-amino acids can serve most effectively as model structures of protein conformations if they are studied in aqueous solution. Fasman and Blout (1960) attempted to prepare a water-soluble, nonionic, high-molecular-weight, synthetic polypeptide, composed of one optical isomer. Their studies were carried out on poly-L-serine since hydroxyl side groups usually facilitate water solubility. However the poly-L-

* This research was generously supported by grants from the National Institutes of Health (RG8974) and from the National Science Foundation (GB256).

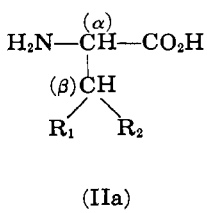
† National Institutes of Health predoctoral fellow, 1963–1964.

serine was prepared from its acetyl derivative, and when the protecting group was removed some racemization occurred which caused the polyserine to be water soluble. Optically pure poly-L-serine was recently prepared (Bohak and Katchalski, 1963) from poly-*O*-benzyl-L-serine by debenzylation with anhydrous hydrogen bromide in dioxane. It was found to be insoluble in water and soluble in hot dichloroacetic acid, trifluoroacetic acid, and 7.0–8.5 M aqueous lithium bromide. Their studies in solution were limited since the polymer is precipitated when the concentration of lithium bromide is increased above 8.5 M or decreased below 7.0 M. They showed that poly-L-serine has a β-conformation in the solid state and does not have an α-helix conformation in the solvent systems investigated.

Berger *et al.* (1961, 1962), and Kulkarni and Blout (1962) succeeded in preparing a water-soluble, nonionic, high-molecular-weight, optically pure, synthetic polypeptide. In the latter case, poly-γ-*N*-[2-morpholinylethyl]-α-L-glutamamide was prepared and found to be in a random conformation in water. However, the polypeptide undergoes a transformation from the random form in water to a helical conformation in aqueous solutions containing more than 50% methanol. They also showed that a copolypeptide of poly-γ-*N*-[2-morpholinylethyl]-α-L-glutamamide with L-methionine is almost completely helical in water when 40% of L-methionine is present in the copolymer.

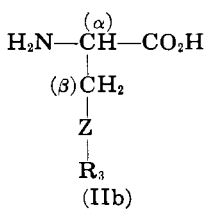
We now wish to report the synthesis and conformational studies of a new polypeptide, poly-δ-hydroxy-L-α-aminovaleric acid (XI), which is helical in dilute solutions of aqueous lithium bromide and in a highly fluorinated solvent, hexafluoroacetone trihydrate, hitherto not used to study polypeptides in solution.

Blout *et al.* (1960) suggested that there are two broad classes of α-amino acids: (I) those which form α-helical structures and (II) those which form either random or β structures. The non-helix-forming polypeptides (II) are in turn of two types: (IIa) those with steric factors that prevent helix formation (R_1 and R_2 disubstitution on the β-carbon atom) and (IIb) those with heteroatoms (oxygen or sulfur) attached directly to the β-carbon atom.



(IIa)

$R_1, R_2 \neq H$



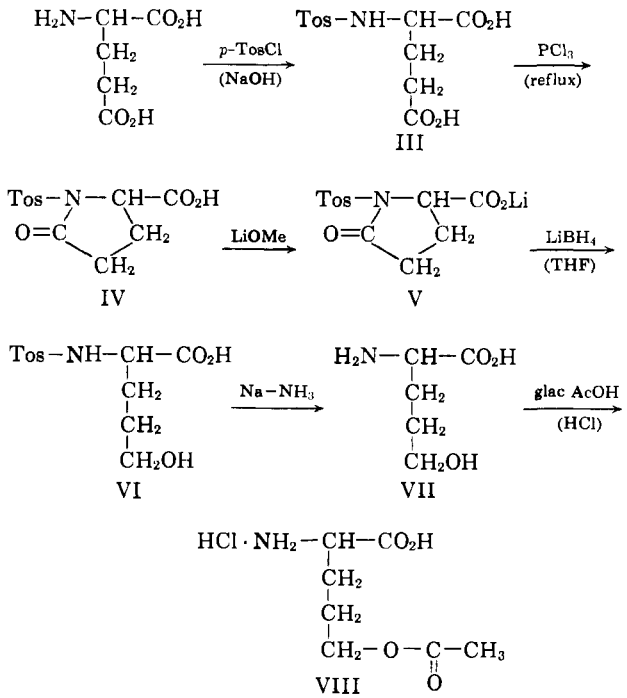
(IIb)

Z = O, S
R_3 = H, alkyl, etc.

