# THE PREPARATION AND PROPERTIES OF SOME 2,4-DINITROPHENYL PEPTIDES

by

James Edward Kruger

A Thesis presented to
the Faculty of Graduate Studies and Research
of the University of Manitoba
in partial fulfillment of the requirements for
the degree of Master of Science
1963

ACKNOWLEDGMENTS

The author wishes to express his appreciation to Dr. J. H. Loudfoot for his kind assistance and encouragement during the course of this work.

Grateful acknowledgments are also extended to the University of Manitoba for the Graduate Student Teaching Assistantship, to the National Research Council of Canada for a summer grant-in-aid of research, and to Mr. J. Travis for a photographic reproduction of the paper electrophoresis apparatus, obtained from his thesis.

## ABSTRACT

2,4-Dinitrophenyl glycylglycine, 2,4-dinitrophenyl glycyl-L-alanine, 2,4-dinitrophenyl glycyl-L-valine, and 2,4-dinitrophenyl-glycyl-L-leucine were prepared in order to study their properties. Two methods of preparation were used, each giving an identical and satisfactory product for any particular 2,4-dinitrophenyl dipeptide.

The melting points, neutral equivalents, specific rotations, electrophoretic behaviours, and absorption spectra of the above compounds were determined.

Electrophoretic behaviour and absorption spectra of some 2,4-dinitrophenyl peptides with a varying number of glycyl residues were also determined.

TABLE OF CONTENTS

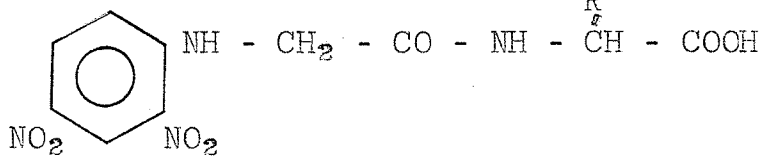
# LIST OF FIGURES

# INTRODUCTION

In a 2,4-dinitrophenyl (DNP)[1] peptide, the DNP radical is linked to the peptide through the α-amino group of the N-terminal amino acid residue. The N-terminal amino acid residue is that residue whose α-carboxyl group is involved in peptide linkage with the α-amino group of the adjoining amino acid, thus leaving the α-amino group of the N-terminal amino acid residue free. This "labelling" of the N-terminal amino acid residue is part of an important technique in protein chemistry by which the amino acid sequence of a protein or peptide may be determined.

The present study was undertaken in order to obtain more knowledge about the properties of certain DNP-peptides.

The melting points, neutral equivalents, specific rotations, electrophoretic behaviour, and absorption spectra were determined for four DNP-dipeptides whose structural formula is shown below.




Differences between the DNP-dipeptides are in the R group as follows:-

[1]DNP will be used to designate the 2,4-dinitrophenyl radical.

| R | DNP-dipeptide |
|---|---|
| -H | DNP-glycylglycine |
| $-CH_3$ | DNP-glycyl-L-alanine |
| $-CH(CH_3)_2$ | DNP-glycyl-L-valine |
| $-CH_2-CH(CH_3)_2$ | DNP-glycyl-L-leucine |

In addition, the electrophoretic behaviour and absorption spectra were determined for DNP-peptides with a varying number of glycyl residues.

In order to study the properties of these DNP-peptides it was first necessary to prepare them. Towards this end two methods of preparation were used.

# LITERATURE REVIEW

Prior to 1945 DNP derivatives of proteins, peptides, and amino acids had been prepared by the method of Abderhalden and Blumberg (10, 12) using 1-chloro-2,4-dinitrobenzene which reacts with the free amino group. In 1945 Sanger (1) found that 1-fluoro-2,4-dinitrobenzene (FDNB)[1] was a more suitable reagent, reacting at a lower temperature and pH, and thus minimizing hydrolysis.

Sanger's preparation of a DNP-amino acid is as follows:-

The amino acid and sodium bicarbonate are dissolved in water. To this is added a solution containing a two fold excess of FDNB dissolved in alcohol. The mixture is shaken for two hours at room temperature, concentrated to remove ethanol, water added, and the solution extracted with ether to remove excess FDNB. The aqueous solution is then acidified with hydrochloric acid and a yellow product precipitates. These reaction conditions have been used almost universally up to the present time.

Under certain circumstances, however, other reaction conditions may prove to be more advantageous. For example, in the preparation of small (microgram) quantities of DNP-peptides, trimethyl amine (4) or trimethyl amine carbonate (5) is sometimes substituted for the sodium

[1]FDNB will be used to designate 1-fluoro-2,4-dinitrobenzene

bicarbonate. Upon completion of the reaction, these reagents can then be conveniently removed by evaporation.

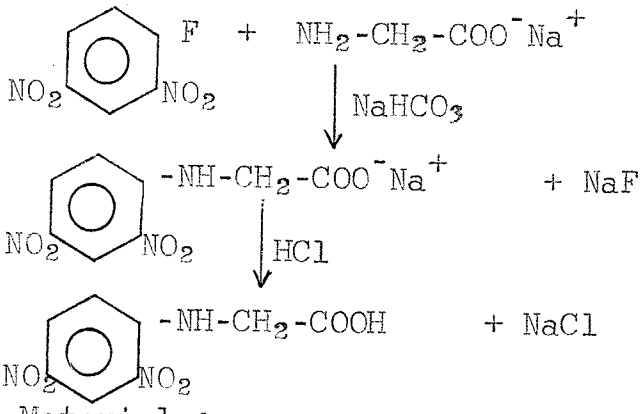
Because DNP-amino acids are commonly used as reference compounds, many of their properties, such as melting points, specific rotations, and absorption spectra are well known (2, 7, 8). However, equally abundant information about the properties of DNP-peptides is not known. These compounds are usually characterized by chromatography and/or hydrolysis with subsequent identification of the parts and not by their precise chemical and physical properties. For example, Rhinesmith et al. (6) prepared DNP-valyl peptides for chromatographic and hydrolytic studies but had no other interest in the compounds.

# EXPERIMENTAL AND RESULTS

## Preparation of Starting Materials

### 2,4-Dinitrophenyl Glycine

DNP-glycine was required for the preparation of DNP-glycyl chloride. It was prepared according to the method of Sanger (1) except that one equivalent of FDNB was used per equivalent of amino acid, as reported by Levy and Chung (3). The equations for the overall reaction are shown below.




Materials:-

| | |
|---|---|
| glycine | 2.00 grams (0.026 mole) |
| FDNB | 5.00 grams (0.026 mole) |
| sodium bicarbonate | 19.4 grams |
| water | 180 c.c. |
| ethyl alcohol (95%) | 210 c.c. |

Glycine and sodium bicarbonate were dissolved in the water. FDNB was dissolved in the alcohol and this

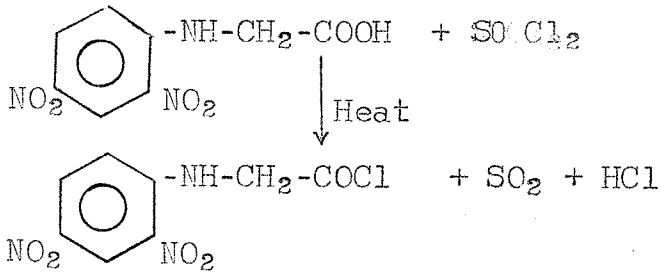
solution was then added to the glycine bicarbonate solution. The mixture was stirred for two hours at room temperature. As the reaction proceeded, a deep yellow color developed. At a low temperature (less than 40°), the mixture was vacuum distilled to remove alcohol, and then acidified with concentrated hydrochloric acid (24 c.c.), which precipitated a yellow crystalline solid. The solid was filtered with suction, washed with ice-cold water to remove excess hydrochloric acid, stored for 15-20 hours in a vacuum dessicator, and heated at 100° to constant weight.

