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PREPARATION AND PROPERTIES

OF SOME

SYNTHETIC POLYPEPTIDES

BY

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## ACKNOWLEDGMENT

The author wishes to express her deep gratitude and indebtedness to Professor C. Richard Zobel who suggested the problem and whose inspiration and indefatigable encouragement, continuous interest and council made the completion of the present problem possible.

## ABSTRACTS

In this thesis the preparation, determination of molecular weight and examination of the spectra of some synthetic polymers and their anhydrides is described.

These include 1) poly- $\gamma$ -benzyl-L-glutamate

2) poly-DL-phenyl alanine

3) phenyl alanine anhydride

The preparation of the above polymers involves a chain polymerization reaction of the carboxy amino acid anhydride. In the case of phenyl alanine the anhydride was not available and its synthesis from the amino acid was carried out.

The molecular weight of the resulting polymers was determined in relation to the initiator and solvent used for polymerization. In case of phenyl alanine the viscosity measurements gave relative values but for the poly- $\gamma$ -benzyl-L-glutamate absolute values could be obtained by using a calibration curve prepared by other workers.

The third aspect of the problem was to identify these compounds by observing their spectra. The wave numbers of the bands were determined from a standard curve obtained by calibrating the instrument against known bands of atmospheric water and  $\text{CO}_2$ . This part enables us to classify the polymer in relation to its molecular configuration as belonging to the  $\alpha$  or  $\beta$  form.

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## INTRODUCTION

Because of their biological importance, proteins are of interest to many types of research investigators. However, until very recently little has been known concerning the physical structure of these molecules. Chemical investigations have already established the fact that proteins are linear polymers of  $\alpha$ -amino acids joined through the peptide link, but the precise structure of the molecule has completely eluded explanation.

By investigating the structure of compounds related to proteins - by virtue of their possessing the peptide link or some other protein - like component - light has been shed on the structure of proteins.

Although the methods of investigation have been manifold it seems only fair to say that the bulk of the important results have come from X-ray and model building investigations. And infrared spectroscopic methods have played a secondary role in verifying the results obtained by these methods.

Of particular interest in these investigations have been the synthetic polypeptides because of their close resemblance to proteins, per se. It has been found that in addition to the close chemical correspondence between the two classes of molecules there is also a close

resemblance between their physical structures. This has been most fortunate because the synthetic origin of the polypeptides makes possible a closer control of the experimental variables and hence complications can be reduced to a minimum. This has meant that a large amount of effort has been directed to the methods for synthesis of these polypeptides and an examination of their structure.

For these reasons an introductory study of the preparation, properties and spectra of these compounds has been attempted. The details of the procedure and results are given in the following report.

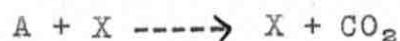
## PREPARATION OF POLYMERS

### INTRODUCTION

The synthesis of poly dl phenyl alanine and the polymerization of poly  $\gamma$  benzyl L glutamate are presented in this section and the variables involved in the reactions are discussed as well.

Polymerization of amino acids was first established by Fischer (20) who used a stepwise method by which amino acid residues were successively and singly joined to form linear polymers which came out to be of low molecular weights as compared to the natural high molecular weight proteins. This method proved to be time consuming and refinements in the technique led to a new field of research in chemistry.

In a comprehensive review of the chemistry of poly amino acids and their synthesis Katchalski (25) points out that the most suitable method for the preparation of high molecular weight synthetic peptides involves the polymerization of the proper carboxy amino acid anhydride. This reaction can be represented by the general formula



Where A = anhydride

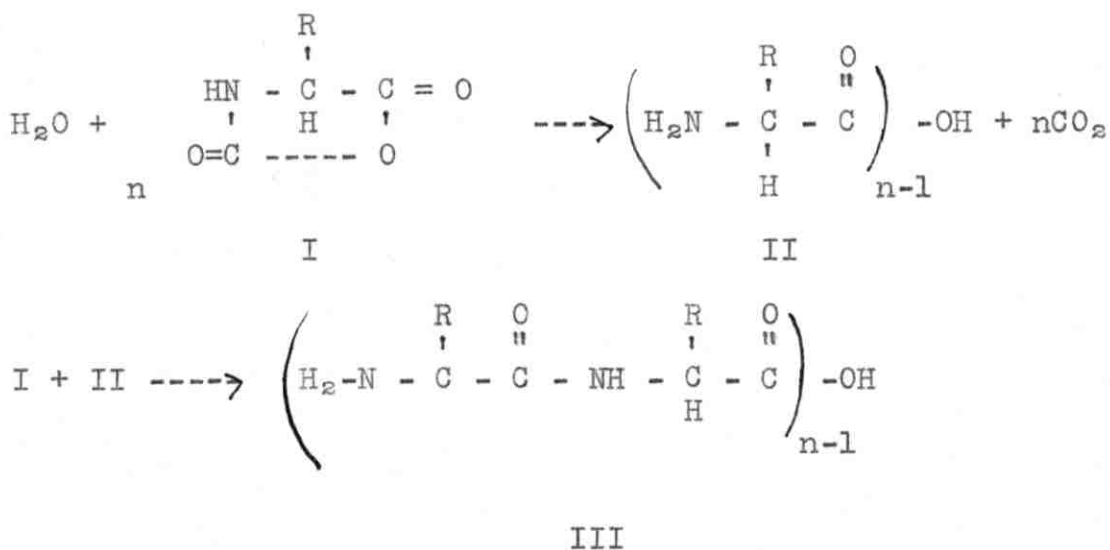
I = initiator

X = polymer

As we can see this polymerization involves

- (i) initiation
- (ii) propagation

with polymers reacting only with the monomer, which is easily attacked by any compound containing an active hydrogen (30). This is illustrated by the following equations.

