Table VII. Half-Wave Potentials[a] and Oxygen Spin Densities of Semiquinones

Quinone	First wave	Second wave	Oxygen Spin Densities		
			Eq 4[b]	Hückel	McLachlen
Pyracyloquinone	−0.738	−1.305	0.06 ± 0.02	0.0478	0.0554
Diketopyracene	−0.908	−1.726	0.29 ± 0.03	0.388	0.414
Acenaphthaquinone	−0.807	−1.656	0.30 ± 0.03	0.356	0.352

[a] *Vs.* a standard calomel electrode. [b] The error derives mainly from the uncertainty in our measurement of the *g* factor.

Half-Wave Potentials. A Sargent Model XV polarograph was converted to a three-electrode, controlled potential instrument by utilizing the output of its motor-driven slide-wire potentiometer as the control potential input of a battery-operated, solid-state potentiostat based on operational amplifier circuitry.[31] The polarographic current was passed through the load resistor network of the Sargent XV and was recorded on its 2.5-mv strip-chart recorder.

The polarographic cell was a water-jacketed, all glass assembly with a 10-cc sample compartment. The anode was a platinum spiral. The reference electrode capillary tip was within 1 cm of the mercury drop. The reference electrode bridge was filled with supporting electrolyte and was separated from the aqueous sce compartment by a fine porosity sintered-glass disk, a renewable electrolyte bridge containing 0.1 *M* tetrabutylammonium iodide in DMSO, and a second sintered-glass disk.

(31) W. M. Schwarz and I. Shain, *Anal. Chem.*, **35**, 1770 (1963).

The sample solution was deaerated with high-purity nitrogen (Linde). Measurements were made at 25.0 ± 0.1°. Half-wave potentials were reproducible to at least ±5 mv. Reported half-wave potentials are averages of cathodic and anodic scans. The potential of the dropping mercury electrode *vs.* the sce was measured at the cell terminals with a Rubicon portable potentiometer at the beginning and end of each scan. The reported half-wave potentials include the liquid junction potential between the aqueous and DMSO solutions.

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Sequence Peptide Polymers. III. Poly Asp(OH)-Ser(H)-Gly and Other Serine-Containing Polymers[1,2]

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Abstract: Poly Asp(OCH$_3$)-Ser(H)-Gly and poly Asp(OH)-Ser(Ac)-Gly have been prepared from the corresponding tripeptide *p*-nitrophenyl ester hydrobromides with molecular weights from 3000 to 11,000. The sequence Asp-Ser-Gly occurs at the active site of several hydrolytic enzymes and the acetylated polymer is a model of the acylated enzymes which are intermediates in the hydrolysis process. The rate of hydrolysis of the acetyl group is, however, comparable to that of certain simple serine derivatives, and much slower than that of the acylated enzymes. Poly Asp(OH)-Ser(H)-Gly and poly Gly-Ser(H)-Gly have been studied briefly.

Sequence peptides which repeat a portion of the active site of an enzyme are of particular interest for the light they may shed on the catalytic process. The present work is concerned with poly Asp(OCH$_3$)-Ser(H)-Gly and with related polymers.[4,5]

Several quite different lines were explored for the synthesis.[6] The preferred route involves the preparation and polymerization of the nitrophenyl ester (**7**) as shown in eq 1–5.[7] Yields of purified product were 60–80% for each step.

Z-Ser(H)-OH (**2**) + HBr-H-Gly-ONP + DCC + TEA ⟶ Z-Ser(H)-Gly-ONP (**5**) (1)

5 + HBr $\xrightarrow{CH_3NO_2}$ HBr-H-Ser(H)-Gly-ONP (**4**) (2)

Z-Asp(OCH$_3$)-OH + **4** + DCC + TEA ⟶ Z-Asp(OCH$_3$)-Ser(H)-Gly-ONP (**8**) (3)

8 + HBr $\xrightarrow{CH_3NO_2}$ HBr-H-Asp(OCH$_3$)-Ser(H)-Gly-ONP (*cf.* **7**) (4)

(tosylate) (**7**) + base ⟶ poly Asp(OCH$_3$)-Ser(H)-Gly (5)

Z-Asp(OBl)-Ser(H)-Gly-ONP (**12**) + AcOH + Ac$_2$O + HBr ⟶ HBr-H-Asp(OH)-Ser(Ac)-Gly-ONP (**11**) (6)

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(2) Much of this work is taken from the Ph.D. Thesis of Fulton F. Rogers, Jr., Florida State University, 1963.

(3) Public Health Fellow 1962–1964.

(4) Part I: D. F. DeTar M. Gouge, W. Honsberg, and V. Honsberg, *J. Am. Chem. Soc.*, 89, 988 (1967).

(5) D. F. DeTar and N. F. Estrin, *Tetrahedron Letters*, 5985 (1966).

(6) Some of these are discussed in ref 4.

(7) The abbreviations are mostly standard, and are described in detail in footnote 4 of ref 4. Numbers identifying peptides are keyed to the Experimental Section for convenience of cross reference.

Table I. Rotations of Serine Derivatives[a]

Compd	Mol wt	Solvent[b]	No. of samples[c]	$a \times 10^{-6}$ [d]	λ_0, mμ[d]	589 mμ[e]	546 mμ[e]	Error[f]
Z-Asp(OBl)-OH	357.3	7–10 HOAc	3,3	*g*	*g*	42.8	50.0	2.5
Z-Ser(H)-OH	239.2	0.3–1 EtOAc	8,8	15.873 ± 0.6	219.76 ± 9	53.2	63.5	1.0
Z-Ser(H)-OH	239.2	4–6 HOAc	3,3	3.9005 ± 0.2	224.30 ± 13	13.2	15.7	2.5
Z-Ser(H)-OH	239.2	2 DMF	4,4	*h*, *i*		−8.7	−10.1	10.0
Z-Ser(H)-OH	239.2	0.6 H_2O	3,3	−3.5398 ± 1.0[i]	152.95 ± 130[i]	−10.9	−12.9	10.0
H-Ser(H)-OH	105.1	2–5 5 *N* HCl	5,5	3.7429 ± 0.2[j]	267.71 ± 9	13.6[j]	16.5[i]	1.8
Z-Ser(H)-Gly-ONP[k,l]	417.4	2–4 1% HOAc 99% DMF	3,3	−5.4642 ± 0.3[m]	181.53 ± 30	−17.4	−20.6	1.8
HBr-H-Ser(H)-Gly-ONP	364.1	2 CH_3OH	6,7	13.976 ± 0.4[m]	246.49 ± 9	48.8	58.9	0.8
HBr-H-Ser(H)-Gly-ONP	364.1	2 H_2O	1,1	17.804 ± 0.2[m]	230.36 ± 4	60.6	72.7	...
HBr-H-Ser(H)-Gly-ONP	364.1	4 1% HOAc 99% DMF	1,1	20.235 ± 0.2[m]	229.21 ± 4	68.7	82.4	...
TosOH-H-Asp(OCH_3)-Ser(H)-Gly-ONP[n]	584.5	2 H_2O	1,1	−15.530 ± 0.3	255.85 ± 6	−55.2	−66.7	...
Z-Asp(OCH_3)-Ser(H)-Gly-ONP[n]	546.5	2 DMSO	1,1	−2.1555 ± 0.4[m]	335.45 ± 24	−9.2	−11.6	...
Z-Asp(OBl)-Ser(H)-Gly-ONP[n]	622.6	4 10% HOAc 90% DMF	5,5	−4.9746 ± 0.3[m]	298.84 ± 14	−19.3	−23.8	1.1
HBr-H-Asp(OH)-Ser(Ac)-Gly-ONP	521.3	2 H_2O	1,1	−9.9813 ± 0.5[m]	259.34 ± 10	−36	−43	...
HBr-H-Gly-Ser(H)-Gly-ONP	421.2	2 DCA	1,1	−40.571	254.86	−144.0	−174.0	...
Z-Gly-Ser(H)-Gly-ONP	474.4	2 DCA	1,1	−33.267	258.12	−119.0	−144.0	...
Poly Asp(OCH_3)-Ser(H)-Gly[n]	273.2	2 DCA	1,1	−21.974	272.60	−81.0	−98.0	...
Poly Asp(OH)-Ser(Ac)-Gly[n]	301.2	2 DCA	1,1	−13.115	301.37	−51.0	−63.0	...
Poly Gly-Ser(H)-Gly[n]	201.2	2 DCA	1,1	−12.308	269.07	−45.0	−54.0	...

