SYNTHESIS OF TETRAZOLES AS AMINO ACID ANALOGS

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY

Gordon J. Sterken

1960

SYNTHESIS OF TETRAZOLES

AS

AMINO ACID ANALOGS

BY

GORDON J. STERKEN

ABSTRACT

of

A THESIS

Submitted to the School for Advanced Graduate Studies of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Chemistry

1960

Approved Robert M. Hulst

ABSTRACT

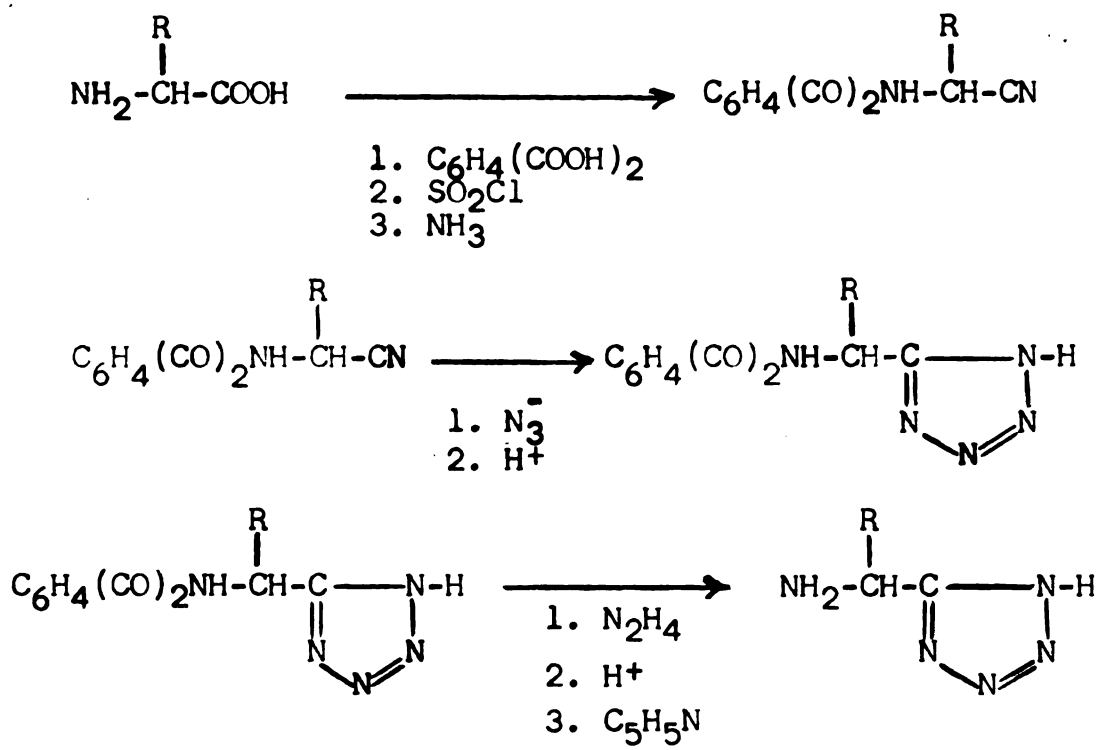
The preparation of compounds which closely resemble essential metabolic intermediates in both structure and chemical reactivity has become the object of increasingly extensive study since the discovery that the utilization of metabolic substrates in many biochemical processes could be inhibited by the addition of a structural analog of the substrate to the enzyme-substrate system. The present work describes the preparation of a number of 5-aminoalkyltetrazoles and demonstrates a chemical similarity between these compounds and the amino acid metabolit es to which they may be structurally related. The 5-aminoalkyltetrazoles whose preparation is described are:

1. 5-(1'-aminopropyl)tetrazole
2. 5-(1'-amino-2'methylpropyl)tetrazole
3. 5-(1'-amino-3'-methylbutyl)tetrazole
4. 5-(1'-amino-1'-methylethyl)tetrazole
5. 5-(1'-aminobenzyl)tetrazole
6. 5-(3'-aminopropyl)tetrazole

1,3-Bis-(5'-tetrazolyl)propane, the tetrazole analog of glutaric acid, was also prepared.

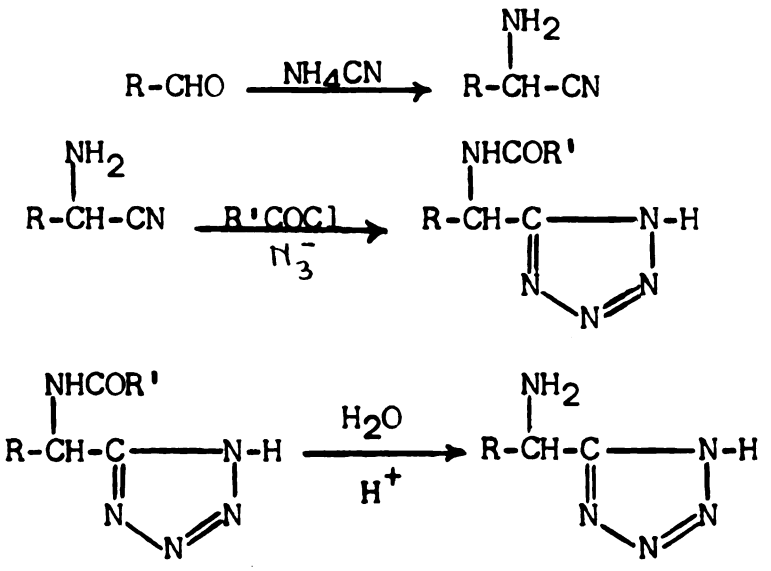
The synthesis of the first three of these compounds was accomplished by two different procedures, in order to provide experimental verification of their assigned structure. The first of these methods utilized the alpha-amino

acid counterpart of the desired tetrazole as a starting material from which an alpha-phthalimidonitrile was prepared. The alpha-phthalimidonitrile was allowed to interact with a salt of hydrazoic acid, forming a 5-(1'-phthalimidoalkyl)-tetrazole which was subsequently converted to a 5-(1'-amino-alkyl)tetrazole by hydrazinolysis.



R: $-C_2H_5$, $-CH(CH_3)_2$, $-CH_2CH(CH_3)_2$.

The related acylaminonitrile was prepared from the appropriate aldehyde, in the alternate method, by interaction with ammonium cyanide and acylation of the resulting alpha-aminonitrile. The acylaminonitrile was then allowed to interact with an azide to form a 5-(1'-acylaminoalkyl) tetrazole, from which the 5-(1'-aminoalkyl)tetrazole was obtained by hydrolysis.



R: $-C_2H_5$, $-CH(CH_3)_2$, $-CH_2CH(CH_3)_2$.

R': $-C_6H_5$, $-CH_3$.

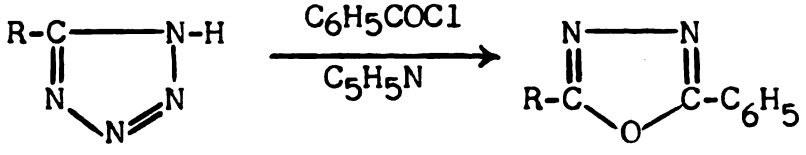
A comparison of the products obtained from the two different procedures established the identity of the 5-(1'-aminoalkyl)tetrazoles.

5-(1'-Amino-1'-methylethyl)tetrazole and 5-(1'-aminobenzyl)tetrazole were prepared from acetone and benzaldehyde, respectively, by the second procedure described above, and 5-(3'-aminopropyl)tetrazole was prepared from gamma-chlorobutyronitrile by interaction with potassium phthalimide to give gamma-phthalimidonitrile, which was then allowed to react with an azide to form the tetrazole. The preparation of 1,3-bis-(5'-tetrazolyl)propane was accomplished by the interaction of glutaronitrile with an azide.

The tetrazole analogs of the six amino acids prepared in this manner were characterized by preparation of their acyl and benzoyl derivatives, and by their reaction with

phenyl isocyanate to form substituted ureas. The pK_1 and pK_2 values of the six aminoalkyltetrazoles were determined by titration with standard acid and base in aqueous solution at 25° C.

In order to provide further confirmation of the identity of 5-aminomethyltetrazole, 5-(1'-aminopropyl)tetrazole, 5-(1'-amino-2'-methylpropyl)tetrazole, 5-(1'-amino-1'-methylethyl)tetrazole, 5-(1'-aminobenzyl)tetrazole, 5-(3'-aminopropyl)tetrazole, and 1,3-bis-(5'-tetrazolyl)propane, these compounds were allowed to react with benzoyl chloride and pyridine to form 2-substituted-5-phenyl-1,3,4-oxadiazoles.



The probable mechanism of this reaction is discussed in Part II.

Hydrolytic degradation of the 1,3,4-oxadiazoles was effected by heating with dilute hydrochloric acid. This degradation was shown, by isolation of the products of hydrolysis, to result in the formation of hydrazine dihydrochloride, benzoic acid, and the appropriate amino acid hydrochloride.

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The writer would like to express his thanks to Doctor Robert M. Herbst for the invaluable guidance, helpful advice and encouragement that he contributed toward the completion of this work.

Thanks are also extended to the Schering Corporation, who provided financial assistance through their Fellowship Program during the academic year of 1958-1959.

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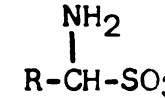
INTRODUCTION

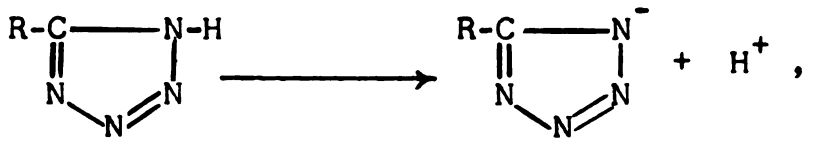
A great deal of interest has been demonstrated in recent years in the development of compounds which have a close chemical and structural similarity to essential metabolites of the living cell, such as amino acids, vitamins, and hormones. Quite frequently, it has been found that a slight alteration of the molecular structure of a physiologically active compound has led to the appearance, upon administration of the analog to living organisms, of symptoms associated with a deficiency of the unaltered material. Structural analogs which possess this characteristic are known as antimetabolites, and the antagonism which they demonstrate toward their physiologically active counterparts is thought to be associated with an inhibitory effect exerted by the analog upon the enzyme systems involved in the metabolism of the original compounds.

Since the utilization of amino acids as metabolites by all living organisms constitutes an area of fundamental importance in the study of biochemical processes, a number of attempts have been made to prepare structurally related metabolic antagonists of these materials. One of the first and most successful of these investigations was the study of a series of alpha-amino sulfonic acids,

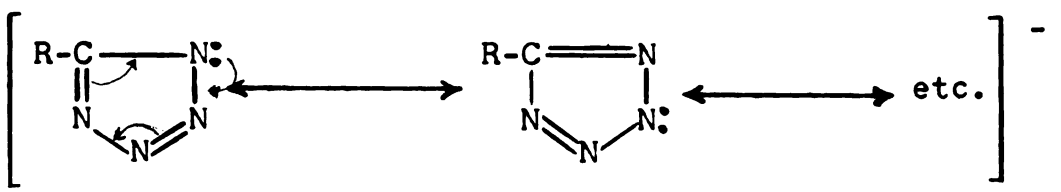


as potential antimetabolites for alpha-amino acids. Preparation of the sulfonic acid analogs of glycine, c-phenylglycine, valine, alanine, and leucine has been accomplished and some specific inhibition of these compounds for the analogous amino acids was reported (1,2). The optical antipodes of both L-serine and L-leucine have been shown to exhibit an antagonism to the action of these metabolites, and the replacement of one of the hydrogen atoms on a terminal methyl group of valine by a chlorine atom has been demonstrated to result in a weak inhibition of bacterial growth, which is reversed by the addition of valine to the culture medium (3,4).

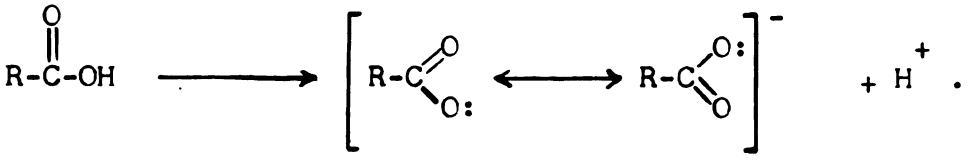
The ability of the tetrazole ring, when substituted only in the 5 position, to dissociate in aqueous solution, providing a tetrazole anion and a proton,



is a well established concept. (5,6,7). This behavior may be readily explained by attributing the acidity of these compounds to resonance stabilization of the anion formed,

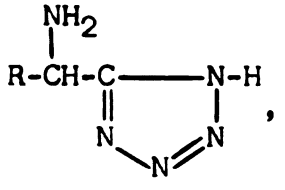


just as the acidity of organic carboxylic acids may be accounted for in a similar manner,



A comparison of the apparent dissociation constants of a representative group of 5-substituted tetrazoles has shown the acidity of these compounds to parallel closely their carboxylic acid counterparts (8).

As a consequence of their acidic behavior in aqueous solution, a number of 5-substituted tetrazoles have been investigated as potential antagonists for acidic metabolites to which they correspond structurally. The preparation of a series of 5-(1'-aminoalkyl)tetrazoles,



has recently been described by McManus (9), who has successfully prepared the tetrazole analogs of glycine, D,L-alanine, beta-alanine, D,L-phenylalanine, D,L-tryptophan, and a number of physiologically active organic acids. These compounds are of considerable chemical and physiological interest, since both their chemical reactivity and molecular construction possess a marked resemblance to active metabolic

intermediates. The structural geometry of a typical alpha-amino acid and its tetrazole counterpart is similar except for possible effects due to the relative size of the carboxyl and tetrazolyl groups. From a consideration of the bond distances and angles involved, these groups may be expected to have similar steric requirements. The work done by McManus (9) indicates that the pK_1 and pK_2 values of glycine, alanine, beta-alanine, and phenylalanine are of the same order of magnitude as the dissociation constants of the tetrazole analogs of these compounds.

The initial purpose of the present work was threefold: first, to extend the series of tetrazole analogs of amino acids to include a number of additional potential amino acid antagonists; second, to investigate the characteristic physical and chemical properties of these compounds; and third, to develop a more convenient method for the synthesis of suitably blocked aminonitriles as precursors of 5-(1'-aminoalkyl)-tetrazoles.

A series of five 5-(1'-aminoalkyl)tetrazoles has been prepared, corresponding to the following alpha-amino acids:

1. D,L-alpha-aminobutyric acid
2. D,L-valine
3. D,L-leucine
4. alpha-aminoisobutyric acid
5. D,L-C-phenylglycine

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The first three of these compounds were prepared by two different methods of synthesis in order to establish unequivocally the structure of the final product. 5-(3'-Aminopropyl)tetrazole was also synthesized as a potential antimetabolite of gamma-aminobutyric acid. A discussion of the preparation and characteristics of these 5-(aminoalkyl)-tetrazoles, including a description of the experimental procedures used, constitutes Part I of this thesis.

When an attempt was made to prepare the benzoyl derivative of 5-(3'-aminopropyl)tetrazole, the percentage composition of the product isolated corresponded to the empirical formula, $C_{18}H_{17}N_3O_2$, rather than to the formula, $C_{11}H_{13}N_5O$, of the expected product. An explanation of this behavior, based on the work of Huisgen, Sauer, and Sturm (10), is developed in Part II of this thesis. Application of the reaction to a number of other 5-(aminoalkyl)tetrazoles and the use of this reaction for verification of the structure of the 5-(aminoalkyl)tetrazoles is also discussed in Part II.

HISTORICAL

The first synthesis of a 5-aminoalkyltetrazole to be reported in the literature was the preparation of 5-(2'-aminoethyl)tetrazole hydrochloride from beta-benzamidopropionitrile by Ainsworth (11) in 1953. Three different methods of synthesis were used to obtain the material. Ainsworth originally prepared the compound by a quantitative conversion of the corresponding nitrile to an iminoether hydrochloride by the method of McElvain and Nelson (12). Conversion of the iminoether hydrochloride to 5-(2'-benzamidoethyl) tetrazole was accomplished by the procedure which Oberhummer (13) used in 1933 to prepare 5-methyltetrazole. Hydrolysis of the benzamido compound with concentrated hydrochloric acid gave the 5-(2'-aminoethyl)tetrazole hydrochloride.

Oberhummer's procedure, which was the earliest preparative technique given in the literature for the synthesis of a 5-alkyltetrazole, was an adaptation of the method developed by Pinner (14) in 1897 for the synthesis of 5-aryltetrazoles. Pinner's work was based on the reaction of iminoether hydrochlorides with hydrazine to form amidrazone hydrochlorides, which were subsequently converted by diazotization with nitrous acid to imidyl azides. The latter cyclized to form the tetrazoles. The procedure was modified by Oberhummer only to the extent that amyl nitrite

rather than sodium nitrite was used in the diazotization step.

A second method of synthesis used by Ainsworth to prepare 5-(2'-aminoethyl)tetrazole hydrochloride involved interaction of beta-benzamidopropionitrile and hydrazoic acid in boiling xylene, followed by hydrolysis of the resulting 5-(2'-benzamidoethyl)tetrazole with concentrated hydrochloric acid as in the first method.

The use of hydrazoic acid in xylene as a means of preparing tetrazoles from nitriles is a modification of a procedure developed in 1950 by Mihina and Herbst (6) in which the nitrile was heated for a prolonged period in a sealed tube with a solution of hydrazoic acid in benzene. This technique was successfully used to produce both 5-alkyl and 5-aryltetrazoles in good yields from the corresponding alkyl or aryl cyanides, but possessed the disadvantage of requiring a pressurized reaction system. An improved procedure has since been developed by Herbst and Wilson (7) for the preparation of 5-aryltetrazoles in which the desired product is obtained by treatment of the aryl cyanide with hydrazoic acid that is generated in situ in refluxing butyl alcohol from sodium azide and glacial acetic acid.

The third technique which Ainsworth employed to prepare 5-(2'-aminoethyl)tetrazole hydrochloride involved the reaction of ethyl beta-benzamidopropionimidate hydrochloride directly with a solution of hydrazoic acid in glacial acetic

acid, and subsequent acid hydrolysis of the product.

In 1956, Behringer and Kohl (15) reported the preparation of the hydrochlorides of 5-(2'-aminoethyl)tetrazole and 5-aminomethyltetrazole from beta-phthalimidopropionitrile and acetamidoacetonitrile, respectively, by refluxing the nitriles with a threefold excess of aluminum azide in tetrahydrofuran. The aluminum azide was generated in situ in the reaction medium by the addition of a solution of anhydrous aluminum chloride in tetrahydrofuran to a suspension of the nitrile and sodium azide in the same solvent. The reaction gave the acylaminoalkyltetrazoles as product in 80% yield, as compared to an average 10% yield by the methods which Ainsworth used.

A modification of the method of Behringer and Kohl was reported by Finnegan, Henry, and Loftquist (16) in 1958. In this procedure, a mixture of the nitrile, ammonium chloride, and sodium azide was heated in dimethylformamide for varying periods of time, depending upon the nitrile used. After the solvent was removed under diminished pressure, the tetrazole was liberated from its ammonium salt by treatment of an aqueous solution of the residue with dilute mineral acid. The yields obtained were comparable to those reported by Behringer and Kohl, and the process had the advantage of being readily adaptable to large scale work. It was found useful for the preparation of both 5-alkyl and 5-aryltetrazoles, and the reaction time was generally shorter than

required by the technique of Behringer and Kohl. One of the compounds which Finnegan and his co-workers described was 5-(4'-aminophenyl)tetrazole, an analog of the physiologically important para-aminobenzoic acid.

The synthesis of tetrazoles as potential antimetabolites has been extensively investigated by McManus (9), who prepared the tetrazole analogs of glycine, D,L-alanine, beta-alanine, D,L-phenylalanine, D,L-tryptophan, para-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, as well as a number of related compounds. The five 5-aminoalkyltetrazoles were characterized by the preparation of their acetyl, benzoyl, and phenylurea derivatives, and, with the exception of the tryptophan analog, by the determination of their pK_1 and pK_2 values, using potentiometric titration. For the synthesis of the acylaminonitrile precursors of these tetrazoles, McManus employed three different methods. In one procedure, the phthalimidonitrile was obtained from the corresponding amino acid by fusion with phthalic anhydride and conversion of the phthalimido acid into the acid chloride and amide as intermediates. A second technique involved the conversion of an alpha-haloacyl halide to a 1-benzyl-5-aminoalkyltetrazole by the procedure of Harvill, Herbst, and Schreiner (17), and catalytic debenzylation of the product with hydrogen. The phenylalanine and tryptophan analogs

were prepared in a third way; the alkylation of ethyl acetamidocyanoacetate, formation of the tetrazole from the resulting alkylated ethyl acetamidocyanoacetate by a modification of the method of Behringer and Kohl (15), followed by hydrolysis and decarboxylation.

The synthesis of a group of 5-aminoaryltetrazoles was reported in 1958 by Veldstra and co-workers (18,19), who used the method of Mihina and Herbst (6) to obtain a series of tetrazole analogs of physiologically and pharmacologically active carboxylic acids such as para-aminobenzoic acid, 2-hydroxy-4-aminobenzoic acid, picolinic acid, nicotinic and isonicotinic acids, 2,4-dichlorophenoxyacetic acid, and 3-indoleacetic acid. All of these compounds had previously been prepared by McManus (9), using the procedure of Herbst and Wilson (7).

The formation of a 2,5-disubstituted-1,3,4-oxadiazole from a 5-monosubstituted tetrazole was first reported in 1929 by Stolle (20), who reported the formation of 2-acetamido-5-methyl-1,3,4-oxadiazole from 5-acetamidotetrazole on prolonged heating of the tetrazole with acetic anhydride. Stolle proposed that the reaction proceded by elimination of the nitrogen atoms in the 2 and 3 position of the tetrazole ring as nitrogen gas to form a nitrogen diradical, followed by the shift of an acetamido group from the ring carbon to the diradical in a Curtius type rearrangement to give a carbodiimide, which subsequently underwent acylation, tautomerization, and ring closure to form the

1,3,4-oxadiazole.

A similar rearrangement has been described by Herbst and Klingbeil (21), who utilize the mechanism of Stolle to account for the formation of 2-(para-nitrophenylamino)-5-methyl-1,3,4-oxadiazole from either 5-(para-nitrophenylamino)tetrazole or 1-(para-nitrophenyl)-5-aminotetrazole on prolonged heating with acetic anhydride. The structure of this material was established by analysis, identification of the products of hydrolytic decomposition, and independant synthesis of the oxadiazole formed in the reaction.

A recent publication of Huisgen, Sauer, and Sturm (10) described the conversion of 5-phenyltetrazole into 2-(para-nitrophenyl)-5-phenyl-1,3,4-oxadiazole on gentle heating with para-nitrobenzoyl chloride and pyridine. The general character of the reaction was established by conversion of a number of 5-alkyl and 5-aryltetrazoles to oxadiazoles by warming with pyridine and a wide variety of acylating agents such as acetyl, benzoyl, para-toluyl, and succinyl chloride, as well as both acetic and benzoic anhydrides. A mechanism was proposed for the reaction, but no experimental detail was given.

PART I

DISCUSSION

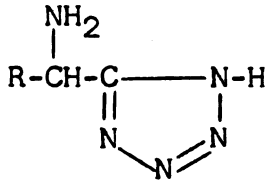
The phenomenon of antagonism between structurally similar metabolites has been known to biochemists for several decades, and has been explained on the basis of the existence of a competition between the similar species for an enzyme or enzyme system with which both are capable of associating. An antagonism of this nature may be demonstrated between sodium and potassium ion in Lactobacillus casei (22), adenosine and cytidine in Neurospora mutants (23), between glycine and alanine in Streptococcus fecalis (24), and in many other cases.

The application of this concept has been extended to include the antagonism of a metabolite by a structurally related non-metabolite, or "antimetabolite". A competitive inhibition of the utilization of the metabolite occurs in the instance where the interaction between the antimetabolite and the affected enzyme system is reversible; a non competitive inhibition results when a high degree of irreversibility is shown in the antimetabolite-enzyme reaction. The investigation of compounds which are either isosteric with biologically active materials or show similar chemical reactivity has received a decided impetus from the considera-

tion that such compounds may possess activity as metabolic antagonists.

The alpha-amino acids, as a consequence of their prominent role in the metabolism of living organisms, have served in a number of instances as a model for the synthesis of potential antimetabolities. Attempts have been made to alter the structural constitution of the amino acid molecule in several different ways, such as by extension or rearrangement of the carbon skeleton of the molecule, replacement of one or more hydrogen atoms by halogen atoms, conversion of the carboxyl group to a carboxamide, or replacement of the carboxyl group by a functional group of related structure and reactivity, such as a sulfonic acid or tetrazolyl moiety (25).

This chemical investigation was initially undertaken for the purpose of preparing a group of 5-(1'-aminoalkyl)-tetrazoles,



as analogs and potential antimetabolites of the corresponding amino acids,

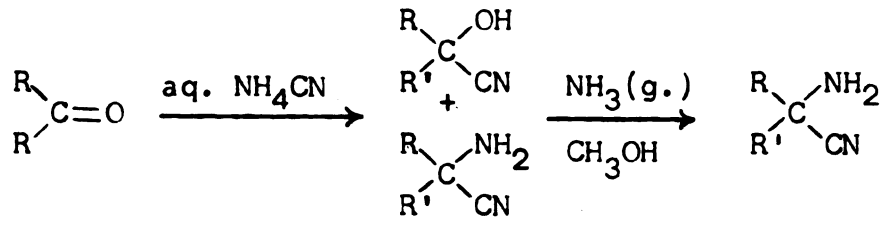
$$\begin{array}{l} NH_2 \\ | \\ R\text{-}CH\text{-}COOH \end{array} ,$$

and to determine the characteristic physical and chemical properties of these compounds for comparison with their amino acid counterparts.