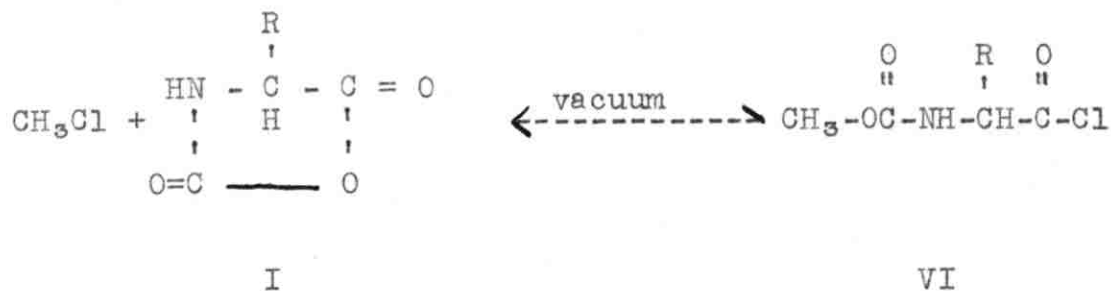
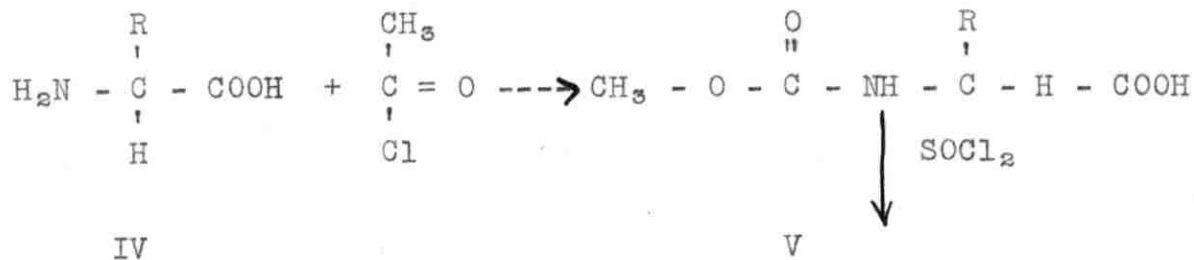


The product of the reaction is determined (32) by

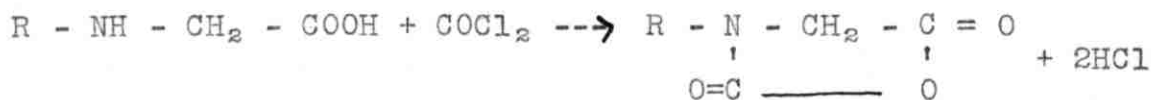
- (i) the supply of monomer molecules
- (ii) the relative concentrations of monomer and initiator represented by the ratio  $\frac{A}{I}$
- (iii) the relative rates of initiation and propagation of the reactions.

The methods for the synthesis of the N-carboxy amino acid anhydride (25), (17), (23) must be presented due to their importance as intermediate products:

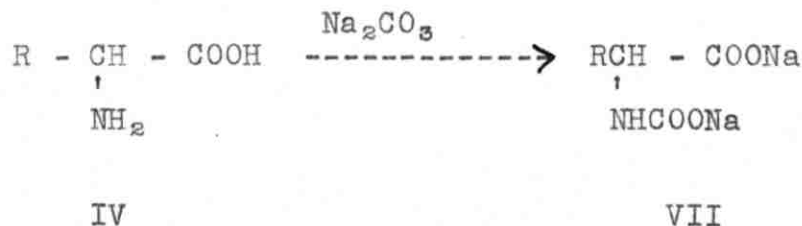
a) Leuch's method where the N carbomethoxy derivatives are treated with thionyl chloride

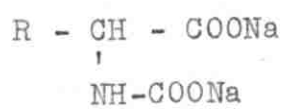


b) Farthing (17) prepared the anhydride from the action of phosgene on the free amino acid in an alkaline aqueous solution:

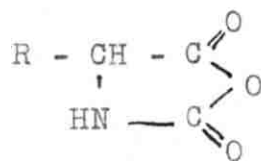
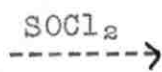


c) Fuchs (23)(18) cyclized the disodium salt of the corresponding carbamic acid with thionyl chloride.





VII

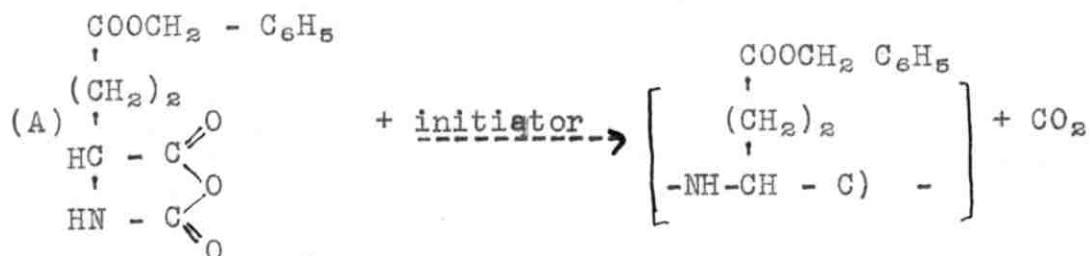


I

EXPERIMENTAL PROCEDURE

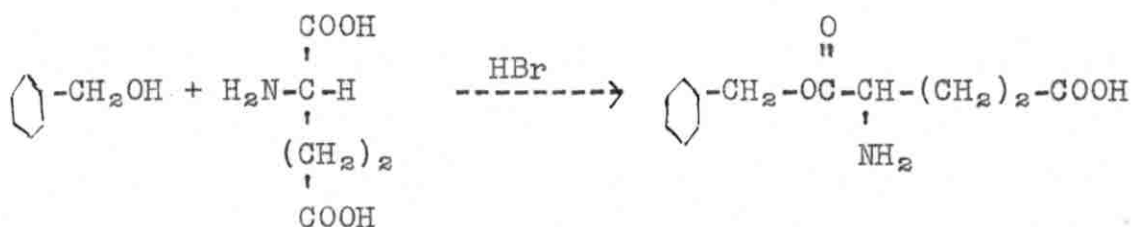
Polymerization of PBG

Benzyl N carboxy L glutamate anhydride was used as our starting material in the polymerization process which follows the scheme (8).



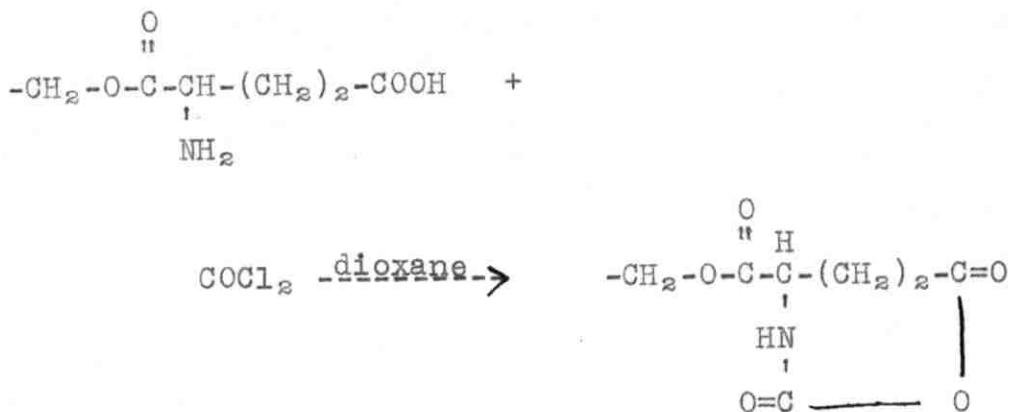
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The  $\gamma$  benzyl N carboxy L glutamate anhydride was prepared by H. Alul in the Chemistry Department at the University of Michigan. The procedure used was that of Blout and Karlson and is illustrated below -