The yield of product was 6.1 grams (94.6% of theory).

Melting point 205°. Literature 205° (10), 206° (11).

2,4-Dinitrophenyl Glycyl Chloride

DNP-glycyl chloride was required as a starting material for one of the methods of preparing DNP-glycyl peptides. It was prepared by reacting DNP-glycine with thionyl chloride. The equation for the reaction is shown below.



Materials:-

| | |
|---|---|
| DNP-glycine | 1.07 grams (0.004 mole) |
| Thionyl chloride (freshly purified) | 11 c.c. |

The glycine and thionyl chloride were heated under reflux on a water bath until a clear solution was obtained (20-40 minutes). The solution was then heated for an additional 30 minutes. Following this excess thionyl chloride was removed by distillation under reduced pressure, using a water bath. Large yellow, needlelike crystals were formed. Throughout the preparation a drierite tube was attached to the condenser in order to exclude moisture.

In some preparations a product of lower purity was obtained initially. Recrystallization from moisture-free benzene was found satisfactory for purification.

The yield of product was 1.15 grams (99.7% of theory). Recrystallized yield was 92.7% of theory.

The melting point was 129-129.5°, and 161-187° mixed with DNP-glycine.

Preparation of 2,4-Dinitrophenyl Dipeptides

Two methods of preparing DNP-glycyl peptides are reported here:

i) By reacting FDNB with the pertinent dipeptide.

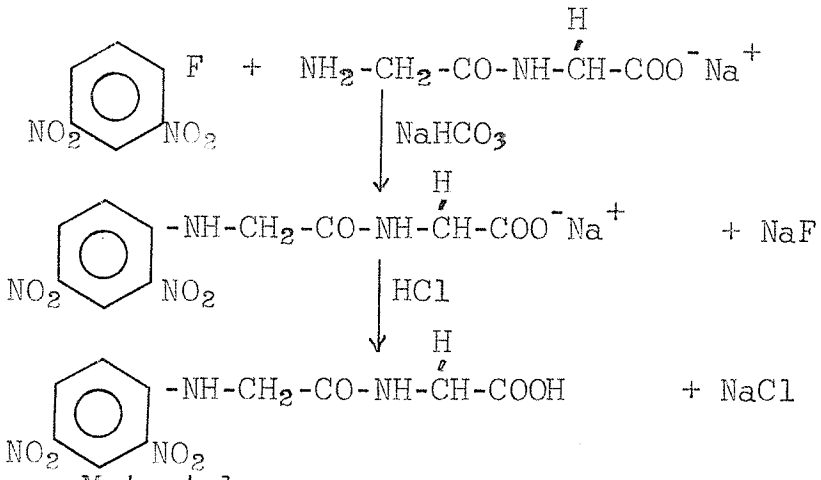
ii) By reacting DNP-glycyl chloride with the pertinent amino acid.

The above order will be followed for describing the preparations of the various DNP-dipeptides.

## 2,4-Dinitrophenyl Glycylglycine

### From the Dipeptide

DNP-glycylglycine was prepared by the method of Sanger (1), suitably modified to obtain a better product. The main modification was the use of equivalent amounts of reactants as reported by Levy and Chung (3). The equations for the overall reaction are as follows:-




Materials:-

| | |
|---|---|
| glycylglycine | 0.31 gram (0.0023 mole) |
| FDNB | 0.44 gram (0.0023 mole) |
| sodium bicarbonate | 2.98 grams |
| water | 20 c.c. |
| ethyl alcohol (95%) | 15 c.c. |

Glycylglycine and sodium bicarbonate were dissolved in the water. FDNB was dissolved in the alcohol

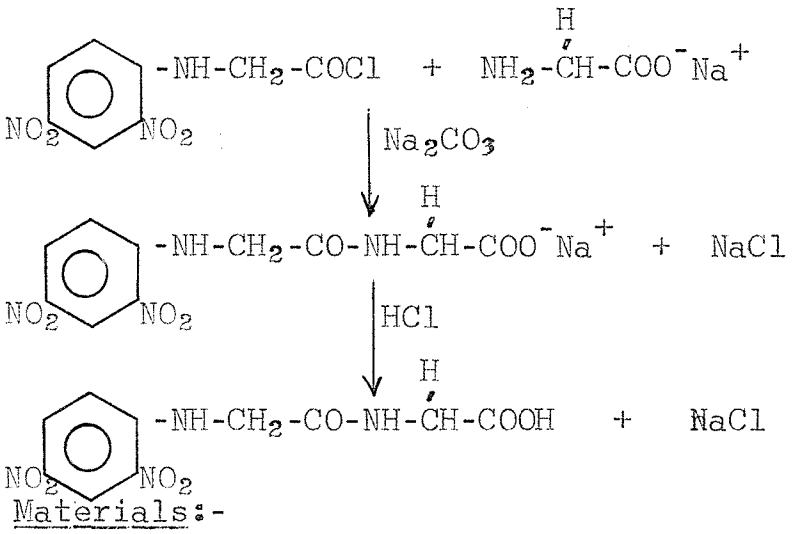
and this solution was then added to the glycylglycine bicarbonate solution. The mixture was stirred for two hours at room temperature followed by a vacuum distillation to remove alcohol. Acidification with concentrated hydrochloric acid (4 c.c.) precipitated a bright yellow solid. The mixture was refrigerated overnight to precipitate additional product. The product was then filtered with suction, washed with iced water to remove excess hydrochloric acid, and dried at 104° to constant weight. Occasionally additional product precipitated out of the filtrate after a period of standing and was treated in an analogous way.

The yield of product was 0.64 gram (91.9% of theory).

The melting point was 189.5-191.5°, and 167-172° when mixed with DNP-glycine.

From 2,4-Dinitrophenyl Glycyl Chloride

Glycine and DNP-glycyl chloride were reacted together in carbonate medium followed by acidification with hydrochloric acid. The equations for the overall reaction are shown below.




Materials:-

| | |
|---|---|
| DNP glycyl chloride | 1.07 grams (0.004 mole) |
| glycine | 0.32 gram (0.004 mole) |
| sodium carbonate | 2.6 grams |
| water | 85 c.c. |
| benzene | 25 c.c. |

Glycine and the sodium carbonate were dissolved in the water and DNP glycyl chloride was dissolved in the benzene. The benzene solution was then slowly added over a two hour period to the aqueous solution with constant stirring at ice-bath temperature. Stirring at ice-bath temperature was continued a further two hours. The mixture was then transferred to a separatory funnel and allowed to stand 2-3/4 hours for separation of the layers. The lower aqueous layer was removed and acidified with 5 c.c. of concentrated hydrochloric acid, precipitating a yellow solid. In order to precipitate product which remained in solution, the mixture was stored

overnight in the refrigerator. The product was next filtered with suction, washed with ice-water to remove excess hydrochloric acid, and heated to constant weight. Recrystallization was affected by dissolving in hot alcohol, adding water to cause turbidity, and cooling slowly. The large yellow crystals were then filtered with suction and dried to constant weight.

The yield of product was 0.67 gram (54.5% of theory).