All of the methods applicable to the synthesis of 5 mono-substituted tetrazoles, including the 5-aminoalkyltetrazoles analogous to alpha-amino acids, are of fundamentally the same type. The preparative techniques cited in the literature for this class of tetrazoles are similar in that they utilize an interaction between hydrazoic acid, or one of its salts, and a nitrile to produce the 5-substituted tetrazole ring system. Consequently, the synthesis of 5-(1'-aminoalkyl)tetrazoles first requires the development of a procedure for the preparation of the corresponding alpha-aminonitrile, with the amino group blocked by a suitable acylating agent.

A plausible and relatively short route to the alpha-aminonitriles appeared to be the interaction of ammonium cyanide with the appropriate aldehydes or ketones under the conditions used in the Strecker synthesis of amino acids. This reaction has been shown to result in an equilibrium mixture of the cyanohydrin of the carbonyl compound and an alpha-aminonitrile of related structure. The yield of alpha-aminonitrile may be increased by dissolving the mixed

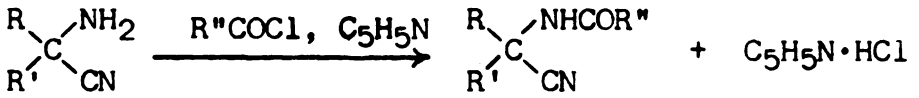
reaction product in methanol and saturating the solution with dry gaseous ammonia (26).



R: alkyl

R': alkyl or H

Acylation of the alpha-aminonitrile obtained as a product in this sequence of reactions could be accomplished by using an acyl pyridinium salt, such as acetyl or benzoyl pyridinium chloride. These compounds have been shown by Terss and McEwan (27) and Thompson (28) to be powerful acylating agents at low temperatures, and may readily be obtained by mixing equimolecular quantities of the acyl halide and pyridine.



The exploration and development of this experimental procedure was undertaken in order to establish its usefulness as a general method for the synthesis of acylated alpha-aminonitriles. The results of this investigation indicate that the method is applicable to propionaldehyde, isobutyraldehyde,

isovaleraldehyde, benzaldehyde, and acetone.

The choice of tetrazole analogs to be investigated was influenced by several factors. The first three 5-(1'-aminoalkyl)tetrazoles prepared were analogs of alpha-aminobutyric acid, valine, and leucine. These compounds were selected for study because of the recognized activity of their amino acid counterparts as metabolites for numerous biological systems. Valine and leucine are among the eight amino acids essential to man, and alpha-aminobutyric acid is known to be associated with the metabolism of methionine, threonine, serine, and glutamic acid.

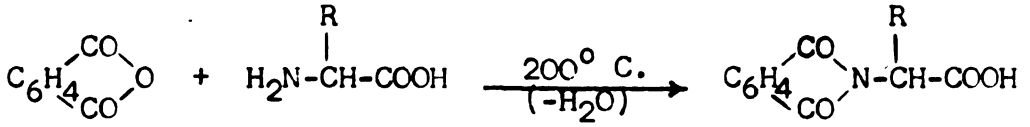
The tetrazole analogs of C-phenylglycine and alpha-aminoisobutyric acid were prepared in order to establish that the preparation of acylated aminonitrile precursors of these compounds from benzaldehyde and acetone, respectively, was feasible. This indicates that the reaction sequence may be used as a general method and is applicable to a variety of carbonyl compounds.

5-(3'-Aminopropyl)tetrazole was the only compound prepared that was not analogous to an alpha-amino acid. gamma-Aminobutyric acid, the carboxylic acid to which it corresponds, is associated with the metabolic breakdown of glutamic acid in brain tissue. The synthesis of the tetrazole was therefore undertaken in order to investigate its possible activity as an antimetabolite. Moreover, since this compound is isomeric with both 5-(1'-aminopropyl)tetrazole and 5-(1'-amino-1'-methylethyl)tetrazole, it was thought that a comparison

of the physical and chemical properties of these three compounds might be of interest.

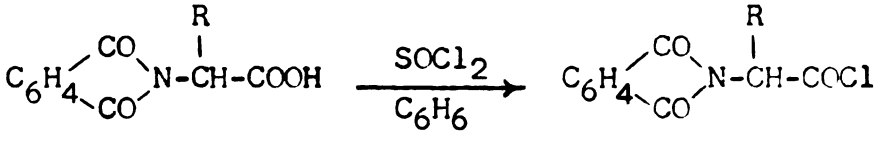
An alternate series of reactions was used to prepare the acylated aminonitriles required for the synthesis of the tetrazole analogs of D,L-alpha-aminobutyric acid, D,L-valine, and D,L-leucine, making it possible to verify the structure of these compounds by comparison of the final products obtained from the two procedures. The racemic alpha-amino acids, which served as starting materials, were subjected to a stepwise sequence of four reactions to give the corresponding alpha-phthalimidonitriles. A brief discussion of each of these reactions will illustrate this alternate method of synthesis.

First, the alpha-amino acid was fused with an equivalent quantity of phthalic anhydride, with the formation of an alpha-phthalimido acid.

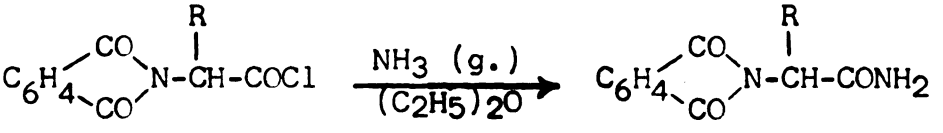


This acylation technique provides a convenient means of "blocking" the reactive amino group. The phthalimido acids are high melting, crystalline products which contain no N-H bond, such as is present in the acylation products obtained with acetic anhydride or benzoyl chloride.

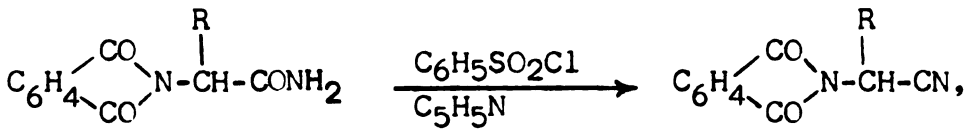
The resulting alpha-phthalimido acid was converted to the acid chloride by reaction with thionyl chloride in a benzene solution.



Conversion of the acid chloride to the alpha-phthalimido-amide was accomplished by saturating an ethereal solution of the acid chloride with gaseous ammonia.



The method of Stephans, Bianco, and Pilgrim (29) was used to convert the alpha-phthalimido amide to the desired nitrile. Dehydration of the amide was accomplished by interaction of the amide with benzene sulfonyl chloride in pyridine. This reaction is ideally suited for dehydration of optically active amides in view of the mildness of the experimental conditions employed.



A comparison of the two methods used to prepare the acylated alpha-aminonitrile intermediates just discussed led to the following conclusions concerning their relative merits and disadvantages:

1. In terms of overall yield, the procedure using the racemic alpha-amino acids as starting materials (hereafter refered to as scheme A for convenience) was superior to the preparation of the corresponding acylated alpha-amino-nitriles from aliphatic aldehydes by the modified Strecker reaction (designated for future reference as scheme B). The average yield of alpha-phthalimidonitrile obtained from alpha-aminobutyric acid, valine, and leucine by scheme A was 61%, as compared with an average 9% yield of acylated alpha-amino-nitrile from propionaldehyde, isobutyraldehyde, and isovaler-aldehyde when scheme B was used.

2. As a preparative technique, scheme B possesses several advantages in terms of convenience and generality of application. The reaction sequence in scheme B involved only two steps, in contrast to the four steps required when the amino acid is the starting material. A comparison of the cost and availability of the starting materials used for the two methods is generally favorable to scheme B, especially

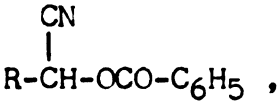
where acetone, rather than alpha-aminoisobutyric acid, may be used as a starting material.

The low yield of acylaminonitrile in scheme B may be attributed to the formation of undesired by-products when the aldehyde or ketone used reacted with ammonium cyanide. The assumption that this step, rather than the acylation step which followed, was responsible for the small amount of acylaminonitrile isolated received support from several sources.

When acetone reacted with ammonium cyanide, a 30% yield of alpha-aminoisobutyronitrile was recovered from the reaction mixture, whereas treatment of alpha-aminoisobutyronitrile with acetyl chloride and pyridine resulted in an 84% yield of alpha-acetamidoisobutyronitrile. Similarly, only a 13% yield of alpha-aminophenylacetonitrile was isolated, as the hydrochloride, from the reaction of benzaldehyde and ammonium cyanide, in contrast to a 68% yield of alpha-acetamidophenylacetonitrile obtained upon acylation of alpha-aminophenylacetonitrile hydrochloride with acetyl chloride and pyridine. These two cases, which represent the only attempts made to isolate the free alpha-aminonitrile intermediates, strongly indicate that the low yields obtained from scheme B are due to competing reactions, such as cyanohydrin formation, when the carbonyl compound and ammonium cyanide were mixed.

The presence of a cyanohydrin in the reaction mixture obtained by interaction of propionaldehyde or isobutyraldehyde with ammonium cyanide, and then ammonia, is shown by the

fact that acylation of each of these reaction mixtures with benzoyl chloride and pyridine resulted in the formation of a considerable quantity of alpha-cyanoalkylbenzoates,



R: CH_3CH_2-, $(CH_3)_2CH$-

in addition to the alpha-benzamidonitriles expected as products. Benzoylation of the propionaldehyde-ammonium cyanide reaction mixture gave the alpha-benzamidobutyronitrile as a solid, and the benzoate of the cyanohydrin as a liquid that was purified by distillation. The amount of alpha-cyanopropylbenzoate obtained accounted for 10% of the propionaldehyde used as starting material. A 15% yield of alpha-cyanoisobutylbenzoate was similarly isolated from the benzoylation of the isobutyraldehyde-ammonium cyanide reaction mixture by distillation of the filtrate after the expected product, alpha-benzamidoisovaleronitrile, had been recovered as a solid.

Several other side reactions are possible in the synthesis of alpha-acylaminonitriles by scheme B. The formation of a secondary amine by the interaction of two molecules of alpha-aminonitrile, according to the following equation,

$$2\ R_2C(CN)NH_2 \longrightarrow (R_2CCN)_2NH + NH_3$$

has been postulated by Lapworth and Coker (30). It is also conceivable that the aminonitrile formed as product could react with the carbonyl compound used as starting material to form an aldimine,

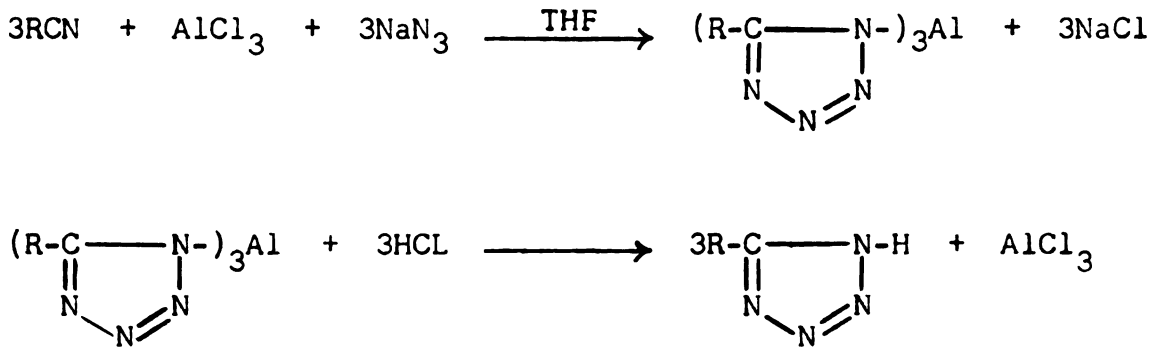
$$R_2C(CN)NH_2 + O{=}C\begin{matrix}R\\R\end{matrix} \longrightarrow R_2C(CN)N{=}C\begin{matrix}R\\R\end{matrix},$$

thereby materially decreasing the yield.

In view of these possibilities, it is not too surprising that the yield of alpha-aminonitrile obtained was only about 10% of the theoretical amount. In this regard, it should also be pointed out that the Strecker synthesis of amino acids is not known to be a high yield procedure; when carried out under optimum conditions with pure reagents, gaseous hydrogen cyanide and ammonia, the average yield is only about 70% (30).

The conversion of the alpha-acylaminonitriles to 5-acylaminoalkyltetrazoles was accomplished in two different ways. The principle method of preparation used was a modification of the technique developed by Behringer and Kohl (15) which involved interaction of anhydrous aluminum chloride in tetrahydrofuran with a suspension of the nitrile and sodium azide in the same solvent. The free tetrazole was liberated

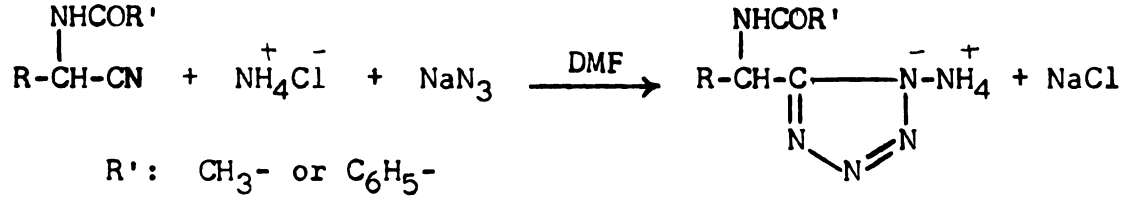
from its aluminum salt by the addition of mineral acid.



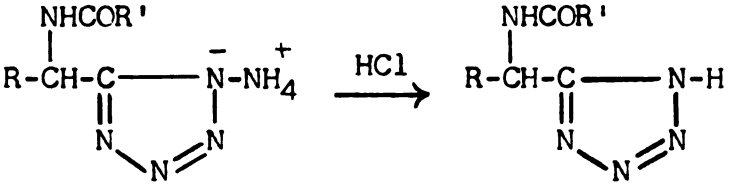
As a means of forcing the chemical equilibrium in the direction of tetrazole formation, Behringer and Kohl used a ratio of three equivalents of azide ion per mole of nitrile. This resulted in the liberation of two moles of hydrazoic acid per mole of product when the reaction mixture was acidified. In order to minimize the amount of hydrazoic acid formed while still maintaining the 3:1 ratio of azide ion to nitrile, the technique of Behringer and Kohl was modified by McManus (9), who isolated the aluminum tetrazolate and washed it free of excess azide ion prior to acidification to liberate the free tetrazole. This was possible because the aluminum salts of 5-acylaminoalkyltetrazoles are negligibly soluble in water, permitting their isolation from the reaction mixture by displacement of the lower boiling tetrahydrofuran with water, and subsequent filtration of the resulting aqueous suspension of the aluminum tetrazolate.

An alternate technique used for the conversion of

several acylaminonitriles to the corresponding 5-acylaminoalkyltetrazoles was based on a procedure that Finnegan, Henry, and Loftquist (16) described in 1958. In this procedure, equivalent quantities of the nitrile, sodium azide, and ammonium chloride are heated in dimethylformamide.



The 5-acylaminoalkyltetrazole was liberated from its ammonium salt in aqueous solution by treatment with mineral acid.



The results obtained by application of the two preparative techniques to specific acylaminonitriles are summarized in Table I.

The preparation of 5-(1'-benzamido-2'-methylpropyl)-tetrazole from alpha-benzamidoisovaleronitrile was carried out by using both the aluminum azide and the ammonium azide

procedure in an attempt to determine which method gave a better yield of benzamidoalkyltetrazole. Equimolecular quantities of the same sample of alpha-benzamidoisovaleronitrile were used for the reaction, the reaction time was the same, and the difference in reaction temperature was never greater than 10° C. for the two cases. Under these conditions, the ammonium azide procedure gave a 78% yield of pure 5-(1'-benzamido-2'-methylpropyl)tetrazole, compared with a 53% yield of the same material obtained by using aluminum azide as the effective reagent.

In general, the ammonium azide method was found to be a more convenient technique for the preparation of 5-substituted tetrazoles than the aluminum azide method because, when ammonium azide was used, the reagents could be directly combined and did not require either preliminary solution, an extended mixing time, or particular care to maintain an anhydrous reaction medium. The isolation and purification of the 5-substituted tetrazoles formed by the ammonium azide method was sometimes complicated, however, by the formation of an oil, which crystallized to give the desired product only after prolonged cooling.

TABLE I

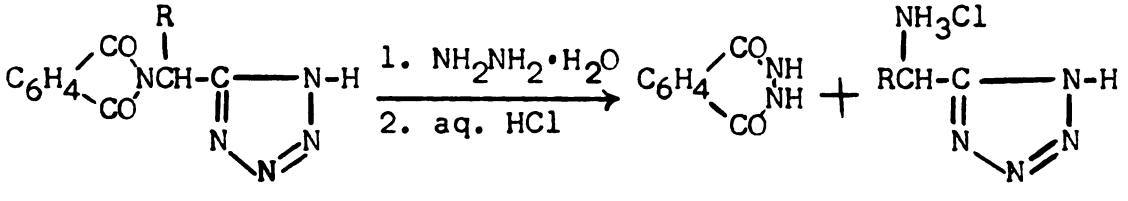
A summary of yields obtained in the conversion of acylaminonitriles to tetrazoles.

Nitrile	Effective Reagent	
	$Al(N_3)_3$	NH_4N_3
Scheme A:		
alpha-phthalimidobutyronitrile	70%	
alpha-phthalimidoisovaleronitrile	67%	
alpha-phthalimidoisocapronitrile	66%	73%
gamma-phthalimidobutyronitrile [a]	91%	
Scheme B:		
alpha-benzamidobutyronitrile	56%	
alpha-benzamidoisovaleronitrile	53%	78%
alpha-acetamidoisocapronitrile		21% [b]
alpha-acetamidoisobutyronitrile		54%
alpha-acetamidophenylacetonitrile	74%	

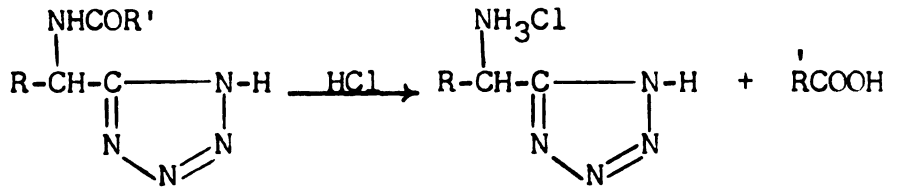
a) Prepared from gamma-chlorobutyronitrile

b) Yield of 5-(1'-amino-1'-methylethyl)tetrazole. Intermediate acylaminotetrazole was not isolated.

After the acylaminoalkyltetrazoles had been prepared, the next step in the synthesis of the desired aminoalkyltetrazoles was the removal of the acyl group from the molecule. In the case of the phthalimidoalkyltetrazoles this was accomplished by hydrazinolysis of the phthalimido group, according to the procedure described by Sheehan and Bolhofer (32). A solid addition complex is first formed by interaction of the phthalimidoalkyltetrazole with a slight excess of hydrazine hydrate (85%) in alcoholic solution. The complex is decomposed by treatment with an equivalent amount of dilute hydrochloric acid to give the aminoalkyltetrazole hydrochloride and phthalhydrazide.

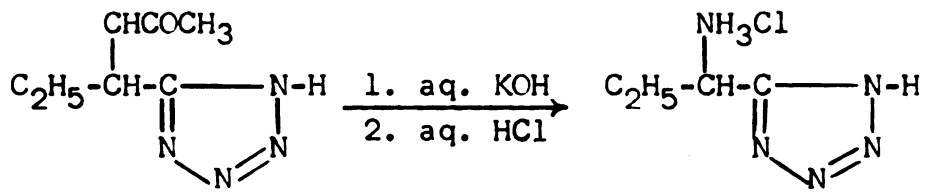


Hydrolysis of the acetyl and benzoyl derivatives of the 5-alkylaminotetrazoles prepared by scheme B was accomplished in all but one case by refluxing these compounds with 6N hydrochloric acid. Concentrated hydrochloric acid (12N) was employed for the hydrolysis of 5-(alpha-acetamidobenzyl)-tetrazole, the C-phenylglycine analog.

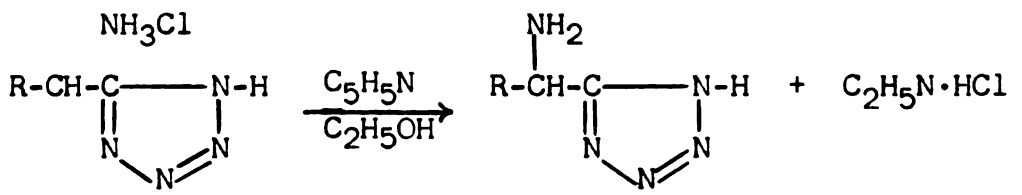


R': CH_3-, C_6H_5-

The hydrolysis of 5-(1'-acetamidopropyl)tetrazole was accomplished by heating with 15% aqueous potassium hydroxide and acidifying the reaction mixture with hydrochloric acid to obtain 5-(1'-aminopropyl)tetrazole as the hydrochloride.



The final step in the preparation of the series of 5-(1'-aminoalkyl)tetrazoles was the treatment of the 5-(amino-alkyl)tetrazole hydrochloride in ethanol solution with a molar equivalent of pyridine. Upon cooling the solution, the free tetrazole slowly precipitated; sometimes several days were required for maximum precipitation.



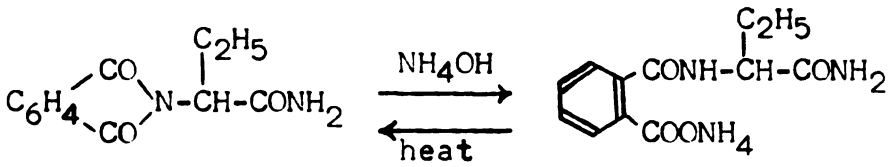
When an attempt was made to prepare 5-(3'aminopropyl)-tetrazole from its hydrochloride in this manner, however, 5-(3'-aminopropyl)tetrazole hydrochloride was recovered unchanged upon cooling the alcoholic solution. This indicated that pyridine is intermediate in basic strength between the 5-(1'-aminoalkyl)tetrazoles and 5-(3'-aminopropyl)-tetrazole and is not basic enough to compete with the terminal amino group of the latter compound for the available proton in alcoholic solution. The free 5-(3-aminopropyl)-tetrazole was prepared by the addition of exactly one molar equivalent of sodium hydroxide to the aqueous solution of 5-(3'aminopropyl)tetrazole hydrochloride, evaporating the reaction mixture to dryness, and extracting the solid residue with absolute ethanol. Evaporation of the alcoholic extract to dryness gave a solid residue which, after several recrystallizations from 95% ethanol to remove the last traces of sodium chloride, yielded a pure sample of 5-(3'-aminopropyl) tetrazole.

In most instances, the application of scheme A and scheme B to the preparation of individual tetrazole analogs of amino acids requires no elaboration. However, in several cases, the results obtained require some additional comment. A brief discussion of these results and their theoretical implications follows.

The alpha-phthalimidoacyl chlorides obtained from alpha-aminobutyric acid, valine and leucine by scheme A are new

compounds. Purification of these compounds by distillation at diminished pressure, failed to yield products of analytical purity. However, their elemental analyses varied only 1-2% from the theoretical values. In the case of alpha-phthalimidobutyryl chloride, a 94% yield of the corresponding amide was isolated by treatment of an ethereal solution of the distilled acid chloride with gaseous ammonia. The structure of the alpha-phthalimidoacyl chlorides is indirectly verified by the facts that both the alpha-phthalimido acids from which they were prepared and the alpha-phthalimidoamides resulting from their reaction with ammonia possess physical properties identical with those previously reported or have percentage compositions which conform to the theoretical values.

In the preparation of alpha-phthalimidobutyramide from alpha-phthalimidobutryl chloride, the addition of the acid chloride to a cold solution of aqueous ammonia gave a product that decomposed at its melting point (198-200° C.) with evolution of ammonia. When the melt was allowed to cool, the solid remelted sharply at 181° C. This is interpreted as an indication that the phthalimido group suffered a base catalyzed hydrolysis on contact with aqueous ammonia to form an ammonium salt which decomposed on heating with loss of ammonia to reform the phthalimide ring.