It was therefore not surprising to learn that poly-L-serine, poly-*O*-acetyl-L-serine, and poly-*O*-benzyl-L-serine do not form helical structures since they fall into class (IIb). In order to be assured that the hydroxyl function in the side chain does not destroy all helical structure, we prepared optically pure poly-δ-hydroxy-L-α-aminovaleric acid.

Results and Discussion

Synthesis of Poly-δ-hydroxy-L-α-aminovaleric Acid.—The synthesis of poly-δ-hydroxy-L-α-aminovaleric acid involves several unique problems. δ-Hydroxy-DL-α-aminovaleric acid has been prepared from butyrolactone (Plieninger, 1950) and 2,3-dihydrofuran (Gaudry, 1951). The racemic compounds from these preparations were resolved by asymmetric hydrolysis of the *N*-chloroacetyl derivative (Greenstein, 1952; Berlinquet and Gaudry, 1952). Since optically pure polypeptides are essential, we thought it necessary to devise a synthetic scheme that does not depend on resolution. This was achieved by using optically pure L-glutamic acid as the starting material. Scheme I summarizes the reactions we carried out to prepare δ-hydroxy-L-α-aminovaleric acid (VII). This was shown to possess essentially the same degree of optical purity as the product obtained by enzymatic resolution of the racemic material.



SCHEME I.—PREPARATION OF δ-HYDROXY-L-α-AMINOVALERIC ACID AND δ-HYDROXY-*O*-ACETYL-L-α-AMINOVALERIC ACID.

In order to prepare a polymer from δ-hydroxy-L-α-aminovaleric acid it is necessary to block the hydroxyl function with a suitable protecting group. Although DL-serine-*N*-carboxyanhydride and L-serine-*N*-carboxyanhydride have been successfully synthesized (Fasman and Blout, 1960) it was found that high-molecular-weight polymers could not be obtained from these monomers. Several different protecting groups have been used for hydroxyl functions. Derivatives of serine such as *O*-acetyl were used almost exclusively in polypeptide synthesis (Fasman and Blout, 1960; Sheehan *et al.*, 1956).

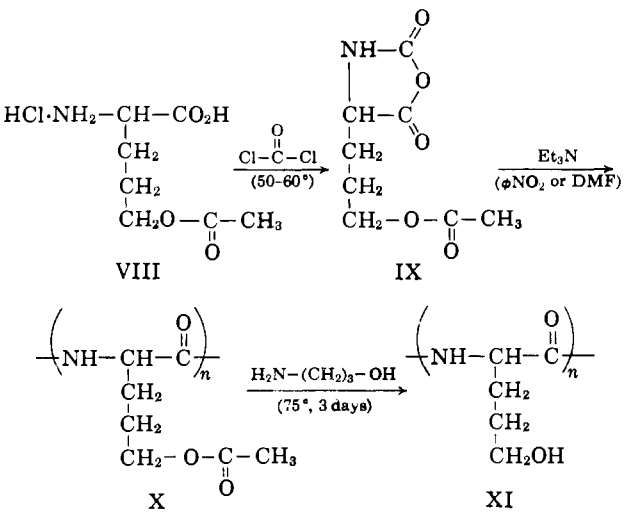
Recently Bohak and Katchalski (1963) showed that deacetylation of poly-*O*-acetyl-L-serine in alkaline medium gives rise to a significant amount of racemization. They also showed that the *O*-benzyl derivative of poly-L-serine could be removed under acidic conditions without racemization. However, *O*-benzyl-L-serine was prepared by a resolution of the racemic compound since direct preparations from L-serine were unsuccessful (Marvel *et al.*, 1940; Okawa and Tani, 1954; Wuensch and Fuerst, 1962).

We undertook the direct preparation of acid-sensitive protected derivatives of L-serine and DL-serine and met with failure. These attempts involved the initial conversion of serine to its copper complex. When the complex was treated with carbobenzoxy chloride or *p*-nitrocarbobenzoxy chloride, the protected chelates were successfully isolated.

Treatment of the complex with benzyl bromide, benzyl chloride, or benzyl iodide under a variety of

reaction conditions failed to give the copper chelate of *O*-benzylserine. This entire approach to the problem of protecting the hydroxyl function by this technique was abandoned when it was discovered that all methods for breaking the chelates also remove the protecting groups.

