PHYSICO-CHEMICAL STUDIES

ON

SEVERAL SYNTHETIC POLYPEPTIDES

BY

ABDU B. BARDAWIL

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Thanks are due to Dr. A. Elliott of the Courtaulds Laboratory who kindly offered us a sample of poly dl alanine.

ABSTRACT

In this thesis, the preparation, determination of molecular weight and investigation of the spectra of some synthetic polypeptides are described.

Poly dl alanine was prepared by polymerization of the dl alanine anhydride. The anhydride having been prepared by the reaction of the free amino acid with carbonyl chloride.

Poly dl tyrosine was prepared by the polymerization of O, carbobenzoxy dl tyrosine anhydride to yield poly O, carbobenzoxy dl tyrosine from which the protective group was removed by treatment with a glacial acetic acid solution of HBr. This procedure is analogous to the one used by Katchalski and Sela for the preparation of poly l tyrosine[14].

Attempts to synthesize poly dl methionine failed in that it proved impossible to prepare the anhydride intermediate.

By the amino nitrogen end group analysis method it was possible to show that one of the prepared samples of poly dl alanine was of low molecular weight. Viscosity measurements confirmed the fact that the other samples of the same polymer were also of low molecular weight.

The spectra of the two polymers, as well as the intermediates used in the preparation of poly dl tyrosine, were obtained. An analysis of the bands in the spectrum showed good agreement with the results of previous investigators.

Since the spectrum of poly dl tyrosine has not been published the spectrum obtained in these investigations could not be confirmed but a number of the more prominant bands were tentatively identified.

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INTRODUCTION

Inspite of the biological importance of proteins, little was known about their structure until quite recently. The reason for this is apparent when one considers the size of the protein molecule. Their molecular weight may be any where in the range of a few thousand to several millions. Consequently, the difficulty involved in trying to specify the location of all the various groups is only to be expected. The fact that most of the proteins are not crystalline in nature, together with the complexity due to their large size and large number of different side chains limits the scope of application of X-ray analysis. Also, great difficulties are encountered in synthesizing large molecules that can be utilized as model compounds for comparison with proteins. The results obtained from chemical studies indicated that proteins are linear polymers of α-amino acids containing polypeptide chains as their major constituent. This furnishes the synthetic polypeptides with particular interest since they may be considered as simple models of proteins. The extensive studies carried out on these polymers has shed considerable light on the structure of proteins, per se.

By means of X-ray analysis, Astbury and his colleagues[1] found that the polypeptide chains - in some fibrous proteins - are not in a simple extended form but rather are folded in

some regular manner. The biological activity of proteins is closely connected with the folding of its constituent polypeptide chains and the denaturation process of proteins is now known to be accompanied by the destruction of the polypeptide folding (proteins become biologically inactive when they are denatured).

Therefore, the study of the changes of the folding in synthetic polypeptides (caused by the rupturing of the bonds that stabilize the folding) may be of considerable help in explaining the activity of proteins. However, it is obvious that no polypeptide can be an adequate model of proteins, for the behaviour of the latter is determined both by the configuration of the back bone (i.e. by the folding of the polypeptide chain) and by the character of the side groups. Also, it is not permissible to assume that the folding of the synthetic material is necessarily the same as in proteins. Nevertheless, synthetic polymers are of some help in the determination of protein structure.

It is now known that polypeptides can be obtained in an α (folded) and β (extended) form. The folds in these polymers are believed to be regular and held by hydrogen bonds between the hydrogen on the amino nitrogen and the oxygen of the carboxyl group. The tendency of the polypeptide to assume the configuration of minimum energy enhances the degree of folding. The primary folds of proteins may be of a similar kind but the secondary folds may involve

other kind of forces, e.g. chemical cross linkages such as disulfide bonds. If the polypeptide (or protein) dissolves in a liquid which can form sufficiently strong hydrogen bonds with either >NH or >CO groups, the interpeptide hydrogen bonds are broken and the regular (primary) folding of the chain is lost. If this happens to proteins they are denatured. (This would not be expected in solutions in non-polar solvents).

With the exception of poly-glutamic acid, most of the synthetic polypeptides that have been prepared and studied by various workers in this field have been polypeptides made from amino acids with inert side chains. Consequently, it was felt that the preparation and study of polymers of tyrosine and methionine would perhaps be of value as model compounds for studies on protein structure. Furthermore, since these compounds have not been very fully investigated it was felt that an analysis of their infrared spectra would be of some value, per se.

Unfortunately, it was found that poly-dl-methionine could not be synthesized. Poly-dl-tyrosine was successfully synthesized and its synthesis and the analysis of its spectra (as well as the spectra of the intermediates prepared in the course of its synthesis) constitute the main portion of this thesis.

In addition an attempt was made to synthesize a high molecular weight sample of poly-dl- alanine so that its solution properties could be studied. Since this polymer

is water soluble and exhibits an α-β transformation, a study of the mechanism of this transformation could aid in an understanding of the process of denaturation in proteins. It was found, however, that the samples prepared here were of low molecular weight and could not be used for the desired studies. The preparation and determination of the molecular weights of several samples of this polymer are also discussed in this thesis.

PREPARATION OF THE POLYMERS

THEORY

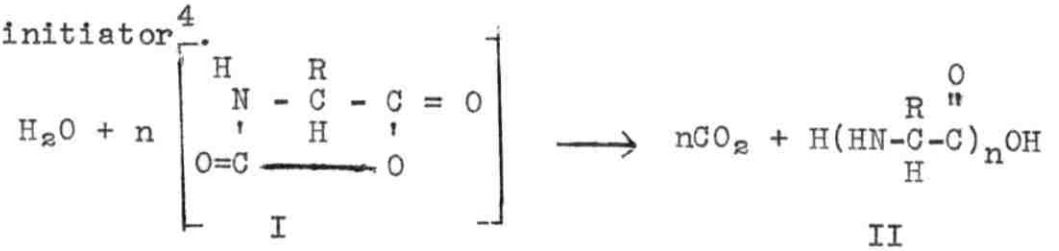
Fischer[2] was the first to polymerize amino acids by the stepwise method, in which amino acid residues were, singly and successively joined together. The polymers obtained by this method were, invariably, of low molecular weight.

Later, Katchalski[3] suggested the use of the amino acid anhydride for synthesizing polypeptides. The polymerization process involves two steps:

1) Initiation

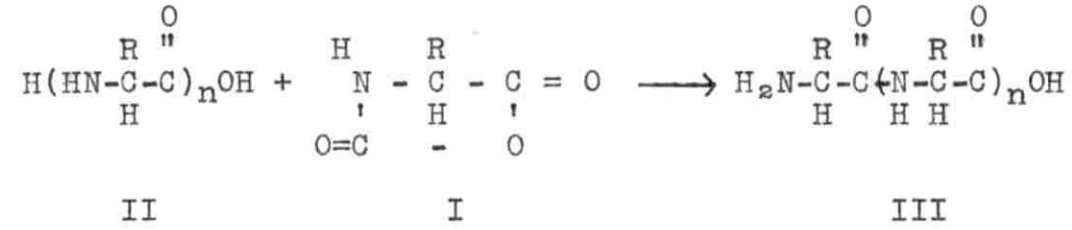
2) Propagation

In the first step, the monomer (anhydride), is initiated to couple with another monomer by a proper initiator. Any compound that has an active hydrogen can serve as an initiator[4].



