OPTICALLY ACTIVE TETRAZOLE ANALOGS OF AMINO ACIDS

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ABSTRACT

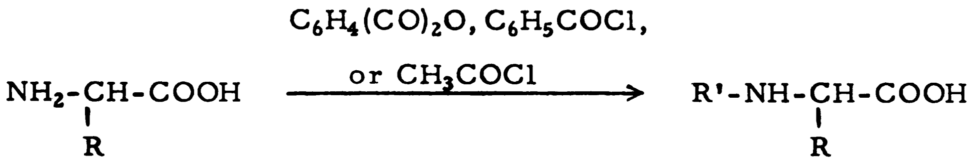
OPTICALLY ACTIVE TETRAZOLE ANALOGS OF AMINO ACIDS

by Vincent T. D'Orazio

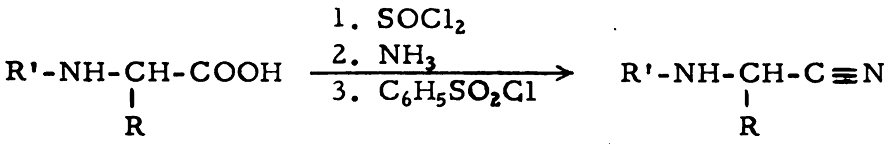
The synthesis of compounds which are similar in structure and reactivity to compounds that play an important role in metabolic processes of living organisms has been the object of extensive study. The concept of antagonism is defined as a competition between metabolites and structurally similar metabolites, or structurally related non-metabolites (antimetabolites) for an enzyme system. Since many amino acids are essential for the development and maintenance of the organism, they have been used as model systems in the synthesis of potential antimetabolites.

Because of the acidic nature of 5-monosubstituted tetrazoles, their use as potential antimetabolites for amino acids appeared promising. McManus (1) and Sterken (2) prepared a series of tetrazole analogs of D, L-amino acids. Since all of the amino acids obtained from proteins are of the L-configuration, it was considered germane to extend this series to include the synthesis of the tetrazole analogs of L-amino acids and determine some characteristic physical properties for comparison with the corresponding amino acids.

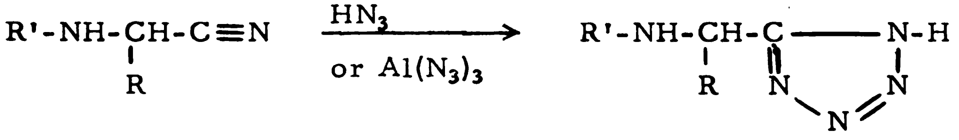
The tetrazole analogs of L-phenylalanine and L-tyrosine were prepared through interaction of the appropriate acylated aminonitriles with hydrazoic acid or aluminum azide. The acylated aminonitriles were synthesized from the corresponding L-amino acids.



$$NH_2\text{-}\underset{R}{\underset{|}{CH}}\text{-}COOH \xrightarrow[]{C_6H_4(CO)_2O,\ C_6H_5COCl,\ \text{or } CH_3COCl} R'\text{-}NH\text{-}\underset{R}{\underset{|}{CH}}\text{-}COOH$$



$$R'\text{-}NH\text{-}\underset{R}{\underset{|}{CH}}\text{-}COOH \xrightarrow[3.\ C_6H_5SO_2Cl]{1.\ SOCl_2 \quad 2.\ NH_3} R'\text{-}NH\text{-}\underset{R}{\underset{|}{CH}}\text{-}C\equiv N$$

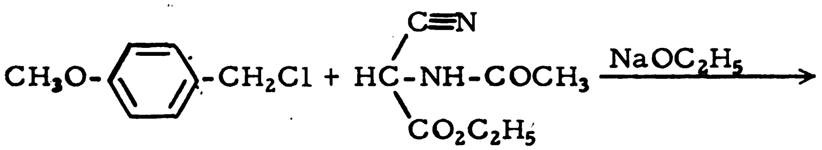


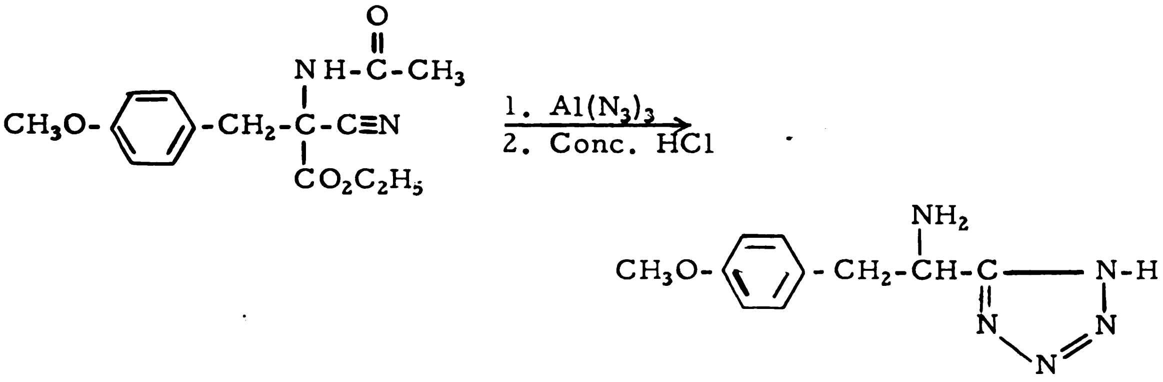
R: $-C_6H_5$, $-C_6H_4OH$

R': $C_6H_4(CO)_2-$, C_6H_5CO-, CH_3CO-

Acid hydrolysis of 5-benzoyl and 5-acetylaminoalkyltetrazole gave rise to tetrazole analogs of D, L-phenylalanine and L-tyrosine. The phthaloylaminoalkyltetrazole was converted to the tetrazole analog of L-phenylalanine by hydrazinolysis.

Alkylation of ethyl acetamidocyanoacetate with anisyl chloride followed by treatment with aluminum azide, and subsequent acid hydrolysis gave rise to the tetrazole analog of O-methyl-D, L-tyrosine.





The structure and configuration of the tetrazole analogs were verified by conversion to the 2, 5-disubstituted 1, 3, 4-oxadiazoles and subsequent acid hydrolysis afforded the original L-amino acids.

Many of the biological properties of amino acids are related to their physical properties. The pK values of the tetrazole analogs were determined by potentiometric titration and found to be in close agreement with their amino acid counterparts. Also, the degree of optical activity is affected by the acidity or alkalinity of the solution. The specific rotations of L-phenylalamine and L-tyrosine in predetermined amounts of acid and base were compared to those of the tetrazole analogs.

LITERATURE CITED

1. J. McManus, J. Org. Chem., 24, 1643 (1959).

2. G. Sterken, "Synthesis of Tetrazoles as Amino Acid Analogs," Ph.D. thesis, Michigan State University, 1960.

OPTICALLY ACTIVE TETRAZOLE ANALOGS OF AMINO ACIDS

By

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To Connie
for her everenduring patience
and understanding

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INTRODUCTION

Amino acids play a vital role in the metabolic processes of living organisms. A close metabolic relationship exists between two structurally similar amino acids; phenylalanine, and tyrosine. Phenylalanine is an essential amino acid, while tyrosine is not, even though tyrosine serves as a precursor for such physiologically important compounds as adrenaline and thyroxine. It has been demonstrated that phenylalanine can be converted to tyrosine in the body. The concept of antagonism between structurally similar metabolites, or between a metabolite and a structurally related non-metabolite is explained on the existence of a competition between these compounds for an enzyme system. These metabolic antagonists, or antimetabolites, have been important as chemotherapeutic agents in the treatment of disease.

Many compounds have been synthesized which are related chemically and structurally to essential metabolites. Usually these structural alterations are in the form of adding substituents to the molecule, or replacing a functional group with one that has a related structure and reactivity.

McManus (3) and Sterken (19) prepared a series of tetrazole analogs of D, L-amino acids. Since all amino acids obtained from proteins are of the L configuration, it was considered cogent to extend this series to include the synthesis of the tetrazole analogs of L-amino acids, and determine some characteristic physical properties of these compounds for comparison with their amino acid counterparts.

The tetrazole analogs of L-phenylalanine and L-tyrosine were prepared through interaction of the appropriate nitriles with hydrazoic acid or aluminum azide. The nitriles were synthesized from optically active amino acids in which the amino group had been acylated with a phthaloyl, benzoyl, or acetyl group. It was found that the procedure which employed the benzoyl group for blocking the amino moiety was the most convenient preparative method for general use. The tetrazole analog of O-methyl-D, L-tyrosine was prepared by alkylating ethyl acetamidocyanoacetate with anisyl chloride. Interaction of the nitrile with aluminum azide afforded the tetrazole, and subsequent acid hydrolysis gave rise to the desired product.

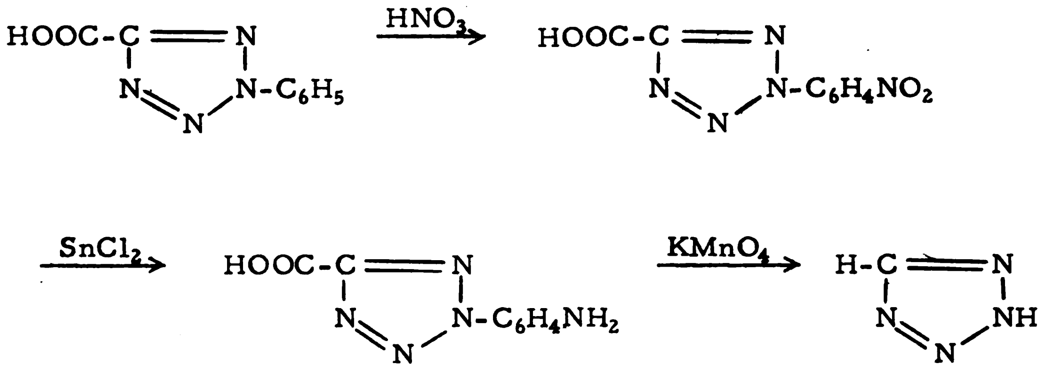
In order to verify the structure and configuration of the tetrazole analogs, the 2, 5-disubstituted 1, 3, 4-oxadiazoles were prepared from the tetrazole analogs. Subsequent acid hydrolysis of the oxadiazoles afforded the original L-amino acids.

Many of the biological properties of amino acids are related to their physical properties. The ability of an amino acid to act as both a proton donor and a proton acceptor has long been recognized. The pK values for the tetrazole analogs were determined by potentiometric titration and found to be in close agreement with the corresponding amino acids.

The degree of optical activity of amino acids is affected by the acidity or alkalinity of the solution. Lutz and Jirgensons (20, 21) determined the specific rotations of a series of naturally occurring amino acids in acid and base. From the curves obtained by plotting the specific rotation against the amount of acid or base, they were able to establish the configuration of these amino acids. Although the analogous curves for the tetrazole analogs were inverted compared to those of the amino acids, it appears that the rotation curves of the tetrazole analogs can be utilized for configuration assignment.

HISTORICAL

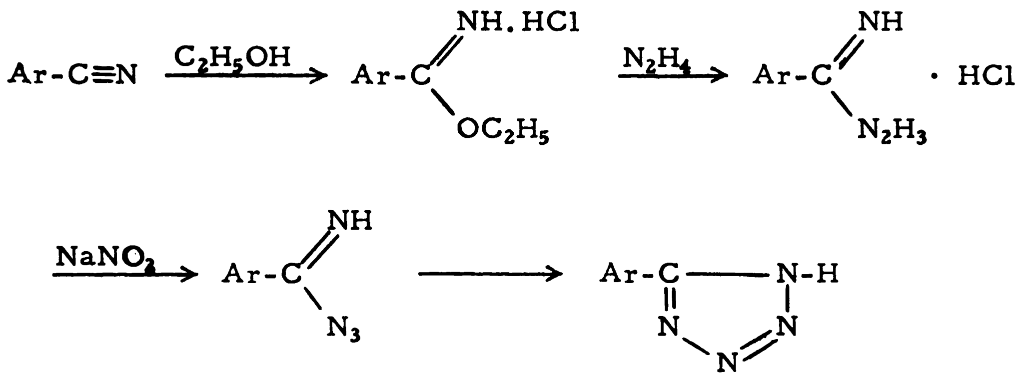
The first synthesis of a tetrazole reported in the literature was carried out by Bladin (24, 40) in 1885. 2-Phenyl-5-cyanotetrazole was synthesized from dicyanophenylhydrazine on treatment with nitrous acid. Hydrolysis of the cyano group followed by decarboxylation resulted in the formation of 2-phenyltetrazole. Bladin (25) also synthesized tetrazole itself from 2-phenyl-5-carboxytetrazole by the following scheme:



The stability of the tetrazole ring was illustrated by its inertness to potassium permanganate.

In 1897 Pinner (27) prepared 5-substituted tetrazoles from nitriles by formation of the iminoether hydrochlorides. Treatment of the iminoether hydrochlorides with hydrazine gave the amidrazone hydrochlorides, which were subsequently converted by diazotization with nitrous acid to the imidyl azides.

The latter cyclized to form the tetrazoles. The scheme may be illustrated as follows:



Ainsworth (28) prepared 5-(2'-aminoethyl)tetrazole from β-benzamidopropionitrile by three different methods. The first method was similar to the scheme used by Pinner (27). However, amyl nitrite was used as the diazotizing agent in place of sodium nitrite for the preparation of the imide azide. In the second method Ainsworth adapted a procedure developed by Mihina and Herbst (29). β-Benzamidopropionitrile was heated with hydrazoic acid in xylene. The third technique involved the reaction of hydrazoic acid in glacial acetic acid with the appropriate iminoether hydrochloride.

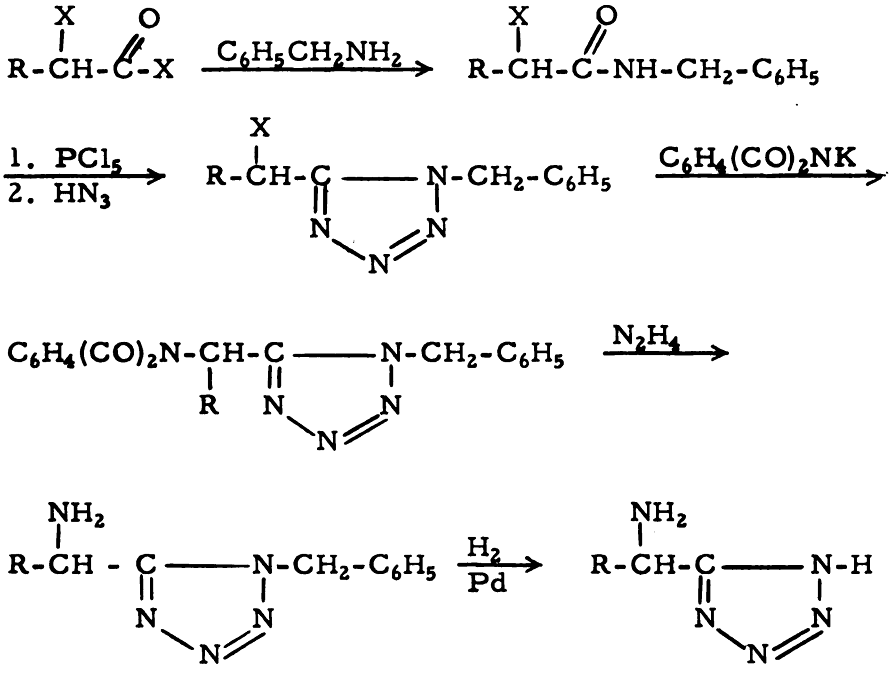
Herbst and Wilson (30) prepared 5-aryltetrazoles by treating the aryl cyanide with hydrazoic acid generated in situ from sodium azide and glacial acetic acid in boiling butyl alcohol.

The preparation of tetrazoles by reaction of aluminum azide generated in situ with nitriles was reported by Behringer and Kohl (31) in 1956. The nitrile was refluxed in dry tetrahydrofuran containing anhydrous aluminum chloride and sodium azide. The yields of the resulting acylaminoalkyltetrazoles were 80-90%.

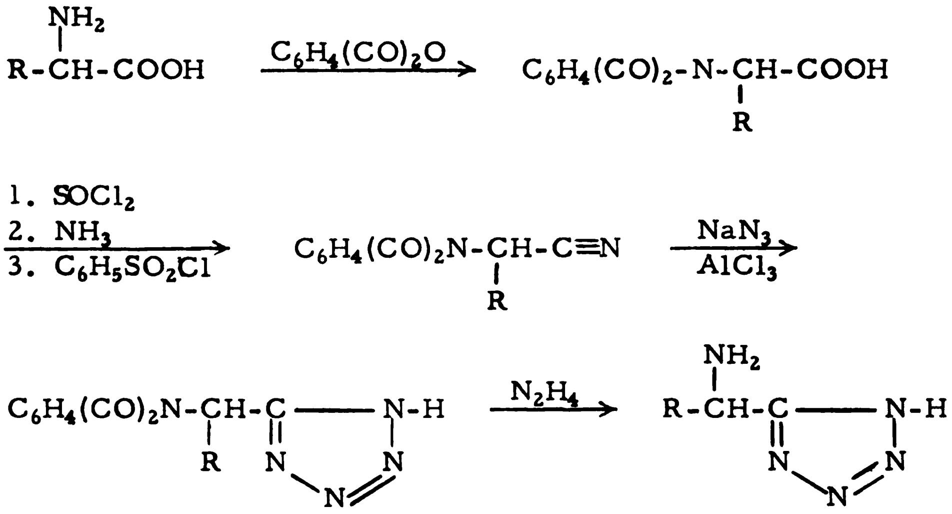
In 1958 Finnegan and co-workers (32) introduced the use of ammonium azide for the synthesis of tetrazoles. In their procedure the nitrile, ammonium chloride, and sodium azide were heated in dimethylformamide. The ammonium salt of the tetrazole was converted to the free tetrazole by treatment with dilute acid. The yields were higher and the reaction time shorter than the aluminum azide and hydrazoic acid methods.

McManus (3) prepared the first tetrazole analogs of amino acids in 1958. The tetrazole analogs of glycine, D, L-alanine, β-alanine, D, L-phenylalanine, D, L-tryptophane, ρ-aminobenzoic acid, 2-hydroxy-4-aminobenzoic acid, picolinic acid, nicotinic acid, isonicotinic acid, 2, 4-dichlorophenoxyacetic acid, 2, 4, 5-trichlorophenoxyacetic acid, 3, 4, 5-trimethoxybenzoic acid, and 3-indoleacetic acid were prepared by three methods.

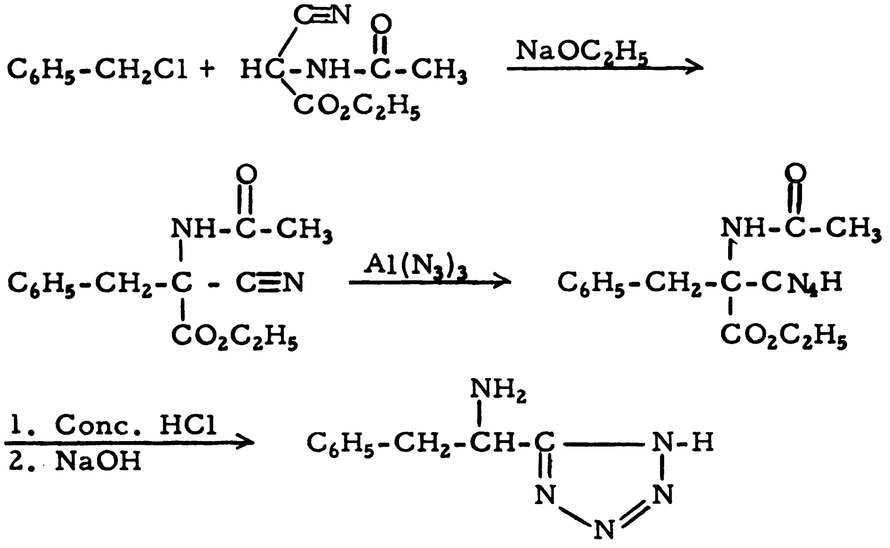
In the first scheme the α-haloacyl halide was converted to the N-benzyl-α-haloamide by reaction with benzylamine. The 1-benzyl-5-α-haloalkyltetrazole was synthesized by the interaction of the α-haloamide with phosphorus pentachloride to give the imino chloride followed by treatment with hydrazoic acid. Reaction with potassium phthalimide gave rise to the 1-benzyl-5-α-phthalimidoalkyltetrazole and subsequent treatment with ethanolic hydrazine resulted in the formation of 1-benzyl-α-aminoalkyl tetrazole. The benzyl group was then removed by hydrogenolysis.



In the second scheme the amino acid was fused with phthalic anhydride. The N-phthaloyl amino acid was converted to the nitrile by way of the acid chloride and amide. Reaction of the nitrile with aluminum azide followed by hydrazinolysis of the phthaloyl compound gave rise to tetrazole analogs of alanine, β-alanine, and D, L-phenylalanine.



In a third technique McManus (3) alkylated ethyl-α-acetamidocyanoacetate with benzyl chloride and gramine. The cyano compounds were then converted to the tetrazoles and following hydrolysis the tetrazole analogs of D, L-phenylalanine and tryptophane were obtained.



Sterken (19) prepared the tetrazole analogs of α-aminobutyric acid, α-aminoisobutyric acid, γ-aminobutyric acid, valine, leucine, and C-phenylglycine by methods similar to those employed by McManus.

The formation of a 2,5-disubstituted-1,3,4-oxadiazole from a 5-substituted tetrazole was first reported by Stolle (33) in 1929. On heating acetic anhydride with 5-aminotetrazole the 2-acetamido-5-methyl-1,3,4-oxadiazole resulted. Stolle proposed a mechanism involving the elimination of nitrogen from the 2 and 3 positions of the tetrazole ring, formation of a nitrogen diradical, followed by the shift of the acetamido group from the ring carbon to the diradical in a Curtius type rearrangement to give a carbodiimide. The carbodiimide then underwent acylation, tautomerization, and ring closure to form the 1,3,4-oxadiazole.

Huisgen and co-workers (34) synthesized 2-(ρ-nitrophenyl)-5-phenyl-1,3,4-oxadiazole from the reaction of 5-phenyltetrazole with ρ-nitrobenzoyl chloride and pyridine. Huisgen extended the scope of the reaction by converting a number of 5-alkyl and 5-aryltetrazoles to their respective oxadiazoles by heating with pyridine and a wide variety of acylating agents such as acetyl, benzoyl, ρ-toluyl, and succinyl chlorides as well as acetic and benzoic anhydrides. Huisgen's proposed mechanism for the formation of the oxadiazole is based on the assumption that acylation occurs at the 2 position of the tetrazole ring.

The theory of amphoteric dissociation of amino acids has long been recognized. Hitchcock (35), Bjerrum (36), and Edsall (37, 38) have determined the ionization constants for many amino acids. Recently Edsall and co-workers (22) worked out a complete ionization scheme for tyrosine and proposed the scheme as illustrated in Figure 1. Since each of the three functional groups can exist in either the ionized or unionized form, there are eight possible forms for tyrosine in going from the protonated form to the dianion. Edsall also determined the ionization constants for tyrosine by two different methods; direct titration, and calculation from "microconstants." The micro-constants are the individual interrelationships between the eight forms.

The pK_1 and pK_2 values for the tetrazole analogs of some amino acids were determined by McManus (3) and Sterken (19) and were found to be in close agreement with ρK values of the respective amino acids.

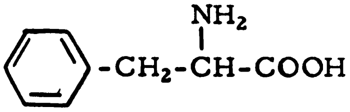
The influence of pH on the optical rotation of optically active electrolytes, with the resulting changes in disassociation, has been extensively studied. Lutz and Jirgensons (20, 21) determined the optical rotations of a series of naturally occurring amino acids dissolved in various amounts of acid and base. From a plot of the specific rotation against the moles of acid and base, Lutz and Jirgensons predicted the configuration of the amino acid. This was done by examining the acid branch of the optical rotation curve. If, with increasing acid concentration, it was found that the rotation changed toward the positive direction, the amino acid was the L-antipode. If the rotation became more negative, the amino acid was assigned the D-configuration. In this manner configurations were assigned to naturally occurring glutamic acid, arginine, lysine, ornithine, histidine, alanine, tyrosine, aspartic acid, leucine, tryptophane, proline, hydroxyproline, and dioxyphenylalanine.

PART I

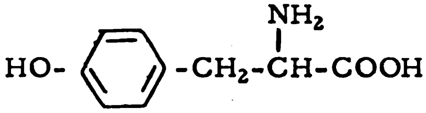
TETRAZOLE ANALOGS OF AMINO ACIDS

DISCUSSION

The close metabolic relationship of phenylalanine (I) and tyrosine (II) might be expected from the similarity in their chemical structure.



I



II

Numerous studies have been carried out to illustrate this relationship. By use of isotope techniques (48, 10) it was shown that phenylalanine can be converted to tyrosine in mammals. This finding is in accord with the earlier work of Rose (44) on amino acids, which are essential to man. He demonstrated that while phenylalanine was indispensable, tyrosine was not, and that the requirement for phenylalanine both in the rat and man is markedly reduced by the inclusion of tyrosine in the diet (11).

Some microorganisms, such as Neurospora can also convert phenylalanine to tyrosine (46). In addition, several mutant strains of Escherichia coli are capable of carrying out a reversible interconversion of phenylalanine and tyrosine, since either amino acid will permit their growth on an otherwise amino acid-free medium. In the mammal, tyrosine also serves as the precursor of the hormones adrelin, noradrelin, and thyroxine. Gurin and Delluva's work (45) using phenylalanine labeled with tritium in the benzene ring and C^{14} in the α-carbon illustrated that this amino acid is converted to adrenaline in the rat.

The concept of antagonism between structurally similar metabolites is explained on the basis of the existence of a competition between the similar species for an enzyme or enzyme system with which both are capable of associating. The application of this concept has been extended to include the antagonism of a metabolite by a structurally related non-metabolite, or "antimetabolite." When interaction between the antimetabolite and the enzyme is reversible a competitive inhibition in the utilization of a metabolite results. Non-competitive inhibition occurs when the antimetabolite-enzyme reaction is irreversible.

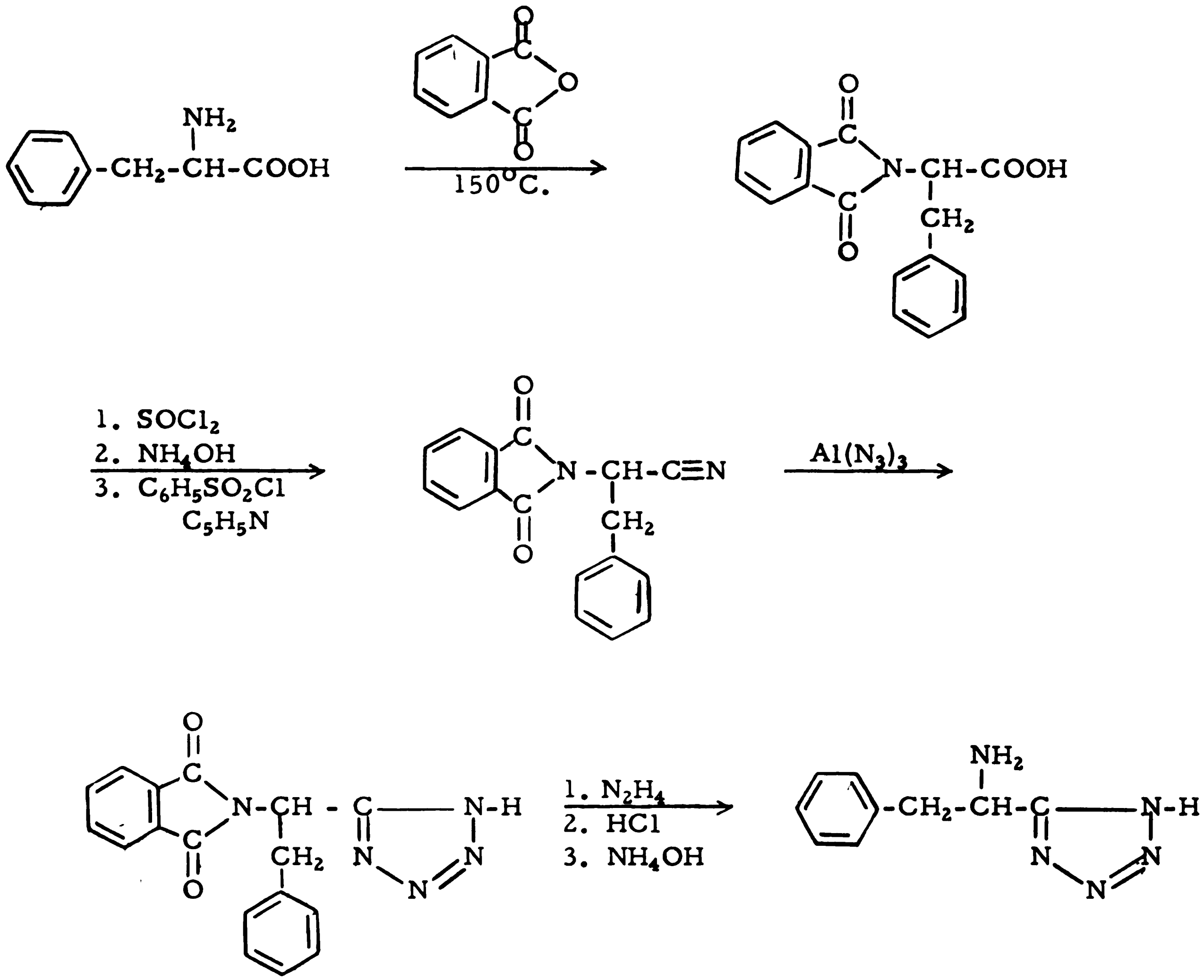
Since the amino acids play such a vital and universal role in metabolic processes of living organisms, they have been used as model systems in the synthesis of potential antimetabolites. Phenylalanine has been investigated thoroughly in this respect. Various alterations in the molecular structure of phenylalanine have produced antagonism to naturally occurring phenylalanine in bacterial growth. These alterations are in the form of adding substituents to the amino group or the ring, replacing the benzene moiety with other ring systems, and replacing the carboxyl group with a functional group of related structure and reactivity, such as the tetrazolyl moiety (43).