The melting point was 191-192°, 191-192.5° when mixed with DNP-glycylglycine prepared from the dipeptide, and 172-181° when mixed with DNP-glycine.

The molecular weight determined by the Rast method was 296. The neutral equivalent was 299.4. Theoretical molecular weight is 298.22.

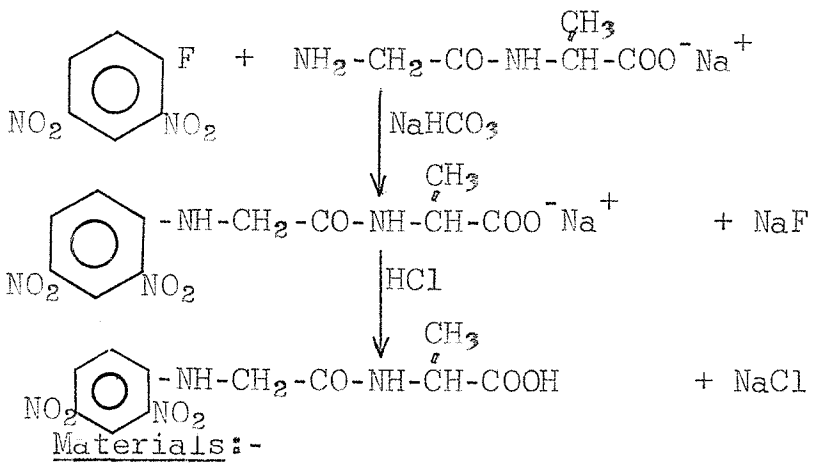
Analysis: calculated for $C_{10}H_{10}N_4O_7$: C, 40.03%; H, 3.38%; N, 18.79%. Found: C, 39.44%; H, 3.64%; N, 16.72%.

All analyses were performed by Geller Laboratories, Bardonia, New York.

2,4-Dinitrophenyl Glycyl-L-Alanine

From the Dipeptide

The equations for the overall reaction are as follows:




Materials:-

| | | |
|---|---|---|
| glycyl-L-alanine | 0.24 gram | (0.0016 mole) |
| FDNB | 0.31 gram | (0.0016 mole) |
| sodium bicarbonate | 1.4 grams | |
| water | 15 c.c. | |
| ethyl alcohol (95%) | 20 c.c. | |

The preparation was carried out in the same manner as DNP-glycylglycine prepared from the dipeptide. Drying of the product was modified by storing overnight in a desiccator containing phosphorus pentoxide, followed by heating at 110° to constant weight. The yellow crystalline product was usually of such purity as not to require recrystallization.

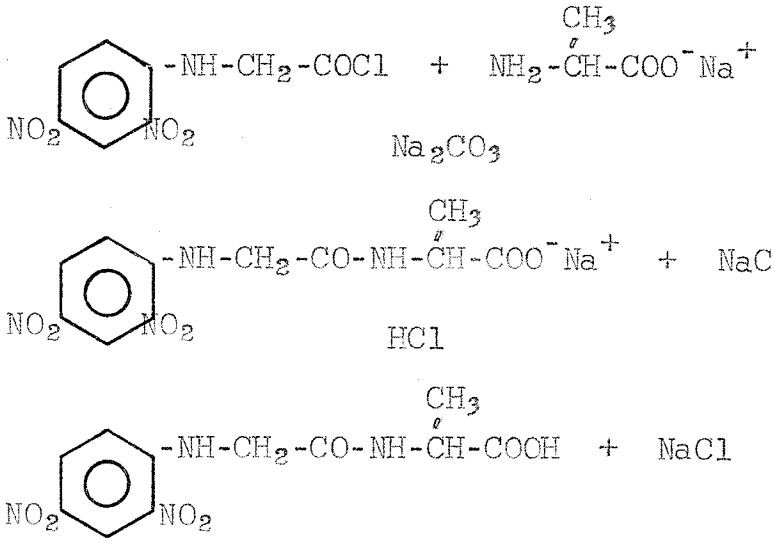
The yield of product was 0.402 gram (75.6% of theory).

The melting point was 175-176.5°, and 164-173° when mixed with DNP-glycine.

$[\alpha]_D^{22}$ = -23.8° (C = 2.2% in 95% ethanol). A Kern Full-Circle polarimeter and 1-dm. tube were used for determining the specific rotations of all the DNP-peptides.

From 2,4-Dinitrophenyl Glycyl Chloride

The equations for the overall reaction are shown below.



Materials:-

| | |
|---|---|
| DNP-glycyl chloride | 1.09 grams (0.004 mole) |
| L-alanine | 0.38 gram (0.004 mole) |
| sodium carbonate | 2.6 grams |
| water | 75 c.c. |
| benzene | 20 c.c. |

The preparation was carried out in the same manner as DNP-glycylglycine prepared from DNP-glycyl-chloride. As the reaction mixture was being acidified, a tarry

material formed and was removed manually. The product was recrystallized by dissolving in alcohol, adding a large volume of water (600-700 c.c.), and evaporating the solution down slowly at room temperature in an evaporating dish. Various crops were filtered off as evaporation proceeded, and if still found to be impure, were subjected to the same treatment again. A red ring of impure product always formed around the upper edges of the evaporating dish and was rejected. Recrystallization from hot alcohol when tried was found detrimental to both yield and quality.

The yield of product was 0.60 gram (44.6% of theory).

The melting point was 175-176.5°, 175-176° when mixed with DNP-glycyl-L-alanine prepared from the dipeptide, and 158-170° when mixed with DNP-glycine.

The neutral equivalent was 317.1. Theoretical molecular weight is 312.24.

$[\alpha]_D^{22}$ = -23.4° (C = 2.77% in 95% ethanol)

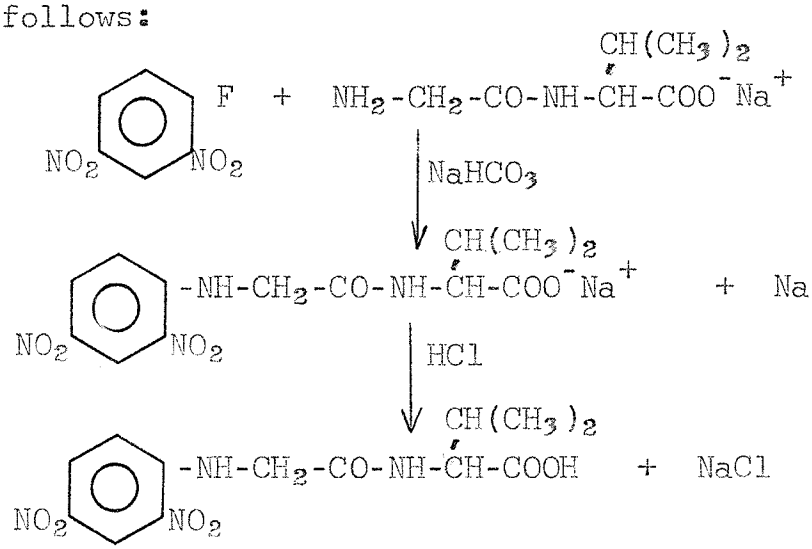
Analysis: Calculated for $C_{11}H_{12}N_4O_7$: C, 42.31%; H, 3.87%; N, 17.98%.

Found: C, 41.75%; H, 3.77%; N, 18.01%.