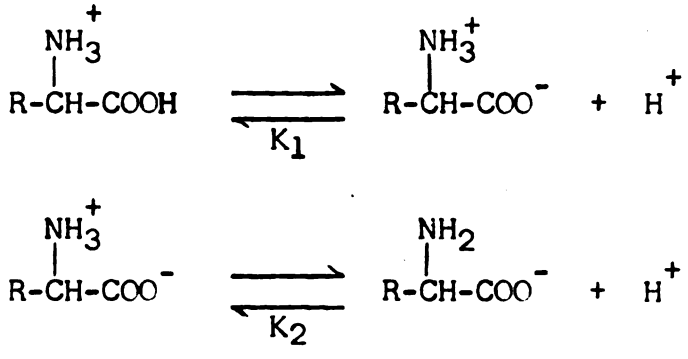


Evidence to support this assumption was provided by warming a small amount of the solid, m.p. 198-200° C., with an aqueous solution of sodium hydroxide. The immediate liberation of ammonia indicated that an ammonium salt was present. Conversion of the ammonium salt to the amide was accomplished smoothly by fusing the high melting material and maintaining it at a temperature above its melting point until the evolution of ammonia was complete. All of the other alpha-phthalimidoacyl chlorides used in scheme A were prepared without difficulty by the addition of gaseous ammonia to an ethereal solution of the acid chloride under anhydrous conditions.

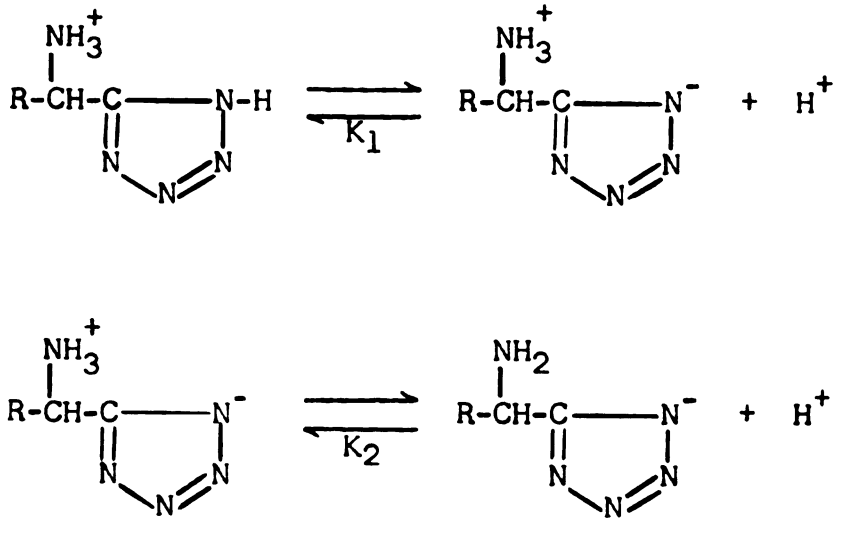
The synthesis of 5-(1'-amino-3'-methylbutyl)tetrazole, the analog of leucine, was undertaken by interaction of the appropriate phthalimidonitrile with ammonium azide in dimethylformamide and with aluminum azide in tetrahydrofuran. This was the only instance in which a 5-(1'-phthalimidoalkyl)-tetrazole was prepared by the ammonium azide method. The phthalimidoalkyltetrazole obtained by the ammonium azide method melted at 82-85° C., while that prepared with aluminum azide in tetrahydrofuran melted at 130° C. Both products gave the same 5-(1'-amino-3'-methylbutyl)tetrazole upon removal of the phthalyl group with hydrazine. Elemental

analysis and neutralization equivalents of the low melting material were in close agreement with the calculated values. Unfortunately, an analytical sample of the high melting material was accidentally lost so that no explanation of the anomal y is availabie.

An important physical property of amino acids is their abiiity to act as both a proton donor and a proton acceptor in aqueous soiution. This tendency may be quantitatively measured by titration of the amino acid hydrochiorides with a standard base. Two equivalents of base are required for each mole of hydrochioride present, as a consequence of the following equilibria:



The 5-aminoalkyltetrazoles are similarly amphoteric in nature, exhibiting the related equilibria:



The pK_1 and pK_2 values of the new tetrazole analogs of amino acids have been determined by titration of these compounds in aqueous solution with standard solutions of hydrochloric acid and sodium hydroxide. A summary of the results obtained, including a comparison with the pK_1 and pK_2 values for the corresponding amino acids, is given in Table II and Table III. The data for the individual titrations of the tetrazoles are included along with a typical titration curve, in Appendix I.

An examination of Tables II and III shows that the chemical behavior of the 5-aminoalkyltetrazoles shows a close similarity to their amino acid counterparts. This comparison may be better illustrated by taking the antilogarithm of the difference in pK value between the amino acid and the corresponding tetrazole as a measure of the relative strength of

the acidic and basic functional groups present in the two species. For example, Table II indicates that the proton associated with the carboxyl group of alpha-aminobutyric acid is 4.5 times more acidic than the proton associated with the tetrazolyl group of its analog, 5-(1'-aminopropyl) tetrazole. In the same manner, Table III indicates that the amino group of alpha-aminobutyric acid is 8.0 times more basic than the corresponding amino group of 5-(1'-amino-propyl)tetrazole. The use of this index of relative acidic and basic strength permits direct comparison of the acidity of the carboxyl and tetrazolyl groups by using a function of the equilibrium constant, K_1, as well as a comparison of the indirect effect which these groups have on the basicity of the neighboring amino group.

TABLE II

Apparent pK_1 values of some 5-aminoalkyltetrazoles and corresponding amino acids in aqueous solution at 25° C.

Compound	pK_1	ΔpK_1	Relative Acidity
5-(1'-aminopropyl)tetrazole	3.20	0.65	4.5
alpha-aminobutyric acid [a]	2.55		
5-(1'-amino-2'-methylpropyl)-tetrazole	2.98	0.66	4.6
valine [a]	2.32		
5-(1'-amino-3'-methylbutyl)-tetrazole	3.25	0.89	7.8
leucine [a]	2.36		
5-(1'amino-1'-methylethyl)-tetrazole	3.10	0.74	5.5
alpha-aminoisobutyric acid [b]	2.36		
5-(3'-aminopropyl)tetrazole	4.68	0.45	2.8
gamma-aminobutyric acid [a]	4.23		
5-(1'-aminobenzyl)tetrazole [c]	2.98		

a- E. Cohn and J. Edsall, Proteins, Amino Acids, and Peptides, Reinhold Publishing Corp., New York, N.Y., pp. 84,99.

b- Thermodynamic values, determined by P.K. Smith et. al., J. Biol. Chem., 122, 109 (1937).

c- The pK_1 of C-phenylglycine has not been determined.

TABLE III

Apparent pK_2 values of some 5-aminoalkyltetrazoles and corresponding amino acids in aqueous solution at 25° C.

Compound	pK_2	ΔpK_2	Relative Bacisity
5-(1'-aminopropyl)tetrazole	8.80	0.90	8.0
alpha-aminobutyric acid [a]	9.60		
5-(1'-amino-2'-methylpropyl)-tetrazole	8.62	1.00	10.0
valine [a]	9.62		
5-(1'-amino-3'-methylbutyl)-tetrazole	8.81	0.79	6.2
leucine [a]	9.60		
5-(1'-amino-1'-methylethyl)-tetrazole	8.88	1.32	20.9
alpha-aminoisobutyric acid [b]	10.20		
5-(3'-aminopropyl)tetrazole	10.32	0.11	1.3
gamma-aminobutyric acid [a]	10.43		
5-(1'-aminobenzyl)tetrazole [c]	7.69		

a- E. Cohn and J. Edsall, Proteins, Amino Acids, and Peptides, Reinhold Publishing Corp., New York, N.Y., pp 84, 99.

b- Thermodynamic values, determined by P.K. Smith et. al., J. Biol. Chem., 122, 109 (1937).

c- The pK_2 of C-phenylglycine has not been determined.

Experimental (Part 1)

The Preparation of 5-(1'-Aminopropyl)tetrazole.

(alpha-Aminobutyric acid analog)

Scheme A.

(a) alpha-Phthalimidobutyric acid.

A mixture of 51.6 g. (0.5 mole) of D,L-alpha-aminobutyric acid and 75 g. (0.505 mole) of powdered phthalic anhydride was fused at 210° C. in an oil bath and maintained at this temperature for two hours. The resulting product was recrystallized from benzene to give 113.5 g. (97% of theory) of alpha-phthalimidobutyric acid, m.p. 94-95° C.

This compound is reported by Hildesheimer (32) to have a melting point of 94-95° C.

(b) alpha-Phthalimidobutyryl chloride.

One hundred and thirteen and five tenths grams (0.49 mole) of alpha-phthalimidobutyric acid was dissolved in 200 ml. of hot benzene and a solution of 82 g. (0.69 mole) of thionyl chloride in 50 ml. of benzene was added dropwise with stirring over a one hour period. The reaction mixture was heated at reflux temperature for four hours before the benzene and excess thionyl chloride were removed by distillation at atmospheric pressure. The residue was fractionated

at diminished pressure; and the fraction distilling between 177° C. and 181° C. at 7 mm. was collected as product. The alpha-phthalimidobutyryl chloride distilled into the receiver as a light yellow oil; the yield was 111.5 g. (91% of theory). Upon standing, the oil crystallized to form a waxy solid.

(c) alpha-Phthalimidobutyramide.

One hundred and four grams (0.41 mole) of alpha-phthalimidobutyryl chloride was added dropwise to a stirred solution of 200 ml. of cold aqueous ammonium hydroxide (density 0.9) over a forty-five minute period. The reaction mixture was stirred for two hours at room temperature, and filtered. The solid material was suspended in 400 ml. of water, allowed to stand for twelve hours, and again filtered. The precipitate was washed with water until the washings were neutral to litmus paper and then dried at 80° C., to give 88 g. of material, m.p. 198-200° C. with evolution of ammonia. The product was insoluble in benzene, diethyl ether, and carbon tetrachloride, and was only slightly soluble in absolute ethanol. Since the loss of ammonia upon melting indicated that the phthalimido group had undergone a base catalyzed hydrolysis upon prolonged contact with aqueous ammonia, the material was tested for the presence of an ammonium salt. Warming a small amount of the product with a 0.1N aqueous sodium hydroxide solution resulted in the immediate liberation of ammonia, indicating

that the material was probably the ammonium salt.

The remainder of the 88 g. of material was fused at 220° C., and maintained at this temperature for one hour, when the odor of ammonia could no longer be detected above the melt. After the molten reaction mass had cooled to room temperature, the solid was recrystallized from absolute ethanol to give 56 g. (59% of theory) of alpha-phthalimidobutyramide, m.p. 181° C.

Analysis.

Calculated for $C_{12}H_{12}N_2O_3$: C, 62.06; H, 5.21; N, 12.06.

Found: C, 62.12; H, 5.11; N, 12.21.

(d) alpha-Phthalimidobutyramide (Alternate procedure).

A mixture of 25.8 g. (0.25 mole) of D,L-alpha-aminobutyric acid and 37.1 g. (0.25 mole) of powdered phthalic anhydride was fused in an oil bath at 200° C., and maintained at this temperature for two hours. The melt was cooled to 70° C., and 100 ml. of benzene was added; a homogeneous solution formed. Twenty milliliters (0.275 mole) of thionyl chloride was added directly to the solution, which was next heated at reflux temperature for two hours. The benzene and excess thionyl chloride were removed by distillation at atmospheric pressure, and the residual material was fractionated at diminished pressure. The fraction distilling from 140-144° C., at 2 mm., was collected as alpha-phthalimidobutyryl chloride. It weighed 53 g. (91% of theory).

The acid chloride was dissolved in 1 l. of diethyl ether,

cooled in a brine bath, and saturated with dry, gaseous ammonia. The resulting suspension was filtered, washed with ether, dried in the air, and resuspended in 1 l. of cold water. The aqueous suspension was stirred for fifteen minutes and filtered. The solid so obtained was washed with cold water and dried at 110° C. for twelve hours to give 46 g. (94% of theory for this step) of a fine white powder, m.p. 180° C. A mixture melting point of this material with the product obtained from Scheme A, part (c), gave no depression of the melting point.

(e) alpha-Phthalimidobutyronitrile.

Fifty-six grams (0.24 mole) of alpha-phthalimidobutyramide was suspended in 255 ml. of pyridine and 103 ml. (0.8 mole) of benzene sulfonyl chloride was added dropwise with stirring to the suspension over a thirty minute period. The reaction mixture was heated at its reflux temperature for fifteen minutes and cooled in an ice bath for thirty minutes. The cold solution was poured, with stirring, into 800 ml. of water, and the resulting suspension was filtered. The solid was washed with water and recrystallized from methyl alcohol to give 46 g. (89% of theory) of alpha-phthalimidobutyronitrile, m.p. 98-98.5° C.

Analysis.

Calculated for $C_{12}H_{10}N_2O_2$: C, 67.28; H, 4.71; N, 13.08.

Found: C, 67.54; H, 4.92; N, 13.25.

(f) 5-(1'-Phthalimidopropyl)tetrazole.

Thirty-nine and five tenths grams (0.17 mole) of alpha-phthalimidobutyronitrile and 33.2 g. (0.51 mole) of powdered sodium azide (technical grade) were suspended in 150 ml. of anhydrous tetrahydrofuran. A filtered solution of 23 g. (0.17 mole) of anhydrous aluminum chloride in 255 ml. of anhydrous tetrahydrofuran was added dropwise with stirring over a one hour period. The reaction mixture was heated at its reflux temperature with continuous stirring for forty hours, after which the tetrahydrofuran was distilled from the solution while water was added at such a rate that the volume remained constant. The resulting suspension was cooled in an ice bath and filtered. The solid was washed with water and resuspended in 225 ml. of water. One hundred milliliters of concentrated hydrochloric acid was added dropwise to the stirred suspension over a period of fifty minutes, and the reaction mixture was stirred at room temperature for one hour, cooled in an ice bath for thirty minutes, and filtered. The precipitate was washed with water and dried at 80° C. The yield of crude 5-(1'-phthalimidopropyl)tetrazole, m.p. 150-156° C., was 30.6 g. (70% of theory). Recrystallization of this material from absolute ethanol raised the melting point to 168-168.5° C.

Analysis.

Calculated for $C_{12}H_{11}N_5O_2$: C, 56.02; H, 4.31; N, 27.23.

Found: C, 55.72; H, 4.34; N, 27.34.

(g) 5-(1'-Aminopropyl)tetrazole.

Twenty-seven and five tenths grams (0.107 mole) of 5-(1'-phthalimidopropyl)tetrazole was dissolved in 250 ml. of absolute ethanol and 6.4 ml. (0.105 mole) of 85% hydrazine hydrate was added to the solution. The reaction mixture was boiled under reflux with stirring for five hours, 100 ml. of solvent was removed by distillation at atmospheric pressure, and the suspension was cooled to -15° C. and filtered. The precipitate was dried at 80° C. to give 21.7 g. of fine white powder, m.p. 158° C. with decomposition. This solid was suspended in 135 ml. of water and the suspension was heated to 50° C. Twenty-seven milliliters of concentrated hydrochloric acid was added with stirring, and the reaction mixture was allowed to stand for fifteen minutes. The suspension was filtered, and the filtrate was evaporated to dryness on the steam bath in a stream of air. The crystalline residue was dissolved in sufficient hot absolute ethanol to obtain a clear solution, which was boiled with Norite and filtered into 9 ml. (0.11 mole) of pyridine. The solution was cooled at -15° C. for two days and filtered. The crude 5-(1'-aminopropyl)tetrazole was dried at 80° C.; yield 3.5 g. (28% of theory), m.p. 255-258° C., with decomposition. After recrystallization from aqueous methanol, 2 g. of pure product, m.p. 269-270° C., with decomposition, was obtained.

Analysis.

Calculated for $C_4H_9N_5$: C, 37.78; H, 7.14; N, 55.08.

Found: C, 37.80; H, 7.41; N, 55.27.

Scheme B.

(a) alpha-Benzamidobutyronitrile.

Eighty-seven grams (1.5 moles) of propionaldehyde was dissolved in 100 ml. of diethyl ether and added to a filtered solution of 100 g. (1.87 moles) of ammonium chloride in 250 ml. of water. The mixture was cooled in a brine bath while a solution of 80 g. (1.63 moles) of sodium cyanide in 175 ml. of water was added dropwise with stirring at such a rate that the temperature of the reaction mixture never exceeded 4° C. Stirring and cooling were continued for six hours after the addition was complete. The organic layer was separated from the aqueous phase, and the aqueous phase was extracted with two 100 ml. portions of diethyl ether. The combined extracts were dried over anhydrous sodium sulfate and added dropwise with stirring to a cooled mixture of 121 ml. (1.5 moles) of pyridine, 175 ml. (1.5 moles) of benzoyl chloride, and 100 ml. of diethyl ether. Addition was completed in two hours; the reaction mixture was stirred for two more hours, washed three times with an equal volume of water, twice with 50 ml. portions of a saturated solution of sodium bicarbonate, and twice again with an equal volume of water. The organic layer was dried over anhydrous sodium sulfate, and the ether was removed by distillation at diminished pressure. The residual oil crystallized partially on cooling at -15° C. for three days. The solid material was isolated by filtration and purified by

recrystallization from absolute alcohol to give 19.8 g. (7% of theory) of alpha-benzamidobutyronitrile as a colorless solid, m.p. 106-107° C.

Analysis.

Calculated for $C_{11}H_{12}N_2O$: C, 70.12; H, 6.43; N, 14.89.

Found: C, 69.97; H, 6.30; N, 14.83.

The oily filtrate was distilled at 4 mm. pressure to give 29 g. of a colorless oil which distilled between 117-118° C. and had an index of refraction at 25° C. of 1.5028. This material was identified by its percentage composition as alpha-cyanopropyl benzoate, and accounted for 10% of the propionaldehyde used as starting material.

Analysis.

Calculated for $C_{11}H_{11}NO_2$: C, 69.82; H, 5.86; N, 7.40.

Found; C, 70.05; H, 5.97; N, 7.23.

(b) 5-(1'-Benzamidopropyl)tetrazole.

Twenty-nine and three tenths grams (0.155 mole) of alpha-benzamidobutyronitrile and 23.7 g. (0.365 mole) of powdered sodium azide (technical grade) were suspended in 50 ml. of anhydrous tetrahydrofuran. A filtered solution of 20.8 g. (0.156 mole) of anhydrous aluminum chloride in 180 ml. of the same solvent was added dropwise with stirring over a two hour period. The mixture was heated at reflux temperature with continuous stirring for sixty-three hours, after which the tetrahydrofuran was distilled from the reaction mixture while water was added at such a rate that the

volume remained constant. Fifty milliliters of concentrated hydrochloric acid was added dropwise with stirring to the reaction mixture which was then heated at reflux temperature for two hours and allowed to stand at room temperature for twelve hours. The reaction mixture was evaporated to one half of its original volume on a steam bath, and the hot suspension was filtered. The finely divided colorless solid was recrystallized from absolute ethanol to give 20 g. (56% of theory) of 5-(1'-benzamidopropyl)tetrazole, m.p. 205° C.

Analysis.

Calculated for $C_{11}H_{13}N_5O$: C, 57.13; H, 5.67; N, 30.29.
Found: C, 57.00; H, 5.63; N, 30.58.

(c) 5-(1'-Aminopropyl)tetrazole.

Nineteen grams (0.082 mole) of 5-(1'-benzamidopropyl)-tetrazole was added to a solution of 18.6 g. (0.3 mole) of potassium hydroxide (technical grade pellets) in 100 ml. of water and heated at reflux temperature for twelve hours. Sufficient concentrated hydrochloric acid was added to register pH 1 with universal indicator paper, and the material was cooled to -15° C., and filtered. The filtrate was evaporated to dryness on the steam bath, and the crystalline residue was dissolved in 400 ml. of hot absolute ethanol. Five milliliters (0.062 mole) of pyridine was added to the clear alcoholic solution. After storing at -15° C. for three days, the solid was separated by

filtration to give 2.35 g. (23% of theory) of 5-(1'-aminopropyl)-tetrazole, m.p. 264° C. A mixture melting point of this material with 5-(1'-aminopropyl)tetrazole prepared by scheme A showed no significant depression.

Derivatives of 5-(1'-aminopropyl)tetrazole

5-(1'-Acetamidopropyl)tetrazole.

Six hundred and thirty-six milligrams (0.005 mole) of 5-(1'-aminopropyl)tetrazole and 0.6 g. (0.006 mole) of acetic anhydride were boiled under reflux for six hours in 10 ml. of glacial acetic acid. The excess acetic acid was removed by warming the reaction mixture *in vacuo* on a steam bath, and the residue was allowed to cool to room temperature. The residual viscous oil crystallized after 30 ml. of amyl acetate was added and the mixture was warmed and stirred. Recrystallization of this solid from amyl acetate gave 5-(1'-acetamidopropyl)tetrazole, m.p. 126-127° C.

Analysis.

Calculated for $C_6H_{11}N_5O$: C, 42.59; H, 6.55; N, 41.40.

Found: C, 42.76; H, 6.54; N, 41.54.

5-(1'-Benzamidopropyl)tetrazole.

The preparation of this compound from *alpha*-benzamidobutyronitrile is described in section (b) of scheme B for the preparation of 5-(1'-aminopropyl)tetrazole. It was obtained by recrystallization of the crude product from absolute ethanol as fine needles, m.p. 205° C.

Analysis.

Calculated for $C_{11}H_{13}N_5O$: C, 57.13; H, 5.67; N, 30.29.

Found: C, 57.00; H, 5.63; N, 30.58.

N-Phenyl-N'-1-(5'-tetrazolyl)propylurea.

Six hundred and thirty-six milligrams (0.005 mole) of 5-(1'-aminopropyl)tetrazole was dissolved in 15 ml. of 0.5N aqueous potassium hydroxide solution, 0.8 g. (0.007 mole) of phenyl isocyanate was added, and the mixture was shaken for one hour at room temperature. The reaction mixture was filtered, and the filtrate was acidified to pH 4-5 with concentrated hydrochloric acid. The precipitate which formed was removed by filtration and recrystallized from water to give N-phenyl-N'-1-(5'-tetrazolyl)propylurea as fine colorless needles, m.p. 199.5° C.

Analysis.

Calculated for $C_{11}H_{14}N_6O$: C, 53.64; H, 5.73; N, 34.13.

Found: C, 53.77; H, 5.97; N, 34.21.

The Preparation of 5-(1'-Amino-2'-methylpropyl)tetrazole.

(Valine analog)

Scheme A.

(a) alpha-Phthalimidoisovaleric acid.

A mixture of 58.6 g. (0.5 mole) of D,L valine and 74 g. (0.5 mole) of powdered phthalic anhydride was fused in an oil bath at 200° C. and maintained at this temperature for two hours. The melt was cooled to room temperature and dissolved in 200 ml. of hot benzene. A 10 ml. portion of this solution was boiled with Norite, filtered, and cooled at 5° C. for several hours. The solid material was separated by filtration, recrystallized again from benzene, and dried at 80° C. to give 5.25 g. (85% of theory, based on the portion taken) of alpha-phthalimidoisovaleric acid, m.p. 104-105° C.

Analysis.

Calculated for $C_{13}H_{13}NO_4$: C, 63.15; H, 5.30; N, 5.67.

Found: C, 63.33; H, 5.48; N, 5.81.

(b) alpha-Phthalimidoisovaleryl chloride.

A solution of 50 ml. (0.69 mole) of thionyl chloride in 50 ml. of benzene was added dropwise with stirring to the 190 ml. of the benzene solution of alpha-phthalimidoisovaleric acid in part (a) above. The reaction mixture was heated at reflux temperature for three and one half hours. The benzene and excess thionyl chloride were removed

from the reaction mixture by distillation at atmospheric pressure. The residual material was distilled at diminished pressure, and the fraction distilling from 152-155° C., at 2 mm., was collected as product. The alpha-phthalimidoisovaleryl chloride distilled into the receiver as a light yellow oil, weighing 90.4 g. (72% of theory).

(c) alpha-Phthalimidoisovaleramide.

A solution of 90.4 g. (0.314 mole) of alpha-phthalimidoisovaleryl chloride in 400 ml. of diethyl ether was cooled in an ice bath and saturated with dry ammonia. The resulting suspension was filtered and the solid was washed with ether and dried at 80° C. This solid was suspended in 200 ml. of cold water, filtered, and dried at 80° C., to give 74.8 g. of crude amide, m.p. 173-174° C. Upon recrystallization of this material from absolute ethanol, 69.5 g. (83% of theory) of alpha-phthalimidoisovaleramide was obtained as a colorless powder, m.p. 175-176° C.

Analysis.

Calculated for $C_{13}H_{14}N_2O_3$: C, 63.40; H, 5.73; N, 11.38.

Found: C, 63.45; H, 5.69; N, 11.33.

(d) alpha-Phthalimidoisovaleronitrile.

Sixty-seven grams (0.274 mole) of alpha-phthalimidoisovaleramide was suspended in 340 ml. of pyridine, and 135 ml. (1.05 moles) of benzene sulfonyl chloride was added dropwise to the stirred suspension over a thirty minute period. The reaction mixture was heated at reflux

temperature for twenty minutes and allowed to stand for twelve hours. It was cooled in an ice bath for thirty minutes and the cold solution was poured, with stirring, into 1 liter of water. The resulting suspension was filtered, and the precipitate was washed with water and recrystallized twice from methyl alcohol to give 49 g. (79% of theory) of alpha-phthalimidoisovaleronitrile as a granular solid, m.p. 66° C.

Analysis.

Calculated for $C_{13}H_{12}N_2O_2$: C, 68.41; H, 5.30; N, 12.28.

Found: C, 68.59; H, 5.51; N, 12.09.

(e) 5-(1'-Phthalimido-2'-methylpropyl)tetrazole.

