SYNTHESIS OF TETRAZOLES

AS

POTENTIAL ANTIMETABOLITES

BY

JAMES M. MCMANUS

A THESIS

Submitted to the School of 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

1958

ACKNOWLEDGMENT

The writer wishes to express his appreciation to Doctor Robert M. Herbst for his most helpful counsel and guidance during the course of this work.

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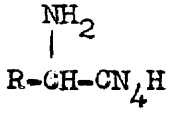
ABSTRACT

In many instances the success of chemotherapy in the treatment of both infectious and noninfectious diseases depends upon an antagonism between the chemotherapeutic agent used and a metabolite of the parasitic organism or foreign substance in the patient's system. One of the direct methods of antagonism involves competitive inhibition of an enzyme or tissue receptor associated with a metabolite by a structurally related antagonist. The resemblance of the antagonist to the metabolite may be through a general over all shape of the molecule or the similarity may be more refined and require the presence of one or more functional groups properly spaced within the structure.

Because of the acidic nature of 5-monosubstituted tetrazoles (1,2,3) it appeared that the investigation of such compounds as possible antimetabolites for certain physiologically active carboxylic acids might prove fruitful.

Accordingly, the tetrazole analogues (I) of glycine, D,L-alanine, β-alanine, D,L-phenylalanine and D,L-tryptophane have been prepared.

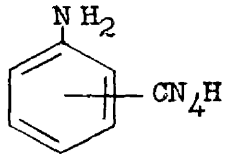


I

Three different routes have been developed for the synthesis of these 5-aminoalkyltetrazoles: 1. from α-haloacyl halides by way of the N-benzyl-α-haloamides, 2. from acyl amino acids by way of their nitriles, 3. from an appropriately alkylated ethyl acetamidocyanoacetate.

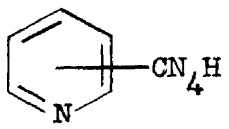
The pK values of all the 5-aminoalkyltetrazoles, excepting the tryptophane analogue, have been determined potentiometrically and shown to be analogous to those of the corresponding amino acids.

The three isomeric 5-nitrophenyltetrazoles and 5-(2'-hydroxy-4'-nitrophenyl)tetrazole have been prepared from the corresponding benzonitriles. Reduction of the 5-nitrophenyltetrazoles resulted in the formation of the corresponding 5-aminophenyltetrazoles which included the analogues (II) of <u>para</u>-aminobenzoic acid, <u>meta</u>- aminobenzoic acid and <u>para</u>-aminosalicylic acid.



II

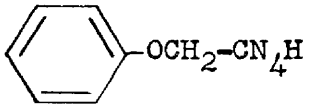
The three isomeric 5-pyridyltetrazoles (III) and 2,6-di(5'-tetrazolyl)pyridine have been prepared as analogues of the corresponding pyridine carboxylic acids by the interaction of the cyanopyridine with sodium azide and glacial acetic acid.



III

Reduction of the 5-pyridyltetrazoles has led to the synthesis of the corresponding 5-piperidyltetrazoles.

A group of six substituted phenoxyacetonitriles has been prepared from the corresponding phenols and chloroacetonitrile. By interaction with hydrazoic acid these were converted into substituted 5-phenoxymethyltetrazoles (IV) including the tetrazole analogues of 2,4-D and



IV

2,4,5-T. Several 5-phenoxymethyltetrazoles have been prepared by an alternate method that involved interaction of substituted phenols with 1-benzyl-5-chloromethyltetrazole and subsequent catalytic debenzylation of the resulting 1-benzyl-5-phenoxymethyltetrazoles.

The tetrazole analogue of 3-indoleacetic acid has also been prepared by the interaction of 3-indolylacetonitrile with aluminum azide.

The interaction of 3,4,5-trimethoxybenzonitrile with sodium azide and glacial acetic acid has led to the formation of 5-(3',4',5'-trimethoxyphenyl)tetrazole. Alkylation of this compound with dialkylaminoalkyl halides was used to prepare three 2-(dialkylaminoalkyl)-5-(3',4',5'-trimethoxyphenyl)tetrazoles which have been characterized as the hydrochlorides.

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1. E. Oliveri - Manadalá, Gazz. chim. ital., 4, II, 175 (1914); C.A., 9, 884 (1915).

2. J. Mihina and R. Herbst, J. Org. Chem., 15, 1082 (1950)

3. R. Herbst and K. Wilson, J. Org. Chem., 22, 1142 (1957).

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INTRODUCTION

In many instances the success of chemotherapy in the treatment of both infectious and noninfectious diseases depends upon an antagonism between the chemotherapeutic agent used and a metabolite of the parasitic organism or foreign substances in the patient's system. Since synthesis and utilization of metabolites or metabolic products are essential to the maintenance and production of living cells, any interference with these processes would result in the death of the organism.

One of the direct methods of antagonism involves competitive inhibition of an enzyme or tissue receptor associated with a metabolite by a structurally related antagonist. The resemblance of the antagonist to the metabolite may be through a general over all shape of the molecule or the similarity may be more refined and require the presence of one or more functional groups properly spaced within the structure. These functional groups are believed to be capable of associating with the prosthetic or reactive group of the enzyme normally available for the metabolite.

One of the most outstanding and well known examples of structural antagonism applied in chemotherapy is that shown for bacterial para-aminobenzoic acid by para-aminobenzenesulfonamide. It is suggested that the inhibition of the utilization of para-aminobenzoic acid in bacteria prevents its incorporation into folic acid, a vitally needed component for growth and reproduction.

Because of the acidic nature of 5-monosubstituted tetrazoles (1,2,3) it appeared that the investigation of such compounds as possible antimetabolites for certain physiologically active carboxylic acids might prove fruitful. This is the first reported instance where 5-monosubstituted tetrazoles have been prepared as analogues of biologically active carboxylic acids.

Accordingly, the present work involves the synthesis of tetrazoles modeled after various classes of metabolic and physiologically active acids and derivatives in which the carboxyl group is replaced by a tetrazolyl group substituted in the 5 position. For convenience and clarity the discussion and experimental are divided into the classes of carboxylic acids used as models for the tetrazole syntheses as follows:

Part I. Tetrazole Analogues of Amino Acids.

Part II. Tetrazole Analogues of Active Aminobenzoic Acid Derivatives.

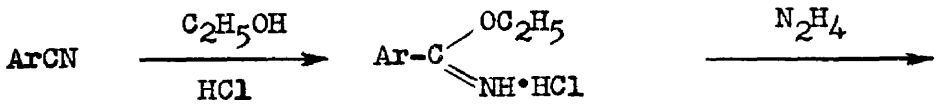
Part III. Tetrazole Analogues of Nicotinic Acid and Other Pyridine Carboxylic Acids.

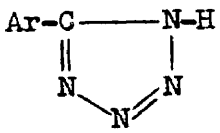
Part IV. Tetrazole Analogues of Plant Auxins.

Part V. Disubstituted Tetrazoles as Analogues of Active Basic Esters.

HISTORICAL

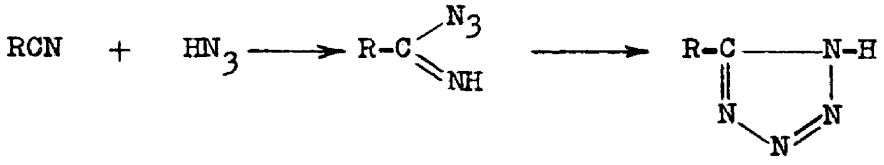
Monosubstituted tetrazoles with the substituent in the 5 position have been prepared from nitriles by a variety of procedures. The conversion of aryl cyanides to 5-aryltetrazoles by way of the imino ethers, amidrazones and imide azides in a stepwise manner was developed by Pinner (4) and may be illustrated schematically as follows:





It has also been shown that 5-aryltetrazoles may be prepared in a convenient manner and in excellent yields through the reaction of aryl cyanides with equivalent amounts of sodium azide and glacial acetic acid in refluxing normal butyl alcohol (3).

The formation of either 5-alkyl or aryltetrazoles has been reported by Mihina and Herbst (2) to proceed in a single step from the respective cyanides by heating the nitrile with a benzene solution of hydrazoic acid in a sealed tube. This is believed to proceed through the imide azide as follows:



More recently a variety of 5-substituted tetrazoles have been prepared by reaction of the cyano compound with an excess of aluminum azide, generated in situ from aluminum chloride and sodium azide in refluxing tetrahydrofuran. (5)

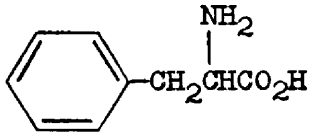
PART I

TETRAZOLE ANALOGUES OF AMINO ACIDS

Discussion

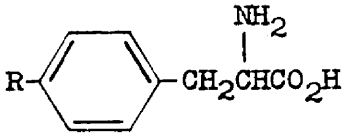
Numerous examples of metabolite antagonism have been reported for compounds which are analogues of naturally occurring α-amino acids.

One of the most thoroughly investigated of these amino acids is phenylalanine (I). Various changes in its molecular structure have



I

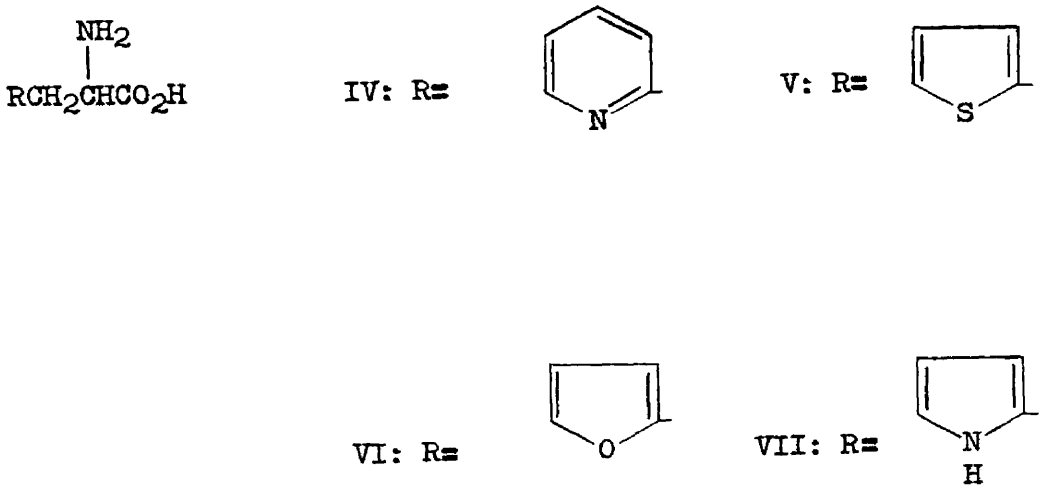
been shown to transform it into an antagonist of the naturally occurring phenylalanine in bacterial growth. Among these changes sufficient to reverse the nutritional effect of this amino acid are the introduction of an amino group (II) (6) or a fluoro group (III) (7) <u>para</u> to the alanine moiety in the benzenoid portion of the structure.



II: R= NH_2

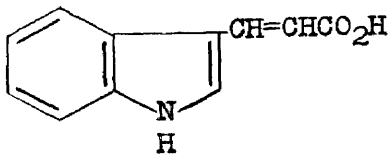
III: R= F

Substitution of certain heterocyclic rings for the phenyl group, such as 2-pyridyl (IV) (8), 2-thienyl (V) (9), 2-furyl (VI) (10), and 2-pyrryl (VII) (11) has also resulted in analogues which have a



specific antagonism for phenylalanine.

Antimetabolites for tryptophane, an essential amino acid for man, are found in 5-methyltryptophane (12) and β-indoleacrylic acid (VIII) (13).



VIII

These changes necessary to develop antimetabolite activity are not restricted to any one portion of the amino acid structure; replacement of the carboxy group by a sulfonic acid residue has led to the synthesis of analogues of glycine, valine, alanine and leucine (14). In some cases these amino sulfonic acids have shown specific inhibition of the use of those amino acids of which they were the analogues as measured by bacterial growth (14).

It appeared fruitful, therefore, in view of this encouraging evidence, to prepare analogues of naturally occurring amino acids in which a tetrazolyl group was substituted for the carboxy group as the acidic portion of the amino acid molecule.

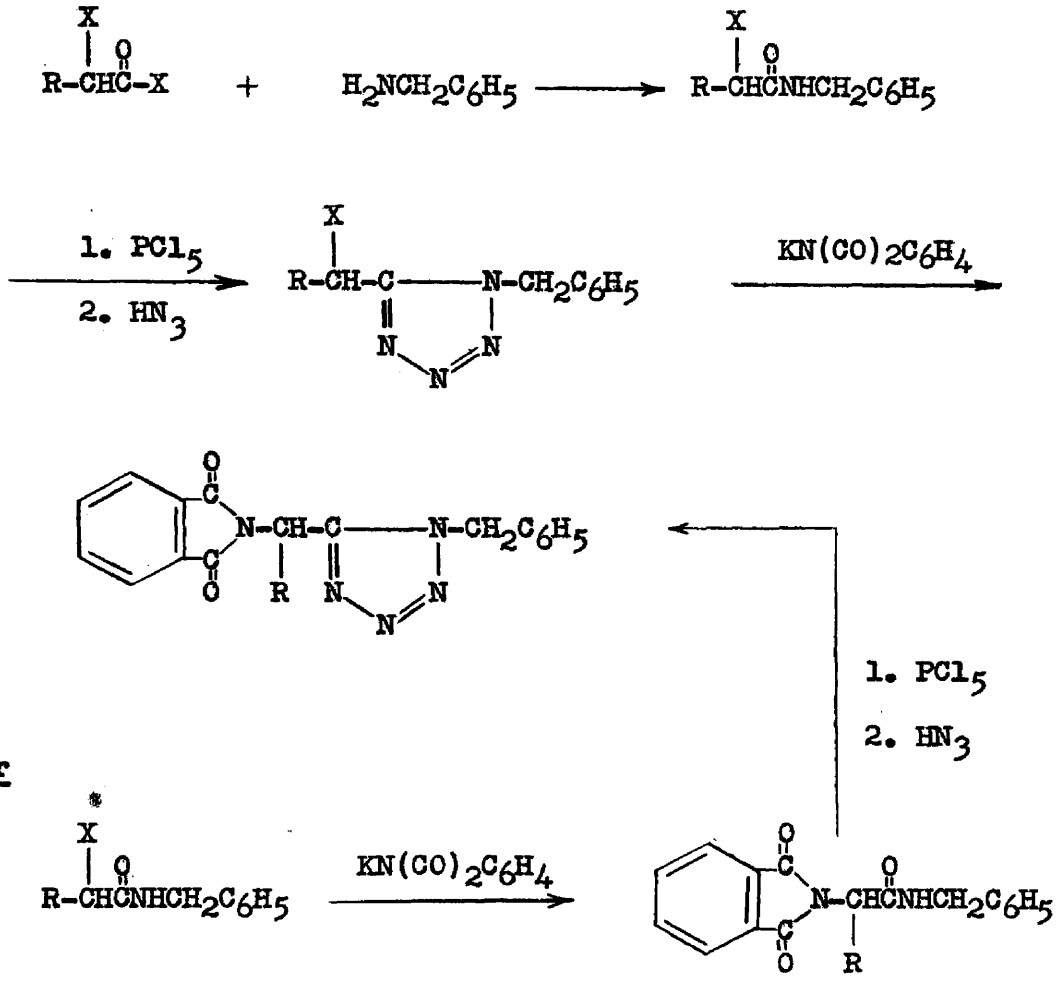
Five different amino acid analogues were prepared: the analogues of glycine, D,L-alanine, β-alanine, D,L-phenylalanine and D,L-tryptophane. The synthesis of each aminoalkyltetrazole, with the exception of the tryptophane analogue, was carried out by two different methods, making it possible to corroborate the structure of the final product from one sequence of reactions by a second unequivocal synthesis. Other advantages of using certain specific synthetic methods will be mentioned in the discussion of that specific scheme of preparation.

The three general methods used in the preparation of the 5-aminoalkyltetrazoles are designated, for simplicity, schemes A, B and C as follows:

Scheme A

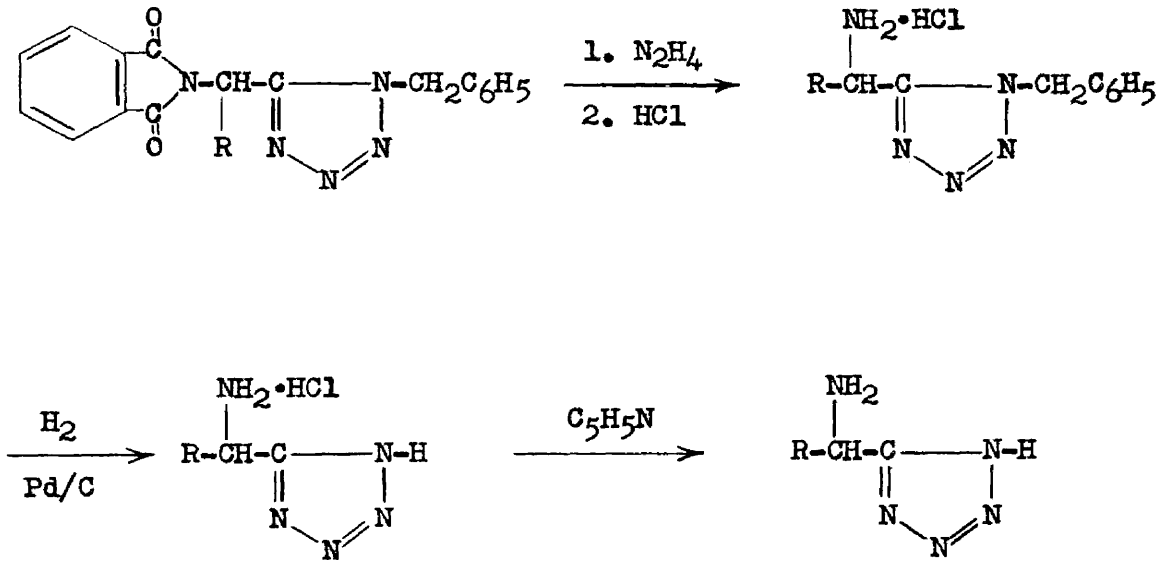
This sequence of stepwise conversions involved the initial reaction of an α-haloacyl halide with benzylamine forming the N-benzyl-α-haloamide. Subsequent conversion of the α-haloamide with phosphorus pentachloride to the imino chloride followed by treatment with hydrazoic acid gave rise to a 1-benzyl-5-α-haloalkyltetrazole. The haloalkyltetrazole was then caused to react with potassium phthalimide forming the 1-benzyl-5-α-phthalimidoalkyltetrazole. Because of the irritating effects of the α-haloalkyltetrazoles on the mucous

membranes it was found more advantageous to first form the α-phthalimidoamide from potassium phthalimide and the α-haloamide. The product was then treated with phosphorus pentachloride and hydrazoic acid. These sequences of reactions may be represented schematically as follows:



The phthalyl residue was conveniently removed from the 1-benzyl-5-α-phthalimidoalkyltetrazole by treatment with hydrazine. The resulting 1-benzyl-5-α-aminoalkyltetrazole was isolated as the hydrochloride.

Treatment of the 1-benzyl-5-α-aminoalkyltetrazole with hydrogen in the presence of palladium on charcoal effected debenzylation of the compound. Liberation of the 5-α-aminoalkyltetrazole from the hydrochloride was accomplished by treatment of an alcohol solution of the amine hydrochloride with a slight excess of pyridine. In cases where the basicity of the amine was too great to allow this type of exchange to be carried out, silver oxide was used to prepare the free amine from the hydrochloride.

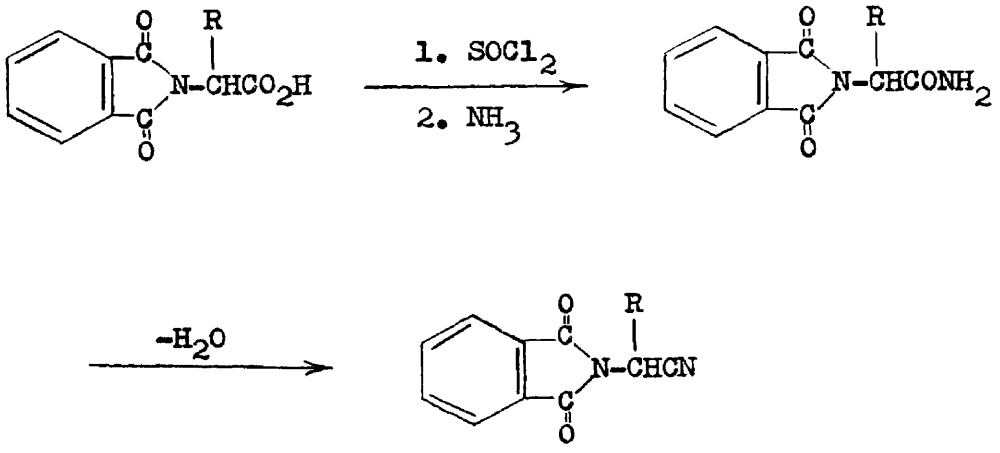


Preparation of the 5-aminoalkyltetrazoles by this series of reactions involved, (1) synthesis of the tetrazole ring from an amide group and (2) formation of the final product by an unambiguous series of reactions.