[a] See ref 4 for description of procedures and for definition of terms. All amino acids are of the L configuration. Within the limits of the technique all compounds shown in this table are optically pure. The molecular weight of the polymer is the residue weight. [b] The first number is the concentration in weight per cent. DMF is dimethylformamide. DMSO is dimethyl sulfoxide. DCA is dichloroacetic acid. [c] The first number is the number of independent samples; the second is the total number of rotations run. [d] Drude parameters. With these parameters it is a simple matter to run off the rotations at any wavelength within 589–365 mμ by use of a deck calculator. The results so obtained may be considered to be averages of our measurements and have precisions of the order of 0.1% and accuracies as specified in the last column. Also note that this is the per cent error, not the absolute error. The molecular weight is given to facilitate calculation of specific rotations. The large error limits of a and λ_0 are the result of their correlation. [e] Molar rotations. [f] Estimated per cent standard deviation of the calculated molar rotations = $100s/Mn$; s is the standard deviation of the observed values; n, number of rotations run. Rotations run from 589 to 365 mμ unless noted. [g] Does not fit Drude; Moffitt (the Moffitt equation is used here merely as an empirical three-term Drude expression) parameters: $a_0 = 354.49$, $b_0 = -227.77$, $\lambda_0 = 200.00$ (chosen arbitrarily). Any value of λ_0 from 100 to 240 mμ is almost as good providing the correct corresponding values of a_0 and b_0 are used. [h] Does not follow Drude. Moffitt (the Moffitt equation is used here merely as an empirical three-term Drude expression) $\pm a_0 = -47.748\,105$, $b_0 = 32.491 \pm 159$, $\lambda_0 = 246.51 \pm 200$. [i] The large standard deviations indicate that a wide selection of parameters work equally well. The cited values reproduce the calculated values to within about 0.1%, and these calculated values taken as averages of the observed values have a standard deviation of 10%. The large error is due to the fact that the observed α values were small. [j] 105% of the values obtained on hydrolysis of Z-Ser(H)-OH. See text. [k] Optical purity by hydrolysis with 5 *N* HCl, 100°, 15 hr; rotation in 5 *N* HCl (*c* 2). [l] 100.9% (av of 2). [m] 589–435 mμ. [n] For optical purity see Table II.

The use of the *p*-nitrophenyl group as a carboxyl-blocking group required an extensive study of factors which affect the yield of product.[2,8] One preparative detail that should be mentioned is an unconventional order of mixing reagents: a solution of the acid and triethylamine is added to a suspension of the hydrobromide in a solution of the carbodiimide. Required rates of addition depend on the choice of solvent and are related to the rate of solution of the salt. It may also be noted that diisopropylcarbodiimide can often be used to advantage where the peptide product is difficult to separate from the very insoluble dicyclohexylurea.

The removal of the benzyloxycarbonyl group was carried out with hydrogen bromide in nitromethane or in dioxane solution since with acetic acid the serine was partly esterified. The tripeptide hydrobromide is difficult to purify but is readily converted to the *p*-toluenesulfonate **7** by mixing it with triethylammonium *p*-toluenesulfonate in methanolic solution.

(8) D. F. DeTar, R. Silverstein, and F. F. Rogers, Jr., *J. Am. Chem. Soc.*, 88, 1024 (1966).

Polymerization was carried out in dimethyl sulfoxide or in dimethylformamide using the equivalent amount of triethylamine or of sodium *p*-nitrophenoxide as the base. The polymer was precipitated with chloroform or with methanol and was extracted with chloroform and with methanol. Some samples were dialyzed against water.

The optical purity of the intermediates and of the polymers was subjected to a careful study. For the peptides two criteria were used, the repeatability of the observed rotations for samples prepared by different techniques (summarized in Table I) and the rotations obtained after complete acid hydrolysis under carefully defined conditions. The hydrolysis technique has a repeatability of about 2%, and there is a further uncertainty of 2% in the rotation values assigned to L-serine. L-Z-Ser-OH provided a definitive serine standard subject to the assumption that loss of optical activity upon hydrolysis was the same as for serine itself (5%). It was possible to show that small amounts of DL-Z-Ser-OH can be removed from L-Z-Ser-OH by recrystallization from ethyl acetate.

The use of hydrolysis to estimate the optical purity of

peptides containing both serine and aspartic acid is necessarily less accurate, but the results were still excellent. In Table II are given the expected molar rotations of aspartic acid and of serine appropriate to the hydrolysis conditions along with the rotations found for the tripeptides. Agreement is within the 3–4% limits specified.