Thirty grams (0.131 mole) of alpha-phthalimidoisovaleronitrile and 25.7 g. (0.394 mole) of powdered sodium azide (technical grade) were suspended in 120 ml. of dry tetrahydrofuran. A filtered solution of 18 g. (0.35 mole) of anhydrous aluminum chloride in 200 ml. of the same solvent was added dropwise with stirring over a one hour period. The mixture was heated at reflux temperature with continuous stirring for forty hours, after which the tetrahydrofuran was distilled from the reaction mixture while water was added at such a rate that the volume remained constant. The resulting suspension was allowed to stand for two days, cooled in an ice bath, and filtered. The solid was washed with water, resuspended in 200 ml. of water, and cooled in an ice bath while 100 ml. of concentrated hydrochloric acid

was added dropwise with stirring during thirty minutes. The reaction mixture was cooled with continuous stirring in an ice bath for six hours, and filtered. The solid was dried for twelve hours at 80° C. leaving 30.1 g. of crude tetrazole. Recrystallization of the crude product from absolute ethanol gave 22 g. (67% of theory) of 5-(1'-phthalimido-2'-methylpropyl)tetrazole as fine, colorless needles, m.p. 166-167° C.

Analysis.

Calculated for $C_{13}H_{13}N_5O_2$: C, 57.55; H, 4.83; N, 25.82.
Found: C, 57.75; H, 4.78; N, 25.79.

(f) 5-(1'-Amino-2'-methylpropyl)tetrazole.

Twenty-one grams (0.078 mole) of 5-(1'-phthalimido-2'-methylpropyl)tetrazole was heated at reflux temperature with 200 ml. of absolute ethanol and 7 ml. (0.115 mole) of hydrazine hydrate (85%) for four and one half hours. The suspension was cooled in a brine bath and filtered to give a fine, colorless powder, m.p. 266° C. This solid was suspended in 150 ml. of water, and the suspension was heated to 55° C. Twenty-eight milliliters of concentrated hydrochloric acid was added with stirring, the suspension was allowed to stand for twenty minutes, and then filtered. The filtrate was evaporated to dryness on a steam bath and the crystalline residue was heated with 400 ml. of absolute ethanol and filtered. The filtrate was treated with 11 ml. (0.135 mole) of pyridine, and the solution was cooled for

forty-eight hours at -15° C. The solid which had separated was filtered and dried at 80° C. to give 4.5 g. (41% of theory) of crude product, m.p. 256° C. Upon recrystallization of the crude material from water, 2 g. of 5-(1'amino-2'-methylpropyl)tetrazole monohydrate, m.p. 263° C. with decomposition, was obtained as cubic crystals. Desiccation of the monohydrate over anhydrous calcium chloride at 110° C. and 2 mm. pressure for twenty-four hours resulted in the loss of 6.29% of the original weight of the sample with the formation of anhydrous 5-(1'-amino-2'-methylpropyl)tetrazole, m.p. 267° C.

Analysis.(monohydrate)

Calculated for $C_5H_{13}N_5O$: C, 37.72; H, 8.23; N, 44.00.

Found: C, 37.28; H, 8.13; N, 43.60.

Analysis.(anhydrous material)

Calculated for $C_5H_{11}N_5$: C, 42.53; H, 7.86; N, 49.61.

Found: C, 42.36; H, 7.98; N, 49.42.

Scheme B.

(a) alpha-Benzamidoisovaleronitrile.

One hundred and thirty-six milliliters (1.5 moles) of freshly distilled isobutyraldehyde was added to a filtered solution of 100 g. (1.87 moles) of ammonium chloride in 250 ml. of water. The solution was cooled in a brine bath while a solution of 80 g. (1.63 moles) of sodium cyanide in 125 ml. of water was added dropwise with stirring at such a rate that the temperature of the reaction mixture never exceeded 4° C. Stirring and cooling were continued for twelve hours after the addition of sodium cyanide was complete. The organic layer was separated from the aqueous phase, and the aqueous phase was extracted with two 100 ml. portions of diethyl ether. The combined extracts were dried over anhydrous sodium sulfate, filtered, and added dropwise with stirring to a cooled mixture of 121 ml. (1.5 moles) of pyridine and 174 ml. (1.5 moles) of benzoyl chloride. Addition was complete in one hour and the reaction mixture was stirred for four hours, diluted with an equal volume of water, and filtered. The solid so obtained was washed with water and recrystallized from 95% ethanol to give 30 g. (10% of theory) of alpha-benzamidoisovaleronitrile as fine, colorless crystals, m.p. 108° C.

Analysis.

Calculated for $C_{12}H_{14}N_2O$: C, 71.26; H, 6.98; N, 13.85.

Found: C, 70.02; H, 7.00; N, 14.03.

The filtrate, which consisted of an aqueous phase and an oily layer, was extracted with two 100 ml. portions of diethyl ether. The ethereal extracts were combined, and the ether was removed by evaporation on a steam bath. The residual oil was dried over anhydrous sodium sulfate and distilled at diminished pressure. The fraction distilling from 106-108° C., at 3 mm., was collected; the yield was 46 g. Elemental analysis of the material showed it to have the composition of alpha-cyanoisobutylbenzoate. The amount isolated accounted for 15% of the isobutyraldehyde used as starting material.

Analysis.

Calculated for $C_{12}H_{13}NO_2$: C, 70.91; H, 6.45; N, 6.89.

Found: C, 70.64; H, 6.52; N, 7.05.

(b) 5-(1'-Benzamido-2'-methylpropyl)tetrazole.

Method 1 (using the procedure of Behringer and Kohl (15).)

Ten and eleven hundredths grams (0.05 mole) of alpha-benzamidoisovaleronitrile and 9.75 g. (0.15 mole) of powdered sodium azide (technical grade) were suspended in 20 ml. of dry tetrahydrofuran. A filtered solution of 6.94 g. (0.052 mole) of anhydrous aluminum chloride in 80 ml. of the same solvent was added dropwise with stirring during twenty minutes. The mixture was heated at reflux temperature with continuous stirring for seventy-two hours, after which the tetrahydrofuran was distilled from the reaction mixture and water added at such a rate that the volume

remained constant. The hot suspension was filtered, the solid washed with water and dried for twelve hours over anhydrous calcium chloride in a vacuum desiccator to give 22.5 g. of crude aluminum salt, m.p. above 260° C. The aluminum salt was suspended in 100 ml. of water, 20 ml. of concentrated hydrochloric acid was added, and the mixture was allowed to stand for thirty minutes at room temperature. The suspension was filtered, the solid was washed with water and dried at 80° C. to give 10.9 g. of crude tetrazole, m.p. 180-185° C. Recrystallization of this material from aqueous ethanol gave 7.1 g. (53% of theory) of crystalline 5-(1'-benzamido-2'-methylpropyl)tetrazole, m.p. 194-196° C.

Method 2 (using the procedure of Finnegan, Henry and Loftquist (16).)

Ten and eleven hundredths grams (0.05 mole) of alpha-benzamidoisovaleronitrile, 3.58 g. (0.055 mole) of powdered sodium azide (technical grade) 2.68 g. (0.51 mole) of ammonium chloride, and 0.1 g. of lithium chloride were suspended in 50 ml. of dimethylformamide. The reaction mixture was heated with stirring on a steam bath for seventy-two hours. The solvent was removed by distillation at diminished pressure, using a water aspirator, and the residue was suspended in 100 ml. of water. After allowing the reaction mixture to stand for thirty minutes, 5 ml. of concentrated hydrochloric acid was added with stirring, and the material was filtered. The solid so obtained was washed with water and

dried for twelve hours over anhydrous calcium chloride in a vacuum desiccator to give 15 g. of crude tetrazole, m.p. 190-191° C. Recrystallization of this material from aqueous ethanol gave 10.34 g. (78% of theory) of crystalline 5-(1'-benzamido-2'-methylpropyl)tetrazole, m.p. 195-196° C. A mixture melting point of this material with the 5-(1'-benzamido-2'-methylpropyl)-tetrazole prepared by method 1 showed no depression.

Analysis.

Calculated for $C_{12}H_{15}N_5O$: C, 58.76; H, 6.16; N, 28.56.
Found: C, 58.95; H, 6.26; N, 28.48.

(c) 5-(1'-Amino-2'-methylpropyl)tetrazole.

A suspension of 33.6 g. (0.137 mole) of 5-(1'-benzamido-2'-methylpropyl)tetrazole in 50 ml. of concentrated hydrochloric acid, and 75 ml. of water was heated at reflux temperature for thirty hours. The reaction mixture was allowed to cool to room temperature, left to stand for twelve hours, and filtered to remove the benzoic acid present. The aqueous filtrate was evaporated to dryness, and the residue was dissolved in sufficient hot absolute ethanol to give a clear solution. The clear alcoholic solution was treated with 11 ml. (0.136 mole) of pyridine, cooled at -15° C. for two hours, and filtered. The solid so obtained was washed with ether and dried at 80° C., to give 5-(1'-amino-2'-methylpropyl)tetrazole as a fine colorless powder, m.p. 266° C. with decomposition. Upon further cool-

ing, a second and third crop of crude product was isolated from the alcoholic mother liquor. The combined material was recrystallized from aqueous ethanol to give 12.5 g. (65% of theory) of 5-(1'-amino-2'-methylpropyl)tetrazole, m.p. 271-272° C. A mixture melting point of this material with the 5-(1'-amino-2'-methylpropyl)tetrazole prepared by scheme A showed no significant depression.

Derivatives of 5-(1'-amino-2'-methylpropyl)tetrazole

5-(1'-Acetamido-2'-methylpropyl)tetrazole.

Seven hundred and six milligrams (0.005 mole) of 5-(1'-amino-2'methylpropyl)tetrazole and 0.6 g (0.006 mole) of acetic anhydride were heated at reflux temperature for four and one half hours in 10 ml. of glacial acetic acid. The excess acetic acid was removed by warming the reaction mixture *in vacuo* on a steam bath, and the material was allowed to cool to room temperature. The crystalline residue was recrystallized from amyl acetate and dried in a vacuum desiccator over anhydrous calcium chloride for twenty-four hours to give 5-(1'-acetamido-2'methylpropyl)tetrazole, m.p. 180° C.

Analysis.

Calculated for $C_7H_{13}N_5O$: C, 45.89; H, 7.15; N, 38.23.

Found: C, 46.00; H, 7.13; N, 38.44.

5-(1'-benzamido-2'-methylpropyl)tetrazole.

The preparation of this compound from *alpha*-benzamido-isovaleronitrile is described in section (b) of scheme B for the preparation of 5-(1'-amino-2'-methylpropyl)tetrazole. It was obtained by recrystallization of the crude product from aqueous ethanol and had a melting point of 195-196° C.

Analysis.

Calculated for $C_{12}H_{15}N_5O$: C, 58.76; H, 6.16; N, 28.58.

Found: C, 58.95; H, 6.26; N, 28.48.

N-Phenyl-N'-1-(5'-tetrazolyl)isobutylurea.

Seven hundred and six milligrams (0.005 mole) of 5-(1'-amino-2'-methylpropyl)tetrazole was dissolved in 15 ml. of 0.5N aqueous potassium hydroxide solution, 0.8 g. (0.007 mole) of phenyl isocyanate was added, and the mixture was shaken at room temperature for one hour. The reaction mixture was filtered, and the filtrate was acidified to pH 4-5 with concentrated hydrochloric acid. The precipitate which formed was removed by filtration and recrystallized from water to give N-phenyl-N'-1-(5'-tetrazolyl)isobutylurea as fine, colorless needles, m.p. 201° C.

Analysis.

Calculated for $C_{12}H_{16}N_6O$: C, 55.37; H, 6.20; N, 32.29.

Found: C, 55.32; H, 6.31; N, 32.32.

The Preparation of 5-(1'-Amino-3'-methylbutyl)tetrazole.

(Leucine analog)

Scheme A.

(a) alpha-Phthalimidoisocaproamide.

A mixture of 62.5 g. (0.476 mole) of D,L-leucine and 71.6 g. (0.484 mole) of powdered phthalic anhydride was heated in an oil bath at 210° C. for three hours. The melt was cooled to room temperature and dissolved in 200 ml. of hot benzene. A solution of 40 ml. (0.55 mole) of freshly distilled thionyl chloride in 40 ml. of benzene was added to the dissolved melt over a 10 minute period. The reaction mixture was heated at reflux temperature for four hours. The benzene and excess thionyl chloride were removed from the reaction mixture by distillation at atmospheric pressure, and the residue was distilled at diminished pressure. The fraction distilling from 192-193° C., at 20 mm., was collected as product. This material was dissolved in 800 ml. of diethyl ether, cooled in an ice bath, and saturated with dry ammonia. The resulting suspension was filtered; the solid was washed thoroughly with ether and dried at 110° C. The solid was suspended in 1 l. of cold water and stirred until a uniform slurry was obtained. After standing for thirty minutes, the slurry was filtered, washed thoroughly with cold water, and the solid dried at 110° C., to give 111 g. of crude amide. Recrystallization of the

crude product from absolute ethanol resulted in 99.2 g. (80% of theory, based on leucine) of alpha-phthalimido-isocaproamide, m.p. 180-181° C.

Analysis.

Calculated for $C_{14}H_{16}N_2O_3$: C, 64.60; H, 6.20; N, 10.76.

Found: C, 64.69; H, 6.40; N, 10.67.

(b) alpha-Phthalimidoisocapronitrile.

Sixty grams (0.23 mole) of alpha-phthalimidoisocaproamide was suspended in 300 ml. of pyridine, and 120 ml. (1.0 mole) of benzene sulfonyl chloride was added dropwise to the stirred suspension over a forty minute period. The reaction mixture was heated at reflux temperature for twenty minutes and cooled in an ice bath. The cold slurry was poured over 800 g. of ice and stirred vigorously until all the ice had melted. The slurry was filtered, the amorphous solid was dissolved in 200 ml. of diethyl ether, and the ethereal solution was washed with water and dried over anhydrous sodium sulfate. After evaporation of the ether on a steam bath, the residual oil was distilled at diminished pressure. The fraction which distilled at 165° C., and 2 mm., was collected as product. This material, which weighed 47.6 g. (85% of theory), solidified upon standing to give fine colorless crystals of alpha-aminoisocapronitrile, m.p. 47-48° C.

Analysis.

Calculated for $C_{14}H_{14}N_2O_2$: C, 69.40; H, 5.83; N, 11.56.

Found: C, 69.35; H, 5.78; N, 11.78.

(c) 5-(1'-Phthalimido-3'-methylbutyl)tetrazole.

Forty-four grams (0.182 mole) of alpha-phthalimidoisocapronitrile and 36 g. (0.554 mole) of powdered sodium azide (technical grade) were suspended in 160 ml. of dry tetrahydrofuran, and a filtered solution of 25 g. (0.187 mole) of anhydrous aluminum chloride in 280 ml. of the same solvent was added dropwise with stirring during ninety minutes. The mixture was heated at reflux temperature with continuous stirring for thirty-six hours, after which the tetrahydrofuran was removed by distillation while water was added at such a rate that the volume remained constant. One hundred and twenty-five milliliters of concentrated hydrochloric acid was added dropwise with stirring to the aqueous suspension over a one hour period, stirring was continued for two hours and forty-five minutes at 0° C., and the suspended solid was separated from the mother liquor by decantation, resuspension in water, and filtration. The gelatinous precipitate so obtained was dissolved in 200 ml. of absolute ethanol, and the alcoholic solution was boiled with Norite and filtered. The filtrate was diluted with diethyl ether, cooled to -15° C., and again filtered. The solid from the second filtration was redissolved in ethyl acetate, precipitated by the addition of petroleum ether,

and filtered. The precipitate was washed with diethyl ether to give 34 g. (66% of theory) of crude 5-(1'-phthalimido-3'-methylbutyl)tetrazole, m.p. 124-127° C. Further recrystallization of this material from an ethyl acetate-petroleum ether solvent system raised the melting point to 130° C.

(d) 5-(1'-Phthalimido-3'-methylbutyl)tetrazole (alternate procedure).

A mixture of 5 g. (0.02 mole) of alpha-phthalimido-isocapronitrile, 1.11 g. (0.021 mole) of ammonium chloride, 1.35 g. (0.02 mole) of sodium azide, and 0.1 g. of lithium chloride in 50 ml. of dimethylformamide was heated at 110° C. for thirty-four hours. The solvent was removed by distillation at diminished pressure (1 mm.), and the residual material was dissolved by shaking with a mixture of 50 ml. of water and 20 ml. of diethyl ether. The ethereal layer was separated from the aqueous phase, and the aqueous phase was acidified to pH 1 with concentrated hydrochloric acid. The ethereal layer was washed with two 20 ml. portions of water and with 40 ml. of 0.25N aqueous sodium hydroxide. The wash liquids were combined and acidified to pH 1 with concentrated hydrochloric acid. Both of the acidic aqueous solutions thus obtained were cooled for five hours at 0° C., and filtered. The precipitates from the two filtrations were combined, and the combined solids were recrystallized from aqueous ethanol to give 4.3 g. (73% of theory) of

crystalline 5-(1'-phthalimido-3'-methylbutyl)tetrazole, m.p. 82-85° C.

Analysis.

Calculated for $C_{14}H_{15}N_5O_2$: C, 58.73; H, 5.30; N; 24.55.

Found: C, 57.90; H, 5.45; N; 24.35.

(e) 5-(1'-Amino-3'-methylbutyl)tetrazole.

A mixture of 32 g. (0.112 mole) of 5-(1'-phthalimido-3'-methylbutyl)tetrazole, m.p. 130° C., 300 ml. of absolute ethanol, and 10.5 ml. (0.172 mole) of hydrazine hydrate (85%) was heated at reflux temperature for eight hours. The suspension was cooled in a brine bath and filtered, and the solid so obtained was resuspended in 200 ml. of water. The aqueous suspension was heated to 55° C., and 50 ml. of concentrated hydrochloric acid was added with stirring. The material was allowed to stand, with occasional stirring, for fifteen minutes. The suspension was filtered, and the filtrate was evaporated to dryness on a steam bath. The crystalline residue, m.p. 196° C., was heated with 500 ml. of absolute ethanol, treated with Norite, and filtered. The filtrate was combined with 16 ml. (0.196 mole) of pyridine, and the solution was cooled to -15° C. for twelve hours and filtered to give 4.5 g. (26% of theory) of crude 5-(1'-amino-3'-methylbutyl)-tetrazole, m.p. 253-256° C. Upon recrystallization from water, the melting point of the product was raised to 276° C.

Analysis.

Calculated for $C_6H_{13}N_5$: C, 46.43; H, 8.44; N, 45.13.

Found: C, 46.25; H, 8.27; N, 44.94.

Scheme B.

(a) alpha-Acetamidoisocapronitrile.

One hundred and thirty-five milliliters (1.33 moles) of freshly distilled isovaleraldehyde was added to a filtered solution of 89 g. (1.66 moles) of ammonium chloride in 225 ml. of water. The solution was cooled in a brine bath, and 120 ml. of diethyl ether was added. A solution of 71 g. (1.45 moles) of sodium cyanide in 160 ml. of water was added dropwise with stirring to the reaction mixture over a three hour period; stirring and cooling were continued for twelve hours after the addition of sodium cyanide was complete. The organic layer was separated from the aqueous phase, and the aqueous phase was extracted with eight 100 ml. portions of diethyl ether. The combined extracts were dried over anhydrous sodium sulfate, filtered, and the solvent removed on a steam bath. The oily residue was dissolved in 300 ml. of methyl alcohol, saturated with dry, gaseous ammonia, and allowed to stand under anhydrous conditions for three days. A stream of dry air was passed through the solution for five hours to remove the excess ammonia and most of the methyl alcohol was removed by evaporation on a steam bath. The resulting oil was added dropwise during one hour to a cooled, stirred slurry of 95 ml. (1.33 moles) of acetyl chloride, and 112 ml. (1.39 moles) of pyridine in 300 ml. of diethyl ether. The reaction mixture was allowed to stand with stirring for four

hours and then was filtered. The filtrate was evaporated on a steam bath to remove the ether, and the residual oil was distilled under diminished pressure. That portion of the material distilling between 74-80° C., 9.86 g. at 2 mm., and between 123-131° C., 31.1 g. at 2 mm., was saved and redistilled separately at the same pressure to give 4 g. of material, b.p. 74-75° C. at 2 mm., and 17.5 g. (9% of theory) of alpha-acetamidoisocapronitrile, b.p. 127-130° C., at 2 mm. The high boiling fraction had an index of refraction at 26° C. of 1.4553. An infra red spectrum of this fraction, using chloroform as a solvent, showed a strong absorption peak at 3.00 microns, indicating the presence of a secondary amide, and a sharp peak at 4.45 microns, indicating the presence of a cyanide group.

(b) 5-(1'Amino-3'-methylbutyl)tetrazole.

Seventeen and one half grams (0.114 mole) of alpha-acetamidoisocapronitrile, 5.93 g. (0.114 mole) of ammonium chloride, 7.84 (0.125 mole) of powdered sodium azide (technical grade), and 0.2 g. of lithium chloride were suspended in 50 ml. of dimethylformamide, and heated with stirring at 105° C., for twenty-five hours. The solvent was removed in vacuo on a steam bath, and the residue was dissolved in 50 ml. of water. The aqueous solution was acidified to a pH of 4-5 with concentrated hydrochloric acid, and cooled in an ice bath. When it was observed that no precipitate formed upon standing, 50 ml. of concentrated hydrochloric

acid was added to the solution, and it was heated at reflux temperature for twelve hours. The reaction mixture was evaporated to dryness, and the residue was extracted with hot absolute ethanol. The alcoholic extract was treated with 10 ml. (0.123 mole) of pyridine and left for several days at a temperature of -15° C. The alcoholic solution was filtered, and the solid so obtained was washed with ether and dried in an oven at 110° C., for five hours, to give 3.6 g. (21% of theory) of crude 5-(1'-amino-3'-methylbutyl)tetrazole, m.p. 268-272° C. When this material was recrystallized from water containing 5% ethanol, and dried at 110° C., the melting point was raised to 275-276° C. A mixture of this material and the 5-(1'-amino-3'-methylbutyl)tetrazole obtained by scheme A showed no depression of the melting point.

Derivatives of 5-(1'-amino-3'-methylbutyl)tetrazole

5-(1'-Acetamido-3'-methylbutyl)tetrazole.

Seven hundred and seventy-one milligrams (0.005 mole) of 5-(1'-amino-3'-methylbutyl)tetrazole and 0.6 g. (0.006 mole) of acetic anhydride were refluxed for one and one half hours in 10 ml. of glacial acetic acid. The excess acetic acid was removed by warming the reaction mixture *in vacuo* on a steam bath, and the residue was allowed to cool to room temperature. The resulting solid was recrystallized from amyl acetate, washed with ether, and dried at 110° C. to give cubic crystals of 5-(1'-acetamido-3'-methylbutyl) tetrazole, m.p. 151-152° C.

Analysis.

Calculated for $C_8H_{15}N_5O$: C, 48.71; H, 7.67; N, 35.51.

Found: C, 48.75; H, 7.50; N, 35.37.

5-(1'-Benzamido-3'-methylbutyl)tetrazole.

Thirty-five hundredths of a gram (0.0025 mole) of benzoyl chloride was added to a solution of 0.3855 g. (0.0025 mole) of 5-(1'-amino-3'-methylbutyl)tetrazole in 5 ml. of 1.25N aqueous sodium hydroxide. The reaction mixture was shaken for one hour at room temperature. The resulting solution was adjusted to pH 4-5 with concentrated hydrochloric acid, and filtered. The solid so obtained was washed with water and ether and dried at 110° C., to give 5-(1'-benzamido-3'-methylbutyl)tetrazole as a colorless

powder, m.p. 165-167° C. The crude product was recrystallized from a 20% ethanol-80% water solvent system to give fine needles, m.p. 169-170° C.

Analysis.

Calculated for $C_{13}H_{17}N_5O$: C, 60.21; H, 6.61; N, 27.01.

Found: C, 60.09; H, 6.65; N, 27.01.

N-Phenyl-N'-1-(5'-tetrazolyl)isoamylurea.

Seven hundred and seventy-one milligrams (0.005 mole) of 5-(1'-amino-3'-methylbutyl)tetrazole was dissolved in 15 ml. of 0.5N aqueous potassium hydroxide solution, 0.8 g. of phenyl isocyanate (0.007 mole) was added, and the mixture was shaken at room temperature for one hour. The reaction mixture was filtered, and the filtrate was acidified to pH 4-5 with concentrated hydrochloric acid. The precipitate was removed by filtration and recrystallized from water to give N-phenyl-N'-1-(5'-tetrazolyl)isoamylurea as fine needles, m.p. 203-204° C.

Analysis.

Calculated for $C_{13}H_{16}N_6O$: C, 56.92; H, 6.61; N, 30.64.

Found: C, 57.08; H, 6.86; N, 30.77.

The Preparation of 5-(1'-Amino-1'-methylethyl)tetrazole.

(alpha-Aminoisobutyric acid analog)

Scheme B.

(a) alpha-aminoisobutyronitrile.