I II

In the second step, the polymer (II) couples with another monomer (I) to form another polymer (III) of larger molecular weight.



II I III

The molecular weight of the product (III) is determined[5] by:

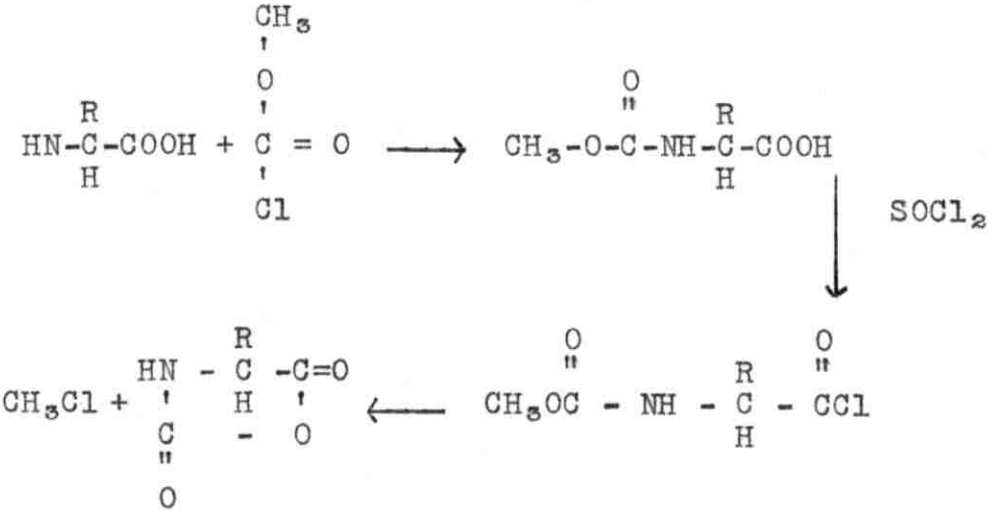
1) The concentration of the monomer, i.e. the anhydride.

2) The relative concentrations of the monomer and initiator, represented by the monomer to initiator ratio.

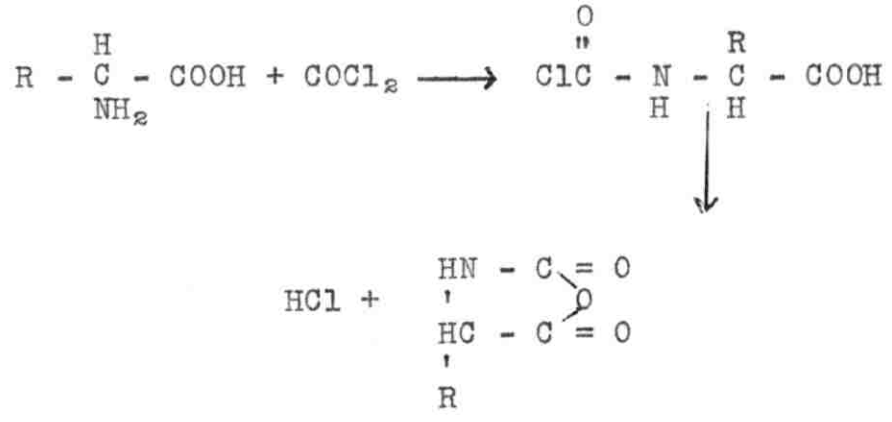
3) The relative rates of initiation and propagation of the reaction.

The three methods generally used for the preparation of N-amino acid anhydrides are:

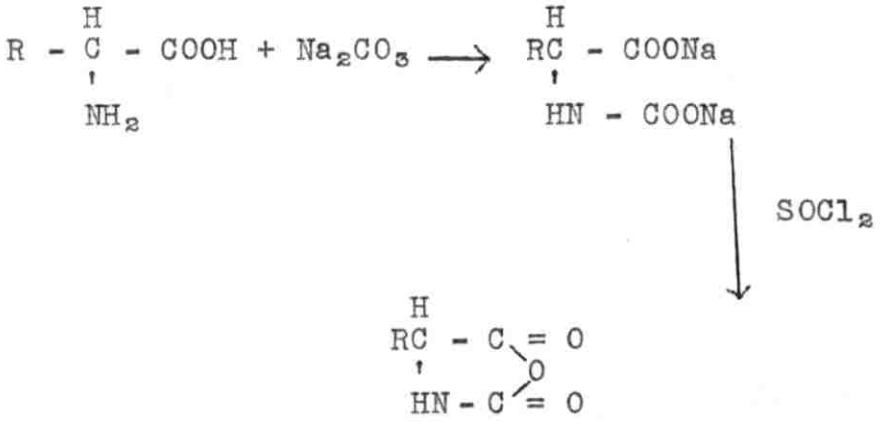
a) Leuch's method[6,7,8], in which the N-carbomethoxy derivative of the amino acid is treated with thionyl chloride:



b) Farthing's method[9], in which phosgene is allowed to react with the free amino acid dissolved in dioxane:



c) Fusch's method[10,11], in which the anhydride is cyclized with thionyl chloride from the disodium salt of the corresponding carbamic acid:

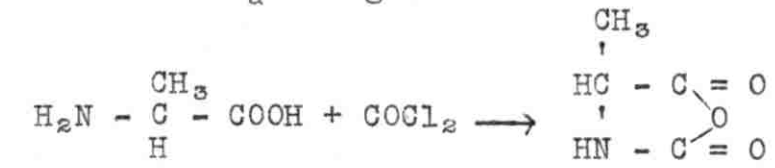


EXPERIMENTAL PROCEDURE

Poly-Dl-Alanine

a) Preparation of the anhydride

The method used for the preparation of the anhydride is that of Farthing[9].



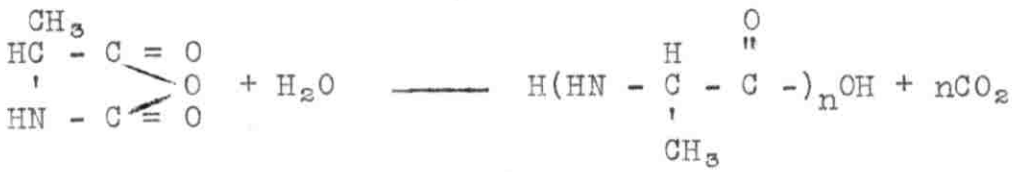
Five grams of dl alanine was suspended in dry dioxane (180 mls) at 38 - 40°C. Phosgene was passed through the solution for five hours until most of the amino acid dissolved. Excess phosgene was removed by passing dry air through the solution for thirty five hours.

After the undissolved amino acid was removed by filtration, the filtrate was distilled on a water bath (38 - 40°C) under reduced pressure (22 mm of Hg). The yellowish residual solution, 4-methyl-dl - 2:5 dione (anhydride) was dried in a vacuum desiccator over phosphorous pentoxide.