Table II. Optical Purity of Peptides and of Polymers

	Molar rotation in 5 *N* HCl (*c* 2) at λ, mμ			
	589	578	546	435
H-Asp(OH)-OH[a]	31.8	33.2	37.9	66.7
H-Ser(H)-OH[a]	13.0	13.6	15.7	30.3
Sum: for comparison with peptides	44.8	46.8	53.6	97.0
TosOH-H-Asp(OCH_3)-Ser(H)-Gly-ONP	45.2[e]	47.2	54.0	97.0
Z-Asp(OCH_3)-Ser(H)-Gly-ONP	45.3[f]	47.5	54.6	97.1
Z-Asp(OBl)-Ser(H)-Gly-ONP	46.1[e]	48.2	55.3	97
Poly Asp(OCH_3)-Ser(H)-Gly[b]	46.8[e]	48.8	55.7	100.8
Poly Asp(OCH_3)-Ser(H)-Gly[c]	46.3	48.4	55.6	100.4
Poly Asp(Im)-Ser(H)-Gly[d]	24.8	26.2	29.9	52.6
Poly Asp(OH)-Ser(Ac)-Gly[g]	47.1	50.3	56.6	101.2
Poly Asp(OH)-Ser(H)-Gly[h]	45.3	48.0	55.0	99.8

[a] These values are *lower* than the respective values reported for pure aspartic acid and for pure serine since they refer to hydrolysates obtained after heating at 100° for 15 hr in 5 *N* hydrochloric acid. This treatment leads to about 3% loss of activity in the aspartic acid and 5% in the serine. [b] Based on polymer purity of 81.6% estimated from elemental analysis. [c] Based on polymer purity of 92.8% estimated from elemental analysis. [d] Based on polymer purity of 89% estimated from elemental analysis. Assuming that the serine is 100% L, the aspartic acid has been racemized by the triethylamine treatment and is 35% L and 65% DL. [e] Averages of two independent samples. [f] Average of five independent samples. [g] Purity 90.6%. [h] Purity 92.0.

Evaluation of the optical purity of the polymers is more difficult for they absorb such solvents as water and chloroform tenaciously, and do not lose solvent completely even on prolonged drying at 100° and 0.1 torr. The reported molar rotations (Table I) are based on the peptide content as estimated from elemental analysis. The error limits are estimated to be about 5–10% for the polymers. Conversion of Tos-OH-H-Asp(OCH_3)-Ser(H)-Gly-ONP to polymeric imide by use of excess triethylamine (third from last line in Table II) led to a low rotation; racemization is presumably limited to the aspartyl residue.

The fact that the polymer is only partly racemic shows that imide formation is faster than racemization. This in turn permits the absence of racemization in the other polymer samples to be taken as a demonstration of the absence of such a process as reversible conversion of ester to imide. Such conversion, if it occurred, would lead to a mixture of α- and β-aspartyl linkages. Removal of the methyl ester of poly Asp(OCH_3)-Ser(H)-Gly by hydrolysis leads to imide formation, and the imide hydrolyzes to a mixture of α- and β-aspartyl linkages.[4,9] An attempt was therefore made to remove the ester group by heating at 85° for 15 hr with lithium iodide in pyridine.[10] The resulting polymer was dark in color and had about half of the ester groups removed.

Proton magnetic resonance of trifluoroacetic acid solutions of peptides and polymers proved most useful in structure verification. Important peak positions are summarized in Table III. Integrated areas agreed well with those expected.

Molecular weights were determined by end-group labeling and by the ultracentrifuge using the Archibald method. The determination of amino end groups by dinitrophenylation using the general procedure of Sanger requires a hydrolysis step and chromatographic isolation of the DNP amino acid. This was carried through on one sample of poly Asp(OCH_3)-Ser(H)-Gly and it was found, as expected, that the only N-terminal residue is aspartic acid. However for estimating molecular weights, this procedure is tedious, involves hydrolytic loss of over half of the DNP-Asp-(OH)-OH, and requires relatively large samples. A shorter procedure was therefore developed which involves spectrophotometric measurements directly on the DNP polymer. Details are given in the Experimental Section. Properties of the polymers are summarized in Table IV. The observed relationship between $\bar{M}_w$ and intrinsic viscosity $[\eta]$ is given by the expression $\log [\eta] = 0.367 \log \bar{M}_w - 2.061$ with a variation in $[\eta]$ from 0.15 to 0.28 and of $\bar{M}_w$ from 2500 to 12,000. For condensation polymerization of the type used here the $\bar{M}_w/\bar{M}_n$ is expected to be about 2.[11] Low values are believed to reflect in part the presence of cyclic material, a known by-product in these reactions.[4]

The rates of hydrolysis of the methyl ester and of the imide were investigated briefly at constant pH in the pH-Stat. Results were calculated for the rate expression $-d[\text{ester}]/dt = k_2[\text{ester}][OH^-]$ and are only approximate since the reaction was somewhat less than pseudo first order in ester. Since the imide gave parallel results, the rate-limiting step is hydrolysis of the imide. At pH 9 in 0.4 *M* sodium chloride the initial rate constant for both ester and imide is about 200 l. $mole^{-1}$ sec^{-1} at 25°. This value is comparable to that reported for Z-Asp(OBl)-Ser(H)-NH_2 (BENCASA),[9] and the polymeric structure seems to be providing no large special effect. It might be expected that there would be a retarding electrostatic effect of the neighboring carboxylate groups on the rate,[12] but this aspect has not been investigated.

The polymers were titrated quantitatively both before and after a very brief hydrolysis with 0.1 *N* sodium hydroxide and gave satisfactory agreement with theory. Values were corrected for polymer contents of 80–95% based on elemental analysis. The results are summarized in Table V.

The rate of hydrolysis of the acetate group of poly Asp(OH)-Ser(Ac)-Gly is of interest because of its close relationship to the acetylated form of chymotrypsin, trypsin, and other hydrolytic enzymes.[13] The second-order rate constant at pH 11.9 was found to be 0.1 l. $mole^{-1}$ sec^{-1}. This may be compared with 0.07

(9) S. A. Bernhard, A. Berger, J. H. Carter, E. Katchalski, M. Sela, and Y. Shalitin, *J. Am. Chem. Soc.*, 84, 2421 (1962).

(10) F. Elsinger, J. Schreiber, and A. Eschenmoser, *Helv. Chim. Acta*, 43, 113 (1960).

(11) P. J. Flory, "Principles of Polymer Chemistry," Cornell University Press, Ithaca, N. Y., 1953, p 325.

(12) (a) See Table I of ref 9; (b) A. Katchalsky and J. Feitelson, *J. Polymer Sci.*, 13, 385 (1954).

(13) Reviewed by T. Wieland and H. Determan, *Ann. Rev. Biochem.*, 35, 676 (1966).