We decided that the acetyl derivative is the most suitable protecting group. The resulting protected polypeptide can be deacetylated by a new technique using nearly neutral conditions by amidolysis with 3-aminopropanol. Scheme II summarizes the reactions carried out to prepare poly-δ-hydroxy-L-α-aminovaleric acid.



SCHEME II.—PREPARATION OF POLY-δ-HYDROXY-*O*-ACETYL-L-α-AMINOVALERIC ACID AND POLY-δ-HYDROXY-L-α-AMINOVALERIC ACID.

δ-Hydroxy-*O*-acetyl-L-α-aminovaleric acid (X) was polymerized to a reasonably high DP in nitrobenzene using triethylamine as the initiator (A/I = 28) $[\eta]_{c\to o}$ = 0.53 in dimethylformamide. The polymer is soluble in dichloroacetic acid, dimethylformamide, and trifluoroethanol. Deacetylation of the polypeptide to form poly-δ-hydroxy-L-α-aminovaleric acid (XI) was carried out by two methods. Method A involves the treatment of a solution of poly-δ-hydroxy-*O*-acetyl-L-α-aminovaleric acid in nitrobenzene with 1.2 equivalents of methanolic sodium methoxide. The resulting polypeptide was characterized as a monohydrate of poly-δ-hydroxy-L-α-aminovaleric acid. It was completely soluble in dichloroacetic acid and very slightly soluble in water. This slight solubility in water may be an indication of some racemization since it was shown (as noted above) that the homologous optically pure poly-L-serine is completely insoluble in water (Bohak and Katchalski, 1963). However, when small amounts of DL-serine residues are incorporated in the chain, the polypeptide becomes markedly soluble in water (Fasman and Blout, 1960).

Bohak and Katchalski (1963) reported that exhaustive deacetylation of poly-*O*-acetyl-L-serine with hydrazine does not cause racemization and yields a water-insoluble poly-L-serine. However, they reported partial hydrazinolysis of the peptide backbone. We succeeded in preparing poly-δ-hydroxy-L-α-aminovaleric acid by deacetylation with 3-aminopropanol. The polymer prepared by this method is a hemihydrate and is completely insoluble in water. It is also soluble in dichloroacetic acid, 0.75–9.8 M aqueous lithium bromide, and exhibits an intrinsic viscosity, $[\eta]_{c\to o}$ = 0.14 in dichloroacetic acid. The polypeptide precipitates from solution at concentrations of lithium bromide less than 0.75 M.

Conformational Studies of Poly-δ-hydroxy-O-acetyl-L-α-aminovaleric Acid.—Optical rotatory dispersion (ORD)[1] studies of poly-δ-hydroxy-*O*-acetyl-L-α-aminovaleric acid were carried out in dimethylformamide, trifluoroethanol, and dichloroacetic acid. We based conformational assignments in part on two parameters, λ_c and b_0, derived from ORD measurements which have been very useful for detecting helicity in other similar polypeptides. As the fraction of helicity for a polypeptide increases, the b_0 approaches a theoretical value of −630 for the right-handed helix (Moffitt, 1956a,b). At the same time the λ_c plot from the Drude equation becomes nonlinear (Yang and Doty, 1957). It has also been empirically shown that most polypeptides have a conformationally dependent Cotton effect with a trough at 233 mμ and an inflection point at 225 mμ (Simmons *et al.*, 1961). Therefore we possess an additional method for determining helicity which involves direct measurement of the Cotton effect at 233 mμ. For poly-γ-benzyl-L-glutamate in a fully helical conformation this Cotton effect is negative with a residue rotation, $[R']_{233}$ = −12,700. When $[R']_{233}$ = −1800 the L-configuration polypeptide is assumed to be in a fully random conformation. Table I summarizes the optical rotatory properties of poly-δ-hydroxy-*O*-acetyl-L-α-aminovaleric acid.

TABLE I

SPECIFIC ROTATIONS AND DERIVED CONSTANTS FOR POLY-δ-HYDROXY-*O*-ACETYL-L-α-AMINOVALERIC ACID AT 25.0°

Solvent	$[\alpha]^{25}_{546}$	b_0	λ_c	$[R']_{233}$
Dimethylformamide	+10.1[a]	−534	Nonlinear	
Trifluoroethanol	−26.5	−757	Nonlinear	−15,400
Dichloroacetic acid	−31.4	+95.9		

[a] This value was measured at 550 mμ.