Scheme B

The reaction of the phthalyl derivative of an α-amino acid with

thionyl chloride followed by treatment with ammonia gas or concentrated ammonium hydroxide resulted in the formation of an ∝-phthalimidoamide. Dehydration of the amide caused the formation of an ∝-phthalimidonitrile.



Preparation of the 5-∝-phthalimidoalkyltetrazole from the corresponding nitrile was effected by an adaptation of the method of Behringer and Kohl (5) which involved conversion of the cyano compound into a 5-substituted tetrazole using aluminum azide, generated *in situ* from sodium azide and aluminum chloride in refluxing tetrahydrofuran. The procedure suggested by Behringer and Kohl for working up the reaction mixture, which consisted of the addition of dilute hydrochloric acid, evaporation to dryness *in vacuo* and extraction of the product with acetone, was replaced by a modified procedure which involved distillation of the tetrahydrofuran from the reaction mixture and simultaneous addition of water at such a rate that the volume of the mixture remained constant. The insoluble aluminum salt which remained after all the tetrahydrofuran had been removed was then treated with

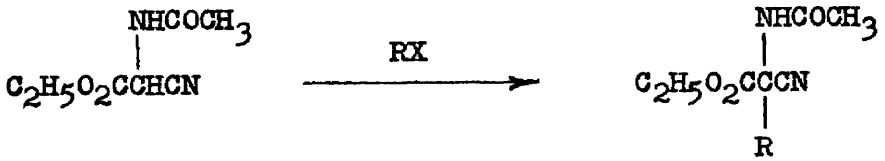
dilute hydrochloric acid. The free, insoluble tetrazole was liberated from the aluminum salt in this way.

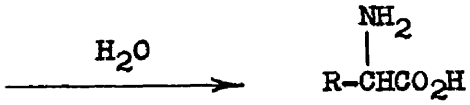
Removal of the phthalyl moiety with hydrazine resulted in the formation of the 5-α-aminoalkyltetrazole hydrochloride from which the free amine was generated by treatment with silver oxide or pyridine.

Starting with an amino acid, for which this route is adapted, offers the possibility of converting an optically active amino acid into an optically active tetrazole analogue.

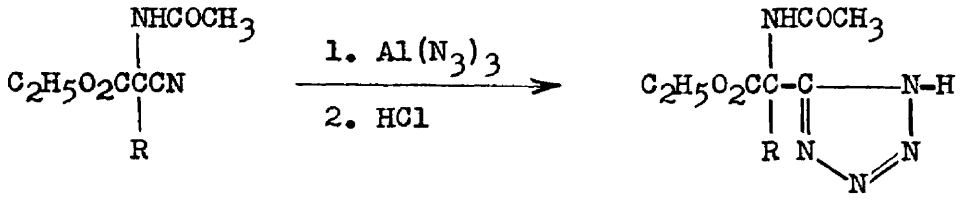
Scheme C

One synthetic method used in the preparation of various α-amino acids consists of the alkylation of ethyl acetamidocyanoacetate followed by acidic or basic hydrolysis to the amino acid.





Treatment of the alkylated ethyl acetamidocyanoacetate intermediate with aluminum azide according to the modified procedure of Behringer and Kohl, which has been previously discussed, resulted in the conversion of the nitrile into a 5-monosubstituted tetrazole.



Either a stepwise hydrolysis of the functional groups or a single step acid hydrolysis gave rise to the ∝-amino acid analogue.

This route to 5-∝-aminoalkyltetrazoles offers a minimum number of steps and makes use of intermediate compounds common to amino acid synthesis.

The synthesis of the individual amino acid analogues is considered in the remaining portion of this discussion; mention is made of any noteworthy differences or anomalies in the reaction sequence or in the isolation of the final product.

5-∝-Aminomethyltetrazole - The glycine analogue was conveniently prepared by scheme A and scheme B.

Since glycine is not optically active the pursuit of scheme B starting from the amino acid offered no advantage over starting from some more easily available intermediate. For this reason the synthesis of 5-∝-aminomethyltetrazole by scheme B was initiated with phthalimidoacetonitrile prepared by the alkylation of potassium phthalimide with chloroacetonitrile.

D,L-5-∝-Aminoethyltetrazole - The synthesis of the tetrazole analogue of D,L-alanine from ∝-bromopropionyl bromide was effected using scheme A and from D,L-alanine according to scheme B.

In the synthetic route following scheme A difficulties experienced

in handling the low melting, mucous membrane irritating α-chloromethyltetrazole in the glycine analogue synthesis prompted the conversion of the N-benzyl-α-bromopropionamide into the N-benzyl-α-phthalimidopropionamide and this subsequently into 1-benzyl-5-α-phthalimidoethyltetrazole, thereby avoiding the formation of the α-haloalkyltetrazole. The remaining reactions in this series were carried out without further departure from the sequence as previously discussed.

5-β-Aminoethyltetrazole - The analogue of β-alanine was synthesized following the procedures of scheme A and scheme B.

During preparation of the product by scheme A, the reaction of N-benzyl-β-bromopropionamide with potassium phthalimide was found to result in dehydrohalogenation of the β-haloamide. The necessary adaptation in that portion of the synthesis to compensate for this difficulty consisted in treating the acid chloride of phthalyl-β-alanine with benzylamine to form N-benzyl-β-phthalimidopropionamide. The subsequent reactions involved in scheme A were followed without further changes.

Synthesis of the final product following scheme B was carried out starting from β-phthalimidopropionitrile, obtained by the cyanoethylation of phthalimide with acrylonitrile. The remainder of the synthesis was effected using the stepwise conversions previously discussed for this synthetic route.

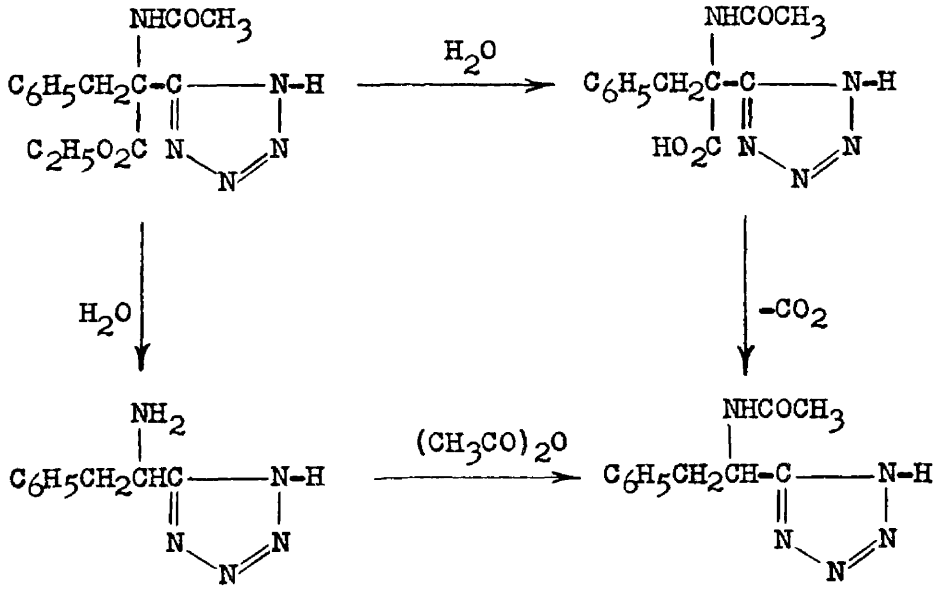
Comparison of the final products from each scheme of preparation for the glycine, D,L-alanine, and β-alanine analogues was made by melting point and mixture melting point.

The analogues thus far mentioned were soluble in water, aqueous acids and aqueous alkalies and exhibited high melting points accompanied by decomposition. The aminoalkyltetrazoles are insoluble in acetone, ethanol and non-polar solvents.

Using methods applicable to the characterization of amino acids, the acetyl, benzoyl and phenylurea derivatives were prepared.

5-α-Aminophenylethyltetrazole - Schemes B and C were used as the synthetic routes for the preparation of the phenylalanine analogue.

The intermediate product, ethyl α-acetamido-α-(5-tetrazolyl)-β-phenylpropionate, obtained by following scheme C, was subjected to both stepwise degradation and single step acid hydrolysis.



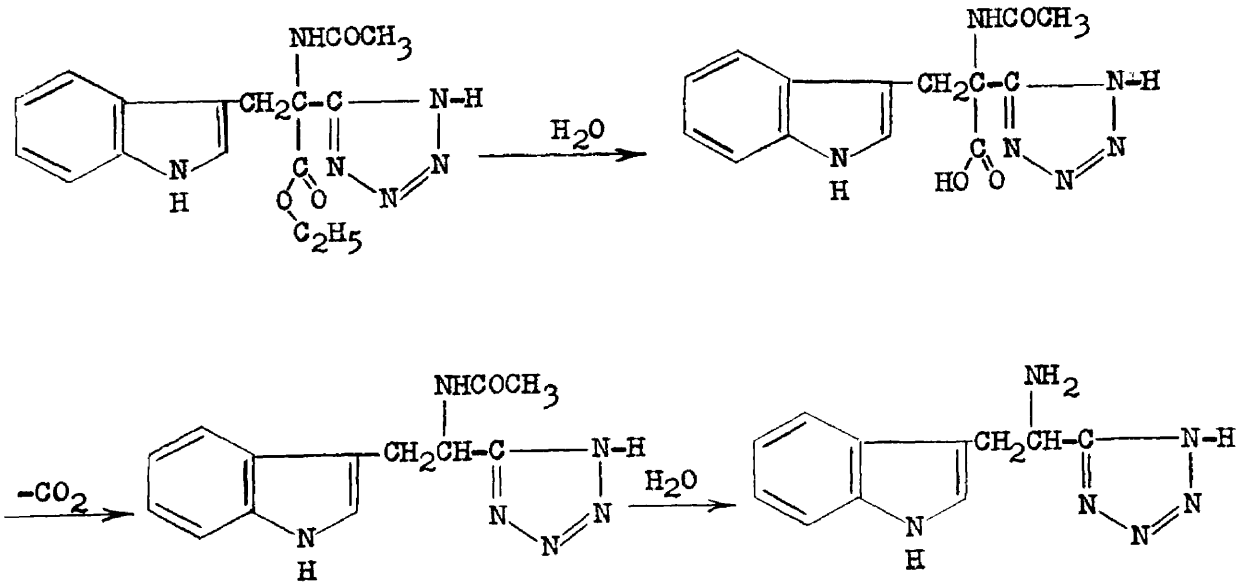
Similar products prepared by scheme B or scheme C were shown to be identical by melting point and mixture melting point determinations.

The D,L-phenylalanine analogue which possessed a high melting point accompanied by decomposition was soluble in aqueous acids and aqueous alkalies and insoluble in ethanol and non-polar solvents. It was soluble in hot water and crystallized as the solution was cooled.

The acetyl, benzoyl and phenylurea derivatives were used to characterize the 5-α-aminophenylethyltetrazole.

5-(α-Aminoβ-(3'-indolyl)ethyl)tetrazole - Scheme C was the only synthetic route used for the preparation of the tryptophane analogue.

The product formed from the reaction of ethyl α-acetamido-α-cyano-β-(3-indolyl)propionate with aluminum azide was degraded to the tryptophane analogue in a stepwise manner using sodium hydroxide as represented schematically below.



In an attempt to further substantiate the similarity of 5-alkylaminotetrazoles to α-amino acids the apparent pK_1 and pK_2 values for the amino acid analogues were determined potentiometrically in aqueous solution at 25°C.

A comparison of the pK_1 values of the amino acids with the tetrazole analogues indicated that the tetrazole group behaves as a weaker acid than the carboxyl group of the amino acid. This would be anticipitated from the work of Mihina and Herbst (2) which showed that 5-alkyltetrazoles were weaker acids than the corresponding carboxylic acids. Examination of the pK_2 values of the aminoalkyltetrazoles suggests that the basicity of the amino group is less than that of the amino group in the amino acids series.

The pK_1 values of the aminoalkyltetrazoles decrease in the order: 5-(β-aminoethyl)tetrazole > 5-(α-aminoethyl)tetrazole > 5-aminomethyltetrazole > 5-(α-amino-β-phenylethyl)tetrazole. This order parallels that in the amino acid series without exception. Examination of the pK_2 values shows a similar parallel order in the basicity of the amino group of the aminoalkyltetrazoles as compared to the amino group of the amino acids. The pK_2 values decrease in the order: 5-(β-aminoethyl)tetrazole > 5-(α-aminoethyl)tetrazole > 5-aminomethyltetrazole > 5-(α-amino-β-phenethyl)tetrazole.

These results are summarized in Table I and the data are collected in Appendix I.

TABLE I

APPARENT pK VALUES OF SOME 5-AMINOALKYLTETRAZOLES AND CORRESPONDING AMINO ACIDS IN AQUEOUS SOLUTION AT 25°C.

Tetrazole	pK_1	pK_2	Amino Acid[1] Analogue	pK_1	pK_2
5-Aminomethyl	2.62	8.54	Glycine	2.34	9.60
5-α-Aminoethyl	2.63	8.77	D,L-Alanine	2.34	9.69
5-β-Aminoethyl	3.99	9.58	β-Alanine	3.60	10.19
5-α-Amino-β-phenylethyl	1.93	8.18	D,L-Phenyl-alanine	1.83	9.13

[1]The pK values for the amino acids were obtained from reference (28).

Experimental[1,2]

The Preparation of 5-Aminomethyltetrazole

(Glycine Analogue)

Scheme A.

(a) N-Benzyl-α-chloroacetamide. Chloroacetylchloride (100 g., 0.88 mole) was slowly added to 188.5 g. (1.76 moles) of benzylamine dissolved in 1 liter of benzene. After the addition was complete the mixture was stirred over night. The reaction mixture was then filtered, the solid suspended in water and again filtered. The benzene solution was concentrated to a small volume, chilled and filtered. This solid was combined with the water insoluble material and the combined product recrystallized from benzene. This gave 106 g. (66% of theory) of N-benzyl-α-chloroactamide, m.p. 92-93.5°C.

This compound is reported by Jacobs and Heidelberger (15) to have a melting point of 93.5-94.5°C.

(b) 1-Benzyl-5-chloromethyltetrazole. To 98.6 g. (0.543 mole) of N-benzyl chloroacetamide suspended in 1 liter of benzene was added in portions 113 g. (0.543 mole) of phosphorus pentachloride. The mixture was allowed to stir at room temperature for six hours after which it

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[1] Microanalyses by Micro-Tech Laboratories, Skokie, Illinois.

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

was concentrated to a volume of 500 ml. under reduced pressure. To the concentrated solution 38.1 g. (0.885 mole) of hydrazoic acid in 300 ml. of xylene was added with cooling. After allowing the mixture to stir overnight at room temperature it was heated to reflux temperature until hydrogen chloride evolution ceased. Removal of the solvent under vacuum left a thick syrup to which was added 500 g. of ice and water. Filtering gave a brown solid which was further purified by recrystallization from an isopropyl alcohol-normal heptane mixture. The product weighed 55.2 g. (49% of theory) and melted at 66-67.5°C.

This compound is described by Harvill, Herbst and Schreiner (16), who reported the melting point as 67-68°C.

(c) 1-Benzyl-5-phthalimidomethyltetrazole. A mixture of 21 g. (0.1 mole) of 1-benzyl-5-chloromethyltetrazole and 21 g. (0.114 mole) of potassium phthalimide was refluxed together in 250 ml. of dry xylene for five hours. The hot solution was filtered with suction and the filtrate cooled. The precipitate, which was filtered and dried, weighed 26.4 g. (83% of theory). The product was further purified by recrystallization from toluene, m.p. 132-133°C.

Analysis. Calculated for $C_{17}H_{13}N_5O_2$: C, 63.94; H, 4.10; N, 21.93. Found: C, 64.07; H, 4.23; N, 22.16.

(d) 1-Benzyl-5-aminomethyltetrazole Hydrochloride. To 10.6 g. (0.033 mole) of 1-benzyl-5-phthalimidomethyltetrazole suspended in 120 ml. of absolute ethanol was added 33 ml. of a one molar solution of hydrazine hydrate in ethanol (0.033 mole) and the resulting mixture refluxed with continued stirring for three hours. The mixture was then

evaporated to dryness and treated with 75 ml. of a 2N hydrochloric acid for ten minutes at 50°C. The resulting suspension was filtered and the filtrate evaporated to dryness.

Recrystallization of the residual solid from an isopropyl alcohol-water mixture gave 4.0 g. (53% of theory) of the product, m.p. 228-229°C.

Analysis. Calculated for $C_9H_{12}ClN_5$: C, 47.90; H, 5.36; Cl, 15.71; N, 31.04. Found: C, 48.11; H, 5.25; Cl, 15.71; N, 31.16.

(e) 5-Aminomethyltetrazole. 1-Benzyl-5-aminomethyltetrazole hydrochloride (10.5 g., 0.047 mole) was dissolved in a mixture of 200 ml. of absolute ethanol and 30 ml. of water. After addition of 2.5 g. of 5% palladium on charcoal the mixture was shaken in a hydrogen atmosphere at an initial pressure of 47 p.s.i. and heated to maintain a temperature of 60°C. After twenty-four hours the catalyst was removed by filtration and the filtrate evaporated to dryness in vacuo. This gave 6.2 g. (97% of theory) of the crude amine hydrochloride.

The crude 5-aminomethyltetrazole hydrochloride (6.1 g., 0.045 mole) was dissolved in 100 ml. of absolute ethanol to which was then added 3.56 g. (0.045 mole) of pyridine. On allowing the solution to stand in an ice chest overnight there was deposited 3.8 g. (85% of theory) of a crystalline solid. The product was further purified by dissolving it in a small amount of water and adding enough absolute ethanol to give a 95% ethanol solution. This solution was allowed to stand in an ice chest overnight allowing the pure product to separate, m.p. 267°C., with decomposition.

Analysis. Calculated for $C_2N_5H_5$: C, 24.24; H, 5.09; N, 70.68. Found: C, 24.49; H, 5.17; N, 70.55.

Scheme B.

(a) Phthalimidoacetonitrile. Potassium phthalimide (110.4 g., 0.6 mole) was added to 450 ml. of distilled dimethylformamide containing 40.8 g. (0.54 mole) of chloroacetonitrile. The resulting wine colored solution was stirred at room temperature for one hour and then at steam bath temperature for two hours. The resulting mixture was transferred to a three liter beaker and 600 ml. of chloroform added followed by 1500 ml. of water. The organic layer was separated and the aqueous portion washed with an additional 300 ml. of chloroform. The two organic layers were then combined, washed with 300 ml. of 0.2 N sodium hydroxide solution and then with 300 ml. of water. Evaporation of the chloroform after drying the solution with sodium sulfate, gave a solid residue.

Recrystallization of the product gave 70.8 g. (63.5% of theory) of a colorless crystalline material melting at 127.5-128.5°C.

Analysis. Calculated for $C_{10}H_6N_2O_2$: N, 15.05. Found: N, 15.32.

This compound is reported by Sonn and Falkenheim (17) to melt at 124-125°C.

(b) 5-(Phthalimidomethyl)tetrazole. To 46.5 g. (0.25 mole) of phthalimidoacetonitrile and 50 g. of sodium azide suspended in 100 ml. of dry tetrahydrofuran was added 35 g. (0.26 mole) of anhydrous aluminum chloride dissolved in 300 ml. of the same solvent. The mixture was then heated at reflux temperature for twenty-four hours

with continuous stirring. At the end of this time the tetrahydrofuran was distilled out of the reaction mixture and water added at such a rate that the volume remained constant. The resulting residual suspension was cooled and the solid filtered. The solid was in turn suspended in 450 ml. of water to which was then added 50 ml. of concentrated hydrochloric acid. The resulting mixture was stirred for one hour at room temperature, filtered and the solid dried. The crude product weighed 50.6 g. (88.5% of theory), m.p. 233.5-235°C., with decomposition. Recrystallization from a mixture of ethyl acetate-ethanol raised the melting point to 234-235°C., with decomposition.

Analysis. Calculated for $C_{11}H_9N_5O_2$: C, 52.40; H, 3.08; N, 30.56. Found: C, 52.44; H, 2.98; N, 30.76.

(c) 5-Aminomethyltetrazole. A suspension of 45.8 g. (0.2 mole) of 5-(phthalimidomethyl)tetrazole in 300 ml. of absolute ethanol was treated with 200 ml. of a one molar solution of hydrazine hydrate in ethanol and the resulting mixture stirred at reflux temperature for three hours. The mixture was then cooled in an ice-box over night and filtered. The solid was suspended in 450 ml. of a 2N hydrochloric acid for fifteen minutes at 50-55°C. The resulting precipitate was filtered. The filtrate was evaporated to dryness in vacuo leaving 23.6 g. of the crude 5-aminomethyltetrazole hydrochloride. This residue was dissolved in absolute ethanol, treated with 23.7 g. (0.3 mole) of pyridine and chilled to -5°C. for thirty-six hours. The colorless, crystalline solid which formed was filtered, washed with alcohol and dried, giving 14.6 g. (73.7% of theory) of 5-aminomethyltetrazole, m.p. 268.5°C., with

decomposition. This product was identical with the material described on page 20, obtained by following Scheme A.