The structure of the anhydride was confirmed by the polymerization of a sample of the compound. The polymer gave a positive Biuret test indicating the presence of the peptide linkage.

b) Preparation of the polymer

The preparation of poly-dl-alanine involves a chain polymerization reaction of the N-carboxy amino acid anhydride with a suitable initiator.



A number of samples of the dl-alanine anhydride were polymerized in several different ways.

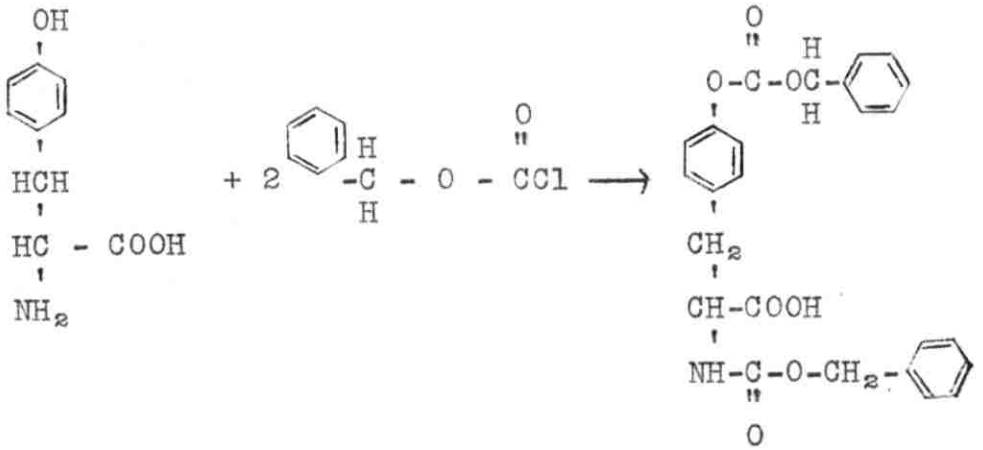
One sample was left in a vacuum desiccator over P_2O_5 for three months, after which time, a very viscous solution was obtained. The solution solidified immediately when exposed to air.

Other samples of the anhydride were polymerized by using water, benzene and aniline as initiators. When a sample of the anhydride was heated to 40-45°C in the presence of traces of water, polymerization occured more rapidly than at room temperature.

The polymers obtained were recrystallized from a 1:1 ethyl acetate - petroleum ether mixture. All of them gave a positive Biuret test. Poly dl alanine was found soluble in water, dichloroacetic acid, formic acid and 40% ethyl alcohol - water solution.

Poly-Dl-Tyrosine

The hydrogen atom of the phenol group of the amino acid (I) (dl-tyrosine) is first protected by forming the O, N-dicarbobenzoxy derivative (II). This is done by allowing the amino acid to react with benzyl chloroformate:



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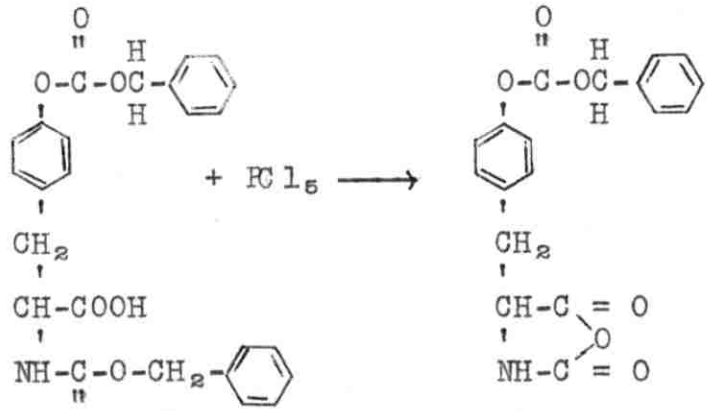
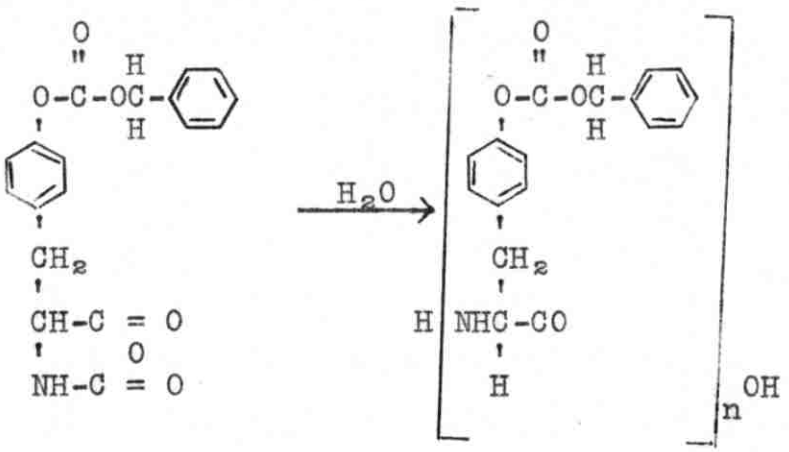
(II) reacts with phosphorous pentachloride to form the O- carbobenzoxy-dl-tyrosine anhydride (III).



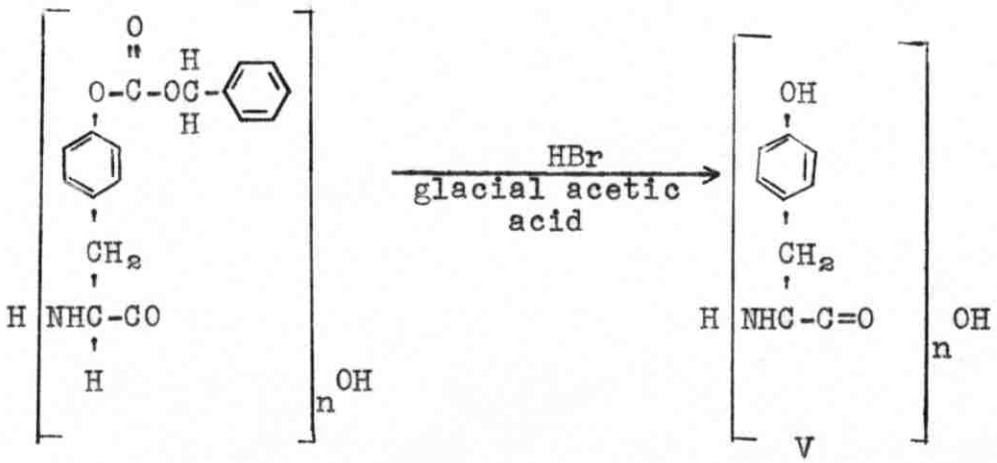
III

The anhydride (III) polymerizes in the presence of water as an initiator, to give poly O- carbobenzoxy-dl-tyrosine (IV).