Two hundred and twenty-one milliliters (3.0 moles) of acetone was added to a filtered solution of 200 g. (3.7 moles) of ammonium chloride in 500 ml. of water. The solution was cooled in a brine bath, while a solution of 160 g. (3.2 moles) of sodium cyanide in 350 ml. of water was added dropwise with stirring at such a rate that the temperature of the reaction mixture never exceeded 5° C. The material was stirred for one hour after all the cyanide had been added and was then left to stand for twelve hours at room temperature. The organic layer was separated from the aqueous phase, and the aqueous phase was extracted with seven 250 ml. portions of diethyl ether. The combined ethereal extracts were evaporated to a volume of 200 ml., combined with the organic layer first separated from the reaction mixture, and the combined solutions dried over anhydrous sodium sulfate. The remaining ether was evaporated, and the residue was diluted with 800 ml. of methyl alcohol. The alcoholic solution was cooled in a brine bath, saturated with dry, gaseous ammonia, and left to stand at

room temperature for three days. The excess ammonia was removed by passing a stream of dry air through the alcoholic solution for six hours, and the methyl alcohol was removed by distillation under diminished pressure. The residual material was distilled through a Vigreux column under diminished pressure, and the fraction distilling from 67-70° C., at 28 mm., weighing 82.2 g. (30% of theory), was collected as alpha-aminoisobutyronitrile. This compound is reported by Gulwitsch and Wasmus (33) to have a boiling point of 49-50° C., at 12 mm. pressure.

(b) alpha-Acetamidoisobutyronitrile.

Eighty grams (0.95 mole) of alpha-aminoisobutyronitrile was mixed with 52 ml. (0.65 mole) of pyridine and 100 ml. of diethyl ether, and the solution was cooled to 4° C. in a brine bath. A solution of 46 ml. (0.65 mole) of acetyl chloride in 60 ml. of diethyl ether was added dropwise with stirring at such a rate that the temperature never exceeded 25° C. The reaction mixture was allowed to stand for twelve hours, filtered, and the solid washed with ether. This solid was extracted with diethyl ether in a Soxhlet apparatus for forty-eight hours. Upon cooling of the ethereal extract, 68 g. of crude alpha-acetamidoisobutyronitrile, m.p. 98-100° C., (84% of theory, based on acetyl chloride) separated. The crude product was recrystallized from a 70% diisopropyl-30% ethanol solvent system to give, after drying over anhydrous calcium chloride, 57.8 g. of alpha-acetamido-

isobutyronitrile, m.p. 103-104° C. This compound is reported by Hellsing (34) to melt at 106° C.

(c) 5-(1'-Acetamido-1'-methylethyl)tetrazole.

Fourteen and sixty-seven hundredths grams (0.116 mole) of alpha-acetamidoisobutyronitrile, 8.32 g. (0.128 mole) of powdered sodium azide (technical grade), 6.22 g. (0.116 mole) of ammonium chloride, and 0.1 g. of lithium chloride were suspended in 100 ml. of dimethylformamide. The reaction mixture was heated with stirring on a steam bath for thirty-six hours. The solvent was removed by distillation under diminished pressure, and the residual material was left to stand for forty-eight hours in the refrigerator. The oily supernatant liquid was separated from the solid residue by decantation, and the solid was dissolved in 50 ml. of water and filtered. The filtrate was treated with sufficient concentrated hydrochloric acid (several drops) to give an acidic reaction with Congo Red paper. No precipitation was noted. The acidified filtrate was evaporated to dryness, and the crystalline residue was extracted with hot absolute ethanol. The alcoholic extract was combined with the oily supernatent first separated from the reaction mixture, and the combined solutions were boiled with Norite, filtered, and cooled at -15° C., for twelve hours. The material was filtered, and the solid product was dried in a vacuum desiccator for two hours to give 10.48 g. (54% of theory) of crude 5-(1'-acetamido-1'-methyl-

ethyl)tetrazole, m.p. 151-153° C. Recrystallization of the crude product from a 25% ethanol-75% diisopropyl ether solvent system raised the melting point to 161-162° C.

Analysis.

Calculated for $C_6H_{11}N_5O$: C, 42.59; H, 6.55; N, 41.40.
Found: C, 42.54; H, 6.56; N, 41.48.

(d) 5-(1'-Amino-1'-methylethyl)tetrazole.

Eight and forty-seven hundredths grams (0.05 mole) of 5-(1'-acetamido-1'methylethyl)tetrazole was added to 50 ml. of 6N hydrochloric acid and heated at reflux temperature for thirty-five hours. The resulting solution was evaporated to dryness on a steam bath. The crystalline residue was heated with 50 ml. of absolute ethanol and filtered. The hot alcoholic solution was combined with 5 ml. (0.062 mole) of pyridine, and the material was cooled at -15° C., for several days. The resulting suspension was filtered, and the solid so obtained was washed with ethanol and dried for four hours at 100° C., to give 1.92 g. (33% of theory) of 5-(1'-amino-1'-methylethyl)-tetrazole, m.p. 256.5° C., with decomposition.

Analysis.

Calculated for $C_4H_9N_5$: C, 37.78; H, 7.14; N, 55.08.
Found: C, 37.59; H, 7.02; N, 55.01.

Derivatives of 5-(1'-Amino-1'-methylethyl)tetrazole.

5-(1'-Acetamido-1'-methylethyl)tetrazole.

The preparation of this compound from alpha-acetamido-isobutyronitrile is described in section (c) of scheme B for the preparation of 5-(1'-amino-1'-methylethyl)tetrazole. It was purified by recrystallization of the crude product from a 25% ethanol-75% diisopropyl ether solvent system and had a melting point of 161-162° C.

Analysis.

Calculated for $C_6H_{11}N_5O$: C, 42.59; H, 6.55; N, 41.40.

Found: C, 42.54; H, 6.56; N, 41.48.

5-(1'-Benzamido-1'-methylethyl)tetrazole.

Thirty-five hundredths of a gram (0.0025 mole) of benzoyl chloride was added to a solution of 0.318 g. (0.0025 mole) of 5-(1'-amino-1'-methylethyl)tetrazole in 5 ml. of 1.25N aqueous sodium hydroxide, and the reaction mixture was shaken for one hour at room temperature. The resulting solution was adjusted to a pH of 4-5 with concentrated hydrochloric acid. The precipitate was collected on a suction filter, washed with water and ether, and dried at 110° C. to give 5-(1'-benzamido-1'-methylethyl)tetrazole as a colorless powder, m.p. 203-204° C. The product was recrystallized from a 10% ethanol-90% water solvent system to give cubic crystals, m.p. 204-205° C.

Analysis.

Calculated for $C_{11}H_{13}N_5O$: C, 57.13; H, 5.67; N, 30.29.

Found: C, 57.16; H, 5.56; N, 30.28.

N-Phenyl-N'-1-(5'-tetrazolyl)isopropylurea.

Six hundred and thirty-six milligrams (0.005 mole) of 5-(1'-amino-1'-methylethyl)tetrazole was dissolved in 15 ml. of 0.5N aqueous potassium hydroxide solution, 0.8 g. (0.007 mole) of phenyl isocyanate was added, and the mixture was shaken at room temperature for one hour. The reaction mixture was filtered, and the filtrate was acidified to pH 4-5 with concentrated hydrochloric acid. The precipitate which formed was separated by filtration and recrystallized from water to give N-phenyl-N'-1-(5'-tetrazolyl)isopropylurea, m.p. 186° C., as fine needles.

Analysis.

Calculated for $C_{11}H_{14}N_6O$: C, 53.64; H, 5.73; N, 34.13.

Found: C, 53.57; H, 5.78; N, 34.26.

The Preparation of 5-(alpha-Aminobenzyl)tetrazole.

(C-Phenylglycine analog)

Scheme B.

(a) alpha-Aminophenylacetonitrile hydrochloride.

One hundred and fifty-two milliliters (1.5 moles) of freshly distilled benzaldehyde was mixed with 100 ml. of diethyl ether and added to a filtered solution of 100 g. (1.87 moles) of ammonium chloride in 250 ml. of water. The solution was cooled in a brine bath, and a solution of 80 g. (1.63 moles) of sodium cyanide in 175 ml. of water was added dropwise with stirring at such a rate that the temperature of the reaction mixture never exceeded 10° C. Stirring and cooling were continued for four hours after the addition of sodium cyanide was complete. The organic layer was separated from the aqueous phase, and the aqueous phase was extracted twice with 100 ml. portions of diethyl ether. The combined extracts were dried over anhydrous sodium sulfate and filtered. The filtrate was cooled in an ice bath and saturated with dry, gaseous hydrogen chloride. The ethereal suspension was filtered, and the solid was dissolved in a minimum quantity of hot absolute ethanol, boiled with Norite, filtered, and reprecipitated by the addition of diethyl ether. The resulting suspension was filtered and the solid

was washed with diethyl ether to give 31 g. (13% of theory) of alpha-aminophenylacetonitrile hydrochloride, m.p. 175° C. This compound is reported by Minovici (35) to melt at 173° C.

(b) alpha-Acetamidophenylacetonitrile.

Thirty grams (0.178 mole) of alpha-aminophenylacetonitrile hydrochloride was suspended in a mixture of 36 ml. (0.45 mole) of pyridine and 100 ml. of diethyl ether, and 15.6 ml. (0.22 mole) of acetyl chloride was added dropwise to the stirred solution. The reaction mixture was allowed to stand for five hours when 100 ml. of water was added to it with stirring. The organic layer was separated, washed with two 100 ml. portions of water, filtered, and dried over anhydrous sodium sulfate. The ether was removed from the organic layer by evaporation, and the residue was recrystallized from absolute ethanol and dried at 80° C., to give 21 g. (68% of theory) of crude alpha-acetamidophenylacetonitrile, m.p. 105-109° C. A small portion of this material was recrystallized from a mixture of equal volumes of petroleum ether (30-60° C.) and ethyl acetate, raising the melting point to 111-112° C. This compound is reported by Reihlen (36) to melt at 113° C.

(c) 5-(alpha-Acetamidobenzyl)tetrazole.

Nineteen and two tenths grams (0.11 mole) of alpha-acetamidophenylacetonitrile and 16.8 g. (0.26 mole) of

powdered sodium azide (technical grade) were suspended in 40 ml. of dry tetrahydrofuran, and a filtered solution of 15 g. (0.112 mole) of anhydrous aluminum chloride in 160 ml. of the same solvent was added dropwise with stirring during seventy-five minutes. The mixture was heated at reflux temperature with continuous stirring for thirty-six hours, after which the tetrahydrofuran was distilled from the reaction mixture while water was added at such a rate that the volume remained constant. The reaction mixture was cooled in an ice bath and filtered. The solid was resuspended in 50 ml. of water and cooled in an ice bath. Fifty milliliters of concentrated hydrochloric acid was added dropwise with stirring to the cold suspension; stirring and cooling were continued for two and one half hours. The material was filtered, and the solid was washed first with 100 ml. of 6N hydrochloric acid and then with 100 ml. of water. The light grey solid was dried at 80° C. for twelve hours to give 17.7 g. (74% of theory) of crude 5-(alpha-acetamidobenzyl)tetrazole, m.p. 202-206° C. Upon recrystallization of a small portion of the crude material from aqueous ethanol, the melting point was raised to 206° C.

Analysis.

Calculated for $C_{10}H_{11}N_5O$: C, 55.29; H, 5.10; N, 32.24.
Found: C, 55.38; H, 5.14; N, 32.04.

(d) 5-(alpha-Aminobenzyl)tetrazole.

Seventeen and seven tenths grams (0.082 mole) of 5-

(alpha-acetamidobenzyl)tetrazole was heated at reflux temperature with 100 ml. of concentrated hydrochloric acid for three hours. The resulting solution was decolorized with Norite, filtered while hot, and evaporated to dryness on the steam bath. The crystalline residue was taken up in sufficient hot absolute ethanol to effect complete solution, and the alcoholic solution was treated with Norite and filtered into 10 ml. (0.122 mole) of pyridine. The solution was cooled at -15° C. for one hour and filtered, and the resulting product was washed with cold absolute ethanol and dried at 80° C. for twelve hours to give 5.52 g. (31% of theory) of crude 5-(alpha-aminobenzyl)tetrazole, m.p. 244° C. Since the product was found to be only very slightly soluble in most common organic solvents, as well as in water, purification of the crude material was accomplished by leaching it with successive portions of aqueous ethanol, ethyl acetate, isopropyl ether, and diethyl ether. Upon drying the residual solid as before, 4.17 g. of 5-(alpha-aminobenzyl)tetrazole was obtained, m.p. 258-259° C., with decomposition.

Analysis.

Calculated for $C_8H_9N_5$: C, 54.84; H, 5.18; N, 39.98.

Found: C, 54.89; H, 5.20; N, 40.09.

Derivatives of 5-(alpha-Aminobenzyl)tetrazole.

5-(alpha-Acetamidobenzyl)tetrazole.

The preparation of this compound from alpha-acetamidophenylacetonitrile is described in section (c) of scheme B for the preparation of 5-(alpha-aminobenzyl)tetrazole. It was obtained from the crude product by recrystallization from aqueous ethanol, and melted at 206° C.

Analysis.

Calculated for $C_{10}H_{11}N_5O$: C, 55.29; H, 5.10; N, 32.24.
Found: C, 55.38; H, 5.14; N, 32.04.

5-(alpha-Benzamidobenzyl)tetrazole.

Seven tenths of a gram (0.005 mole) of benzoyl chloride was added to a solution of 0.876 g. (0.005 mole) of 5-(alpha-aminobenzyl)tetrazole in 10 ml. of 1.25N aqueous sodium hydroxide, and the reaction mixture was shaken for one hour at room temperature. The resulting solution was adjusted to pH 4-5 with concentrated hydrochloric acid and the precipitate collected on a filter. The solid was washed with water and dried to give a fine, colorless powder, m.p. 217-218° C. A portion of the crude product was recrystallized from aqueous ethanol to give 5-(alpha-benzamidobenzyl)tetrazole, m.p. 219-220° C.

Analysis.

Calculated for $C_{15}H_{13}N_5O$: C, 64.50; H, 4.69; N, 25.08.
Found: C, 64.50; H, 5.01; N, 24.86.

N-Phenyl-N'-alpha-(5'-tetrazolyl)benzylurea.

Eight hundred and seventy-six milligrams (0.005 mole) of 5-(alpha-aminobenzyl)tetrazole was dissolved in 15 ml. of 0.5N aqueous potassium hydroxide solution, 0.8 g. (0.007 mole) of phenyl isocyanate was added and the mixture was shaken at room temperature for one hour. The reaction mixture was filtered and the filtrate acidified to pH 4-5 with concentrated hydrochloric acid. The precipitate which formed was removed by filtration and recrystallized from water to give N-phenyl-N'-alpha-(5'-tetrazolyl)benzylurea, m.p. 196° C.

Analysis.

Calculated for $C_{15}H_{14}N_6O$: C, 61.21; H, 4.80; N, 28.56.

Found: C, 61.28; H, 5.01; N, 28.46.

The Preparation of 5-(3'-Aminopropyl)tetrazole.

(gamma-Aminobutyric acid analog)

Scheme C.

(a) gamma-Phthalimidobutyronitrile.

One hundred and sixty-two grams (0.87 mole) of potassium phthalimide was dissolved in 650 ml. of dimethylformamide, and 73 g. (0.82 mole) of gamma-chlorobutyronitrile was added dropwise with stirring over a one hour period. The reaction mixture was allowed to stir for twelve hours at room temperature, then heated to 90° C., and maintained at that temperature for one hour. The material was cooled to room temperature and filtered. The filtrate was poured into 900 ml. of chloroform, and the resulting solution was extracted with two 1500 ml. portions of water. The combined aqueous extracts were washed with two 300 ml. portions of chloroform, and all the chloroform solutions were combined. The combined chloroform solutions were washed with 600 ml. of 0.2N aqueous sodium hydroxide solution, and then with 600 ml. of water. The solution was dried over anhydrous sodium sulfate and the chloroform was removed by distillation. The residue was triturated with diethyl ether and filtered. The solid so obtained was recrystallized from ethyl acetate and dried in a vacuum desiccator for several days to give 108 g. (66% of theory)

of gamma-phthalimidobutyronitrile, m.p. 80-81° C. This compound is reported by Gabriel (37) to have a melting point of 80.5-81.5° C.

(b) 5-(3'-Phthalimidopropyl)tetrazole.

One hundred and six tenths grams (0.5 mole) of gamma-phthalimidobutyronitrile and 97.5 g. (1.5 moles) of powdered sodium azide (technical grade) were suspended in 200 ml. of dry tetrahydrofuran, and a filtered solution of 71.4 g. (0.52 mole) of anhydrous aluminum chloride in 600 ml. of the same solvent was added dropwise with stirring over a two hour period. The mixture was heated with continuous stirring at reflux temperature for seventy-two hours, after which the tetrahydrofuran was distilled from the reaction mixture while water was added at such a rate that the volume remained constant. The reaction mixture was cooled to room temperature and allowed to stand for twelve hours. One hundred milliliters of concentrated hydrochloric acid was added dropwise with stirring to the reaction mixture over a thirty minute period. Stirring was continued for two hours while the material was cooled in a brine bath. The reaction mixture was filtered, and the solid so obtained was washed thoroughly with water and dried for twelve hours at 80° C., to give 111.5 g. (91% of theory) of crude 5-(3'-phthalimidopropyl)tetrazole, m.p. 200-202° C. Recrystallization of the crude material from absolute ethanol raised the melting point to 202-203° C.

Analysis.

Calculated for $C_{12}H_{11}N_5O_2$: C, 56.02; H, 4.31; N, 27.23.

Found: C, 56.34; H, 4.39; N, 27.28.

(c) 5-(3'-Aminopropyl)tetrazole hydrochloride.

One hundred and ten grams (0.428 mole) of 5-(3'-phthalimidopropyl)tetrazole was dissolved in 620 ml. of absolute ethanol, and 45 ml. (0.74 mole) of hydrazine hydrate (85%) was added in small portions with stirring to the refluxing solution. The material was maintained at reflux temperature with stirring for five minutes and allowed to stand at room temperature for twelve hours. An additional 400 ml. of hot absolute ethanol was added to the semisolid reaction mass, and the material was heated with stirring at reflux temperature for one hour. The reaction mixture was allowed to cool to room temperature and was filtered. The filtrate was then treated with five ml. of hydrazine hydrate, heated at reflux temperature for four hours, cooled to -5° C., and filtered. The solid so obtained was combined with the solid from the first filtration, and the filtrate was discarded. The combined solids were dried in an oven at 80° C. for twelve hours and added with stirring to a solution of 200 ml. of concentrated hydrochloric acid in 800 ml. of water which had been heated to 65° C. The resulting suspension was stirred without further heating for twenty minutes and filtered to remove the precipitate of phthalhydrazide. The filtrate was evaporated to dryness on the steam bath in a

stream of air, to give a crystalline residue of 73 g. of crude 5-(3'-aminopropyl)tetrazole hydrochloride, m.p. 145-167° C. This material was recrystallized once from absolute ethanol and once from a 95% ethanol-5% pyridine solvent system to remove the hydrazine dihydrochloride present as an impurity. The yield of 5-(3'-aminopropyl)tetrazole hydrochloride was 28 g. (40% of theory), m.p. 152-154° C. A further recrystallization of a small portion of this material from ethanol raised the melting point to 153-154° C.

Analysis.

Calculated for $C_4H_{10}N_5Cl$: C, 29.36; H, 6.16; N, 42.81. Cl, 21.67

Found: C, 29.50; H, 6.09; N, 42.72. Cl, 21.88

(d) 5-(3'-Aminopropyl)tetrazole.

To 13.557 g. (0.08286 mole) of 5-(3'-aminopropyl)tetrazole hydrochloride was added 170.6 ml. (0.08286 mole) of 0.4813N aqueous sodium hydroxide solution, and the resulting solution was warmed and filtered to remove a minute quantity of undissolved material. The filtrate was evaporated to dryness on a steam bath and dried over anhydrous calcium chloride at 3 mm. pressure for twelve hours to give 16.886 g. of crystalline material. This material was extracted with absolute ethanol in a Soxhlet extractor for seventy-two hours, and the alcoholic extract was evaporated to dryness to give 4.204 g. (40% of theory) of crude

5-(3'-aminopropyl)tetrazole, m.p. 251° C. Several recrystallizations of this material from 95% ethanol raised the melting point to 263° C.

Analysis.

Calculated for $C_4H_9N_5$: C, 37.78; H, 7.14; N, 55.08.

Found: C, 37.84; H, 7.09; N, 55.20.

Derivatives of 5-(3'-Aminopropyl)tetrazole.

5-(3'-Acetamidopropyl)tetrazole.

A solution of 0.636 g. (0.005 mole) of 5-(3'-aminopropyl)tetrazole and 0.6 g. (0.006 mole) of acetic anhydride in 10 ml. of glacial acetic acid was heated at reflux temperature for five hours. The excess acetic acid was removed by warming the reaction mixture *in vacuo* on the steam bath, and the crystalline residue was recrystallized from amyl acetate to give 5-(3'-acetamidopropyl)tetrazole, m.p. 111-112° C.

Analysis.

Calculated for $C_6H_{11}N_5O$: C, 42.59; H, 6.55; N, 41.40.

Found: C, 42.72; H, 6.50; N, 41.43.

5-(3'-Benzamidopropyl)tetrazole.

Thirty-five hundredths of a gram (0.0025 mole) of benzoyl chloride was added to a solution of 0.318 g. (0.0025 mole) of 5-(3'-aminopropyl)tetrazole in 5 ml. of 1.25N aqueous sodium hydroxide, and the reaction mixture was shaken for one hour at room temperature. The resulting solution was adjusted to pH 4-5 with concentrated hydrochloric acid and the precipitate collected on a filter. The solid was washed with water and ether and dried for an hour at 110° C., to give crude 5-(3'-benzamidopropyl)tetrazole, m.p. 125-127° C. The crude material was recrystallized from water, raising the melting point to 128.5° C.

Analysis.

Calculated for $C_{11}H_{13}N_5O$: C, 57.13; H, 5.67; N, 30.29.

Found: C, 57.27; H, 5.65; N, 30.17.

The determination of the apparent pK values of some 5-aminoalkyltetrazoles.

The apparent pK values of the 5-aminoalkyltetrazoles were determined by titration of weighed samples of the tetrazoles in aqueous solution with standard hydrochloric acid and sodium hydroxide solutions. The samples were placed in a three necked 250 ml. flask, dissolved in 125 ml. of water, and titrated in a thermostated bath at 25 $\pm$ 1° C. The pH was determined after each addition of titrant with a Model H-2 Beckman pH meter.

The pK_1 values of the 5-aminoalkyltetrazoles were determined by taking the pH of the solution at the point of half neutralization of the tetrazole, as calculated from the normality of the acid used as a titrant and the molecular weight of the tetrazole. The pK_1 of 5-(3'-aminopropyl)tetrazole was also determined by titration of the hydrochloride with standard base; using the pH at the point of half neutralization, as determined from the break in the curve denoting the addition of one equivalent of base, as the pK_1 value.

The pK_2 values of the 5-aminoalkyltetrazoles were determined as the pH of the solution at the point of half neutralization of the free tetrazole with standard base, using the break in the titration curve to denote the addition of one equivalent of base. The titration curves in all cases exhibited the form normally obtained for an amino acid.

Part II

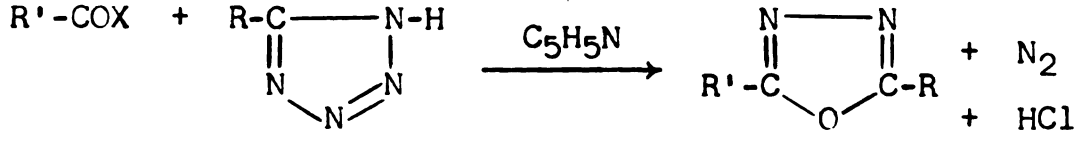
DISCUSSION

After the tetrazole analogs of the amino acids chosen for study in Part I had been prepared, an investigation of the chemical properties of these compounds was undertaken. The possible reactions of 5-aminoalkyltetrazoles may be divided, for the sake of convience, into three catagories:

1. Reactions involving the amino group
2. Reactions involving the tetrazolyl group
3. Reactions involving substitutuion or rearrangement in the alkyl side chain.

Since it appeared reasonable to expect that the reactions of the amino group would dominate the chemistry of the 5-aminoalkyltetrazoles, in view of the well established tendency for this group to undergo a wide variety of characteristic chemical reactions, techniques such as acetylation with acetic anhydride, benzoylation under Schotten-Bauman conditions, and the formation of phenyl substituted ureas by reaction with phenyl isocyanate were investigated as a means of preparing derivatives of the 5-aminoalkyltetrazoles. All of these efforts, which are reported in the experimental section of Part I, were successful, confirming the observation of McManus (9) that these reactions are characteristic of 5-aminoalkyltetrazoles.

The reactions of 5-aminoalkyltetrazoles which directly involve the tetrazolyl group are considerably more limited in scope than those of category 1. In contrast to the versatile reactivity of the carboxyl group of an amino acid, which can react by displacement of its hydroxyl group to form a wide variety of derivatives, the tetrazolyl group of a 5-aminoalkyltetrazole reacts, in most cases, simply as a proton donor to form salts with bases. An important exception to this behavior, however, was discovered by Huisgen, Sauer, and Sturm (10) in 1958, who found that 5-substituted tetrazoles would react, in the presence of pyridine, with acyl halides to form 2,5-disubstituted 1,3,4-oxadiazoles.