Benzyl alcohol    Glutamic acid

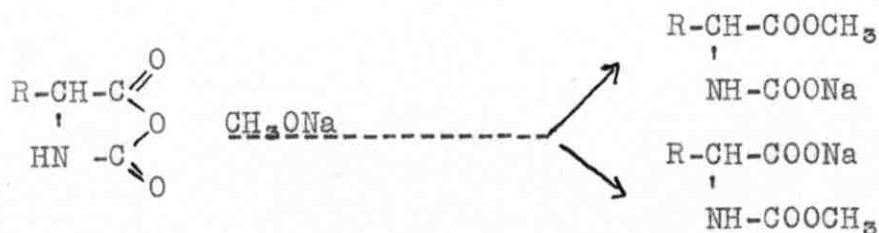
Benzyl glutamate



Benzyl carboxy glutamate anhydride

Three polymerizations were carried out using dioxane as the solvent and varying the nature of the initiator which was triethyl amine in two cases and sodium hydroxide in absolute methanol in the third case. The anhydride to initiator ratio was  $\frac{A}{I} = 100$  in all cases.

The anhydride was added at  $-30^\circ\text{C}$  to the solution made up of solvent and initiator and sufficient time was allowed for the reaction to proceed to completion. The required time was about three days. The end of the reaction was determined by determining the amount of unreacted anhydride by titration with sodium methoxide using thymol blue as an indicator. The reaction taking place during the titration is the following (8), (25)

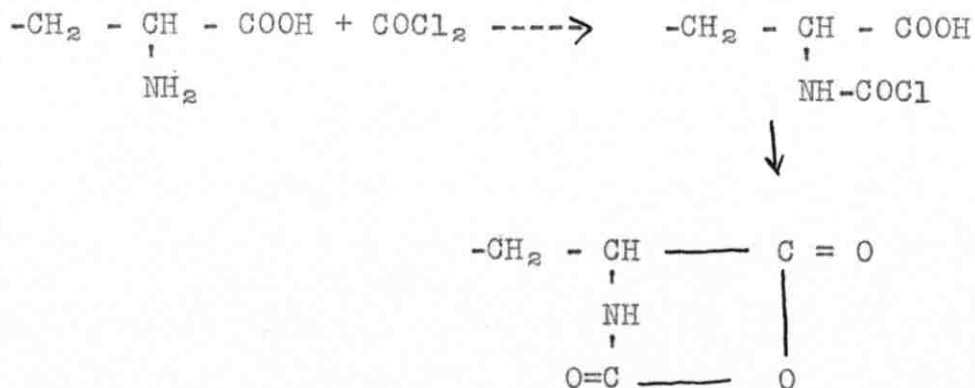




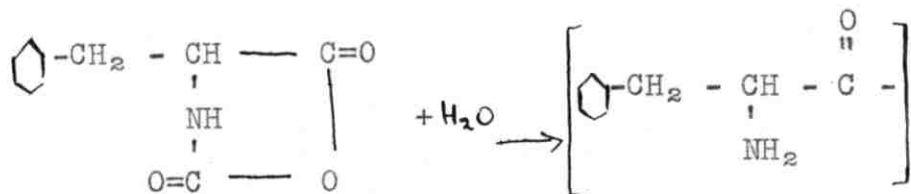
When titration measurement showed that the reaction had gone to completion (ca. 80%), precipitation was effected from a 95% ethanol solution. The white crystals that separated out were insoluble in water, but soluble in benzene, chloroform and dichloro acetic acid. The products obtained in polymerization were of different appearance. Those obtained by initiation with triethyl amine in dioxane were white powderish sample whereas the material obtained with NaOH initiation was a solid resilient film. The molecular weight of these polymers was determined by viscosity measurements which will be described in another section.

#### Synthesis of Poly-dl-phenyl alanine

The general synthetic method followed (18), (25), (17), (27), (19) was described in detail by Farthing (17). It involves passing phosgene directly into a suspension of dl phenyl alanine in dioxane at 35-40°C until the powdered amino acid goes into solution. This generally requires about 4 - 6 hours.



Dry air was passed into the solution to remove the excess of phosgene and the dioxane was removed by vacuum distillation at 40°C. which leaves the anhydride crystals in the flask. These compounds polymerize readily by the chain mechanism already presented which can be initiated by water, amines or any compounds with an active hydrogen



The white crystals were recrystallized from petroleum ether and ethyl acetate. On cooling the solution, an immediate precipitate was obtained which was filtered out and dried. When the ethyl acetate - petroleum ether solution was allowed to stand overnight a second solid portion precipitated out. This material was also filtered and dried. An examination of the first product showed it to be insoluble in several organic solvents (i.e., chloroform, benzene, etc..) but to be soluble in water. Its melting point was ca. 225 - 227°C. This clearly indicated that the material obtained was not the anhydride desired. Addition of AgNO<sub>3</sub> to a sample of the material dissolved in water gave an immediate white curdy precipitate - AgCl. Hence it was presumed that the compound obtained was the hydrochloride of phenyl alanine rather than the anhydride.

The second portion of material obtained (by precipitation from the solvent after standing overnight) was also insoluble in most organic reagents as well as in water. It appeared to decompose rather than melt at ca. 217°C. An investigation of the spectrum of this compound showed it to be poly-dl-phenyl alanine (here after designated poly-dl-phenyl alanine I). It thus appeared that due to presence of excessive moisture in the reagents during the course of the reaction the anhydride was never obtained, but immediately polymerized to poly-dl-phenyl alanine. Although this latter material was somewhat contaminated with the hydrochloride the two could be separated by washing with water due to their different solubilities.

At this point it was decided to repeat the experiment to get the anhydride which could be used to obtain the polymer under controlled conditions. By improving the techniques in drying the reagents and by immediate recrystallization of the solid compound obtained after the removal of dioxane solution the anhydride crystals were obtained. This was confirmed by the melting point which came out to be 126 - 127°C as well as by the spectra which will be described later.

Two ways by which the poly-dl-phenyl alanine was prepared are discussed

(1) the anhydride was added to a solution containing dioxane as a solvent and triethylamine as an initiator.

(ii) the polymerization of anhydride was carried out in benzene solution where the water contained in benzene is sufficient to start the reaction.