IV

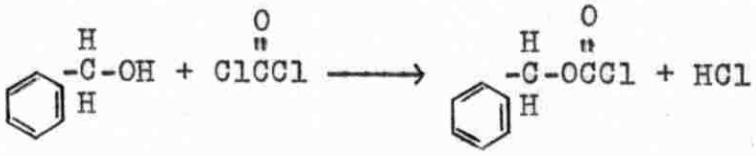
Poly-dl-tyrosine (V) is obtained by treating poly O-carbobenzoxy-dl-tyrosine (IV) with 33% HBr in glacial acetic acid.



V

Benzyl chloroformate is, therefore, an essential reagent for the preparation of poly-dl-tyrosine and poly dl methionine. This compound decomposes easily and, so, must be prepared freshly before its use in the laboratory.

a) Benzyl Chloroformate[12]



Dry toluene (about 540 g) was placed in a three liter round bottomed flask, which was stoppered with a two-hole rubber stopper: one of the holes was connected to the phosgene tank while the other was connected to a flask containing dry toluene. This flask was connected to another flask containing 10% NaOH. The exit gases were led to open air.

The reaction flask was cooled to 0°C in an ice-bath, and phosgene was allowed to bubble through the toluene for about seven hours (until approximately 105 g of the gas were absorbed by the s olvent). Then benzyl alcohol (105 g) was added to the reaction solution through a separatory funnel. The alcohol was added rapidly while shaking the reaction mixture gently. The solution was left for fifteen hours at room temperature..

Excess phosgene and the by-product, hydrochloric acid, were driven out of the solution by passing first nitrogen and then dry air through the system.

The solution was heated to a temperature less than 60°C. in a water bath and distilled under reduced pressure (25 mm of Hg). About 300 g of toluene were removed by distillation, the rest remained with t he benzyl chloroformate in t he reaction flask, (toluene does not interfere in the preparation of the derivatives).

The product was not purified through ordinary distillation because of the decomposition of benzyl chloroformate

at higher temperatures[13].

b) Preparation of Poly-Dl-Tyrosine

(i) Preparation of O, N- dicarbobenzoxy/dl tyrosine[14]

Dl-tyrosine (8 g) was dissolved in 2N sodium hydroxide (42 mls) and the solution was cooled to 0°C. Benzyl chloroformate (35 g) and 4N sodium hydroxide (30 mls) were then added, simultaneously, at different intervals for a period of one hour, and the reaction mixture stirred vigorously after each addition.

The solution was left for 50 minutes at room temperature and the creamy, oily product obtained was extracted twice with 100 ml portions of ether. The extracts were discarded and the aqueous layer was acidified with 4N hydrochloric acid to the congo red end point (pH 3.0). The product was extracted several times with ether and the combined extracts were dried over anhydrous sodium sulfate. The ether solution was evaporated and the white residue was recrystallized from benzene.

The compound obtained melted at 128 - 134°C., and was found to be soluble in chloroform, ethyl acetate, ether and boiling benzene. It gave a negative Millon test (indicating the absence of the phenol group). The neutralization equivalent of the compound was determined by the titration in ethanolic solution with aqueous sodium hydroxide using phenolphthalein as an indicator. The neutralization equivalent was found to be 447 as compared with the

theoretical value 449.

(ii) Preparation of O, carbobenzoxy-dl-tyrosine anhydride

The procedure followed for the preparation of O, carbobenzoxy-dl-tyrosine anhydride, poly O, carbobenzoxy-dl-tyrosine and poly-dl-tyrosine is analogous to Katchalski's[14] method for the preparation of l-derivatives of the same compounds.

O, N-dicarbobenzoxy-dl-tyrosine (3.5 g) was dissolved in anhydrous benzene (50 mls) and the solution quickly cooled to 0°C. Two grams of phosphorous pentachloride were added to the cold solution and the mixture shaken for 15 minutes. After shaking it was filtered to remove any undissolved phosphorus pentachloride. The filtrate was heated to 50°C and was shaken for half an hour after which it was left at room temperature for five hours.

Precipitation of the anhydride was induced by the addition of 100 mls of petroleum ether to the solution. The compound was recrystallized from benzene and then dried in vacuo over H_2SO_4 and KOH.

The anhydride (III) melted at 75 - 77°C with the evolution of gas and was found to be soluble in benzene and ethyl acetate. It was insoluble in petroleum ether.

The structure of the anhydride was confirmed by polymerizing a sample of the compound. The product gave a positive Biuret test.

(iii) Preparation of poly O-carbobenzoxy-dl-tyrosine

Poly O-carbobenzoxy-dl-tyrosine was obtained by refluxing a benzene solution of the anhydride (2 g of the anhydride in 30 mls of benzene) for forty two hours. The traces of water present in the benzene played the role of an initiator.

After refluxing the solution was cooled to room temperature and petroleum ether (150 mls) was added. The white fibrous precipitate formed was purified by dissolving it in hot dichloroacetic acid and pouring the acid solution into ice-cold water. The polymer (IV) was removed by filtration and was washed with cold water and dried in vacuo over H_2SO_4 and KOH.

Compound IV gave a negative Millon test and a positive Biuret test.

(iv) Preparation of poly-dl-tyrosine

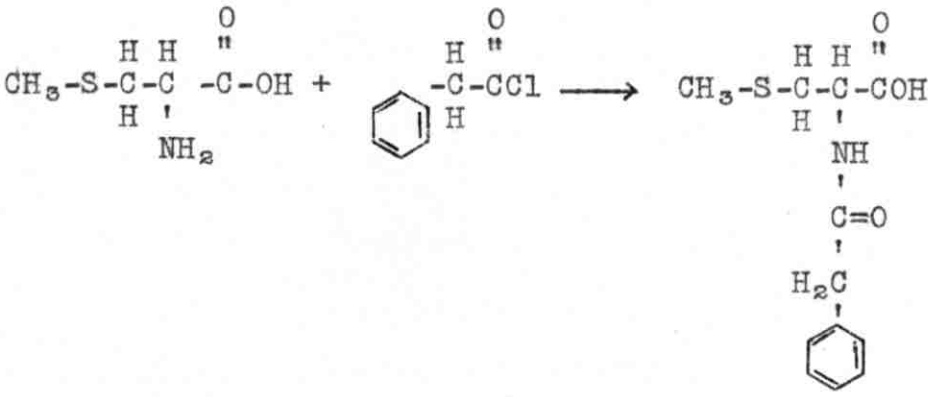
To remove the carbobenzoxy protective groups from the carbobenzoxy polymer, 0.7 g of poly O-carbobenzoxy-dl-tyrosine were added to 10 g of a 30% HBr solution in glacial acetic acid. The polymer went into solution with the evolution of gas. After the solution had stood for one hour at room temperature, anhydrous ether (150 mls) was added. The white precipitate which was formed was redissolved in 5 mls of absolute ethyl alcohol, and then the solution was poured into ice-cold water to reprecipitate the polymer.

The poly-dl-tyrosine obtained gave a positive Biuret test and a positive Millon test.

Poly-Dl-Methionine

a) N-carbobenzoxy-dl-methionine

The method used in preparing N,- carbobenzoxy-dl-methionine is similar to that of Carpenter[15]. However, instead of using p, nitrobenzylchloroformate, benzylchloroformate was used in this preparation.