Derivatives

5-Acetylaminomethyltetrazole. One and five-tenths grams (0.015 mole) of 5-aminomethyltetrazole was added to 10 ml. of glacial acetic acid containing 1.53 g. (0.015 mole) of acetic anhydride and the mixture refluxed for two hours. The solvent was removed under vacuum and the residue recrystallized from amyl acetate, m.p. 159.5-161°C.

Analysis. Calculated for $C_4H_7N_5O$: C, 34.04; H, 4.99; N, 49.63. Found: C, 34.15; H, 4.94; N, 49.59.

5-Benzoylaminomethyltetrazole. To 15 ml. of water containing 1.0 g. of sodium hydroxide was added 0.99 g. (0.01 mole) of 5-aminomethyltetrazole. When a complete solution had been attained 1.4 g. (0.01 mole) of benzoyl chloride was added and the mixture shaken for one hour. The resulting solution was then neutralized with dilute hydrochloric acid and adjusted to a pH of 4-5. The precipitate which formed was filtered and recrystallized from water, m.p. 229.5-230°C., with decomposition.

Analysis. Calculated for $C_9H_9N_5O$: C, 53.20; H, 4.47; N, 34.47. Found: C, 53.37; H, 4.45; N, 34.30.

N-Phenyl-N'-(5-tetrazolylmethyl)urea. One gram (0.01 mole) of 5-aminomethyltetrazole was dissolved in 12 ml. of 1N potassium hydroxide solution to which was then added 15 ml. of water. One and six-tenths grams of phenyl isocyanate was then added and the mixture shaken

for one hour. The solution was filtered and the filtrate acidified to a pH of 4-5 with dilute hydrochloric acid. The precipitate which formed was filtered and recrystallized from water, m.p. 194.5-195°C., with decomposition.

Analysis. Calculated for $C_9H_{10}N_6O$: C, 49.53; H, 4.62; N, 38.52. Found: C, 49.60; H, 4.81; N, 38.53.

The Preparation of 5-α-Aminoethyltetrazole (Alanine Analogue)

Scheme A.

(a) N-Benzyl-α-bromopropionamide. One hundred grams of α-bromopropionyl bromide (0.463 mole) was added to 99.1 g. of benzylamine dissolved in one liter of dry benzene over a period of thirty minutes and the resulting mixture allowed to stir overnight. The mixture was then filtered, the solid washed with water and the filtrate concentrated and cooled. The residue from the water wash and the solid which crystallized from the benzene concentrate were combined and recrystallized several times from benzene to give 58.1 g. (51.8% of theory) of product, m.p. 91.5-92.5°C.

Kushner and co-workers (18) report a melting point of 93.5-94.5°C., for this compound.

Analysis. Calculated for $C_{10}H_{12}BrNO$: Br, 33.01; N, 5.79. Found: Br, 33.10; N, 5.99.

(b) N-Benzyl-α-phthalimidopropionamide. Twenty two and two-

tenths grams (0.12 mole) of potassium phthalimide was added to 24.2 g. (0.1 mole) of N-benzyl-α-bromopropionamide dissolved in 75 ml. of dimethylformamide and the mixture heated on a steam bath for one hour with continued stirring. The mixture was then transferred to a 500 ml. beaker, 100 ml. of chloroform was added and the resulting mixture treated with 250 ml. of water. The organic layer was separated and the aqueous layer washed with 50 ml. of chloroform. The organic layer was combined with the chloroform wash, treated first with 50 ml. of a 0.2N sodium hydroxide solution and then with 50 ml. of water and then evaporated in a stream of dry air. The residue which remained was dried at 70°C., to give 23.4 g. (76.0% of theory) of product, m.p. 130-138°C. Recrystallization from toluene gave 18.0 g. (58.5% of theory) of product melting at 140-141°C. The analytical sample melted at 141-142°C.

Analysis. Calculated for $C_{18}H_{16}N_2O_3$: C, 70.12; H, 5.23; N, 9.09. Found: C, 70.13; H, 5.34; N, 9.23.

(c) 1-Benzyl-5-(α-phthalimidoethyl)tetrazole. Six and two-tenths grams (0.02 mole) of N-benzyl-α-phthalimidopropionamide suspended in 100 ml. of dry benzene was treated with 4.16 g. (0.02 mole) of powdered phosphorus pentachloride. After a few minutes of stirring the solution became clear. After two hours of continued stirring 2.78 g. (0.065 mole) of hydrazoic acid dissolved in 20 ml. of dry benzene was added. After a short time a colorless precipitate started to form. Stirring was continued for two hours at room temperature and for two hours at reflux temperature. The flask was then cooled until the temperature of the solution was 5°C., and the precipitate was filtered.

The solid, after washing with water and drying, amounted to 5.9 g. (88.1% of theory), m.p. 147°C. The analytical sample was recrystallized from toluene, m.p. 145.7-146.7°C.

Analysis. Calculated for $C_{18}H_{15}N_5O_2$: C, 64.85; H, 4.54; N, 21.01. Found: C, 64.95; H, 4.54; N, 20.92.

(d) 1-Benzyl-5-(α-aminoethyl)tetrazole Hydrochloride. Five and three-tenths grams (0.016 mole) of 1-benzyl-5-(α-phthalimidoethyl)-tetrazole was refluxed with 16 ml. of a one molar solution of hydrazine hydrate in absolute ethanol, to which had been added 50 ml. of absolute ethanol, for a period of three hours with continued stirring. After the mixture had been evaporated to dryness in vacuo the residue was digested with 40 ml. of 2N hydrochloric acid for 10-15 minutes at 40-50°C. The precipitate which remained was filtered and the filtrate evaporated to dryness. The crude product, 3.5 g., was recrystallized several times from isopropyl alcohol to give 2.3 g. (60.5% of theory) of pure product, m.p. 184-184.5°C.

Analysis. Calculated for $C_{10}H_{14}ClN_5$: C, 50.10; H, 5.89; Cl, 14.79; N, 29.22. Found: C, 50.04; H, 5.89; Cl, 14.92; N, 29.16.

(e) 5-α-Aminoethyltetrazole. Five-tenths grams (0.0021 mole) of 1-benzyl-5-(α-aminoethyl)tetrazole was dissolved in a mixture of 10 ml. of water and 65 ml. of absolute ethanol and the mixture shaken with 0.5 g. of palladium on charcoal in a hydrogen atmosphere at an initial pressure of 40 p.s.i. and temperature of 50-55°C. After twenty-four hours the catalyst was filtered and the filtrate evaporated to dryness. The residue which remained was dissolved in 5 ml. of hot ethanol and

treated with 0.5 ml. of pyridine. Cooling overnight in an ice-box resulted in the formation of a colorless precipitate which was filtered and washed with absolute ethanol. The product amounted to 110 mg., m.p. 267-268°C., with decomposition.

A mixture melting point with an analytical sample of 5-α-aminoethyltetrazole prepared according to Scheme B, page 29, showed no depression.

Scheme B

(a) Phthalyl alanine. Fifty three and four-tenths grams (0.6 mole) of alanine was mixed with 88.9 g. (0.6 mole) of phthalic anhydride and the mixture heated in an oil bath for one hour at 160-170°C. The melt was cooled and recrystallized from water to give 130.1 g. (98.7% of theory) of the product, m.p. 158-159°C.

A melting point of 160-162°C., is reported for this compound by Gabriel (19).

(b) α-Phthalimidopropionamide. To a stirred suspension of 68.5 g. (0.334 mole) of phthalyl alanine in 500 ml. of dry benzene was added 61.3 g. (0.52 mole) of thionyl chloride. The mixture was then heated on a steam bath with continued stirring until a clear solution formed. The yellow solution which resulted after two hours of heating was then cooled to 15°C., and ammonia gas slowly bubbled into the solution until a precipitate ceased to form. The solid precipitate was filtered, dried and suspended in a liter of water. Filtration and drying of the solid gave 46.5 g. (64% of theory) of the desired product, m.p. 209-210°C.

No further purification was carried out.

This compound is reported by Radde (20) to have a melting point of 211-212°C.

(c) α-Phthalimidopropionitrile. Forty four and five-tenths grams (0.204 mole) of α-phthalimidopropionamide was refluxed with 200 ml. of pyridine and 120 ml. of benzenesulfonyl chloride for ten minutes. The reaction mixture was then cooled and poured into ice and water. After standing for twenty minutes the solid precipitate was filtered and dried and amounted to 40.9 g. (100% of theory), m.p. 129-130°C. Recrystallization from methanol gave a colorless crystalline solid, m.p. 136-138°C.

This material is reported by Radde (20) to melt at 139-140°C.

(d) 5-α-Phthalimidoethyltetrazole. To 49.3 g. (0.246 mole) of α-phthalimidopropionitrile and 48 g. (0.738 mole) of sodium azide suspended in 100 ml. of dry tetrahydrofuran was added 32.7 g. (0.246 mole) of anhydrous aluminum chloride dissolved in 300 ml. of dry tetrahydrofuran. The resulting mixture was refluxed with continued stirring for twenty-four hours. At the end of this time the tetrahydrofuran was distilled from the reaction mixture and water added at such a rate that the volume remained the same. The resulting suspension was filtered, the solid resuspended in 400 ml. of water and treated with 50 ml. of concentrated hydrochloric acid. After stirring at room temperature for one hour the solid was filtered and dried to give 54.3 g. (90.7% of theory) of product, m.p. 229-231°C. A portion of the product was recrystallized from aqueous ethanol, m.p. 230-231°C., with decomp-

osition.

Analysis. Calculated for $C_{11}H_9N_5O_2$: C, 54.32; H, 3.73; N, 28.80. Found: C, 54.29; H, 3.75; N, 28.78.

(c) 5-α-Aminoethyltetrazole. To 37.1 g. (0.153 mole) of 5-α-phthalimidoethyltetrazole suspended in 400 ml. of absolute ethanol was added 153 ml. of a one molar ethanol solution of hydrazine and the resulting mixture stirred under continued reflux for three hours. At the end of this time the mixture was evaporated to dryness and the residue treated with 400 ml. of 2N hydrochloric acid for fifteen minutes at 55°C. The resulting suspension was filtered and the filtrate, in turn, evaporated to dryness.

The crude hydrochloride, 20.0 g., was dissolved in 100 ml. of absolute ethanol and treated with 18 g. of pyridine. After the mixture had remained in an ice box for thirty-six hours it was filtered. The dried solid amounted to 7.2 g. (40.4% of theory), m.p. 260-262°C., with decomposition.

The product was purified by dissolving it in a minimum amount of water and reprecipitating it by the addition of sufficient absolute ethanol to make a 90% alcohol solution, m.p. 272-273°C., with decomposition.

Analysis. Calculated for $C_3H_7N_5$: C, 31.85; H, 6.24; N, 61.91. Found: C, 31.79; H, 6.10; N, 62.09.

Derivatives

5-α-Acetylaminoethyltetrazole. One and thirteen-hundredths grams (0.01 mole) of 5-α-aminoethyltetrazole and 1.02 g. (0.01 mole) of acetic anhydride were refluxed for one hour in 10 ml. of glacial acetic acid. The excess acetic acid was removed in a vacuum and the solid residue recrystallized from amyl acetate, m.p. 145-145.5°C.

Analysis. Calculated for $C_5H_9N_5O$: C, 38.70; H, 5.85; N, 45.14. Found: C, 38.69; H, 5.71; N, 45.13.

5-α-Benzoylaminoethyltetrazole. To 1.13 g. (0.01 mole) of 5-α-aminoethyltetrazole dissolved in 20 ml. of water containing 0.9 g. of sodium hydroxide was added 1.4 g. (0.01 mole) of benzoyl chloride. The resulting mixture was shaken several times over a period of one hour. The solution was then filtered and the filtrate acidified to a pH of 4-5 with dilute hydrochloric acid. The solid which formed was filtered and recrystallized from water. The product melted at 176-177°C., then solidified and remelted at 199-200°C.

Analysis. Calculated for $C_{10}H_{11}N_5O$: C, 55.29; H, 5.11; N, 32.24. Found: C, 55.49; H, 5.09; N, 32.43.

N-Phenyl-N'-(α-5-tetrazolylethyl)urea. One and thirteen-hundredths grams (0.01 mole) of 5-α-aminoethyltetrazole was dissolved in 12 ml. of a 1N potassium hydroxide solution to which was then added 18 ml. of water. One and two-tenths grams of phenyl isocyanate was added and the mixture shaken several times over a period of two hours. After this time the solution was filtered and the filtrate acidified to pH 4

with dilute hydrochloric acid. The precipitate which formed was filtered and recrystallized from water, m.p. 184-185°C., with decomposition.

Analysis. Calculated for $C_{10}H_{12}N_6O$: C, 51.71; H, 5.21; N, 36.20. Found: C, 51.88; H, 5.35; N, 36.22.

The Preparation of 5-(β-Aminoethyl)tetrazole (β-Alanine Analogue)

Scheme A.

(a) Phthalyl β-alanine. A mixture of 35.6 g. (0.4 mole) of β-alanine and 59.3 g. (0.4 mole) of phthalic anhydride was fused at 180°C. for a period of thirty minutes. At the end of this time the contents of the flask were removed and recrystallized from water. This gave 80.0 g. (91.4% of theory) of the product melting at 149-150°C.

This compound was reported by Gabriel (19) to melt at 150-151°C.

(b) β-Phthalimidopropionyl Chloride. To 39.1 g. (0.179 mole) of phthalyl-β-alanine suspended in 500 ml. of dry benzene was added 35.4 g. (0.3 mole) of thionyl chloride. The resulting mixture was heated just under reflux temperature with continued stirring for three hours. After this time the solution was concentrated in a vacuum until the excess thionyl chloride and all the benzene had been removed. Trituration of the residue with petroleum ether gave a colorless crystalline material which was filtered and dried. The acid chloride weighed 38.6 g. (90.9% of theory), m.p. 105-106.5°C.

This compound is reported by Gabriel (21) to melt at 107-108°C.

(c) N-Benzyl-β-phthalimidopropionamide. To 34.9 g. (0.326 mole) of benzylamine in 500 ml. of dry benzene was added 38.6 g. (0.163 mole) of β-phthalimidopropionyl chloride in small portions over a period of twenty minutes. The reaction mixture was kept at room temperature by means of an ice-water bath. After allowing the mixture to stir overnight the precipitate was filtered, dried and digested with 750 ml. of water for two hours. Filtering and drying again gave 50.9 g. (quantitative yield) of the product, m.p. 194-197°C.

Recrystallization from absolute ethanol gave 41.0 g. (81.7% of theory) of pure product melting at 198-199.5°C.; the analytical sample melted at 198-198.5°C.

Analysis. Calculated for $C_{18}H_{16}N_2O_3$: C, 70.12; H, 5.23; N, 9.09. Found: C, 69.98; H, 5.03; N, 9.16.

(d) 1-Benzyl-5-β-Phthalimidoethyltetrazole. To 35.1 g. (0.114 mole) of N-benzyl-β-phthalimidopropionamide suspended in 500 ml. of dry benzene was added 23.8 g. (0.114 mole) of phosphorus pentachloride. The mixture was stirred at 60°C. for a period of two hours during which time the solution became homogeneous. To the slightly yellow solution was then added 14.8 g. (0.344 mole) of hydrazoic acid in 106 ml. of dry benzene. The solution was stirred for two hours at room temperature, followed by two hours at reflux temperature. The resulting suspension was cooled and filtered. After drying the residue it was digested in water and again filtered and dried to give 22.8 g. (60.0% of theory) of crude product, m.p. 153.5-157°C. The benzene filtrate was concentrated in half its volume, cooled and filtered. The dried precipitate amounted

to 5.1 g. (13.4% of theory), m.p. 148.5-153°C.

Recrystallization of the combined solids from toluene gave 25.5 g. (67.2% of theory) of product, m.p. 156.5-158°C. The analytical sample melted at 159-159.5°C.

Analysis. Calculated for $C_{18}H_{15}N_5O_2$: C, 64.85; H, 4.54; N, 21.01. Found: C, 65.03; H, 4.33; N, 21.19.

(e) 1-Benzyl-5-(β-aminoethyl)tetrazole Hydrochloride. To 20.5 g. (0.062 mole) of 1-benzyl-5-β-phthalimidoethyltetrazole in 250 ml. of ethanol was added 62 ml. of a one molar ethanol solution of hydrazine. The mixture was stirred under reflux for three hours and then evaporated to dryness. The residue was then treated with 200 ml. of 2N hydrochloric acid at 50-55°C. for ten minutes followed by filtration. Evaporation of the filtrate gave 11.1 g. (74.8% of theory) of the crude, colorless product melting at 126-129°C.

Recrystallization from a mixture of water and isopropyl alcohol raised the melting point to 138.5-139.5°C.

Analysis. Calculated for $C_{10}H_{14}ClN_5$: C, 50.10; H, 5.89; Cl, 14.79; N, 29.22. Found: C, 49.88; H, 6.00; Cl, 14.98; N, 28.97.

(f) 5-β-Aminoethyltetrazole Hydrochloride. Four and eight-tenths grams (0.02 mole) of 1-benzyl-5-β-aminoethyltetrazole hydrochloride was dissolved in a mixture of 100 ml. of absolute ethanol and 15 ml. of water. After addition of 1.2 g. of 5% palladium on charcoal the mixture was shaken in an atmosphere of hydrogen at 50-60°C., at an initial pressure of 50 p.s.i. for twenty-four hours. The mixture was then filtered and the filtrate evaporated to dryness; the residue was

recrystallized from ethanol-ether, and gave 1.1 g. (36.8% of theory) of product, m.p. 127.5-128.8°C.

Analysis. Calculated for $C_3H_8ClN_5$: C, 24.09; H, 5.39; Cl, 23.70; N, 46.82. Found: C, 24.09; and 24.24; H, 5.54 and 5.45; Cl, 23.51; N, 46.69.

Scheme B

(a) 5-β-Phthalimidoethyltetrazole. To 50 g. (0.25 mole) of β-phthalimidopropionitrile (22) and 50 g. of sodium azide suspended in 100 ml. of dry tetrahydrofuran was added 35 g. (0.26 mole) of anhydrous aluminum chloride dissolved in 300 ml. of dry tetrahydrofuran. The resulting mixture was refluxed for twenty-four hours with continued stirring. At the end of this time the tetrahydrofuran was distilled from the mixture and water added at such a rate that the volume of the contents of the flask remained the same. The resulting suspension was filtered and the solid resuspended in 450 ml. of water and treated with 50 ml. of concentrated hydrochloric acid. After one hour of stirring this was filtered and the solid dried to give 57.3 g. (94.2% of theory) of crude product, m.p. 240-244.5°C., with decomposition.

Recrystallization from a mixture of ethanol and ethyl acetate raised the melting point to 249.5-250.5°C., with decomposition.

Analysis. Calculated for $C_{11}H_9N_5O_2$: C, 54.32; H, 3.73; N, 28.80. Found: C, 54.33; H, 3.93; N, 28.99.

This compound is reported by Behringer and Kohl (5) to melt at 241°C.

(b) 5-β-Aminoethyltetrazole Hydrochloride. To 24.3 g. (0.1 mole) of 5-β-phthalimidoethyltetrazole suspended in 360 ml. of absolute ethanol was added 100 ml. of a one molar solution of hydrazine hydrate in ethanol and the resulting mixture refluxed with continued stirring for three hours. The solvent was removed in a vacuum and the dry residue treated with 225 ml. of 2N hydrochloric acid at 50-55°C. for a period of ten minutes. The resulting mixture was filtered and the filtrate evaporated to dryness on a steam bath in a jet of dry air. The residue, 13.3 g. (89.3% of theory), was recrystallized from ethanol, m.p. 130-132°C.

The melting point for this compound is reported as 128-129°C. by Ainsworth (23) and 132°C. by Behringer and Kohl (5).

(c) 5-β-Aminoethyltetrazole. A solution of 7.45 g. (0.05 mole) of 5-β-aminoethyltetrazole hydrochloride was dissolved in 65 ml. of water to which was then added 6.1 g. (0.0264 mole) of powdered silver oxide. The resulting mixture was protected from light and allowed to stir for twenty-eight hours. The suspension was filtered and the filtrate was saturated with hydrogen sulfide. The resulting black precipitate of silver sulfide was removed by filtration and the filtrate, after treatment with Norite, was evaporated to a small volume. Trituration with acetone gave a colorless precipitate which was further purified by dissolving in a small amount of water followed by precipitation with acetone. The pure product weighed 3.0 g. (58.3% of theory) and melted at 223-224°C., with decomposition.

Analysis. Calculated for $C_3H_7N_5$: C, 31.85; H, 6.24; N, 61.91. Found: C, 32.11; H, 6.32; N, 62.02.

Derivatives

5-(β-Acetylaminoethyl)tetrazole. One and thirteen-hundredths grams (0.01 mole) of 5-(β-aminoethyl)tetrazole was added to 10 ml. of glacial acetic acid containing 1.02 g. (0.01 mole) of acetic anhydride and the mixture refluxed for one hour. Removal of the solvent in a vacuum left a solid residue which was recrystallized from amyl acetate, m.p. 202-203°C.