From Table I it can be concluded that poly-δ-hydroxy-*O*-acetyl-L-α-aminovaleric acid is in a fully helical conformation in dimethylformamide and trifluoroethanol. The unusually large values of b_0 and $[R']_{233}$ (−757 and −15,400, respectively) in trifluoroethanol have been observed for other helical polypeptides in this solvent (M. Goodman and M. A. Stake, unpublished results). In dichloroacetic acid (a helix-breaking solvent) the b_0 value of +96 indicates that most of the secondary structure has been destroyed.

Conformational Studies of Poly-δ-hydroxy-L-α-aminovaleric Acid.—Optical rotatory dispersion studies of poly-δ-hydroxy-L-α-aminovaleric acid (XI) were carried out only on the sample obtained from amidolysis of the *O*-acetyl precursor in hexafluoroacetone trihydrate, 0.75–9.8 M aqueous lithium bromide, and dichloroacetic acid. In hexafluoroacetone trihydrate, the b_0, λ_c, and $[R']_{233}$ values are −607, nonlinear, and −11,600, respectively.

These results indicate that poly-δ-hydroxy-L-α-aminovaleric acid is in a fully helical conformation in hexafluoroacetone trihydrate.[2] This secondary structure is almost completely destroyed in dichloroacetic acid where b_0 = +33. Measurements of b_0 and $[R']_{233}$ were also carried out in aqueous lithium bromide at polymer concentrations between 0.19 and 0.23% at

[1] Abbreviation used in this work: ORD, optical rotatory dispersion.

[2] Our laboratory is currently engaged in the study of hexafluoroacetone trihydrate and other fluorinated solvents on polypeptide solubility and structure. These results will be reported elsewhere.

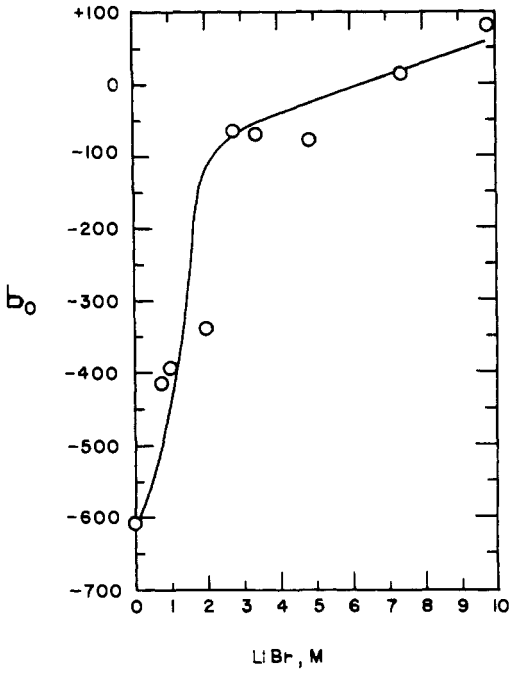


FIG. 1.—The plot of b_0 versus molarity of lithium bromide. The measurement at 0.0 M LiBr was taken in hexafluoroacetone trihydrate.

25.0°. The polymers were dissolved in a small measured volume of 9.8 M aqueous lithium bromide and diluted with water to give the required concentrations. When dilutions exceed 0.75 M LiBr, the polymer precipitates from solution. Figures 1 and 2 depict the change in b_0 and $[R']_{233}$ with molarity of lithium bromide. It can be seen that there is a transition from the random to the helical conformation at about 2 M LiBr. In 2.75 M LiBr the b_0 and $[R']_{233}$ values are, respectively, −65 and −4700; in 2.0 M LiBr the values are −340 and −7380, respectively. At lower salt concentrations the trend toward increased helicity continues: in 0.75 M LiBr the b_0 and $[R']_{233}$ values were −414 and −11,300, respectively.

We also observed a slight blue shift in the Cotton-effect trough as the polypeptide went from the random coil to the helical conformation.[3] Therefore, in 9.8 M, 9.2 M, and 7.4 M LiBr solutions the Cotton-effect trough appeared at 235 mμ. At LiBr concentrations ≤4.9 M the trough appeared at 233 mμ.