The amino acid (15 g) was dissolved in 4N sodium hydroxide (25 mls). This solution was cooled to 0°C. and benzyl chloroformate and 4N sodium hydroxide were added at intervals with vigorous shaking for one hour until a total of 50 g of benzylchloroformate and 25 mls of sodium hydroxide had been added. After standing for two hours at room temperature the alkaline solution was acidified with concentrated hydrochloric acid and extracted with ethyl acetate. The organic phase was washed with 1N HCl and water and was then extracted with 1N sodium bicarbonate. The alkaline layer was washed with ethyl acetate before it was

acidified again with concentrated hydrochloric acid. The precipitate formed was collected by filtration, and was recrystallized from an n-amyl acetate - n-hexane mixture.

The compound melted at 107 - 109°C and was found to be soluble in ethanol, amyl acetate and ethyl acetate. Its neutralization equivalent was found equal to 285.6 ; the theoretical value is 283.

b) Dl-methionine anhydride

Attempts were made to form the dl-methionine anhydride from its N-carbobenzoxy derivative by allowing the derivative to react with phosphorous pentachloride. The carbobenzoxy derivative of dl-methionine was dissolved in different solvents, such as boiling benzene, amyl acetate and ethyl acetate, and the solution was cooled to zero before adding phosphorous pentachloride. In most of the cases, the original compound was recovered and no product was obtained. All these attempts failed to give the dl-methionine anhydride.

MOLECULAR WEIGHT DETERMINATION

INTRODUCTION

Although either physical or chemical methods may be used for the determination of the molecular weight of synthetic polypeptides, not all methods will give results expressed in terms of absolute weights. The most commonly applied physical methods depend upon the measurement of osmotic pressures, light scattering, sedementation rates and the viscosity of dilute solutions of polypeptide samples. The results obtained with these various methods are not usually the same due to the diverse solubility properties of the poly amino acids. And in particular because the samples involved possess a range of molecular weights so that in reality only average values are obtained. Depending upon the physical variable measured the resultant molecular weight obtained may be a number average value or a weight average value.

Eirich et al[16] determined the molecular weight of poly-ε-N-carbobenzoxy-l-lysine from sedimentation rate, osmotic pressure and viscosity measurements. Osmotic pressure measurements gave a molecular weight value of 6.5×10^3, in NN-dimethyl formamide and 26×10^3 in benzyl alcohol. These results emphasize the fact that the choice of solvent for the determination of the molecular weight of a polypeptide is of great importance.

The physical methods find extensive use in cases where only relative measurements are required. In this work, viscosity measurements were carried out on polymers of dl alanine to determine the relative molecular weight of several samples of this polymer. The chemical methods for the determination of the molecular weight of polypeptides depend unpon the chemical reactivity of the end groups of the amino acids and consequently they are sometimes known as end group analysis methods. The two principal chemical methods are: the Van Slyke Amino Nitrogen Method[17] and the 2:4 Dinitro Fluoro Benzene Derivative Method (DNFB)[18]. The use of the Van Slyke Method was at first limited to water-soluble proteins until Doherty and Ogg[19] developed a modified procedure by means of which the molecular weight of water-insoluble materials could be determined. This method is similar to Van Slyke's original method in that the molecular weight of the poly amino acid is determined by measuring quantitatively the volume of nitrogen gas liberated when nitrous acid is allowed to react with a sample of the polymer.

The second method (DNFB derivative) involves a coupling reaction between the free amino group of the polymer and DNFB (one molecule of DNFB to each amino group). The product of this coupling reaction is a coloured substance. In order to determine the molecular weight of the polymer, a measured sample of its DNFB derivative is first hydrolyzed

(acid hydrolysis); then the number of amino group which are coupled with DNFB is determined by a colourimetric comparison of the hydrolized polymer sample with a standard prepared from the DNFB derivative of the free amino acid. Since each polymeric molecule terminates with an amino group the number of these groups must give a measure of the molecular weight of the polymer.

The molecular weight of synthetic polypeptides can also be determined through potentiometric titration[20]. Another method, is by accurate measurement of the anhydride to initiator ratio.

MOLECULAR WEIGHT OF POLY-DL-ALANINE

a) Relative Molecular Weight Measurements - Viscosity

The relative molecular weights of different samples of poly-dl-alanine were determined by viscosity measurements. Inspite of the simplicity of this method (from the experimental point of view) there are a number of variables that have to be taken into account, e.g. temperature and solvent. Unless a calibration curve is available viscosity measurements can not give more than relative molecular weights.

(i) Theory

Viscosity can be defined as that property in fluids, which opposes the relative motion of adjacent portions of the fluid and can be considered as a type of internal friction. If a liquid with a coefficient of viscosity (η) flows with a uniform velocity at a rate of v cm in t sec., through a narrow tube of radius r cm and length l cm, under a driving pressure of p dynes/cm, then:

$$\eta = \frac{pr^4t}{8vl} \qquad 21$$

From this equation it is clear that unless the variables are known, or unless the viscometer used is calibrated with a liquid of known η, then no absolute values for η are to be expected. All the above variables are, with the exception of p, dependent on the viscometer. The pressure (p) however, can be replaced by ghd, where h is the height of the liquid

in the capillary in cm, g is the gravitational attraction (981 cm/sec^2) and d is the density of the solution used in gm/ml.

By substituting ghd for p in the above equation the relative coefficients of viscosity (η_1, η_2) of two different solutions of different densities (d_1, d_2) can be determined. If the measurements are made in the same viscometer:

$$\frac{\eta_1}{\eta_2} = \frac{d_1 t_1}{d_2 t_2}$$

where t_1 and t_2 are the rates of flow of solution (1) and (2) respectively.

The viscosity of a solution is usually expressed in terms of its specific viscosity (η_{sp}) which is defined as:

$$\eta_{sp} = \frac{\eta_{obs} - \eta_o}{\eta_o}$$

where η_o is the viscosity coefficient of the solvent. A still more useful term is the intrinsic viscosity $[\eta]$;

$$[\eta] = \frac{\eta_{sp}}{c}$$

where c is the concentration of the solute in grams per liter of solvent.

Staundinger[22] related $[\eta]$ to the molecular weight of the polymer

$$[\eta] = Km\, \bar{M}^a$$

where Km and a are constants dependent on the substance used.

For long molecules a is usually unity.

Because of the variation of $\frac{n_{sp}}{c}$ with c, n is usually obtained by extrapolating the graph $\frac{n_{sp}}{c}$ vs c to zero concentration. This gives the value of the intrinsic viscosity at infinite dilution.

(ii) Procedure

The viscosity of four solutions (dichloroacetic acid) of equal concentration of four different polymer samples of dl-alanine was determined in an Ubbelholde viscometer. The rates of flow of these samples were recorded at a temperature of 26.5°C. The densities of the solution were determined with a picnometer.

(iii) Results

From the relation -

$$\frac{n_1}{n_2} = \frac{d_1 t_1}{d_2 t_2}$$

it is clear that the relative viscosity, and hence the relative molecular weight, can be obtained by comparing the $d_i t_i$ products for all the samples.