Table III. Proton Magnetic Resonance Absorption in Peptide Intermediates and Polymers[a]

	Origin of absorption						
		CH_2			CH		
	NH	Asp	Ser	Gly	Asp	Ser	Other
Poly Ser(H)-Gly	488[c]		258[c]	258[c]		293[c]	
Poly Asp(Im)-Gly-Gly	478[c]	196[c]	(278)[d]	259[b]	295[c]		
Poly Asp(Im)-Ser(H)-Gly	490[c]	200[c]	264[b]	264[h]	318[c]	318[c]	*e*
Poly Asp(OH)-Ser(Ac)-Gly	488[c]	194[c]	280[b]	261[b]	310[c]	310[c]	136[f]
Poly Asp(OCH_3)-Gly-Gly	482[c]	190[c]		262[h]	313[c]		233[g]
Poly Asp(OCH_3)-Ser(H)-Gly	492[c]	190[c]	260[c]	260[h]	313[c]	297[c]	233[g]
Poly Gly-Ser(H)-Gly	486[c]		261[b]	261[b]		297[c]	
Z-Ser(H)-OH			259 (4)[h]			285[h]	
Z-Ser(H)-Gly-ONP	488		260(4)[h]	273(5)[h]		290[h]	
Z-Asp(OCH_3)-Ser(H)-Gly-ONP	485[c]	189(6)[h]	258[b]	273(5)[h]	302[c]	302[c]	230[g]
HBr-H-Asp(OH)-Ser(Ac)-Gly-ONP	486[c] 520[c]	209(6)[h]	284(4)[h]	275(5)[d]	308[c]	308[c]	136[f]
TosOH-H-Asp(OCH_3)-Ser(H)-Gly-ONP	488[c] 521[c]	206(6)[h]	262(4)[h]	276(5)[h]	305[c]	305[c]	236[g]
Z-Asp(OBl)-Ser(H)-Gly-ONP	485[c]	193(6)[h]	250[c]	269(5)[h]	298[c]	298[c]	

[a] Values reported are cycles per second from tetramethylsilane reference. The solvent was trifluoroacetic acid; a Varian A-60 spectrometer was used. [b] Broad simple peak. [c] Complex broad peak; approximate center. [d] CH_2 of the internal glycine. [e] No methoxyl peak detectable. [f] Sharp singlet of CH_3CO. [g] Sharp singlet of OCH_3. [h] Doublet (*J* value in parentheses).

Table IV. Summary of Molecular Weight Data on Polymers

Sample	Polymer	% N[a]	$\bar{M}_n$[b]	$\bar{M}_w$[c]	$[\eta]$[d]	$\bar{M}_w/\bar{M}_n$[e]
47	Asp(OCH_3)-Ser(H)-Gly	13.58	4700[f]	11,000	0.266	2.3
81	Asp(OCH_3)-Ser(H)-Gly	14.46	8900[f,g]	10,000	0.251	1.1
70	Asp(OCH_3)-Ser(H)-Gly	13.50	4400[f]	6,800	0.225	1.5
50	Asp(OCH_3)-Ser(H)-Gly	13.46	4000[f]	7,500	0.234	1.9
137	Asp(OCH_3)-Ser(H)-Gly	13.00	4000[i]	7,600	0.218	1.9
92	Asp(OCH_3)-Ser(H)-Gly	12.79	1800[j]	3,700	0.165	2.1
102A	Asp(OCH_3)-Ser(H)-Gly	12.45	5800[h]	7,600	0.244	1.3
102B	Asp(OCH_3)-Ser(H)-Gly	11.70	3400[h]	2,500	0.154	0.7
61	Asp(OCH_3)-Ser(H)-Gly	13.38	5700[h]	4,900	0.208	0.9
112	Asp(Im)-Ser(H)-Gly	15.31[k]	3200[h]	5,000	0.163	
124	Asp(OH)-Ser(Ac)-Gly	12.15[l]	3800	6,300	0.168	
181	Asp(OH)-Ser(Ac)-Gly	12.52[l]	...	5,300	...	...
185	Asp(OH)-Ser(H)-Gly	15.67[n]	...	3,900	...	...
147	Asp(0.5Im)-Ser(H)-Gly[m]	14.82[o]	8100	4,900	0.184	
95	Gly-Ser(H)-Gly	18.22[p]	4700[r]	...	0.286	
115	Gly-Ser(H)-Gly	18.95[p]	3200[r]	...	0.183	
141	Ser(H)-Gly	16.76[q]	1800[r]		0.145	

[a] Theoretical 15.38. Lower values are believed due to presence of solvent, and this has been demonstrated in some cases. [b] By dinitrophenylation, corrected for amount of polymer present in a given sample as based on % N. [c] By Archibald technique, ultracentrifuge. [d] In dichloroacetic acid at 30°. [e] Based on 20% standard deviation of $\bar{M}_w$ and 20% for $\bar{M}_n$, expected error is 0.55. Average of first seven is 1.73 with standard deviation of 0.54 per value or 0.2 for the average. Average of all nine is 1.52 with standard deviation of 0.57 per value or 0.2 for the average. [f] Using sodium *p*-nitrophenoxide. [g] Dialyzed. [h] Using triethylamine. [i] Using triethylamine plus 6.7 *M* urea. [j] Collected from miscellaneous preparations. [k] Theory 17.42. [l] Theory 13.95. [m] From attempted pyridine–LiI hydrolysis. [n] Theory 16.21. [o] Theory 16.81. [p] Theory 20.89. [q] Theory 19.44. [r] Small insoluble residue present in DNP run.

Table V. Titration of Polymers[a]

Polymer	Moles of OH^-/mole of residue Before hydrolysis	After hydrolysis
Poly Asp(OH)-Ser(Ac)-Gly	0.96, 0.99, 0.95	2.00
Poly Asp(Im)-Ser(H)-Gly	0.061, 0.062	1.01, 0.99
Poly Asp(OCH_3)-Ser(H)-Gly	0.07, 0.04, 0.05	1.10, 0.99
Poly Asp(OCH_3)-Gly-Gly	*b*	1.07
Poly Asp(OH)-Ser(H)-Gly	0.78	...

[a] Values are based on polymer content based on elemental analysis. Titrations before hydrolysis used as end point pH 7.4. Values after hydrolysis were obtained by treatment with a known excess of 0.1 *N* sodium hydroxide at 50° for 5 min and back titration with 0.2 *N* hydrochloric acid. [b] Insufficiently soluble.

for benzyl acetate and 0.006 for ethyl acetate, in solvents containing some alcohol.[14]

(14) "Tables of Chemical Kinetics," National Bureau of Standards Circular 510, U. S. Government Printing Office, Washington, D. C., 1951, p 101.

Experimental Section[15-18]

1. Z-Asp(OBl)-OH.[19] The following preparation has been repeated many times. A mixture of 70 g of Z-Asp(OH)-OH, 350 ml of benzene, 10 g of $TosOH \cdot H_2O$, and 100 ml of benzyl alcohol was refluxed until no more water was collected in a trap. The cooled mixture was shaken with 10 g of magnesium oxide for 10 min and filtered. Benzene and most of the benzyl alcohol were removed *in vacuo*. Trituration of the resulting oil with 200 ml of hexane gave 112 g of crystalline Z-Asp(OBl)-OBl, mp 61–63°.