In the first case the  $\frac{A}{I} = 200$ .

The procedure by which polymerization takes place is the same as the one presented for the synthesis of poly-benzyl glutamate. Enough time was allowed for the reaction to go to completion and was about four days. The end of the reaction was determined by titration of the anhydride as before. The precipitation of the poly-dl-phenyl alanine when using dioxane as a solvent was possible with 95% ethanol solution. In the solution using benzene as a solvent the polymer precipitated out of solution and was separated by filtration.

The spectra of the poly-dl-phenyl alanine was determined and this will be presented in another section.

#### Reagents

Thymol blue solution (6)

0.5 grams were dissolved in 100 cc. of methanol

Sodium methoxide (6)

Three grams of metallic sodium were dissolved in one liter of methyl alcohol and the solution was titrated with standard acid.

Triethyl amine (8)

was refluxed with metallic sodium and distilled.

Dioxane (8)

Dried over metallic sodium.

Methyl alcohol

dried over calcium oxide.

Ethyl acetate

dried over calcium chloride.

Petroleum ether

dried over metallic sodium.

Phosgene

from Matheson Coleman & Bell.

Dichloroacetic acid

Fischer's pure.

#### DISCUSSION

From the results obtained above with poly-dl-phenyl alanine it is apparent that the presence of water in reagents results in lower molecular weight polymers. (See viscosity data). This is presumably due to the presence of an increased number of active centers about which chain initiation can begin. The same effect would be shown by decreasing the anhydride to initiator ratio. This makes it clear then that it is important to exclude the absorption of atmospheric water during the course of the reaction.

Furthermore, from the fact that precipitation of the polymer occurred in benzene, whereas, the dioxane solution became viscous - the material remaining in solution - it

would seem that the latter solvent is better for polymerization. And higher molecular weight samples could be obtained.

DETERMINATION OF THE VISCOSITY OF HIGH  
MOLECULAR WEIGHT POLYPEPTIDES

INTRODUCTION

The simplest way to determine the molecular weight of a substance is to measure its effect on the viscosity of a solvent. However, a number of important points must be kept in mind when using this technique. Molecular weights determined in this way are essentially weight average molecular weights and as such emphasize the higher molecular weight fraction; hence caution must be applied when comparing these results with those obtained by other methods.

In systems possessing interacting groups such as the carboxyl and amino groups in polypeptides, it is important to minimize aggregative effects. In a number of cases (25) it is not at all certain that such aggregation has been avoided and hence deceptively high molecular weights are obtained. The use of non interacting solvents such as benzene is not recommended for this reason. The measurements made here were carried out on solutions of the various polypeptide polymers in dichloro acetic acid. This no doubt eliminates all interference due to solute - solute interaction but may give rise to some solute - solvent interaction whose effect on the results cannot be anticipated. Molecular weights obtained by viscosity measurements are only relative values and can give absolute values only when a calibration curve is

available. Doty et al (13) have made a rather extensive investigation of molecular weight relations for poly benzyl-1-glutamate and have published a curve (see fig. 1) showing the relation between viscosity and molecular weights. By using this curve it is possible to ascertain the molecular weight of our samples by measuring the viscosity under appropriate conditions. Doty's curve was prepared by determining the intrinsic viscosity of samples whose molecular weight had been determined by light scattering. It might be mentioned that, strictly speaking, light scattering methods yield a weight average molecular weight which is not precisely the same as the viscosity average molecular weight (21).

For the samples of poly-dl-phenyl alanine the viscosity measurements are only of value in showing the relative molecular weights among the various samples.

Theory:

Poiseuille derived a Law (12) that gives a relation between viscosity  $\eta$ , volume of the liquid  $v$  flowing in unit time, pressure  $p$ , radius of the tube  $r$  and length of the tube  $l$

$$\eta = \frac{pr^4}{8vl}$$

This equation offers experimental difficulties in determining the absolute viscosity of compounds so that measurement of viscosities of solutions is made relative to the



viscosity of a known standard solution represented by the general equation:

$$\frac{\eta_1}{\eta_0} = \frac{d_1 t_1}{d_0 t_0}$$

The Ubbelohde viscometer eliminates the necessity of density measurements since the measurements are made under controlled air pressure (11).

#### Nomenclature

- $\eta$  = viscosity of solution
- $\eta_0$  = viscosity of solvent
- $n_{sp}$  = specific viscosity
- $\frac{n_{sp}}{C}$  = intrinsic viscosity
- $d_1$  = density of solution
- $d_0$  = density of solvent
- $t_1$  = time of flow of solution
- $t_0$  = time of flow of solvent
- $\rho$  = density of liquid in Stoke's Law
- $K_m$  = characteristic constant
- $C$  = concentration in gm/liter
- $M$  = molecular weight

Units: The absolute unit of viscosity is the poise which is defined (11)(32) as the viscosity of a material which requires a shearing force of 1 dyne/cm<sup>2</sup> to maintain a velocity gradient of 1 cm/sec between two planes 1 cm apart.

Application:

Viscosity as defined is dependent on the resistance of a fluid to shear. In solutions, the viscosity is affected by solute molecules. This is represented by Stoke's law

$$\frac{n_1}{n_2} = \frac{(\rho_{\text{sphere}} - \rho_{\text{fluid}(1)}) t_1}{(\rho_{\text{sphere}} - \rho_{\text{fluid}(2)}) t_2}$$

By starting with the relation  $F = ma$ , Newton was able to show that viscosity is proportional to mass (Newtonian flow). This holds for solutions of low concentration and small particles (and in particular spherical particles). In cases where the assumptions are not valid we get non Newtonian flow and the relation between viscosity and molecular weight is indefinite.

Staudinger (11) assumed that for linear structure, the molecular weight is proportional to length of polymer.

$$[\eta] = K_m M$$

where  $[\eta] = \frac{n_{sp}}{C}$

$$n_{sp} = \frac{n}{n_0} - 1$$

so that  $M = \frac{K_m}{[\eta]}$

The value of  $\frac{n_{sp}}{C}$  as function of  $C$  is not constant and to compensate for this variation, reduced viscosity at

infinite dilution is used; hence,  $K_m M = [\eta] \lim_{c \rightarrow 0}$

For high molecular weight polymers expressing a non linear relation, is frequently more accurate, (9) -

$$[\eta] = KM^\alpha$$

which reduces to a linear relation when the exponent is unity.

To overcome the difficulties arising from molecular size and shape, viscosity measurements are taken at low concentrations. The intrinsic viscosity at infinite dilution is obtained by extrapolating the line  $\frac{\eta_{sp}}{C}$  vs.  $C$  to  $C = 0$ .