Table 1 gives the average rates of flow, the densities and the product dt for the different solutions used.

Table 1

Sample	1	2	3	4
density in grs/mls	1.5676	1.5531	1.5593	1.5537
average rate of flow t in sec	385.8	394.4	378.1	387.8
dt in $\frac{\text{grs x sec}}{\text{ml}}$	604.7	612.5	589.6	602.5

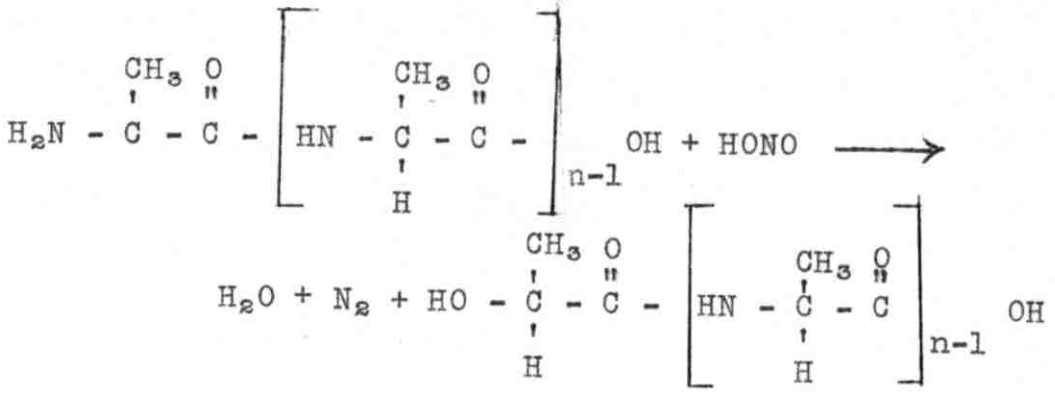
The third row in Table 1 shows that the four polymer samples have approximately equal molecular weights.

The absolute value of the molecular weight of sample number four was determined by the V_an Slyke method as described in the following section.

b) Absolute Measurement - V_an Slyke

(i) Theory

The method which was used to determine the absolute value of the molecular weight of poly-dl-alanine is the Van Slyke method. This method involves the reaction of nitrous acid with the amino group of the polymer.



The pressure of the liberated nitrogen gas can be measured (in terms of mm of Hg) and the molecular weight of the polymer can then be obtained by multiplying this pressure by a factor, which is a function of the temperature and of the apparatus used. Tables of these factors are usually provided with the instrument.

(ii) Procedure

The Van Slyke apparatus used was an old one, and was lacking an essential piece, which is the Hample pipette. So it was necessary to modify the usual procedure[23].

Five millimeters of an aqueous solution of the polymer, plus one milliliter of glacial acetic acid were introduced into the instrument and the solution was shaken for two minutes. Nitrous acid (2.5 mls) was then added and the reaction was left for three minutes at 20°C. For the last 1.5 minutes the solution was shaken. The reading of the mercury manometer (attached to the instrument) was recorded as p_1. Twenty milliliters of potassium permanganate were then introduced inorder to absorb the evolved nitrogen. The

solution was shaken for 1.5 minutes, after which time 1.5 mls of the solution were driven out of the apparatus and the manometer reading was recorded as p_o. The difference $p_1 - p_o$ gives the pressure of the nitrogen gas liberated from a measured sample of the polymer.

(iii) Results

The apparatus was first calibrated against samples of dl alanine and the results obtained (for six trials) for the molecular weight of the amino acid were found to deviate by less than 8% from the true value for the molecular weight of dl alanine (molecular weight, found experimentally, is 96, as compared with the theoretical value for the molecular weight is 89).

The average value (three trials) for the molecular weight of a sample of poly_dl_alanine was found to be 1750.

Although the value obtained for the molecular weight may not be considered to be precise it is certainly true that the molecular weight of the sample was low. The viscosity values show that this will be true also for all the samples prepared. Consequently the samples could not be used in studies on the α-β transformation since low molecular weight polymers can not be completely converted to the α configuration. Further support for this conclusion was gained from an analysis of the infrared spectrum of this polymer and the data will be given in another section.

INFRARED SPECTRA OF POLYMERS

INTRODUCTION

The internal energy of a molecular system may be subdivided into three component parts: the electronic energy arising from the distribution of the electrons in the potential field of the nuclei, the vibrational energy arising from the oscillatory motions of the nuclei and the rotational energy. The absorption of energy by such a system results in an alternation in any one or all three of these component terms. By means of spectroscopic methods it is possible to observe the energy changes occuring in a molecular system. Large changes in energy result from electronic transitions and give rise to spectra in the visible or ultraviolet regions. Vibrational and / or rotational transitions result in small changes in energy and hence give rise to spectra in the infrared region. Consequently, an examination of the infrared spectrum of a compound will give information concerning the molecular motions that take place.

The application of spectroscopic methods to the study of protein structure commenced with the work of Wright[24], Blout[25], Edsall[26], Darmon[27] and Buswell et al[28,29].

In these studies the absorption bands characteristic of the hydrogen bonded NH groups and carbonyl groups, as well as some other components in the protein molecule were identified. Also, it was found that due to the similarity of

their backbone structure, polypeptides and proteins have similar spectra arising from the absorption of the amide linkage $-\overset{\overset{O}{\|}}{C}-\underset{H}{N}-$ [30]. This fact emphasized the usefulness of synthetic polypeptides as an aid in the s tudy of the structure of proteins.

Infrared spectra give information concerning the configuration of molecules. Since synthetic polypeptides are capable of assuming two different molecular configurations (the α - or folded configuration and the β or extended configuration) it was expected, and has been found, that certain differences occur in the infrared absorption spectra of the two forms[31,32]. The principal differences occur in the location of the bands that are associated with the peptide link although other minor differences have also been noted.

The transformation of α to β was found by Ambrose and Elliott[32,33] to cause a lowering (about 30 cm^{-1}) of the C=O stretching and NH complex deformation frequencies.

According to Elliott[34], films of high molecular weight poly_dl_alanine (D.p. = 500) give an α peak due to the C=O stretching mode of vibration at 1661 cm^{-1} and do not have an absorption band at 1629 cm^{-1} corresponding to the β form. But when such sample is boiled in water an insoluble gel forms which gives rise to two absorption maxima, one at 1661 cm^{-1} (due to C=O stretching in the α form) and the

other at 1629 cm^{-1} (due to the C=O carbonyl stretching in the β form). The second peak (1629 cm^{-1}) disappears and only the α peak (1661 cm^{-1}) is observed, when the gel is dissolved in formic acid. Also the α form of poly dl alanine shows the NH complex deformation peak at 1539 cm^{-1} while the β form shows it at 1515 cm^{-1}.

The work done in the Courtaulds laboratory by Ambrose and Elliott[32,33,34,35,36] proved that the solvent plays an important role in this α - β transformation. Furthermore, investigation by Blout et al[37] on the spectra of many samples of poly-γ-benzyl-l-glutamate of known molecular weight, showed that high molecular weight polypeptides tend to assume the α form while the low molecular weight samples assume the β form. This seems to be true regardless of the solvents used if the molecular weights are sufficiently high or low respectively. This means, therefore, that infrared studies may be used to obtain a very approximate indication of the molecular weight of a polypeptide sample.