McManus (3) and Sterken (19) prepared a series of tetrazole analogs of amino acids. It was considered germane to extend this series to include optically active tetrazole analogs of phenylalanine and tyrosine, and determine some characteristic physical properties for comparison with the corresponding amino acids.

The most common and widely used technique for the synthesis of 5-substituted tetrazoles is through interaction of a nitrile with hydrazoic acid or one of its salts. Three general schemes were used for the preparation of nitriles from the corresponding L-amino acids with the amino group suitably blocked. A fourth scheme employed, consisted of alkylating ethyl acetamidocyanoacetate. A brief outline of each scheme is given.

Scheme A

This method involved the use of the phthaloyl group as the amino protecting group. L-phenylalanine was fused with phthalic anhydride in an oil bath at 145-150°C. The temperature must be kept below 150°C. to prevent racemization. The N-phthaloyl amino acid is then converted to the acid chloride with thionyl chloride, and subsequently to the amide with ammonium hydroxide. The amide was dehydrated with benzenesulfonyl chloride in the presence of pyridine to give the nitrile. It was possible to use a three to one molar ratio of benzenesulfonyl chloride to amide without appreciably affecting the yield. A cleaner reaction resulted by using the smaller amount of benzenesulfonyl chloride than that used by Peterson and Nieman (2) to prepare the nitrile. Treatment of the nitrile with aluminum azide, generated *in situ* from sodium azide and anhydrous aluminum chloride in dry tetrahydrofuran, gave the tetrazole. It was noted that difficulty was encountered in obtaining a clean, solid tetrazole if the aluminum chloride-tetrahydrofuran mixture became dark. By cooling the tetrahydrofuran, then adding it slowly to the aluminum chloride cooled in an ice bath, only a slight yellow discoloration appeared. The result was a more workable product. The aluminum salt of the tetrazole was suspended in 1 N hydrochloric acid liberating the free tetrazole. The phthaloyl group was removed by hydrazinolysis giving the tetrazole analog of L-phenylalanine. This scheme may be outlined as follows:

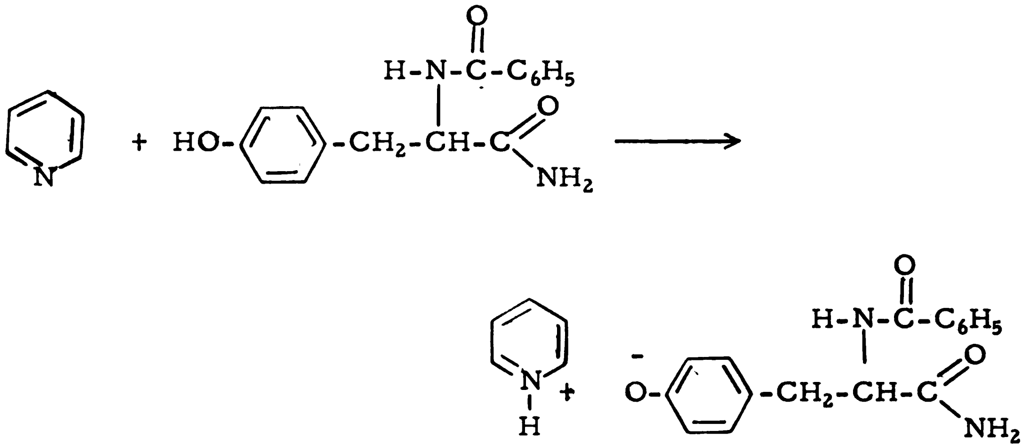


Attempts to apply the use of the phtholyl group as a suitable amino blocking group for tyrosine were unsuccessful. Fusions with L-tyrosine and phthalic anhydride were attempted at 150°C., 175°C., and 200°C. for 30-45 minutes. At the higher temperature the reaction appeared to take place from the fact that water condensed in the upper part of the flask, but efforts to obtain the desired product from the melt were without success.

Scheme B

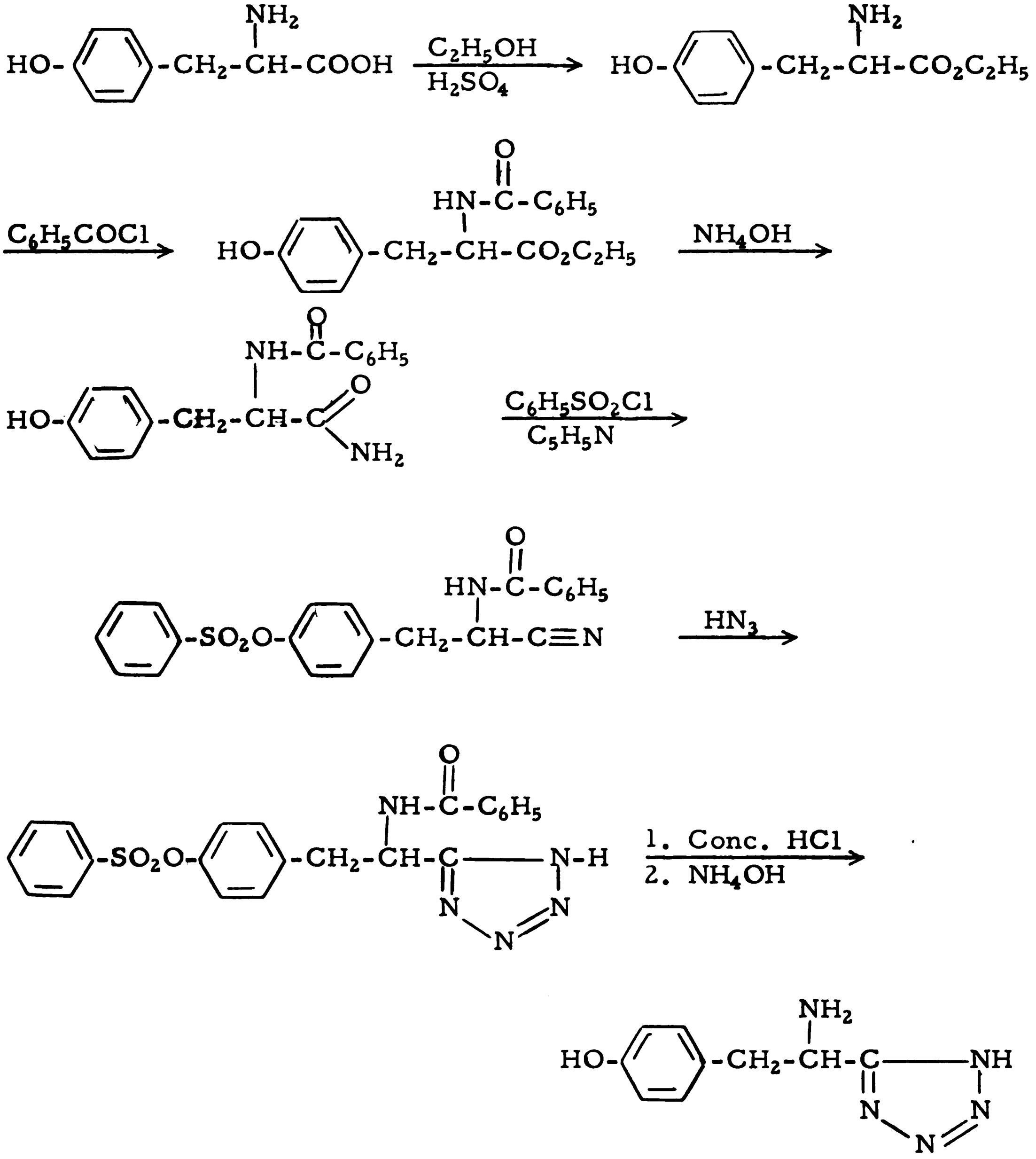
The tetrazole analogs of D, L-phenylalanine and L-tyrosine were synthesized from the alpha-benzamidonitriles. L-tyrosine was converted to the ester since the benzoylation of the amino group was found to occur more readily for the ester than the amino acid. Ammonolysis of the ester gave the amide.

On addition of pyridine to the amide of N-benzoyltyrosine, a precipitate formed which was believed to be the pyridinium phenoxide salt.



Since the phenoxide anion is a stronger nucleophile than the amide, reaction of benzenesulfonyl chloride with the phenoxide anion would occur first. Reaction between equal molar amounts of benzenesulfonyl chloride and N-benzoyltyrosine amide resulted in the formation of L-α-benzamido-β-(ρ-benzenesulfonyloxyphenyl)propionamide. When the molar ratio of benzenesulfonyl chloride to N-benzoyltyrosine amide was 3 to 1, L-α-benzamido-β-(ρ-benzenesulfonyloxyphenyl)propionitrile was formed.

Formation of the tetrazole from hydrazoic acid generated in situ from sodium azide and glacial acetic acid, resulted in a cleaner product while maintaining high yields. Another advantage found in using hydrazoic acid was that the work up of the product was simplified. The reaction mixture was evaporated to dryness leaving only the tetrazole and its sodium salt in the residue. Acidification resulted in a tetrazole of high purity. The free tetrazole was obtained by acid or base hydrolysis of the benzamido moiety. These compounds were found to be difficultly hydrolyzed, requiring 24-48 hours of refluxing. Hydrolysis with concentrated hydrochloric acid is preferred to basic hydrolysis, since the progress of the reaction can be followed by formation of the soluble amine hydrochloride, and the possibility of racemerization is precluded. Refluxing is continued until all of the benzamido tetrazole goes into solution. The scheme is outlined as follows:

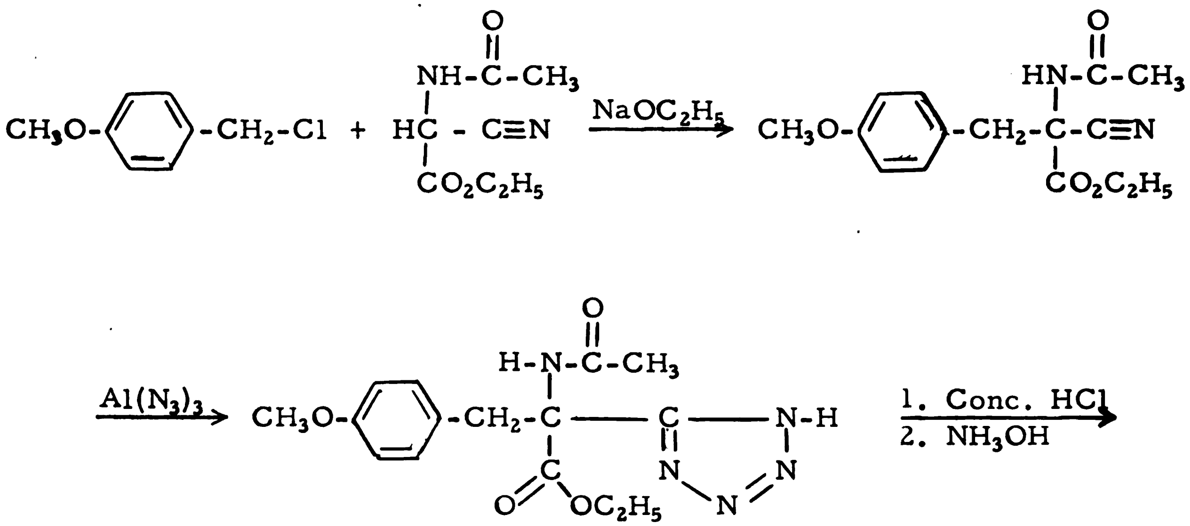


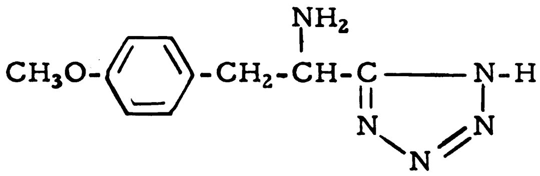
Scheme C

This method is essentially the same as scheme B except that the acylation of the amino group is accomplished with acetic anhydride. The scheme was found to be readily adaptable for the preparation of the tetrazole analog of D, L-phenylalanine, but when applied to the synthesis of the tetrazole analog of L-tyrosine, many difficulties were encountered. The acetylated derivatives of L-tyrosine were found to be very water soluble and difficult to crystallize, except for N-acetyl tyrosine ethyl ester. A great deal of difficulty was experienced in trying to get the acetylated nitrile to crystallize. In this respect the benzoylated compounds are more desirable for synthetic purposes. However, the 5-acetylaminoalkyltetrazoles were more easily hydrolyzed and the acetic acid produced can be removed by evaporation.

Scheme D

The alkylation of acetylaminomalonic ester and ethyl acetamidocyanoacetate has been a classical method for the synthesis of amino acids. However, its application is limited to the synthesis of D, L-amino acids. In the synthesis of tetrazole analogs of amino acids by the alkylation of ethyl acetamidocyanoacetate, only a minimum number of steps is required. The procedure is outlined as follows:





Anisyl chloride is prepared by the interaction of concentrated hydrochloric and anisyl alcohol. The chloride should be used as quickly as possible and not stored. Several minor explosions have been reported on storing the material overnight (50). Following alkylation of ethyl acetamidocyanoacetate with anisyl chloride, the product was converted to the tetrazole with aluminum azide. Either a stepwise hydrolysis of the functional groups, or a single step acid hydrolysis, gave rise to the tetrazole analog of O-methyl-D, L-tyrosine. Attempts to obtain the tetrazole analog of D, L-tyrosine by refluxing the O-methyl compound with 57% hydroiodic acid were unsuccessful.

EXPERIMENTAL[1,2,3]

The Preparation of D, L-5-(α-Amino-β-phenylethyl)tetrazole
(D, L-Phenylalanine Analog)

Scheme A

(a) D, L-α-Phthalimido-β-phenylpropionic Acid

A mixture of 25.0 g. (0.152 mole) of D, L-phenylalanine and 22.4 g. (0.152 mole) of phthalic anhydride was fused in an oil bath at a temperature of 180° for a period of forty-five minutes. After allowing the resulting melt to cool to room temperature, it was dissolved in 100 ml. of methyl alcohol, filtered, and 100 ml. of hot water added. The solution was then heated and allowed to crystallize. The solid phthalimido derivative was filtered and dried to give 27.1 g. (92% of theory) of product, m.p. 175-176° C.

J. Sheenan and V. Frank (1) reported this compound to melt at 177.5-179° C.

(b) D, L-α-Phthalimido-β-phenylpropionyl Chloride

To a refluxing solution of 25.0 g. (0.0848 mole) of D, L-α-phthalimido-β-phenylpropionic acid suspended in 250 ml. of dry benzene was added 10.7 g. (0.09 mole) of thionyl chloride with stirring over a period of ten minutes. Refluxing was continued for an additional hour and during this

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[1] Microanalysis by Microtech Laboratories, Skokie, Illinois.

[2] Melting points were taken in open capillary tubes and are uncorrected.

[3] All infrared spectra taken as Nujol mulls.

period 5.0 g. of thionyl chloride was added to insure complete reaction. The reaction mixture becomes homogeneous since the acid chloride is soluble in benzene. The benzene and excess thionyl chloride were removed under reduced pressure on a steam bath; 100 ml. of fresh benzene was added to the residue and again evaporated. The yield of crude acid chloride was 26.0 g. (98% of theory), m.p. 133-136°.

Sheenen and Frank (1) reported a melting point of 124-126° C. for the acid chloride.

(c) D, L-α-Phthalimido-β-phenylpropionamide

Twenty-five grams (0.0794 mole) of D, L-α-phthalimido-β-phenylpropionyl chloride was added to 100 ml. of cold ammonium hydroxide and stirred for thirty minutes. The solid was filtered, washed with cold water, and dried to give 21.1 g. of product (92% of theory), m.p. 232-233° C.

This compound was reported by Peterson and Nieman (2) to melt at 236-237° C.

(d) D, L-α-Phthalimido-β-phenylpropionitrile

To 20.0 g. (0.068 mole) of D, L-α-amino-β-phenylpropionamide suspended in 72.5 ml. of pyridine was added 36.0 g. (0.204 mole) of benzenesulfonyl chloride and the resulting mixture was refluxed for ten minutes. After cooling to room temperature, the solution was poured into ice water. The precipitate which formed was filtered, washed with cold water, and dried. The crude nitrile weighed 18.2 g. (97% yield), m.p. 127-129° C. After recrystallization from methyl alcohol, the melting point was raised to 134-135° C.

Peterson and Nieman (2) reported a melting point of 134-135° C. for this compound.

(e) D, L-5-(α-Phthalimido-β-phenylethyl)tetrazole

To 17.0 g. (0.0616 mole) of D, L-α-phthalimido-β-phenylpropionitrile and 12.0 g. (0.185 mole) of sodium azide placed in a 300 ml. 3-necked round bottom flask equipped with stirrer and condenser was added 9.1 g. (0.068 mole) of anhydrous aluminum chloride dissolved in 150 ml. of dry tetrahydrofuran. The dissolution of aluminum chloride in tetrahydrofuran is best accomplished by slowly adding cold tetrahydrofuran to the aluminum chloride which is in a flask placed in an ice bath. The nitrile, sodium azide, aluminum chloride mixture was refluxed for a period of twenty-four hours. The tetrahydrofuran was then distilled out of the reaction mixture and water added at such a rate that the volume of the mixture remained constant. The solid residue was filtered, washed with water, and dried. The finely ground solid was suspended in 175 ml. of water, and 20 ml. of concentrated hydrochloric acid added. The precipitate was filtered, washed with water, and dried. The yield of tetrazole was 19.2 g. (98% of theory), m.p. 213-214°C.

This compound, prepared in a similar manner is reported by McManus (3) to have a melting point of 212.5-213° C.

(f) D, L-5-(α-Amino-β-phenylethyl)tetrazole

To 18.0 g. (0.0565 mole) of D, L-5-(α-phthalimido-β-phenylethyl)-tetrazole suspended in 100 ml. of absolute ethanol was added 56.5 ml. of 1 M ethanolic hydrazine solution. The mixture was refluxed for two hours, cooled to room temperature, then evaporated to dryness. The residue was treated with 140 ml. of 2 N hydrochloric acid and warmed on a steam bath at 50-55° C. for ten minutes. The mixture was filtered and the filtrate was neutralized to a pH of 5 with concentrated ammonium hydroxide. The resulting precipitate was filtered, washed with cold water, and dried. The product weighed 8.5 g. (80% yield), m.p. 270-271° C. with decomposition.

McManus (3) reported a melting point of 271-272° C. with decomposition for this compound.

Scheme B

(a) N-Benzoyl-D, L-phenylalanine

To 32.0 g. (0.194 mole) of D, L-phenylalanine placed in a 3-necked 300 ml. round bottom flask equipped with a stirrer was added 194 ml. of 1 N sodium hydroxide. The flask was immersed in an icewater bath to maintain the temperature at 1° C. or lower. Benzoyl chloride (27.3 g., 0.194 mole) was added and, simultaneously, at a rate four times faster 97 ml. of 2 N sodium hydroxide was added. After complete addition of the reagents, the mixture was stirred for an additional thirty minutes. If an appreciable amount of solid material separated, a little water was added. The solution was then filtered and the filtrate acidified with cooling to Congo red. The precipitate was filtered, washed with water, and dried *in vacuo* over phosphorus pentoxide. The yield was 45.5 g. (87% of theory), m.p. 184-186° C.

This compound was prepared in a similar manner by Steiger (4) who reported a melting point of 186° C.

(b) D, L-Ethyl-α-benzamido-β-phenylpropionate

N-Benzoyl-D, L-phenylalanine (42.0 g., 0.156 mole) was suspended in 200 ml. of absolute ethanol, and 3 ml. of concentrated sulfuric acid was added. After refluxing for two hours, the solution was filtered and the filtrate was evaporated to dryness under reduced pressure. The solid product was then washed several times with cold water and recrystallized from 50% ethanol. After drying at 70°C., the ester weighed 45.6 g. (99% of theory), m.p. 94-95.5° C.

Max (5) reported this compound to melt at 95-95.5° C.

(c) D, L-α-Benzamido-β-phenylpropionamide

To 40.0 g. (0.135 mole) of N-benzoyl-D, L-phenylalanine ethyl ester dissolved in 50 ml. of acetone was added 100 ml. of concentrated aqueous ammonium hydroxide. The solution was allowed to stand at room temperature for two days with occasional stirring. The solution was then evaporated to dryness and the residue washed several times with cold water. The crude product weighed 32.5 g. (93% of theory), m.p. 195-197° C. Recrystallization from chloroform raised the melting point to 198° C.

A melting point of 198° C. for this compound was reported by Max (5).

(d) D, L-α-Benzamido-β-phenylpropionitrile

To 20.0 g. (0.0747 mole) of D, L-α-benzamido-β-phenylpropionamide and 105 ml. of pyridine placed in a 250 ml. flask, was added 39.5 g. (0.244 mole) of benzenesulfonyl chloride and the mixture refluxed gently for ten minutes. The resulting solution was allowed to cool to room temperature and poured into ice water. Upon standing in the refrigerator overnight, a yellow precipitate resulted which was filtered, washed with cold water, and dried. The crude nitrile weighed 18.1 g. (97% yield) and had a melting point of 130-134°C. Recrystallization from chloroform-petroleum ether raised the melting point to 148° C.

Kjaer (6) reported the melting point for the nitrile to be 150° C.

(e) D, L-5-(α-Benzamido-β-phenylethyl)tetrazole

To 10.0 g. (0.040 mole) of D, L-α-benzamido-β-phenylpropionitrile and 7.8 g. (0.12 mole) of sodium azide was added 5.9 g. (0.044 mole) of anhydrous aluminum chloride dissolved in 150 ml. of dry tetrahydrofuran. After refluxing the solution for a period of twenty-four hours, the tetrahydrofuran was distilled out of the reaction mixture while water was added at such a rate that the volume of the mixture remained constant. The solid was filtered, washed with water, and dried. The finely ground solid was suspended in 175 ml. of water and 20 ml. of concentrated hydrochloric acid added. The precipitate was then filtered, washed with water, and dried. The product weighed 10.8 g. (92% of theory) and melted at 232-233° C.

McManus (3) reported this compound to have a melting point of 233-234° C.

(f) D, L-5-(α-Amino-β-phenylethyl)tetrazole

To 8.0 g. (0.0273 mole) of D, L-5-(α-benzamido-β-phenylethyl)-tetrazole was added 100 ml. of concentrated hydrochloric acid and the mixture refluxed for twenty-four hours. The clear solution was allowed to cool to 0° C. and the resulting precipitate filtered; 3.26 g. of material having a melting point of 119-120° C. was collected. When mixed with an authenic sample of benzoic acid, the melting point of the product showed no depression. The filtrate was adjusted to pH 5 by addition of aqueous ammonium hydroxide and the resulting precipitate filtered, washed with water, and dried. The weight of the tetrazole was 4.2 g. (81% of theory); m.p. 272-273° C. with decomposition.

A mixture melting point with a sample of D, L-5-(α-amino-β-phenylethyl)tetrazole prepared by Scheme A showed no depression.

Scheme C

(a) N-Acetyl-D, L-phenylalanine

D, L-Phenylalanine (30.5 g., 0.1845 mole) was dissolved in 92.5 ml. of 2 N sodium hydroxide and 100 ml. of water. The solution was cooled in an ice bath and 48.8 ml. of acetic anhydride and 488 ml. of 2 N sodium hydroxide were added in eight equal portions with vigorous shaking and cooling between additions. The mixture was then allowed to stand at room temperature for forty-five minutes.

Addition of 182 ml. of 6 N sulfuric acid caused precipitation of the product which was filtered, washed with water, and dried, yielding 33.7 g. (89% of theory) of N-acetyl-D, L-phenylalanine, m.p. 150-152° C.

The compound is reported by du Vigneaud and Meyer (7) to have a melting point of 151-152° C.

(b) D, L-Ethyl-α-acetamido-β-phenylpropionate

To a solution of 30.0 g. (0.145 mole) of N-acetyl-D, L-phenylalanine dissolved in 200 ml. of absolute ethanol was added 3 ml. of concentrated sulfuric acid. After refluxing the mixture for two hours, the alcohol was evaporated under reduced pressure and the solid residue was washed thoroughly with cold water, filtered, and dried over phosphorus pentoxide *in vacuo*. The yield was 34.6 g. (91%), m.p. 60-61°C.

The same compound prepared in another manner was reported to have a melting point of 67° C. by Ashley and Harington (8).

(c) D, L-α-Acetamido-β-phenylpropionamide

To 29.2 g. (0.125 mole) of N-Acetyl-D, L-phenylalanine ethyl ester dissolved in 100 ml. of acetone was added 200 ml. of concentrated aqueous ammonium hydroxide. The mixture was allowed to stand at room temperature for two days with occasional stirring. The solution was then evaporated to dryness and the residue washed with cold water. The yield of amide was 18.0 g. (70%), m.p. 143-145° C. The melting point was raised to 159-162° C. after recrystallization from 50% ethanol. A melting point of 161.5° C. was reported by Bergmann (13).

(d) D, L-α-Acetamido-β-phenylpropionitrile

To 16.0 g. (0.0778 mole) of D, L-α-acetyl-β-phenylpropionamide and 116 ml. of pyridine was added 41.0 g. (0.233 mole) benzenesulfonyl chloride and the mixture refluxed for ten minutes. After cooling to room temperature, the solution was poured into water and allowed to stand in the refrigerator overnight. The resulting precipitate was filtered, washed with water and dried. The yield of crude nitrile was 12.8 g. (88% of theoretical), m.p. 99-100° C. After several recrystallizations from chloroform-petroleum ether, the melting point was raised to 108.5-109.5° C.

Analysis.

Calculated for $C_{11}H_{12}N_2O$: C, 70.19; H, 6.43; N, 14.89.

Found: C, 70.03; H, 6.33; N, 15.11.

(e) D, L-5-(α-Acetamido-β-phenylethyl)tetrazole

To 10.0 g. (0.0533 mole) of D, L-5-α-acetamido-β-phenylpropionitrile and 10.4 g. (0.160 mole) of sodium azide was added 7.9 g. (0.059 mole) of anhydrous aluminum chloride dissolved in 150 ml. of dry tetrahydrofuran. After refluxing for twenty-four hours, the tetrahydrofuran was distilled out of the reaction mixture while water was added at such a rate that the volume of the mixture remained constant. The aluminum salt of the tetrazole, which separated as a solid, was filtered and dried. The finely ground crude tetrazole salt was suspended in 175 ml. of water, and 20 ml. of concentrated hydrochloric acid added. The precipitate was filtered, washed with water, and dried. The yield of tetrazole was 10.1 g. (82% of theory), m.p. 226-227° C.

McManus reported this compound to have a melting point of 224.5-225.5° C.

(f) D, L-5-(α-Amino-β-phenylethyl)tetrazole

To 8.0 g. (0.0346 mole) of D, L-5-(α-acetamido-β-phenylethyl)-tetrazole was added 100 ml. of concentrated hydrochloric acid and the mixture refluxed for twelve hours. The clear solution was filtered. The filtrate was adjusted to pH 5 by addition of concentrated ammonium hydroxide and the resulting precipitate was filtered, washed with water, and dried to give 5.0 g. (78% of theory) of product, m.p. 270-271° C. with decomposition.

A mixture melting point with an authenic sample of D, L-5-(α-amino-β-phenylethyl)tetrazole showed no depression.

The Preparation of L-5-(α-Amino-β-phenylethyl)tetrazole
(L-Phenylalanine Analog)

Scheme A.

(a) L-α-Phthalimido-β-phenylpropionic acid

A mixture of 16.5 g. (0.1 mole) of L-phenylalanine[1,2] and 14.8 g. (0.1 mole) of phthalic anhydride was fused in an oil bath at a temperature of 145-150° C. for thirty minutes. The melt was then allowed to cool to room temperature and dissolved in 50 ml. of methanol. A small amount of unreacted amino acid was filtered off and 50 ml. of water added to the filtrate. The solution was then heated and allowed to crystallize. A total of 27.3 g. (93% of theory) of product was obtained which had a melting point of 177-179° C.; $[\alpha]_D^{25} = -206^\circ$, C = 1.92 in absolute ethanol.

This compound was reported by Sheehan (9) to have a melting point of 183-185° C., $[\alpha]_D^{25} = -212^\circ$, C = 1.92 in absolute ethanol.

(b) L-α-Phthalimido-β-phenylpropionyl chloride

To a refluxing suspension of 25.0 g. (0.0848 mole) of L-α-phthalimido-β-phenylpropionic acid in 100 ml. of dry benzene was added 11.9 g. (0.1 mole) of thionyl chloride with stirring over a period of

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[1] Nutritional Biochemicals Corp.

[2] $[\alpha]_D^{25} = -34.9^\circ$, C = 1.936 in H_2O.

ten minutes. The reaction mixture was heated at reflux temperature for an additional hour during which 5.0 g. more thionyl chloride was added to insure complete reaction. The benzene and excess thionyl chloride were removed from the clear solution under reduced pressure. The residue was dissolved in 50 ml. of fresh benzene which was again removed under reduced pressure. The crude acid chloride weighed 26.3 g. (99% of theory) and had a melting point of 83-85° C., $[\alpha]_D^{25} = -198^\circ$, C = 2.256 in benzene.

Sheenan (9) reported a melting point of 83-84° C., $[\alpha]_D^{25.5} = -197^\circ$, C = 2.256 in benzene.