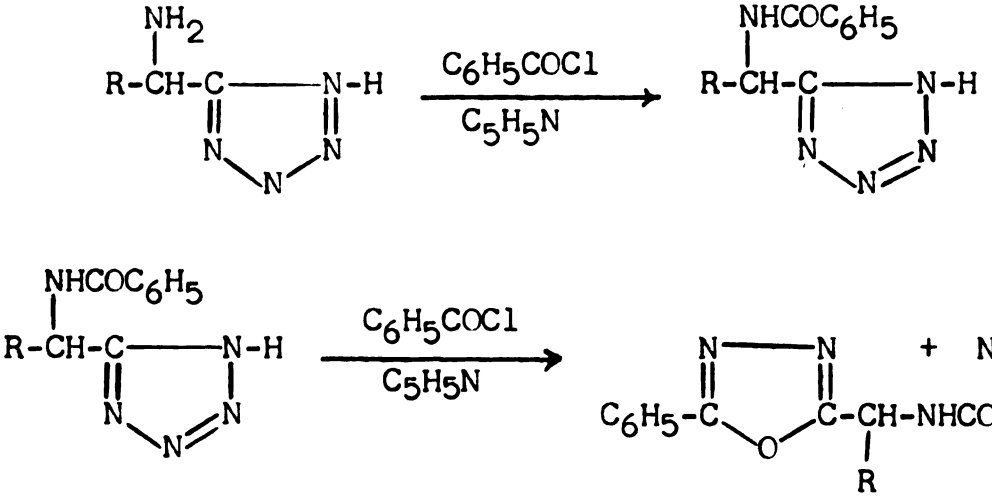
R: CH_3-, iC_3H_7-, C_6H_{13}-, C_6H_5-, (p)$NO_2C_6H_4$-, $C_6H_5CH_2$-, etc.
R': CH_3-, C_6H_5-, (p)$NO_2C_6H_4$-, etc.

The reaction was shown to occur in good yield under mild conditions, and the 1,3,4-oxadiazoles obtained were described as high melting, crystalline compounds whose structure could be verified by identification of the products of acid hydrolysis.

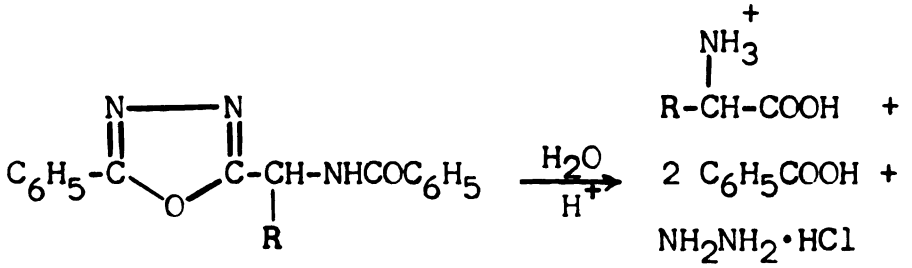
On the basis of these results, the preparation of 1,3,4-oxadiazoles seemed to present a promising method, based

on a reaction of the tetrazolyl group, by which derivatives of the tetrazole analogs of amino acids could be obtained. This method appeared especially attractive in view of the fact that the products of hydrolytic degradation of the 1,3,4-oxadiazoles would be expected to include the original amino acids used as starting materials in the preparation of 5-alkylaminotetrazoles. The following sequence of reactions illustrates the formation and subsequent hydrolysis of the 1,3,4-oxadiazole derivatives:

Formation.



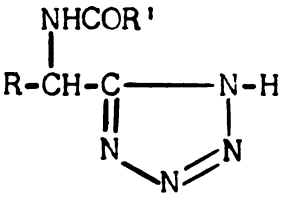
Hydrolysis.



An attempt was made to convert the 5-aminoalkyltetrazoles described in Part I to the corresponding 1,3,4-oxadiazoles by subjecting them to benzoylation in the presence of pyridine as suggested by Huisgen, Sauer, and Sturm (10). The conversion was successfully accomplished for all of these compounds except the leucine analog, 5-(1'-amino-3'-methylbutyl)tetrazole, which formed only 5-(1'-benzamido-3'-methylbutyl)tetrazole on interaction with benzoyl chloride and pyridine.

The mechanism involved in the formation of a 2,5-disubstituted 1,3,4-oxadiazole from a 5-substituted tetrazole by interaction with an acid chloride and pyridine merits some elaboration at this point, since the conversion involves an elimination and rearrangement which are not intuitively apparent upon consideration of the structures of the starting material and final product. Two mechanisms have been proposed which account for this phenomenon, both involving a rapid sequence of interrelated reactions. A brief account of each is given next, followed by a discussion of their relative merits.

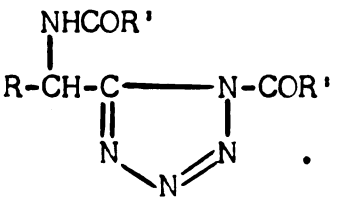
The first step in the conversion of a 5-aminoalkyltetrazole to a 1,3,4-oxadiazole is presumed to be acylation of the primary amino group to give a 5-acylaminoalkyltetrazole.



R: alkyl

R': CH_3-, C_6H_5-, etc.

Next, a second molecule of acid chloride could attack the 5-acylaminoalkyltetrazole at the number one nitrogen atom of the tetrazole ring to form

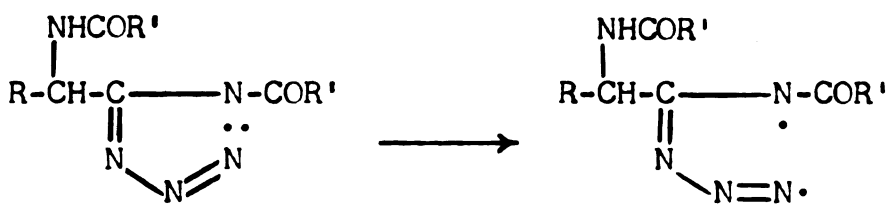


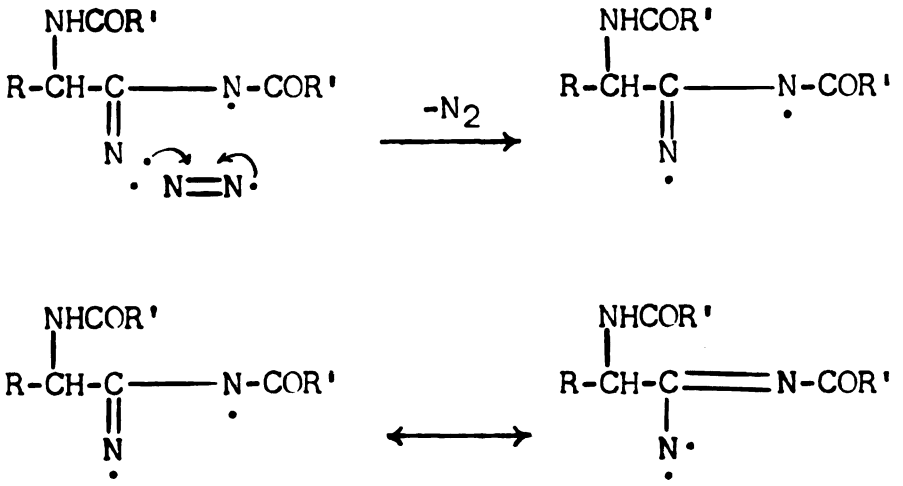
R: alkyl

R': CH_3-, C_6H_5-, etc.

Here, the presence of pyridine in the reaction mixture is assumed to provide sufficient driving force to accomplish the direct acylation of the tetrazole nucleus.

The second acylation would act to weaken the aromatic character of the tetrazole ring system by providing an "electron sink" for the pi electrons responsible for its resonance stabilization. This could, in turn, lead to a rupture of the bond between the number one and number two nitrogen atoms of the tetrazole ring system, followed by the elimination of the number two and number three ring nitrogens as a nitrogen molecule, as shown by the following sequence of reactions:

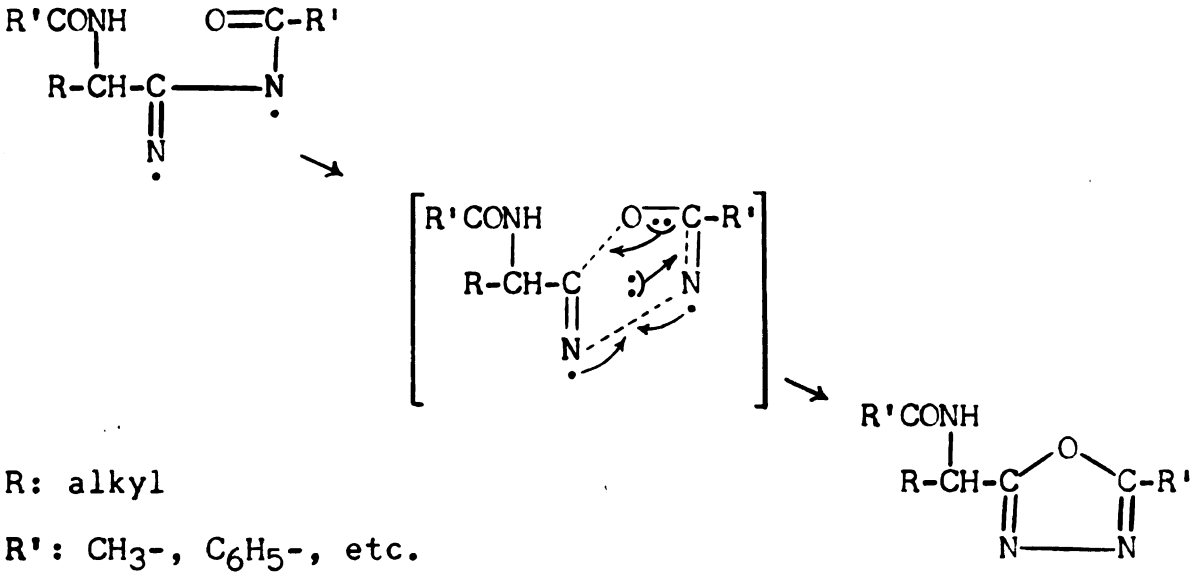




R: alkyl

R': CH_3-, C_6H_5-, etc.

The nitrogen diradical formed as a consequence of the loss of a molecule of nitrogen from the tetrazole ring could next form a 1,3,4-oxadiazole ring by undergoing a series of electronic shifts. A concerted mechanism for this rearrangement might be represented in the following way:

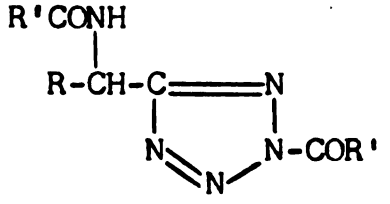


R: alkyl

R': CH_3-, C_6H_5-, etc.

This explanation of the formation of a 2,5-disubstituted 1,3,4-oxadiazole from a 5-substituted tetrazole is essentially the same as that proposed by Stolle (20) to account for the formation of 2-acetamido-5-methyl-1,3,4-oxadiazole upon prolonged heating of 5-aminotetrazole with acetic anhydride. It is based on the assumption that acylation of the tetrazole nucleus occurs at the 1 position.

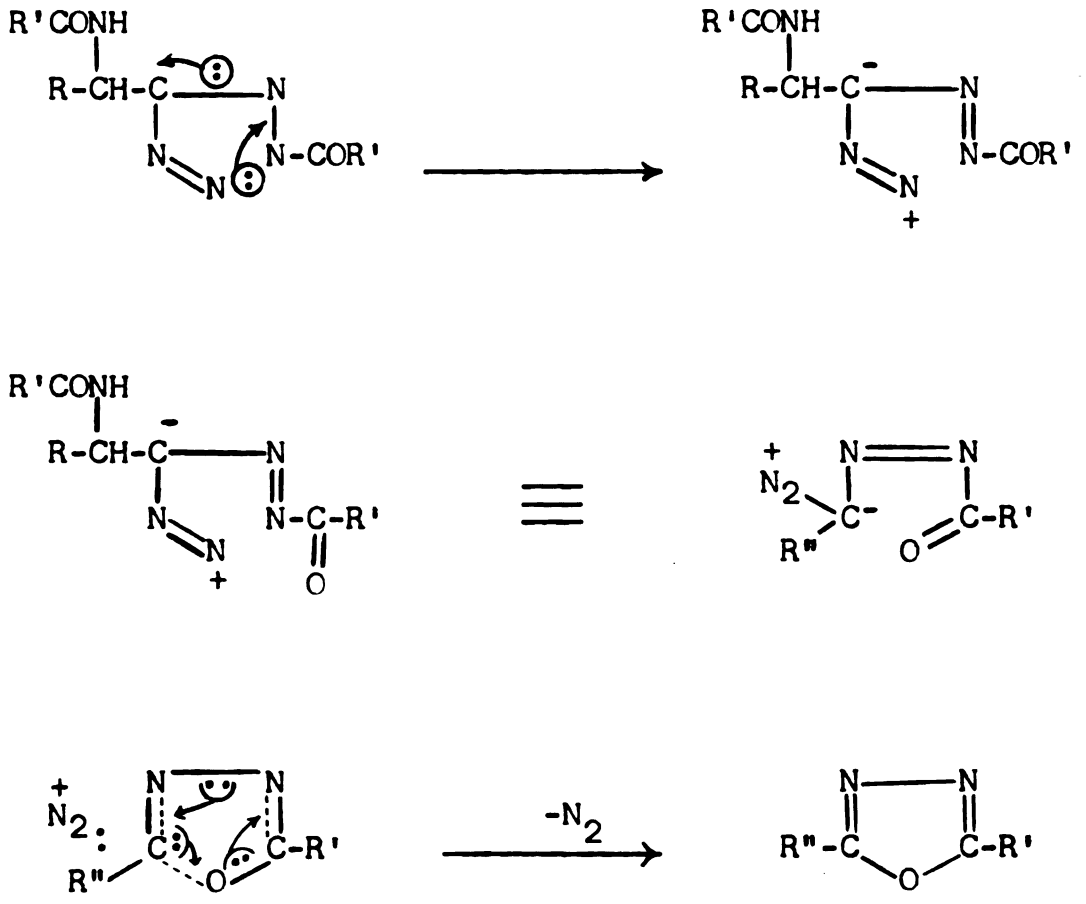
Huisgen, Sauer, and Sturm (10) have proposed an alternate mechanism for the conversion of 5-substituted tetrazoles to the corresponding 1,3,4-oxadiazoles in acylating media by assuming that acylation of the tetrazole ring occurs at the 2 position, leading to an intermediate of the following type:



R: alkyl

R': CH_3-, C_6H_5-, etc.

This compound could react to become a 1,3,4-oxadiazole by heterolytic clevage of the bond between the number two and number three nitrogen atoms of the tetrazole ring, followed by elimination of the number three and number four ring nitrogen atoms as a nitrogen molecule and simultaneous electronic rearrangement.



R: alkyl

R': CH_3-, C_6H_5-, etc.

R": R'CONHCH-
|
R

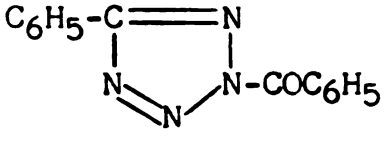
The essential difference between these two reaction mechanisms is that Stolle proposed that the initial attack of the acyl group occurs at the 1 position of a 1-H tetrazole while Huisgen, Sauer, and Sturm maintained that acylation of the tetrazole nucleus first takes place at the 2-position of a 2-H tetrazole. This question is not likely to be resolved by ordinary chemical means, since

(1) the final product is the same in both cases

(2) the hydrogen atom associated with the tetrazolyl group has been shown by a number of investigators (38,39) to exist in tautomeric equilibrium between the 1 and 2 positions of the tetrazole ring, and

(3) the transformation involves a series of rapid chemical equilibria practically equivalent to a concerted process.

Huisgen, Sauer, and Sturm (10) claim to have isolated a benzoyl-5-phenyltetrazole by the interaction of 5-phenyltetrazole, benzoyl chloride, and pyridine. Although they assign the structure of 2-benzoyl-5-phenyltetrazole to this compound, no experimental data are given to substantiate this assumption.



5-Aminomethyltetrazole (9), the analog of glycine, reacted on heating with pyridine and benzoyl chloride to form 2-benzamidomethyl-5-phenyl-1,3,4-oxadiazole. Glutaronitrile was converted to 1,3-bis-(5'-tetrazolyl)propane by treatment with ammonium azide, using the method developed by Finnegan, Henry, and Loftquist (16). Treatment of 1,3-bis-(5'-tetrazolyl)propane with pyridine and benzoyl chloride under the

same experimental conditions used to prepare the other 1,3,4-oxadiazoles resulted in the isolation of 1,3-bis-(5'-phenyl-1',3',4'-oxadiazolyl)propane from the reaction mixture, indicating that both of the tetrazolyl groups present in the molecule are susceptible to the tetrazole-oxadiazole transformation. These examples provide additional support to the conclusion that the reaction of a 5-substituted tetrazole with pyridine and benzoyl chloride to form a 2-substituted 5-phenyl-1,3,4-oxadiazole may be considered as a general reaction of this class of compounds.

The apparent pK_a value of 1,3-bis-(5'-tetrazolyl)propane was determined in aqueous solution at 25° C. by titration with standard sodium hydroxide solution. The pK_a was found to be 5.30, as compared with authentic glutaric acid, which has a pK_a of 4.34 (40). The data for this titration may be found in Appendix I.

In order to establish definitely the structure of the 1,3,4-oxadiazoles prepared from the tetrazole analogs of glycine, D,L-alpha-aminoisobutyric acid, D,L-C-phenylglycine, gamma-aminobutyric acid, and glutaric acid, these oxadiazoles were hydrolyzed by prolonged treatment with an excess of hot hydrochloric acid. The respective hydrolysates were cooled to 5° C., filtered to remove any benzoic acid present, and evaporated to dryness. The solid residues remaining after the evaporation were extracted with absolute ethanol to give a crystalline solid, which consisted largely of hydrazine dihydrochloride, and an alcoholic extract from which the

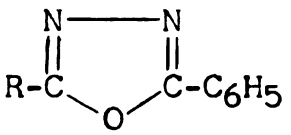
hydrochloride of the original amino acid could be recovered. The identity of these degradation products was verified by mixture melting points with authentic samples. Table IV summarizes the results obtained by this procedure.

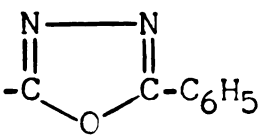
Hydrolysis of the oxadiazole derived from the tetrazole analog of D,L-valine was carried out in an open beaker, and it is assumed that no benzoic acid was isolated in this case because it had all sublimed during the course of the hydrolysis. The glycine and D,L-C-phenylglycine hydrochlorides could not be obtained in crystalline form, and consequently their presence in the hydrolysates from these two degradations was not verified by the mixture melting point technique.

No attempt was made to investigate any reactions of the 5-aminoalkyltetrazoles involving substitution or rearrangement in the alkyl side chain (Category 3). Very little is known regarding reactions of this type and they are considered to fall outside the scope of this thesis.

TABLE IV

Products obtained by the hydrolytic degradation of some 2,5-disubstituted 1,3,4-oxadiazoles



R-	Hydrolytic Degradation Products		
	C_6H_5COOH	$N_2H_4 \cdot 2HCl$	$R-CH(NH_3Cl)-COOH$
$-NHCH_2COC_6H_5$	+	+	
$-CH(CH(CH_3)_2)NHCOC_6H_5$		+	+
$-C(CH_3)_2NHCOC_6H_5$	+	+	+
$-CH(C_6H_5)NHCOC_6H_5$	+	+	
$-CH_2CH_2CH_2NHCOC_6H_5$	+	+	+
$-(CH_2)_3-$ 	+	+	+

Experimental (Part 2)

I. The Reaction of 5-Substituted Tetrazoles with Benzoyl Chloride and Pyridine.

5-Aminomethyltetrazole (Glycine Analog)

(a) 5-Aminomethyltetrazole.

This compound was prepared from glycine in a 21% overall yield by converting the glycine to phthalimidoacetonitrile by the same sequence of reactions employed to prepare the phthalimidonitriles of D,L-alpha-aminobutyric acid, D,L-valine, and D,L-leucine, followed by the conversion of the phthalimidoacetonitrile to 5-aminomethyltetrazole according to the procedure described by McManus (9). The crude product was recrystallized from water as a colorless powder, m.p. 254-256° C.

Analysis.

Calculated for $C_2H_5N_5$: C, 24.24; H, 5.09; N, 70.68.

Found: C, 24.50; H, 5.09: N, 70.86.

(b) 2-Benzamidomethyl-5-phenyl-1,3,4-oxadiazole.

A mixture of 0.496 g. (0.005 mole) of 5-aminomethyltetrazole, 1.2 ml. (0.01 mole) of benzoyl chloride, 4 ml. (0.05 mole) of pyridine, and 10 ml. of benzene was heated at reflux temperature for seven hours. The reaction mixture

was diluted with 20 ml. of benzene and the benzene solution was washed successively with 20 ml. portions of 0.2N aqueous sodium hydroxide, 0.1N hydrochloric acid, and water. The final washing with water resulted in the formation of a precipitate in the benzene layer, which was isolated by filtration. This solid was recrystallized from 95% ethanol to give 2-benzamidomethyl-5-phenyl-1,3,4-oxadiazole as fine needles, m.p. 141-142° C.

Analysis.

Calculated for $C_{16}H_{13}N_3O_2$: C, 68.80; H, 4.69; N, 15.05.
Found: C, 68.88; H, 4.66; N, 14.88.

5-(1'-Aminopropyl)tetrazole
(alpha-Aminobutyric acid Analog)

(c) 2-(1'-Benzamidopropyl)-5-phenyl-1,3,4-oxadiazole.

A mixture of 0.318 g. (0.0025 mole) of 5-(1'-aminopropyl)tetrazole, 0.6 ml. (0.005 mole) of benzoyl chloride, 2 ml. (0.025 mole) of pyridine, and 5 ml. of benzene was heated at reflux temperature for ten hours. The solution was diluted with 10 ml. of benzene and washed with successive 10 ml. portions of 0.1N aqueous sodium hydroxide, 0.1N hydrochloric acid, and water. The benzene layer was dried over anhydrous sodium sulfate and evaporated to dryness on a steam bath. The solid residue was recrystallized twice from absolute ethanol to give 2-(1'-benzamidopropyl)-5-phenyl-1,3,4-oxadiazole as a fine colorless powder, m.p. 102-103° C.

Analysis.

Calculated for $C_{18}H_{17}N_3O_2$: C, 70.34; H, 5.58; N, 13.67.

Found: C, 70.52; H, 5.71; N, 13.48.

5-(1'-Amino-2'-methylpropyl)tetrazole

(Valine Analog)

(d) 2-(1'-Benzamido-2'methylpropyl)-5-phenyl-1,3,4-oxadiazole from 5-(1'-amino-2'-methylpropyl)tetrazole.

A mixture of 0.353 g. (0.0025 mole) of 5-(1'-amino-2'-methylpropyl)tetrazole, 0.6 ml. (0.005 mole) of benzoyl chloride, 2 ml. (0.025 mole) of pyridine, and 5 ml. of benzene was heated at reflux temperature for eight hours. The material was diluted with 10 ml. of benzene, and washed with successive 10 ml. portions of 0.1N aqueous sodium hydroxide, 0.1N hydrochloric acid, and water. The benzene layer was dried over anhydrous sodium sulfate and evaporated to dryness on a steam bath. The solid residue was recrystallized from absolute ethanol to give 2-(1'-benzamido-2'-methylpropyl)-5-phenyl-1,3,4-oxadiazole as a fine colorless powder, m.p. 141° C.

Analysis.

Calculated for $C_{19}H_{19}N_3O_2$: C, 71.00; H, 5.96; N, 13.08.

Found: C, 71.14; H, 6.02; N, 12.96.

(e) 2-(1'-Benzamido-2'-methylpropyl)-5-phenyl-1,3,4-oxadiazole from 5-(1'-benzamido-2'-methylpropyl)tetrazole.

A mixture of 4.18 g. (0.017 mole) of 5-(1'-benzamido-

2'-methylpropyl)tetrazole, 3 ml. (0.025 mole) of benzoyl chloride, 10 ml. (0.125 mole) of pyridine, and 20 ml. of benzene was heated at reflux temperature for twenty-three hours, allowed to cool to room temperature, and filtered. The benzene solution was washed with one 100 ml. and two 50 ml. portions of water. The benzene solution was dried over anhydrous sodium sulfate and evaporated to approximately one fourth its original volume on a steam bath. The concentrated solution was cooled in an ice bath, but no precipitate formed. The cold solution was poured into twice its volume of a 10% aqueous solution of sodium carbonate and the material was stirred vigorously for thirty minutes. The crystalline precipitate which formed was isolated by filtration, washed with water, and dried at 110° C. to give 3.82 g. (70% of theory) of crude 2-(1'-benzamido-2'-methylpropyl)-5-phenyl-1,3,4-oxadiazole, m.p. 133-136° C. After two recrystallizations from absolute ethanol, the melting point of the product was 141° C. A mixture melting point of this material and the 2-(1'-benzamido-2'-methylpropyl)-5-phenyl-1,3,4-oxadiazole prepared from 5-(1'-amino-2'-methylpropyl) tetrazole in part (d) showed no depression.

5-(1'-Amino-3'-methylbutyl)tetrazole

(Leucine Analog)

(f) Attempted preparation of 2-(1'-benzamido-3'-methylbutyl)-5-phenyl-1,3,4-oxadiazole.