Analysis. Calculated for $C_5H_9N_5O$: C, 38.70; H, 5.85; N, 45.14. Found: C, 38.81; H, 6.06; N, 45.28.

5-(β-Benzoylaminoethyl)tetrazole. One and thirteen-hundredths grams (0.01 moles) of 5-(β-aminoethyl)tetrazole was added to 20 ml. of water containing 0.8 g. (0.01 mole) of sodium hydroxide. To the resulting solution was then added 1.4 g. (0.01 mole) of benzoyl chloride and the mixture shaken for one hour. The solution was then neutralized with dilute hydrochloric acid and adjusted to a pH of 4-5. The resulting precipitate was filtered and recrystallized twice from water, m.p. 200.5-201.0°C., with decomposition.

This compound is reported by Ainsworth (23) to melt at 206°C.

Analysis. Calculated for $C_{10}H_{11}N_5O$: C, 55.29; H, 5.11; N, 32.24. Found: C, 55.13; H, 5.16; N, 32.27.

N-Phenyl-N'-(β-5-tetrazolylethyl)urea. One and thirteen-hundredths grams (0.01 mole) of 5-(β-aminoethyl)tetrazole was dissolved in 12 ml.

of 1N potassium hydroxide solution to which was then added 18 ml. of water. To the resulting solution was added 1.2 g. of phenyl isocyanate and the mixture shaken for one hour. The suspension was filtered and the filtrate acidified to a pH of 4-5 with dilute hydrochloric acid. The precipitate which formed was filtered and recrystallized from aqueous alcohol, m.p. 199-199.5°C., with decomposition.

Analysis. Calculated for $C_{10}H_{12}N_6O$: C, 51.71; H, 5.21; N, 36.20. Found: C, 51.93; H, 5.43; N, 36.33.

The Preparation of 5-(α-Aminophenylethyl)tetrazole (Phenylalanine Analogue)

Scheme B

(a) α-Phthalimido-β-phenylpropionyl Chloride. D,L-Phenylalanine (25 g., 0.152 mole), was fused with 22.4 g. (0.152 mole) of phthalic anhydride at 145-150°C. for a period of thirty minutes. The resulting melt was poured into ice and water with stirring and the solid phthalimido derivative filtered and dried to give 43.5 g. (97.4% of theory) of product, m.p. 174-176°C.

Without further purification the crude acid was added to 500 ml. of dry benzene containing 31.0 g. (0.149 mole) of finely ground phosphorus pentachloride and the resulting mixture heated to 50-55°C. on a steam bath with continued stirring for one hour. After this time the solvent and phosphorus oxychloride were removed in a vacuum and the residue triturated with petroleum ether. The solid which formed was filtered and dried. The yield of acid chloride was 43.9 g. (96.5% of theory),

m.p. 134-136°C.

Sheehan and Frank (24) reported the acid to melt at 177.5-179°C., and the acid chloride to melt at 124-126°C.

(b) α-Phthalimido-β-phenylpropionamide. α-Phthalimido-β-phenylpropionyl chloride (10 g., 0.03 mole) was added all at once to 50 ml. of a cold ammonium hydroxide solution and stirred with continued cooling for thirty minutes. The amide, which separated as a solid was filtered and dried at 70°C. giving 9.0 g. (96% of theory) of crude product, m.p. 231-233°C. The product was not purified further but used in the crude state.

The pure amide is reported to melt at 236-237°C. by Peterson and Nieman (25).

(c) α-Phthalimido-β-phenylpropionitrile. To 19.5 ml. of benzenesulfonyl chloride and 32.6 ml. of pyridine was added 9.0 g. (0.032 mole) of α-phthalimido-β-phenylpropionamide and the resulting mixture refluxed for ten minutes. The mixture was allowed to cool and was poured into ice and water. The precipitate which formed was filtered and dried. The product weighed 8.1 g., m.p. 127-130°C., and was not further purified for use in the next reaction.

This compound, prepared in exactly the same manner, is reported by Niemann and Peterson (25) to melt at 134-135°C. when pure.

(d) 5-(α-Phthalimido-β-phenylethyl)tetrazole. To 8.1 g. (0.0305 mole) of α-phthalimido-β-phenylpropionitrile and 6.5 g. (0.1 mole) of sodium azide was added 75 ml. of dry tetrahydrofuran containing 4.4 g. (0.033 mole) of anhydrous aluminum chloride. The resulting

mixture was stirred under reflux for a period of twenty-four hours. The tetrahydrofuran was then distilled out of the reaction mixture and water added at such a rate that the volume of the mixture remained constant. The solid material was then filtered off, resuspended in 175 ml. of water and treated with 20 ml. of concentrated hydrochloric acid. The mixture was filtered and the solid product washed with water and dried. The yield of tetrazole was 9.2 g. (94.5% of theory), m.p. 212-213°C.

Recrystallization from ethyl acetate raised the melting point to 212.5-213°C., with slight decomposition.

Analysis. Calculated for $C_{17}H_{13}N_5O_2$: C, 63.94; H, 4.10; N, 21.94. Found: C, 63.97; H, 4.39; N, 22.19.

(e) 5-(α-Amino-β-phenylethyl)tetrazole. Five grams (0.0162 mole) of 5-(α-phthalimido-β-phenylethyl)tetrazole was added to 16.2 ml. of a one molar ethanolic solution of hydrazine followed by treatment with 50 ml. of absolute ethanol. The resulting mixture was refluxed for two hours, allowed to cool and then evaporated to dryness. The residue was treated with 40 ml. of 2N hydrochloric acid and warmed for ten minutes at 50-55°C. The mixture was then filtered and the filtrate neutralized to a pH of 5. The resulting precipitate was filtered, washed with cold water and dried. The yield of product was 2.5 g. (81.5% of theory), m.p. 271-272°C., with decomposition.

Mixture melting points with an analytical sample of 5-(α-amino-β-phenylethyl)tetrazole prepared by an alternate method, showed no depression.

Scheme C.

(a) Ethyl α-acetamido-α-cyano-β-phenylpropionate. To a solution of 4.6 g. (0.2 mole) of sodium in 100 ml. of absolute ethanol was added 34.0 g. (0.2 mole) of ethyl acetamidocyanoacetate.[1] The resulting solution was heated and stirred while 26.6 g. (0.215 mole) of benzyl chloride was added over a period of fifteen minutes. Refluxing and stirring were continued for two hours after which the mixture was cooled and poured on to 500 g. of ice and water. The syrup which resulted was separated by decantation of the water and dissolved in a benzene-cyclohexane mixture. Drying by azeotropic distillation followed by cooling and scratching gave a light tan precipitate which was filtered and dried. The yield of crude product was 31.4 g. (59.6% of theory), m.p. 129.5-130°C.

Recrystallization from aqueous alcohol raised the melting point to 130-131°C.

The compound is reported by Albertson and Tullar (26) to melt at 134°C.

(b) Ethyl α-acetamido-α-(5-tetrazolyl)-β-phenylpropionate and 5-(α-Amino-β-phenylethyl)tetrazole. To 67.8 g. (0.256 mole) of ethyl α-acetamido-α-cyano-β-phenylpropionate and 49.9 g. (0.768 mole) of sodium azide was added 34.1 g. (0.256 mole) of anhydrous aluminum chloride dissolved in 550 ml. of dry tetrahydrofuran. The resulting mixture was refluxed with continued stirring for twenty-four

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[1] Winthrop Laboratories

hours. At the end of this time the tetrahydrofuran was distilled out of the reaction mixture and water added at such a rate that the volume remained constant. The suspended aluminum salt was filtered and dried. The filtrate was made acid to Congo red and cooled overnight. The precipitate which formed was filtered and dried to give 8.0 g. of crude product. Recrystallization from aqueous alcohol gave the pure ethyl α-acetamido-α-(5-tetrazoyl)-β-phenylpropionate, m.p. 147.5-148.5°C.

Analysis. Calculated for $C_{14}H_{17}N_5O_3$: C, 55.43; H, 5.65; N, 23.09. Found: C, 55.41; H, 5.40; N, 23.09.

The dried aluminum salt was refluxed with 450 ml. of concentrated hydrochloric acid for three hours and the mixture evaporated almost to dryness. The residue was taken up in 250 ml. of 95% ethanol and treated with 30 grams of pyridine. Cooling of the mixture overnight in an ice-box resulted in the formation of a crystalline precipitate of 5-(α-amino-β-phenylethyl)tetrazole. The yield after filtering and drying was 25.9 g., m.p. 261.5-263°C., with decomposition. Recrystallization from water raised the melting point to 270.5-271.5°C., with decomposition.

Analysis. Calculated for $C_9H_{11}N_5$: C, 57.12; H, 5.86; N, 37.02. Found: C, 56.85; H, 5.95; N, 37.24.

(c) <u>α-Acetamido-α-(5-tetrazolyl)-β-phenylpropionic acid.</u> One and five-tenths grams (0.005 mole) of ethyl α-acetamido-α-(5-tetrazolyl)-β-phenylpropionate was added to 16 ml. of water containing 0.8 g. of sodium hydroxide and the mixture refluxed for a period of one hour. The resulting solution was filtered and the filtrate acidified

with dilute hydrochloric acid to a pH of 2-3. Scratching initiated the formation of a colorless crystalline precipitate. After allowing the mixture to stand overnight in an ice-box the solid was filtered and air dried. The product melted at approximately 110°C. with decomposition, resolidified and melted again at 224-225°C.

The compound was purified by dissolving it in tetrahydrofuran, decolorizing the solution with Norite and reprecipitating with petroleum ether.

Analysis. Calculated for $C_{12}H_{13}N_5O_3$: C, 52.36; H, 4.76; N, 25.45. Found: C, 52.68; H, 5.00; N, 25.55.

(d) 5-(α-Acetamido-β-phenylethyl)tetrazole. Twenty milligrams of α-acetamido-α-(5-tetrazolyl)-β-phenylpropionic acid was heated at 170°C.; after recrystallization from water it melted at 226°C.

A mixture melting point with the product formed by the acetylation of 5-(α-amino-β-phenylethyl)tetrazole, page 43, showed no depression.

(e) 5-(α-Amino-β-phenylethyl)tetrazole. Two grams (0.0066 mole) of ethyl α-acetamido-α-(5-tetrazolyl)-β-phenylpropionate was refluxed in 25 ml. of concentrated hydrochloric acid for two hours. At the end of this time the pH of the solution was adjusted to approximately 5 with ammonium hydroxide at which point a precipitate started to form; at a pH of 6 the precipitation was complete. Filtered and dried, the solid weighed, 1.2 g. (96% of theory). The material was recrystallized from water to give 0.9 g. (72% of theory), of pure product, m.p. 276-277°C., with decomposition.

A mixture melting point with an analytical sample of 5-(α-amino-

β-phenylethyl)tetrazole prepared by a different method showed no depression.

Derivatives

5-(α-Acetamido-β-phenylethyl)tetrazole. To 20 ml. of glacial acetic acid was added 1.89 g. (0.01 mole) of 5-(α-amino-β-phenylethyl)tetrazole and 1.1 g. of acetic anhydride and the mixture refluxed for one hour. The solution was cooled and poured on to 50 g. of ice and water. The resulting solid was filtered and recrystallized from aqueous ethanol to give 1.75 g. of acetyl derivative, m.p. 224.5-225.5°C.

Analysis. Calculated for $C_{11}H_{13}N_5O$: C, 57.13; H, 5.67; N, 30.29. Found: C, 57.17; H, 5.79; N, 30.15.

5-(α-Benzoylamino-β-phenylethyl)tetrazole. To 0.94 g. (0.005 mole) of 5-(α-amino-β-phenylethyl)tetrazole dissolved in 10 ml. of water containing 0.45 g. of sodium hydroxide was added 0.7 g. (0.005 mole) of benzoyl chloride. After shaking the mixture several times over a period of two hours the mixture was filtered and the filtrate acidified with dilute hydrochloric acid to a pH of 4-5. The precipitate which formed was recrystallized several times from aqueous ethanol, m.p. 234-235° C., with decomposition.

Analysis. Calculated for $C_{16}H_{15}N_5O$: C, 65.51; H, 5.15; N, 23.88. Found: C, 65.52; H, 5.00; N, 23.97.

N-Phenyl-N'-(α-(5-tetrazolyl)-β-phenylethyl)urea. One and nine-tenths grams (0.01 mole) of 5-(α-amino-β-phenylethyl)tetrazole was dissolved in 12 ml. of a 1N potassium hydroxide solution and then

diluted with 18 ml. of water. One and two-tenths grams of phenyl isocyanate was added to the solution and the mixture shaken several times over a period of two hours. The resulting mixture was then filtered and the filtrate acidified to a pH of 4-5 with dilute hydrochloric acid. The precipitate was filtered and recrystallized from aqueous ethanol, m.p. 188.5-189.5°C., with decomposition.

Analysis. Calculated for $C_{16}H_{16}N_6O$: C, 62.32; H, 5.23; N, 27.26. Found: C, 62.40; H, 5.24; N, 27.42.

The Preparation of 5-(α-Amino-β-(3'-indolyl)ethyl)tetrazole

(Tryptophane Analogue)

Scheme C

(a) Ethyl α-acetamido-α-cyano-β-(3-indolyl)propionate. In a one liter three necked flask fitted with a mechanical stirer, reflux condenser and nitrogen inlet was placed 5.1 g. of dry powdered sodium hydroxide and 400 ml. of dry toluene. The temperature was raised to 90-95°C., and a mixture of 75 g. (0.432 mole) of gramine (27) and 73.3 g. (0.432 mole) of ethyl acetamidocyanoacetate was added over a period of twenty minutes. After the addition was complete the reaction started and dimethylamine commenced to be liberated. The temperature was raised to the reflux point and held at this point for twelve hours during which time stirring and nitrogen perfusion were continued.

The reaction mixture was filtered while still warm and the solid air dried. Suspension of the dried solid in water followed by filtration and drying at 50°C., gave 76.4 g. (59.2% of theory) of the

product, m.p. 193-195°C. Recrystallization from aqueous ethanol or normal butyl alcohol raised the melting point to 195.5-197°C.

This compound is reported by Albertson and Tullar (26) to melt at 196-198°C.

(b) Ethyl α-acetamido-α-(5-tetrazolyl)-β-(3'-indolyl)propionate. To 19.5 g. (0.3 mole) of sodium azide in 50 ml. of dry tetrahydrofuran was added 13.3 g. (0.1 mole) of anhydrous aluminum chloride dissolved in 200 ml. of the same solvent. The resulting mixture was heated to reflux temperature with continued stirring for one hour. After allowing the mixture to cool to room temperature 29.9 g. (0.1 mole) of ethyl α-acetamido-α-cyano-β-(3-indolyl)propionate was added and refluxing and stirring continued for an additional twenty-four hours. At the end of this time 150 ml. of water was added to the reaction mixture followed by cooling with an ice-water bath to 5°C. To the cooled mixture was added 50 ml. of concentrated hydrochloric acid over a period of twenty minutes. The contents of the flask were then poured into 300 ml. of ether and the organic layer separated. The aqueous layer was washed with 100 ml. of a mixture of equal volumes of ether and tetrahydrofuran, the organic layers combined, washed with 100 ml. of water and dried over sodium sulfate.

Removal of the organic solvent in vacuo left a solid residue which was dissolved in 500 ml. of boiling chloroform. Upon cooling in an ice-water bath there was deposited a crystalline precipitate which was filtered and dried. Concentration of the mother liquor yielded an additional batch of product. Combination of the solids gave 11.1 grams (32.5% of theory), of the desired product, m.p. 178-181°C.

Recrystallization from chloroform raised the melting point to 183.5-185°C., with slight decomposition.

Analysis. Calculated for $C_{16}H_{18}N_6O_3$: C, 56.13; H, 5.30; N, 24.55. Found: C, 56.00; H, 5.29; N, 24.54.

(c) <u>α-Acetamido-α-(5'-tetrazolyl)-β-(3-indolyl)propionic acid.</u> Six and eight-tenths grams (0.02 mole) of ethyl α-acetamido-α-(5'-tetrazolyl)-β-(3-indolyl)propionate and 3.2 g. (0.08 mole) of sodium hydroxide were heated in 32 ml. of water at reflux temperature for four hours. The resulting solution was treated with Norite, filtered and acidified with 8.4 ml. of concentrated hydrochloric acid (0.10 mole). Continued cooling and scratching caused the formation of a crystalline precipitate. After allowing the mixture to remain overnight in an ice-box the solid was filtered and washed with a small amount of cold water. Drying gave 4.0 g. (64% of theory) of the product, m.p. 146-150°C., with decomposition.

Recrystallization from water raised the melting point to 153-155°C., with decomposition. The product crystallized as a dihydrate.

Analysis. Calculated for $C_{14}H_{14}N_6O_3 \cdot 2H_2O$: N, 23.99. Found: N, 24.15 and 23.89.

Calculated for $C_{14}H_{14}N_6O_3$: N, 26.74. Found: N, 26.65.

(d) <u>5-(α-Acetamido-β-(3'-indolyl)ethyl)tetrazole.</u> Seven and four-tenths grams (0.024 mole) of α-acetamido-α-(5'-tetrazolyl)-β-(3-indolyl)propionic acid was heated at reflux temperature in 200 ml. of water for two and a half hours. The resulting solution was then treated with Norite and filtered while hot. Cooling of the filtrate

caused the formation of a precipitate which when filtered and dried amounted to 5.8 g. (91.4% of theory), m.p. 220.5-221.5°C., with decomposition.

Recrystallization from water raised the melting point to 223-223.5°C., with decomposition.

Analysis. Calculated for $C_{13}H_{14}N_6O$: C, 57.76; H, 5.22; N, 31.10. Found: C, 57.87; H, 5.26; N, 31.18.

(e) 5-(α-Amino-β-(3'-indolyl)ethyl)tetrazole. One and three-tenths grams (0.005 mole) of 5-(α-acetamido-β-(3'-indolyl)ethyl)-tetrazole was dissolved in 16 ml. of water containing 1.3 g. of sodium hydroxide and the solution refluxed for twelve hours. Without allowing the solution to cool to any extent 2.7 ml. of concentrated hydrochloric acid was added all at once and the pH adjusted to 5 with dilute ammonium hydroxide solution. Cooling and scratching caused the formation of a crystalline precipitate which was filtered and dried. The product weighed 0.8 g. (70% of theory), m.p. 246-249°C., with decomposition. Recrystallization from water raised the melting point to 268.5-269°C., with decomposition.

Analysis. Calculated for $C_{11}H_{12}N_6$: C, 57.88; H, 5.30; N, 36.82. Found: C, 57.83; H, 5.47; N, 36.92.

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 in aqueous solution with standard sodium hydroxide and hydrochloric acid solutions. The weighed samples were

transferred to a three necked, 250 ml. flask and dissolved in 100-125 ml. of water. The solution was then titrated in a thermostated bath at 25 ± 1°C. The pH was determined after each addition of titrant with a Beckman pH Meter, Model H-2. From these data the region of half neutralization was plotted on a large scale and the best straight line drawn. The pH at the mid-point of this line was determined from the plot and assigned the value of the pK of the functional group being titrated. The titration curves in each instance exhibited the form normally obtained for an amino acid.

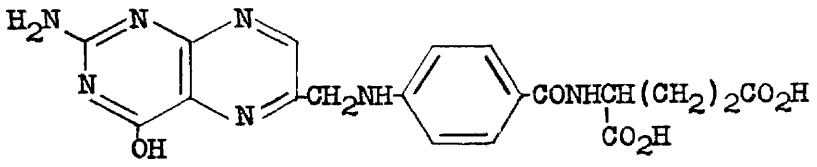
Comparison of the pK_1 and pK_2 values of the amino acids and the tetrazole analogues are presented in Table I. The data for the potentiometric titrations are recorded in Appendix I.

PART II

TETRAZOLE ANALOGUES OF ACTIVE AMINOBENZOIC ACID DERIVATIVES

Discussion

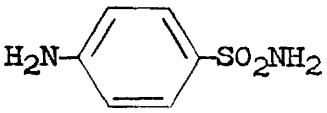
During recent years two aminobenzoic acid derivatives have been prominent in chemotherapy. Para-aminobenzoic acid itself plays a unique role in metabolism as a portion of folic acid (I), an essential material for the synthesis of nucleic acids.



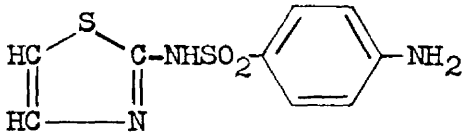
I

Evidence that certain drugs structurally related to para-aminobenzoic acid could inhibit its incorporation into folic acid (29) resulted from the application of the antimetabolite concept in chemotherapy as a means of combating bacterial invasion of the body.

Those drugs which have been most effective in interfering with the utilization of para aminobenzoic acid have been shown to be related to sulfanilamide (II) (30). One of the best bacteriostatic drugs to result from extensive investigations is sulfathiazole (III) (31), which is most widely used in the therapy of staphyloccoccal and pneumococcal infections.