The dibenzyl ester (112 g) was dissolved in a mixture of 1200 ml of dioxane and 500 ml of water and to this was added a mixture of 120 ml of 2 *N* sodium hydroxide solution, 700 ml of water, and 1200 ml of dioxane. After 24 hr at room temperature the pH was brought to 5.5 by adding a few drops of concentrated hydrochloric acid. Solvents were removed on the rotary evaporator, and the residue was

(15) Nitrogen analyses and rotations by Mrs. Lillian Ross.
(16) We are indebted to Carla Howard for the ultracentrifuge runs.
(17) Peptides are in indexing order, amino acids first, then dipeptides, tripeptides, polymers, alphabetic order from amino acid.
(18) For assays and general procedures see ref 4.
(19) This preparation was carried out by Drs. W. Honsberg and Frank Gilmore.

taken up in 240 ml of 1 *N* aqueous sodium bicarbonate and extracted with ether to remove unreacted diester. Acidification gave an oil which crystallized, 66 g (74%). Recrystallization from benzene gave 60 g (67%) of product, mp 102–103°. Further recrystallization with 60% recovery gave material with mp 106–108° (lit.[20,21] 108°). The infrared spectrum (137, oil) showed bands at: 1740, 1730, 1705, 1695, 1645, and 1530 cm^{-1}.

2. Z-Ser(H)-OH.[22,23] This product has been prepared using three methods for maintaining basicity: sodium bicarbonate, gradual addition of sodium hydroxide, and magnesium oxide. The use of magnesium oxide is best for large-scale runs.

A mixture of 125 g of magnesium oxide, 2000 ml of water, and 105 g of serine was stirred and cooled to 5°, and 800 ml of ether was added. Then 264 g of benzyl chlorocarbonate was added, one-fourth immediately, one-half over 10 min, and one-fourth over 20 min. The temperature was maintained at 5° throughout and for an additional 2.5 hr. Filtration required a filter aid (Celite 535, a diatomaceous earth). The ether layer of the filtrate was separated and the aqueous layer extracted with three 600-ml portions of ether and then slowly added to 110 ml of cold concentrated hydrochloric acid. The product separated as fine white crystals, 191 g, mp 98–100°. Extraction of the filtrate gave another 21 g, mp 103–105°.

The product was further purified by recrystallization from 550 ml of ethyl acetate and then from 500 ml of water to give eventually 162 g of product, mp 114–116°. Ethyl acetate is a convenient solvent for estimating optical purity (see Table I).

3. Z-Asp(OCH_3)-Ser(Ac)-OH. A mixture of 8.04 g of Z-Asp(OCH_3)-ONP, 3.67 g of HCl-H-Ser(Ac)-OH, and 6.06 g of triethylamine in 50 ml of acetone was allowed to stand for 18 hr at 20°. The solvent was evaporated; the residue was taken up in water, and the solution acidified with hydrochloric acid and extracted with ethyl acetate six times. The ethyl acetate was dried, reduced in volume to 60 ml, and diluted with 120 ml of hexane to give 7.1 g (87%) of white crystals, mp 132–134°. Recrystallization from the same solvent gave mp 141–142°.

Anal. Calcd for $C_{18}H_{22}N_2O_9$: C, 52.68; H, 5.40; N, 6.83; mol wt, 410.4. Found: C, 52.6; H, 5.60; N, 7.00; mol wt (vapor phase osmometer, ethanol), 389, 395.

4. HBr-H-Ser(H)-Gly-ONP. In 1500 ml of methylene chloride (freshly distilled from phosphorus pentoxide) was suspended 84.4 g of Z-Ser(H)-Gly-ONP. Dry hydrogen bromide was introduced at a rapid rate. Within 3 min the suspension thickened and stirring became difficult. After 15 min evolution of carbon dioxide began. The hydrogen bromide was introduced for another 1.5 hr, during which time the thick mixture transformed into an easily stirred suspension. Reaction was judged complete when no material less dense than methylene chloride was present. Dry air was then passed through for 0.5 hr and then the product was filtered and washed with several 200-ml portions of acetonitrile; yield 66.0 g (90%), mp 170.5–171.5°. The product can be recrystallized by solution in a minimum quantity of hot DMF followed by a tenfold excess of hot acetonitrile, mp 173–173.5°. Only one spot was found with thin layer chromatography on acidic silica gel using 20% methanol in methylene chloride.

Anal. Calcd for $C_{11}H_{14}N_3O_6Br$: C, 36.30; H, 3.88; N, 11.55; O, 26.38; Br, 21.90; ONP, 37.94. Found: C, 36.28; H, 3.87; N, 11.53, 11.57; O, 26.65; Br, 22.11; ONP, 37.6, 38.0.

5. Z-Ser(H)-Gly-ONP. A detailed study of this reaction has been reported by Rogers.[2] To a solution of 49.5 g (0.24 mole) of dicyclohexylcarbodiimide in 600 ml of acetonitrile cooled to 5° was added 66.6 g (0.24 mole) of HBr-H-Gly-ONP. The suspension was vigorously stirred and immediate addition begun of a solution of 59.6 g (0.25 mole) of Z-Ser-OH and 31.8 ml (0.23 mole) of triethylamine in 200 ml of acetonitrile. The rate of addition was adjusted to require 8 min. The mixture was stirred at ice-bath temperature for 1 hr with addition of 400 ml of acetonitrile to facilitate stirring. After 2 hr more at room temperature, the mixture was filtered. The solid was stirred with a 250-ml portion of warm (40°) dimethylformamide, and the insoluble portion was extracted with another 150-ml portion. The DMF solutions were allowed to stand for several minutes at room temperature to allow precipitation of traces of DCU. Upon addition of 300 ml of cold 0.01 *N* HCl followed by 200 ml of water, the product precipitated and was collected after about 30 min, 68 g, mp 161–162°. The acetonitrile filtrate yielded a second crop upon evaporation to dryness and trituration of the residue with 100 ml of methanol, 7.5 g, mp 157–158°. The total crude yield was 75%. Recrystallization from 350 ml of 1:3 DMF–methanol gave 62.3 g (83% recovery) of colorless material, mp 170–171°.

Thin layer chromatography on acidic silica gel with 4% methanol in methylene chloride gave a single yellow spot when sprayed with dilute alkali.

Anal. Calcd for $C_{19}H_{19}N_3O_8$: C, 54.67; H; 4.60; N, 10.07; O, 30.65; ONP, 33.09. Found (2 samples): C, 54.30, 54.48; H, 4.67, 4.53; N, 10.12, 10.58; O, 30.65, 30.24; ONP 33.2, 32.7.

6. HBr-H-Asp(OH)-Ser(Ac)-Gly-ONP. A freshly prepared 3.8 *M* solution of hydrogen bromide in acetic acid (50 ml) plus 2.5 ml of acetic anhydride was placed in a pressure-equalized addition funnel and added rapidly with stirring to 6.25 g of Z-Asp(OBl)-Ser(H)-Gly-ONP. The material dissolved and during the next 15 min gas evolution (CO_2) was observed. After a total reaction time of 4 hr, 100 ml of ether was added. This caused precipitation of a gum. The solvent was decanted and 100 ml more ether was added. The solvent was decanted from the partly crystalline material. This was dissolved in 30 ml of acetonitrile and ether added to precipitate a sticky product. The residue was again dissolved in 30 ml of warm acetonitrile and then began to crystallize; yield 2.4 g of nonhydroscopic white powder, mp 141–142°. Extraction with 40 ml of hot acetonitrile gave 2.2 g of material, mp 143–144.5°.