## 2,4-Dinitrophenyl Glycyl-L-Valine

### From the dipeptide

The equations for the overall reactions are as follows:




Materials:-

| | |
|---|---|
| glycyl-L-valine | 0.145 gram (0.0008 mole) |
| FDNB | 0.154 gram (0.0008 mole) |
| sodium bicarbonate | 0.73 gram |
| water | 7 c.c. |
| ethyl alcohol (95%) | 10 c.c. |

The preparation was carried out in the same manner as DNP-glycylglycine and DNP-glycyl-L-alanine prepared from their respective dipeptides. The product was purified in the same way as DNP-glycyl-L-alanine prepared from the acid chloride.

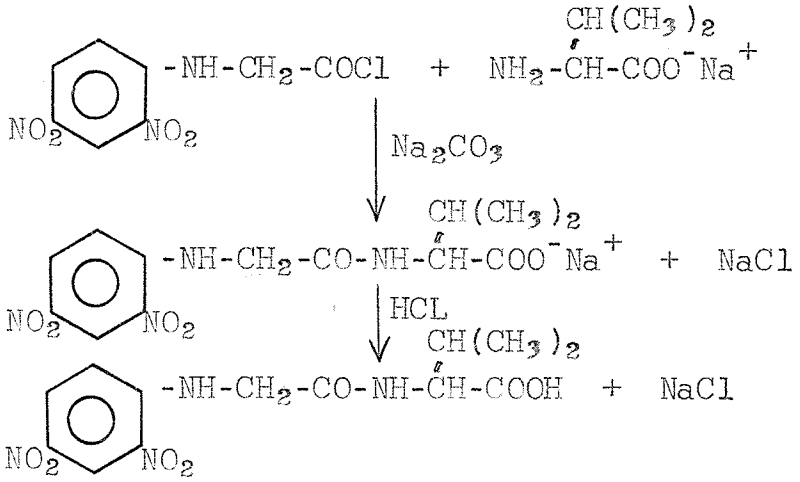
The yield of product was 0.14 gram (47.7% of theory).

The melting point was 132-134°, and 134-164° when mixed with DNP-glycine.

$[\alpha]_D^{22}$ = -22.05° (C = 1.20%in 95% ethanol)

From 2,4-Dinitrophenyl Glycyl Chloride

The equations for the overall reaction are shown below.




Materials:-

| | |
|---|---|
| DNP-glycyl chloride | 0.94 gram (0.0036 mole) |
| L-valine | 0.42 gram (0.0036 mole) |
| sodium carbonate | 2.6 grams |
| water | 75 c.c. |
| benzene | 23 c.c. |

The procedure used for the preparation of the other DNP-dipeptides from their acid chlorides was adopted here. Purification was carried out in the same manner as

DNP-glycyl-L-alanine prepared from the acid chloride.

The yield of product was 0.55 gram (44.4% of theory).

The melting point was 130-131°C, 129.5-131° when mixed with DNP-glycyl-L-valine prepared from the dipeptide, and 132-168° when mixed with DNP-glycine.

The neutral equivalent was 337. Theoretical molecular weight is 340.29

$[\alpha]_D^{22}$ = -20.4° (C = 1.2% in 95% ethanol)

Analysis: Calculated for $C_{13}H_{16}N_4O_7$:

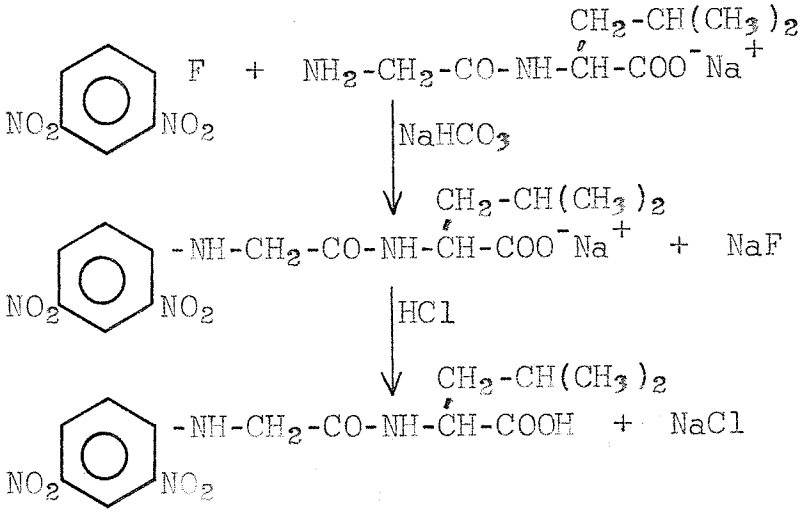
C, 45.59%; H, 4.74%; N, 16.38%

Found: C, 45.29%; H, 4.96%; N, 14.9%.

2,4 Dinitrophenyl Glycyl-L-Leucine

From the Dipeptide

The equations for the overall reaction are shown below.



Materials:-

| | |
|---|---|
| glycyl-L-leucine | 0.115 gram (0.006 mole) |
| FDNB | 0.127 gram (0.006 mole) |
| sodium bicarbonate | 0.548 gram |
| water | 5 c.c. |
| ethyl alcohol (95%) | 15 c.c. |

The preparation was carried out in the same manner as the other DNP-dipeptides prepared from the dipeptide. The product was purified by the method used for purifying DNP-glycyl-L-valine prepared from the dipeptide. Drying of the product was modified by storing it overnight in a vacuum desiccator, followed by heating to constant weight at a low temperature (less than 80°).

The yield of product was 0.11 gram (48.5% of theory).

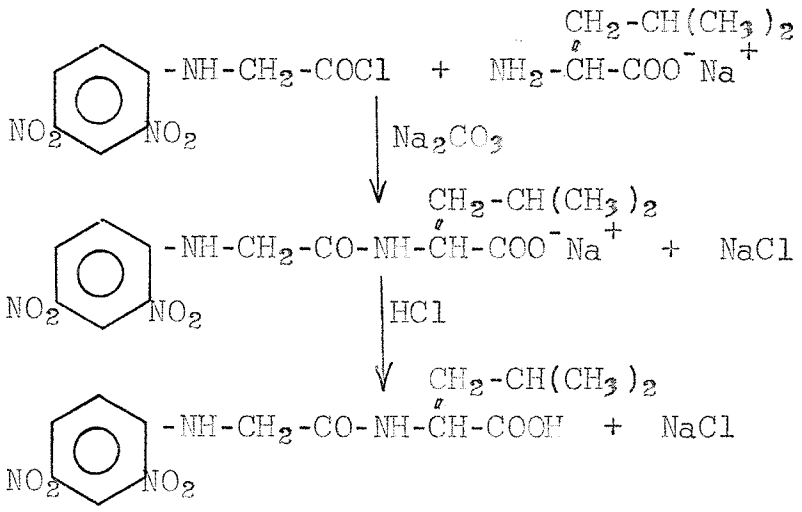
The melting point was 98°-100°, and 164-182° when mixed with DNP-glycine.

$[\alpha]_D^{22} = -29.85°$ (C = 1.00% in 95% ethanol)

Just before the DNP-glycyl-L-leucine actually melted, the crystal structure seemed to collapse internally, suggesting the possibility that it was a hydrate.

From DNP-Glycyl Chloride

The equations for the overall reaction are as follows:




Materials:-

| | |
|---|---|
| DNP-glycyl chloride | 0.947 gram (0.0036 mole) |
| L-leucine | 0.478 gram (0.0036 mole) |
| sodium carbonate | 2.6 grams |
| water | 75 c.c. |
| benzene | 21 c.c. |

The preparation was carried out in the same manner as the other DNP-peptides prepared from the acid chloride. The product was purified by the method used for purifying DNP-glycyl-L-alanine and DNP-glycyl-L-valine prepared from the acid chloride. If the product was only slightly impure, it could be purified by dissolving in a minimum of cold alcohol, adding enough water to cause turbidity, followed by slow evaporation of the solution down to small bulk at room temperature.