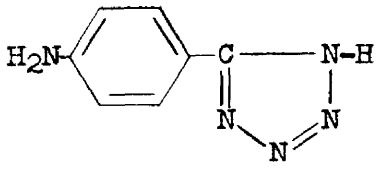
II



III

The second derivative of aminobenzoic acid, important for its tuberculostatic activity (32), is 2-hydroxy-4-aminobenzoic acid commonly referred to as para-aminosalicylic acid.

In view of the similarity of 5-monosubstituted tetrazoles to carboxylic acids, the synthesis of tetrazoles related to para-aminobenzoic acid and 2-hydroxy-4-aminobenzoic acid was undertaken. The respective nitrophenyltetrazoles were readily synthesized from substituted benzonitriles. Reduction of the nitrophenyltetrazoles led to the desired aminophenyltetrazoles. Accordingly, 5-(4'-aminophenyl)tetrazole (IV) was obtained in good yields through the reduction of 5-(4'-nitrophenyl)-tetrazole using tin and hydrochloric acid.



IV

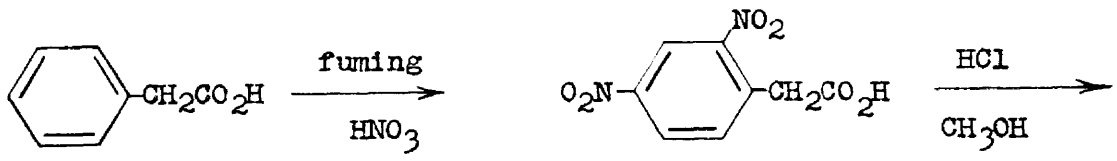
5-(3'-Aminophenyl)tetrazole, also prepared for evaluation as an antimetabolite for para-aminobenzoic acid, was synthesized from the corresponding nitro compound using either zinc and hydrochloric acid or hydrogen and platinum oxide catalyst as reducing agents. 5-(2'-Hydroxy-4'-aminophenyl)tetrazole, the analogue of para-aminosalicylic acid, was prepared from 5-(2'-hydroxy-4'-nitrophenyl)tetrazole by reduction with hydrogen and platinum oxide.

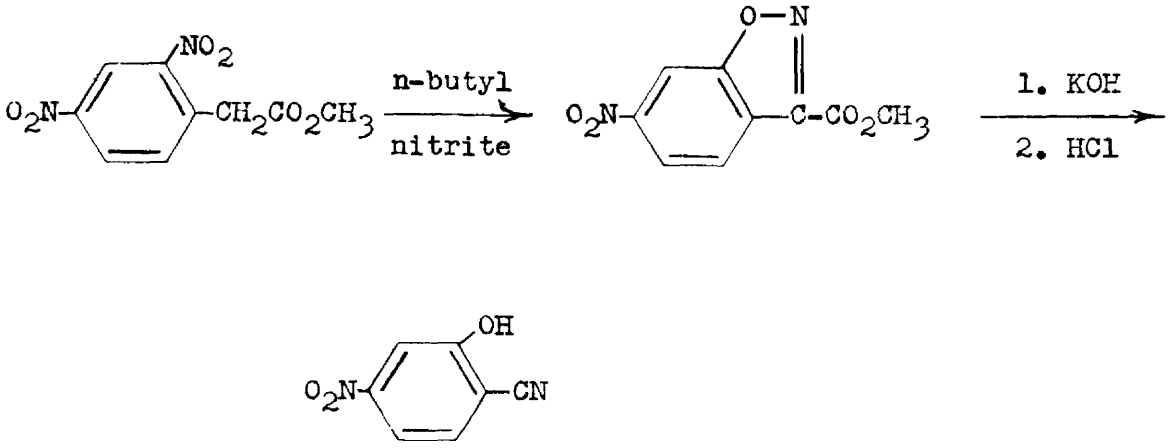
The high melting aminophenyltetrazoles, which could be readily recrystallized from water or aqueous ethanol, possess amphoteric character; they are readily soluble in both aqueous acids and aqueous alkalies.

The acetyl derivatives, used to characterize the aminophenyltetrazoles, were prepared by refluxing the respective amines with glacial acetic acid containing an equivalent amount of acetic anhydride.

The nitrophenyltetrazoles used as intermediates for the aminophenyltetrazole syntheses were prepared from the corresponding nitrobenzonitriles according to the procedures of Herbst and Wilson (3) by heating the aryl cyanides with sodium azide and glacial acetic acid in normal butyl alcohol at reflux temperature for several days.

With the exception of 2-hydroxy-4-nitrobenzonitrile the nitrobenzonitriles were commercially available. 2-Hydroxy-4-nitrobenzonitrile was synthesized by a series of reactions starting with phenylacetic acid (33) which may be illustrated schematically as follows:





5-(4'-Aminophenyl)tetrazole, 5-(3'-aminophenyl)tetrazole and their derivatives, as well as 5-(2'-hydroxy-4'-aminophenyl)tetrazole were submitted for pharmacological screening.

Experimental

The Preparation of Nitrophenyltetrazoles

5-(2'-Nitrophenyl)tetrazole. Ortho-nitrobenzonitrile (25 g., 0.17 mole), 16.2 g. (0.25 mole) of sodium azide and 15 g. (0.25 mole) of glacial acetic acid were added to 100 ml. of normal butyl alcohol and the mixture refluxed for four days. At the end of this time an additional 5 g. of sodium azide and 10 g. of glacial acetic acid were added and the heating continued for an additional two days. The contents of the flask were then transferred to a larger flask, 200 ml. of water added and the mixture distilled until all the alcohol had been removed. The solution was filtered, and the filtrate treated with concentrated hydrochloric until a precipitate no longer formed. The solid was filtered and dried; the yield of crude product was 12.2 g. (37.6% of theory).

Recrystallization from water gave a colorless crystalline product (needles) melting at 159.5-161°C.

Analysis. Calculated for $C_7H_5N_5O_2$: C, 43.99; H, 2.64; N, 36.64. Found: C, 44.13; H, 2.84; N, 36.37.

5-(3'-Nitrophenyl)tetrazole.

(a) Preparation from a Benzene Solution of Hydrazoic Acid. Meta-nitrobenzonitrile (15.6 g., 0.106 mole) was added to 50 ml. of xylene containing 6.8 g. of hydrazoic acid and the mixture sealed in a Pyrex combustion tube which in turn was heated for 125 hours at 140-145°C. At the end of this time the tube was cooled and opened, and the contents

washed out with a small amount of benzene. Chilling of the benzene-xylene mixture gave a crystalline material which was filtered and dried. Recrystallization of the product from water, using Norite to decolorize the solution, gave 8.6 g. (39.0% of theory) of a crystalline solid melting at 150.5-151.5°C.

Analysis. Calculated for $C_7H_5N_5O_2$: C, 43.99; H, 2.64; N, 36.64. Found: C, 44.19; H, 2.68; N, 36.93.

This compound is reported by Lossen (34) to melt at 145°C.

(b) Preparation from Sodium Azide and Glacial Acetic Acid. Meta-nitrobenzonitrile, (32.5 g., 0.25 mole), 22 g. of sodium azide and 20 g. of glacial acetic acid were heated at reflux temperature in 100 ml. of normal butyl alcohol for four days. At the end of this time an additional 5 g. of sodium azide and 10 g. of glacial acetic acid were added and the refluxing continued for two more days. The reaction mixture was then poured into a larger flask, 300 ml. of water added and the mixture distilled until all the alcohol had been removed. Cooling of the aqueous, residual solution caused the separation of a pale yellow solid which when filtered and recrystallized from water, using Norite to remove the color, gave 41.8 g. (87.5% of theory) of the pale, yellow product (needles), m.p. 150.5-151.5°C.

A mixture melting point with the product formed from the sealed tube reaction of meta-nitrobenzonitrile and hydrazoic acid showed no depression.

5-(4'-Nitrophenyl)tetrazole

(a) Preparation from a Benzene Solution of Hydrazoic Acid. Para-

nitrobenzonitrile (15.6 g., 0.106 mole) was added to 50 ml. of xylene containing 6.8 g. (0.158 mole) of hydrazoic acid and the mixture sealed in a Pyrex combustion tube. The tube and its contents were then heated at 140-145°C. for 125 hours. After this time the tube was allowed to cool, opened and the contents washed out with a small amount of benzene. The solid material was filtered and recrystallized from absolute ethanol until the colorless, crystalline product melted at 218.5-219°C.

The crude yield amounted to 16.6 g. (77% of theory).

Analysis. Calculated for $C_7H_5N_5O_2$: C, 43.99; H, 2.64; N, 36.64. Found: C, 44.15; H, 2.70; N, 36.74.

This compound is reported by Pinner (35) to melt at 219°C.

(b) Preparation from Sodium Azide and Glacial Acetic Acid. Para-nitrobenzonitrile (32.5 g., 0.25 mole), 22 g. of sodium azide and 20 g. of glacial acetic acid were added to 100 ml. of normal butyl alcohol and the mixture heated at reflux temperature for four days. At the end of this time an additional 5 g. of sodium azide and 10 g. of glacial acetic acid were added and the heating continued for two more days. The reaction mixture was then transferred to a larger flask, 300 ml. of water added and the mixture distilled until all the alcohol had been removed. On cooling the clear solution a yellow precipitate formed which was filtered and dried. The solid was then dissolved in a minimum amount of hot water and treated with concentrated hydrochloric acid until the solution was acid to Congo red paper. During the acidification a precipitate formed which, after cooling, was filtered and dried.

This crude product amounted to 37.0 g. (77.8% of theory), and could be further purified by recrystallization from water, m.p. 218.5-219°C.

A mixture melting point determination with the product from the reaction of para-nitrobenzonitrile and hydrazoic acid in a sealed tube showed no depression.

5-(2'-Hydroxy-4'-nitrophenyl)tetrazole. 2-Hydroxy-4-nitrobenzonitrile (33) (41.0 g., 0.25 mole), 22 g. of sodium azide and 20 g. of glacial acetic acid were heated at reflux temperature in 100 ml. of normal butyl alcohol for six days. At the end of this time the reaction mixture was transferred to a larger flask, 300 ml. of water added and distilled until 250 ml. of distillate had been collected. On cooling a solid crystallized from the residual solution; the solid was filtered and recrystallized twice from water to give 53.5 g. (86.6% of theory) of pure product.

Analytical data indicates that this was the sodium salt of the corresponding tetrazole.

Analysis. Calculated for $C_7H_4N_5O_3Na$: N, 30.57. Found: N, 30.37 and 30.14.

The sodium salt was converted quantitatively into the free tetrazole by treating a hot aqueous solution of the sodium salt with concentrated hydrochloric acid until it was acid to Congo red paper. The mixture was then cooled, filtered and the product recrystallized from ethanol, m.p. 283-283.5°C., with decomposition.

Analysis. Calculated for $C_7H_5N_5O_3$: C, 40.59; H, 2.43; N, 33.81. Found: C, 40.72; H, 2.82; N, 33.72.

The Preparation of Aminophenyltetrazoles

5-(3'-Aminophenyl)tetrazole. Concentrated hydrochloric acid

(85 ml.) was added to a mixture of 17.2 g. (0.09 mole) of 5-(3'-nitrophenyl)tetrazole and 35 g. of granular tin contained in a 300 ml., three necked flask. The reaction started almost immediately and cooling was necessary. After stirring the reaction mixture for an additional fifteen minutes the clear solution was decanted from the unreacted tin which was then washed with a small amount of water. The washings were combined with the decanted acid solution and the resulting solution made basic with concentrated ammonium hydroxide. The tin hydroxide was filtered and the filtrate made acid with glacial acetic acid until a precipitate no longer formed on the addition of more acid.

The crude 5-(3'-aminophenyl)tetrazole was filtered and dried giving 11.5 g. (79.5% of theory) of a colorless crystalline solid which may be further purified by recrystallization from water, m.p. 199-200°C.

Analysis. Calculated for $C_7H_7N_5$: C, 52.17; H, 4.38; N, 43.46. Found: C, 52.12; H, 4.49; N, 43.11.

5-(4'-Aminophenyl)tetrazole

(a) Reduction Using Tin and Hydrochloric Acid. 5-(4'-Nitrophenyl)-tetrazole (17.2 g., 0.09 mole) and 35 g. of granular tin in a 300 ml. two necked flask were treated with 75 ml. of concentrated hydrochloric acid. Cooling was necessary once the reaction started. When the reaction had subsided, the clear hot solution was stirred for an additional fifteen minutes and then decanted from the unreacted tin. The tin was washed with a small amount of water and the washings combined with the hydrochloric acid solution. The acid solution was made strongly basic with concentrated ammonium hydroxide, the stannous hydroxide filtered

and washed with dilute ammonium hydroxide and the basic solution neutralized with glacial acetic acid until a precipitate no longer formed.

Filtration and drying of the solid gave 12.7 g. (87.5% of theory) of the crude product, which may be further purified by recrystallization from ethanol-water mixture, m.p. 267°C., with decomposition.

A mixture melting point with the product from the catalytic reduction of the corresponding nitro compound showed no depression in the decomposition point.

(b) Reduction Using Platinum Oxide and Hydrogen. 5-(4'-Nitrophenyl)-tetrazole (7.3 g., 0.0382 mole), suspended in 150 ml. of glacial acetic acid, was shaken with 150 mg. of platinum oxide in a hydrogen atmosphere at an initial pressure of 48.5 p.s.i. At the end of twenty-four hours the theoretical amount of hydrogen had been taken up and the chilled suspension of catalyst and a white solid was filtered with suction. The solids were treated with hot ethanol, the platinum filtered off and the alcohol removed in a vacuum. The solid residue was recrystallized from ethanol-water mixture, using Norite to decolorize the solution, giving 5.0 g. (81.5% of theory) of 5-(4'-aminophenyl)tetrazole as colorless needles melting with decomposition at 267°C.

Analysis. Calculated for $C_7H_7N_5$: C, 52.17; H, 4.38; N, 43.46. Found: C, 51.93; H, 4.34; N, 43.29.

5-(2'-Hydroxy-4'-aminophenyl)tetrazole. The hydrated sodium salt of 5-(2'-hydroxy-4'-nitrophenyl)tetrazole (24.7 g., 0.10 mole) was suspended in 150 ml. of water with 250 mg. of platinum oxide and

hydrogenated at an initial pressure of 50 p.s.i. After twenty-four hours the reaction was complete and the catalyst was filtered off. The filtrate was heated with a small amount of a sodium hydrosulfite solution to remove any traces of orange color. This was followed by treatment with Norite and filtration. Concentrated hydrochloric acid was then added to the cooled filtrate until a precipitate no longer formed. Filtering and drying gave a colorless, crystalline solid which amounted to 13.0 g. (73.5% of theory), m.p. 261-262°C., with decomposition. Further recrystallization from water did not change the decomposition point.

Analysis. Calculated for $C_7H_7N_5O$: C, 47.45; H, 3.99; N, 39.55. Found: C, 47.32; H, 4.17; N, 39.72.

Derivatives

5-(2'-Acetylaminophenyl)tetrazole. 5-(2'-Nitrophenyl)tetrazole (7.7 g., 0.040 mole) was mixed with 15.7 g. of tin and the mixture treated with 35 ml. of concentrated hydrochloric acid; cooling was necessary once the reaction started. When the reaction had subsided the clear solution was decanted from the unreacted tin. The tin was washed with a small amount of water and the washings combined with the hydrochloric acid solution. The acid solution was made strongly basic with ammonium hydroxide and the tin hydroxide filtered. The excess ammonium hydroxide was neutralized with glacial acetic acid and the solution adjusted to a pH of 5. Continued cooling did not precipitate the aminophenyltetrazole.

The slightly acid solution was then evaporated to dryness and the

residue refluxed with 4.08 g. (0.040 mole) of acetic anhydride in 25 ml. of glacial acetic acid. After two hours the mixture was cooled and poured into ice and water. This provided the crude product as a brown solid. Recrystallization from water, using Norite to decolorize the solution, gave the pure product as a colorless solid, m.p. 176-177°C.

Analysis. Calculated for $C_9H_9N_5O$: C, 53.19; H, 4.46; N, 34.47. Found: C, 52.87; H, 4.44; N, 34.37.

5-(3'-Acetylaminophenyl)tetrazole. 5-(3'-Aminophenyl)tetrazole (7.0 g., 0.0435 mole) was refluxed with 5.0 g. of acetic anhydride in 30 ml. of glacial acetic acid for two hours. At the end of this time the mixture was poured into 50 ml. of ice and water and the solid filtered. Recrystallization of the product from water, using Norite to decolorize the solution, gave 5.7 g. (65% of theory) of the acetyl derivative as a colorless crystalline solid melting with decomposition at 254-255°C.

Analysis. Calculated for $C_9H_9N_5O$: C, 53.19; H, 4.46; N, 34.47. Found: C, 53.22; H, 4.58; N, 34.50.

5-(4'-Acetylaminophenyl)tetrazole. 5-(4'-Aminophenyl)tetrazole (10 g., 0.062 mole) was refluxed with 7.0 g. of acetic anhydride in 30 ml. of glacial acetic acid for a period of two hours. The mixture was then poured into 50 ml. of ice and water and the solid material filtered. The dried crude product amounted to 12.5 g. (99% of theory) and was further recrystallized with difficulty from glacial acetic acid giving a colorless powder melting with decomposition at 278°C.

Analysis. Calculated for $C_9H_9N_5O$: C, 53.19; H, 4.46; N, 34.47. Found: C, 53.09; H, 4.68; N, 34.27.

5-(2'-Hydroxy-4'-acetylaminophenyl)tetrazole. 5-(2'-Hydroxy-4'-aminophenyl)tetrazole (2.66 g., 0.015 mole) was heated with 1.5 g. of acetic anhydride in 15 ml. of glacial acetic acid at reflux temperature for two hours. The solution was then poured into 50 ml. of ice and water, the solid filtered and oven dried at 70°C. This gave 3.2 g. (97.5% of theory) of a cream colored material melting with decomposition at 277.5°C. Further recrystallization from water, in which it is difficulty soluble, gave a colorless, crystalline product (needles), m.p. 281-282°C., with decomposition.

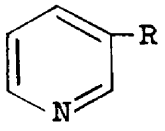
Analysis. Calculated for $C_9H_9N_5O_2$: C, 49.31; H, 4.14; N, 31.95. Found: C, 49.15; H, 4.31; N, 32.06.

PART III

TETRAZOLE ANALOGUES OF NICOTINIC ACID AND OTHER PYRIDINE CARBOXYLIC ACIDS

Discussion

One of the first instances of vitamin antagonism was that observed in the case of pyridine-3-sulfonamide (I) (36) for niacin (II) and niacinamide (III).

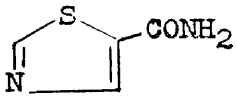


I: R= SO_2NH_2

II: R= CO_2H

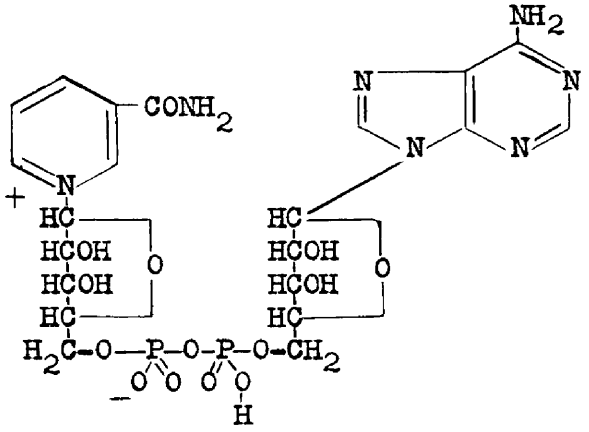
III: R= $CONH_2$

3-Acetylpyridine (37) and thiazole-5-carboxamide (IV) (38) have also been reported to exhibit nicotinic acid antagonism.



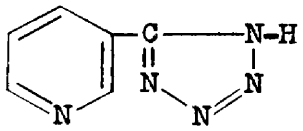
IV

Niacin is important in both animal and bacterial metabolism and exists as a portion of coenzyme I (V) and coenzyme II, both essential enzymes in biological oxidations.



V

The possibility that 3-(5'-tetrazolyl)pyridine (VI) might act as



VI

a nicotinic acid antagonist prompted its synthesis, as well as that of the two other positional isomers, the 2- and 4-(5'-tetrazolyl)pyridines.

The procedure for the synthesis was that employed by Herbst and Wilson (3) for the preparation of 5-aryltetrazoles and consisted of heating the respective cyanopyridines with sodium azide and glacial acetic acid in refluxing normal butyl alcohol. Although the method requires continuous heating at reflux temperature for six days, the yields were, in every case, over 90% of theory. It was found in

several runs that reducing the heating period to three or four days did not lower the yields to any extent.

In all the preparations of the pyridyltetrazoles, replacement of the butyl alcohol solvent by water, during the working up of the reaction mixture, resulted in a clear solution of the sodium salt of the tetrazole. Acidification of this solution with concentrated hydrochloric acid followed by cooling and filtration, provided the corresponding pyridyltetrazole in a relatively pure state. Further purification was carried out by recrystallization from water, in which the tetrazoles were moderately soluble when hot and insoluble when cold.