(c) L-α-Phthalimido-β-phenylpropionamide

L-α-Phthalimido-β-phenylpropionyl chloride (25.0 g., 0.080 mole) was added to 200 ml. of cold concentrated ammonium hydroxide and stirred for thirty minutes. The solid was filtered, washed with cold water, and dried to give 21.6 g. (92% of theory) of amide, m.p. 214-216° C. Recrystallization from 95% ethanol raised the melting point to 217-218° C. Peterson and Nieman (2) reported the melting point to be 226-227.5° C.

(d) L-α-Phthalimido-β-phenylpropionitrile

To 20.0 g. (0.0681 mole) of L-α-phthalimido-β-phenylpropionamide suspended in 72.5 ml. of pyridine was added 36.0 g. (0.204 mole) of benzenesulfonyl chloride and the resulting mixture was refluxed gently for ten minutes. After cooling to room temperature, the solution was poured into ice water. The precipitate which formed was filtered, washed with cold water, and dried. The crude nitrile weighed 18.7 g. (99% of theory), m.p. 146-149° C. Recrystallization from methanol raised the melting point to 150-151° C., $[\alpha]_D^{25} = -102^\circ$, C = 2 in $CHCl_3$.

A melting point of 150-153.2° C. and $[\alpha]_D^{25} = -103^\circ$, C = 2% in $CHCl_3$, was reported by Peterson and Nieman (2).

(e) L-5-(α-Phthalimido-β-phenylethyl)tetrazole

To 16.2 g. (0.059 mole) of L-α-phthalimido-β-phenylpropionitrile and 11.5 g. (0.177 mole) of sodium azide was added 8.7 g. (0.065 mole) of anhydrous aluminum chloride dissolved in 150 ml. of dry tetrahydrofuran. After refluxing for twenty-four hours, the tetrahydrofuran was distilled out of the reaction flask and water added at such a rate that the volume remained constant. The solid product was filtered and dried. The finely ground crude tetrazole salt was suspended in 175 ml. of water and 20 ml. of concentrated hydrochloric acid added. The precipitate was filtered, washed with water, and dried. The crude product weighed 16.9 g. (90% of theory) and melted at 170-178° C. The melting point was raised to 182-183° C. after recrystallization from chloroform-petroleum ether. $[\alpha]_D^{25} = -139.9^\circ$, C = 4 in absolute ethanol.

Analysis.

Calculated for $C_{17}H_{13}N_5O_2$: C, 63.94; H, 4.10; N, 21.93.
Found: C, 63.81; H, 3.88; N, 22.17.

(f) L-5-(α-Amino-β-phenylethyl)tetrazole

To 14.0 g. (0.0439 mole) of L-5-(α-phthalimido-β-phenylethyl)-tetrazole suspended in 100 ml. of absolute ethanol was added 43.9 ml. of a 1 M ethanolic hydrazine solution. The mixture was refluxed for two hours, cooled to room temperature, then evaporated to dryness. The residue was treated with 109 ml. of 2 N hydrochloric acid and warmed on a steam bath at 50-55° C. for ten minutes. The mixture was filtered and the filtrate adjusted to pH 5 with concentrated ammonium hydroxide. The resulting precipitate was filtered, washed with cold water, and dried. The product weighed 6.0 g. (72% of theory), m.p. 287° C. with decomposition, $[\alpha]_D^{25} = +38.0^\circ$, C = 0.945 in H_2O.

Analysis.

Calculated for $C_9H_{11}N_5$: C, 57.12; H, 5.86; N, 37.02.
Found: C, 57.20; H, 5.85; N, 36.88.

The Preparation of L-5-[α-Amino-β-(ρ-hydroxyphenyl)ethyl]tetrazole (L-Tyrosine Analog)

Scheme B

(a) L-Tyrosine ethyl ester

One hundred grams (0.552 mole) of L-tyrosine was placed in a 500 ml. round bottom flask containing 300 ml. of absolute ethanol. The suspension was treated with hydrogen chloride gas until all of the tyrosine dissolved. After addition of 3 ml. of concentrated sulfuric acid, the solution was refluxed for 6 hours. The excess ethanol was removed under reduced pressure and the residue dissolved in 200 ml. of water; the solution was filtered, the filtrate adjusted to pH 8 with concentrated ammonium hydroxide. The ester which separated as a crystalline solid was filtered with suction, washed several times with cold water, and dried at 80° C. The crude ester weighed 105.0 g. (92% of theory), m.p. 99-102° C. Recrystallization from ethyl acetate-petroleum ether, gave a melting point of 103-104°C., $[\alpha]_D^{25} = +17.40^\circ$, C = 4.0 in absolute ethanol.

Fischer (12) reported the ester to have a melting point of 108-109° C., $[\alpha]_D^{20} = +20.40^\circ$, C = 4.85% in absolute ethanol.

(b) L-Ethyl-α-benzamido-β-(ρ-hydroxyphenyl)propionate

A solution of 103.4 g. (0.495 mole) of L-tyrosine ethyl ester in 750 ml. of chloroform was cooled to 0° C. Benzoyl chloride (70.3 g., 0.50 mole) was added in seven portions with vigorous shaking between additions. The temperature was maintained below 10° C. After all of the benzoyl chloride had been added, 250 ml. of 2 N sodium carbonate (0.50 eq.) was added and the mixture shaken until no more carbon dioxide was evolved. The chloroform layer was then separated, washed several times with cold water, and evaporated to dryness. The yield of crude product was 149.6 g. (96% of theory), m.p. 115-118° C. Recrystallization from chloroform-petroleum ether raised the melting point to 120-121° C., $[\alpha]_D^{25} = -22.5°$, C = 3.7 in absolute ethanol. Bergel and Lewis (14) reported a melting point of 122-123° C. and $[\alpha]_D^{20} = -23.7°$, C = 3.7 in absolute ethanol.

(c) L-α-Benzamido-β-(ρ-hydroxyphenyl)propionamide

To a solution of 143.0 g. (0.457 mole) of L-ethyl-β-benzamido-β-(ρ-hydroxyphenyl)propionate dissolved in 200 ml. of acetone was added 300 ml. of concentrated aqueous ammonia. The solution was allowed to stand at room temperature for two days with occasional stirring. The precipitate which formed was collected and the filtrate was evaporated to dryness *in vacuo* without heating. The combined solid products were suspended in 500 ml. of cold water and the pH adjusted to 4 with concentrated hydrochloric acid. The crude amide was filtered, dried, and weighed; the yield was 98.0 g. (76% theory), m.p. 198-200° C. A melting point of 204-205° C. was obtained after recrystallization from 50% ethanol. $[\alpha]_D^{25} = -22.9°$, C = 1 in methanol.

Bergman and Fruton (15) reported this compound to have a melting point of 198° C. and $[\alpha]_D^{23} = -24.6°$, C = 4.6% in methanol.

Analysis.

Calculated for $C_{16}H_{16}N_2O_3$:	C, 67.59; H, 5.67; N, 9.86.
Found:	C, 67.51; H, 5.68; N, 10.07.

(d) L-α-Benzamido-β-(ρ-benzenesulfonyloxyphenyl)propionamide

L-α-Benzamido-β-(ρ-hydroxphenyl)propionamide (14.0 g., 0.0493 mole) was dissolved in 50 ml. of pyridine. A precipitate of the pyridinium phenoxide salt quickly formed. Benzenesulfonyl chloride (9.5 g., 0.054 mole) was added, the mixture shaken vigorously for 10 minutes, and refluxed gently for 10 minutes. After allowing the solution to cool to room temperature, it was poured into cold water and placed in a refrigerator overnight. The precipitate which formed was collected on a filter, washed with cold water, and dried. The product weighed 10.5 g. (50% of theory), m.p. 210-213° C. Recrystallization from 95% ethanol raised the melting point to 218-219° C. The product was insoluble in aqueous sodium hydroxide and gave a positive qualitative test for sulfur.

Analysis.

Calculated for $C_{22}H_{20}N_2O_5S$: N, 6.60.

Found: N, 6.82.

(e) L-α-Benzamido-β-(ρ-benzenesulfonyloxyphenyl)propionitrile

To a suspension of 60.0 g. (0.2115 mole) of L-α-benzamido-β-(ρ-hydroxyphenyl)propionamide in 90 ml. of pyridine, 111.6 g. (0.63 mole) of benzenesulfonyl chloride was added. The mixture was refluxed for ten minutes, cooled to room temperature, then poured into cold water and allowed to stand overnight in the refrigerator. The solid that had separated was dissolved in 60% ethanol and was cooled overnight in the refrigerator. The precipitate was collected on a filter and dried, m.p. 116-119° C. The yield of crude nitrile was 54.3 g. (63% of theory). Several recrystallizations from 60% ethanol raised the melting point to 124-125° C., $[\alpha]_D^{25} = -8.3°$, C = 4.0 in chloroform. A positive qualitative test for sulfur was obtained and the compound was insoluble in aqueous sodium hydroxide.

Analysis.

Calculated for $C_{22}H_{18}N_2O_4S$: C, 65.01; H, 4.46; N, 6.89; S, 7.89.

Found: C, 64.93; H, 4.63; N, 6.84; S, 7.77.

(f) L-5-[α-Benzamido-β-(ρ-benzenesulfonyloxyphenyl)ethyl]tetrazole

L-α-Benzamido-β-(ρ-benzenesulfonyloxyphenyl)propionitrile (34.2 g., 0.084 mole) was placed in a 500 ml. 3-necked round bottom flask equipped with a condenser and a stirrer. Sodium azide (10.4 g., 0.16 mole), glacial acetic acid (9.6 g., 0.16 mole), and n-butyl alcohol (150 ml.) were added and the mixture refluxed for two days. The mixture was then evaporated under reduced pressure in the hood. The residue was suspended in 500 ml. of cold water, and then acidified to Congo red with hydrochloric acid. The precipitate was collected and washed several times with cold water. The product gave a positive qualitative test for sulfur and was soluble in sodium hydroxide solution. The yield of crude tetrazole was 36.6 g. (97%), m.p. 204-6° C. Recrystallization from ethyl acetate containing a small amount of ethanol gave a product melting at 209.5-210.5° C., $[\alpha]_D^{25} = -55.5^\circ$, C = 2.0 in 1 M sodium hydroxide.

<u>Analysis</u>.

Calculated for $C_{22}H_{19}N_5O_4S$: C, 58.78; H, 4.26; N, 15.58; S, 7.13.
Found: C, 58.74; H, 4.31; N, 15.44; S, 7.20.

(g) L-5-[α-Amino-β-(ρ-hydroxyphenyl)ethyl]tetrazole

A suspension of 33.4 g. (0.0744 mole) of L-5-[α-benzamido-β-(ρ-benzenesulfonyloxyphenyl)ethyl]tetrazole in 250 ml. of concentrated hydrochloric acid was boiled under reflux for forty-eight hours. The clear solution was cooled slowly to 0° C. and the precipitate of benzoic acid collected. The precipitate weighed 8.8 g. (97% of theory), m.p. 119-120° C. A mixture melting point with an authenic sample of benzoic acid showed no depression. The filtrate was evaporated to dryness in an air stream, dissolved in 200 ml. of cold water, and adjusted to pH 6 with concentrated ammonium hydroxide. The yield of product was 14.8 g. (97% of theory), m.p. 295° C. with decomposition. Recrystallization from water raised the melting point of the product to 302° C. with decomposition, $[\alpha]_D^{25} = +33.8^\circ$, C = 4.0 in 2 N hydrochloric acid.

Analysis.

Calculated for $C_9H_{11}N_5O$: C, 52.67; H, 5.40; N, 34.13.

Found: C, 52.20; H, 5.41; N, 33.88.

Scheme C

(a) N-Acetyl-L-tyrosine

L-Tyrosine (45.0 g., 0.2485 mole) was dissolved in 127.5 ml. of 2 N sodium hydroxide and 75 ml. of water. The solution was cooled in an ice bath and 60 ml. of acetic anhydride and 600 ml. of 2 N sodium hydroxide were added in eight equal portions with vigorous shaking. After standing at room temperature for forty-five minutes, 251.7 ml. of 6 N sulfuric was added. The solution was then evaporated to dryness and the residue extracted with 80% acetone. The sodium sulfate was filtered off and the acetone extract evaporated *in vacuo* to a thick syrup. A small amount of water was added to the syrup (about 25 ml.) and the acetyltyrosine crystallized on standing in the refrigerator overnight. The yield of product was 12.1 g. (66% of theory), m.p. 147-149° C.

du Vigneaud (7) reported a melting point of 152-154° C.

(b) L-Ethyl-α-acetamido-β-(ρ-hydroxyphenyl)propionate

N-Acetyl-L-tyrosine (11.0 g., 0.0494 mole) was dissolved in 100 ml. of absolute ethanol, 1 ml. of concentrated sulfuric acid was added, and the solution heated under reflux for two hours. The solution was then evaporated to a thick syrup, 200 ml. of water added, and the mixture thoroughly shaken. On standing in the refrigerator for several hours a precipitate formed. The solid was filtered, washed several times with cold water, and dried *in vacuo* over phosphorus pentoxide. The crude ester melted at 76-79° C. and the yield was 7.8 g. (64% of theory). A melting point of 79-80° C. was reported by Kaufman and co-workers (16) for this compound.

(c) L-α-Acetamido-β-(ρ-hydroxyphenyl)propionamide

L-Ethyl-α-acetamido-β-(ρ-hydroxyphenyl)propionate (7.0 g., 0.0284 mole) was dissolved in 25 ml. of acetone and 100 ml. of concentrated aqueous ammonium hydroxide added. After allowing the mixture to stand at room temperature for two days with occasional stirring, the solution was then evaporated to dryness in vacuo without heating. The residue was suspended in water and adjusted to pH 3. The yield of amide was 4.9 g. (80% of theory), m.p. 220-221° C. Recrystallization resulted in a product melting at 221-222° C.

Analysis.

Calculated for $C_{11}H_{14}N_2O_3$: C, 59.44; H, 6.35; N, 12.60.

Found: C, 59.43; H, 6.50; N, 12.62.

This compound was reported by Kaufman and Neurath (17) to have a melting point of 222-224° C.

(d) L-α-Acetamido-β-(ρ-benzenesulfonyloxyphenyl)propionitrile

L-α-Acetamido-β-(ρ-hydroxyphenyl)propionamide (4.0 g., 0.018 mole) was suspended in 13.3 ml. of pyridine and 9.5 g. (0.054 mole) of benzenesulfonyl chloride was added. The mixture was refluxed gently for ten minutes, cooled to room temperature, and poured into cold water. After standing in the refrigerator overnight, the water was decanted and the residue was dissolved in 70% ethanol and refrigerated overnight. The crystalline solid was filtered and recrystallized from 70% ethanol. Four and a half grams (73% yield) of nitrile was obtained. Its melting point was 84-85° C. A positive qualitative test for sulfur indicated that the benzenesulfonyate had been formed.

Analysis.

Calculated for $C_{17}H_{16}N_2O_4S$: C, 59.30; H, 4.68; N, 8.13; S, 9.30.

Found: C, 59.22; H, 4.64; N, 7.93; S, 9.39.

(e) L-5-[α-Acetamido-β-(ρ-benzenesulfonyloxyphenyl)ethyl]tetrazole

L-α-Acetamido-β-(ρ-benzenesulfonyloxyphenyl)propionitrile (4.0 g., 0.0116 mole) was placed in a 100 ml. round bottom flask equipped with a condenser and a stirrer. After addition of 1.51 g. (0.0232 mole) of sodium azide, 1.39 g. (0.0232 mole) of glacial acetic acid, and 50 ml. of n-butyl alcohol, the mixture was refluxed for two days. The reaction mixture was evaporated to dryness under reduced pressure in the hood, the residue suspended in 200 ml. of cold water, and then acidified with hydrochloric acid to Congo red. The precipitate of crude tetrazole was collected on a filter, washed several times with cold water, and dried. The yield of crude tetrazole was 4.5 g. (100% of theory), m.p. 205-207° C. Recrystallization from ethyl acetate containing 25% ethanol raised the melting point to 210-211° C. A positive qualitative test for sulfur was obtained.

Analysis.

Calculated for $C_{17}H_{17}N_5O_4S$:	C, 52.70; H, 4.42; N, 18.08; S, 8.28.
Found:	C, 52.71; H, 4.42; N, 18.02; S, 8.19.

(f) L-5-[α-Amino-β-(ρ-hydroxyphenyl)ethyl]tetrazole

A suspension of 4.0 g. (0.0103 mole) of L-5-[α-acetamido-β-(ρ-benzenesulfonyloxyphenyl)ethyl]tetrazole in 100 ml. of concentrated hydrochloric acid was heated under reflux for two days. The clear solution was filtered and the filtrate was evaporated to dryness, dissolved in 100 ml. of water, and adjusted to pH 6 with aqueous ammonia. The product separated from the solution as a crystalline solid; the yield was 2.0 g. (94% of theory), m.p. 297° C. with decomposition. Recrystallization of this material from water raised the melting point to 302° C. with decomposition. Mixture melting point with a pure sample of 5-[α-amino-β-(ρ-hydroxyphenyl)ethyl]tetrazole prepared by an alternate method showed no depression. Also, the infrared spectrum was identical with that of authenic 5-[α-amino-β-(ρ-hydroxyphenyl)-ethyl]tetrazole.

The Preparation of D, L-5[α-Amino-β-(ρ-methoxyphenyl)ethyl]tetrazole
(O-Methyl-D, L-Tyrosine Analog)

Scheme D

(a) ρ-Methoxybenzyl chloride (anisyl chloride)

In a 100 ml. round bottom flask fitted with a stirrer, was placed 13.8 g., 12.5 ml. (0.1 mole) of anisyl alcohol and 24.8 ml. (0.3 mole) of concentrated hydrochloric acid. After stirring for ten minutes, the solution is transferred to a separatory funnel and the lower layer of anisyl chloride separated, dried over 20 g. of magnesium sulfate for thirty minutes, and filtered. This material cannot be stored for prolonged periods and should be used as quickly as possible. The procedure for preparing anisyl chloride is similar to that described by Rorig and co-workers (18). Without further purification the crude anisyl chloride was used for the preparation of ethyl-α-acetamido-α-cyano-β-(ρ-methoxyphenyl)propionate.

(b) D, L-Ethyl-α-acetamido-α-cyano-β-(ρ-methoxyphenyl)propionate

To a solution of 2.4 g. (0.1 mole) of sodium in 100 ml. of dry absolute ethanol was added 17.0 g. (0.1 mole) of ethyl acetamidocyanoacetate.[1] ρ-Methoxybenzyl chloride (15.6 g., 0.1 mole) was added dropwise over a period of fifteen minutes with stirring. Refluxing and stirring were continued for an additional two hours. The thick tan suspension was poured into cold water and the tan solid which precipitated was filtered, washed with water, and dried yielding 24.8 g. (86% of theory) of crude product, m.p. 160-163° C. Recrystallization from

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[1] Aldrich Chemical Co.

50% ethanol afforded a white, crystalline material which melted at 165.5-166° C.

Analysis.

Calculated for $C_{15}H_{18}N_2O_4$: C, 62.05; H, 6.25; N, 9.70.

Found: C, 62.04; H, 6.16; N, 9.36.

(c) D, L-Ethyl-α-acetamido-α-(5-tetrazolyl)-β-(ρ-methoxyphenyl)propionate

To 26.1 g. (0.09 mole) of ethyl-α-acetamido-α-cyano-β-(ρ-methoxyphenyl)propionate and 17.5 g. (0.27 mole) of sodium azide was added 13.3 g. (0.10 mole) of anhydrous aluminum chloride dissolved in 150 ml. of dry tetrahydrofuran. After the resulting mixture was refluxed for twenty-four hours, the tetrahydrofuran was distilled from the reaction mixture and water was added at such a rate as to keep the volume constant. The aluminum salt of the tetrazole that had separated from the aqueous solution was filtered and dried. The filtrate was acidified and on cooling in the refrigerator overnight the small amount of tetrazole which precipitated was filtered, washed with water, and dried.

The aluminum salt of the tetrazole was finely ground and suspended in 175 ml. of water and 20 ml. of concentrated hydrochloric acid. The tetrazole was filtered, washed with cold water, and dried. The combined products weighed 23.0 g. (77% of theory). Recrystallization from benzene gave a melting point of 137.5-138° C.

Analysis.

Calculated for $C_{15}H_{19}N_5O_4$: C, 54.04; H, 5.75; N, 21.01.

Found: C, 53.99; H, 5.64; N, 20.93.

(d) D, L-α-Acetamido-α-(5-tetrazolyl)-β-(ρ-methoxyphenyl)-propionic acid

D, L-Ethyl-α-acetamido-α-(5-tetrazolyl)-β-(ρ-methoxyphenyl)-propionate (5.0 g., 0.015 mole) was added to 48 ml. of water containing

2.4 g. (0.06 mole) of sodium hydroxide and the mixture refluxed for one hour. The resulting solution was filtered, the filtrate cooled, and acidified to Congo red. The mixture was cooled overnight in the refrigerator and the precipitate which formed was filtered and dried. The crude acid had a melting point of 109-111° C. with gas evolution, solidified, and remelted at 162-163° C. Recrystallization attempts with ethyl acetate-petroleum ether caused a lowering of the melting point. Further attempts at recrystallization gave a product having a melting point of 155-157° C. which was shown to be identical with D, L-5-[α-acetamido-β-(ρ-methoxyphenyl)ethyl]tetrazole by mixture melting point and infrared spectrum.

In view of the difficulty encountered in recrystallization of the material, apparently due to decarboxylation, a sample of crude product was submitted for analysis after drying at room temperature in a desiccator over P_2O_5.

<u>Analysis</u>.

Calculated for $C_{13}H_{15}N_5O_4 \cdot 2H_2O$: N, 20.52; H_2O, 11.43.

Found: N, 20.56; H_2O, 11.34.

The amount of water of hydration in the crude product was determined by drying a sample to constant weight at 78° C. under reduced pressure over phosphorus pentoxide. The dried material melted at 104° C.

(e) <u>D, L-5-[α-Acetamido-β-(ρ-methoxyphenyl)ethyl]tetrazole</u>

D, L-α-Acetamido-α-(5-tetrazolyl)-β-(ρ-methoxyphenyl)propionic acid (2.0 g., 0.0066 mole) was heated in an oil bath at 140° C. with gas evolution for fifteen minutes. The residue weighing 1.7 g. (100% of theory) had a melting point of 162-163° C. Recrystallization from aqueous ethanol resulted in a melting point of 156-157° C.

Analysis.

Calculated for $C_{12}H_{15}N_5O_2$: C, 55.16; H, 5.79; N, 26.81.

Found: C, 55.25; H, 5.97; N, 26.89.

(f) D, L-5-[α-Amino-β-(ρ-methoxyphenyl)ethyl]tetrazole

D, L-Ethyl-α-acetamido-α-(5-tetrazolyl)-β-(ρ-methoxyphenyl)-propionate (18.0 g., 0.0541 mole) was refluxed with 200 ml. of concentrated hydrochloric acid for three hours. The solution was evaporated almost to dryness, then dissolved in 100 ml. of water. The solution was then filtered and the filtrate adjusted to pH 6 with concentrated ammonium hydroxide. The precipitate was filtered and dried yielding 9.2 g. (78% of theory) of product, m.p. 273° C. with decomposition. Recrystallization from water raised the melting point to 298° C. with decomposition.

Analysis.

Calculated for $C_{10}H_{13}N_5O$: C, 54.78; H, 5.98; N, 31.95.

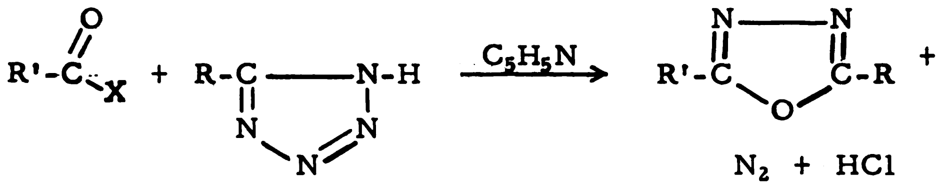
Found: C, 54.73; H, 6.05; N, 32.22.

PART II

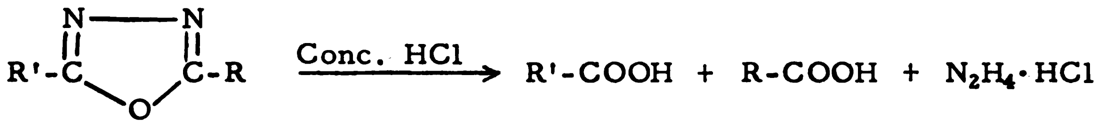
PROOF OF STRUCTURE AND CONFIGURATION OF TETRAZOLE ANALOGS OF AMINO ACIDS

DISCUSSION

Huisgen (34) and co-workers found that 5-substituted tetrazoles would react, in the presence of pyridine, with a variety of acyl halides to form 2, 5-disubstituted 1, 3, 4-oxadiazoles.

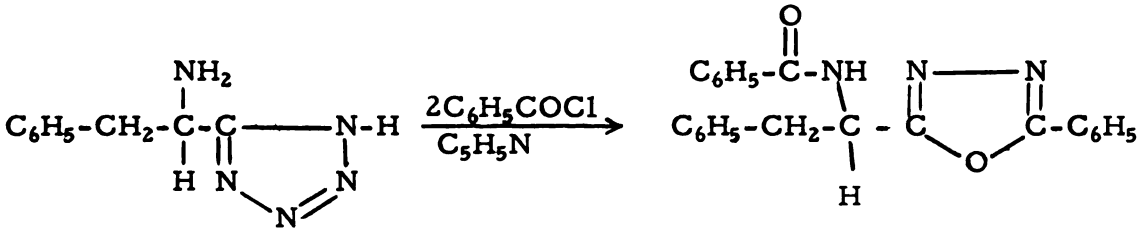


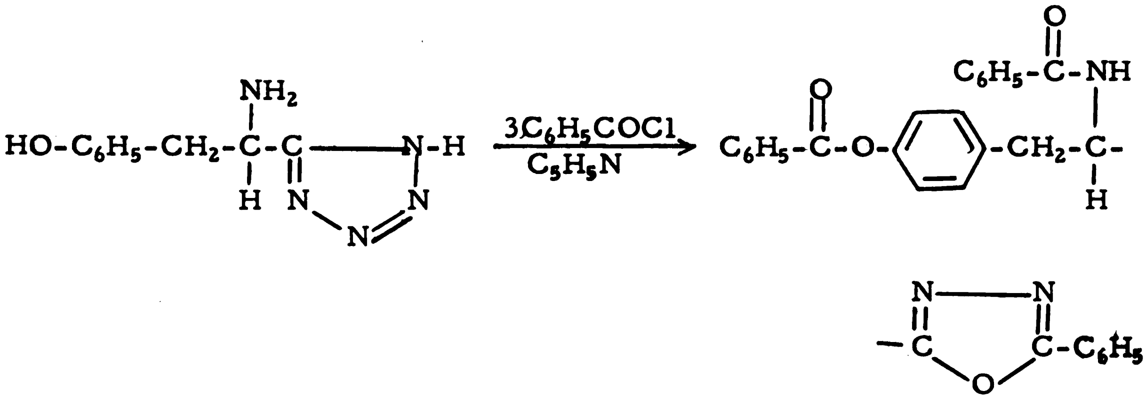
The oxadiazole was then degraded by refluxing with concentrated hydrochloric acid.



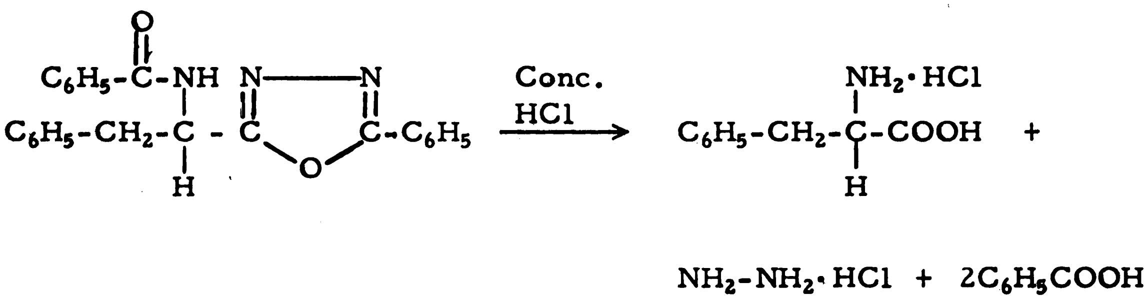
It therefore appeared fruitful to synthesize the oxadiazoles from the tetrazole analogs of the amino acids prepared in Part I. Their subsequent hydrolysis would give the original amino acids used as starting materials. In the case of the optically active amino acids, this technique would be a method to prove their configuration, since the amino acids recovered from the tetrazoles should have the same rotation as the original amino acids.