EXPERIMENTAL

Materials.—L-Glutamic acid was purchased from the Mann Research Laboratories. Benzene and dioxane (Brothers Chemical Corp., Orange, N. J.) were refluxed over, distilled from, and stored over sodium ribbon. Dimethylformamide was fractionally distilled at reduced pressure. Triethylamine (Matheson, Coleman and Bell) was distilled from calcium hydride at atmospheric pressure. Trifluoroethanol (Columbia Organic Chemical Co., Columbia, S. C.), and dichloroacetic acid (Fisher, purified) were used without further purification. Hexafluoroacetone trihydrate was kindly furnished by the Allied Chemical Corp. Chloroform (Brothers Chemical) was purified by refluxing over and distillation from phosphorus pentoxide. Tetrahydrofuran (Matheson, Coleman and Bell) was purified by standing over potassium hydroxide, decantation, and distillation from lithium aluminum hydride.

[3] This effect was first observed by M. Goodman and I. G. Rosen in a different series of measurements.

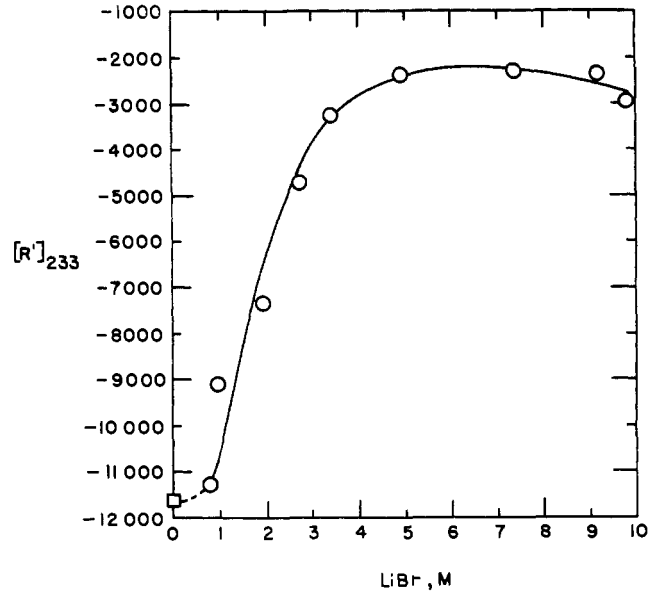


FIG. 2.—The plot of $[R']_{233}$ versus molarity of lithium bromide. The measurement at 0.0 M LiBr was taken in hexafluoroacetone trihydrate. The points at 9.8, 9.2, and 7.4 M LiBr were observed at 235 mμ.

Optical Rotatory Dispersion.—Measurements of b_0 were carried out on the Rudolph Model 80 photoelectric polarimeter equipped with a constant-temperature bath set at 25.0 ± 0.5° and a xenon light source (Hanovia 150-w 901-Cl source). Measurements of b_0 were computed using λ = 589–370 mμ and path length = 2 dm. The ultraviolet-rotatory-dispersion measurements in the 233-mμ region and some b_0 measurements were carried out on the Bendix-Ericsson Model B automatic recording spectropolarimeter equipped with a xenon light source as before. The jacketed cells (prepared by Optical Cell Co., Inc., Brentwood, Md.) had path lengths of 1.0–5.0 mm and were held at 25.0 ± 0.3° by means of a constant-temperature bath. Residue rotations were corrected for the refractive index of the solvent at 589 mμ. The concentration of the sample used for the b_0 measurements was the same as that used for determination of $[R']_{233}$.

Synthetic Procedures

N-p-Toluenesulfonyl-L-glutamic Acid (III).—This compound was prepared by the method of Rudinger *et al.* (1959) from L-glutamic acid and crude *p*-toluenesulfonyl chloride. The reaction mixture afforded an 80% yield of product with mp 123.5–126.0°. Recrystallization from water gave a product with mp 127.5–128.2°.

Anhydrous 1-p-Toluenesulfonyl-L-pyrrolid-5-one-2-carboxylic Acid (IV).—This material was prepared by a modification of the procedure of Rudinger *et al.* (1959). A mixture of dry, finely powdered *N-p*-toluenesulfonyl-L-glutamic acid (III) (30 g, 0.099 mole) in dry chloroform (300 ml) was placed in a 500-ml round-bottomed 1-necked flask equipped with a distilling column and head, heating mantle, and magnetic stirrer. The reaction took place in a well-ventilated hood. Phosphorus trichloride (10 ml, 15.7 g, 0.11 mole) was added cautiously and the suspension gently refluxed until the solution cleared (5 minutes). The solvent was removed under reduced pressure and the glassy residue was triturated with 100 ml of water and decanted, and the process was repeated twice more. Cooling overnight at −5° afforded 24.6 g (82%) of white crystalline monohydrate, mp 69–73° decomp. The monohydrate

was converted to anhydrous 1-*p*-toluenesulfonyl-L-pyrrolid-5-one-2-carboxylic acid by azeotropic distillation with benzene containing a small volume of ethanol to dissolve the monohydrate. On cooling overnight at −5° the anhydrous product was obtained almost quantitatively; mp 126.5–128.5° decomp (Rudinger [1954a], reported mp 129–130°).