Anal. Calcd for $C_{17}H_{21}N_4O_{10}Br$: C, 39.17; H, 4.06; N, 10.75; O, 30.69; Br, 15.33; ONP, 26.50. Found: C, 38.72; H, 4.11; N, 10.61; O, 30.58; Br, 15.37; ONP, 25.9.

7. TosOH-H-Asp(OCH_3)-Ser(H)-Gly-ONP. Z-Asp(OCH_3)-Ser(H)-Gly-ONP (20 g) was placed in a 1-l. flask fitted for introduction of hydrogen bromide and 400 ml of reagent grade methylene chloride was added. During 1.5 hr, a stream of hydrogen bromide was introduced. A thick suspension formed and this changed to a fine suspension. The product was filtered (hygroscopic) and washed with two portions of methylene chloride and then with two portions of ether. It was then dissolved in 200 ml of methanol and precipitated with 1500 ml of ether. The material solidified upon standing overnight at 0°, 15.9 g of material with an ill-defined melting point. Attempts to get better material were not successful.

It was found practical to form the toluenesulfonate. The above product was dissolved in 75 ml of methanol and 30 g of triethylammonium *p*-toluenesulfonate was added. Removal of solvent on the rotary evaporator gave an oil. This was dissolved in 200 ml of acetonitrile; the solvent was removed and redissolved in 150 ml of acetonitrile. Upon scratching with a glass rod crystals separated; yield 13.8 g, mp 145–147°. The salt was dissolved in a mixture of 10 ml of hot dimethylformamide plus 10 ml of acetonitrile. Addition of 100 ml of hot acetonitrile gave an immediate separation of crystals, 12.4 g (58%), mp 150–151°. Chromatography (thin layer) on acidic silica gel G using 20% methanol in methylene chloride showed a single spot with alkaline spray.

Anal. Calcd for $C_{23}H_{28}N_4O_{12}S$: C, 47.25; H, 4.83; N, 9.58; O, 32.84; S, 5.49; OCH_3, 5.31; ONP, 23.6. Found: C, 46.91; H, 5.03; N, 9.44; O, 32.69; S, 5.45; OCH_3 5.64; ONP, 23.3. Three other samples gave N, 9.30, 9.30, 9.36; ONP, 24.0, 23.3, 23.2.

8. Z-Asp(OCH_3)-Ser(H)-Gly-ONP. (This preparation was carried out more than 20 times.)[2] This is a modification of the preparation of Z-Ser(H)-Gly-ONP. To a stirred solution of 22.4 g (0.108 mole) of DCC in 600 ml of acetonitrile, cooled in an ice bath, was added 37.4 g (0.103 mole) of HBr-H-Ser(H)-Gly-ONP and then there was added a solution of 33.3 g (0.118 mole) of Z-Asp(OCH_3)-OH and 14.4 ml (0.103 mole) of triethylamine in 200 ml of acetonitrile at a uniform rate over a period of 1 hr. The proportions of reagents, their purity, and the rate of addition all affect the yield of product.

The resulting thick suspension was filtered, and the solid was extracted with two 100-ml portions of dimethylformamide containing 1 ml of 0.2 *N* HCl. The combined filtrates were concentrated to 300 ml in the rotary evaporator (flask temperature 40°). This was stirred while 300 ml of a slurry of ice in 0.01 *N* HCl was added slowly. This gave a moderately rapid precipitation of a white product. Then 500 ml of ice water was added and the material collected, 33.4 g, mp 176–179°.

The urea was extracted with an additional 100 ml of dimethylformamide and the product precipitated with 200 ml of ice water. The product was converted to a manageable solid by addition of 100 ml of methanol, yield 16.3 g, mp 183–185°, total yield 88%. The combined crops were stirred with 250 ml of hot methanol for 30 min and filtered, 46.5 g (78%), mp 186–188°. For analysis the material was recrystallized from ethyl acetate to give a product, mp 187–188°. This gave a single spot (alkaline spray) upon thin layer

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chromatography on acid silica gel G with 5% methanol in methylene chloride.

Anal. Calcd for $C_{24}H_{26}N_4O_{11}$: C, 52.75; H, 4.80, N, 10.26; O, 32.22; OCH_3, 5.68; ONP, 25.3. Found (2 samples): 52.77, 52.69; H, 4.86; 4.96; N, 10.24, 10.23; O, 32.45, 34.20; OCH_3, 6.00, 5.90; ONP, 24.8, 25.2.

9. Z-Asp(OCH_3)-Ser(Ac)-Gly-OBl. A mixture of 3.7 g of Z-Asp(OCH_3)-Ser(Ac)-OH, 3.04 g of TosOH-H-Gly-OBl, 3.81 g of CMC, 1.25 ml of triethylamine, and 50 ml of chloroform was stirred for 3 hr at 20°. The solvent was evaporated; the residue was extracted with acetone and the insoluble urea removed by filtration. The acetone was removed, and the residue was taken up in ethyl acetate, washed with water, 1 *N* sodium bicarbonate, 3% hydrochloric acid, and water, and dried over magnesium sulfate. Addition of hexane caused precipitation of 2.5 g (50%) of product, mp 122–125°. Recrystallization from ethyl acetate–hexane gave mp 135–136°.

Anal. Calcd for $C_{27}H_{31}N_3O_{10}$: C, 58.16; H, 5.60; N, 7.54; mol wt, 557.5. Found: C, 58.1; H, 5.41; N, 7.08, 7.50, 7.60; mol wt (osmometer, ethanol), 530, 561.

10. Z-Gly-Ser(H)-Gly-ONP. This preparation was similar to that of Z-Asp(OCH_3)-Ser(H)-Gly-ONP: to 7.15 g of DCC in 150 ml of acetonitrile was added 12.2 g of HBr-H-Ser(H)-Gly-ONP, cooled to ice temperature, then a solution of 7.83 g of Z-Gly-OH and 4.62 ml of triethylamine in 60 ml of acetonitrile was added over 1 hr. The infrared curve indicated 8% remaining DCC and anhydride peaks at 1830 cm^{-1}. After another 30 min only 2% of DCC remained, and the anhydride peaks remained. One milliliter of 1 *N* HCl was added; the urea was removed by filtration and washed with 25 ml, and twice the volume of iced methanol (pH 1) was added to give 4.25 g of product, mp 178–180°. Another 7.4 g was obtained from the urea by extraction with 25 ml of DMF and precipitation by addition of 50 ml of iced methanol, total yield 67%. The former fraction was taken up in 8 ml of hot DMF and precipitated with 40 ml of hot acetonitrile to give 3.1 g of material, mp 195°. The latter had mp 195–196° as isolated.