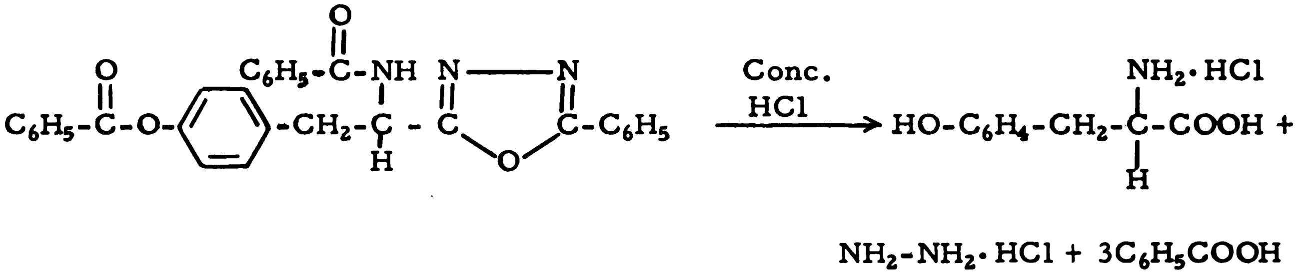
Formation





Hydrolysis

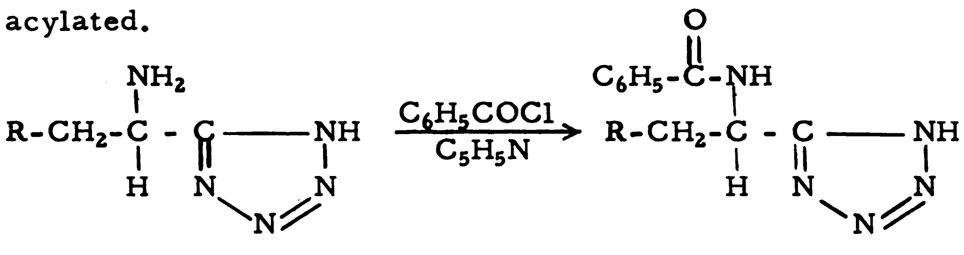




The amino acids obtained on hydrolysis of the oxadiazoles prepared from the tetrazole analog of L-phenylalanine and L-tyrosine did have the same sign of rotation indicating that the tetrazole analogs were of the L-configuration, but their rotations were not of the same magnitude as those of the original amino acids. It appeared that during the formation of the oxadiazole, some racemization may have occurred. It is known that in the presence of base, the hydrogen at the asymmetric carbon of optically active acylated amino acids is labile and racemerization can occur (7). A similar situation could account for the partial racemerization of the optically active tetrazoles or oxadiazoles in pyridine. The melting points of the recrystallized amino acids, obtained on hydrolysis of the oxadiazoles, were lower than both the L and D, L amino acids. However, their infrared spectra were identical with those obtained from authenic samples of L-phenylalanine and L-tyrosine.

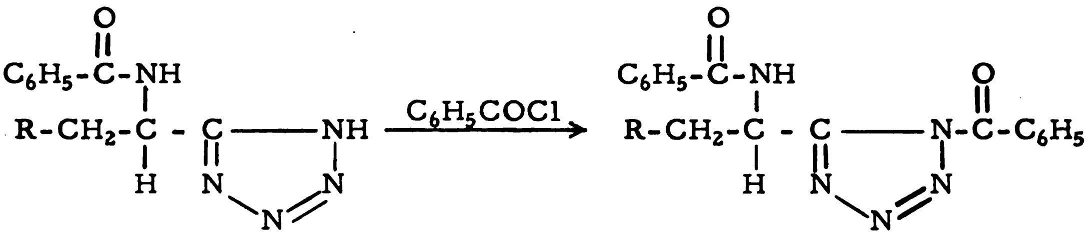
Stolle (33) and Huisgen (34) have proposed mechanisms for the formation of 2, 5-disubstituted 1, 3, 4-oxadiazoles from 5-substituted tetrazoles. Stolle's mechanism was proposed to explain the formation of 2-acetamido-5-methyl-1, 3, 4-oxadiazole from the interaction of acetic anhydride with 5-aminotetrazole. It is based on the assumption that acylation occurs at the one position of the tetrazole ring. These mechanisms, as adapted to the formation of 2, 5-disubstituted 1, 3, 4-oxadiazoles from tetrazole analogs of phenylalanine and tyrosine, are as follows.

In the first step of Stolle's mechanism the amino group is acylated.

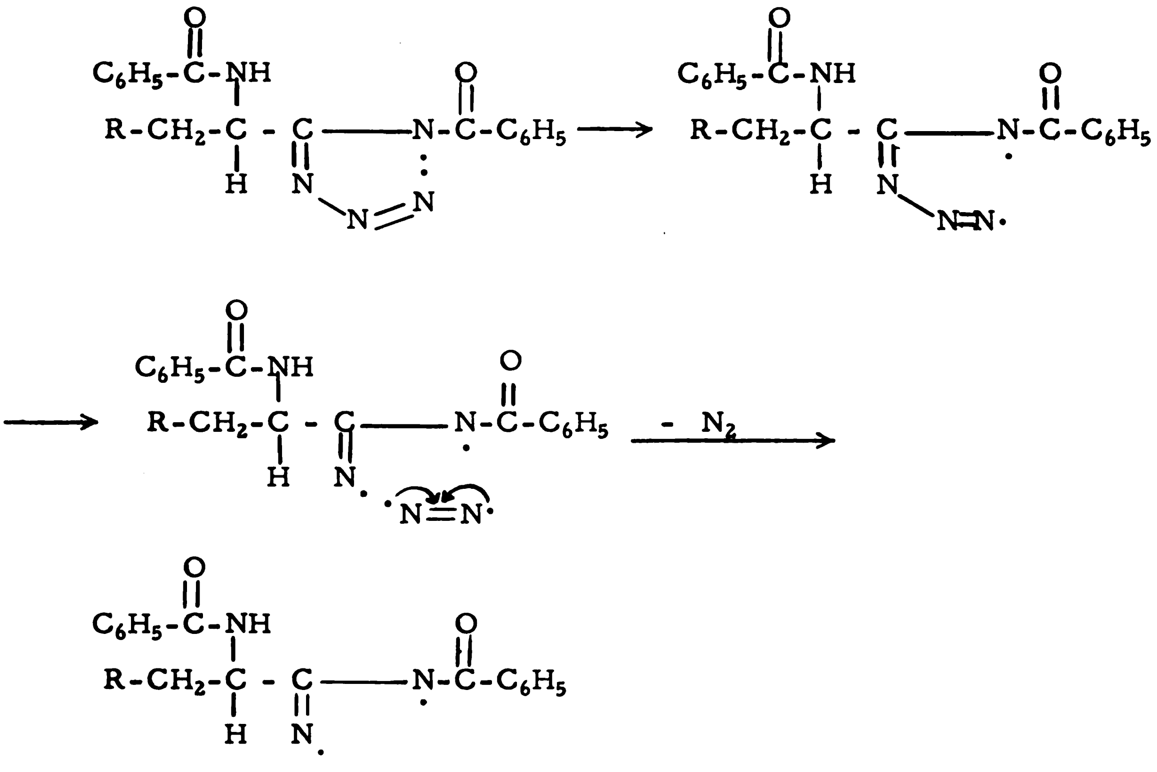


R: $-C_6H_5$, $-C_6H_4OH$

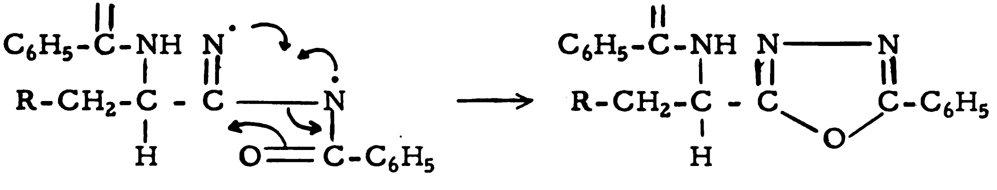
This is followed by acylation at the one position of the tetrazole ring by a second molecule of benzoyl chloride.



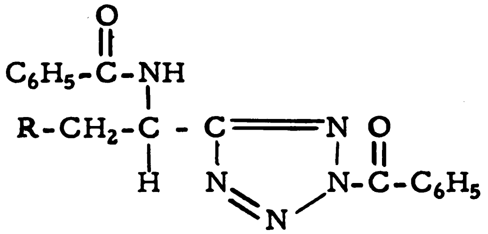
Next, a diradical would result from the rupture of the bond between the number one and two nitrogens followed by the elimination of number two and three nitrogens as a nitrogen molecule.



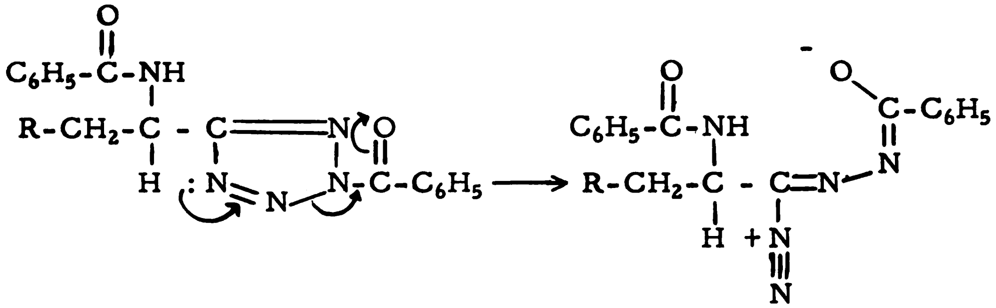
This nitrogen diradical would then give rise to the 1, 3, 4-oxadiazole.



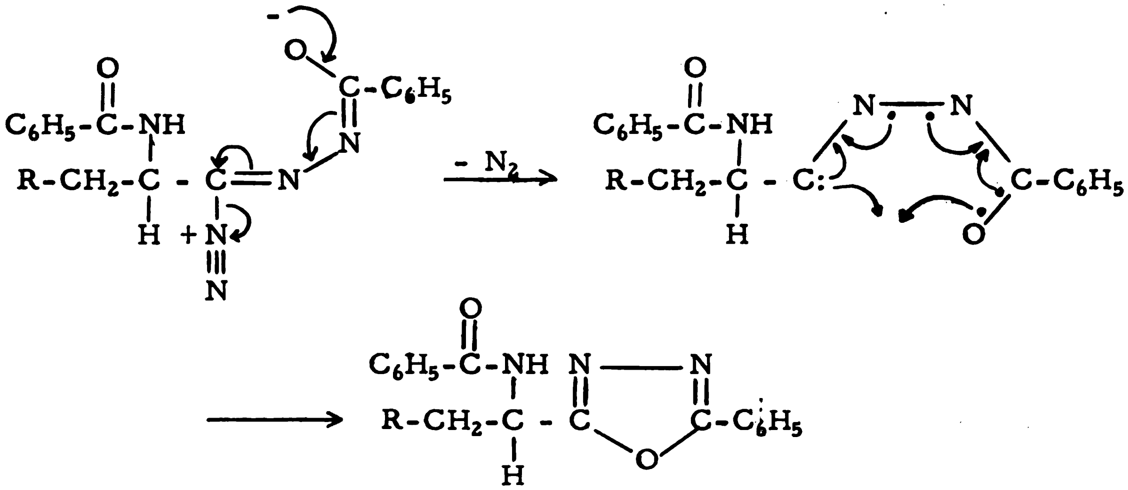
The mechanism proposed by Huisgen (34) assumes acylation takes place at the number two nitrogen.



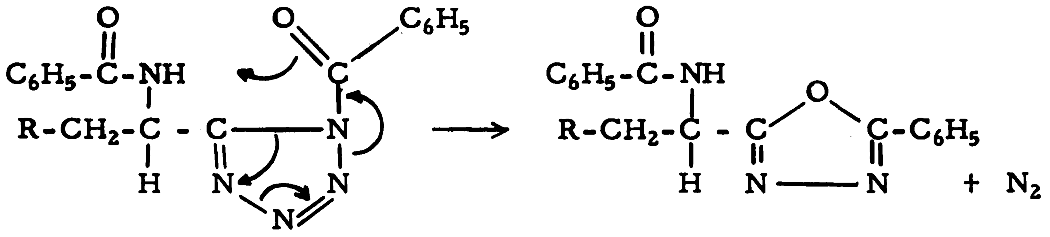
Bond cleavage occurs between the number two and three nitrogens and this is followed by elimination of a molecule of nitrogen from the number three and four nitrogens.

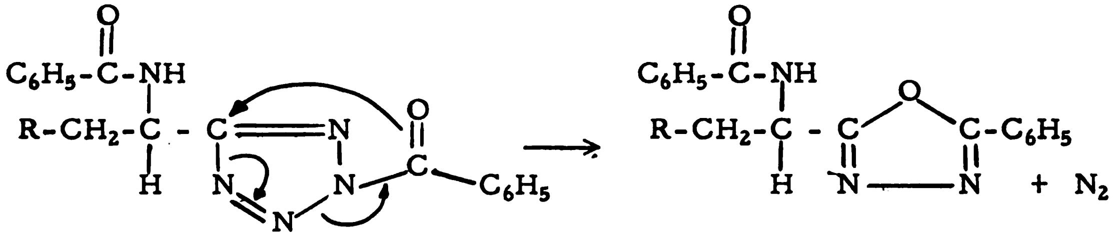


Elimination of nitrogen, formation of the carbine, and cyclization would give rise to the oxadiazole.

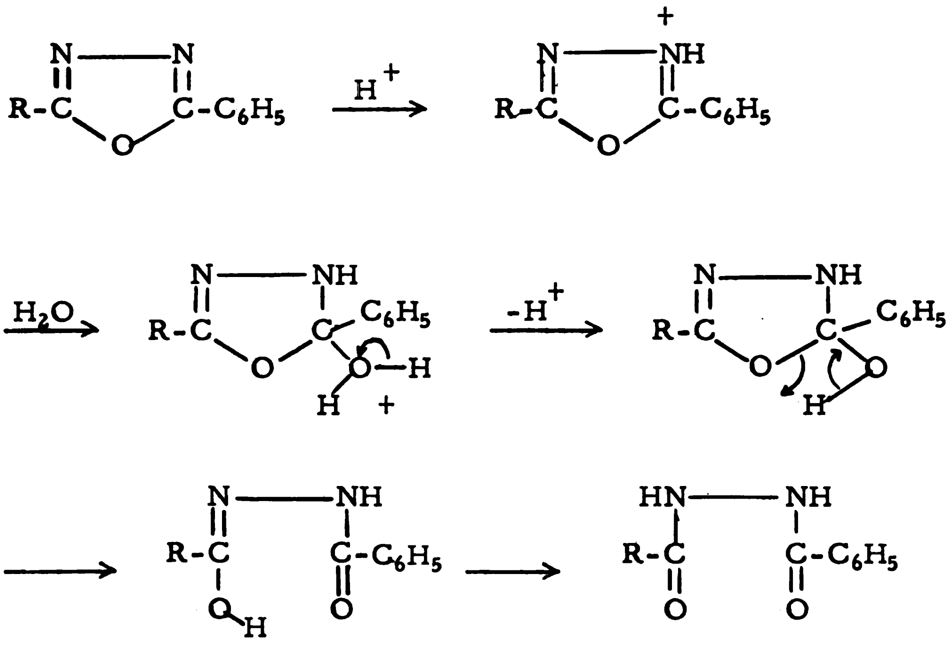


A concerted mechanism can be postulated for the formation of oxadiazole from tetrazoles, acylated at either the one or two positions.

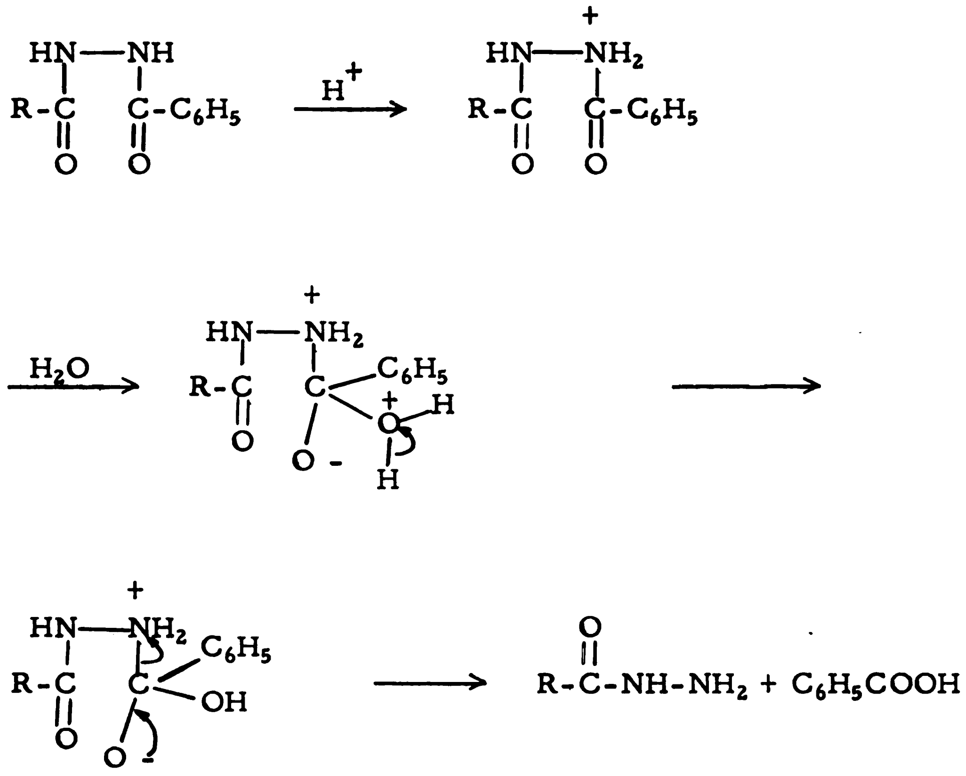




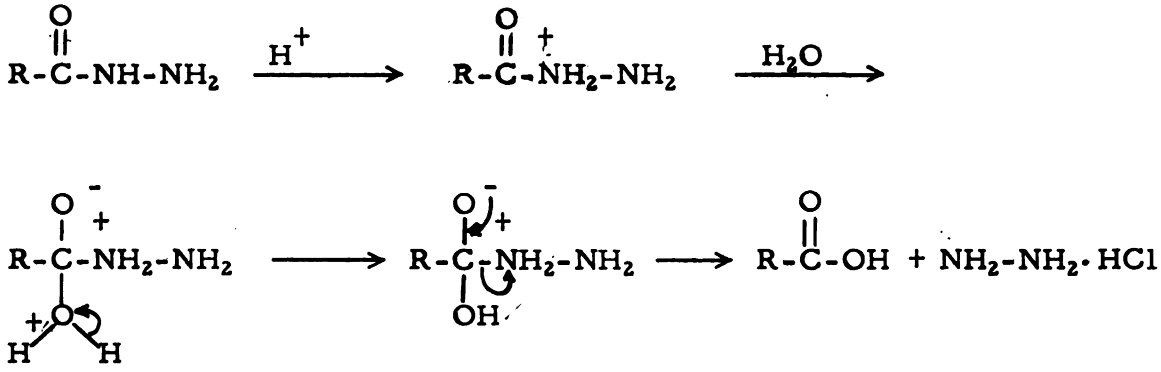
The degradation of 2-acylaminoalkyloxadiazoles with concentrated hydrochloric acid to give amino acid hydrochlorides, may proceed in the following manner. Protonation of the nitrogen, attack of a water molecule, followed by elimination of a hydrogen ion would give rise to a diacylhydrazine.



A repetition of the same sequence of reactions as previously outlined would result in the formation of the hydrazide and benzoic acid.



Protonation of the hydrazide with subsequent attack by water would give rise to hydrazine hydrochloride and the amino acid hydrochloride.



EXPERIMENTAL[1]

A. Synthesis of 2-Substituted-5-Phenyl-1,3,4-Oxadiazoles From Tetrazole Analogs of Amino Acids

D,L-5-(α-Amino-β-phenylethyl)tetrazole

(D, L-Phenylalanine analog)

(a) D,L-5-(α-Amino-β-phenylethyl)tetrazole

This compound was prepared from D,L-phenylalanine by converting the amino acid to α-phthalimido-β-phenylpropionitrile. The nitrile was then converted to D,L-5-(α-amino-β-phenylethyl)tetrazole according to the procedure described in Part I. The crude product was recrystallized from water after which it melted at 278° C. with decomposition.

(b) D,L-2-(1'-Benzamido-2'-phenylethyl)-5-phenyl-1,3,4-ozadiazole

A mixture of 3.78 g. (0.02 mole) of D,L-5-(α-amino-β-phenylethyl)-tetrazole, 5.60 g. (0.04 mole) of benzoyl chloride, 16 ml. (0.2 mole) of pyridine, and 25 ml. of dry benzene was refluxed for ten hours. A small amount of oxadiazole precipitated from the benzene solution. The solution was evaporated to dryness and the residue recrystallized several times from absolute ethanol. Six grams (81% of theory) of oxadiazole was collected, m.p. 167.5-168° C.

Analysis

Calculated for $C_{23}H_{19}N_3O_2$: C, 74.78; H, 5.18; N, 11.38.

Found: C, 74.78; H, 5.16; N, 11.30.

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[1] All infrared spectra taken as Nujol mulls.

L-5-(α-Amino-β-phenylethyl)tetrazole

(L-Phenylalanine analog)

(a) L-5-(α-Amino-β-phenylethyl)tetrazole

The procedure used for the preparation of L-5-(α-amino-β-phenylethyl)tetrazole was the same as that used for the preparation of the D, L-5-(α-amino-β-phenylethyl)tetrazole. The melting point of pure tetrazole was 287° C. with decomposition, $[\alpha]_D^{25} = +38.0°$, C = .945 in H_2O.

(b) L-2-(1'-Benzamido-2'-phenylethyl)-5-phenyl-1, 3, 4-oxadiazole

A mixture of 1.50 g. (0.008 mole) of L-5-(α-amino-β-phenylethyl)-tetrazole, 2.53 g. (0.018 mole) benzoyl chloride, 6.4 ml. of pyridine, and 25 ml. of benzene was refluxed for three hours. The solution was evaporated to dryness and the residue recrystallized from absolute ethanol, m.p. 191-192° C., $[\alpha]_D^{25} = -14.9°$, C = 5.0 in chloroform.

Analysis.

Calculated for $C_{23}H_{19}N_3O_2$:	C, 74.78; H, 5.18; N, 11.38.
Found:	C, 75.03; H, 5.28; N, 11.36.

L-5-[α-Amino-β-(ρ-hydroxyphenyl)ethyl]tetrazole

(L-Tyrosine Analog)

(a) L-5-[α-Amino-β-(ρ-hydroxyphenyl)ethyl]tetrazole

This compound was prepared as described in Part I by converting L-tyrosine to the N-benzoyl nitrile and subsequently to the tetrazole followed by removal of the protecting benzoyl group by hydrolysis. The pure tetrazole had a melting point of 302° C. with decomposition, $[\alpha]_D^{25} = +33.8°$, C = 4.0 in 2 N hydrochloric acid.

(b) L-2-[1'-Benzamido-2'-(ρ-benzoyloxyphenyl)ethyl]-5-phenyl-1, 3, 4- oxadiazole

A mixture of 2.05 g. (0.01 mole) of L-5-[α-amino-β-(ρ-hydroxyphenyl)ethyl]tetrazole, 4.60 g. (0.033 mole) benzoyl chloride, 8 ml. of pyridine, and 30 ml. of dry benzene was refluxed for five hours. The solution was then evaporated to dryness and the residue washed with cold absolute ethanol. The product was insoluble in sodium hydroxide. The yield of oxadiazole was 3.8 g. (80% of theory), m.p. 202-203° C. after recrystallization from absolute ethanol. $[\alpha]_D^{25} = -19.8^\circ$, C = 2.0 in methylene chloride.

Analysis.

Calculated for $C_{30}H_{23}N_3O_4$: C, 73.61; H, 4.74; N, 8.58.

Found: C, 73.73; H, 4.87; N, 8.74.

B. Acid Hydrolysis of 2-Substituted-5-Phenyl-1,3,4-Oxadiazoles as Proof of Structure and Configuration

(a) Hydrolysis of D,L-2-(1'-Benzamido-2'-phenylethyl)-5-phenyl-1,3,4-oxadiazole

Three grams (0.0082 mole) of D,L-2-(1'-benzamido-2'-phenylethyl)-5-phenyl-1,3,4-oxidiazole was added to 25 ml. of concentrated hydrochloric acid and the mixture refluxed for twelve hours. The clear solution was cooled to 0° C. and the precipitate collected on a filter. The solid weighed 1.95 g. (98% of theory) and had a melting point of 119-120° C. Recrystallization from water raised the melting point to 120-121° C. and a mixture melting point with an authenic sample of benzoic acid showed no depression. The filtrate was then evaporated to dryness, the residue dissolved in 25 ml. of water, and the resulting solution neutralized to pH 6 with concentrated ammonium hydroxide. The precipitate was filtered, dried, and weighed. The yield of D,L-phenylalanine was 1.35 g. (80% of theory), m.p. 310-314° C. with decomposition. Its infrared spectrum was identical with that of an authenic sample of D,L-phenylalanine.

(b) Hydrolysis of L-2-(1'-benzamido-2'-phenylethyl)-5-phenyl-1,3,4-oxadiazole

L-2-(1'-benzamido-2'-phenylethyl)-5-phenyl-1,3,4-oxadiazole (2.0 g., 0.0054 mole) was suspended in 25 ml. of concentrated hydrochloric acid and refluxed for twelve hours. The clear solution was cooled to 0° C. and the precipitate collected on a filter. The solid weighed 625 mg. (95% of theory), m.p. 117-119° C. Recrystallization from water raised the melting point to 119-120° C., and when mixed with authenic benzoic acid, no depression of the melting point was observed. The filtrate was evaporated to dryness, the residue was dissolved in 25 ml. of water, and the solution was neutralized to pH 6 with concentrated ammonium hydroxide. After cooling overnight in the refrigerator, the precipitate was collected on a filter, dried, and weighed. The yield of L-phenylalanine, m.p. 234° C. with decomposition, was 615 mg. (69% of theory). After three recrystallizations from water, the melting point was raised to 254° C. with decomposition, $[\alpha]_D^{25} = -4.1^\circ$, C = 1.096 in water. Its infrared spectrum was identical with authenic L-phenylalanine.

(c) Hydrolysis of L-2-[1'-benzamido-2'-(ρ-benzoyloxyphenyl)-ethyl]-5-phenyl-1,3,4-oxadiazole

To 2.4 g. (0.0049 mole) of L-2-[1'-benzamido-2'-(ρ-benzoyloxyphenyl)ethyl]-5-phenyl-1,3,4-oxadiazole was added 50 ml. of concentrated hydrochloric acid and the mixture refluxed for forty-eight hours. The clear solution was cooled to 0° C. and the resulting precipitate collected on a filter, washed with cold water, and dried. The solid weighed 1.78 g. (98% of theory) and melted at 118-119° C. After recrystallization from water, the melting point was raised to 120-121° C. There was no depression in melting point when the product was mixed with an authenic sample of benzoic acid. The filtrate was evaporated to dryness, the residue dissolved in 50 ml. of water, and the pH of the solution adjusted to 6 with concentrated ammonium hydroxide. The precipitate was filtered, washed with cold water, and dried. The yield of L-tyrosine was 775 mg. (82% of theory), m.p. 269-272° C. with decomposition. After recrystallization from water, the melting point was 285-287° C. with decomposition, $[\alpha]_D^{25} = -6.9°$, C = 3.54 in 1 N hydrochloric acid. Its infrared spectrum was identical with that of an authenic sample of L-tyrosine.

PART III

OPTICAL ROTATION AND pK DETERMINATIONS OF TETRAZOLE ANALOGS OF AMINO ACIDS

DISCUSSION

Many of the biological properties attributed to amino acids are related to their physical properties. Two of these physical properties, which play an important role, are the amphoteric nature of amino acids, and the fact that all naturally occurring amino acids, except glycine, are optically active.

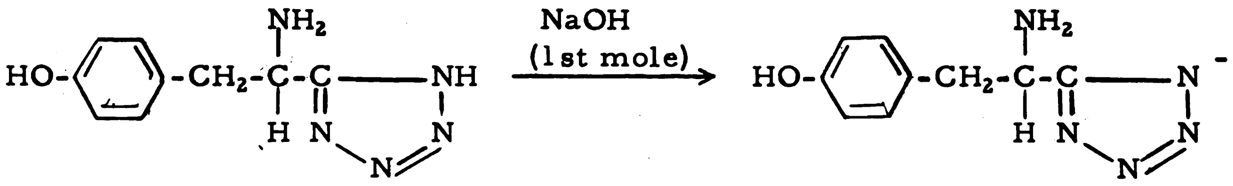
One of the most important influences, which affect the degree of optical activity of amino acids, is the acidity or alkalinity of the solution. The influence of pH on the rotation of optically active electrolytes, with the resulting changes in dissociation, has been extensively studied. Usually the specific rotation of the neutral molecule differs from that of the free ions and in some instances it has been possible to correlate the rotation curves with the dissociation constants of the substance.

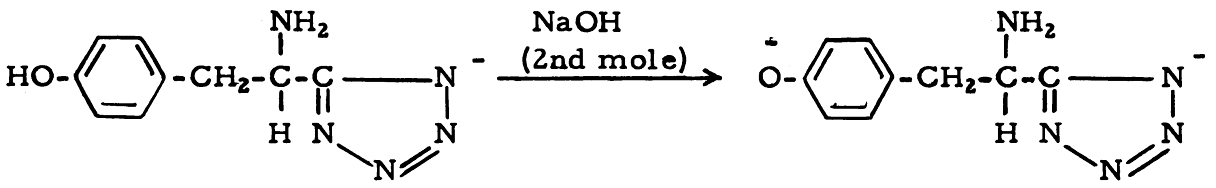
Lutz and Jirgensons (20, 21) determined the specific rotations of a series of naturally occurring amino acids dissolved in predetermined amounts of acid and base. From a plot of the specific rotation and the moles of acid or base, Lutz and Jirgensons predicted the configuration of the amino acids by examining the acid branch of the optical rotation curve. If, with increasing acid concentration, it was found that the rotation changed toward the positive direction, the amino acid was assigned the L-configuration. If the rotation became more negative, the D-configuration was assigned to the amino acid. In this way configurations were assigned to naturally occurring glutamic acid, arginine, lysine, ornithine, histidine, alanine, tyrosine, aspartic adid, leucine, tryptophane, proline, hydroxyproline, and dioxyphenylalanine.