Lithium 1-p-Toluenesulfonyl-L-pyrrolid-5-one-2-carboxylate (V).—Anhydrous 1-*p*-toluenesulfonyl-L-pyrrolid-5-one-2-carboxylic acid (IV) (4.25 g, 0.015 mole) was dissolved in 50 ml of anhydrous methanol. To this, lithium ribbon (0.104 g, 0.015 mole) which was reacted with 50 ml of anhydrous methanol was added with stirring. Stirring continued for 10 minutes, after which the solution was evaporated to dryness leaving 4.33 g (100%) of the salt; mp >275°.

δ-Hydroxy-L-α-tosylaminovaleric Acid (VI).—A mixture of the lithium salt (V) (2.90 g, 0.010 mole) in dry tetrahydrofuran (40 ml) was placed in a 500-ml round-bottomed 3-necked flask equipped with a reflux condenser, dropping funnel, heating mantle, and magnetic stirrer. Lithium borohydride (0.78 g, 0.042 mole) in dry tetrahydrofuran (20 ml) was added dropwise with stirring over a period of 10 minutes. An additional 20 ml of tetrahydrofuran was used for rinsing the dropping funnel. The reaction mixture was stirred and refluxed overnight, after which the solvent was removed under reduced pressure. The residue was diluted in 40 ml of water and acidified by careful addition of 5 M HCl to *p*H 2. After cooling at 0° there was obtained a yield of 1.87 g (65%) of white crystals. Crystallization from benzene-ethanol gave a product having mp 162.0–162.5° decomp $[\alpha]_D^{25}$ = 16.92 (*c* = 1.6, absolute ethanol); neutralization equivalent = 291 (theory = 287).

Anal. Calcd for $C_{12}H_{17}NSO_5$: C, 50.16; H, 5.97; N, 4.88. Found: C, 50.57; H, 6.23; N, 4.73.

The reduction was also carried out in slightly lower yield by generating lithium borohydride *in situ* from potassium borohydride–lithium chloride.

δ-Hydroxy-L-α-aminovaleric Acid (VII).—This compound was prepared by a modification of the procedure used by Rudinger (1954b) for the detosylation of L-glutamine. δ-Hydroxy-L-α-tosylaminovaleric acid (VI) (5.74 g, 0.02 mole) was placed in a 3-necked round-bottomed flask equipped with a mechanical stirrer and glass paddle, and fitted with two guard tubes filled with sodium hydroxide pellets. Liquid ammonia (200 ml) was added carefully and the solution was stirred until clear. Sodium (~3 g, ~0.130 g-atom) was added gradually in thin slices to the rapidly stirring solution. The end of the reaction was indicated by a dark-blue coloration stable for more than 3 minutes. Solid ammonium acetate was added to this blue solution until the blue coloration disappeared. The ammonia was allowed to evaporate by continued stirring at room temperature. The last traces of ammonia were removed by evacuating the flask at the water aspirator and warming to 40°. The dry residue was dissolved in 40 ml of ice water and stirred for 1 hour with 20 g of a carboxylate-ion exchanger (Amberlite IRC-50) in the form of its ammonium salt. The exchanger was removed by filtration and washed with deionized water. The combined aqueous extracts were evaporated *in vacuo* at less than 40° to a volume of 30 ml. Barium acetate (1 M) (30 ml) was added and the precipitate removed by the combined processes of centrifugation and decantation. The precipitate was washed with two more portions of deionized water (25 ml each). The aqueous extracts were combined and freeze dried to a small volume. A large excess of absolute ethanol was added and the solution was cooled overnight at 0°. Filtration and drying afforded 1.39 g (52%) of white crystalline amino acid which gave a positive ninhydrin-spot test. After two more crystallizations from ethanol-water, material with mp 220–220.5° decomp (uncor) was obtained (lit. [J. Rudinger, private communication] reported mp 221–222° decomp). $[\alpha]_D^{25}$ = 28.2° (*c* = 1.9, 6 M HCl); $[M]_D^{25}$ = 37.5° (*c* = 1.9, 6 M HCl) (lit. [Greenstein and Winitz, 1961] reported $[M]_D^{25}$ = 38.3° [*c* = 2, 5 M HCl] and [Berlinquet and Gaudry, 1952] $[\alpha]_D^{23}$ = 28.8° [*c* = 2, 6 M HCl]) for product prepared by enzymatic resolution of the stereoisomers.