A mixture of 0.648 g. (0.0025 mole) of 5-(1'-amino-3'-methylbutyl)tetrazole, 0.6 ml. (0.005 mole) of benzoyl chloride, 4 ml. (0.05 mole) of pyridine, and 10 ml. of benzene was heated at reflux temperature for four hours. The reaction mixture was cooled to room temperature, diluted with 20 ml. of benzene, and washed with successive 20 ml. portions of 0.1N aqueous sodium hydroxide, 0.1N hydrochloric acid, and water. The benzene solution was dried over anhydrous sodium sulfate and evaporated to dryness on a steam bath but only a negligible quantity of residual solid remained in the beaker. Upon acidification of the sodium hydroxide wash solution, a precipitate formed, which was isolated by filtration, washed with water, and recrystallized from aqueous ethanol to give a small quantity of fine colorless needles, m.p. 167-168° C. A mixture melting point of this material and the 5-(1'-benzamido-3'-methylbutyl) tetrazole prepared from 5-(1'-amino-3'-methylbutyl)tetrazole showed no depression.

Analysis.

Calculated for $C_{13}H_{17}N_5O$: N, 27.01.

Found: N, 27.01.

5-(1'-Amino-1'-methylethyl)tetrazole

(alpha-Aminoisobutyric acid Analog)

(g) 2-(1'-Benzamido-1'-methylethyl)-5-phenyl-1,3,4-oxadiazole.

A mixture of 0.636 g. (0.005 mole) of 5-(1'-amino-1'-methylethyl)tetrazole, 1.2 ml. (0.01 mole) of benzoyl chloride, 4 ml. (0.05 mole) of pyridine, and 10 ml. of benzene was heated at reflux temperature for ten hours. After the reaction mixture cooled to room temperature, it was diluted with 20 ml. of benzene. The benzene solution was washed with successive 20 ml. portions of 0.1N aqueous sodium hydroxide, 0.1N hydrochloric acid, and water. After drying the benzene solution over anhydrous sodium sulfate, the solvent was removed by evaporation on a steam bath. The residual solid was recrystallized from absolute ethanol to give 2-(1'-benzamido-1'-methylethyl)-5-phenyl-1,3,4-oxadiazole, m.p. 145° C.

Analysis.

Calculated for $C_{18}H_{17}N_3O_2$: C, 70.34; H, 5.58; N, 13.67.

Found: C, 70.41; H, 5.69; N, 13.40.

5-(alpha-Aminobenzyl)tetrazole

(C-Phenylglycine Analog)

(h) 2-(alpha-Benzamidobenzyl)-5-phenyl-1,3,4-oxadiazole.

A mixture of 0.876 g. (0.005 mole) of 5-(alpha-aminobenzyl)tetrazole, 1.2 ml. (0.01 mole) of benzoyl chloride, 2 ml. (0.025 mole) of pyridine, and 10 ml. of benzene was

heated at reflux temperature for ten hours. The material was cooled to room temperature and diluted with 20 ml. of benzene. The benzene solution was washed with successive 20 ml. portions of water, 0.1N aqueous sodium hydroxide, 0.1N hydrochloric acid, and water. The benzene solution was dried over anhydrous sodium sulfate and evaporated to dryness on a steam bath. The solid residue was recrystallized from absolute ethanol to give 2-(alpha-benzamidobenzyl)-5-phenyl-1,3,4-oxadiazole, m.p. 179-180° C.

Analysis.

Calculated for $C_{22}H_{17}N_3O_2$: C, 74.35; H, 4.82; N, 11.83.

Found: C, 74.13; H, 4.88; N, 11.91.

5-(3'-Aminopropyl)tetrazole
(gamma-Aminobutyric acid Analog)

(i) 2-(3'-Benzamidopropyl)-5-phenyl-1,3,4-oxadiazole.

A mixture of 3.2 g. (0.02 mole) of 5-(3'-aminopropyl)-tetrazole hydrochloride, 3 ml. (0.025 mole) of benzoyl chloride, 10 ml. (0.125 mole) of pyridine, and 20 ml. of benzene was heated at reflux temperature for eight hours. The reaction mixture was cooled to room temperature and poured into 200 ml. of water. The benzene layer was separated and the aqueous layer was extracted with 20 ml. of benzene. The combined benzene extracts were washed successively with 10 ml. portions of 10% aqueous sodium carbonate solution and water and dried over anhydrous sodium sulfate. The

benzene solution was evaporated to one half of its original volume on a steam bath and cooled in an ice bath. The precipitate was isolated by filtration and recrystallized from a 50% benzene-50% hexane solvent system and dried at 80° C. to give 3.4 g. (89% of theory, based on benzoyl chloride) of 2-(3'-benzamidopropyl)-5-phenyl-1,3,4-oxadiazole as fine needles, m.p. 113° C.

Analysis.

Calculated for $C_{18}H_{17}N_3O_2$: C, 70.34; H, 5.58; N, 13.67.

Found: C, 70.59; H, 5.88; N, 13.76.

1,3-Bis-(5'-tetrazolyl)propane

(Glutaric acid Analogue)

(j) 1,3-Bis-(5'-tetrazolyl)propane.

Twenty-three and five tenths grams (0.25 mole) of glutaronitrile, 35.75 g. (0.55 mole) of powdered sodium azide (technical grade), 26.75 g. (0.5 mole) of ammonium chloride, and 0.2 g. of lithium chloride were suspended in 125 ml. of dimethylformamide and heated with stirring at 105° C. for one hundred and twenty hours. The dimethylformamide was removed from the reaction mixture by distillation at diminished pressure from a steam bath, using a water aspirator. The residual solid was dissolved in water and acidified to pH 4 with concentrated hydrochloric acid. The resulting precipitate was isolated by filtration, washed with water, and dried at 80° C. The crude material was recrystal-

lized from water, washed with small amounts of acetone and diethyl ether, and dried at 80° C. to give 37.8 g. (84% of theory) of 1,3-bis-(5-tetrazolyl)propane, m.p. 192-193° C.

Analysis.

Calculated for $C_5H_8N_8$: C, 33.33; H, 4.48; N, 62.20.
Found: C, 33.32; H, 4.67; N, 62.31.

(k) 1,3-Bis-(5'-phenyl-1',3',4'-oxadiazolyl)propane.

A mixture of 4.5 g. (0.025 mole) of 1,3-bis-(5'-tetrazolyl)propane, 5.8 ml. (0.05 mole) of benzolyl chloride, 4.1 ml. (0.05 mole) of pyridine, and 50 ml. of benzene was heated at reflux temperature for ten hours. The reaction mixture was allowed to cool to room temperature, and the solid which had separated was collected by filtration. The filtrate was washed with successive 50 ml. portions of 0.2N aqueous sodium hydroxide, 0.2N hydrochloric acid, and water, and the solid was washed separately with the same sequence of solutions. The benzene filtrate was dried over anhydrous sodium sulfate and evaporated to dryness on a steam bath in a stream of air. The residue from this evaporation and the solid obtained from the filtration were combined and recrystallized from absolute ethanol to give 7.0 g. (87% of theory) of 1,3-bis-(2'-phenyl-1',3',4'-oxadiazolyl)propane, m.p. 133-134° C.

Analysis.

Calculated for $C_{19}H_{16}N_4O_2$: C, 68.66; H, 4.85; N, 16.68.
Found: C, 68.56; H, 4.72; N, 16.64.

II. Acid Hydrolysis of 2-Substituted 5-Phenyl-1,3,4-Oxadiazoles as a Proof of Structure.

(a) Hydrolysis of 2-(1'-benzamidomethyl)-5-phenyl-1,3,4-oxadiazole.

Two tenths of a gram (0.0007 mole) of 2-(1'-benzamidomethyl)-5-phenyl-1,3,4-oxadiazole was added to 20 ml. of 2N hydrochloric acid, and the material was heated at reflux temperature for five hours. The reaction mixture was cooled to 5° C. and filtered. The solid so obtained was recrystallized from water to give a small quantity of fine needles, m.p. 119-120° C., which showed no depression of the melting point when mixed with an authentic sample of benzoic acid. The filtrate from the acid hydrolysis was evaporated to dryness, and the crystalline residue was extracted several times with cold ethanol and washed with diethyl ether to give a residue of cubic crystals, m.p. 197-198° C., which showed no depression of the melting point when mixed with an authentic sample of hydrazine dihydrochloride. The alcoholic extract was evaporated to dryness in a stream of air to give a small amount of oil which resisted repeated attempts to obtain it in crystalline form. A green flame resulted when a copper wire was dipped in the oil and held over a microburner, indicating the presence of a halogen in the material.

(b) Hydrolysis of 2-(1'-Benzamido-2'-methylpropyl)-5-phenyl-1,3,4-oxadiazole.

A mixture of 1.2 g. (0.0037 mole) of 2-(1'-benzamido-2'-methylpropyl)-5-phenyl-1,3,4-oxadiazole, 5 ml. of concentrated hydrochloric acid, and 50 ml. of water was heated on a hot plate in an open beaker for twenty hours, adding 1N hydrochloric acid periodically to maintain an adequate volume of solution. The reaction mixture was concentrated to a volume of about 10 ml. and filtered while still warm. The solid so obtained was dried at 110° C. to give 0.113 g. of crystalline material, m.p. 185-189° C. When this material was washed with diethyl ether, the melting point was raised to 189° C., and the purified material showed no depression of the melting point when mixed with an authentic sample of D,L-valine hydrochloride. The aqueous filtrate from the hydrolysis was evaporated to dryness, and the crystalline residue was extracted with absolute ethanol to leave, after air drying, 0.18 g. of crystalline material, m.p. 194-196° C.; the melting point of which was not depressed on admixture of an authentic sample of hydrazine dihydrochloride. The alcoholic extract was evaporated to dryness at room temperature to give a small quantity of gummy residue which gave a deep purple color when dissolved in water, buffered to a pH of 5.5 with acetate, and heated with an alcoholic solution of ninhydrin.

(c) Hydrolysis of 2-(1'-Benzamido-1'-methylethyl)-5-phenyl-1,3,4-oxadiazole.

Two tenths of a gram (0.0007 mole) of 2-(1'-benzamido-1'-methylethyl)-5-phenyl-1,3,4-oxadiazole was added to 20 ml. of 2N hydrochloric acid, and the material was heated at reflux temperature for five hours. The reaction mixture was cooled to 5° C., and was filtered. The solid so obtained was recrystallized from water to give a small quantity of fine needles, m.p. 120-121° C., which showed no depression of the melting point when mixed with an authentic sample of benzoic acid. The filtrate from the acid hydrolysis was evaporated to dryness, and the crystalline residue was extracted several times with cold absolute ethanol and washed with diethyl ether to give a crystalline solid, m.p. 198° C., which showed no depression of the melting point when mixed with an authentic sample of hydrazine dihydrochloride. The alcoholic extract was evaporated to dryness at a diminished pressure of 2 mm. to give a white powder, m.p. 224-227° C., which melted at 228-229° C. when mixed with an authentic sample of alpha-aminoisobutyric acid hydrochloride.

(d) Hydrolysis of 2-(alpha-Benzamidobenzyl)-5-phenyl-1,3,4-oxadiazole.

Two tenths of a gram (0.00056 mole) of 2-(alpha-benzamidobenzyl)-5-phenyl-1,3,4-oxadiazole was added to 20 ml. of N hydrochloric acid, and the mixture was heated at reflux

temperature for five hours. The reaction mixture was cooled to 5° C., and was filtered. The solid so obtained was recrystallized from water to give a small quantity of fine needles, m.p. 120-121° C., which showed no depression of the melting point when mixed with an authentic sample of benzoic acid. The filtrate from the acid hydrolysis was evaporated to dryness, and the crystalline residue was extracted several times with cold absolute ethanol and washed with diethyl ether to give a crystalline material, m.p. 195-196° C., which melted at 196-198° C. when mixed with an authentic sample of hydrazine dihydrochloride. Evaporation of the alcoholic extract resulted in the isolation of an oily film which proved to be too small a quantity for further investigation.

(e) Hydrolysis of 2-(3'-benzamidopropyl)-5-phenyl-1,3,4-oxadiazole.

Two tenths of a gram of 2-(3'-benzamidopropyl)-5-phenyl-1,3,4-oxadiazole was heated with a solution of 5 ml. of concentrated hydrochloric acid in 20 ml. of water on a hot plate in an open beaker for two hours. The solution was cooled to 5° C., and filtered. The solid so obtained was recrystallized from water to give fine needles, m.p. 121-122° C., which showed no depression of the melting point when mixed with an authentic sample of benzoic acid. The aqueous filtrate from the acid hydrolysis was evaporated to dryness. The residue was triturated with diethyl ether and filtered.

The solid so obtained was extracted with cold absolute ethanol to give a crystalline material, m.p. 197° C.; mixture melting point with an authentic sample of hydrazine dihydrochloride, 196-197° C. Upon evaporation of the alcoholic extract at room temperature in a stream of air, an amorphous solid was obtained which was recrystallized from ethyl acetate to give a small amount of crystalline material, m.p. 134-136° C., which showed no depression of the melting point when mixed with an authentic sample of gamma-aminobutyric acid hydrochloride.

(f) Hydrolysis of 1,3-Bis-(5'-phenyl-1',3',4'-oxadiazolyl)-propane obtained from the tetrazole analog of glutaric acid.

A mixture of 1.89 g. (0.0057 mole) of 1,3-bis-(5'-phenyl-1',3',4'-oxadiazolyl)propane, 8 ml. of concentrated hydrochloric acid, and 20 ml. of water was heated at reflux temperature for ten hours, cooled to 5° C., and filtered. The solid so obtained was washed with water and dried at 50° C. to give 1.15 g. (0.0094 mole) of benzoic acid, m.p. 121-122° C. The filtrate was evaporated to dryness, and the crystalline residue was triturated with diethyl ether and filtered. The insoluble residue was washed with diethyl ether and dried at 80° C. to give 0.52 g. (0.005 mole) of hydrazine dihydrochloride, m.p. 198-200° C. The ethereal solutions were combined and evaporated to dryness on the steam bath in a stream of air to give a crystalline solid, m.p. 96-97° C., which showed no depression of the

melting point when mixed with an authentic sample of glutaric acid.

SUMMARY

1. A series of three 5-(1'-aminoalkyl)tetrazoles (5-(1'-aminopropyl)tetrazole, 5-(1'-amino-2'-methylpropyl)tetrazole, and 5-(1'-amino-3'-methylbutyl)tetrazole) has been prepared by two different methods. These compounds, which are the tetrazole analogs of D,L-alpha-aminobutyric acid, D,L-valine, and D,L-leucine, were characterized by the preparation of their acetyl, benzoyl, and phenylurea derivatives.

2. The tetrazole analogs of alpha-aminoisobutyric acid, 5-(1'-amino-1'-methylethyl)tetrazole, and of C-phenylglycine, 5-(alpha-aminobenzyl)tetrazole, have been prepared from acetone and benzaldehyde, respectively. The acetyl, benzoyl, and phenylurea derivatives of these tetrazoles have been prepared.

3. The tetrazole analog of gamma-aminobutyric acid, 5-(3'-aminopropyl)tetrazole, has been prepared and was characterized by the preparation of its acetyl and benzoyl derivatives. The hydrochloride of this tetrazole is described.

4. The apparent pK_1 and pK_2 values of all of the 5-aminoalkyltetrazoles prepared have been determined in aqueous solution at 25° C., and are shown to be comparable to those of the corresponding amino acids.

5. The tetrazole analog of glutaric acid, 1,3-bis-(5'-tetrazolyl)propane, has been prepared from glutaronitrile.

6. A series of seven 2-substituted-5-phenyl-1,3,4-oxadiazoles has been prepared from 5-aminoalkyltetrazoles by treatment of these compounds with pyridine and benzoyl chloride. The oxadiazoles prepared were 2-benzamidomethyl-5-phenyl-1,3,4-oxadiazole, 2-(1'-benzamidopropyl)-5-phenyl-1,3,4-oxadiazole, 2-(1'-benzamido-2'-methylpropyl)-5-phenyl-1,3,4-oxadiazole, 2-(1'-benzamido-1'-methylethyl)-5-phenyl-1,3,4-oxadiazole, 2-(alpha-benzamidobenzyl)-5-phenyl-1,3,4-oxadiazole, 2-(3'-benzamidopropyl)-5-phenyl-1,3,4-oxadiazole, and 1,3-bis-(5'-phenyl-1',3',4'-oxadiazolyl)propane.

7. The structures of the 1,3,4-oxadiazoles derived from the tetrazole analogs of D,L-alpha-aminoisobutyric acid, D,L-C-phenylglycine, and glutaric acid have been established by hydrolytic degradation of these compounds and identification of all of the resulting degradative fragments.

8. The infra red spectra of 5-(1'-aminopropyl)tetrazole, 5-(1'-amino-2'-methylpropyl)tetrazole, and 5-(1'-amino-3'-methylbutyl)tetrazole are reported.

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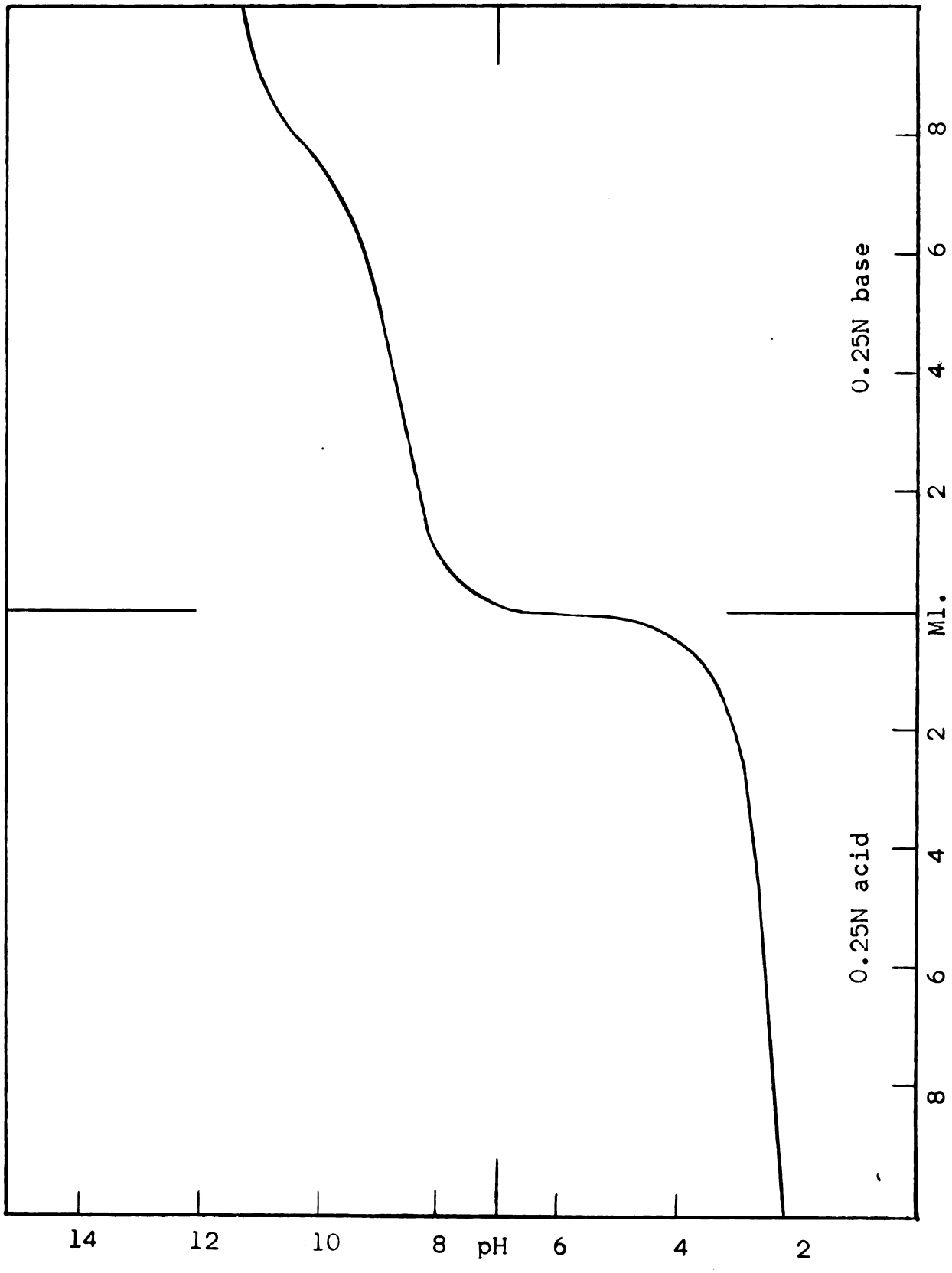
APPENDIX I

Data for the titration of some 5-aminoalkyltetrazoles with standard acid and base

Titration curve of a typical 5-(1'-aminoalkyl)tetrazole.
(5-(1'-Aminopropyl)tetrazole)



Data for the titration of 5-(1'-aminopropyl)tetrazole with standard hydrochloric acid at 25° C.

Sample weight: 0.1187 g. Acid strength: 0.2576N

Ml.	pH
0.00	6.18
0.50	3.80
1.00	3.50
1.50	3.30
1.75	3.21
2.00	3.13
2.50	2.99
3.00	2.90
3.50	2.82
4.00	2.75
4.50	2.69
5.00	2.63
5.50	2.58
6.00	2.52
6.50	2.47
7.00	2.43
8.00	2.35
9.00	2.30
10.00	2.24

pK_1 (calculated): 3.20

Data for the titration of 5-(1'-amino-2'-methylpropyl)-tetrazole with standard hydrochloric acid at 25° C.

Sample weight: 0.3159 g. (weighed as monohydrate) Acid strength: 0.2576N

Ml.	pH
0.00	6.15
0.50	4.01
1.00	3.70
1.50	3.50
2.00	3.37
2.50	3.25
3.00	3.15
3.50	3.05
3.75	3.00
3.85	2.98
4.00	2.96
4.50	2.87
5.00	2.80
5.50	2.74
6.00	2.70
6.50	2.66
7.00	2.62
7.50	2.59
7.75	2.57
8.00	2.55
8.50	2.52
9.00	2.49
10.00	2.44

pK_1 (calculated): 2.98

Data for the titration of 5-(1'-amino-3'-methylbutyl)-tetrazole with standard hydrochloric acid at 25° C.

Sample weight: 0.1740 g. Acid strength: 0.2576N

Ml.	pH
0.00	6.23
0.50	3.90
1.00	3.60
1.50	3.41
2.00	3.29
2.25	3.23
2.50	3.18
3.00	3.07
3.50	2.97
4.00	2.88
4.50	2.80
5.00	2.75
5.50	2.70
6.00	2.66
6.50	2.63
7.00	2.60
8.00	2.55
9.00	2.50
10.00	2.45

pK_1 (calculated): 3.25

Data for the titration of 5-(1'-amino-1'-methylethyl)-tetrazole with standard hydrochloric acid at 25° C.

Sample weight: 0.2579 g. Acid strength: 0.2576N

Ml.	pH
0.00	6.10
0.50	4.01
1.00	3.70
1.50	3.51
2.00	3.38
2.50	3.31
3.00	3.24
3.50	3.16
3.75	3.12
4.00	3.10
4.25	3.08
4.50	3.06
5.00	3.01
5.50	2.96
6.00	2.91
6.50	2.87
7.00	2.83
7.50	2.81
7.75	2.80
8.00	2.79
8.50	2.77
9.00	2.75
9.50	2.73
10.00	2.71

pK_1 (calculated): 3.10

Data for the titration of 5-(1'-aminobenzyl)tetrazole with standard hydrochloric acid at 25° C.

Sample weight: 0.3597 g. Acid Strength: 0.2576N

Ml.	pH
0.00	5.27
0.50	4.00
1.00	3.67
1.50	3.47
2.00	3.32
2.50	3.21
3.00	3.13
3.50	3.05
3.75	3.01
4.00	2.98
4.25	2.95
4.50	2.90
5.00	2.83
5.50	2.78
6.00	2.72
6.50	2.70
7.00	2.67
7.50	2.63
7.75	2.61
8.00	2.59
8.25	2.58
8.50	2.57
9.00	2.55
9.50	2.53
10.00	2.51

pK_1 (calculated): 2.98

Data for the titration of 5-(3'-aminopropyl)tetrazole with standard hydrochloric acid at 25° C.

Sample weight: 0.2643 g. Acid strength: 0.2576N

Ml.	pH
0.00	7.39
0.50	5.80
1.00	5.50
1.50	5.32
2.00	5.21
2.50	5.04
3.00	4.87
3.50	4.79
3.75	4.73
4.00	4.66
4.25	4.61
4.50	4.55
5.00	4.43
5.50	4.30
6.00	4.18
6.50	4.01
7.00	3.82
7.50	3.60
8.00	3.30
8.50	3.05
9.00	2.92
9.50	2.80
10.00	2.70

pK_1 (from curve): 4.65

Calculated equivalent weight: 128.2

Theoretical equivalent weight: 127.2

Data for the titration of 5-(1'-aminopropyl)tetrazole with standard sodium hydroxide at 25° C.