In early preparations the product was dried by heating at 85° to constant weight. Due to slight

decomposition, a better method later adopted was overnight dessication in an Abderhalden drying apparatus at very low pressure, using phosphorus pentoxide as dessicant.

The yield of product was 0.77 gram (59.9% of theory).

The melting point was 98-100°, 98-99.5° when mixed with DNP-glycyl-L-leucine prepared from the dipeptide, and 178-190° when mixed with DNP-glycine.

The neutral equivalent was 364.1. Theoretical molecular weight is 354.30.

$[\alpha]_D^{22} = -29.09^{\circ}$ (C = 1.0%in 95% ethanol)

Analysis: calculated for $C_{14}H_{20}N_4O_8$: C, 45.16%; H, 5.41%; N, 15.05%.

Found: C, 45.82%; H, 5.24%; N, 15.11%.

The analysis was calculated on the basis of a monohydrate.

## Paper Electrophoresis of 2,4-Dinitrophenyl Peptides

Electrophoresis was carried out on a Reco Model E-800-2 electrophoresis unit. This apparatus is shown in Figure 1. The apparatus is designed for either horizontal or hanging open strip electrophoresis. Whatman No. 1 paper strips of dimensions 50 x 20 cm. and 0.02 molar sodium borate pH 9.21 were used in all experiments.

### 2,4-Dinitrophenyl Dipeptides

A closed strip technique was first tried in order to test the feasibility of using the method of Abraham and Newton (9) which is applicable to some DNP-amino acids. The paper was moistened with buffer and the excess blotted off as much as possible.

DNP-glycylglycine and DNP-glycyl-L-leucine, as well as a mixture of the two, were dissolved in alcohol and spotted to the centre of the paper with a capillary dropper. The strip was then placed across the platform surface and the ends dipped in buffer. Glass plates were placed on top of the strip. A potential of 500 volts was applied for 5-1/2 hours with no noticeable separation. In addition, the extreme saturation of the paper with buffer gave a large diffusion of the spots. Hence, this technique was abandoned in

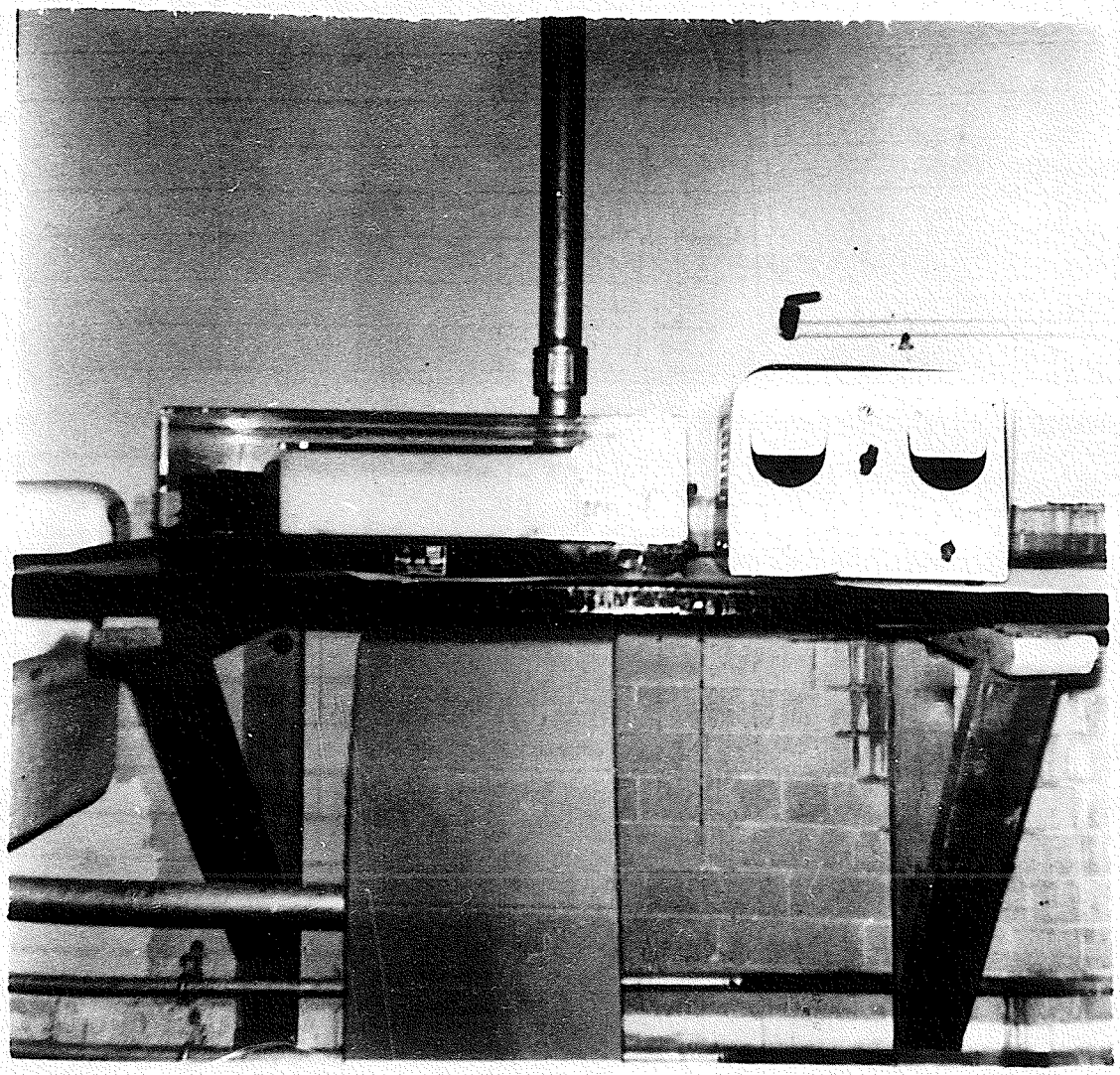


Figure 1.

Paper electrophoresis apparatus (general)

favor of the hanging open strip technique in which the paper was elevated about one inch above the center of the platform surface. Employing the same conditions and sample spots, no separation was observed after 5 hours, and the spots had migrated only a slight distance towards the anode. The probable explanation for the slight distance travelled was that the heat generated by the current was only partially dissipated by the water-cooled platform. Evaporation thus caused a migration of the buffer to the apex and the sample spots, which originated at this point, had to move against this buffer ''current''. To overcome this, modifications were made, giving much better results. The apex of the hanging strip was moved very close to the anode end of the apparatus and the spots were applied close to the cathode. The spots would now move with the buffer flow. To minimize evaporation, a lower field strength of 300 volts (current of 3-5 milliamperes) was used, as well as a longer electrophoretic time (20-22 hours). A problem that arose was capillary rise of solvent up the paper strip especially from the cathode side. It was minimized by stopping the electrophoresis from time to time and stretching the paper tautly across the apparatus.