All the pyridyltetrazoles were solids which exhibited decomposition at their melting points. They displayed typical amphoteric character being soluble in both aqueous acids and alkalies and possessed an isoelectric point at a pH of about 5. Their solubilities in water, although considerably less than that of the pyridine carboxylic acids, followed the same order as that observed in the carboxylic acid series, i.e., 2-isomer > 3-isomer > 4-isomer.

The pyridyltetrazoles were shown to undergo normal reduction in glacial acetic acid using hydrogen and a platinum oxide catalyst to the respective piperidyltetrazoles.

Since the reduction of the pyridine ring to a piperidyl structure was accompanied by the formation of a more basic secondary amino group, the physical characteristics of the piperidyltetrazoles differed markedly from those of the starting pyridyltetrazoles. In addition to behaving as amphoteric substances the piperidyltetrazoles had higher

decomposition points and an increased water-solubility.

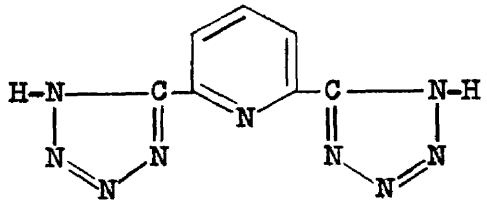
Purification of the three isomeric piperidyltetrazoles was accomplished by dissolving the material in a minimum amount of water, treating with Norite and after filtering, precipitating with acetone.

The acetyl derivatives of the piperidyltetrazoles were prepared conveniently and in moderately good yields by refluxing them in glacial acetic acid containing an equivalent amount of acetic anhydride.

The acetylpiperidyltetrazoles were also prepared without isolation of the intermediate piperidyltetrazole by reduction of the respective pyridyltetrazoles in glacial acetic acid, filtration of the reaction mixture from the catalyst and subsequent refluxing of the filtrate with the required amount of acetic anhydride.

The acetylpiperidyltetrazoles, which could be purified by recrystallization from water or isopropyl alcohol, exhibited moderately high melting points.

Because of its possible antagonism toward 2,6-pyridinedicarboxylic acid, a material involved in spore formation, 2,6-di(5'-tetrazolyl)-pyridine (VIII) was also prepared.



VIII

The synthesis was carried out under the conditions suggested for the pyridyltetrazoles. Although the reaction mixture of the 2,6-dicyanopyridine, glacial acetic acid and sodium azide in butyl alcohol showed no apparent change in physical appearance after one day of refluxing, the reaction period was extended for four days.

Acidification of the clear aqueous solution after the butyl alcohol had been displaced by water, gave a copious precipitate of the corresponding 2,6-di(5'-tetrazolyl)pyridine in a quantitative yield.

Attempts to purify the product by recrystallization from various solvents was not applicable to a very large quantity and the method of dissolving the product in base followed by treatment with Norite and reacidification provided purification of the crude product. Comparison of the decomposition points of samples purified in this manner with that of the analytical sample which was recrystallized from a large volume of water shows no appreciable difference.

The ditetrazolylpyridine, although insoluble in most solvents, dissolves in strong acids and aqueous alkalies. It melts with spontaneous decomposition which expells the material out of the top of the capillary tube.

The pyridyl-, piperidyl- and acetylpiperidyltetrazoles were submitted for screening along with the 2,6-di(5'-tetrazolyl)pyridine.

Experimental

The Preparation of 5-Pyridyltetrazoles

5-(2'-Pyridyl)tetrazole. 2-Cyanopyridine (26 g., 0.25 mole), 20 g. (0.33 mole) of glacial acetic acid and 22 g. (0.33 mole) of sodium azide were added to 100 ml. of normal butyl alcohol and the mixture heated at reflux temperature for a period of four days. At this time an additional 5 g. of sodium azide and 10 g. of glacial acetic acid were added and the heating continued for two more days. The contents of the flask were transferred to a 500 ml. flask, 300 ml. of water was added and the mixture distilled until 300 ml. of distillate had collected. The residual solution was then treated with concentrated hydrochloric acid until a precipitate no longer formed.

The solid was filtered, recrystallized from water and finally oven dried at 70°C. to give 33.4 g. (91% of theory) of a colorless crystalline solid melting at 211-211.5°C.

Analysis. Calculated for $C_6H_5N_5$: C, 48.98; H, 3.43; N, 47.60. Found: C, 49.16; H, 3.67; N, 47.81.

5-(3'-Pyridyl)tetrazole. 3-Cyanopyridine (26 g., 0.25 mole), 22 g. (0.33 mole) of sodium azide and 20 g. (0.33 mole) of glacial acetic acid were heated in 100 ml. of normal butyl alcohol at reflux temperature for a period of four days. At the end of this time an additional 5 g. of sodium azide and 10 g. of glacial acetic acid were added and the refluxing continued for two additional days. The reaction mixture was then transferred to a 500 ml. flask, 300 ml. of water added and the mixture distilled until 300 ml. of distillate had been collected.

Concentrated hydrochloric acid was added to the residual aqueous solution until a precipitate no longer formed. The solid was filtered, recrystallized from water and dried giving 33.3 g. (90.5% of theory) of a colorless crystalline solid melting at 234-235°C.

Analysis. Calculated for $C_6H_5N_5$: C, 48.98; H, 3.43; N, 47.60. Found: C, 49.17; H, 3.42; N, 47.66.

5-(4'-Pyridyl)tetrazole. 4-Cyanopyridine (26 g., 0.25 mole), 22 g. (0.33 mole) of sodium azide and 20 g. (0.33 mole of glacial acetic acid were added to 100 ml. of normal butyl alcohol and the mixture heated to reflux temperature for a period of four days. After this time an additional 5 g. of sodium azide and 10 g. of glacial acetic acid were added and the heating continued for two more days. The contents of the flask were then transferred to a 500 ml. flask, 300 ml. of water added and the mixture distilled until all the alcohol had been removed. The residual aqueous solution was then treated with concentrated hydrochloric acid until a precipitate no longer formed on the addition of more acid.

The solid was filtered, recrystallized from water and dried giving 34.3 g. (93.2% of theory) of pure product, m.p. 253-254°C., with decomposition.

Analysis. Calculated for $C_6H_5N_5$: C, 48.98; H, 3.43; N, 47.60. Found: C, 49.19; H, 3.57; N, 47.33.

2,6-Di(5'-tetrazolyl)pyridine. 2,6-Dicyanopyridine (27.5 g., 0.213 mole), 38.2 g. (0.586 mole) of sodium azide and 38 ml. of glacial acetic acid were added to 100 ml. of normal butyl alcohol and the

mixture refluxed for two days. At the end of this time an additional 10 g. of sodium azide and 20 ml. of glacial acetic acid were added and the refluxing continued for an additional two days. The contents of the flask were then transferred to a larger flask, 300 ml. of water added and the mixture distilled until all the alcohol had been removed. Concentrated hydrochloric acid was added to the aqueous residual solution until a precipitate no longer formed on the addition of more acid. The solid was filtered giving 45.6 g. (99.5% of theory) of a colorless crystalline material which decomposed at 287°C.

The crude product was further purified by dissolving in aqueous base and reprecipitating with acid. The analytical sample was recrystallized from water, in which the product is very slightly soluble, m.p. 290°C., with decomposition.

Analysis. Calculated for $C_7H_5N_9$: C, 39.07; H, 2.34; N, 58.59. Found: C, 39.15; H, 2.57; N, 58.60.

The Preparation of 5-Piperidyltetrazoles

5-(2'-Piperidyl)tetrazole. 5-(2'-Pyridyl)tetrazole (11.0 g., 0.075 mole) was suspended in 150 ml. of glacial acetic acid and hydrogenated in a hydrogen atmosphere over 250 mg. of platinum oxide catalyst at an initial pressure of 50 p.s.i.; the reaction was complete in twenty-four hours. The catalyst was filtered from the solution and the filtrate concentrated to a small volume. The addition of ether caused the precipitation of a colorless solid which was further purified by dissolving in water, treating with Norite and precipitating with acetone. This gave 10.5 g. (91.5% of theory) of pure product,

m.p. 287-287.2°C., with decomposition.

Analysis. Calculated for $C_6H_{11}N_5$: C, 47.05; H, 7.24; N, 45.72. Found: C, 47.03; H, 7.12; N, 45.98.

5-(3'-Piperidyl)tetrazole. 5-(3'-Pyridyl)tetrazole (11.7 g., 0.0795 mole) was suspended in 150 ml. of glacial acetic acid and hydrogenated over 300 mg. of platinum oxide catalyst in a hydrogen atmosphere at an initial pressure of 50 p.s.i. After twenty-four hours the reaction was complete and the catalyst was removed by filtration. The filtrate was concentrated to a small volume and ether added to precipitate the product. This gave 12.2 g. (99.7% of theory) of the crude product.

The product was further purified by dissolving in a minumum amount of water and cooling. The precipitate which was formed was filtered and used as the analytical sample, the remainder of the product was precipitated from the aqueous filtrate by the addition of acetone. The colorless crystalline product melted with decomposition at 296-297°C.

Analysis. Calculated for $C_6H_{11}N_5$: C, 47.05; H, 7.24; N, 45.72. Found: C, 47.10; H, 7.31; N, 45.66.

5-(4'-Piperidyl)tetrazole. 5-(4'-Pyridyl)tetrazole (14.7 g., 0.1 mole) was suspended in 200 ml. of glacial acetic acid and shaken with 400 mg. of platinum oxide catalyst in a hydrogen atmosphere at an initial pressure of 50 p.s.i. After twenty-four hours the reaction mixture was filtered. Concentration of the filtrate followed by the addition of ether gave a solid precipitate which was further purified by dissolving in a minimum amount of water, treating with Norite, and

reprecipitating by the addition of acetone to give 13.2 g. (86.3% of theory) of crude product. The product crystallized from water as colorless cubic crystals, which did not decompose at 370°C. but showed shrinking and browning at 237°C.

Analysis. Calculated for $C_6H_{11}N_5$: C, 47.05; H, 7.24; N, 45.72. Found: C, 46.97; H, 7.22; N, 46.03.

The Preparation of Acetylpiperidyltetrazoles

(a) 5-(2'-N-Acetylpiperidyl)tetrazole. 5-(2'-Piperidyl)tetrazole (10 g., 0.066 mole) was refluxed with 6.7 g. (0.066 mole) of acetic anhydride in 30 ml. of glacial acetic acid for a period of two hours. The solvent was then removed in a vacuum and the residue recrystallized from water, decolorizing with Norite, to give 6.0 g. (46.6% of theory) of the colorless crystalline product which melted at 135.4-136.5°C.

Analysis. Calculated for $C_8H_{13}N_5O$: C, 49.22; H, 6.71; N, 35.88. Found: C, 49.14; H, 6.64; N, 35.61.

(b) 5-(2'-Pyridyl)tetrazole (22.5 g., 0.15 mole) was suspended in 150 ml. of glacial acetic acid and shaken with 150 mg. of platinum oxide catalyst in a hydrogen atmosphere at an initial pressure of 50 p.s.i. After the theoretical amount of hydrogen had been taken up the catalyst was removed by filtration. The filtrate was treated directly with 16.3 g. (0.16 mole) of acetic anhydride and the mixture refluxed for two hours. Evaporation of the solvent left 24.6 g. (84.0% of theory) of the crude product which was purified by recrystallization from water, decolorizing the solution with Norite, m.p. 135.5-136.5°C.

A mixture melting point determination with the product formed from the reaction of acetic anhydride and 5-(2'-piperidyl)tetrazole showed no depression.

5-(3'-N-Acetylpiperidyl)tetrazole (a) 5-(3'-Piperidyl)tetrazole (9.0 g., 0.059 mole) was refluxed for two hours in 30 ml. of glacial acetic acid containing 6.0 g. (0.059 mole) of acetic anhydride. At the end of this time the solvent was removed in a vacuum and the residue recrystallized from isopropyl alcohol giving 5.6 g. (48.7% of theory) of the colorless crystalline acetyl derivative which melted at 170-171°C.

Analysis. Calculated for $C_8H_{13}N_5O$: C, 49.22; H, 6.71; N, 35.88. Found: C, 49.45; H, 6.74; N, 36.06.

(b) 5-(3'-pyridyl)tetrazole (22.5 g., 0.15 mole) was suspended in 125 ml. of glacial acetic acid and shaken with 150 mg. of platinum oxide catalyst in a hydrogen atmosphere at an initial pressure of 50 p.s.i. After twenty-four hours the reaction was complete and the catalyst was filtered from the reaction mixture. Sixteen and three-tenths grams (0.16 mole) of acetic anhydride was then added to the filtrate and the solution refluxed for two hours. At the end of this time the solvent was removed in a vacuum and the residue recrystallized from isopropyl alcohol to give the acetyl derivative, m.p. 169-170.5°C. A second recrystallization raised the melting point to 170.5-171°C., and gave 15.5 g. (53% of theory) of the desired product.

A mixture melting point with 5-(3'-acetylpiperidyl)tetrazole, formed from the reaction of acetic anhydride and 5-(3'-piperidyl)-

tetrazole, showed no depression.

5-(4'-N-Acetylpiperidyl)tetrazole (a) 5-(4'-Piperidyl)tetrazole (12.2 g., 0.08 mole) was refluxed with 8.2 g. (0.08 mole) of acetic anhydride in 30 ml. of glacial acetic acid for two hours. The solvent was then removed in a vacuum and the residue recrystallized from isopropyl alcohol giving 9.2 g. (59% of theory) of a colorless crystalline product melting at 156.5-157.5°C.

Analysis. Calculated for $C_8H_{13}N_5O$: C, 49.22; H, 6.71; N, 35.88. Found: C, 49.25; H, 6.84; N, 35.81.

(b) 5-(4'-Pyridyl)tetrazole (22.5 g., 0.15 mole) was suspended with 200 mg. of platinum oxide catalyst in 125 ml. of glacial acetic acid and shaken in an atmosphere of hydrogen at an initial pressure of 50 p.s.i. After twenty-four hours the reaction was complete and the catalyst was filtered from the reaction mixture. Sixteen and three-tenths grams (0.16 mole) of acetic anhydride was added to the filtrate and the solution refluxed for two hours. After this time the solvent was removed in a vacuum and the residue recrystallized from isopropyl alcohol giving 19.7 g. (67.4% of theory) of the product which melted at 156-157°C.

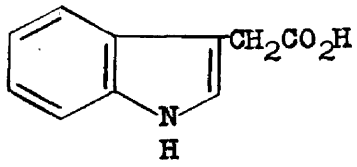
A mixture melting point determination with the product from the reaction of acetic anhydride and 5-(4'-piperidyl)tetrazole showed no depression.

PART IV

TETRAZOLE ANALOGUES OF PLANT AUXINS

Discussion

Isolation and identification of indole-3-acetic acid (I) as a natural growth hormone in plants initiated a search for other substances which could elicit this type of activity. Among those synthetic materials which were shown to stimulate growth was a group of chlorinated chemical compounds derived from phenoxyacetic acid. Varying degrees of activity were demonstrated depending on the number and position of the chlorine atoms in the benzenoid portion of the structure. Of the phenoxyacetic acid derivatives studied, 2,4-dichlorophenoxyacetic acid (2,4-D) (II) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) were shown to be the most active. (39)



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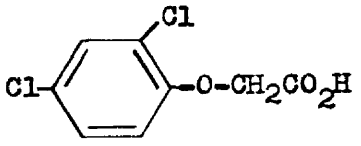


II

Koepfli and his co-workers (40), in an attempt to correlate molecular structure with auxin activity, postulated some of the minimum structural requirements a substance should possess in order to elicit growth stimulation in plants. These included:

a. a ring system as nucleus

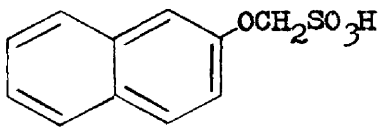
b. a double bond in this ring

c. a side chain

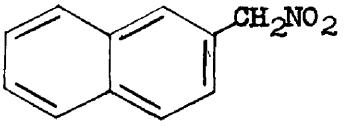
d. a carboxyl group on the side chain at least one carbon removed from the ring

e. a particular space relationship between the ring and the carboxyl group

The requirement that there be a carboxyl group on the side chain finds exception in that the corresponding aldehydes, nitriles, esters and amides also show, to a certain degree, hormonal activity. Exception to the carboxylic acid rule has also been shown by active chemical compounds in which the carboxylic acid group is replaced by a sulfonic acid moiety (III) or a nitro group (IV) (41).



III



IV

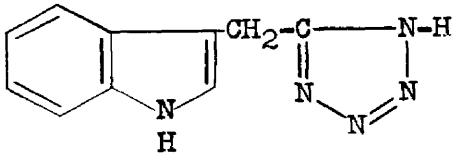
Because of the acidic character of monosubstituted tetrazoles with a substitutent in the 5 position it appeared of interest in this study to incorporate the tetrazole nucleus into the chemical structure of an active plant auxin in place of the carboxyl group.

It was anticipated that the analogue could display several types

of physiological behavior; it could perform similar to a plant hormone and stimulate growth, it could possess activity as a plant growth inhibitor, either by preventing growth through antagonism of the specific hormone of which it is the analogue or by inhibition of the natural plant hormones, or the tetrazole could be totally inactive in either facilitating or inhibiting plant growth.

The carboxylic acid analogues synthesized for this study were those of indole-3-acetic acid and variously substituted phenoxyacetic acids.

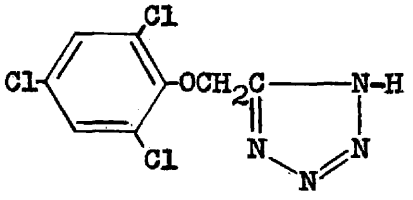
The synthesis of 5-(3'-indolylmethyl)tetrazole (v) was carried out in 88% yields by the reaction of 3-indolylacetonitrile with aluminum azide by adaptation of the procedures of Behringer and Kohl (5).



V

The substituted phenoxymethyltetrazoles were synthesized by two general procedures. The first involved the reaction of the appropriately substituted phenoxyacetonitrile with sodium azide and acetic acid in refluxing normal butyl alcohol following the procedure of Herbst and Wilson (3). This was applicable to the synthesis of the 2,4-dichloro-, 2,4,5-trichloro- and unsubstituted phenoxymethyltetrazoles.

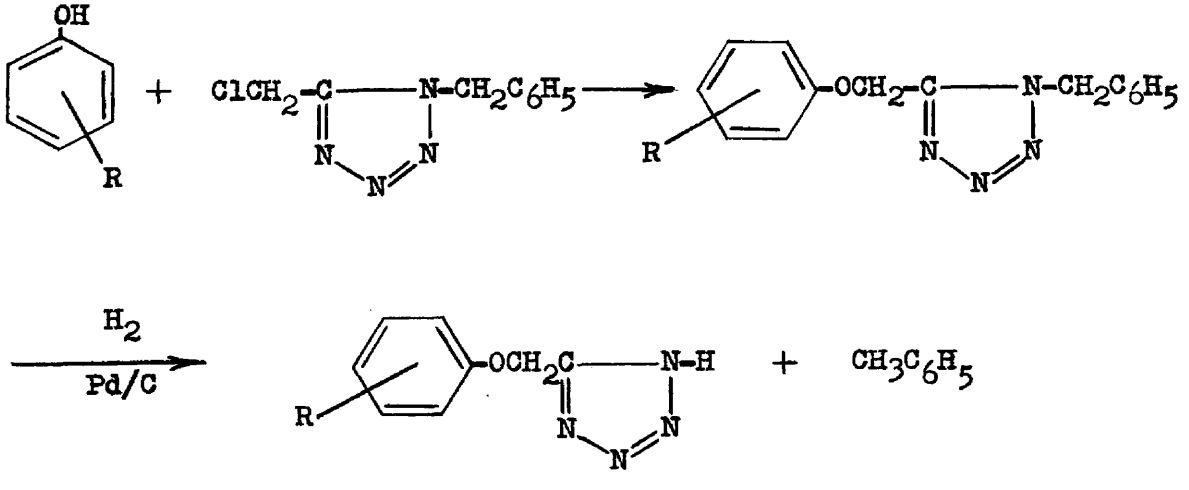
Attempted formation of the corresponding 5-(2',4',6'-trichlorophenoxymethyl)tetrazole (VI) by the use of these conditions resulted in a deep brown coloration of the reaction mixture due to extensive decomposition.



VI

The reaction of the 2-chloro-, 4-chloro- and 2,4,6-trichlorophenoxyacetonitriles with aluminum azide in refluxing tetrahydrofuran, using an adaptation of the procedure of Behringer and Kohl, resulted in very good yields of the corresponding tetrazoles.

The alternate method used for the formation of the phenoxymethyltetrazoles involved the alkylation of the appropriately substituted phenol with 1-benzyl-5-chloromethyltetrazole (16) followed by removal of the benzyl group with hydrogen and palladium on charcoal as illustrated schematically below:



In several instances, namely in the cases of the 2,4-dichloro- and 2,4,6-trichloro- isomers, debenzylation was accompanied by dehalogenation and possibly reduction making identification and comparison with the compounds prepared by the alternate route difficult. Comparisons where made, were done by melting point and mixture melting points.