Sample weight: 0.2524 g. Base strength: 0.2544N

Ml.	pH
0.00	6.13
0.50	7.66
1.00	7.99
1.50	8.19
2.00	8.34
2.50	8.48
3.00	8.59
3.25	8.64
3.50	8.69
3.75	8.74
4.00	8.80
4.25	8.86
4.50	8.92
5.00	9.01
5.50	9.11
6.00	9.26
6.50	9.40
7.00	9.65
7.25	9.75
7.50	9.91
7.75	10.17
8.00	10.40
8.50	10.80
9.00	11.00
10.00	11.25

pK_2 (from curve): 8.80

Calculated equivalent weight: 127.5

Theoretical equivalent weight: 127.2

Data for the titration of 5-(1'-amino-2'-methylpropyl)-tetrazole with standard sodium hydroxide solution at 25° C.

Sample weight: 0.2604 g. Base strength: 0.2544N

Ml.	pH
0.00	6.10
0.50	7.60
1.00	7.94
1.50	8.14
2.00	8.30
2.50	8.44
3.00	8.58
3.25	8.63
3.50	8.70
4.00	8.85
4.50	9.00
5.00	9.18
5.50	9.39
6.00	9.70
6.20	9.91
6.30	10.03
6.35	10.10
6.40	10.18
6.45	10.25
6.50	10.30
7.00	10.83
7.50	11.09
8.00	11.24
9.00	11.39
10.00	11.53

pK_2 (from curve): 8.62

Calculated equivalent weight: 159.2*

Theoretical equivalent weight: 159.4*

* Calculated as the monohydrate

Data for the titration of 5-(1'-amino-3'-methylbutyl)-tetrazole with standard sodium hydroxide at 25° C.

Sample weight: 0.2514 g. Base strength: 0.2514N

Ml.	pH
0.00	6.35
0.50	7.79
1.00	8.10
1.50	8.30
2.00	8.47
2.50	8.61
3.00	8.74
3.25	8.81
3.50	8.88
4.00	9.02
4.50	9.18
5.00	9.33
5.50	9.55
6.00	9.83
6.25	10.08
6.50	10.38
6.75	10.59
7.00	10.78
7.50	11.00
8.00	11.18
8.50	11.27
9.00	11.34
10.00	11.45

pK_2 (from curve): 8.81

Calculated equivalent weight: 153.8

Theoretical equivalent weight: 154.2

Data for the titration of 5-(1'-amino-1'-methylethyl)tetrazole with standard sodium hydroxide at 25° C.

Sample weight: 0.2591 g. Base strength: 0.25144N

Ml.	pH
0.00	6.02
0.50	7.77
1.00	8.09
1.50	8.30
2.00	8.45
2.50	8.59
3.00	8.70
3.50	8.79
3.75	8.83
3.80	8.85
3.90	8.87
4.00	8.88
4.25	8.92
4.50	8.99
5.00	9.10
5.50	9.21
6.00	9.36
6.50	9.51
7.00	9.70
7.50	9.98
7.75	10.18
7.85	10.26
8.00	10.39
8.50	10.76
9.00	11.03
10.00	11.30

pK_2 (from curve): 8.88

Calculated equivalent weight: 127.2

Theoretical equivalent weight: 127.3

Data for the titration of 5-(1'-aminobenzyl)tetrazole with standard sodium hydroxide at 25° C.

Sample weight: 0.3497 g. Base strength: 0.2544N

Ml.	pH
0.00	5.25
1.00	6.95
2.00	7.34
3.00	7.60
3.50	7.70
3.75	7.75
4.00	7.80
4.50	7.90
5.00	8.01
6.00	8.30
7.00	8.70
7.25	8.85
7.50	9.09
7.75	9.43
7.85	9.72
8.00	10.10
8.25	10.52
8.50	10.75
9.00	11.00
10.00	11.25

pK_2 (from curve): 7.69

Calculated equivalent weight: 175.2

Theoretical equivalent weight: 174.9

Data for the titration of 5-(3'-aminopropyl)tetrazole with standard sodium hydroxide at 25° C.

Sample weight: 0.2553 g. Base strength: 0.2544N

Ml.	pH
0.00	7.32
0.50	9.20
1.00	9.54
1.50	9.74
2.00	9.90
3.00	10.15
3.50	10.26
3.75	10.30
4.00	10.36
4.25	10.41
4.50	10.43
5.00	10.54
5.50	10.65
6.00	10.77
6.50	10.89
7.00	11.00
7.50	11.10
8.00	11.19
8.50	11.25
9.00	11.30
9.50	11.35
10.00	11.40

pK_2 (calculated): 10.35

No definite endpoint is detectable from the curve

Data for the titration of 5-(3'-aminopropyl)tetrazole hydrochloride with standard sodium hydroxide at 25° C.

Sample weight: 0.3297 g. Base strength: 0.25$\mu\mu$N

Ml.	pH
0.00	3.39
1.00	3.96
2.00	4.27
3.00	4.51
3.50	4.62
4.00	4.73
4.50	4.85
5.00	4.97
6.00	5.21
7.00	5.63
7.50	6.07
7.75	6.68
7.85	7.71
7.90	8.22
8.00	8.68
8.50	9.32
9.00	9.60
10.00	9.92
11.00	10.15
12.00	10.33
13.00	10.49
14.00	10.58
15.00	10.89
16.00	11.09
17.00	11.20

pK_1 (from curve): 4.72

pK_2 (calculated): 10.30

Calculated equivalent weight (from first endpoint): 166.23

Theoretical equivalent weight: 163.62

Data for the titration of bis-1,3-di-(5'-tetrazolyl)propane with standard sodium hydroxide at 25° C.

Sample weight: 0.1650 g. Base strength: 0.06729N

Ml.	pH
0.00	3.32
5.00	4.49
10.00	4.95
15.00	5.40
20.00	5.85
21.00	5.95
22.00	6.05
23.00	6.23
24.00	6.38
25.00	6.56
26.00	6.87
26.50	7.15
26.75	7.40
27.00	7.86
27.25	9.10
27.35	9.45
27.50	9.79
27.75	10.10
28.00	10.30
28.50	10.50
29.00	10.65
30.00	10.81

Calculated equivalent weight: 89.82

Theoretical equivalent weight: 90.089

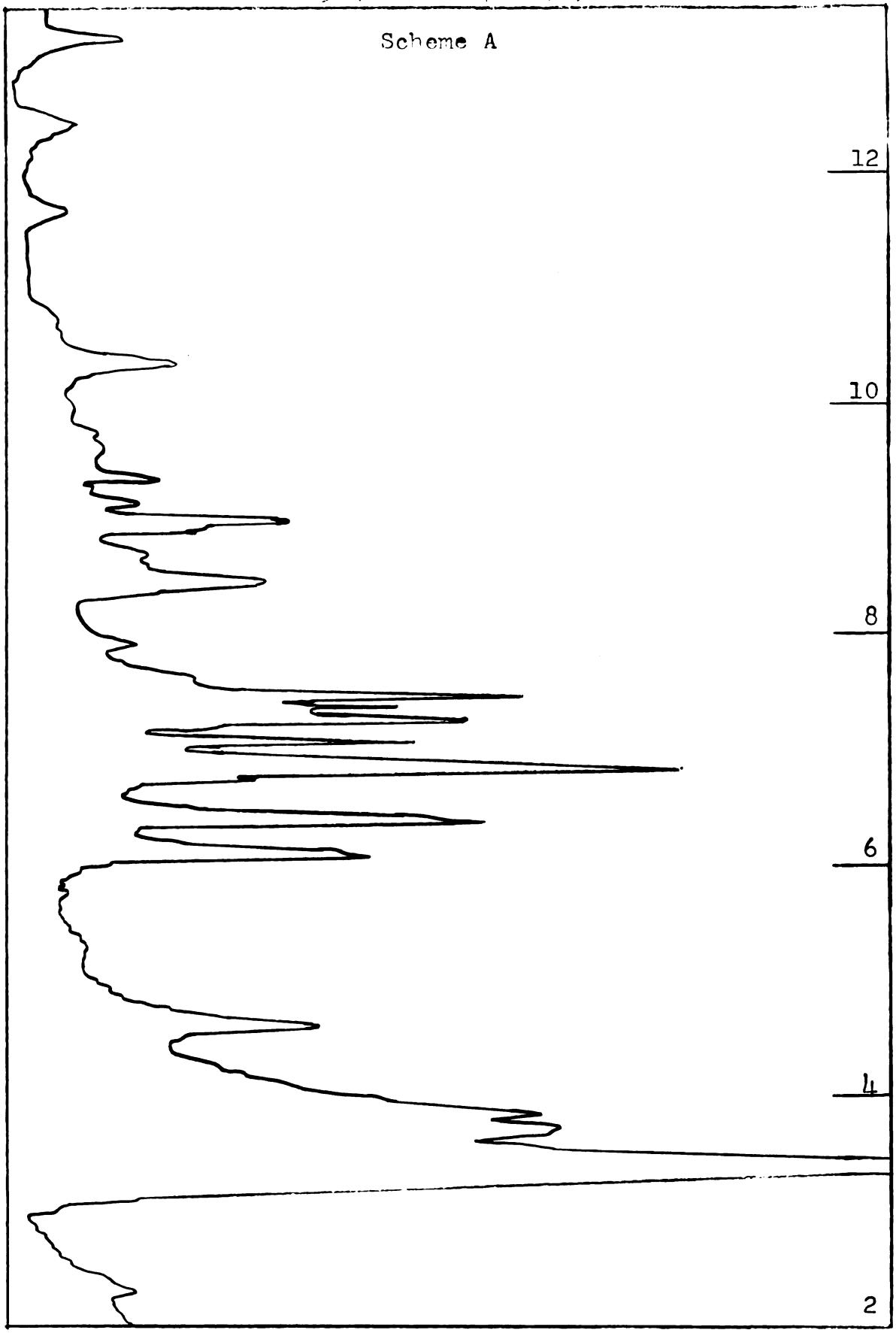
pK_a (from curve): 5.30

pK_a of glutaric acid: 4.34

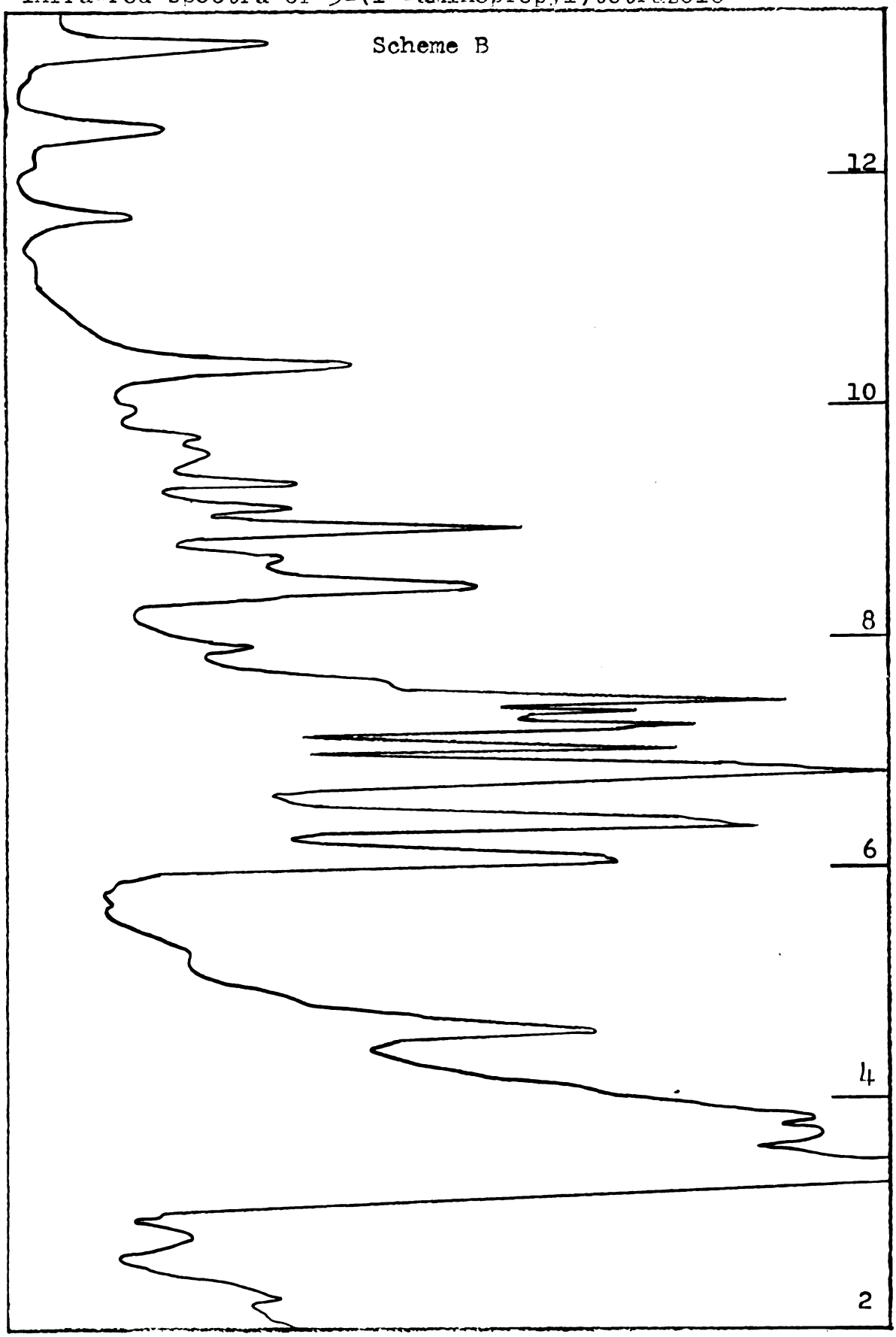
APPENDIX II

Infra red spectra of some 5-(1'-aminoalkyl)tetrazoles (taken as Nujol mulls).

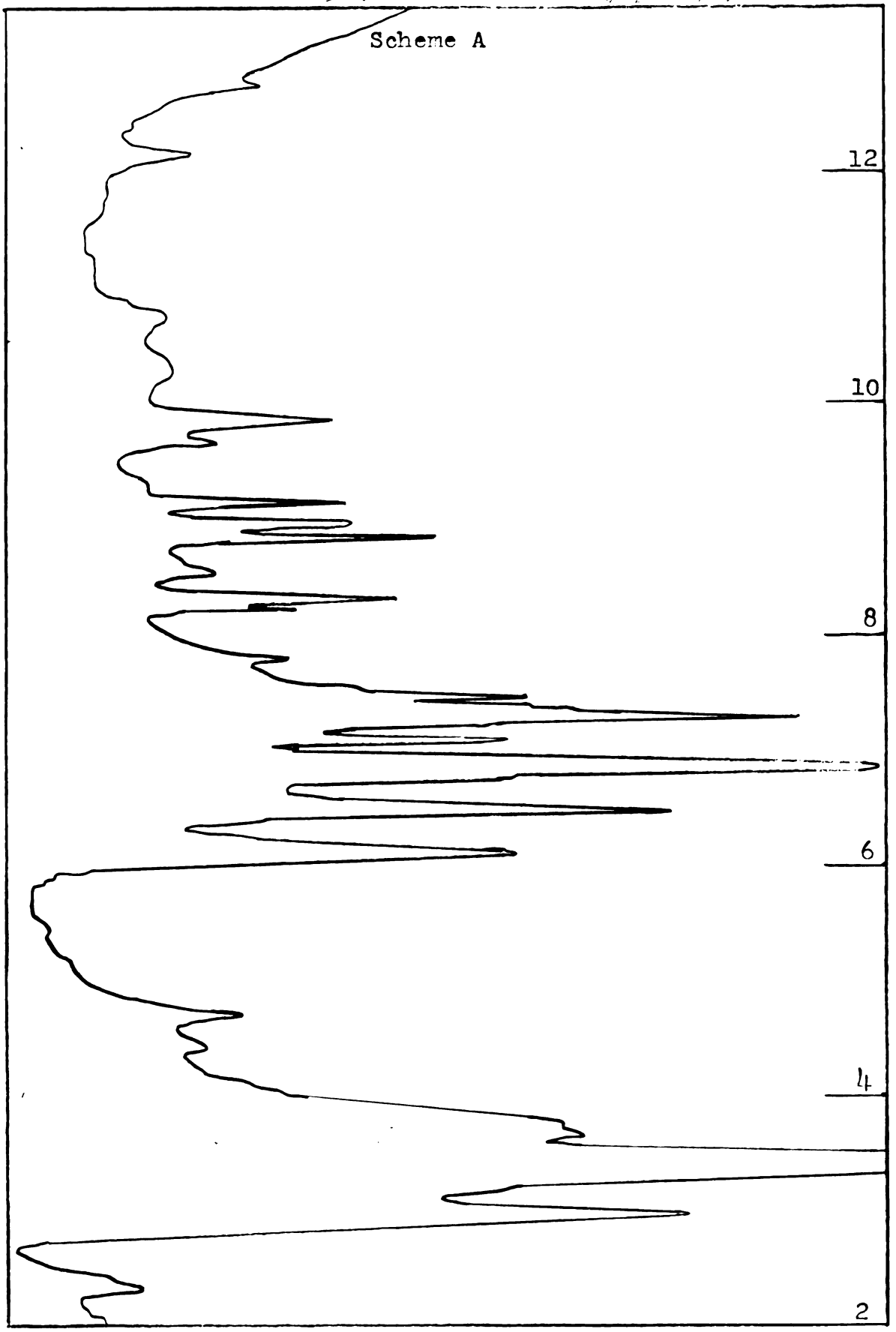
Infra-red spectra of 5-(1'-aminopropyl)tetrazole



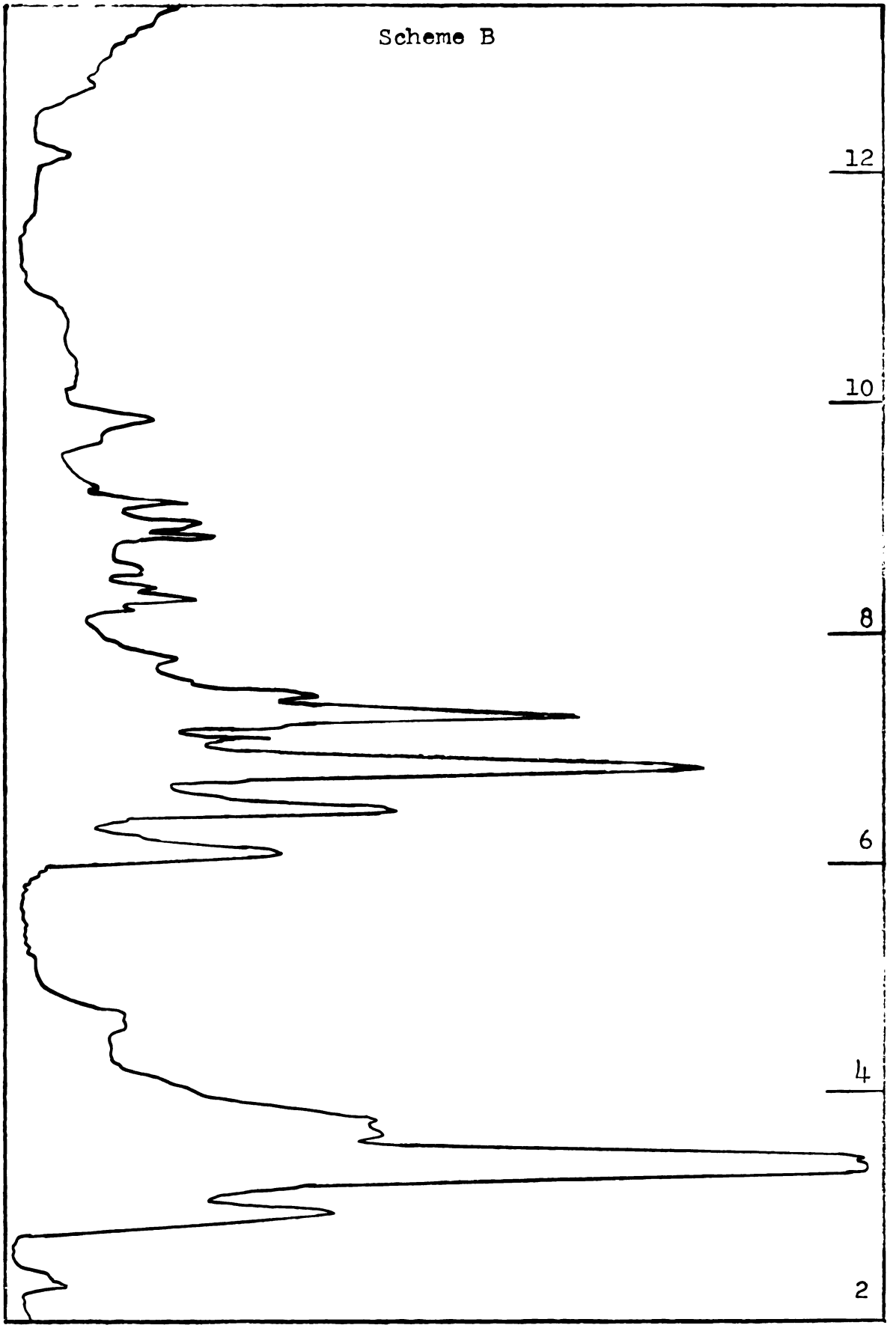
Infra-red spectra of 5-(1'-aminopropyl)tetrazole



Infra-red spectra of 5-(1'-amino-2'-methylpropyl)tetrazole

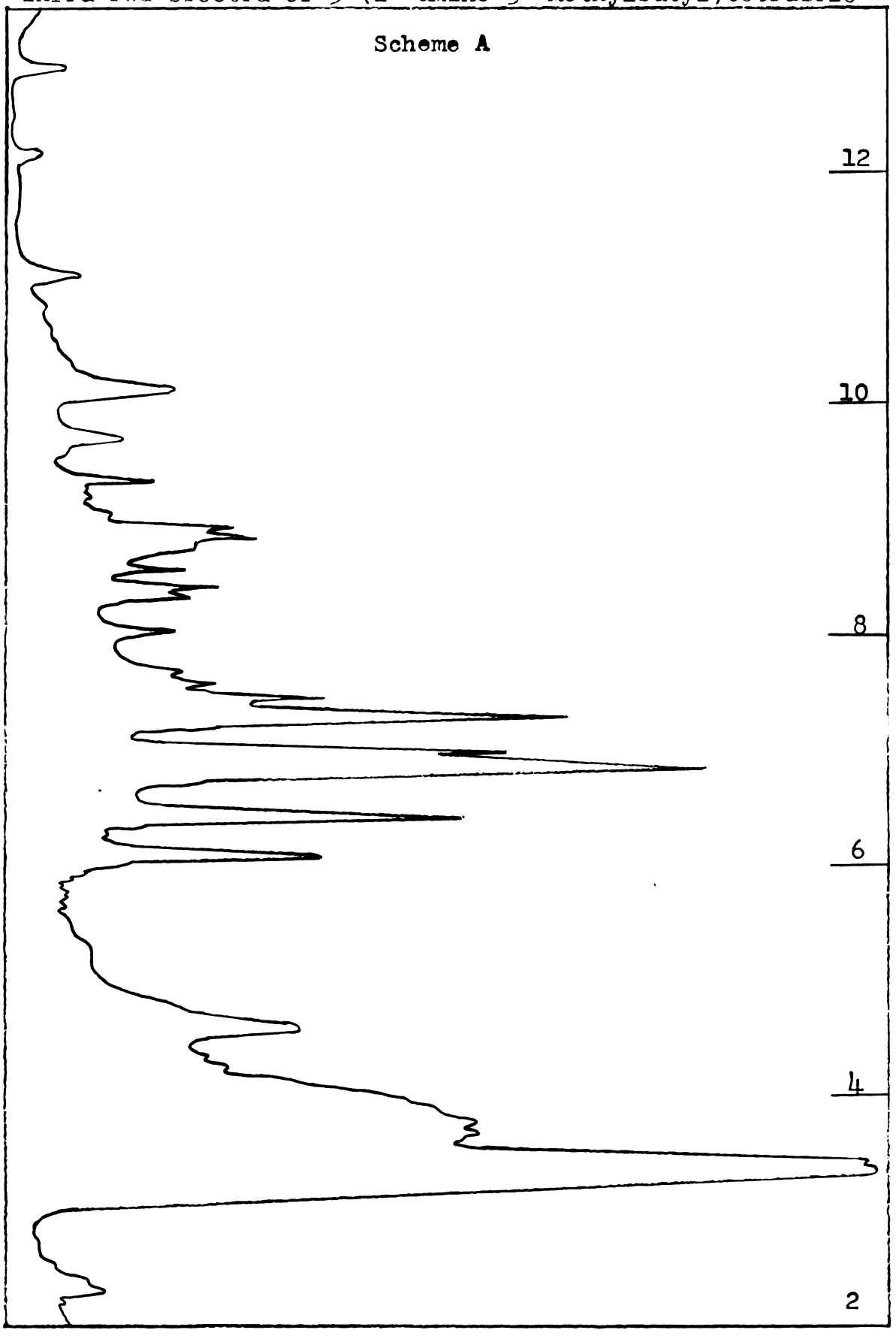


Infra-red spectra of 5-(1'-amino-2'-methylpropyl)tetrazole



Infra-red spectra of 5-(1'-amino-3'-methylbutyl)tetrazole

Scheme A



Infra-red spectra of 5-(1'-amino-3'-methylbutyl)tetrazole

Scheme B