The four DNP-dipeptides which had previously been prepared were now studied electrophoretically under these conditions. Each of the four DNP-dipeptides travelled

a different distance individually and these were as follows:- DNP-glycylglycine 14 cm., DNP-glycyl-L-alanine 13.2 cm., DNP-glycyl-L-valine 10.7 cm., DNP-glycyl-L-leucine 10.2 cm. An electrophoretic pattern illustrating this is shown in Figure 2(a). Various combinations of the DNP-dipeptides gave the following information. DNP-glycylglycine separated only slightly from DNP-glycyl-L-alanine but separated completely from DNP-glycyl-L-valine and DNP-glycyl-L-leucine. DNP-glycyl-L-alanine separated only slightly from DNP-glycyl-L-valine but had a reasonably distinct separation from DNP-glycyl-L-leucine.

DNP-glycyl-L-valine and DNP-glycyl-L-leucine formed only an elongated spot, indicating very little separation. The distances that the separated or partially separated components travelled was close to that which the individual components travelled. A mixture of all four DNP-dipeptides was spotted and electrophoresis resulted in an elongated dumbell shaped form. This showed that DNP-glycylglycine and DNP-glycyl-L-alanine had separated very little from each other but had partially separated from the also indistinct DNP-glycyl-L-valine and DNP-glycyl-L-leucine.

DNP-glycine when present in a mixture of the above peptides separated completely from them. The electrophoretic pattern in Figure 2(b) illustrates this.

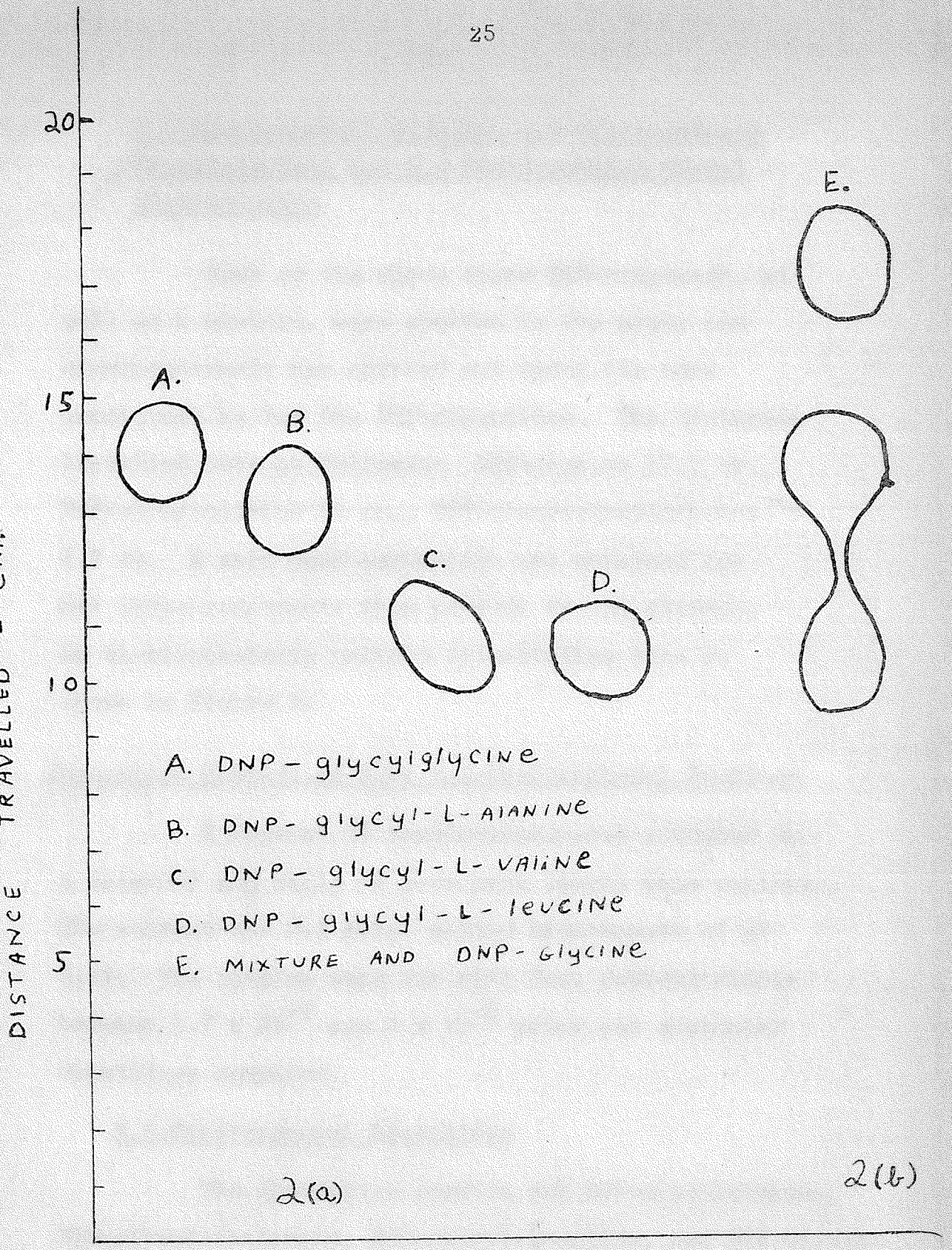



Figure 2

(a) Electrophoretic pattern of individual 2,4-dinitrophenyl dipeptides (solvent 0.02 molar sodium borate pH 9.21) (time 21-22 hours).

(b) Electrophoretic pattern of a mixture of 2,4-dinitrophenyl dipeptides including 2,4-dinitrophenyl glycine (solvent 0.02 molar sodium borate pH 9.21) (time 21-22 hours).

2,4-Dinitrophenyl glycine, 2,4-Dinitrophenyl Glycylglycine, and 2,4-Dinitrophenyl Glycyl glycylglycine

Each of the above three DNP-compounds, as well as a mixture, were spotted to the paper and electrophoresis was carried out under the same conditions as for the DNP-dipeptides. The distances travelled were as follows:- DNP-glycine 17.5 cm., DNP-glycylglycine 14 cm., DNP-glycylglycylglycine 8.8 cm. A very good separation was obtained for all three components when present in the mixture. An electrophoretic pattern illustrating this is shown in Figure 3.

## Absorption Spectra of Some 2,4-Dinitrophenyl Peptides

A Beckman DB spectrophotometer attached to a recorder and cells of 1-cm path length were employed. The solvent was 0.2 molar sodium bicarbonate of pH 8.42. The samples were run with four concentrations between $1.7 \times 10^{-6}$ and $7 \times 10^{-5}$ molar and equimolar quantities compared.

### 2,4-Dinitrophenyl Dipeptides

The absorption spectra for DNP-glycylglycine, DNP-glycyl-L-alanine, DNP-glycyl-L-valine, and DNP-glycyl-L-leucine are shown in Figure 4. The wavelengths of