In an effort to apply this theory to the tetrazole analogs of L-phenylalanine and L-tyrosine, their rotations were determined in specified amounts of acid and base; the results are summarized in Tables 3 and 4. A plot of the specific rotation against the amounts of acid and base was made and represented in Figures 18 and 19. For comparison, the rotations of L-phenylalanine and L-tyrosine in specified amounts of acid and base were also determined; the results summarized in Tables 1 and 2. Figures 16 and 17 represent the plot of the specific rotation against the amounts of acid and base for the amino acids.

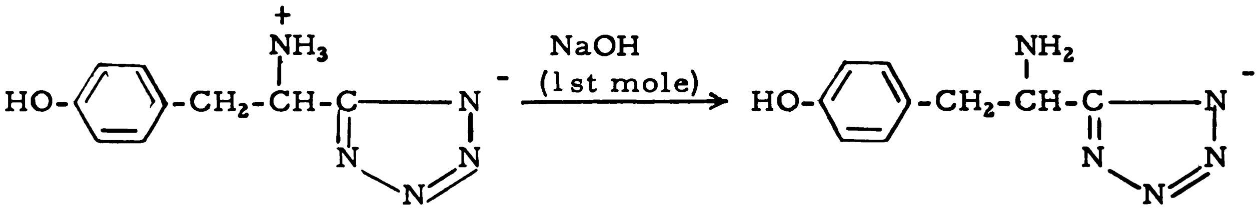
Several intrinsic difficulties were encountered. Tyrosine has a very low water solubility and the measurement of its rotation in water was not possible. Also, tyrosine was only incompletely soluble in less than equal molar quantities of acid or base, so that its rotation under these conditions could not be determined.

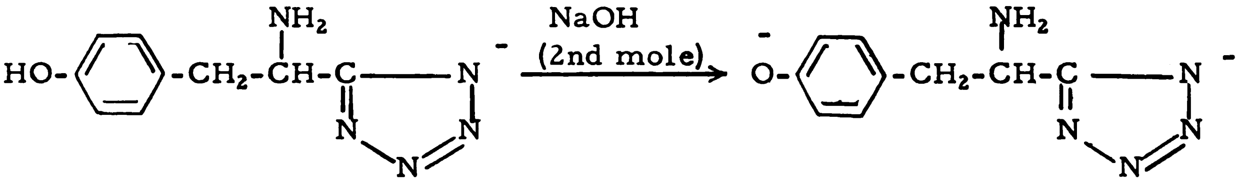
From the characteristic features of the curves, some conclusions were drawn. The curves of the tetrazole analogs of L-phenylalanine and L-tyrosine were essentially of the same shape, having a maximum dextro rotation in water and having a minimum dextro rotation in the acid portion, when the molar ratio of tetrazole to acid was 1 to 1. However, in the base portion of the curve, the tetrazole analog of phenylalanine reached a minimum dextro rotation when the molar ratio of tetrazole to base was 1 to 1, while in the case of the tetrazole analog of tyrosine, the minimum dextro rotation was reached when the molar ratio of tetrazole to base was 1 to 2. This last observation is anomalous, since the second mole of base would be expected to react with the phenol to form the phenoxide anion, which is removed from the asymmetric center and shouldn't affect the rotation appreciably.





If written as the dipolar ion, the overall result is the same.





The curves obtained from the plot of the specific rotations of L-phenylalanine and L-tyrosine against the moles of acid and base were similar to those of Lutz and Jirgensons. A maximum levo rotation was observed for L-phenylalanine in water, and the solutions became less levo rotatory to the point in the acid portion of the curve, where the molar ratio of amino acid to acid was 1 to 1. Thereafter, the levo rotation decreased slightly with increased acid concentration. This is consistent with the theory of Lutz and Jirgensons for L-amino acids. In the base portion of the curve for L-phenylalanine, a decrease in levo rotation was also observed from the maximum levo rotation in water, to the point where the ratio of amino acid to base was 1 to 1. Thereafter, the rotation remained constant with increasing base concentration.

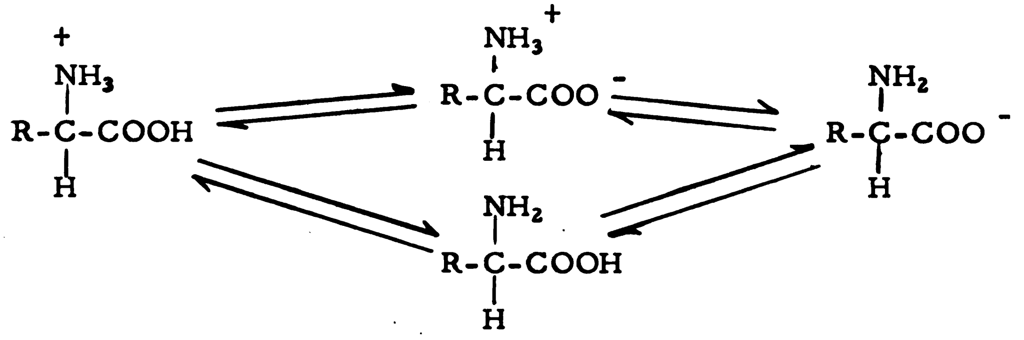
The curve for L-tyrosine was essentially of the same pattern as that of L-phenylalanine except that the minimum levo rotation in base was reached when the molar ratio of sodium hydroxide to tyrosine was 2 to 1.

In comparing the rotation curves for L-phenylalanine and L-tyrosine with their tetrazole analogs, an inverse relationship was noted. A maximum levo rotation was observed for the amino acids in water solutions, while a maximum dextro rotation for water solutions of the tetrazole analogs was noted. Lutz and Jirgensons used the characteristic change in rotation toward the positive direction in the acid portion of the curves, to assign optically active amino acids to the L-configuration. It appears that the characteristic change in rotation toward the negative direction in the acid portion of the curves of the tetrazole analogs, can be used to assign them to the L-configuration.

Conversion of the tetrazole analogs of L-phenylalanine and L-tyrosine to the oxadiazoles, and the subsequent degradation to the amino acids established that the tetrazole analogs belong to the L-series.

The amphoteric nature of amino acids and proteins account for many of their biological properties. The ability of an amino acid or protein to act as both a proton donor and a proton acceptor has long been recognized.

In the case of simple amino acids, that is, those that have one carboxyl group and one amino group, the ionization is as follows:



However, for the polyvalent ampholytes such as tyrosine, the ionization scheme is more complex. Recently Edsall and co-workers (22) determined the complete ionization scheme for tyrosine as illustrated in Figure 1. Since each of the three functional groups can exist in either the ionized form, or the unionized form, there are eight possible forms for tyrosine in going from the protonated form to the dianion.

The ionization constants were determined by two different methods; spectrophotometric measurements and calculation from "microconstants." These microconstants are the individual interrelationships between the eight forms of tyrosine in Figure 1. The carboxyl group is by far the strongest acidic group and the ionic form of the amino and the hydroxyphenyl groups do not affect K_1. Therefore, it was determined by direct titration. However, since the amino and hydroxyphenyl groups are of comparable acidity, the ionization of one will be dependent on the form of the other. The distribution of charges will affect the intensity of electrostatic interactions between the two groups. The pK values for the model compounds O-methyl-tyrosine, tyrosine ethyl ester, tyrosine amide, and phenylalanine were determined by titration, and by use of mathematical treatments, the microconstants for the ionization of tyrosine were calculated. The pK_2 and pK_3 values for tyrosine were calculated from these microconstants. These values were found to be in close agreement to those obtained by Winnek and Schmidt (26).

The tetrazole analogs of the amino acids are also amphoteric and would be expected to exhibit the same equilibria as their amino acid counterparts. In the case of the tetrazole analog of L-phenylalanine, the ionization is simple and is represented in Figure 2. The tetrazole analog of tyrosine presents a more complex case. However, it was noted that the ammonium groups of the tetrazole analogs of amino acids were ten times more acidic than those of the corresponding amino acids (3).

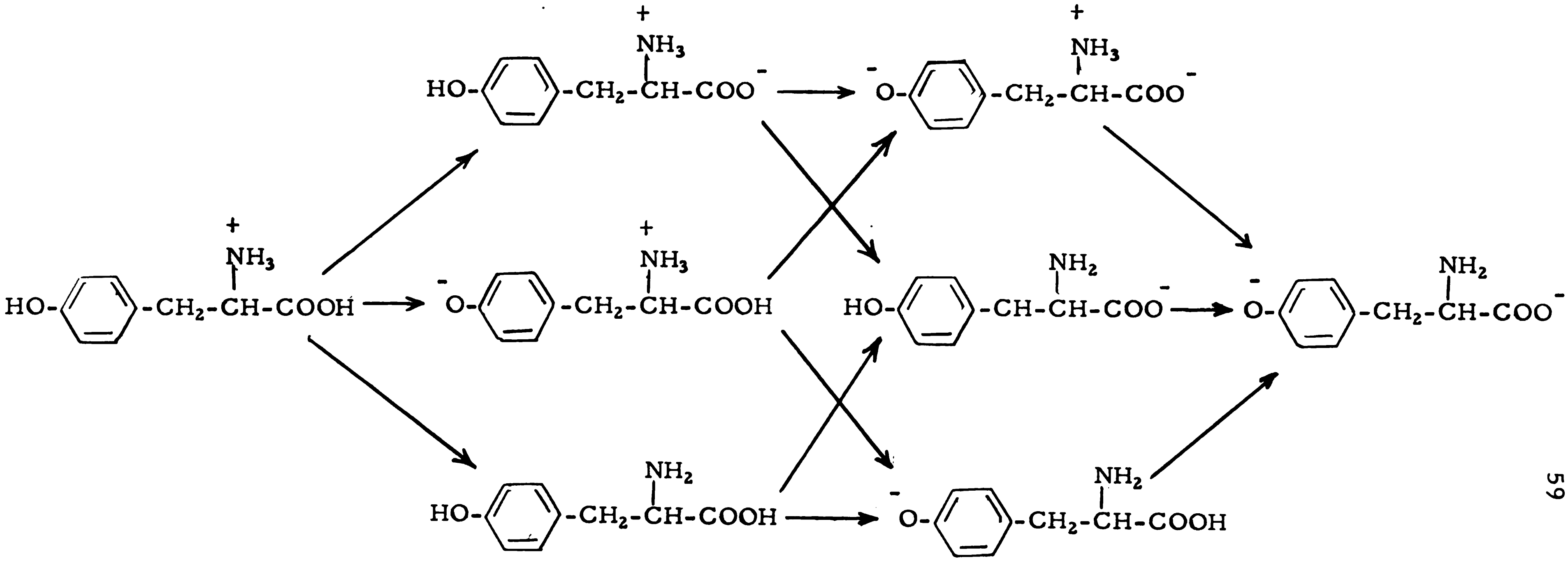


Figure 1. Complete Ionization Scheme for Tyrosine.

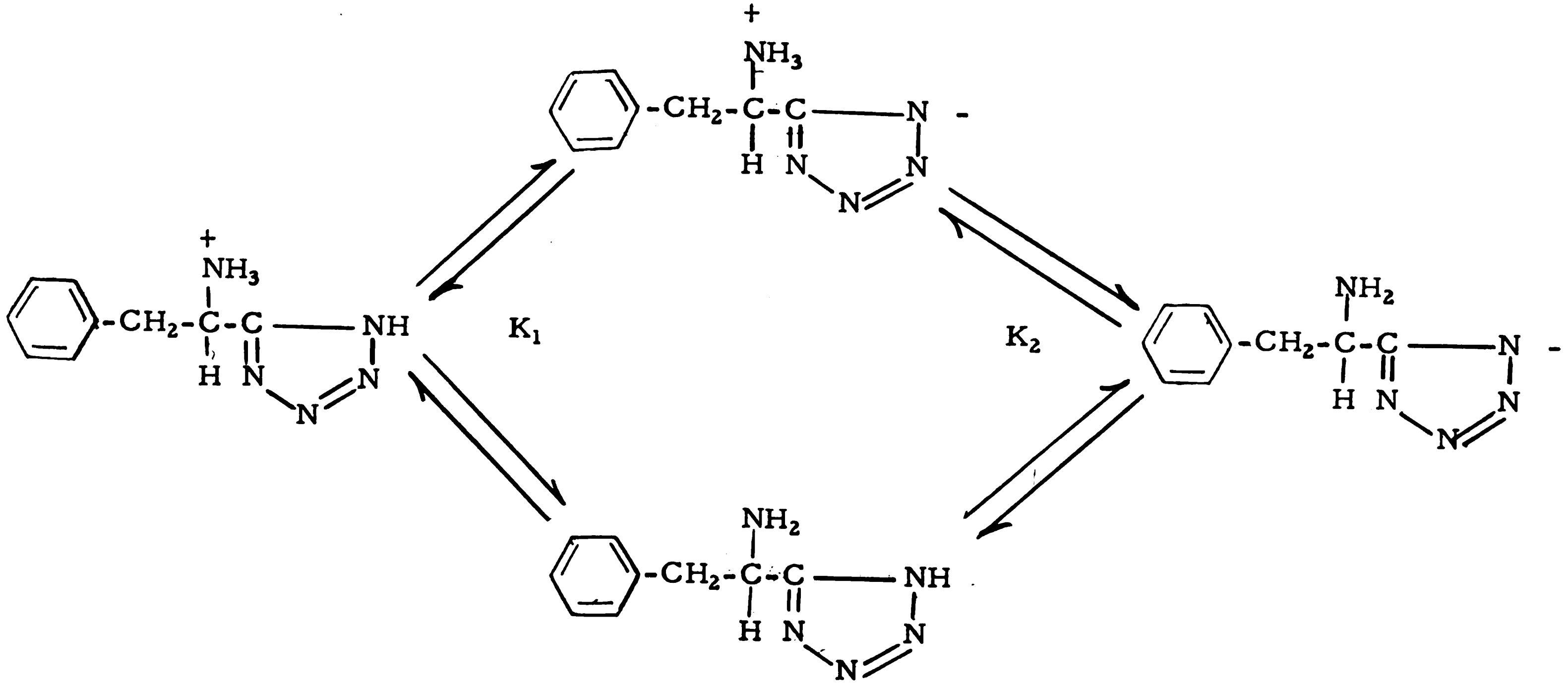


Figure 2. Ionization Scheme of the Tetrazole Analog of L-Phenylalanine.

Therefore, the form of one group would have less of an effect on the ionization of the other. The ionization scheme for the tetrazole analog of tyrosine is represented in Figure 3.

The pK values for the tetrazole analogs of L-phenylalanine, L-tyrosine, and O-methyltyrosine were determined by titration of these compounds in aqueous solution with standard solutions of hydrochloric acid and sodium hydroxide. A summary of the results, including a comparison of the pK values for the corresponding amino acids, is given in Table 11. The data for the titration of each amino acid and its titration curve are found in Part III of the Experimental.

An examination of Table 11 shows that there is a close agreement in the pK values for the amino acids and their tetrazole analogs. This comparison is facilitated by taking the antilogarithm of the difference in the pK value between the amino acid and the corresponding tetrazole, and is a measure of the relative strength of the acidity of the two species. For example, K_1 for L-phenylalanine, which is a measure of the acidity of the carboxyl group, is about 10 times larger than K_1 for the tetrazole analog of L-phenylalanine, where K_1 is a measure of the acidity of the tetrazole group. Similarly, K_2, which is a measure of the acidity of the ammonium group in both compounds, is about 11.5 times larger for the tetrazole analog than for L-phenylalanine. The hydroxyphenyl group of the tetrazole analog is slightly more acidic than that of the amino acid as evidenced by a comparison of the pK_3 values.

The acidity of the carboxyl group would be expected to be greater than that of the tetrazole based on the comparative electronegativities of oxygen and nitrogen. Since the tetrazolyl group is a weaker acid than the carboxyl, the tetrazolate anion is a stronger base than the carboxylate anion, and it exerts a stronger electron withdrawal effect on the ammonium group. This would account for the higher acidity of

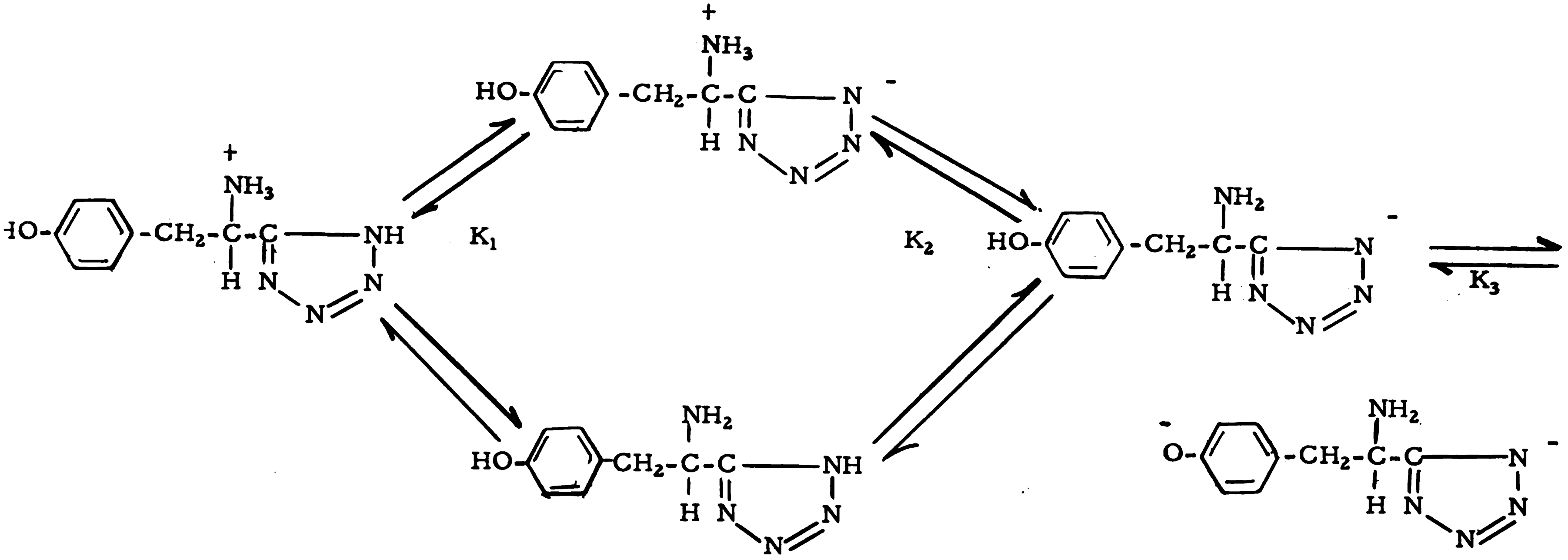


Figure 3. Ionization Scheme for the Tetrazole Analog of L-Tyrosine.

the ammonium moiety in the tetrazole analog as compared to the amino acid. Also, the slightly higher acidity of the hydroxyphenyl group of the tetrazole analog of tyrosine could be explained by the increased electron withdrawal effect of the tetrazolate anion compared to the carboxylate anion.

The reason for the greater acidity of phenylalanine and its tetrazole analog as compared to tyrosine and O-methyltyrosine and their tetrazole analogs is not so apparent. Perhaps the answer may be that the electron release effect of the hydroxyphenyl and methoxyphenyl groups increase slightly the proton affinity of the ammonium and carboxyl groups, thereby making them less acidic (49).

EXPERIMENTAL

A. Specific Rotations of L-Phenylalanine, L-Tyrosine, and Their Tetrazole Analogs in Various Concentrations of Acid and Base[1]

(a) L-Phenylalanine

Samples of L-phenylalanine[2] (1.65 g., 0.01 mole) were dissolved in predetermined amounts of 1 M sodium hydroxide and 1 M hydrochloric acid, and the rotation of the solutions determined. Specific rotations calculated for each concentration of acid and base are given in Table 1.

(b) L-Tyrosine

Samples of L-tyrosine[3] (0.905 g., 0.005 mole) were dissolved in predetermined amounts of 1 M sodium hydroxide and 1 M hydrochloric acid and the rotation of each solution was determined. Specific rotations calculated for each concentration of acid and base are given in Table 2.

(c) L-5-(α-Amino-β-phenylethyl)tetrazole
(L-Phenylalanine Analog)

This compound was prepared by converting L-phenylalanine to L-α-phthalimido-β-phenylpropionitrile. The tetrazole was then synthesized from the nitrile according to the procedure outlined in Part I. The pure tetrazole had a melting point of 287° C. with decomposition, $[\alpha]_D^{25} = +38.0^\circ$, C = .945 in H_2O.

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[1] All measurements were carried out on a Rudolph Precision Polarimeter, Model 70, using a 1 dm. tube and sodium light at 24° C.

[2] Nutritional Biochemicals Corp.

[3] Nutritional Biochemicals Corp.

Table 1. Specific Rotations of L-Phenylalanine in NaOH and HCl. Using 1 M NaOH and 1 M HCl at 24° C., 1.65 g. (0.01 mole) of L-phenylalanine.

Moles of Amino Acid	Moles of Acid or Base	α	$[\alpha]_D^{24}$
1.65 g. (0.01 mole) in	100 ml. H_2O	-1.107°	-33.5°
1	0.5 mole NaOH	-1.165°	-17.7°
1	1.0 mole NaOH	-0.190°	-1.44°
1	2.0 moles NaOH	-0.202°	-1.53°
1	4.0 moles NaOH	-0.338°	-2.56°
1	0.5 mole HCl	-1.618°	-24.4°
1	1.0 mole HCl	-1.447°	-11.0°
1	2.0 mole HCl	-1.118°	-8.5°

Table 2. Specific Rotations of L-Tyrosine in NaOH and HCl. Using 1 M NaOH and 1 M HCl at 24° C., 0.905 g. (0.005 mole) of L-Tyrosine.

Moles of Amino Acid	Moles of Acid or Base	α	$[\alpha]_D^{24}$
1	1 mole NaOH	-0.059°	-32.6°
1	2 moles NaOH	-0.949°	-13.8°
1	1 mole HCl	-0.165°	-45.5°
1	2 moles HCl	-0.890	-12.3°
1	4 moles HCl	-0.789	-10.9°

Solutions containing 0.4725 g. (0.0025 mole) of L-5-(α-amino-β-phenylethyl)tetrazole and predetermined amounts of 0.5 M hydrochloric acid and 0.5 M sodium hydroxide were prepared and their rotations determined. The specific rotations calculated for each solution are given in Table 3.

Table 3. Specific Rotations of L-5-(α-Amino-β-phenylethyl)tetrazole in NaOH and HCl (L-Phenylalanine Analog), Using 0.5 M NaOH and 0.5 M HCl at 24°C., 0.4725 g. (0.0025 mole) of L-5-(α-Amino-β-phenylethyl)tetrazole.

Moles of Tetrazole	Moles of Acid or Base	α	$[\alpha]^{24}_{D}$
0.4725 g. (0.0025 mole) in	50 ml. H_2O	+0.717°	+38.0°
1	0.5 mole NaOH	+0.544°	+28.8°
1	1.0 mole NaOH	+0.724°	+19.1°
1	2.0 moles NaOH	+0.733°	+19.4°
1	4.0 moles NaOH	+0.737°	+19.5°
1	0.5 mole HCl	+0.334°	+35.4°
1	1.0 mole HCl	+1.277°	+33.8°
1	2.0 moles HCl	+1.246°	+33.0°
1	4.0 moles HCl	+1.262°	+33.5°

(d) L-5-[α-Amino-β-(ρ-hydroxyphenyl)ethyl]tetrazole (L-Tyrosine Analog)

This compound was prepared by converting L-tyrosine into L-α-benzamido-β-(ρ-benzenesulfonyloxy)phenylpropionitrile and subsequently to its tetrazole as described in Part I. The pure tetrazole had a melting point of 302° C. with decomposition, $[\alpha]^{25}_{D}$ = +33.8°, C = 4.0 in 2 N HCl.

Solutions of 0.5125 g. (0.0025 mole) of L-5-[α-amino-β-(ρ-hydroxyphenyl)ethyl]tetrazole containing predetermined amounts of 0.5 M sodium hydroxide and 0.5 M hydrochloric acid were prepared and their rotations determined. The specific rotations calculated for each solution are given in Table 4.

Table 4. Specific Rotations of L-5-[α-Amino-β-(ρ-hydroxyphenyl)ethyl]-tetrazole in NaOH and HCl (L-Tyrosine Analog). Using 0.5 M NaOH and 0.5 M HCl at 24°C., 0.5125 g. (0.0025 mole) of L-5-[α-Amino-β-(ρ-hydroxyphenyl)ethyl]tetrazole.

Moles of Tetrazole	Moles of Acid or Base	α	$[\alpha]^{24}_{D}$
0.5125 g. (0.0025 mole) in	1000 ml. H_2O	+0.042°	+41.0°
1	0.5 mole NaOH	+0.056°	+27.4°
1	1.0 mole NaOH	+0.610°	+14.9°
1	2.0 moles NaOH	+0.073°	+1.8°
1	4.0 moles NaOH	+0.171°	+4.2°
1	1.0 mole HCl	+0.713°	+34.8°
1	2.0 moles HCl	+1.430°	+34.9°
1	4.0 moles HCl	+1.437°	+35.0°

B. The Determination of the Apparent pK Values of Some Amino Acids and Their Tetrazole Analogs

The apparent pK values of the 5-aminoalkyltetrazoles were determined by titration of weighed samples of the tetrazole in aqueous solution with standard sodium hydroxide and standard hydrochloric acid solutions. The weighed samples were placed in volumetric flasks and dissolved in the specified amounts of water. They were then transferred to a flask and titrated in a thermoregulated bath at 25° ± 1° C. The pH was determined after each addition of titrant with a Beckman pH meter, model H-2.

The pK_1 values were determined by taking the pH of the solution at the point of half neutralization, as calculated from the normality of the acid used as a titrant, and the molecular weight of the tetrazole.

The pK_2 values were determined by taking the pH of the solution at the point of half neutralization, as calculated from the normality of the base used as a titrant, and the molecular weight of the tetrazole.

The pK_3 value for the hydroxyphenyl group of the L-tyrosine analog was determined by taking the pH of the solution at the half neutralization point between the addition of one and two equivalents of base, as calculated from the normality of the base, and the molecular weight of the tetrazole.

The pK values determined in this fashion were in close agreement with those determined by plotting the pH versus the milliliters of titrant, finding the midpoint of the best straight line drawn at the point of neutralization, and noting the pH at the point at which one-half the amount of titrant required for neutralization was added. The titration curves exhibited a pattern similar to that of most amino acids. The results are recorded in Tables 5-10 and shown graphically in Figures 20-25. Table 11 gives comparative values for the amino acids and their tetrazole analogs.

Table 5. **Potentiometric Titration of L-5-(α-Amino-β-phenylethyl)-tetrazole with Hydrochloric Acid (L-Phenylalanine Analog)**

Concentration	pH	Ml. of 0.10156 N HCl	Apparent pK_1
0.140 g./100 ml. of aqueous solution	6.00	0	
	3.88	0.5	2.83
	3.51	1.0	
	3.30	1.5	
	3.18	2.0	
	2.95	3.0	
	2.80	4.0	
	2.68	5.0	
	2.58	6.0	
	2.49	7.0	
	2.42	8.0	
	2.34	9.0	
	2.29	10.0	
	2.19	12.0	

Table 6. **Potentiometric Titration of L-5-(α-Amino-β-phenylethyl)-tetrazole with Sodium Hydroxide (L-Phenylalanine Analog)**

Concentration	pH	Ml. of 0.10036 N NaOH	Apparent pK_2
0.140 g./100 ml. of aqueous solution	6.00	0	
	6.97	0.5	8.07
	7.30	1.0	
	7.49	1.5	
	7.66	2.0	
	7.92	3.0	
	8.18	4.0	
	8.42	5.0	
	8.80	6.0	
	9.70	7.0	
	10.70	8.0	
	11.00	9.0	
	11.19	10.0	
	11.29	11.0	
	11.35	12.0	
	11.50	15.0	

Table 7. Potentiometric Titration of D, L-5-[α-Amino-β-(p-methoxyphenyl)ethyl]tetrazole with Hydrochloric Acid (O-Methyl-D, L-Tyrosine Analog)

Concentration	pH	Ml. of 0.10156 N HCl	Apparent pK_1
0.150 g./250 ml. of aqueous solution	6.40	0	3.20
	4.15	0.5	
	3.88	1.0	
	3.62	1.5	
	3.51	2.0	
	3.28	3.0	
	3.12	4.0	
	3.00	5.0	
	2.90	6.0	
	2.78	7.0	
	2.70	8.0	
	2.62	9.0	
	2.59	10.0	
	2.50	12.0	
	2.39	15.0	
	2.25	20.0	

Table 8. Potentiometric Titration of D, L-5-[α-Amino-β-(p-methoxy)-phenylethyl]tetrazole with Sodium Hydroxide (O-Methyl-D, L-Tyrosine Analog)

Concentration	pH	Ml. of 0.10036 N NaOH	Apparent pK_2
0.150 g./250 ml. of aqueous solution	6.40	0	8.08
	7.11	0.5	
	7.39	1.0	
	7.59	1.5	
	7.73	2.0	
	8.00	3.0	
	8.25	4.0	
	8.51	5.0	
	8.83	6.0	
	9.23	7.0	
	9.60	8.0	
	9.85	9.0	
	10.06	10.0	
	10.21	11.0	
	10.39	12.0	
	10.78	15.0	

Table 9. Potentiometric Titration of L-5-[α-Amino-β-(p-hydroxyphenyl)-ethyl]tetrazole with Hydrochloric Acid (L-Tyrosine Analog)

Concentration	pH	Ml. of 0.10156 N HCl	Apparent pK_1
0.1051 g./250 ml. of aqueous solution	6.40	0	3.28
	4.03	0.5	
	3.70	1.0	
	3.52	1.5	
	3.39	2.0	
	3.29	3.0	
	3.03	4.0	
	2.84	6.0	
	2.70	8.0	
	2.60	10.0	
	2.51	12.0	
	2.41	14.0	
	2.31	16.0	

Table 10. Potentiometric Titration of L-5-[α-Amino-β-(p-hydroxyphenyl)ethyl]tetrazole with Sodium Hydroxide (L-Tyrosine Analog).