Anal. Calcd for $C_5H_{11}NO_3$: C, 45.10; H, 8.33; N, 10.52. Found: C, 44.98; H, 8.16; N, 10.27.

δ-Hydroxy-O-acetyl-L-α-aminovaleric Acid Hydrochloride (VIII).—This compound was prepared by a modification of the procedure used by Sheehan *et al.* (1956) for *O*-acetyl-L-serine hydrochloride. A solution of δ-hydroxy-L-α-aminovaleric acid (VII) (0.62 g, 0.0047 mole) in 31 ml of fresh glacial acetic acid was placed in a 125-ml 3-necked round-bottomed flask. The solution was suspended in an ice bath and hydrogen chloride was bubbled in over a period of 25 minutes to ensure saturation. The pale-yellow solution was stoppered and stood overnight at room temperature. The reaction mixture was evaporated *in vacuo* at less than 40° to a clear oil. The oil was taken up in 31 ml of glacial acetic acid and saturated again with hydrogen chloride, stoppered, and allowed to stand overnight. The reaction mixture was evaporated to dryness again leaving a solid yellow residue. The residue was taken up in 10 ml of absolute ethanol, precipitated with an excess of anhydrous ether (200 ml) and placed in the refrigerator overnight. Filtration in the dry box afforded 0.82 g (83%) of pale-yellow solid. Crystallization from ethanol-ether gave product with mp 127–128° decomp; $[\alpha]_D^{25}$ = 19.82° (*c* = 1.0, 6 M HCl).

δ-Hydroxy-O-acetyl-L-α-aminovaleric Acid (VIIIa).—It was also possible to prepare the free amino acid by a modification of the procedure described above for the hydrochloride. After the usual reactions with glacial acetic acid and hydrogen chloride the residue obtained on evaporation was diluted in a minimum of deionized water and run through a column containing Amberlite IR-45 ion exchanger. Additional volumes of deionized water were run through the column until the eluate gave a negative ninhydrin-spot test. The large aqueous solution was freeze dried to dryness leaving an 89% yield of amino acid. Crystallization from water-ethanol-ether gave product having mp 219–220° decomp (uncor), $[\alpha]_D^{25}$ = 23.34° (*c* = 1.14, 6 M HCl).

Anal. Calcd for $C_7H_{13}NO_4$: C, 47.99; H, 7.48; N, 8.00. Found: C, 47.70; H, 7.20; N, 8.47.

δ-Hydroxy-O-acetyl-L-α-aminovaleric Acid N-Carboxyanhydride (IX).—This preparation was carried out by a modification of the procedure used by Fasman and Blout (1960) for *O*-acetyl-L-serine *N*-carboxyanhydride. δ-Hydroxy-*O*-acetyl-L-α-aminovaleric acid (VIIIa) (3.30 g, 0.019 mole) was suspended in dry dioxane (135 ml) in a 3-necked 250-ml round bottomed flask equipped with a magnetic stirring apparatus and immersed in an oil bath. The flask was fitted with a gas-inlet tube and a water-jacketed distilling column attached to a toluene–ammonium hydroxide–trap system. The entire apparatus was placed in a well-ventilated hood. The mixture was gently stirred while phosgene was bubbled in for 1.5 hours and the temperature maintained at 50–60°. Nitrogen was then passed through the clear solution for another 1.5 hours. The reaction mixture was evaporated to dryness *in vacuo*. The thick yellow oil that remained resisted all attempts at crystallization. The last traces of solvent were removed by pumping the

oil out *in vacuo* for several hours leaving 3.18 g (83.7%) of a thick pale-yellow oil whose infrared spectrum revealed all the peaks characteristic of an *N*-carboxyanhydride. Compound IX was also prepared as a thick oil from the amino acid hydrochloride (VIII) in 96.3% yield. Its infrared spectrum was identical to that observed when the free amino acid was used as starting material.