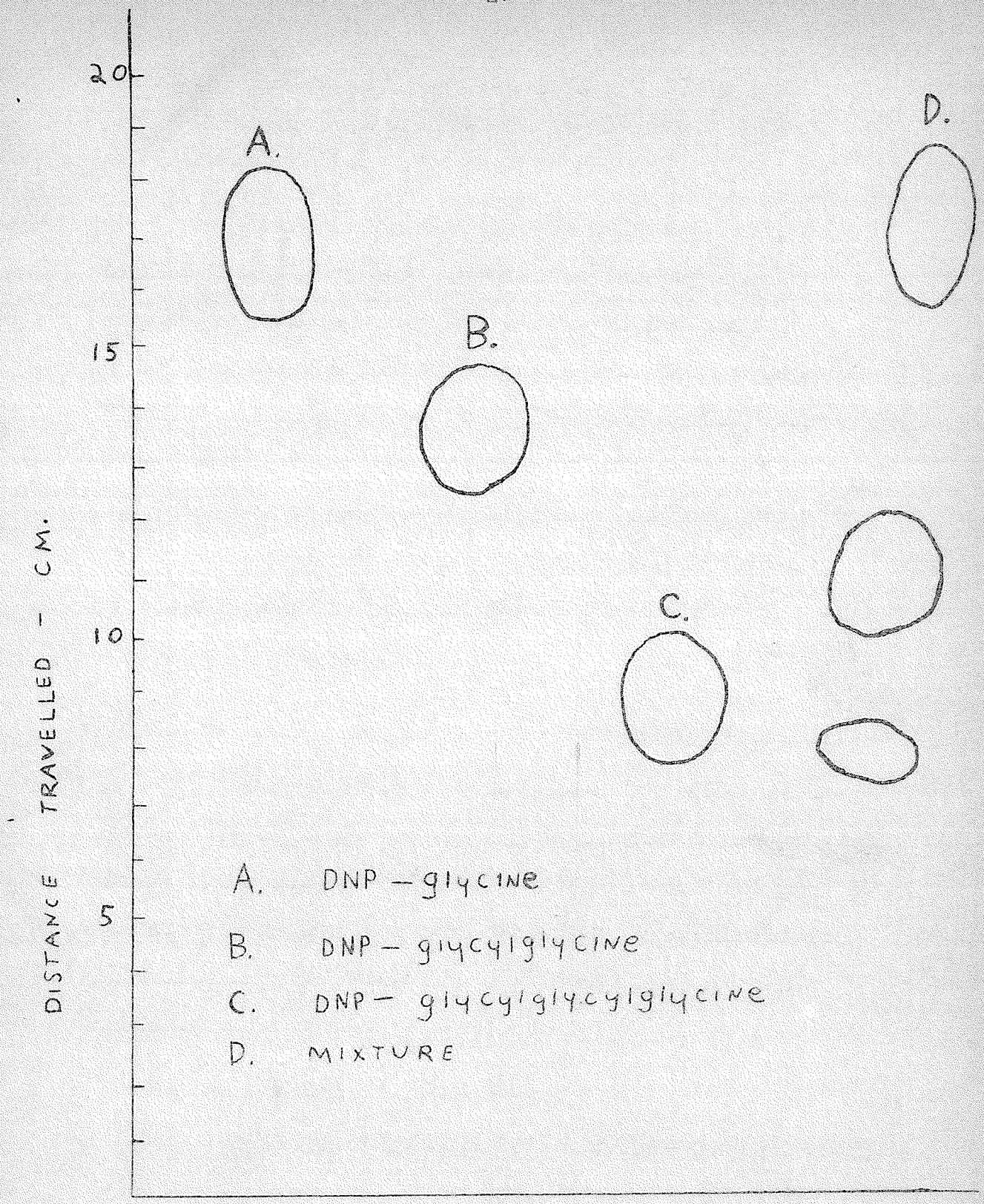
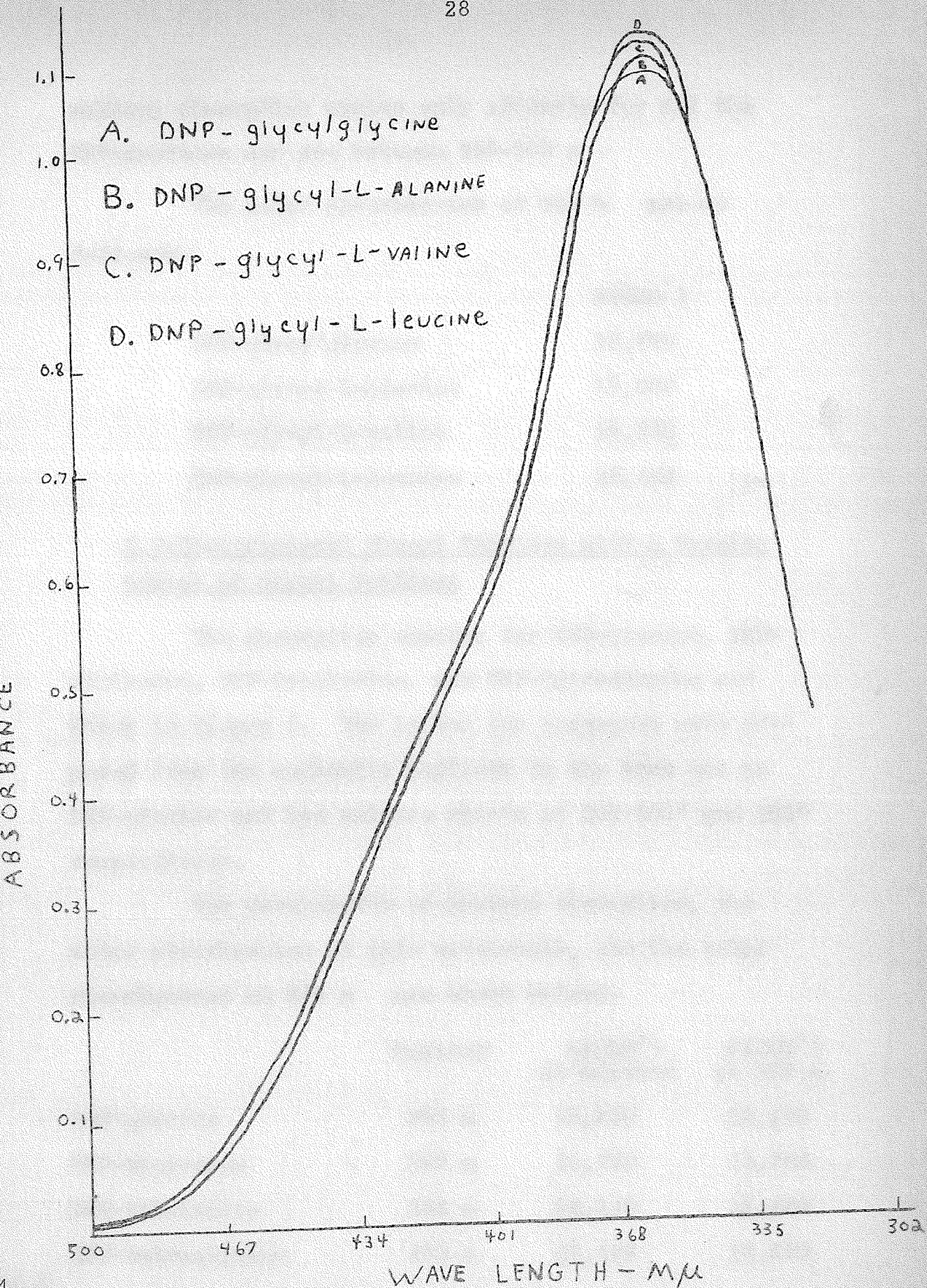



Figure 3

Electrophoretic pattern of 2,4-dinitrophenyl glycine, 2,4-dinitrophenyl glycylglycine, and 2,4-dinitrophenyl glycylglycylglycine individually and in a combined mixture (solvent 0.02 molar sodium borate pH 9.21) (time 21-22 hours).




igure 4

osorption spectra for 2,4-dinitrophenyl dipeptides (solvent 0.2 molar odium bicarbonate) (concentration 7 x $10^{-5}$ molar).

maximum absorption varies only slightly for all the DNP-peptides and are between 356-360 mμ.