This value can be used to determine the molecular weight of the samples of poly- $\gamma$ -benzyl-L-glutamate by using Doty's graph.

## EXPERIMENTAL PROCEDURE

Material used: Three samples of poly- $\gamma$ -benzyl-L-glutamate were prepared by polymerizing the anhydride in dioxane solution using triethyl amine as an initiator in two cases designated as (PBG I & II respectively) and sodium hydroxide in absolute methanol in the third case (PBG III).

Two samples of poly-DL-phenyl alanine were prepared. Sample I was the polymer obtained directly by passing phosgene in phenyl alanine suspended in dioxane while the sample II was the end product of a controlled polymerization of phenyl alanine anhydride in dioxane using triethyl amine as initiator.

### Determination of Molecular Weight of Polymers:

Appropriate amounts of the proper polymer were added to dichloro acetic acid to prepare the required concentrations: 0, 0.25, 0.50, 0.75, 1%. The solutions were allowed to stand in a warm water bath until the polymer dissolved. 7 cc. of the solution were pipetted into a viscometer and the time of passage of the liquid between the two marks was recorded and the intrinsic viscosity calculated.

RESULTS

Table I

Conc.	t <sub>sec</sub>	n <sub>sp</sub>	$\frac{n_{sp}}{C}$
0%	353		
0.25%	376	0.0651	0.260
0.50%	401	0.136	0.272
0.75%	430	0.218	0.291
1%	455	0.288	0.288
		$[\eta]_0 \approx 0.244$	
		Mol. Wt. $\approx 40,000$	

Poly- $\gamma$ -Benzyl-L-Glutamate I

Table II

Conc.	t <sub>sec</sub>	n <sub>sp</sub>	$\frac{n_{sp}}{C}$
0%	353		
0.25%	373	0.055	0.220
0.50%	392	0.110	0.220
0.75%	422	0.191	0.255
1%	452	0.282	0.282
		$[\eta]_0 \approx 0.182$	
		Mol. Wt. $\approx 23,000$	

Poly- $\delta$ -Benzyl-L-Glutamate II

Table III

Conc.	$t_{\text{sec}}$	$n_{\text{sp}}$	$\frac{n_{\text{sp}}}{c}$
0%	301		
0.25%	324	0.0764	0.306
0.50%	350	0.160	0.320
0.75%	376	0.249	0.332
1%	409	0.358	0.358
$n_o$	=	0.286	
Mol. Wt. = 45,000			

Poly- $\gamma$ -Benzyl-L-Glutamate III

Table V

Conc.	t <sub>sec</sub>	n <sub>sp</sub>	$\frac{n_{sp}}{C}$
0%	295		
0.25%	307	0.041	0.0416
0.50%	320	0.082	0.166
0.75%	345	0.170	0.226
1%	354	0.240	0.240
$[\eta] \approx$			0.08

Poly-dl-phenyl alanine II

Table IV

Conc.	t <sub>sec</sub>	n <sub>sp</sub>	$\frac{n_{sp}}{C}$
0%	281		
0.25%	304	0.080	0.0418
0.50%	301	0.07	0.140
0.75%	326	0.160	0.156
1%	331	0.179	0.179
$[\eta] \approx$			0.06

Poly-dl-phenyl alanine I

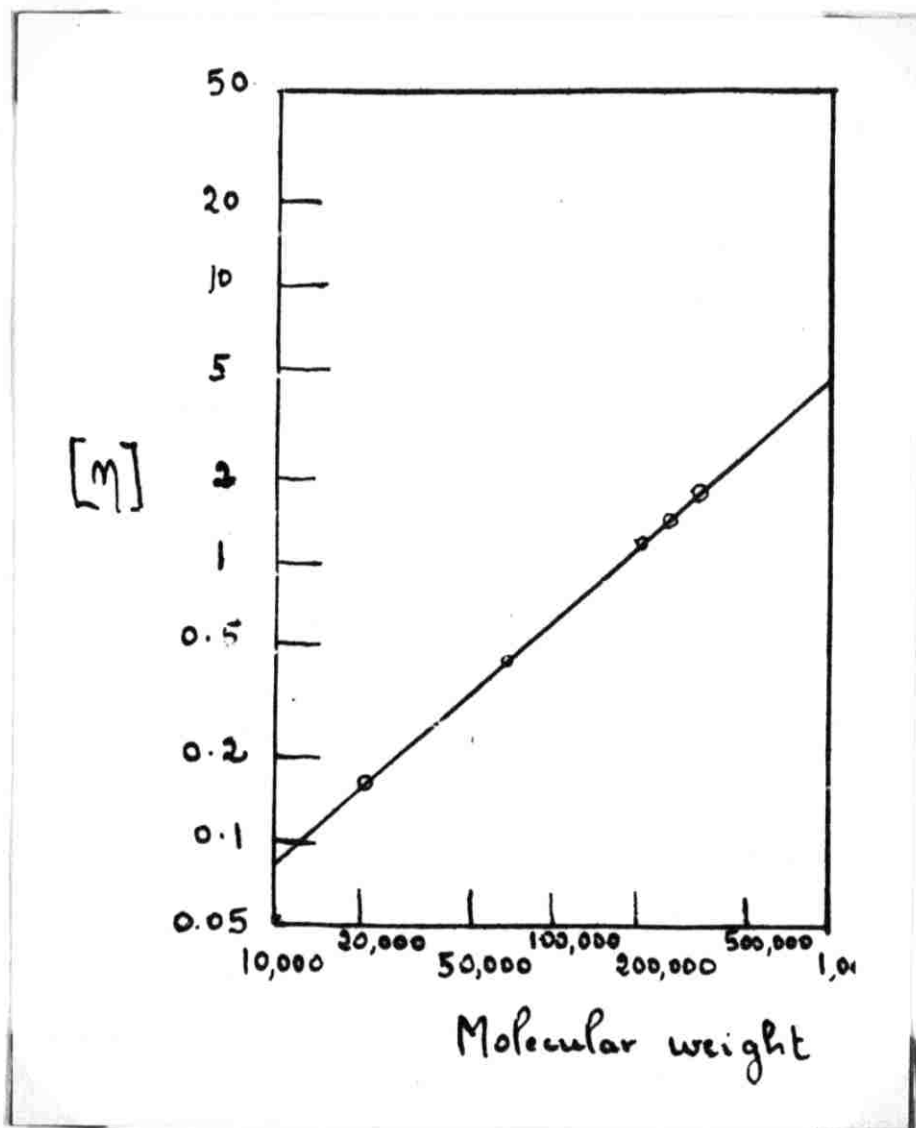


Fig. 1 - Intrinsic Viscosity vs. Molecular Weight for Poly-β-Benzyl-L-Glutamate (from Doty, P., Bradbury, J.H., and Holtzer, A.M., Journ. Amer. Chem. Soc. Vol. 78 (1956) 951)