EXPERIMENTAL PROCEDURE

The instrument used in the infrared study of the different compounds prepared was a Perkin-Elmer Model 112, double pass, single beam Spectrophotometer. A calibration curve was obtained for the instrument by using known bands of atmospheric water vapor and carbon dioxide for the region 4000 - 1300 cm^{-1} [38,41] This calibration curve was checked against a polystyrene film sample provided with the instrument. The results obtained from the calibration curve for the polystyrene sample were less than 1% in error as compared with the reported ones.

To eliminate the absorption bands due to water vapor, the system was flushed continuously with nitrogen. Since this did not completely eliminate the interference from water vapor a blank was also run for every measurement to facilitate the precise determination of the spectra.

The spectroscopic samples were prepared by dissolving the polypeptide in suitable solvents and spreading the solution on AgCl plates. The solvent was then removed by evaporation in an oven.

RESULTS

The results of the spectral investigation are presented on the following pages. The numbers in the tables refer to the position of the maxima of the absorption bands and are given in wave numbers (cm^{-1}).

Poly_dl_alanine

The infrared spectra of poly_dl_alanine was studied extensively by Elliott and the changes in absorption due to the α - β transformation were determined[34,39,40]. In the present study spectroscopic measurements have only been used as an indirect source of information about the molecular weight of the poly_dl_alanine samples prepared.

Films prepared from solutions of poly_dl_alanine (sample number four) in water had absorption bands at both 1660 cm^{-1} and 1630 cm^{-1} corresponding to the α and β configurations respectively.

Films prepared from solutions of the same sample in formic acid and dichloroacetic acid also showed the same peaks. In all of these solutions the 1515 cm^{-1} peak due to β NH complex deformation, was observed, while the NH complex deformation at 1539 cm^{-1} characteristic of the α form was absent.

The conclusion to be drawn from these results is a confirmation of the results obtained from the end group analysis measurements, that the poly_dl_alanine samples

prepared were of low molecular weight.

D_r. A Elliott of the Courtaulds laboratory, England, kindly provided us with a sample of poly-dl-alanine (of unknown molecular weight) which, had been dissolved in formic acid and dialised against water to free it from small molecular weight peptides. It had then been freeze-dried.

The infrared spectra of films of this polymer cast from water, dichloroacetic acid and formic acid solutions gave the 1515 cm^{-1} and 1629 cm^{-1} β peaks.

This again indicates that this sample of poly-dl-alanine is also of low molecular weight.

Poly-dl-tyrosine and its derivatives and N-carbobenzoxy-dl-methionine

The spectra of poly-dl-tyrosine and its derivatives are expected to show certain characteristic features in view the fact that certain functional groups are common to all. All of the compounds prepared contain the phenyl ring and consequently have bands in the region of 1500 cm^{-1} and 1600 cm^{-1}. In many systems containing the aromatic ring these two bands are at or very close to the positions noted. However, the addition of substituents to the ring may be expected to shift the position of both of these bands and para substitution (as in the compounds studied here) generally leads to an increase in these two frequencies. All of the compounds studied have absorption bands in these two regions

and the specific locations and tentative assignments are noted in the tables.

Another band which is very characteristic of the phenyl ring is the =CH. stretching frequency occurring at ca 3040 cm^{-1}. This band has been located for all the compounds studied.

The CH_2 group is also common to all of the compounds studied and its characteristic deformation at ca 1462 cm^{-1} has been observed in all cases except that of methionine. In methionine the band seems to have undergone a shift to somewhat higher frequency, 1473 cm^{-1}. Since this is the only band observed in this region its identification would seem certain, but no explanation is forthcoming as to the rather unusual shift.

The only other group which is common to all of the molecules studied is the peptide group $-\overset{O}{\overset{\|}{C}}-\overset{H}{\overset{|}{N}}-$. In general this group gives rise to several characteristic bands: ca 3300 cm^{-1} due to the hydrogen bonded N-H stretching vibration, ca 1650 cm^{-1} due to the hydrogen bonded C=O stretching vibration, ca 1550 cm^{-1} due to the complex deformation vibration of the hydrogen bonded N-H, and ca 700 cm^{-1} also due to the N-H deformation vibration. The 3300 cm^{-1} band is observed only for poly O-carbobenzoxy-dl-tyrosine and poly-dl-tyrosine. However, in the remaining

three compounds a band is observed at ca 3450 cm^{-1} which is in all likelihood due to the non-hydrogen-bonded NH group. In general free NH groups in amides etc. give rise to absorption in the region 3400 - 3450 cm^{-1}. The carbonyl stretching frequency at ca 1650 cm^{-1} is observed only for poly O,- carbobenzoxy-dl-tyrosine. In all of the remaining compounds a band is noted in the region ca 1675 cm^{-1}. This position corresponds very well with that for a free peptide carbonyl group and is in agreement with the predicted free NH group for these compounds - with the exception of poly dl-tyrosine. This particular molecule presents a very real anomaly in that the data are conflicting. The NH complex deformation mode was observed in all compounds except the O, N-dicarbobenzoxy derivative. In this case two bands are noted but neither in a position that allows for unequivocal identification. The low frequency NH deformation vibration occurs outside of the region investigated in these studies.

Of the large number of remaining bands only a few can be identified. The characteristic ester carbonyl stretching frequency is observed in most instances at ca 1730 cm^{-1}. In the O, N dicarbobenzoxy derivative there is an additional band in this region that can be assigned to the carbonyl of the carboxyllic acid group.

The two bands at 1787 cm^{-1} and 1833 cm^{-1} in O, carbobenzoxy dl tyrosine anhydride serve as positive identification for that compound. It has been found that compounds

containing a five membered ring anhydride have two absorption bands, one in the region 1740 - 1800 cm^{-1} and the other in the region 1800 - 1860 cm^{-1}. The difference in location of these bands is usually of the order of 50 cm^{-1}. In the compound studied here the difference of 46 cm^{-1} is in good agreement with the anticipated value.

In the spectrum of poly-dl-tyrosine the band at 3652 cm^{-1} is good evidence for the free -OH phenol group which usually absorbs in the region 3650 - 3590 cm^{-1}. The occurrence of the bands at 3622 cm^{-1} in several of the other compounds can not be explained, but would not seem to invalidate the assignment of the 3652 cm^{-1} band to the OH absorption.

In the case of N-carbobenzoxy-dl-methionine the only distinctive band that could be identified is the 1375 or 1386 cm^{-1} band arising from the symmetrical CH_3 deformation vibration. A band corresponding to the asymmetric deformation vibration should also be observed at 1450 $\pm$ 20 cm^{-1} but can not be assigned with certainty.

In conclusion it should be pointed out that most of the band assignments have been made on the basis of the extensive tabulations in Bellamy[30] and of other work on proteins and polypeptides[32,33,34,40].