Although derivatives of the substituted phenoxymethyl tetrazoles were not prepared, the 5-(3'-indolylmethyl)tetrazole was characterized as a monopicrate.

All the 5-monosubstituted tetrazoles prepared in this group were solids exhibiting moderately high to high melting points. All were acidic substances readily soluble in aqueous alkalies. 5-(Phenoxymethyl)tetrazole and 5-(3'-indolylmethyl)tetrazole, in addition to being recrystallizable from water, were soluble in acetone and ethanol. The chlorinated phenoxymethyltetrazoles, although insoluble in cold water and very slightly soluble in hot water, could be recrystallized from ethanol, aqueous ethanol or toluene.

The nitriles used as intermediates for the phenoxymethyltetrazole syntheses were prepared from the phenols, chloroacetonitrile and potassium carbonate in refluxing acetone. This method of preparation offered a distinct advantage over those which involved synthesis of the nitrile, either from the acid by way of the acid chloride and amide or from phenoxymethyl chloride and sodium cyanide (42); both of these latter methods involve a series of stepwise conversions starting from the phenol.

Substantiation of the identity of the phenoxyacetonitriles was made by comparison with physical constants in the literature, elemental analysis, and in some cases hydrolysis to the corresponding phenoxyacetic acids.

Experimental

The Preparation of Phenoxyacetonitriles

Phenoxyacetonitrile. A mixture of 23.5 g. (0.25 mole) of phenol, 18.7 g. (0.25 mole) of chloroacetonitrile and 34.5 g. (0.25 mole) of potassium carbonate in 75 ml. of dry acetone was heated at reflux temperature for eight hours. The mixture was then poured into 200 ml. of water containing 10 g. (0.25 mole) of sodium hydroxide and extracted with ether. The ether layer was separated, dried over sodium sulfate and the ether removed in a vacuum. A reddish oil (34.5 g.) which remained when distilled gave 27.2 g. (82% of theory) of a clear oily liquid boiling at 73-76°C. at 1 mm.

This product was previously described by Powell and Adams (43) who reported b.p. 132°C. at 30 mm.

2-Chlorophenoxyacetonitrile. A mixture of 32.0 g. (0.25 mole) of 2-chlorophenol, 18.7 g. (0.25 mole) of chloroacetonitrile and 34.5 g. (0.25 mole) of potassium carbonate in 80 ml. of dry acetone was heated at reflux temperature with continued stirring for twenty hours. After this time the mixture was poured into 200 ml. of water containing 10 g. (0.25 mole) of sodium hydroxide and extracted with ether. On evaporation of the dried ether layer there remained 38.7 g. of a dark oil. On distillation there was obtained a fore run and an after run, 102-107°C. at 1 mm. and 109-114°C. at 1 mm., which together amounted to 12.5 g. The middle fraction which distilled at 107-109°C. at 1 mm. amounted to 18.3 g. (43.8% of theory) and was redistilled to obtain an analytical sample, b.p. 109°C. at 1 mm.

Analysis. Calculated for C_8H_6ClNO: Cl, 21.16; N, 8.36. Found: Cl, 21.07; N, 8.10.

4-Chlorophenoxyacetonitrile. To 100 ml. of dry acetone were added 32.0 g. (0.25 mole) of 4-chlorophenol, 18.7 g. (0.25 mole) of chloroacetonitrile and 34.5 g. (0.25 mole) of potassium carbonate and the resulting mixture refluxed for eighteen hours. The mixture was then poured into 150 ml. of water containing 10 g. (0.25 mole) of sodium hydroxide and extracted with ether. The ether layer was separated, dried over sodium sulfate and the ether removed in a vacuum. The residue consisted of a dark red oil which solidified on standing, 39.0 g. (93% of theory). Recrystallization from petroleum ether gave a colorless crystalline product melting at 46.5-47.5°C.

Analysis. Calculated for C_8H_6ClNO: Cl, 21.16; N, 8.36. Found: Cl, 21.17; N, 8.17.

2,4-Dichlorophenoxyacetonitrile. A mixture of 40 g. (0.25 mole) of 2,4-dichlorophenol, 18.7 g. (0.25 mole) of chloroacetonitrile and 34.6 g. (0.25 mole) of potassium carbonate in 60 ml. of dry acetone was refluxed for twenty hours. The pink colored mixture was then poured into 200 ml. of water containing 10 g. (0.25 mole) of sodium hydroxide and the mixture extracted with ether. The etheral layer was separated, dried with sodium sulfate and the solvent removed in a vacuum. The residue consisted of 43.1 g. (85.2% of theory) of a dirty, white solid which melted at 45-46.5°C. Recrystallization from petroleum ether raised the melting point to 48.5-49°C., (colorless needles).

Analysis. Calculated for $C_8H_5Cl_2NO$: C, 47.55; H, 2.50; Cl, 35.10;

N, 6.93. Found: C, 47.57; H, 2.51; Cl, 35.24; N, 6.81.

This product has been previously reported by Barber, et. al, (42) as melting at 44-46°C.

2,4,5-Trichlorophenoxyacetonitrile. A mixture of 49.4 g. (0.25 mole) of 2,4,5-trichlorophenol, 18.7 g. (0.25 mole) of chloroacetonitrile and 34.5 g. (0.25 mole) of potassium carbonate in 75 ml. of dry acetone was refluxed for twenty-four hours. The mixture was then poured into 200 ml. of water containing 10 g. (0.25 mole) of sodium hydroxide and extracted with ether. The ether layer was separated, dried over sodium sulfate and the solvent removed in a vacuum. The residue consisted of 52.8 g. (88.2% of theory) of a cream colored solid which melted at 86-90°C. Recrystallization from petroleum ether raised the melting point to 91.5-92.5°C.

Analysis. Calculated for $C_8H_4Cl_3NO$: C, 40.63; H, 1.71; Cl, 44.98; N, 5.92. Found: C, 40.88; H, 1.96; Cl, 44.83; N, 5.79.

2,4,6-Trichlorophenoxyacetonitrile. A mixture of 49.4 g. (0.25 mole) of 2,4,6-trichlorophenol, 18.7 g. (0.25 mole) of chloroacetonitrile and 34.5 g. (0.25 mole) of potassium carbonate were refluxed together in 75 ml. of dry acetone for eight hours. The mixture was then poured into 200 ml. of water containing 10 g. of sodium hydroxide and extracted with ether. The ether layer was separated, dried over sodium sulfate and the solvent removed in a vacuum. The residual product consisted of 58.0 g. (98.5% of theory) of a colorless crystalline solid melting at 101-102°C. Recrystallization from ethanol raised the melting point to 102-103°C.

This product was reported by Drain, Peak and Whitmont (44) to melt at 103°C.

Analysis. Calculated for $C_8H_4Cl_3NO$: C, 40.63; H, 1.71; Cl, 44.98; N, 5.92. Found: C, 40.63; H, 1.63; Cl, 44.87; N, 5.68.

Hydrolysis of Phenoxyacetonitriles

Phenoxyacetic acid. Five and three-tenths grams (0.04 mole) of phenoxyacetonitrile was added to 100 ml. of a 25% sodium hydroxide solution and the mixture refluxed for twelve hours. At the end of this time the solution was acidified with dilute hydrochloric acid. The precipitate which formed was filtered and dried; it weighed 4.9 g. (80.5% of theory), m.p. 98-99°C., after recrystallization from water.

Sabenjeff and Dworkowitsch (45) reported a melting point of 97°C. for this compound.

2,4-Dichlorophenoxyacetic acid. Five grams (0.025 mole) of 2,4-dichlorophenoxyacetonitrile was added to 50 ml. of a 25% solution of sodium hydroxide and the resulting mixture refluxed for twelve hours. The precipitate which formed on cooling the reaction mixture was filtered and dissolved in 200 ml. of hot water. Acidification of this solution with dilute hydrochloric acid gave a colorless crystalline precipitate which when filtered and dried melted at 137-138.5°C. Recrystallization of the product from water raised the melting point to 138.5-139°C.

This compound was previously reported to melt at 138°C., by Porkorny (46).

2,4,5-Trichlorophenoxyacetic acid. Four and seven-tenths grams (0.02 mole) of 2,4,5-trichlorophenoxyacetonitrile was added to 60 ml. of a 25% sodium hydroxide solution and the mixture refluxed for twelve hours. At the end of this time the solution was cooled and the precipitate filtered. The solid was dissolved in hot water; a small amount of insoluble material was removed by filtration. Acidification of the filtrate with dilute hydrochloric acid gave a colorless precipitate. Filtration and drying gave 4.3 g. (84.5% of theory) of 2,4,5-trichlorophenoxyacetic acid, m.p. 150.5-152°C., after recrystallization from benzene.

This compound was previously described by Porkorny (46) who reported the melting point as 153°C.

The Preparation of Phenoxymethyltetrazoles

5-(Phenoxymethyl)tetrazole. A mixture of 16.3 g. (0.125 mole) of phenoxyacetonitrile, 11 g. (0.165 mole) of sodium azide and 10 g. (0.165 mole) of glacial acetic acid in 60 ml. of normal butyl alcohol was boiled under reflux for four days. Sodium azide (2.5 g.) and 5 g. of glacial acetic acid were then added and the heating continued for an additional two days. At the end of this time the contents of the flask were transferred to a larger container, 200 ml. of water added and the mixture distilled until all the alcohol had been removed. Acidification of the residual aqueous solution with dilute sulfuric acid gave a colorless precipitate, which when filtered and dried weighed 22.1 g. (quantitative yield).

Recrystallization from water gave colorless crystals melting at

127.5-129°C.

Analysis. Calculated for $C_8H_8ON_4$: C, 54.54; H, 4.58; N, 31.80. Found: C, 54.50; H, 4.65; N, 31.93.

5-(2'-Chlorophenoxymethyl)tetrazole. To a suspension of 16.7 g. (0.1 mole) of 2-chlorophenoxyacetonitrile and 19.5 g. (0.3 mole) of sodium azide in 50 ml. of dry tetrahydrofuran was added 13.3 g. (0.1 mole) of anhydrous aluminum chloride in 160 ml. of the same solvent. The resulting mixture was refluxed with continued stirring for twenty-four hours. At the end of this time the organic solvent was distilled and water added at such a rate that the volume remained constant. After the solid which separated had been filtered, it was suspended in 250 ml. of water, treated with 30 ml. of concentrated hydrochloric acid and the resulting mixture stirred at room temperature for one hour.

Filtering and drying of the precipitate gave 18.8 g. (89.5% of theory) of the product melting at 131-135°C.; this was further purified by recrystallization from an alcohol-water mixture, m.p. 134-135.5°C. The analytical sample was recrystallized from toluene, m.p. 134.5-135.5°C.

Analysis. Calculated for $C_8H_7ClN_4O$: C, 45.62; H, 3.35; Cl, 16.83; N, 26.60. Found: C, 45.92; H, 3.63; Cl, 16.94; N, 26.58.

5-(4'-Chlorophenoxymethyl)tetrazole. To a suspension of 16.7 g. (0.1 mole) of 4-Chlorophenoxyacetonitrile and 19.5 g. (0.3 mole) of sodium azide in 50 ml. of dry tetrahydrofuran was added 13.3 g. (0.1 mole) of anhydrous aluminum chloride dissolved in 160 ml. of dry tetrahydrofuran. The mixture was refluxed with continued stirring

for twenty-four hours. At the end of this time the tetrahydrofuran was distilled from the reaction mixture and water gradually added at such a rate that the volume remained constant. After the solid that separated was filtered, it was suspended in 225 ml. of water, treated with 30 ml. of concentrated hydrochloric acid and the resulting mixture stirred at room temperature for one hour.

Filtering and drying of the solid gave 20.6 g. (98% of theory) of the crude product. This was recrystallized from an alcohol-water mixture to give 13.9 g. (66% of theory) of pure product, m.p. 165-166°C.

Analysis. Calculated for $C_8H_7ClN_4O$: C, 45.62; H, 3.35; Cl, 16.83; N, 26.60. Found: C, 45.72; H, 3.63; Cl, 16.78; N, 26.48.

5-(2',4'-Dichlorophenoxymethyl)tetrazole. A mixture of 25.2 g. (0.125 mole) of 2,4-dichlorophenoxyacetonitrile, 11 g. (0.165 mole) of sodium azide and 10 g. of glacial acetic acid in 60 ml. of normal butyl alcohol was refluxed for four days. At the end of this time an additional 2.5 g. of sodium azide and 5 g. of glacial acetic acid were added and the heating continued for two more days. The contents of the reaction flask were then transferred to a 500 ml. round bottom flask, 200 ml. of water added and the mixture distilled until all the alcohol had been removed. The residual aqueous solution was treated with Norite, filtered and acidified with concentrated hydrochloric acid until a precipitate no longer formed. The solid was filtered and dried; it weighed 25.6 g. (83.7% of theory).

Recrystallization from toluene gave colorless needles melting at 124.5-125.5°C.

Analysis. Calculated for $C_8H_6Cl_2N_4O$: C, 39.21; H, 2.47; Cl, 28.94; N, 22.86. Found: C, 39.40; H, 2.59; Cl, 29.04; N, 22.96.

5-(2',4',5'-Trichlorophenoxymethyl)tetrazole. A mixture of 29.6 g. (0.125 mole) of 2,4,5-trichlorophenoxyacetonitrile, 11 g. (0.165 mole) of sodium azide and 10 g. (0.165 mole) of glacial acetic acid in 60 ml. of normal butyl alcohol was boiled under reflux for four days. An additional 2.5 g. of sodium azide and 5 g. of glacial acetic acid were added and the heating continued for two more days. The entire contents of the flask were transferred to a larger flask, 200 ml. of water added and the mixture distilled until about 175 ml. of distillate had been collected. The hot residual solution was treated with Norite, filtered and acidified with concentrated hydrochloric acid until a precipitate no longer formed.

The solid was filtered and dried giving 25.4 g. (73% of theory) of crude product, m.p. 155-162°C. Recrystallization from toluene gave a colorless crystalline product (plates) which melted at 163.5-165°C.

Analysis. Calculated for $C_8H_5Cl_3N_4O$: C, 34.37; H, 1.80; Cl, 38.06; N, 20.05. Found: C, 34.74; H, 1.77; Cl, 38.28; N, 20.11.

5-(2',4',6'-Trichlorophenoxymethyl)tetrazole. A suspension of 5.8 g. (0.0246 mole) of 2,4,6-trichlorophenoxyacetonitrile and 4.8 g. (0.074 mole) of sodium azide in 30 ml. of dry tetrahydrofuran was treated with 2.98 g. (0.025 mole) of anhydrous aluminum chloride dissolved in 60 ml. of dry tetrahydrofuran. The mixture was refluxed with continued stirring for twenty hours. At the end of this time the organic solvent was distilled and water gradually added at such a

rate that the volume remained constant. After the solid which precipitated had been filtered it was suspended in 150 ml. of water, 20 ml. of concentrated hydrochloric acid was added and the mixture stirred at room temperature for one hour.

The solid was filtered and dried at 70°C., giving 6.6 g. (96.5% of theory) of crude product, m.p. 159-161°C. The product was recrystallized from toluene and then from ethanol, m.p. 164-165°C.

Analysis. Calculated for $C_8H_5Cl_3N_4$: C, 34.37; H, 1.80; Cl, 38.06; N, 20.05. Found: C, 34.62; H, 2.06; Cl, 37.90; N, 20.02.

The Preparation of 1-Benzyl-5-phenoxymethyltetrazoles.

1-Benzyl-5-phenoxymethyltetrazole. A mixture of 8.3 g. (0.04 mole) of 1-benzyl-5-chloromethyltetrazole (16), 4.7 g. (0.05 mole) of phenol and 2.7 g. (0.05 mole) of sodium methylate in 75 ml. of absolute methanol was boiled under reflux for ten hours with continued stirring. The entire contents of the flask was then poured into 150 ml. of water and the solid precipitate filtered. Recrystallization from aqueous methanol gave 3.8 g. (35.9% of theory) of the desired product melting at 66.5-67°C.

Analysis. Calculated for $C_{15}H_{14}N_4O$: C, 67.65; H, 5.30; N, 21.04. Found: C, 67.40; H, 5.36; N, 21.08.

1-Benzyl-5-(2',4'-dichlorophenoxymethyl)tetrazole. A mixture of 8.3 g. (0.04 mole) of 1-benzyl-5-chloromethyltetrazole, 8.15 g. (0.05 mole) of 2,4-dichlorophenol and 2.7 g. (0.05 mole) of sodium methylate was refluxed in 75 ml. of absolute methanol with continued stirring for sixteen hours. At the end of this time the contents of the flask

were added to 150 ml. of water and the precipitate which formed filtered and dried to give 12.4 g. (92.5% of theory) of crude product, m.p. 95-97°C. Recrystallization from methanol gave 8.6 g. (64.2% of theory) of pure product, m.p. 107.5-108°C.

Analysis. Calculated for $C_{15}H_{12}Cl_2N_4$: C, 53.75; H, 3.61; Cl, 21.16; N, 16.72. Found: C, 53.83; H, 3.90; Cl, 21.04; N, 16.76.

1-Benzyl-5-(2',4',5'-trichlorophenoxymethyl)tetrazole. A mixture of 6.93 g. (0.033 mole) of 1-benzyl-5-chloromethyltetrazole, 8.15 g. (0.0416 mole) of 2,4,5-trichlorophenol and 2.24 g. (0.0416 mole) of sodium methylate was refluxed in 75 ml. of absolute methanol with continued stirring for seven hours. The resulting mixture was cooled and poured into 150 ml. of water. The precipitate was filtered and dried giving 10.6 g. (86.2% of theory) of crude product, m.p. 101.5-105°C. Recrystallization from methanol gave 6.4 g. (52% of theory) of the colorless, crystalline product melting at 113.5-114.5°C.

Analysis. Calculated for $C_{15}H_{11}Cl_3N_4$: C, 48.74; H, 3.00; Cl, 28.78; N, 15.16. Found: C, 48.61; H, 3.12; Cl, 28.71; N, 15.31.

1-Benzyl-5-(2',4',6'-trichlorophenoxymethyl)tetrazole. A mixture of 6.93 g. (0.033 mole) of 1-benzyl-5-chloromethyltetrazole, 8.15 g. (0.0416 mole) of 2,4,6-trichlorophenol and 2.2 g. (0.0416 mole) of sodium methylate was refluxed in 75 ml. of absolute methanol with continued stirring for twenty hours. The contents of the flask were then poured into 150 ml. of water and the precipitate which formed filtered, and dried, 12.3 g. (quantitative yield), m.p. 109-111°C. Recrystallization from methanol gave 9.1 g. (74.0% of theory) of the

colorless crystalline product melting at 112-113°C.

Analysis. Calculated for $C_{15}H_{11}Cl_3N_4$: C, 48.74; H, 3.00; Cl, 28.78; N, 15.16. Found: C, 48.75; H, 3.01; Cl, 28.92; N, 14.98.

Debenzylation of 1-Benzyl-5-phenoxymethyltetrazoles

5-(Phenoxymethyl)tetrazole. Two and seven-tenths grams (0.01 mole) of 1-benzyl-5-phenoxymethyltetrazole was shaken with 1 g. of 5% palladium on charcoal in 100 ml. of absolute ethanol in a hydrogen atmosphere at an initial pressure of 50 p.s.i. for twelve hours. The catalyst was then filtered and the solvent removed in a vacuum. The residue which remained was treated with dilute base and filtered. From the alkali insoluble solid 1.3 g. (49%) of starting material was recovered. On acidification of the filtrate with dilute hydrochloric acid a precipitate formed which when filtered and dried gave 400 mg. (43.4% of theory) of the crude product, m.p. 125-127°C.

Recrystallization of the product from water raised the melting point to 127.5-128.5°C.

A mixture melting point with a sample of the same compound prepared from the corresponding nitrile, sodium azide and glacial acetic acid, showed no depression.

5-(2',4',5'-Trichlorophenoxymethyl)tetrazole. One and eight-tenths grams (0.005 mole) of 1-benzyl-5-(2',4',5'-trichlorophenoxymethyl)tetrazole and 1 g. of 5% palladium on charcoal were suspended in 75 ml. of absolute ethanol and shaken in an atmosphere of hydrogen at an initial pressure of 50 p.s.i. for twelve hours. The catalyst was then filtered and washed with warm ethanol; the washings were

combined with the filtrate. Removal of the solvent in a vacuum left a residue which when recrystallized from toluene gave 0.85 g. (61% of theory) of product melting at 149-155°C. Repeated recrystallizations from toluene raised the melting point to 160-162°C.

The mixture melting point (162.5-164°C.), with an analytical sample of the same product, m.p. 163.5-165°C., formed from the reaction of the corresponding nitrile with sodium azide and acetic acid in refluxing butanol, was not markedly depressed.