Concentration	pH	Ml. of 0.10036 N NaOH	Apparent pK_2
0.1051 g./250 ml. of aqueous solution	6.40	0	
	7.38	0.5	8.17
	7.68	1.0	
	7.83	1.5	pK_3
	8.00	2.0	10.05
	8.17	2.5	
	8.31	3.0	
	8.49	3.5	
	8.62	4.0	
	8.88	4.5	
	9.11	5.0	
	9.34	5.5	
	9.55	6.0	
	9.72	6.5	
	9.90	7.0	
	10.00	7.5	
	10.11	8.0	
	10.22	8.5	
	10.30	9.0	
	10.38	9.5	
	10.47	10.0	
	10.51	10.5	
	10.59	11.0	
	10.70	12.0	
	11.00	15.0	
	11.27	20.0	

Table 11. Apparent pK Values of Some Amino Acids and Their Tetrazole Analogs

Compound	pK_1	ΔpK_1	Relative Acidity	pK_2	ΔpK_2	Relative Acidity	pK_3	ΔpK_3	Relative Acidity
L-5-(α-amino-β-phenylethyl)-tetrazole	2.83			8.07	1.06	11.5			
L-phenylalanine (37)	1.83	1.00	10.0	9.13					
D, L-5-(α-amino-β-p-methoxyphenylethyl)tetrazole	3.20			8.08	1.27	13.4			
O-methyl-L-tyrosine (22)	2.21	.99	9.8	9.35					
L-5-(α-amino-β-p-hydroxyphenylethyl)tetrazole	3.27			8.17	1.02	10.5	10.5	.38	2.4
L-tyrosine (41)	2.20	1.07	11.8	9.19			10.43		

SUMMARY

1. The tetrazole analogs of D, L-phenylalanine, L-phenylalanine, and L-tyrosine were prepared by three different routes.

2. The tetrazole analog of O-methyl-D, L-tyrosine was prepared by alkylation of ethyl acetamidocyanoacetate with anisyl chloride.

3. 2-Substituted-5-phenyl-1, 3, 4-oxadiazoles were synthesized from the tetrazole analogs of D, L-phenylalanine, L-phenylalanine, and L-tyrosine.

4. Proof of structure and configuration of the tetrazole analogs of L-phenylalanine and L-tyrosine were accomplished by hydrolysis of their oxadiazoles to L-phenylalanine and L-tyrosine.

5. The apparent pK values for L-phenylalanine, L-tyrosine, and O-methyl-D, L-tyrosine were determined by potentiometric titration and were found to be analogous to those of the corresponding amino acids.

6. The specific rotations of L-phenylalanine, L-tyrosine, and their tetrazole analogs were determined in various concentrations of acid and base.

7. The infrared spectra of a number of new compounds are reported.

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APPENDIX

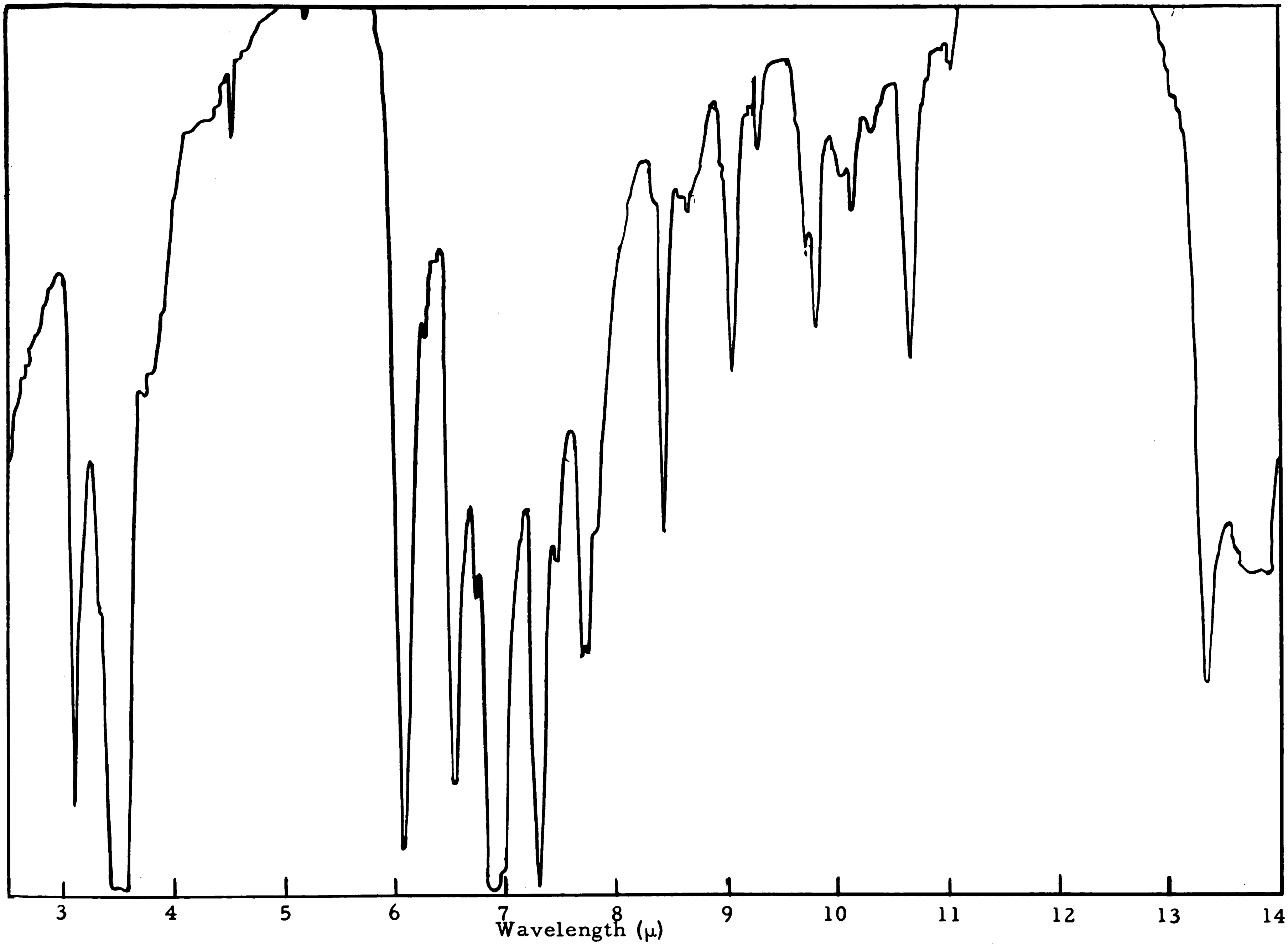

Figure 4. Infrared spectrum of D,L-α-Acetamido-β-phenylpropionitrile.

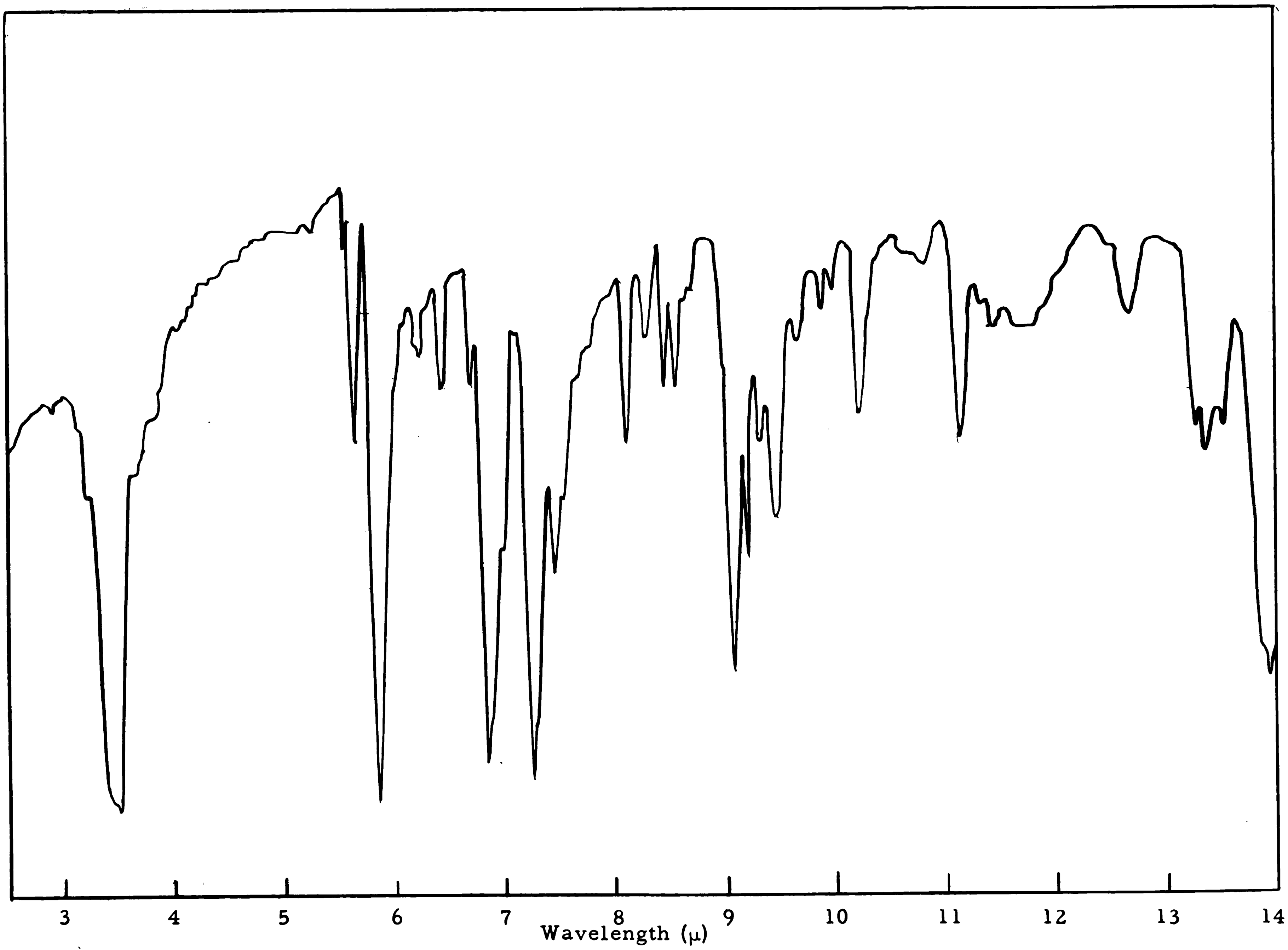

Figure 5. Infrared spectrum of L-5-(α-Phthalimido-β-phenylethyl)tetrazole.

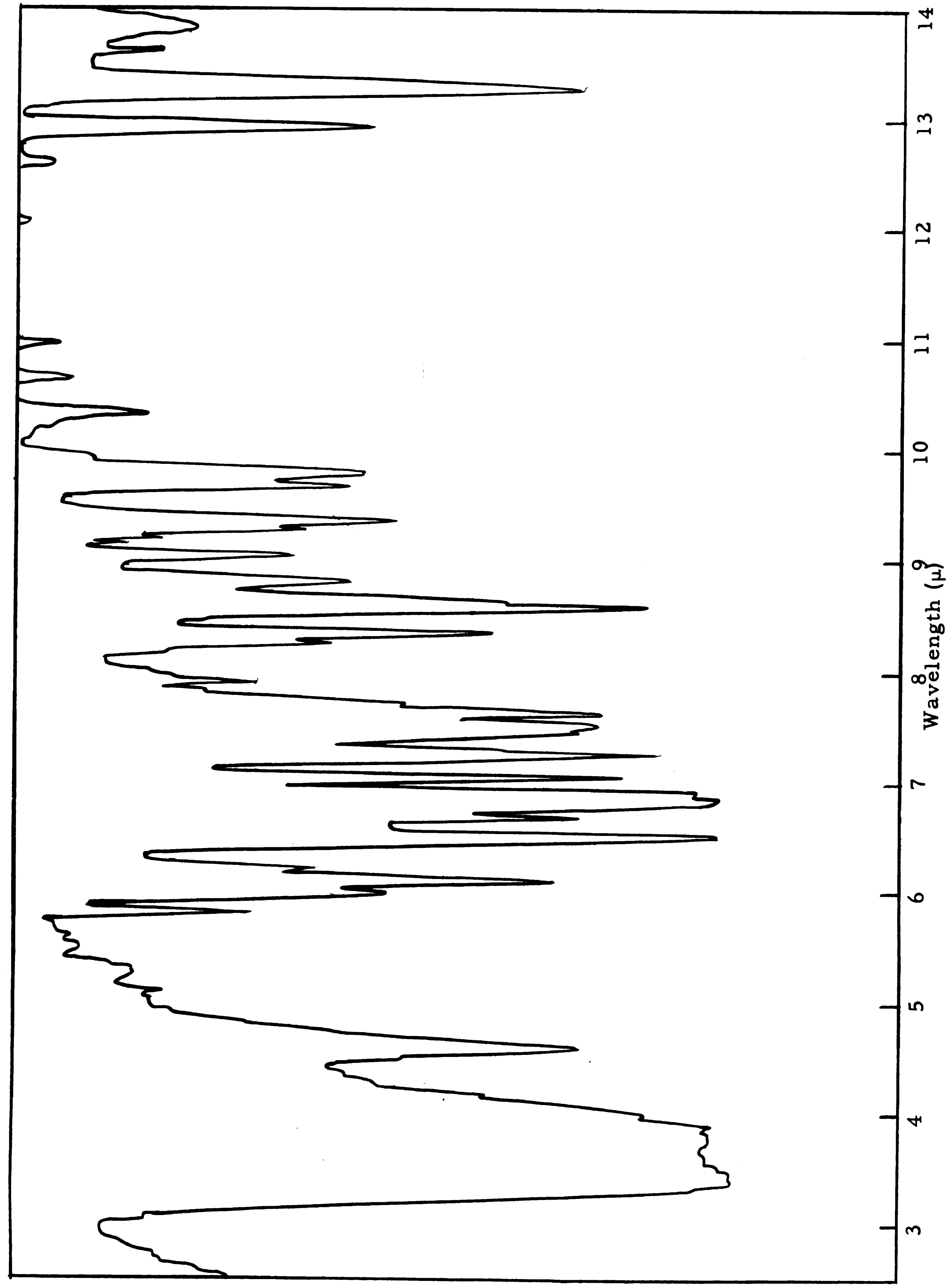

Figure 6. Infrared spectrum of L-5-(α-Amino-β-phenylethyl)tetrazole.

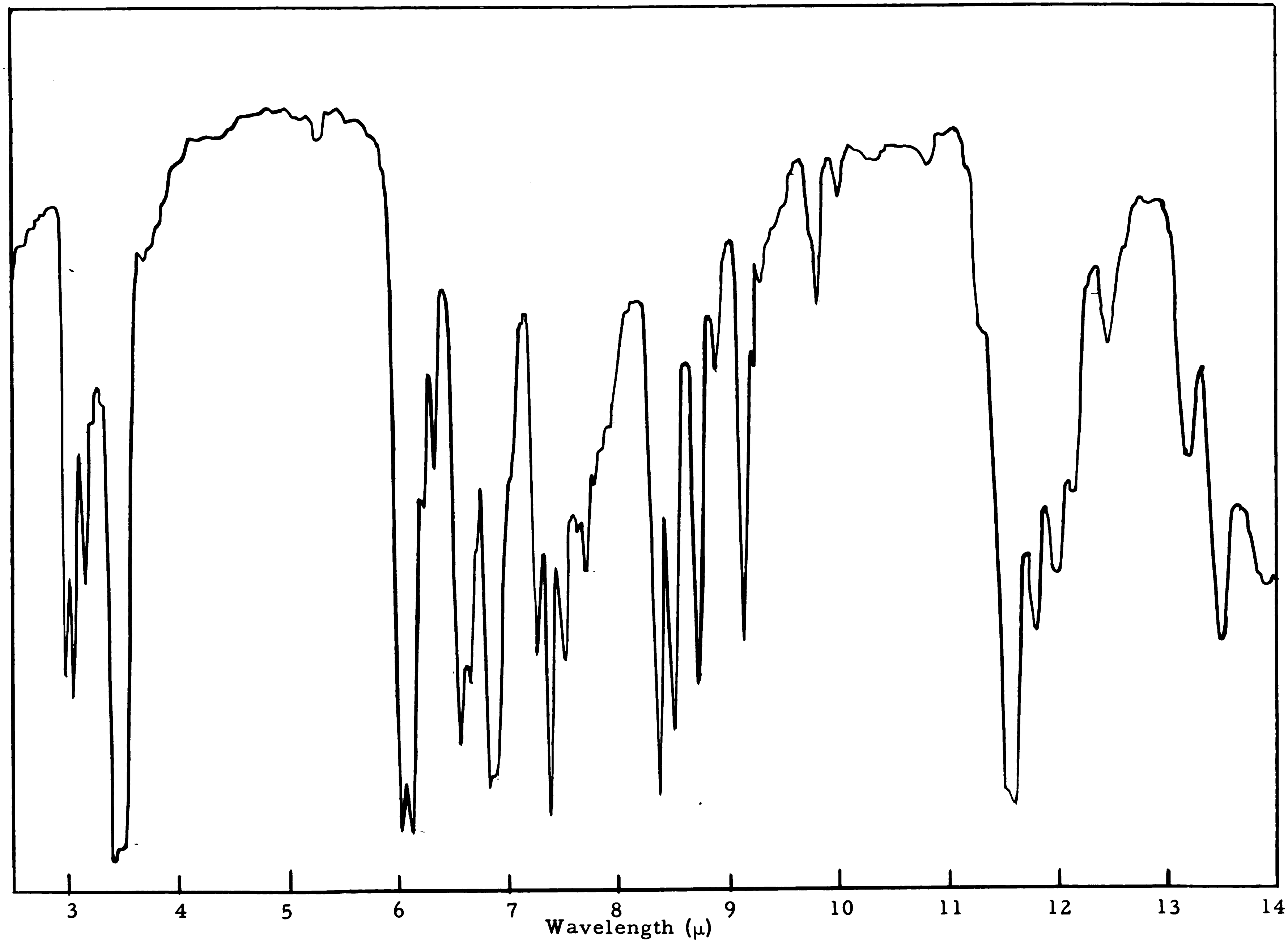

Figure 7. Infrared spectrum of L-α-Benzamido-β-(p-benzenesulfonoxylphenyl)propionamide.

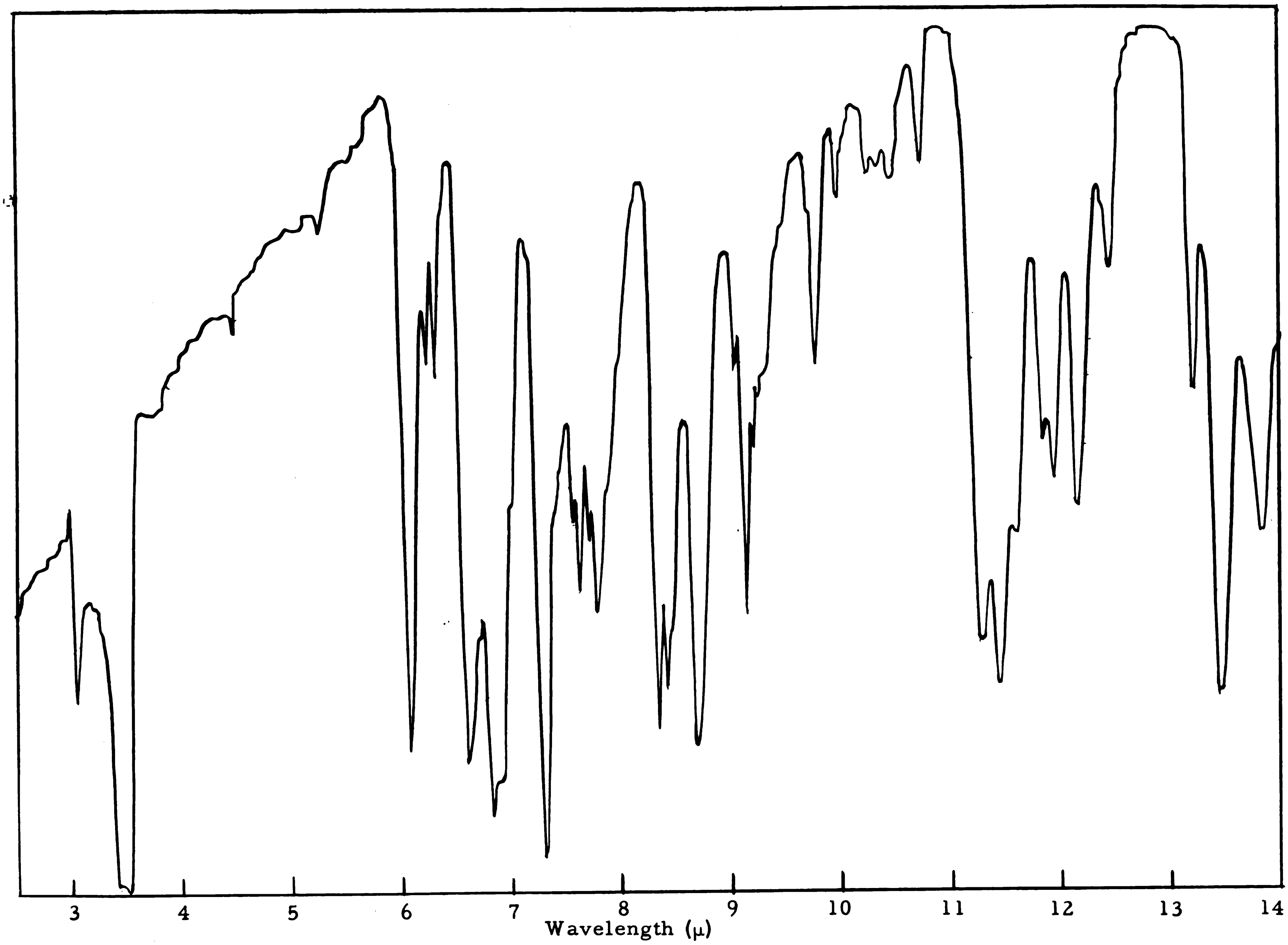

Figure 8. Infrared spectrum of L-α-Benzamido-β-(p-benzenesulfonyloxyphenyl)propionitrile.

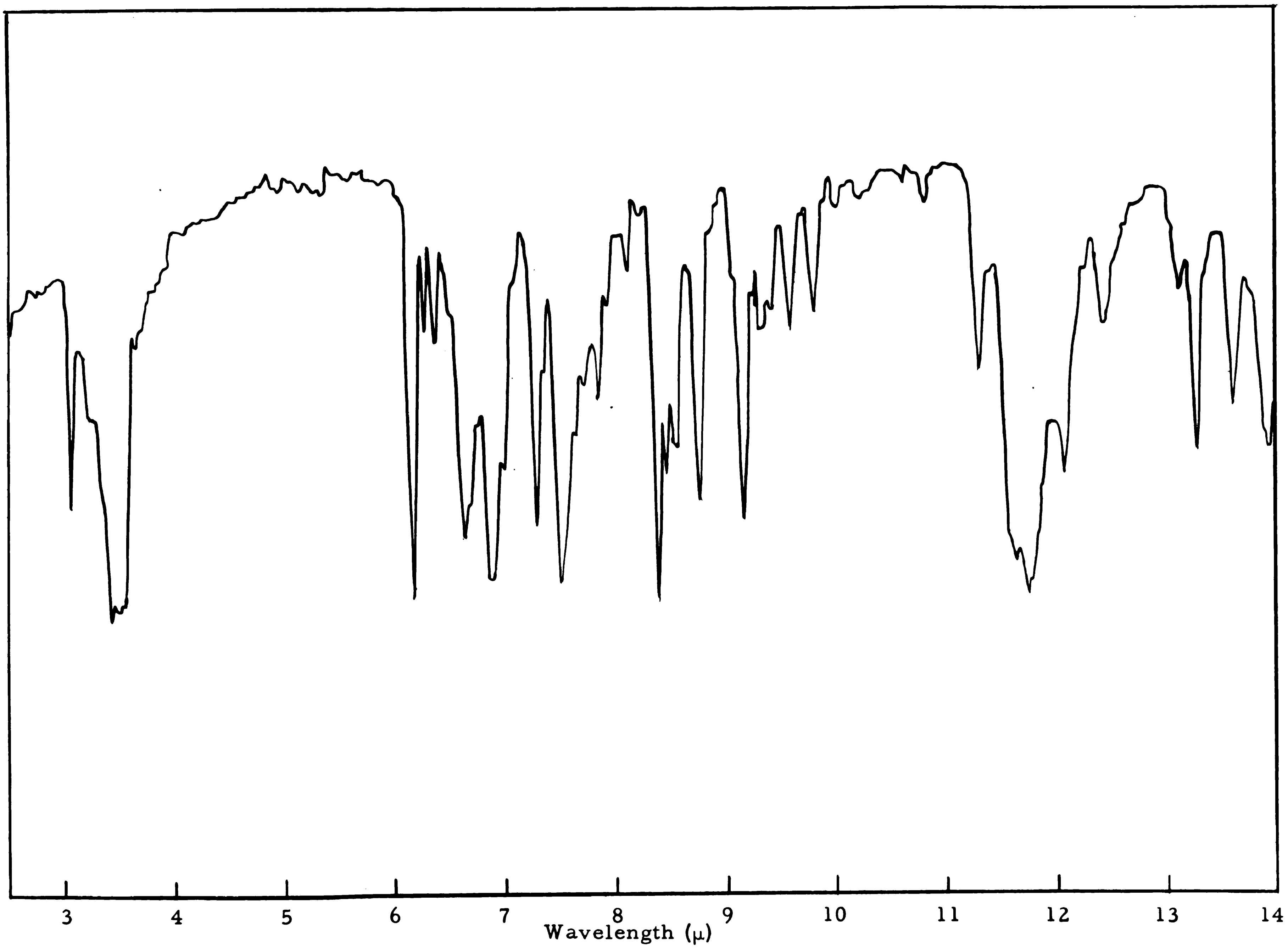

Figure 9. Infrared spectrum of L-5-[α-Benzamido-β-(p-benzenesulfonyloxyphenyl)ethyl]tetrazole.

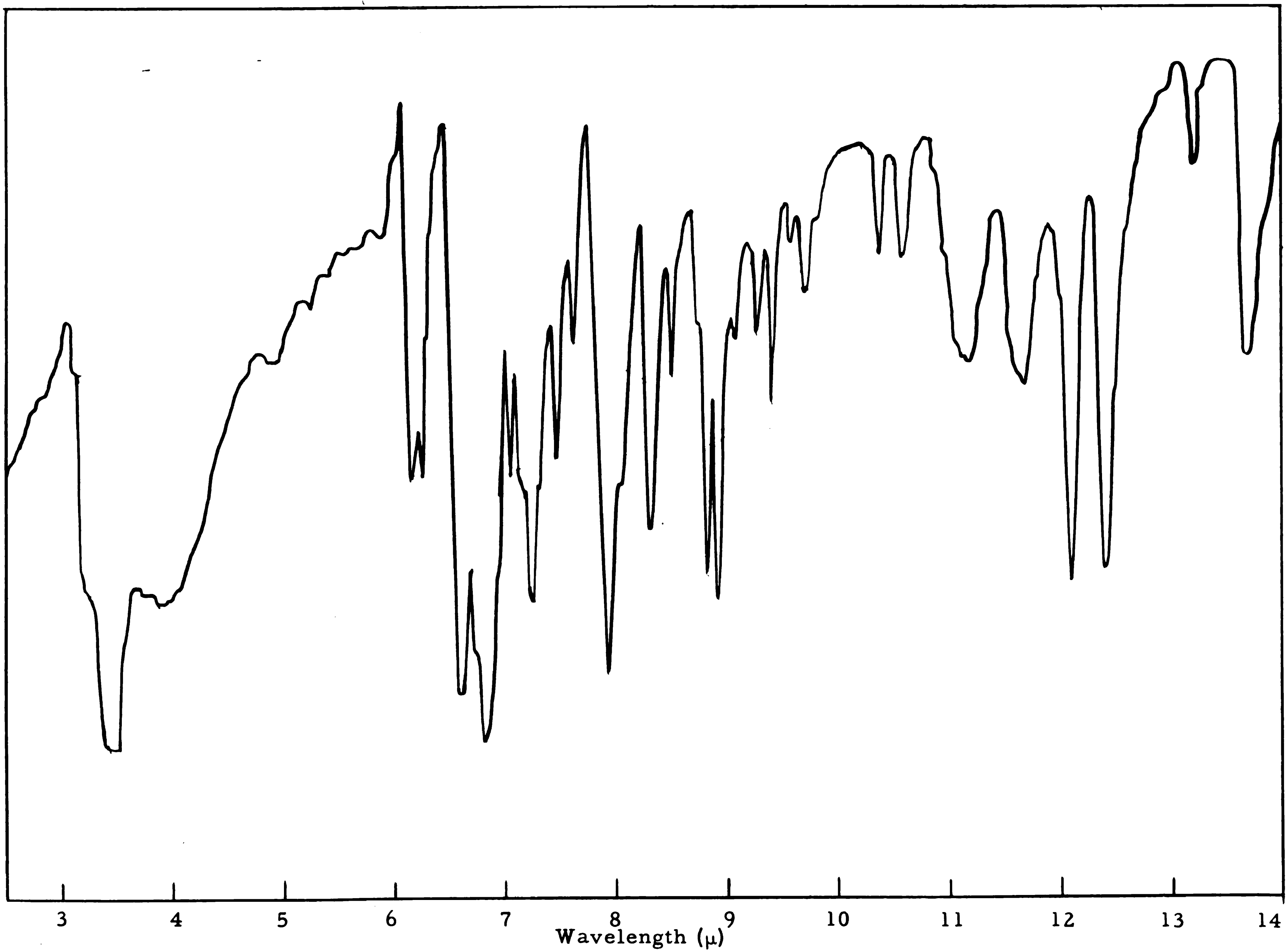

Figure 10. Infrared spectrum of L-5-[α-Amino-β-(p-hydroxyphenyl)ethyl]tetrazole.