Table 2

Absorption Bands

O,N dicarbobenzoxy dl tyrosine	O, carbobenzoxy dl tyrosine anhydride	Poly, O, carbobenzoxy dl tyrosine	Poly dl tyrosine	N, carbobenzoxy dl methionine	Assignment
1335	1341	-	1335	1338	δ CH alkyl
1354	1359	-	-	1359	
1371	1375	1375	1375	1375	δ CH_3
1384	1384	-	-	1386	
1397	1400	1390	1397	-	
1414	1421	-	1411	1419	
1456	1459	1462	1458	1473	δ CH_2
-	1473	1482	1484	-	
1495	1495	-	-	1495	
1506	1510	1507	1503	1507	) ν C=C
1523	1523	1530	1526	1523	)
1539	1543	-	1544	1543	
1575	1562	1558	1560	1562	δ NH
1598	-	1583	1586	-	) ν C=C
-	1623	-	1623	1631	)
1678	1651	1656	1682	1685	) ν C=O amide
1695	1691	-	1692	1695	)
-	1695	-	-	-	
1726	-	1737	1730	1730	) ν C=O acid and ester
1740	-	-	-	-	)
-	1787	-	-	-	) C=O anhydride
-	1833	-	-	-	) C=O
-	2292	-	-	-	
2350	-	-	2322	2372	
-	2552	-	-	-	
-	-	-	2710	-	
-	2754	2754	-	-	
2820	-	-	-	2850	ν CH_2
-	-	2900	-	-	
3030	3050	3052	3040	3052	ν CH phenyl
-	-	3300	3300	-	ν NH amide
3460	3460	-	-	3430	
3622	3622	-	-	3622	
-	-	-	3652	-	OH phenol
3920	3920	-	3920	3920	

SUMMARY

In conclusion it would seem desirable to summarize the results obtained in the present investigation. The work falls naturally into several distinct sections and will be discussed in that manner.

Poly-dl-Alanine

The initial purpose of this work was to prepare a high molecular weight sample of poly-dl-alanine. In some solvents (notably water) this polymer occurs in the α-configuration. In other solvents (such as formic acid) the polymer occurs in the β-configuration. By varying the composition of the solvent it should be possible to cause a transformation from the α — β configuration and it is this phenomenon which was to be studied in the present work. Furthermore, the stability of the α-helical configuration may be expected to vary with temperature and the determination of the transition temperature for this transformation was another object of this work. The two forms, α and β can be identified by differences in their infrared spectron as well as in their optical rotation.

To prepare the samples needed for these studies the carbobenzoxy anhydride of dl-alanine was polymerized in a number of different ways. The anhydride had been prepared by the reaction of dl-alanine with carbonyl chloride.

By utilizing the Van Slyke Amino Nitrogen end group

analysis method, it was possible to determine the average molecular weight of one of the polymers. Then by a comparison of the relative viscosities of the various polymers it was possible to estimate the approximate molecular weights of the other samples. These tests showed that all of the polymers prepared had molecular weights of less than 2000.

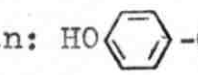
The fact that the polymers of dl-alanine were of low molecular weight could be due to one of several reasons: i) the use of a high concentration of initiator, thus making the anhydride to initiator ratio low. ii) the failure to obtain a pure sample of the monomer prior to polymerization (the sample was contaminated with the solvent used in the preparation of the anhydride, dioxane). iii) the presence of impurities which might act as initiators in the solvents used.

The infrared spectra of the various polymer samples all showed absorption bands at 1515 cm^{-1} and 1629 cm^{-1} as well as bands at 1497 cm^{-1} and 1695 cm^{-1}. The bands at 1515 cm^{-1} and 1629 cm^{-1} are characteristic of the complex NH deformation vibration and the CO stretching vibration in polypeptides in the β-configuration. Hence, it became apparent that it was not possible to obtain samples in the pure α-configuration with the polymers produced in this work. This was not surprising since polypeptides with D.P.'s of 50, 100 or greater are usually required to produce pure α-samples. The polymers produced in these investigations

had D.P.'s of about 25.

The failure to obtain samples of high molecular weight poly-dl-alanine made the study of the $\alpha \rightleftharpoons \beta$ transformation impossible.

Preparation of poly-dl-Tyrosine and poly-dl-Methionine

Another aim of the present work was the preparation and investigation of the infrared spectra of poly-dl-tyrosine and poly-dl-methionine. The reasons for this were: i) most of the polypeptide samples prepared by other investigators have had inert side chains. In proteins, however, many of the side chains are not inert but contain reactive groups and consequently side chain interactions may be an important factor in the stabilization of protein structures. Hence, the study of polypeptides with active groups on the side chains might throw considerable light on the problem of protein structure. The polymers selected were poly-dl-tyrosine (side chain: HO  -CH_2 and poly-dl-methionine (side chain: CH_3-S-CH_2). ii) at the present time very little work has been done on poly-dl-methionine and poly-dl-tyrosine. Hence, their preparation and an investigation of their spectra would be of interest, per se.

The method used for the preparation of poly-dl-tyrosine was essentially the same as that used by Katchalski for the l-isomer. The phenol group was first protected by formation of the O,N-dicarbobenzoxy derivative of tyrosine. The anhydride was then made from this derivative by treatment

with PCl_5. As in the case of poly-dl-alanine, the anhydride was then polymerized. To obtain poly-dl-tyrosine the carbobenzoxy protective group was then removed with HBr in glacial acetic acid solution.

Essentially the same procedure was to be used for the preparation of poly-dl-methionine. However, in this case it was not possible to obtain the anhydride. When PCl_5 was added to solutions of the carbobenzoxy derivative of dl-methionine no reaction took place. The reason for the failure of this reaction was not ascertained. It might, however, be noted that an attempt by Farthing[42] to produce the anhydride of dl-methionine by the direct reaction of the amino acid with carbonyl chloride was also unsuccessful.

Infrared Spectra of Poly-dl-Tyrosine and its Derivatives

In the last section of this thesis the results of an analysis of the infrared spectra of some of the substances prepared in these investigations are presented. The table (p. 36) lists all the bands observed and their assignments. In the accompanying text a detailed discussion of some of the bands is given.

Most of the compounds studied contain the peptide linkage and the bands arising from the vibrations of the various members of that group were identified. The bands arising from the characteristic vibrations of the phenyl ring could also be identified.

In poly-dl-tyrosine it was possible to establish the

existence of the -OH group by its characteristic absorption band. And the carbobenzoxy anhydride of dl-tyrosine was positively identified by the characteristic bands at 1787 cm^{-1} and 1833 cm^{-1}.

Most of the remaining bands could not be positively identified although a number of tentative assignments were made. It is to be hoped that a more extensive analysis of the spectrum of poly-dl-tyrosine would yield more positive information concerning its structure. In particular the spectra should be obtained under a variety of conditions in order to obtain information concerning the possibility of poly-dl-tyrosine existing in more than one configuration. It would be particularly interesting to study the changes produced in the spectra by changes in the dielectric constant of solutions of this polymer.

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