The molar absorbancies at 359 mμ are as follows:-

| | $\epsilon_{M(COO^-)}$ |
|---|---|
| DNP-glycylglycine | 15,700 |
| DNP-glycyl-L-alanine | 16,000 |
| DNP-glycyl-L-valine | 16,280 |
| DNP-glycyl-L-leucine | 16,430 |

2,4-Dinitrophenyl Glycyl Peptides with a Varying number of glycyl residues

The absorption spectra for DNP-glycine, DNP-diglycine, DNP-triglycine, and DNP-tetraglycine are shown in Figure 5. The latter two compounds were prepared from the authentic peptides in the same way as DNP-glycine and had melting points of 202-203° and 208° respectively.

The wavelengths of maximum absorption, the molar absorbancies at this wavelength, and the molar absorbancies at 359 mμ are shown below:-

| | Maximum $\lambda$ | $\epsilon_{M(COO^-)}$ at maximum $\lambda$ | $\epsilon_{M(COO^-)}$ at 359 mμ |
|---|---|---|---|
| DNP-glycine | 366 mμ | 15,610 | 15,140 |
| DNP-diglycine | 357 mμ | 15,700 | 15,700 |
| DNP-triglycine | 352 mμ | 12,140 | 11,880 |
| DNP-tetraglycine | 355 mμ | 13,400 | 13,570 |

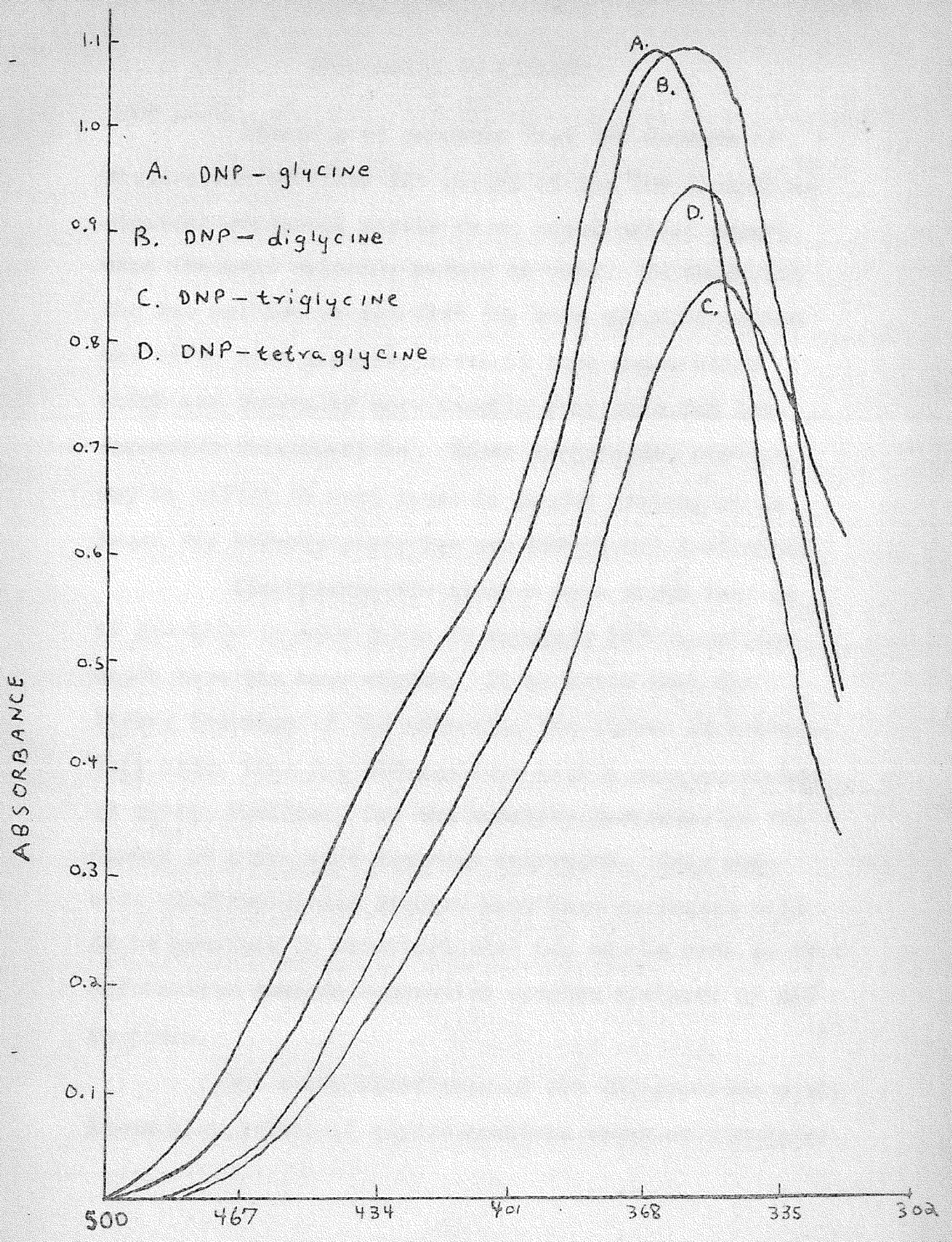



Figure 5

Absorption spectra for 2,4-dinitrophenyl peptides with a varying number of glycyl residues (solvent 0.2 molar sodium bicarbonate) concentration $7 \times 10^{-5}$ molar.

# DISCUSSION OF RESULTS

Identity of products from two methods of preparation confirms the nature of the DNP-dipeptides studied; evidently little or no racemization occurs when the acid chloride method is used. In comparing the two methods we see that the acid chloride method may offer more promise, since it uses amino acids which are generally more readily available and less expensive than peptides. These advantages, however, may be offset in some cases by poorer yields, as is found for DNP-glycylglycine and DNP-glycyl-L-alanine.

Electrophoretic studies have shown that it is possible in some cases to separate DNP-dipeptides which have the same charge. It is noted that the larger the size of the molecule, the slower it travels. This holds true for DNP-peptides with a varying number of glycyl residues, for the mobility decreases as the number of amino acid residues increases. Only when more electrophoretic studies have been performed will it be possible to ascertain what use can be made of this information towards separating complex mixtures of DNP peptides.

The molar absorbancy of the DNP-peptides with increasing number of glycyl residues shows an irregular

behaviour. Whether there is any trend in this irregularity cannot be ascertained with the present number of glycyl residues studied.

# BIBLIOGRAPHY


1. F. Sanger, Biochem. J., 39, 507 (1945)

2. K. R. Rao and H. A. Sober, J. Am. Chem. Soc., 76, 1328 (1954)

3. A. L. Levy and D. Chung, J. Am. Chem. Soc., 77, 2899 (1955)

4. F. Sanger and E.O.P. Thompson, Biochem. J., 53, 353 (1953)

5. I. M. Lockhart and E. P. Abraham, Biochem. J., 58, 633 (1954)

6. S. H. Rhinesmith et al., Anal. Bio., 4, 284 (1962)

7. H. Fraenkel-Conrat, K. J. Harris and A. L. Levy, Methods of Biochemical Analysis, 2, 359 (1955)

8. J. P. Greenstein and M. Winitz, Chem. of the Amino Acids, John Wiley and Sons Inc., Volume 2, 1564

9. P.S.F. Newton and E. P. Abraham, Biochem. J., 53, 604 (1953)

10. E. Abderhalden and P. Blumberg, Hoppe Seyler's Z., 65, 318 (1910)

11. H. M. Rice and F. J. Sowden, Can. J. Chem. 30, 575 (1952)

12. E. Abderhalden and W. Stix, Hoppe-Seyler's Z., 129, 143 (1923)