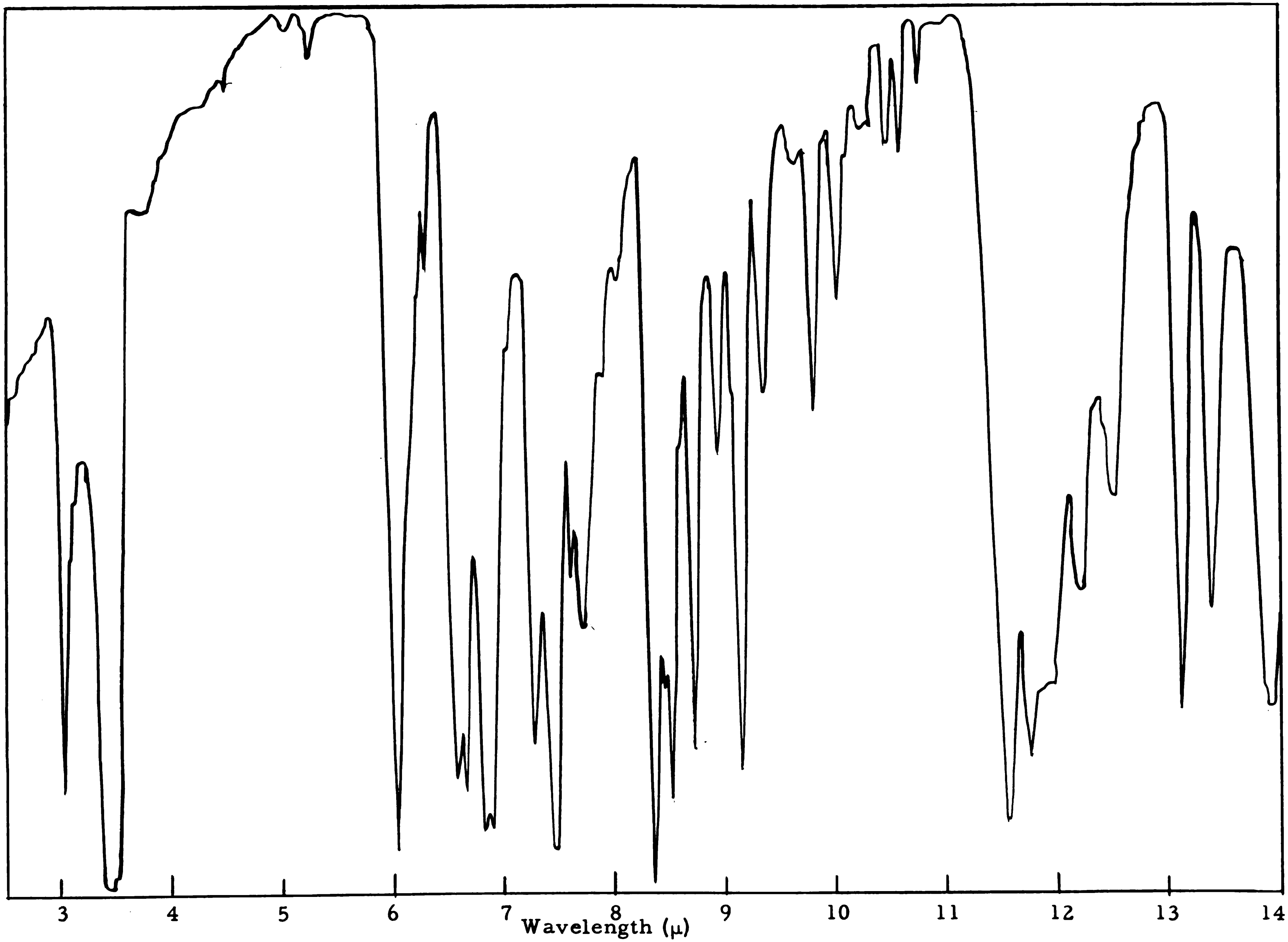

Figure 11. Infrared spectrum of L-α-Acetamido-β-(p-benzenesulfonyloxyphenyl)propionitrile.

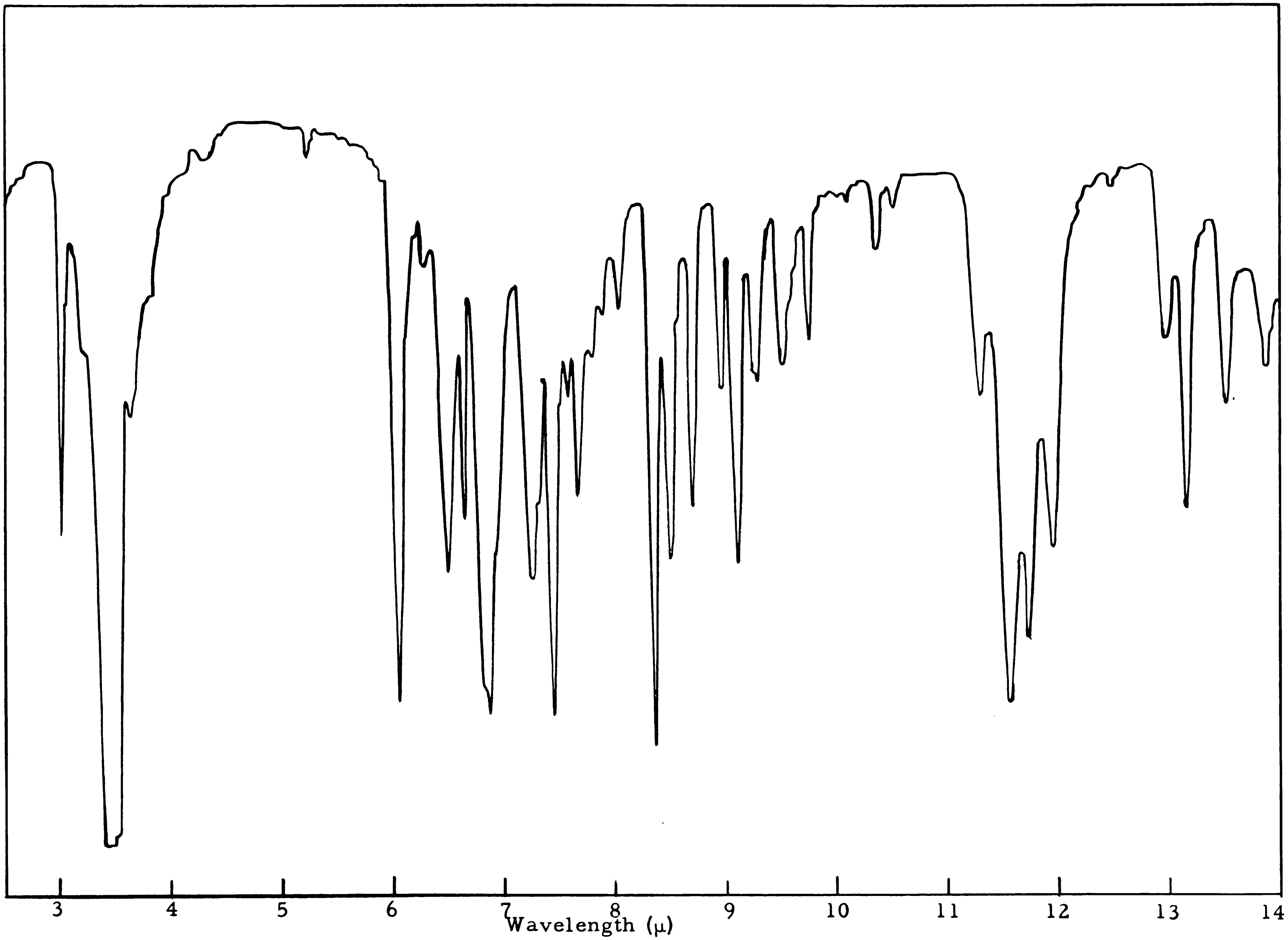

Figure 12. Infrared spectrum of L-5-[α-Acetamido-β-(p-benzenesulfonyloxyphenyl)ethyl]tetrazole.

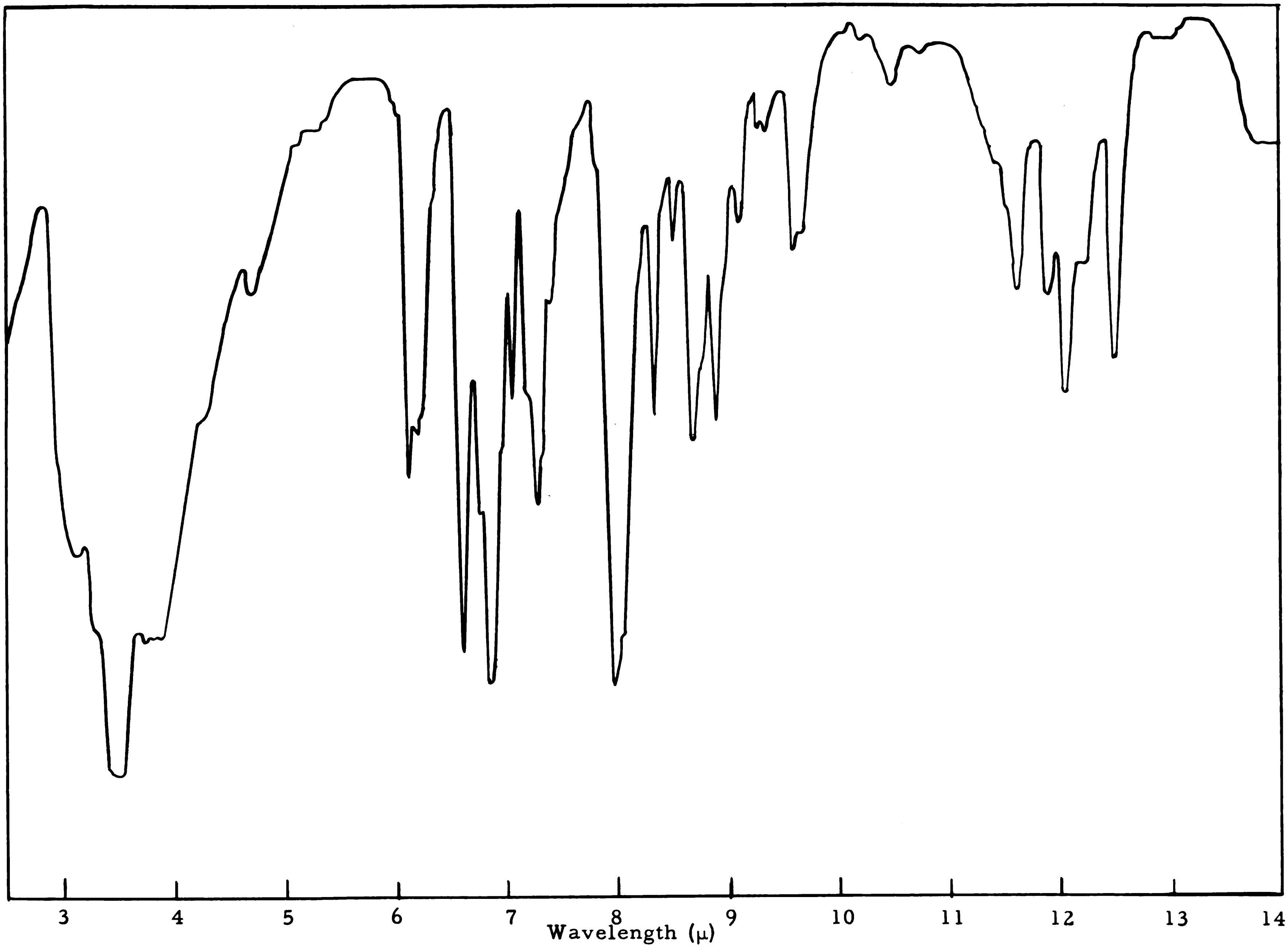

Figure 13. Infrared spectrum of D, L-5-[α-Amino-β-(p-methoxyphenyl)ethyl]tetrazole.

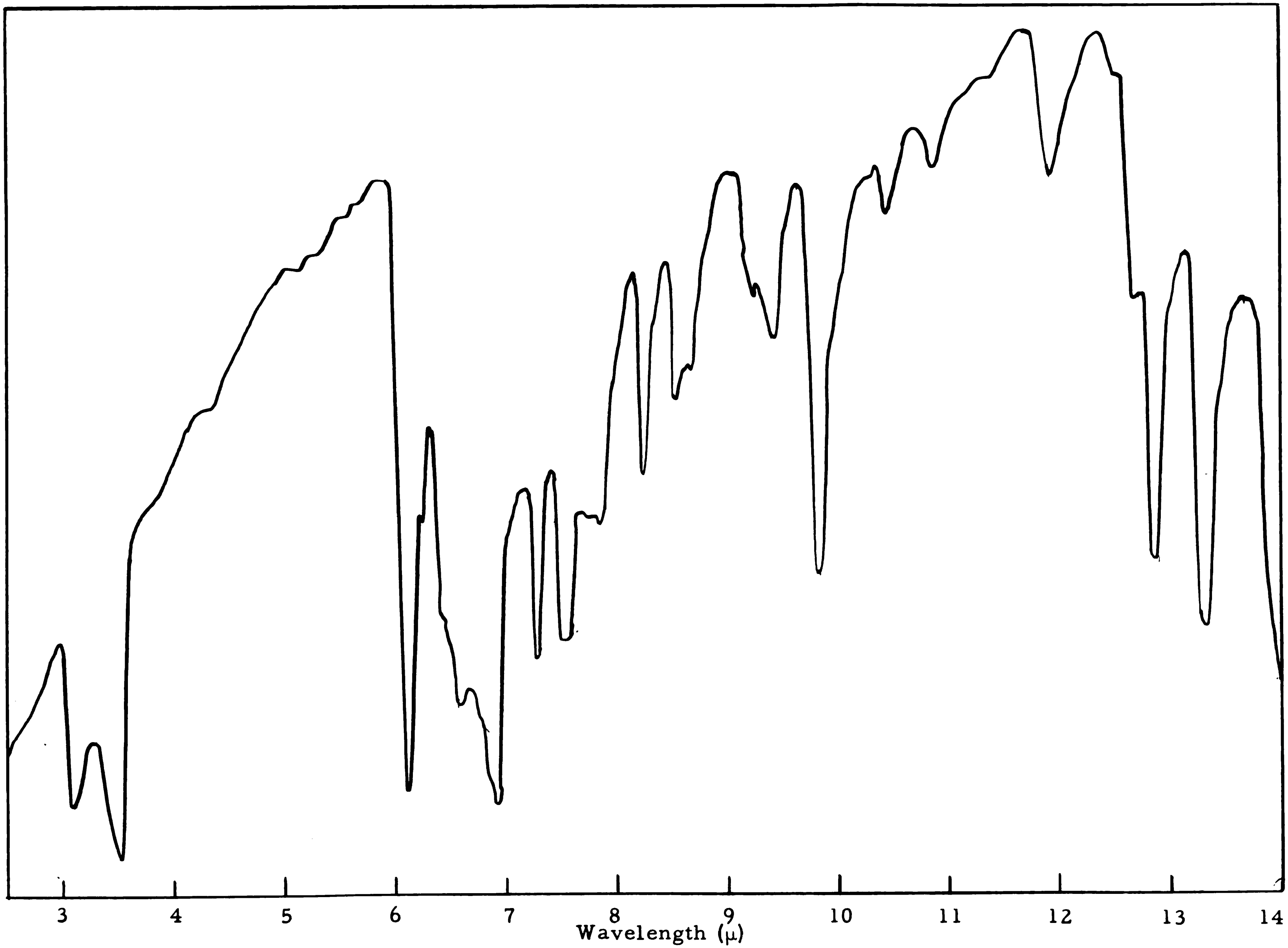

Figure 14. Infrared spectrum of L-2-(1'-Benzamido-2'-phenylethyl-5-phenyl-1,3,4-oxadiazole.

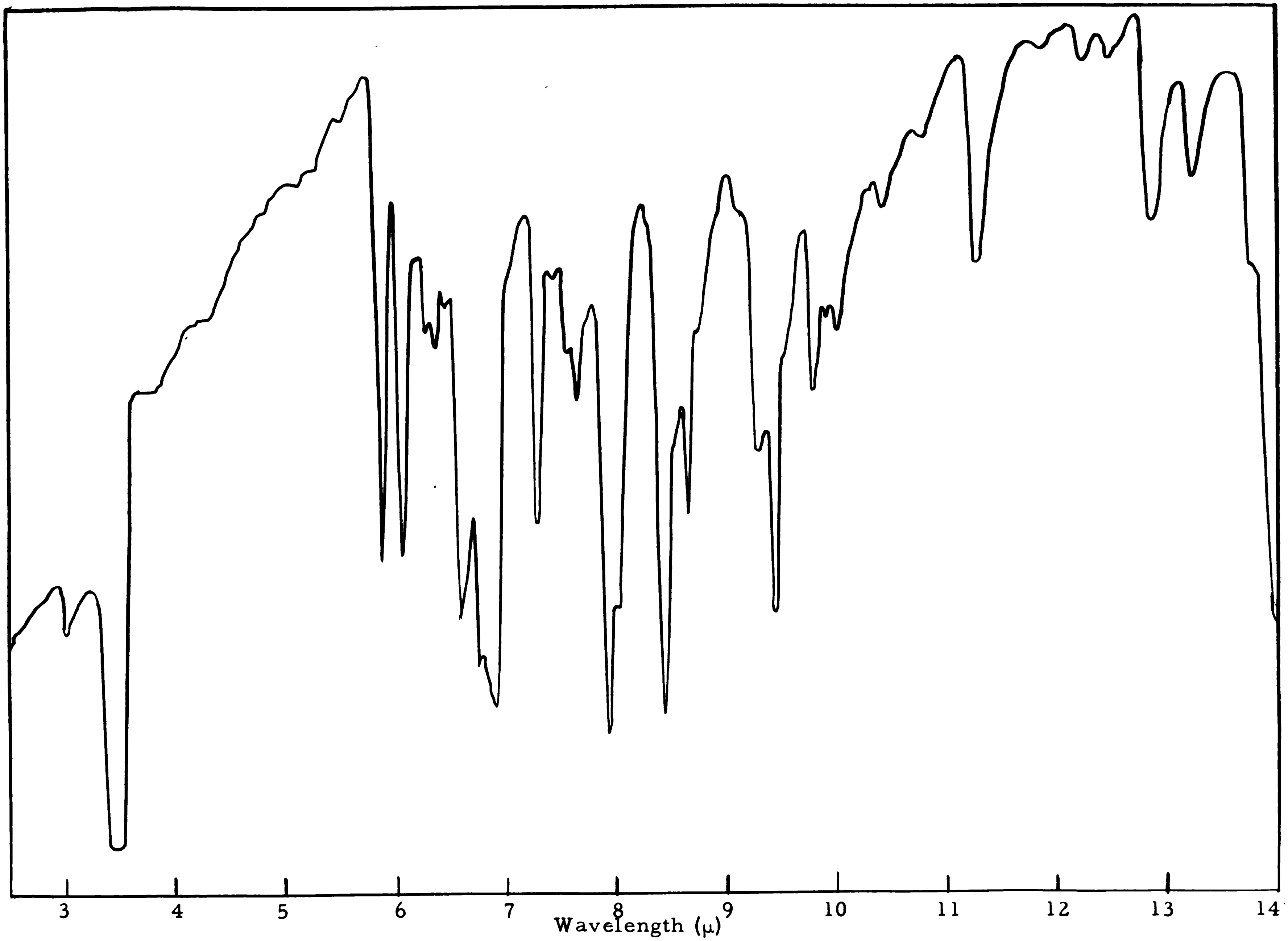

Figure 15. Infrared spectrum of L-2-[1'-Benzamido-2'-(p-benzoyloxyphenyl)ethyl]-5-phenyl-1, 3, 4-oxadiazole.

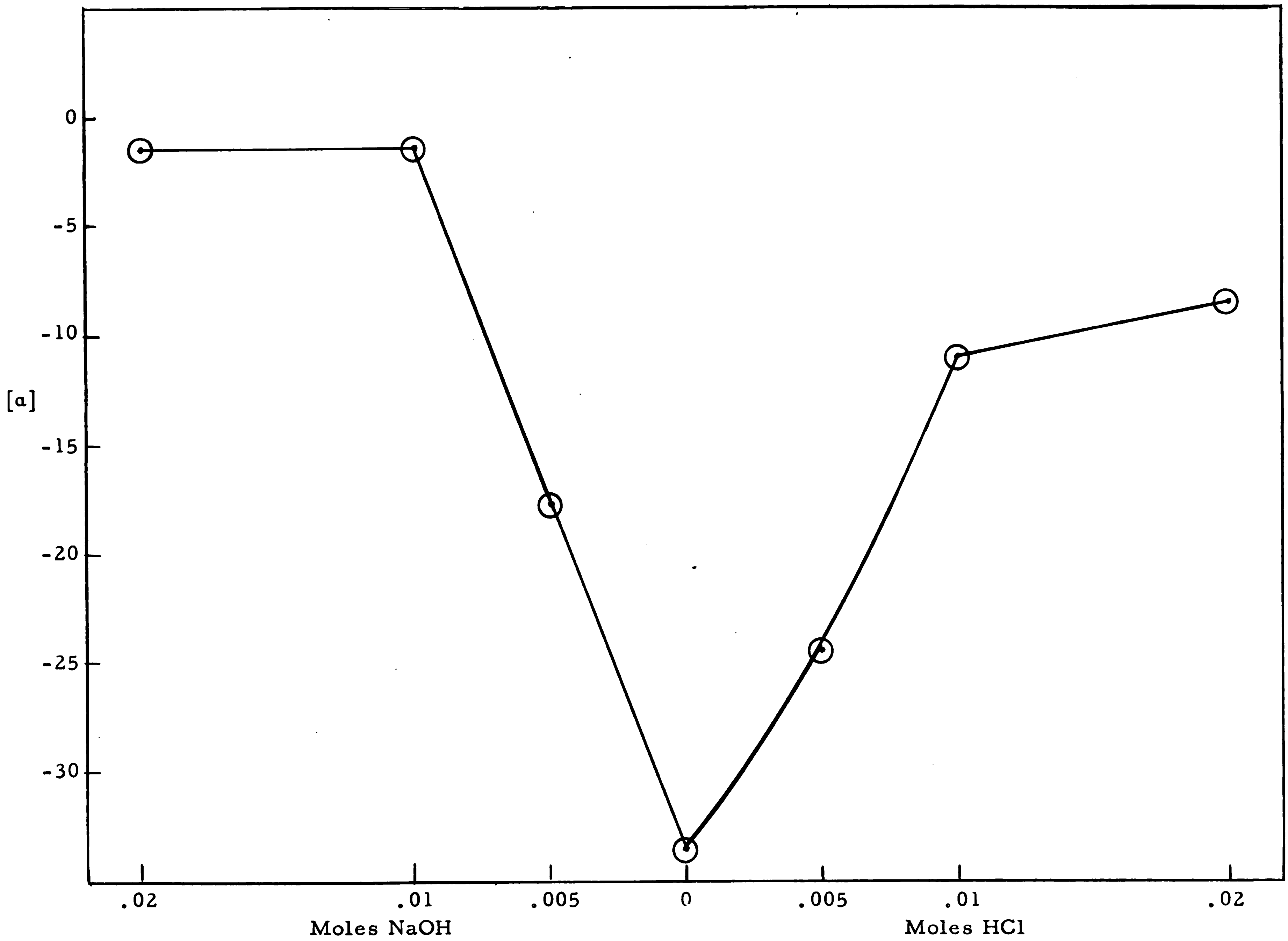

Figure 16. Specific Rotation of L-Phenylalanine in NaOH and HCl.

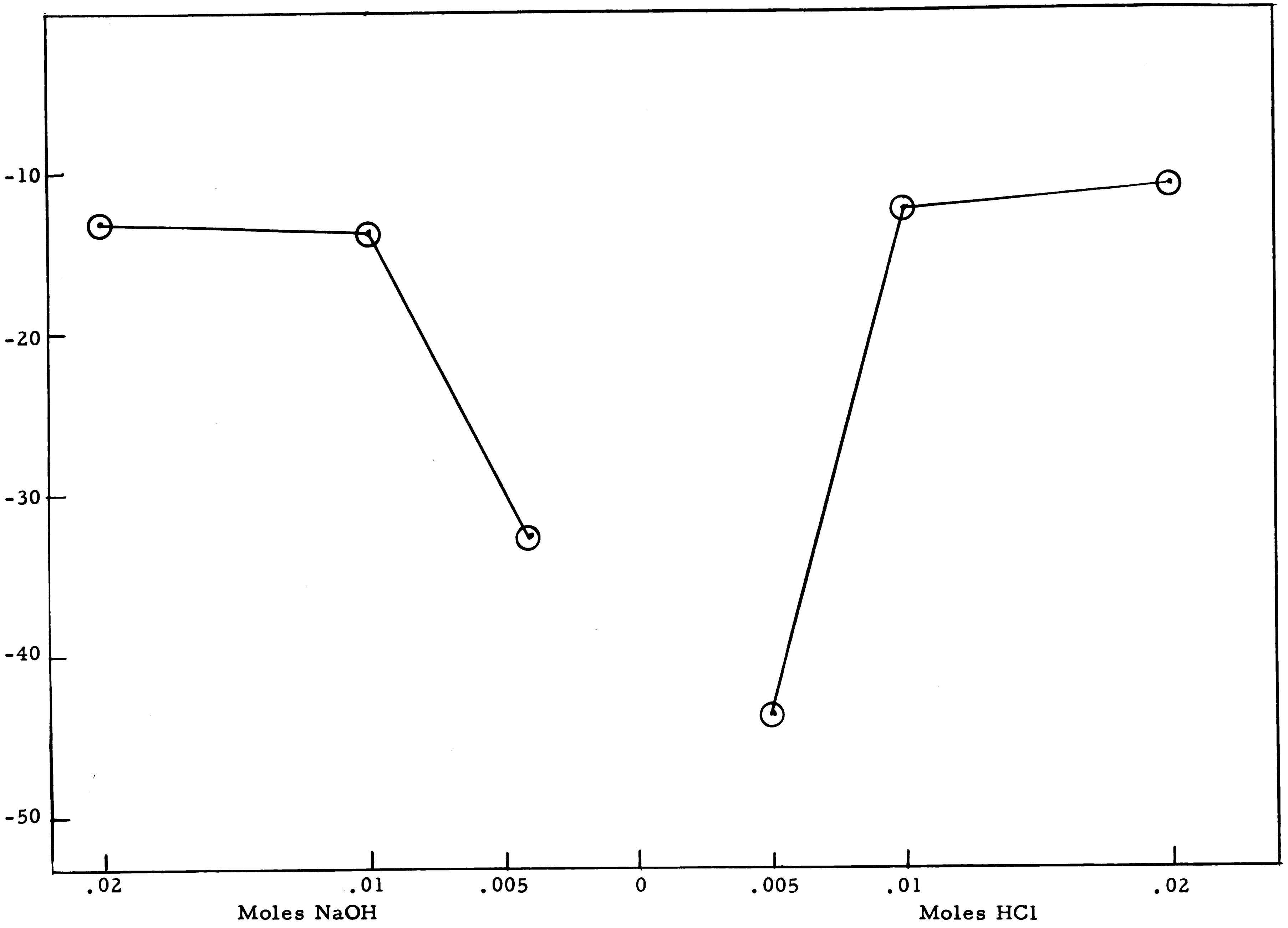

Figure 17. Specific Rotation of L-Tyrosine in NaOH and HCl.