Anal. Calcd for $C_{21}H_{22}N_4O_9$: C, 53.16; H, 4.68; N, 11.81; O, 30.35; ONP, 29.1. Found: C, 53.33; H, 4.80; N, 11.92; O, 30.91; ONP, 29.1.

11. Z-Asp(OBl)-Ser(H)-Gly-ONP. This preparation was carried out similarly to the preparation of Z-Asp(OCH_3)-Ser(H)-Gly-ONP. To 13.6 g of DCC (0.065 mole assuming 98.5% purity) in 200 ml of acetonitrile was added 23.6 g (0.065 mole) of HBr-H-Ser(H)-Gly-ONP and then a solution of 26.6 g (0.074 mole) of Z-Asp(OBl)-OH and 9.8 ml (0.065 mole) of triethylamine in 50 ml of acetonitrile over 75 min. Addition of 150 ml of acetonitrile was necessary to permit stirring. The reaction was allowed to proceed for 1 hr with removal of the ice bath after the addition was complete. An infrared curve of the supernatant liquid showed that 98% of the DCC had disappeared, and there was still a small absorption at 1830 cm^{-1}. The work-up was also similar to that for the methyl ester: 100 ml and then 30 ml of DMF containing 1 ml of 0.1 *N* HCl per 100 ml was used to extract the filter cake. The crude product was dissolved in 80 ml of hot 50:50 dimethylformamide–methanol, filtered to remove a small amount of solid, 300 ml of hot methanol added, and the suspension allowed to cool, yielding 25.2 g of soft needles, mp 161–162°. Recrystallization from a DMF–acetonitrile mixture gave a product which melted at 148° and then resolidified and melted again at 162°. Both forms have the same rotation in DMF. The product gave a single spot (alkaline spray) when chromatographed (tlc) on acidic silica gel G with 5% methanol–methylene chloride.

Anal. Calcd for $C_{30}H_{30}N_4O_{11}$ (two samples for N and ONP): C, 57.87; H, 4.86; N, 9.00; O, 28.27; ONP, 22.2. Found: C, 58.27; H, 4.95; N, 9.18, 9.10; O, 27.83; ONP, 22.2, 21.5.

Polymerization of H-Asp(OCH_3)-Ser(Ac)-Gly-OH. To a solution of 0.2–0.3 g of the tripeptide acid in 5 ml of dimethylformamide, DCC (1–2 equiv) was added. Evaporation of the solvent and extraction with water gave low molecular weight material (about 750, equivalent to 2.5 tripeptide units). This line was not pursued for reasons indicated in the discussion.

Poly Asp(OCH_3)-Ser(H)-Gly. a. Use of Triethylamine and Dimethyl Sulfoxide. A solution of 7.8 g of TosOH-H-Asp(OCH_3)-Ser(H)-Gly-ONP was prepared in 10 ml of dimethyl sulfoxide (DMSO) by slight warming. This was cooled to room temperature and stirred while 1.87 ml of triethylamine was added over a period of 2 min. During the next 10 min an unstirrable gel formed. This was stored for 4 hr, and addition of 10 ml of DMSO redissolved the gel. The mixture was stored in the dark for 4 days and the polymer precipitated by slow addition of 100 ml of chloroform. The polymer was extracted with chloroform, methanol, and ether. It was dried at 50°, then over P_2O_5 at 95° (0.3 μ) for 16 hr to give 3.07 g of polymer (84%).

This material was subjected to further purification by suspension in DMSO (gel) which was centrifuged at 10,000 rpm; this was repeatedly suspended in chloroform and centrifuged. After drying, it was extracted with chloroform in a Soxhlet extractor and dried for 24 hr at 100° and 0.1 mm against P_2O_5.

Anal. Calcd for $C_{10}H_{15}N_3O_6$: C, 43.95; H, 5.53; N, 15.38, O, 35.13; OCH_3, 11.36. Calcd for $C_{10}H_{15}N_3O_6$ plus 8.2% of $CHCl_3$ and 1.8% of inorganic residue: C, 40.3; H, 5.04; N, 13.83, O, 31.6, OCH_3, 10.22; Cl, 7.3; residue, 1.8. Found: C, 39.9; H, 5.07; N, 13.7; O, 32.4; OCH_3, 10.2: Cl, 7.3; residue, 1.8.

b. Use of Sodium *p*-Nitrophenoxide. To a solution of 0.346 g of dried sodium *p*-nitrophenoxide (in oven at 110°) in 2 ml of DMSO was added 1.26 g of TosOH-H-Asp(OCH_3)-Ser(H)-Gly-ONP. Within 30 min the mixture gelled. After 50 hr at room temperature, the polymer was precipitated by addition of 25 ml of methanol. It was washed with methanol and acetonitrile and dried at 70° over P_2O_5 to give 0.44 g of polymer. Several samples were combined and dialyzed against water. After drying, the analysis was: C, 43.7; H, 6.14; N, 14.13; O, 35.99; OCH_3, 9.4.

Rotation of L-Serine. Literature values of the rotation of serine are insufficiently precise to permit a definitive assay of optical purity. Different lots of commercial serine (Nutritional Biochemicals) showed rotations of 80–97% of the values of pure L-serine as determined below and even lower purities were suggested by comparison with certain literature values. Since nitrogen assays were theoretical (13.32 found *vs.* 13.33 theory), the presence of considerable DL-serine was indicated.

Fortunately it was discovered that fairly pure L-Z-Ser(H)-OH can be recrystallized from ethyl acetate to give optically pure material. Among several samples which gave rotations close to those summarized in Table I, one had mp 118–119° when the capillary was placed in the bath at 100° and with the temperature rising 3°/min. A DL sample under these conditions showed mp 124–125°. A series of mixtures was made up containing 5, 10, and 20% of DL-Z-Ser(H)-OH, the remainder being the purest sample of L isomer available. The first crops obtained upon recrystallization from ethyl acetate showed melting points of 116–117, 115–117, and 113–116° and rotations, 100, 98, and 89% of the best values.

Other reference compounds containing serine were available for comparison; HBr-H-Ser(H)-Gly-ONP, *e.g.*, prepared by three different workers in our laboratories had rotations within 1% of the average value. The concordance of rotations of such samples prepared at different times is good evidence that all are optically pure.

The rotation of pure L-serine was estimated by controlled hydrolysis of various serine derivatives with 5 *N* HCl (15 hr at 100°). This procedure gives values with some scatter, about 5% from the average, but with a common average. Similar treatment of serine itself gave a 2% scatter and a 5% loss of activity. The reported values for pure serine in Table I are based on the averages of several different types of samples and on the assumption that the average of the hydrolyzed samples is to be increased by 5% to compensate for loss. These resulting rotation values are appreciably lower than those tabulated by Greenstein and Winitz.[24] We believe the reported values to be too high; they correspond fairly well with our measurements in 1 *N* HCl.