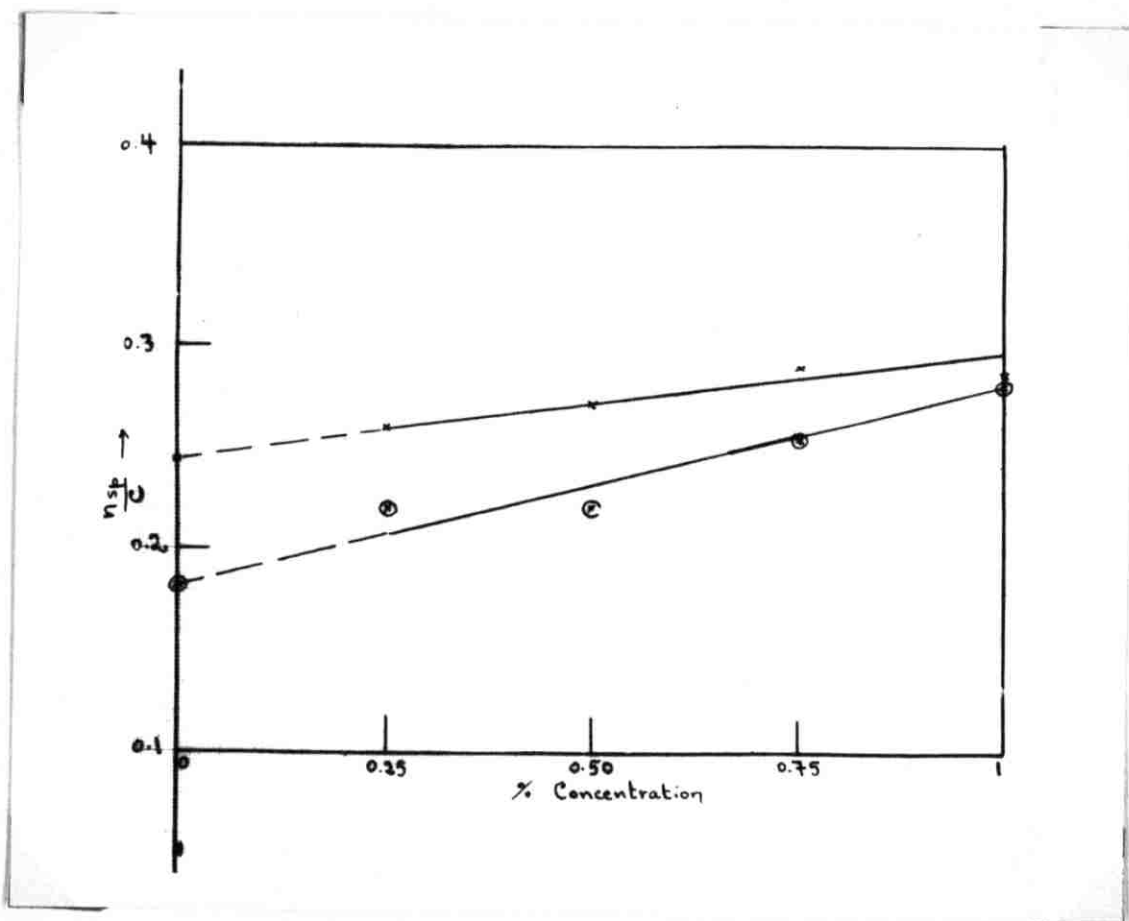


Fig. 2 - Intrinsic Viscosity vs. Percent Concentration for  
Poly- $\gamma$ -Benzyl-L-Glutamate I  $\times \times \times$   
Poly- $\gamma$ -Benzyl-L-Glutamate II  $\circ \circ \circ$

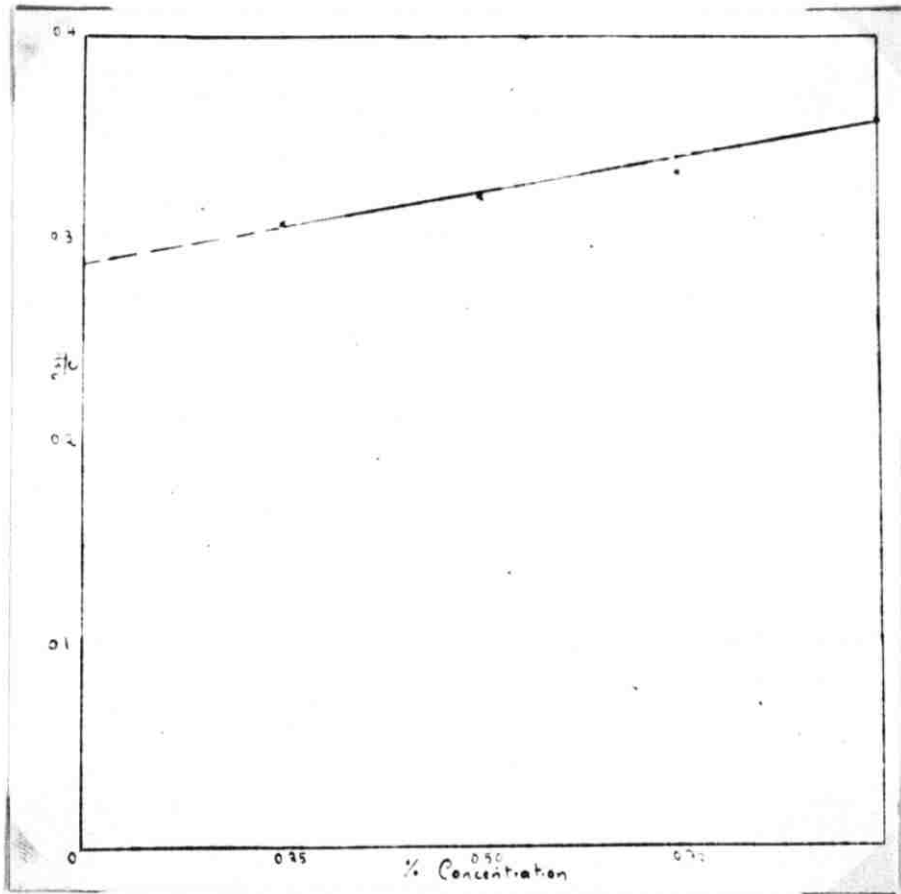


Fig. 3 - Intrinsic Viscosity vs. Percent Concentration for Poly- $\gamma$ -Benzyl-L-Glutamate III