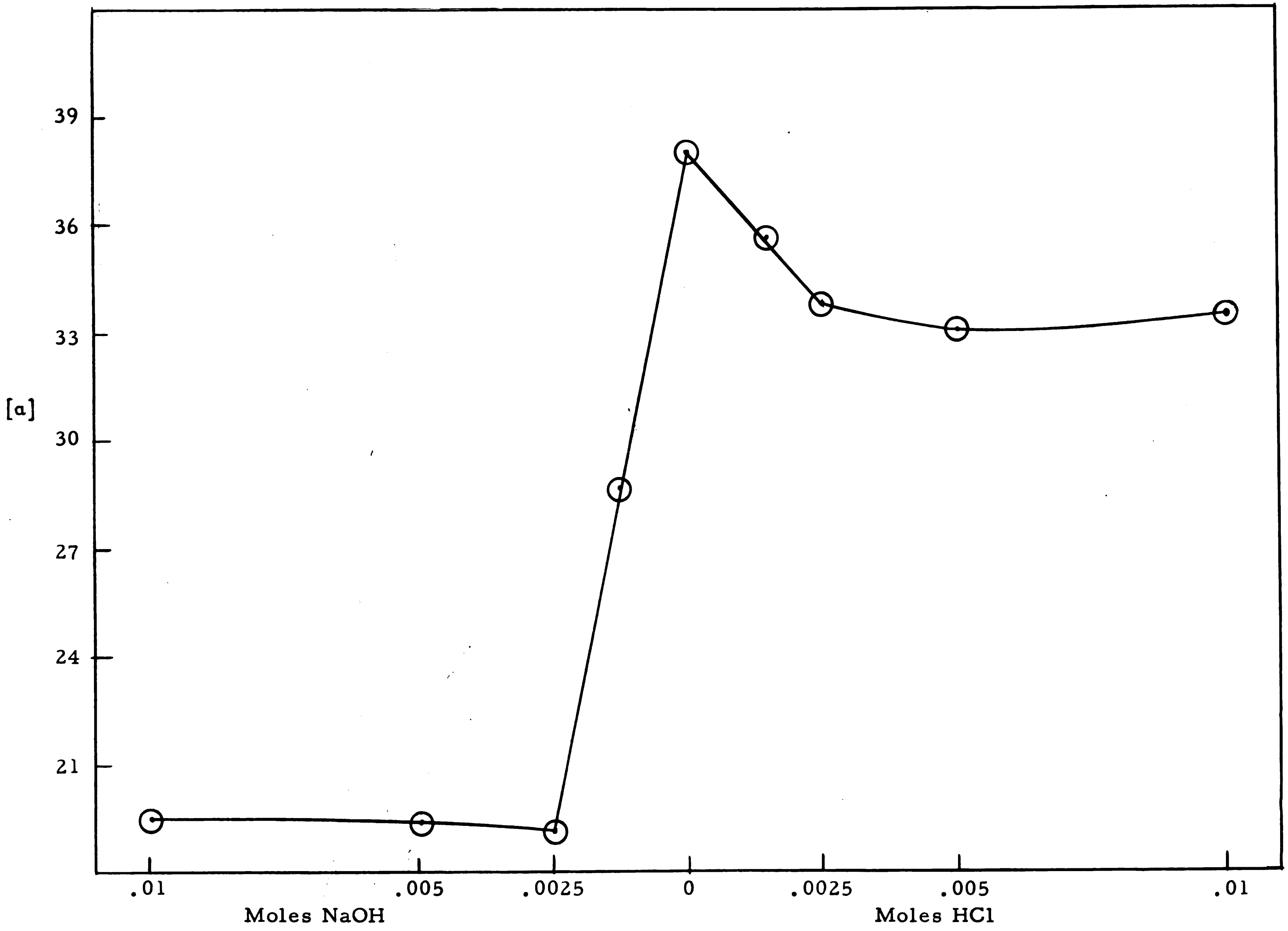

Figure 18. Specific Rotation of L-5-(α-Amino-β-phenyl ethyl) tetrazole in NaOH and HCl.

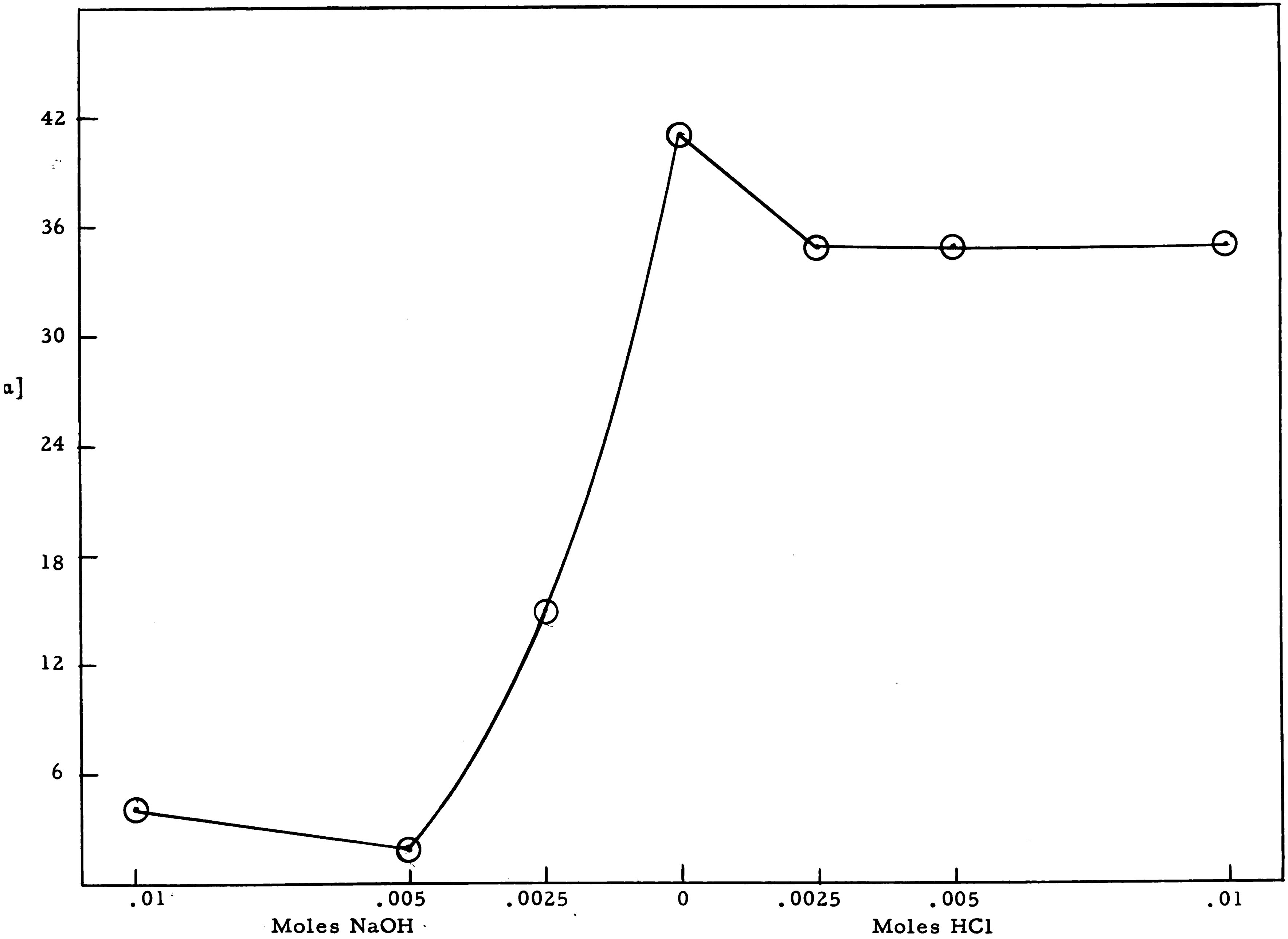

Figure 19. Specific Rotation of L-5-[α-Amino-β-(p-hydroxyphenyl)ethyl]tetrazole in NaOH and HCl.

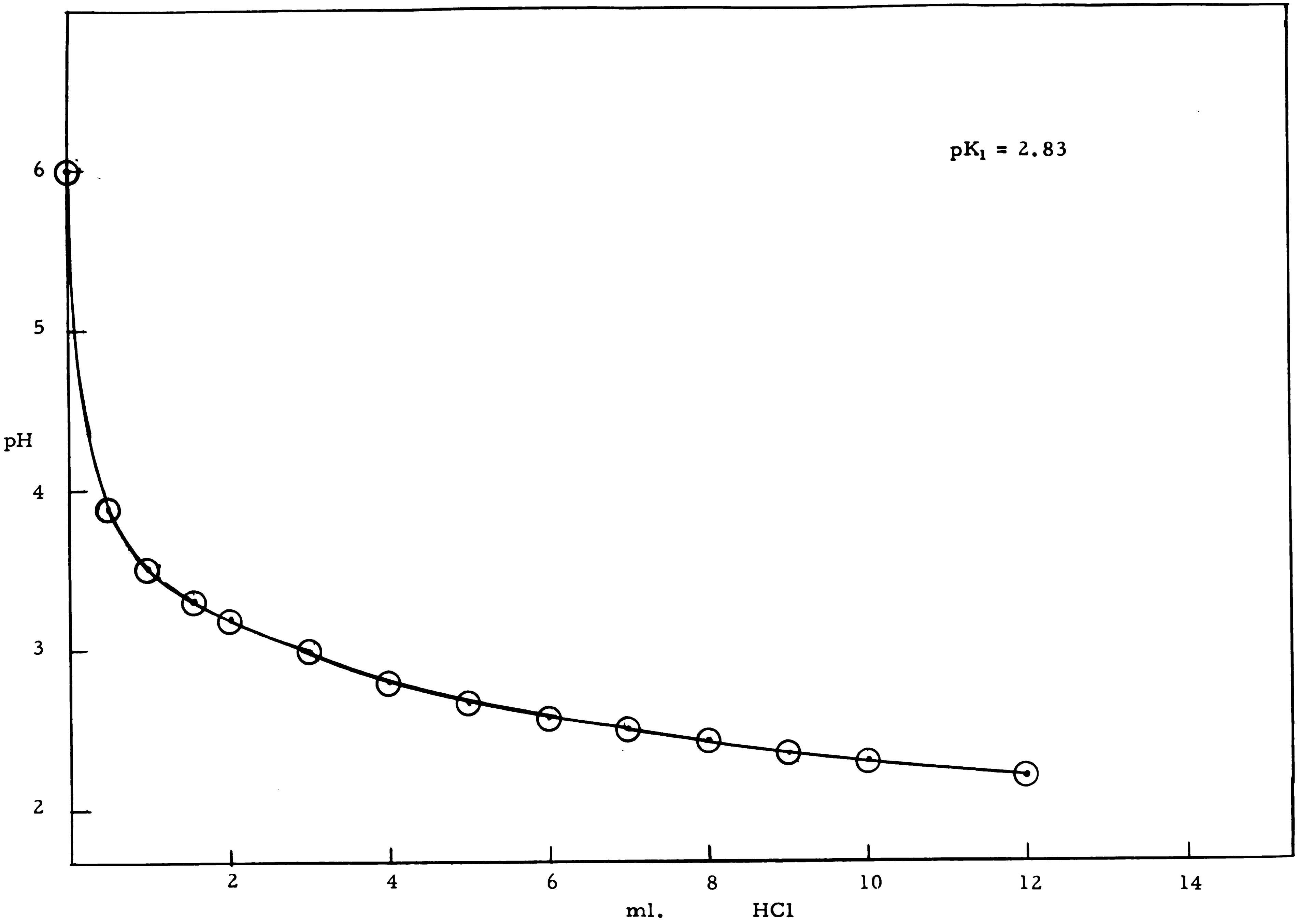

Figure 20. Potentiometric Titration Curve of L-5-(α-Amino-β-phenylethyl)tetrazole in HCl.

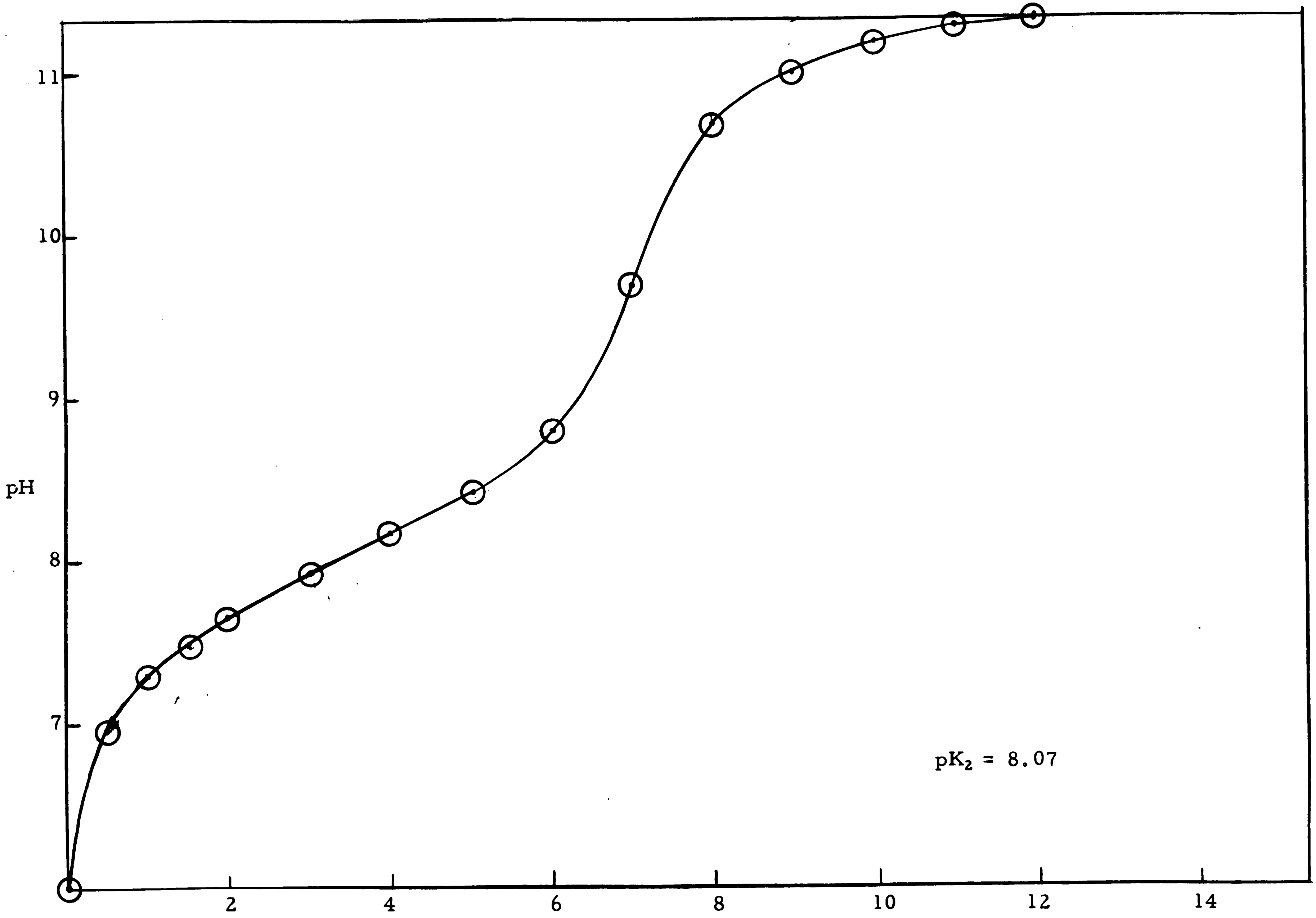

Figure 21. Potentiometric Titration Curve of L-5-(α-Amino-β-phenylethyl)tetrazole in NaOH.

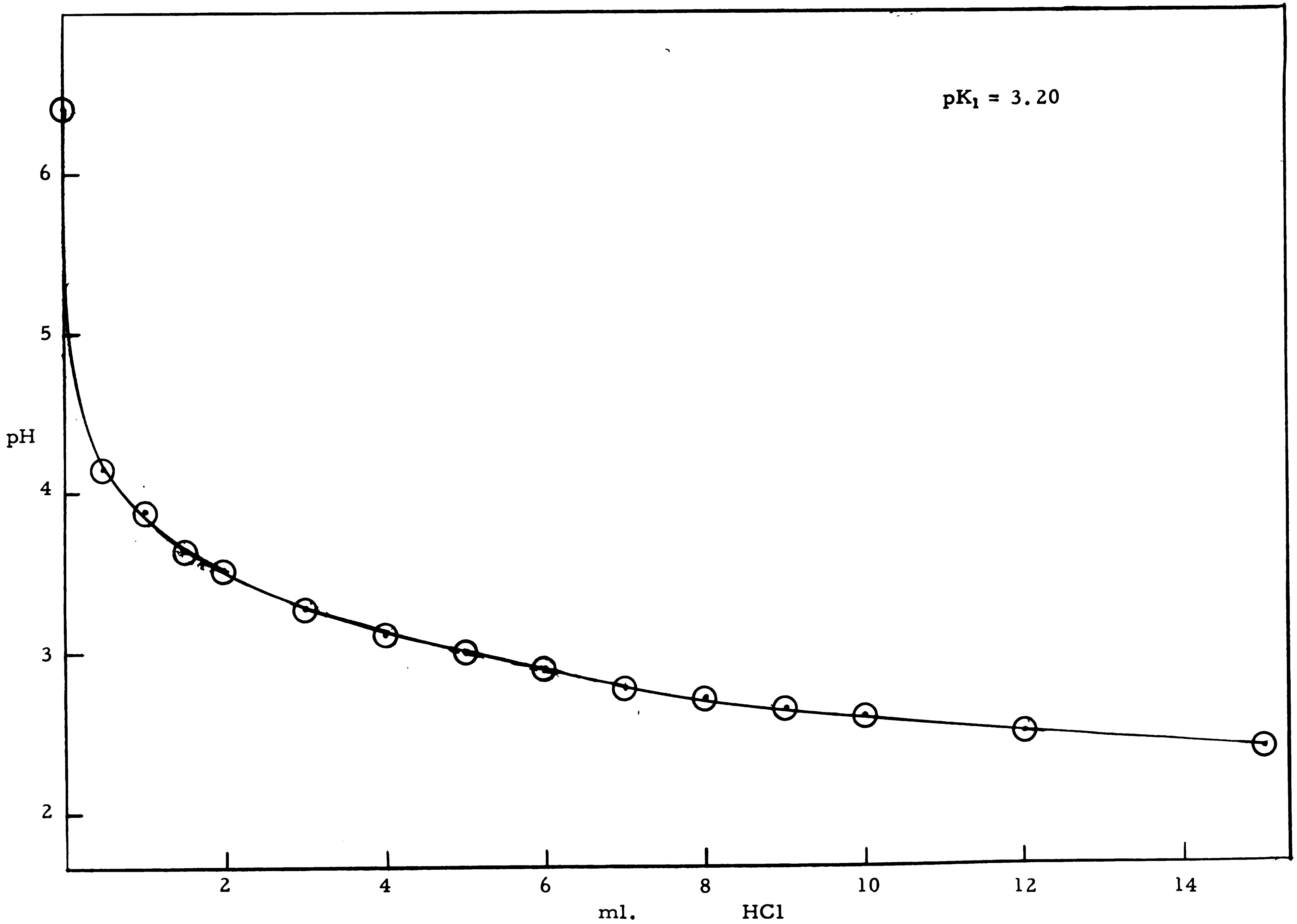

Figure 22. Potentiometric Titration Curve of D, L-5-[α-Amino-β-(p-methoxyphenyl)ethyl]tetrazole in HCl.

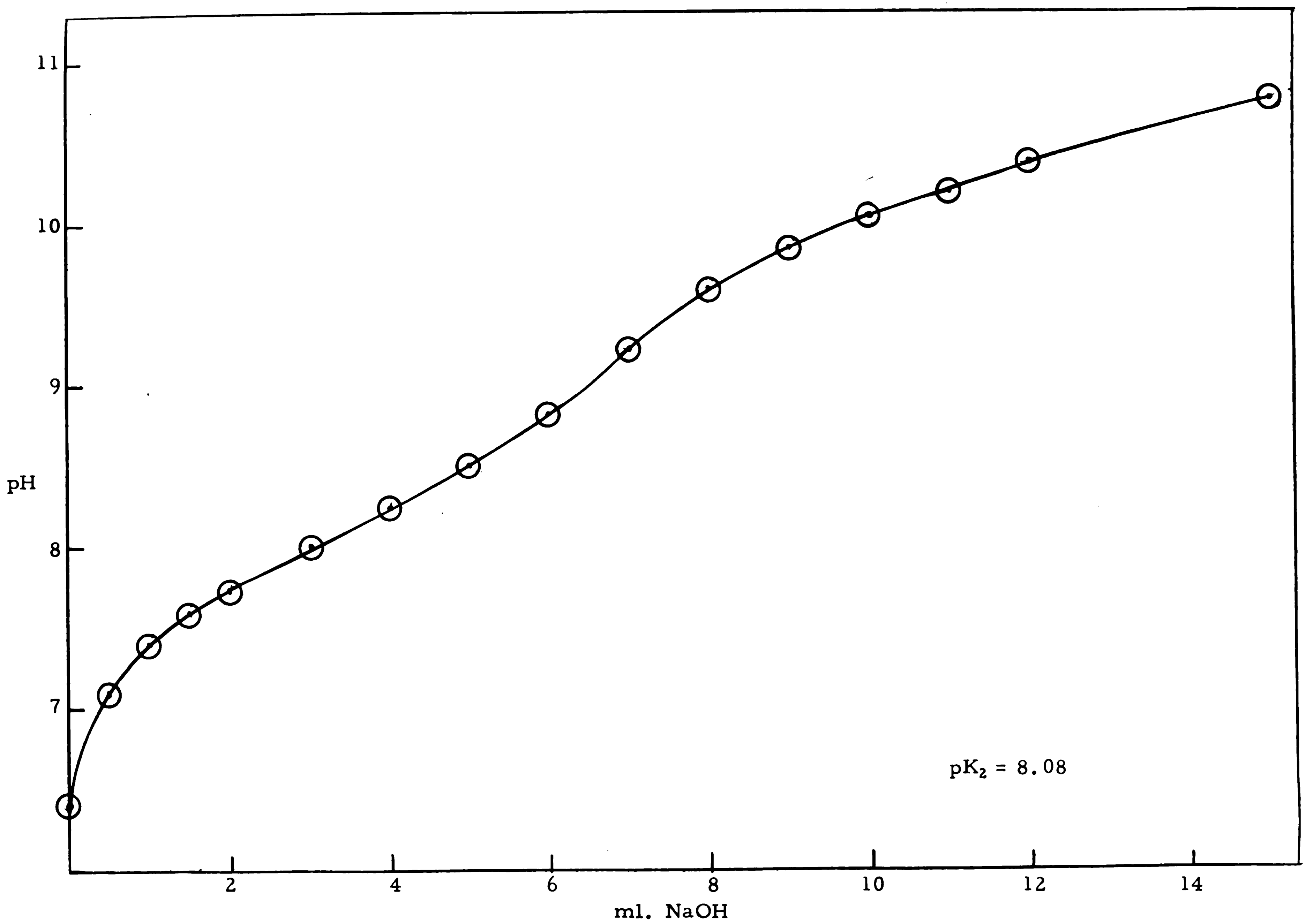

Figure 23. Potentiometric Titration Curve of D, L-5-[α-Amino-β-(p-methoxyphenyl)ethyl]-tetrazole in NaOH.

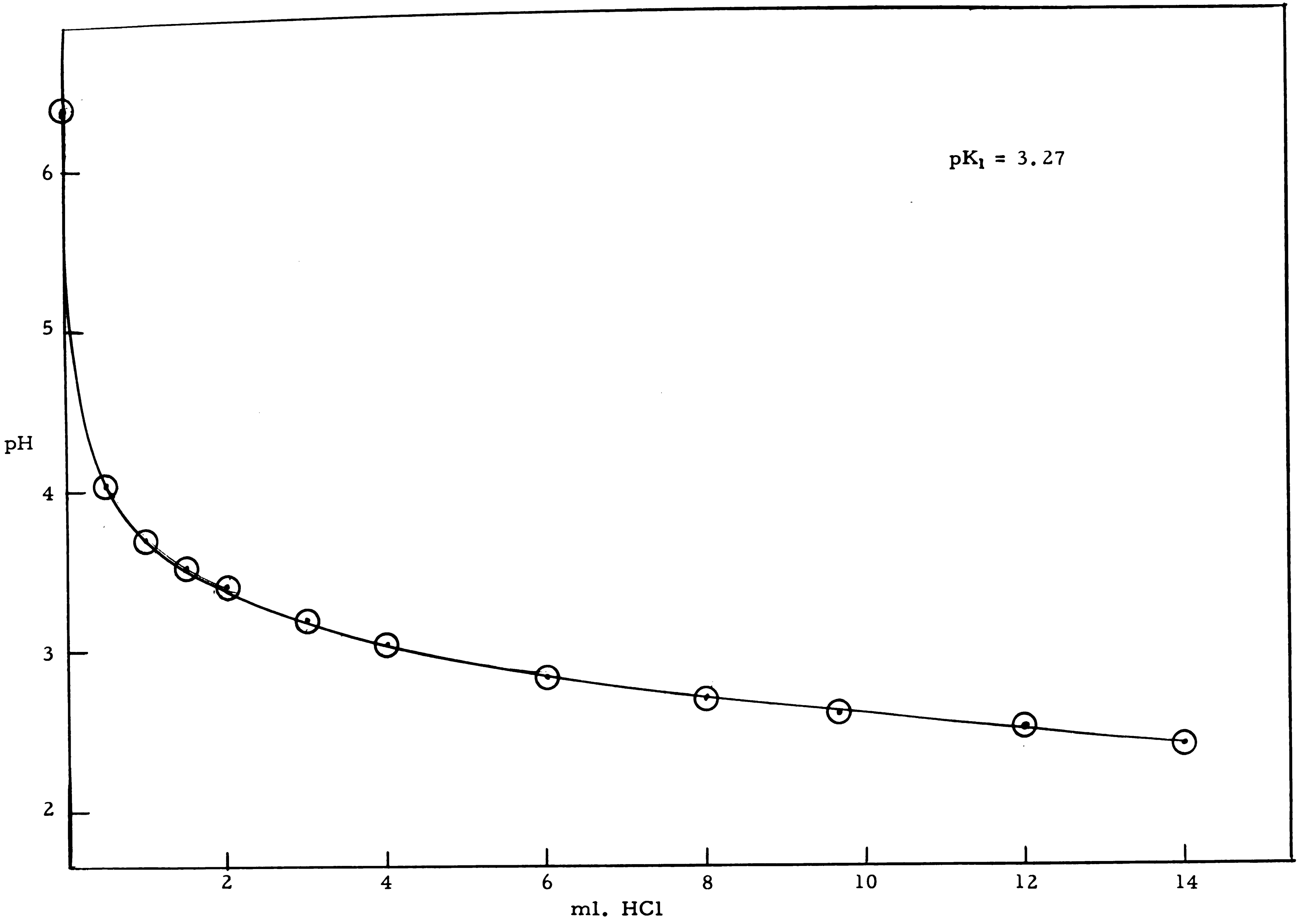

Figure 24. Potentiometric Titration Curve of L-5-[α-Amino-β-(p-hydroxyphenyl)ethyl]tetrazole in HCl.

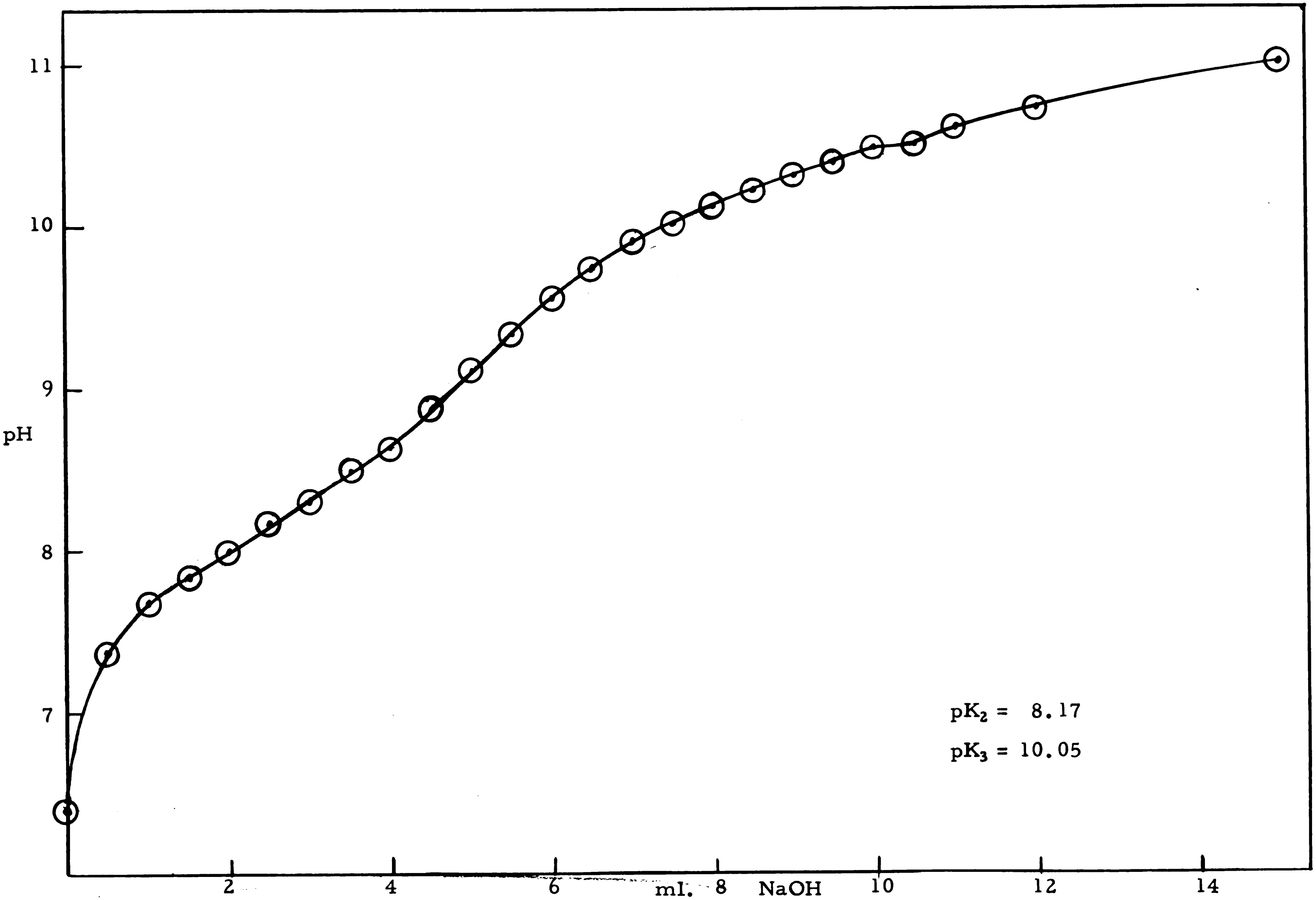

Figure 25. Potentiometric Titration Curve of L-5-[α-Amino-β-(p-hydroxyphenyl)ethyl]tetrazole in NaOH.