Poly-δ-hydroxy-O-acetyl-L-α-aminovaleric Acid (X).—METHOD A.—δ-Hydroxy-*O*-acetyl-L-α-aminovaleric acid *N*-carboxyanhydride (IX) (379 mg, 1.88 mmoles) was dissolved in dry nitrobenzene (7.5 ml) and initiated with triethylamine (0.17 ml) of 0.4 M in benzene (6.8×10^{-2} mmole, $A/I = 28$). The solution was placed in a desiccator over calcium chloride and polymerization was allowed to proceed for 2 days. The viscous reaction mixture was poured into anhydrous ether (60 ml) with stirring. The precipitated polymer was centrifuged and washed several times with ether. The polymer was dried *in vacuo* at 60° yielding 230 mg (77.7%) of an off-white fibrous solid. It was soluble in dichloroacetic acid, dimethylformamide, and trifluoroethanol, and slightly soluble in chloroform. The viscosity was measured in dimethylformamide (c_0 = 0.5%) and gave $[\lambda]_{c\to 0} = 0.53$. This corresponds to a $\overline{DP}$ of about 370 when compared to the poly-γ-benzylglutamate system (Doty *et al.*, 1956).

Anal. Calcd for $C_7H_{11}NO_3$: C, 53.49; H, 7.06; N, 8.91. Found: C, 53.49; H, 7.24; N, 9.07.

METHOD B.—δ-Hydroxy-*O*-acetyl-L-α-aminovaleric Acid *N*-carboxyanhydride was polymerized under identical conditions as described in method A with the exception that dimethylformamide was used as a solvent. A 78% yield of white crystalline polymer was obtained. Infrared spectra of polymers from methods A and B were found to be identical.

Poly-δ-hydroxy-L-α-aminovaleric Acid (XI).—Poly-δ-hydroxy-*O*-acetyl-L-α-aminovaleric acid (X) was deacetylated by (A), the method used by Fasman and Blout (1960) for the L-serine homolog and (B) by amidolysis with 3-aminopropanol in a manner related to those described independently by Berger *et al.* (1961, 1962) and Kulkarni and Blout (1962).

METHOD A.—Poly-δ-hydroxy-*O*-acetyl-L-α-aminovaleric acid (X) (33.4 mg, 0.213 mmole) was dissolved in nitrobenzene (4 ml). Addition of sodium methoxide (1.19 ml of 0.2141 M in methanol, 0.256 mmole) caused the solution to become somewhat cloudy. The solution was stirred for 2 days and poured into anhydrous ether (25 ml). The precipitated polymer was isolated by centrifugation and was washed several times with ether. The residual yellow solid was suspended in 5 ml of deionized water and dialyzed versus water for 10 hours.[4] The suspension was filtered through a medium sintered funnel and the filtrate was lyophilized, yielding a white fluffy powder, weight 7.65 mg, 31.2%. Several milligrams of uncharacterized tan material were removed during the filtration step. The polypeptide was soluble in dichloroacetic acid and slightly soluble in water.

[4] Cellulose dialyzing tubing No. 4465-A2 obtained from Arthur H. Thomas Co.

Anal. Calcd for $C_5H_9NO_2 \cdot H_2O$: C, 45.10; H, 8.33; N, 10.51. Found: C, 45.05; H, 7.66; N, 9.70.

METHOD B.—Poly-δ-hydroxy-*O*-acetyl-L-α-aminovaleric acid (X) (179 mg, 1.14 mmoles) was dissolved in 3-aminopropanol (812 mg, 0.011 mole) in a test tube. The tube was corked and placed in a bath at 75 ± 5°. After 24 hours the resulting gel was stirred and replaced in the bath for an additional 44 hours. The gel was then dissolved in deionized water and dialyzed versus water for 5 days. The cloudy solution was lyophilized yielding 131 mg of white rubbery polymer (86%). Infrared spectra of polymers from methods A and B were found to be identical. The polypepetide was insoluble in water and every solvent tested, with the exception of dichloroacetic acid, hexafluoroacetone trihydrate, and aqueous lithium bromide.

Anal. Calcd for $C_5H_9NO_2 \cdot {}^1/_2\ H_2O$: C, 48.37; H, 8.12; N, 11.28. Found: C, 48.40; H, 7.91; N, 11.41.

ACKNOWLEDGMENT

We wish to thank Dr. Joseph Rudinger for his extremely helpful suggestions and discussion concerning the synthetic routes to our monomers.

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