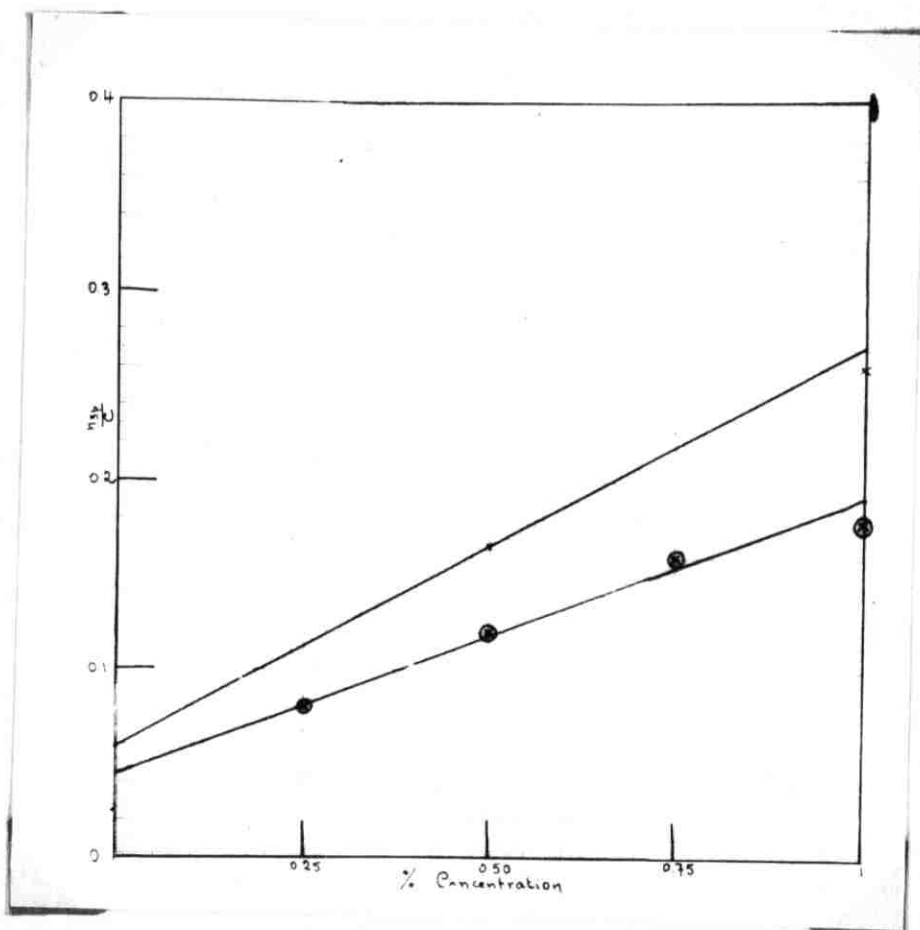


Fig. 4 - Intrinsic Viscosity vs. Percent Concentration for  
Poly-dl-Phenylalanine I    ⊙—⊙—⊙  
Poly dl-Phenylalanine II    ×—×—×

## DISCUSSION

Viscosity is a linear function of concentration in dilute solutions and the curve can be extrapolated to  $C=0$  to get the intrinsic viscosity. With a concentration beyond 8% (24) the viscosity generally will not follow a linear relationship.

One variable that must be very carefully controlled is the temperature at which measurements are carried out. Small variation in temperature can result in significant changes in the viscosity. An increase in temperature causes a decrease in viscosity which may be due to a decrease in the solvation process as well as to coiling or uncoiling of particles.

From the graphs it can be seen that the polymerization of glutamate in dioxane using sodium hydroxide in absolute methanol as initiator resulted in a higher molecular weight compound than was obtained by polymerizing the same sample in dioxane using triethyl amine as initiator. Therefore sodium hydroxide in absolute methanol appears to be a better initiator than triethyl amine. The molecular weight determination of poly- $\gamma$ -benzyl-L-glutamate was possible using Doty's graph where the relation between intrinsic viscosity and molecular weight, determined by light scattering, was available.

No such information was available for poly-DL-phenyl alanine so that the intrinsic viscosity gives only a relative

information about the molecular weights of the two samples. The controlled polymerization resulted in a higher molecular weight compound since the intrinsic viscosity is larger. This may be the result of more active centers being present when the polymerization took place for the first sample of poly-dl-phenyl alanine as was mentioned in the previous section.

## SPECTRA OF POLYMERS

### INTRODUCTION

Due to the similarity of their backbone structures, proteins and polypeptides have similar spectra arising from the repetition of the amide linkage  $\text{O}=\text{C} - \text{N} \begin{matrix} / \\ \backslash \\ \text{H} \end{matrix}$ . However, little information is obtained from spectral investigation about the residues which vary widely in composition.

Interest in the examination of the spectra in the past few years carried out by workers in the Courtaulds Research Laboratory in London (1), (2), (3), (15), (16) and more recently by Blout et al in Boston (7) arises from the fact that the structure of proteins and synthetic polypeptides is still under investigation. The material studied spectroscopically by the Courtaulds people was carefully investigated but determination of the physical properties of the polypeptides were not explored so that the information obtained was incomplete. On the other hand Blout et al investigated the properties of the material used in spectroscopy.

As shown from the spectra of synthetic polypeptides bands at ca. 3300, 1650 and 1550  $\text{cm}^{-1}$  do appear. These are due to NH stretching, CO stretching and a mixture of CN stretching and NH deformation frequencies. The exact location of the bands is a function of molecular configuration which

can be represented by two forms:

(i) the  $\alpha$ -helix or folded form

(ii) the  $\beta$  or extended form.

The next question was to find out the governing factor of the molecular configuration. The results of the investigations done by Courtauld's group implied that the solvent in which the samples were dissolved was the governing factor.

To study this problem Blout et al determined the spectra of series of poly- $\gamma$ -benzyl-L-glutamates of known molecular weights. Their conclusion was that high molecular weight samples exist in the  $\alpha$  form while the low molecular weight samples assume the  $\beta$  form irrespective of the solvent used.

The purpose of our present study was to identify the compounds synthesized (described in the first part of this paper) by an examination of the spectra and to confirm, if possible the results obtained by Blout et al.

## EXPERIMENTAL PROCEDURE

The determination of the spectra of the samples to be discussed was carried out on a Perkin-Elmer Model 112, double pass, single beam spectrophotometer using rock salt optics throughout the entire spectral region  $3500 - 750 \text{ cm}^{-1}$ . A calibration curve was obtained for the instrument by using known bands of atmospheric water and carbon dioxide for the region ca.  $4300 - 1300 \text{ cm}^{-1}$  and ammonia vapor from ca.  $1300 - 1700 \text{ cm}^{-1}$  (14). To obtain the  $\text{NH}_3$  vapor a 10 cm gas absorption cell was evacuated and the vapors from concentrated  $\text{NH}_4\text{OH}$  bottle were passed into the cell.