Identification of End Groups by Dinitrophenylation. The modification by Porter of Sanger's method of identifying end groups was used as a basis for examining one polymer (RC-50-A) both for molecular weight and for identity of the end group. Poly Asp(OCH_3)-Ser(H)-Gly (29.2 mg) and 50 mg of sodium bicarbonate were dissolved in 2 ml of distilled water by warming to 50°. The solution was cooled to room temperature, and a solution of 6.5 mg of dinitrofluorobenzene in 2 ml of ethanol was added. After 3 hr, 0.1 ml of concentrated aqueous ammonia was added and 15 min later the solvent removed. The residue was refluxed for 12 hr with 10 ml of 6 *N* hydrochloric acid, diluted to 25 ml with distilled water, and a 10-ml aliquot taken for assay.

The DNP-aspartic acid and other products were extracted with ethyl acetate, and the residue from the ethyl acetate was taken up in chloroform and chromatographed on a 2 × 0.5 in. column of silica gel which had been treated with a 15% by weight acetic acid–sodium acetate buffer. The first yellow band consisted of dinitro-

(24) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," John Wiley and Sons, Inc., New York, N. Y., 1961, p 116.

aniline as determined by thin layer chromatography on silica gel G, buffered with acetic acid–sodium acetate, and with ethanol–0.22 M ammonia (89:11) as eluent, R_f 0.77. The second yellow band was eluted from the column with ether–methanol–acetic acid (49:49:2.5), and on thin layer chromatography as above had R_f 0.12. This is the value found for DNP-aspartic acid. There were no traces of DNP-serine (R_f 0.36), nor of DNP-glycine (R_f 0.55) in any fraction.

The DNP-aspartic acid was taken up in 25 ml of a sodium borate buffer and absorbance read at 361 mμ. When the above procedure starting with the hydrochloric acid hydrolysis was carried out on pure DNP-aspartic acid, it was found that recovery was only 48%. Losses have been noted before during the hydrolysis steps.

The calculated number-average molecular weight, correcting for the loss, was 7200.

Number-Average Molecular Weights by Dinitrophenylation. An appropriate weight of sample (1–6 mg) was dissolved in 2 ml of 2% sodium bicarbonate, warming if necessary to effect solution. To this was added 2 ml of a freshly prepared 1–3% solution of 2,4-dinitrofluorobenzene in ethanol. After 2 hr at room temperature the solution was transferred quantitatively to a separatory funnel using 6 ml of 3 N hydrochloric acid for rinsing. The solution was extracted with two 10-ml portions of carbon tetrachloride and the aqueous layer filtered through wet filter paper into a 25-ml volumetric flask and diluted to volume with 3 N hydrochloric acid. Absorbance was read at 353 mμ. A blank run was used to correct for absorbance owing to dissolved carbon tetrachloride or other substances. Sample size was chosen to give an absorbance of about 0.8, and an extinction coefficient of 1.59×10^4 l. mole^{-1} cm^{-1} was used. The rationale is that the extinction coefficient of the DNP derivatives may be expected to be relatively insensitive to the amino acid or the peptide to which the DNP is attached. In support of this it was found that five samples of DNP-Asp(OH)-OH in 2% sodium bicarbonate and in hydrochloric acid ranging from 1.2 to 6 N all had an extinction coefficient at 353 mμ of 1.59×10^4 with a standard deviation of 0.022 per run or 0.01 for the average. The value for DNP-Gly-Gly-Gly-OH was 1.60. Values reported at 350 mμ for DNP-Gly-OH (1.55), DNP-Phe-OH (1.57), DNP-Gly-Gly-OH (1.58), DNP-Phe-Val-OH (1.55), and for H-Lys-(DNP)-OH (1.49) are close.[25] (All values are to be multiplied by 10^4.) Properties of the polymers are summarized in Table IV.

(25) F. Sanger, *Biochem. J.*, **39**, 507 (1945); R. R. Porter, *Methods Enzymol.*, 4, 221 (1957).

$$\bar{M}_n = \text{mg of sample} \times 1.59 \times 10^4/(A_{samp} - A_{blank})(\text{volume in milliliters})$$

Weight-Average Molecular Weights from Ultracentrifuge Measurements. The data in this paper are based on the Archibald method using the Beckman-Spinco Model E ultracentrifuge.[26] Runs were made in aqueous solution (mostly in 0.01 M KCl, but sometimes in water alone with comparable results) and at a concentration of 0.5–1%, usually using a Schlieren angle of 70° (50–85°) and a speed of 12,590, 20,410, or 24,630 rpm with photographs at intervals ranging from 10 to 100 min. In most cases only the meniscus values were used.

The principal source of error is the estimation of the intercept at the meniscus, and the error arises both from the problem of accurately identifying the meniscus and from the fading of the pattern. In the present series the meniscus was taken as the boundary as it appeared on the photographic plate, and extrapolation procedures were used to get the $(dc/dx)_0$ value at this point. Plots of several sets of data showed that the arbitrary function log (dc/dx) gave good agreement with the visual estimated of $(dc/dx)_0$ and that parabolic extrapolation was less satisfactory. Errors arising from $(dc/dx)_0$ estimates are the major source of the roughly 10% standard deviation per exposure. Data were processed by the computer program ARCHBD.

The calculated molecular weight is sensitive to the assumed density of the polymer, or of its reciprocal, the partial specific volume $\bar{V}$. Estimates of solution densities at concentrations of 1% with a precision of 1 part in 5000 lead to a 25% error in $\bar{V}$. We therefore used a calculated value for $\bar{V}$ of 0.61 for poly Asp(OCH$_3$)-Ser(H)-Gly.[27] The weight-average molecular weights in this paper have an estimated accuracy of about 20%. All data have been processed in a uniform fashion so that comparisons between samples involve only the standard deviation of the scatter which is 10%. Control runs on ribonuclease gave much sharper curves and satisfactory agreement with published results.

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Halide Ions as Probes for Nuclear Magnetic Resonance Studies of Proteins. The Sulfhydryl Groups of Hemoglobin

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Abstract: Chlorine nmr spectroscopy is used to verify that the unreactive sulfhydryl groups of hemoglobin are incapable of forming structures of the type protein–S–HgCl even when the protein is dissociated in media of high ionic strength or low pH. However, dissociation of hemoglobin into fragments appears to be accompanied by conformational changes that restrict the motion of chlorine ions bound to mercury atoms complexed at the sites of the reactive sulfhydryl groups. The changes in structure and chemical reactivity of hemoglobin in urea solution are complex.

In recent years there has been a great deal of interest in the application of nmr techniques to systems of biological importance.[2] Since the direct application of nmr to proteins in solution is seldom fruitful, several indirect methods involving relaxation effects have been developed.[3,4] Recently Stengle and Baldeschwieler[5] reported a novel technique which involves the use of a halide ion (usually chloride) as a probe for the study of mercury complexes of proteins. In this paper, this

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