A constant energy background, which helps in identifying bands, was obtained by using a slit drive while running the spectra. However, at long wavelengths, the resulting large slit openings caused a decrease in resolving power of the instrument. To eliminate the absorption bands due to water vapor, the system was flushed with nitrogen after closing the openings with AgCl covers. With all these precautions, complete interference from water vapor was not possible which affected the spectra of phenyl alanine in that some of the bands were not identifiable.

The sample was dissolved in an appropriate solvent and spread on an AgCl plate. The solvent was then removed either by evaporation in an oven or by precipitation in ether. The solvent used with the polymers was dichloro



acetic acid except with phenyl alanine sample II and the anhydride of phenyl alanine where the films were prepared from chloroform solution. To determine whether heating the polymer in dichloro acetic acid results in its partial degradation the following tests were carried out:

An aliquot of a 1% solution of poly- $\gamma$ -benzyl-L-Glutamate in dichloro acetic acid was left in a water bath for one week at a temperature of 75° after which a colorimetric determination was carried out using the Biuret test to measure the extent of degradation of the protein (10) where the color shifts from purplish violet to blue as hydrolysis proceeds. A standard containing the same amount of solution was left at room temperature and its polypeptide content determined by the same method. Spectrophotometric readings showed that there was no significant change in the color intensity and maximum wavelength of the two solutions which proved that the solution was not hydrolyzed under these conditions. This is in contrast to an experiment we carried by refluxing the solution in alkali for several hours which resulted in the hydrolysis of the protein as shown by the same colorimetric determination.

To eliminate the possibility of hydrolysis resulting from the Biuret reagent itself (26) which consists of an equal amount of a 10% NaOH solution and a 0.2% CuSO<sub>4</sub>, pH measurements were done on a solution before and after hydrolysis. The results showed that there were a shift in

the pH from 12.6 to 9.52 while it remained the same under the ordinary conditions of the experiment proving that no hydrolysis results from the NaOH of Biuret reagent.

### Results

The results of the spectral investigation are presented on the following pages. The numbers in the tables refer to the maxima of the absorption bands and are given in wave numbers.

#### Assignment of frequencies:

The spectral region of proteins and synthetic polypeptides is divided into three regions (31) ca.  $3300\text{ cm}^{-1}$  designated as region I, ca.  $1600\text{ cm}^{-1}$  region II and about  $1250\text{ cm}^{-1}$  region III, which is the result of the nature of the molecules and the origin of the spectra. The bands in the  $3300\text{ cm}^{-1}$  region result from hydrogenic valence vibrations; those in the  $1600\text{ cm}^{-1}$  region arise from multiple bond valence vibrations and in the lower frequency region, the bands are due to hydrogenic deformation and skeletal vibrations.

Observed Bands in the Infrared Spectrum  
of  
Poly-  $\beta$ -Benzyl-L-Glutamate

I	II	III
3300	3300	3300
3022	3025	3022
2940	2932	2936
1726	1726	1723
1650	1650	1647
1540	1549	1543
1455	1452	1455
1390	1390	1390
1325	1325	1320
1255	1253	1255
1210	1212	1213
1177	1172	1170
1117	1120	1120
1082	1082	1082
1026	1026	1026
1000		1000
963	968	970
912	915	913
836	832	825
792	795	796

Observed Bands in the Infrared Spectrum  
of

<u>Poly-dl-Phenyl Alanine I</u>	<u>Poly-dl-Phenyl Alanine II</u>	<u>dl-phenyl Alanine Anhydride</u>
		3400
3280	3280	
3022	3022	
2936	2936	
		1822
		1773
	1638	
	1560	
		1400
		1360
		1307
1276	1270	1281
1219	1225	1240
1190	1190	1170
1120	1117	1110
1079	1080	1086
		1061
1030	1029	
		996
917	916	924
		900
819	822	

Poly- $\gamma$ -benzyl-L-glutamate

A characteristic band appeared at  $3300\text{ cm}^{-1}$  in all three samples. It is due to N-H valence vibration. The band at  $3022\text{ cm}^{-1}$  was not assigned to any specific group while the band at ca.  $2925\text{ cm}^{-1}$  was ascribed to a C-H valence vibration.

In region II two characteristic bands were observed at ca.  $1726$  and  $1650\text{ cm}^{-1}$  which can be ascribed to ester and carbonyl group valence vibrations respectively. The nature of the  $1545\text{ cm}^{-1}$  band is still not definite, some investigators attributing it to a combination of N-H deformation and C-N valence vibrations (5), (16) (22).

In region III the  $1390\text{ cm}^{-1}$  band is assigned to a C-H deformation vibration. The rest of the bands cannot be assigned with certainty.

Poly-DL-phenylalanine

The bands observed in the spectra of this compound are similar to the glutamate and will not be described again.

DL-phenylalanine anhydride

In region I the N-H valence vibration occurred at ca.  $3400\text{ cm}^{-1}$  as compared to the  $3280\text{ cm}^{-1}$  band in the polymer.

In region II a pair of bands at  $1822\text{ cm}^{-1}$  and  $1773\text{ cm}^{-1}$  was observed. The difference between them ca.  $49\text{ cm}^{-1}$  is in good agreement with five membered cyclic ring anhydride (5). They are attributed to the presence of the carbonyl group in

the anhydride.

In region III the strong band at  $1281\text{ cm}^{-1}$  may be assigned to a C-O-C stretching vibration. Below this region several bands occur but their origin is unknown.

#### DISCUSSION

The presence of a band at  $3300\text{ cm}^{-1}$  is characteristic of the  $\alpha$  configuration. It occurs at  $3280\text{ cm}^{-1}$  in the  $\beta$  form.

Our results show that the three samples of poly- $\gamma$ -benzyl-L-glutamate exist in the  $\alpha$  form. This is in agreement with the results obtained by viscosity measurements. The range was 23000 - 45000. Synthesis of lower molecular weight compounds could presumably be obtained by changing the anhydride - initiator ratio. Samples below a molecular weight of ca. 20,000 would be expected to be in the  $\beta$  configuration.

The bands at  $3280\text{ cm}^{-1}$  indicate that the poly-DL-phenylalanine samples exist in the  $\beta$  form.

Due to presence of NH and CO groups in phenylalanine anhydride, intermolecular hydrogen bonding would be expected. However, the presence of the NH band at  $3400\text{ cm}^{-1}$  shows that such hydrogen bonding does not exist.

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