Attempted debenzylation of 1-Benzyl-5-(2',4'-dichlorophenoxymethyl)tetrazole

One and seven-tenths grams (0.005 mole) of 1-benzyl-5-(2',4'-dichlorophenoxymethyl)tetrazole and 1 g. of 5% palladium on charcoal were shaken in an atmosphere of hydrogen at an initial pressure of 50 p.s.i. while suspended in 100 ml. of absolute ethanol. After twelve hours the catalyst was filtered and washed with warm ethanol. The combined filtrate and washings were evaporated in a vacuum and the residue recrystallized from toluene several times, m.p. 101-102°C.

Attempted debenzylation of 1-Benzyl-5-(2',4',6'-trichlorophenoxymethyl)tetrazole

Three and seven-tenths grams (0.01 mole) of 1-benzyl-5-(2',4',5'-trichlorophenoxymethyl)tetrazole and 1 g. of 5% palladium on charcoal, suspended in 100 ml. of absolute ethanol, were shaken in an atmosphere of hydrogen at an initial pressure of 50 p.s.i. for ten hours. The catalyst was then filtered from the reaction mixture and washed with

warm ethanol. The combined filtrate and washings were evaporated in a vacuum and the residue recrystallized from toluene. In spite of repeated recrystallizations the solid melted over a wide range. The product was then treated with dilute base, filtered and the filtrate acidified. The precipitate formed was recrystallized from toluene, m.p. 109-112°C.

The Preparation of Indolylmethyltetrazole

5-(3'-Indolylmethyl)tetrazole. Seven and eight-tenths grams (0.12 mole) of sodium azide was caused to react with 5.32 g. (0.04 mole) of anhydrous aluminum chloride in 120 ml. of dry refluxing tetrahydrofuran for one hour. To the resulting mixture was added 5.8 g. (0.037 mole) of 3-indolylacetonitrile and the refluxing continued with stirring for an additional twenty-four hours. After this time the tetrahydrofuran was distilled from the reaction mixture and water added at such a rate that the volume of the mixture remained constant. After all the organic solvent had been removed the solid precipitate was filtered, resuspended in 250 ml. of water and treated with sufficient concentrated hydrochloric acid to make the pH of the solution 2. After ten minutes the solid material was filtered and washed with a small amount of water. Drying gave 6.5 g. (88.4% of theory) of the crude product melting at 173-176.5°C., with decomposition. The product was recrystallized from ethylene dichloride and then from water to give 4.5 g. (61.2% of theory) of pure product, m.p. 179-180°C., with decomposition.

Analysis. Calculated for $C_{10}H_9N_5$: C, 60.29; H, 4.55; N, 35.16.

Found: 60.34; H, 4.75; N, 35.00.

The monopicrate was prepared in and recrystallized from water, m.p. 131-132°C.

Analysis. Calculated for $C_{16}H_{12}N_8O_6$: C, 44.86; H, 2.83; N, 26.16. Found: C, 45.52; H, 3.20; N, 25.83.

PART V

DISUBSTITUTED TETRAZOLES AS ANALOGUES OF ACTIVE BASIC ESTERS

Discussion

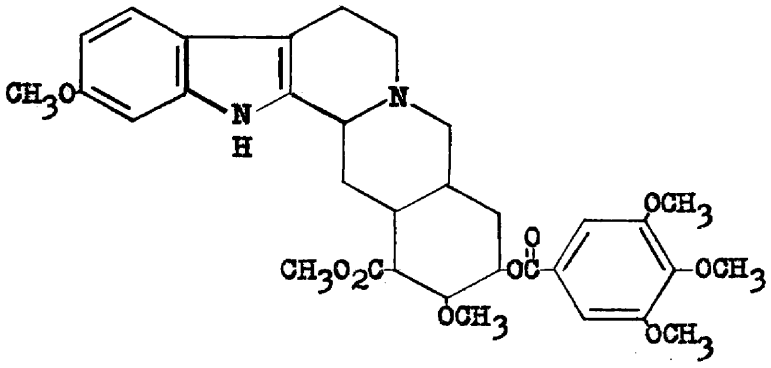
Recognition of the hypotensive action or the blood pressure lowering ability of the extracts of Rauwolfia serpentina resulted in an extensive investigation into the chemistry, pharmacology and medicinal aspects of the Rauwolfia alkaloids.

Reserpine (I), one of the most important alkaloids of this group, which was first isolated in the pure state in 1952, by Schlittler and his co-workers (47), has been shown by pharmacological and clinical investigators not only to be one thousand times as potent a hypotensive agent as the crude drug, but to have the unique property of producing a state of quiesence which has value in the treatment of mental illness.

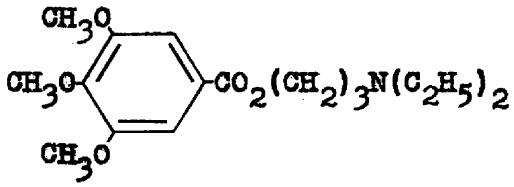
Following the proposal for the structure of reserpine by Schlittler (48) and the confirmation of this structure by an unequivocal synthesis (49), an intense search to determine the active portion of the reserpine molecule showed that replacement of the 3,4,5-trimethoxybenzoyl moiety by other acid groupings either lowered or destroyed its physiological activity (50). This suggested that the pharmacodynamic action was due, not to the portion of the molecule containing the indole ring, but to the remainder of the structure which included the trimethoxybenzoyl ester grouping (51).

In an effort to substantiate this theory Weinberg and Miller prepared a series of dialkylaminoalkyl 3,4,5-trimethoxybenzoates, the

most active of which was diethylamino-n-propyl 3,4,5-trimethoxybenzoate (II).



I

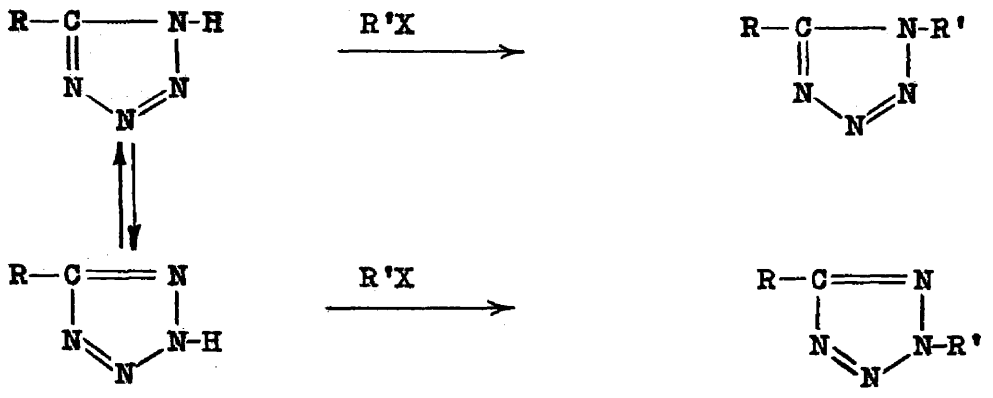


II

In exploring the analogy of 5-monosubstituted tetrazoles to carboxylic acids it was anticipated that alkylation of 5-(3',4',5'-trimethoxyphenyl)tetrazole would lead to a structure similar to those synthesized by Weinberg, in which the ester grouping would be replaced by a disubstituted tetrazole.

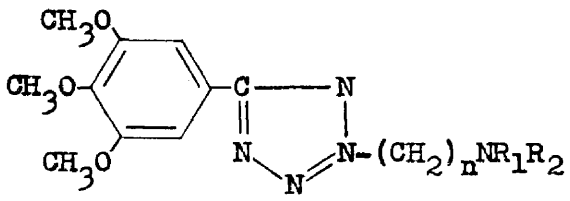
Because of the tautomeric equilibrium which exists in 5-monosubstituted tetrazoles, alkylation could and does lead to the formation

of two isomeric products (52):



Previous investigations carried out on the alkylation of 5-monosubstituted tetrazoles with dialkylaminoalkyl halides have shown that by the use of selective solvents one could isolate the predominating isomer, the 2,5-disubstituted product, free from contamination by the other isomer (53).

Alkylations of the 5-aryltetrazoles were carried out under the conditions suggested by Elpern (53) and the product isolated in such a manner that it provided only the 2,5-disubstituted tetrazole. The 2-dialkylaminoalkyl-5-(3',4',5'-trimethoxyphenyl)tetrazoles (III), which were initially isolated as oils, were readily converted to stable hydrochlorides by bubbling dry hydrogen chloride gas into an etheral solution of the base. The hydrochlorides were used to characterize the products and were also the form most suitable for pharmacological testing purposes.



III

The starting material for this series of preparations, 5-(3',4',5'-trimethoxyphenyl)tetrazole, was synthesized in the following manner: 3,4,5-trimethoxybenzoic acid was converted to the acid chloride and subsequently, by reaction with a concentrated ammonium hydroxide into the corresponding amide in good yields. The amide, in turn, was converted to the nitrile according to the general procedure of Fahrenbach (54). The nitrile was then caused to react with sodium azide and glacial acetic acid in refluxing normal butyl alcohol, following the method of Herbst and Wilson (5), and yielded the corresponding aryltetrazole.

Experimental

3,4,5-Trimethoxybenzamide. The acid chloride formed from the reaction of 94 g. (0.44 mole) of 3,4,5-trimethoxybenzoic acid and 100 ml. of thionyl chloride was added in small portions to 400 ml. of concentrated ammonium hydroxide at 0°C. After two hours of stirring the mixture was allowed to warm up to room temperature and was filtered. The insoluble product after drying, amounted to 74.5 g. (80.3% of theory), m.p. 178-180°C.

Gaebe and Suter (55) report a melting point of 176-177°C., for this compound.

3,4,5-Trimethoxybenzonitrile. Thirty three and five-tenths grams (0.168 mole) of 3,4,5-trimethoxybenzamide was refluxed with 18.4 g. (0.097 mole) of sodium metabisulfite and 59.5 g. of phosphorus oxychloride for one hour. The wine colored solution was then poured carefully onto ice. The grey solid which formed was filtered and dried; the crude yield was 29.8 g. (91.9% of theory), m.p. 93-94.5°C.

Heffter and Capellmann (56) report this compound to melt at 95°C.

5-(3',4',5'-Trimethoxyphenyl)tetrazole. 3,4,5-Trimethoxybenzonitrile (29.8 g., 0.154 mole), 14.95 g. (0.23 mole) of sodium azide and 13.8 g. (0.23 mole) of glacial acetic acid were added to 100 ml. of normal butyl alcohol and the mixture refluxed for four days. At the end of this time an additional 5 g. of sodium azide and 10 g. of glacial acetic acid were added and the heating continued for two more days. The contents of the flask were then transferred to a larger

flask, 250 ml. of water added and the mixture distilled until all the alcohol had been removed. The residual solution was cooled and enough hydrochloric acid added to completely precipitate the tetrazole. The solid was filtered and recrystallized from a water-isopropyl alcohol mixture, 33.9 g. (93.2% of theory), m.p. 199-200°C.

Analysis. Calculated for $C_{10}H_{12}N_4O_3$: C, 50.84; H, 5.12; N, 23.72. Found: C, 51.07; H, 5.22; N, 23.55.

2-(Diethylaminoethyl)-5-(3',4',5'-trimethoxyphenyl)tetrazole Hydrochloride. 5-(3',4',5'-Trimethoxyphenyl)tetrazole (11.8 g., 0.05 mole) and 8.6 g. (0.05 mole) of diethylaminoethyl chloride hydrochloride were suspended in 80 ml. of acetone to which was then added 8.4 g. (0.21 mole) of sodium hydroxide in 7.5 ml. of water. The mixture was heated under reflux with continued stirring for three hours. At the end of this time 50 ml. of water was added, and the mixture extracted with benzene. The benzene layer was separated, dried by azeotropic distillation and the solvent removed in a vacuum. The oily residue which remained was dissolved in 250 ml. of cold ether and dry hydrogen chloride bubbled into the solution until a precipitate no longer formed.

The solid was filtered and dried to give 15.4 g. (83.2% of theory) of crude product. Recrystallization from isopropyl alcohol gave the pure, colorless, crystalline product melting at 147-148°C.

Analysis. Calculated for $C_{16}H_{26}ClN_5O_3$: C, 51.67; H, 7.05; Cl, 9.53; N, 18.84. Found: C, 51.69; H, 6.95; Cl, 9.52; N, 19.05.

2-(3'-Diethylaminopropyl)-5-(3',4',5'-trimethoxyphenyl)tetrazole Hydrochloride. 5-(3',4',5'-Trimethoxyphenyl)tetrazole (18.0 g., 0.076 mole) and 14.5 g. (0.078 mole) of 3-diethylaminopropyl chloride hydrochloride were suspended in 100 ml. of acetone to which was then added 12.6 g. (0.32 mole) of sodium hydroxide in 12 ml. of water. The mixture was refluxed with continued stirring for eight hours. After dilution with 80 ml. of water the mixture was extracted with benzene. The benzene layer was then separated, dried by azeotropic distillation and the solvent removed in a vacuum. The oily residue which remained was dissolved in 300 ml. of cold ether and dry hydrogen chloride bubbled into the solution until a precipitate no longer formed.

The solid was filtered and dried; this gave 27.1 g. (92.5% of theory) of crude product, m.p. 150-155°C. Recrystallization from isopropyl alcohol gave 21.8 g. (74.3% of theory) of a colorless crystalline product melting at 160-161°C.

Analysis. Calculated for $C_{17}H_{28}ClN_5O_3$: C, 52.92; H, 7.31; Cl, 9.19; N, 18.15. Found: C, 52.90; H, 7.25; Cl, 9.41; N, 18.31.

2-(3'-Dimethylaminopropyl)-5-(3',4',5'-trimethoxyphenyl)tetrazole Hydrochloride. 5-(3',4',5'-Trimethoxyphenyl)tetrazole (18.0 g., 0.076 mole) and 12.0 g. (0.076 mole) of 3-dimethylaminopropyl chloride hydrochloride were suspended in 160 ml. of acetone to which was then added 12.6 g. (0.32 mole) of sodium hydroxide dissolved in 12 ml. of water. The resulting mixture was refluxed with continued stirring for eight hours. At the end of this time 80 ml. of water was added and the mixture extracted with benzene. The benzene layer was sep-

arated, dried by azeotropic distillation and the solvent removed in a vacuum. The oily residue which remained was dissolved in 300 ml. of cold ether and dry hydrogen chloride bubbled into the solution until a precipitate no longer formed.

The filtered, dried solid amounted to 13.6 g. (50.3% of theory) after recrystallization from isopropyl alcohol, m.p. 162.5-163.5°C.

Analysis. Calculated for $C_{15}H_{24}ClN_5O_3$: C, 50.34; H, 6.76; Cl, 9.91; N, 19.57. Found: C, 50.17; H, 6.89; Cl, 9.98; N, 19.37.

SUMMARY

1. Three different routes for the synthesis of 5-aminoalkyltetrazoles have been developed to prepare the tetrazole analogues of glycine, D,L-alanine, β-alanine, D,L-phenylalanine and D,L-tryptophane. The acetyl, benzoyl and phenylurea derivatives of the 5-aminoalkyltetrazoles have been prepared and in one instance the hydrochloride has been described.

2. The apparent pK_1 and pK_2 values of the 5-aminoalkyltetrazoles, with the exception of the tryptophane analogue, have been determined and shown to be analogous to those of the corresponding amino acids.

3. Starting from the appropriately substituted nitrobenzonitriles the three isomeric 5-nitrophenyltetrazoles and 5-(2'-hydroxy-4'-nitrophenyl)tetrazole have been prepared.

4. Reduction of the 5-nitrophenyltetrazoles using two different methods has been shown to result in the formation of the corresponding 5-aminophenyltetrazoles which included the tetrazole analogues of para-aminobenzoic acid, meta-aminobenzoic acid and para-aminosalicylic acid. The acetyl derivatives of all the aminophenyltetrazoles have been prepared.

5. The three isomeric 5-(pyridyl)tetrazoles, including the tetrazole analogue of nicotinic acid, have been prepared from the corresponding cyanopyridines. The preparation of 2,6-di(5'-tetrazolyl)-pyridine has also been described.

6. The 5-(pyridyl)tetrazoles, on treatment with hydrogen and platinum oxide, have been reduced to the corresponding 5-(piperidyl) tetrazoles.

7. A group of six substituted phenoxyacetonitriles has been prepared from the corresponding phenols and chloroacetonitrile. The hydrolysis of three of these has been shown to lead to the formation of the corresponding phenoxyacetic acid.

8. A group of six substituted 5-phenoxymethyltetrazoles, including the tetrazole analogues of 2,4D and 2,4,5T, has been prepared from the corresponding nitrile.

9. A group of four 1-benzyl-5-phenoxymethyltetrazoles has been prepared. Debenzylation in two cases has been shown to lead to the formation of the similarly substituted 5-phenoxymethyltetrazole. In the other two cases debenzylation was accompanied by dehalogenation and possibly reduction.

10. The tetrazole analogue of 3-indoleacetic acid, 5-(3'-indolyl)-tetrazole, has been prepared.

11. The interaction of 3,4,5-trimethoxybenzonitrile with hydrazoic acid has led to the formation of 5-(3',4',5'-trimethoxyphenyl)-tetrazole. Alkylation of this compound with dialkylaminoalkyl halides has resulted in the formation of three 2-(dialkylaminoalkyl)-5-(3',4',5'-trimethoxyphenyl)tetrazoles which have been characterized as the hydrochlorides.

APPENDIX I

POTENTIOMETRIC TITRATION DATA

Compound	pH	Ml. of Aq. HCl 0.10175 N	Apparent pK_1
5-Aminomethyltetrazole	5.70	0.0	2.62
0.3184 g./125 ml.	3.65	4.0	
aqueous solution	3.55	5.0	
	3.47	6.0	
	3.39	7.0	
	3.35	8.0	
	3.27	9.0	
	3.21	10.1	
	3.15	11.0	
	3.11	12.0	
	3.05	13.0	
	3.00	14.0	
	2.95	15.0	
	2.92	16.0	
	2.88	17.0	
	2.83	18.0	
	2.80	19.0	
	2.76	20.0	
	2.71	21.0	
	2.63	23.0	
	2.60	24.0	
	2.56	25.0	
	2.52	26.1	
	2.49	27.0	
	2.41	29.0	
	2.38	30.0	
	2.34	31.0	
	2.30	32.0	
	2.25	33.0	
	2.20	35.0	
	2.15	36.0	
	2.12	38.0	
	2.05	40.0	
	2.00	42.0	
	1.98	44.0	

APPENDIX I (Continued)

Compound	pH	Ml. of Aq. HCl 0.10175 N	Apparent pK_1
5-(α-Aminoethyl)tetrazole	5.60	0.0	2.63
0.2950 g./125 ml.	3.92	2.0	
aqueous solution	3.60	4.0	
	3.40	6.0	
	3.10	10.0	
	3.05	11.0	
	3.00	11.5	
	2.95	12.5	
	2.90	13.5	
	2.85	14.5	
	2.80	16.0	
	2.75	17.0	
	2.70	18.0	
	2.65	19.0	
	2.62	20.0	
	2.59	21.0	
	2.55	22.0	
	2.50	23.0	
	2.45	24.0	
	2.43	25.0	
	2.40	26.0	
	2.35	27.0	
	2.33	28.0	
	2.30	29.0	
	2.27	30.0	
	2.23	32.0	
	2.13	36.0	
	2.02	40.0	
	1.95	45.0	
	1.88	50.0	

APPENDIX I (Continued)

Compound	pH	Ml. of Aq. HCl 0.10175 N	Apparent pK_1
5-(β-Aminoethyl)tetrazole 0.2906 g./100 ml. aqueous solution	6.52	0.0	3.99
	5.05	2.0	
	4.85	3.0	
	4.75	4.0	
	4.60	5.0	
	4.45	7.0	
	4.35	8.0	
	4.22	10.0	
	4.15	11.0	
	4.10	12.0	
	4.07	12.5	
	4.03	13.0	
	4.00	13.5	
	3.98	14.0	
	3.92	15.0	
	3.86	16.0	
	3.80	17.0	
	3.72	18.0	
	3.66	19.0	
	3.50	21.0	
	3.43	22.0	
	3.35	23.0	
	3.24	24.0	
	3.13	25.0	
	2.80	28.0	
	2.40	33.0	
	2.28	36.0	
	2.18	39.0	
	2.05	43.0	
	1.98	50.0	

APPENDIX I (Continued)

Compound	pH	Ml. of Aq. HCl 0.10175 N	Apparent pK_1
5-(α-Amino-β-phenyl-ethyl)tetrazole	5.20	0.0	1.93
0.1353 g./100 ml. aqueous solution	3.28	2.0	
	2.90	4.0	
	2.67	6.0	
	2.52	8.0	
	2.36	10.5	
	2.30	12.0	
	2.19	14.5	
	2.14	16.0	
	2.12	16.5	
	2.10	17.0	
	2.05	19.0	
	1.98	22.0	
	1.94	24.0	
	1.90	25.5	
	1.88	27.0	
	1.83	30.0	
	1.78	35.0	
	1.72	40.0	
	1.69	45.0	

APPENDIX I (Continued)

Compound	pH	Ml. of Aq. NaOH 0.09688 N	Apparent pK_2
5-Aminomethyltetrazole	5.75	0.0	8.54
0.3022 g./125 ml.	7.60	3.0	
aqueous solution	7.95	6.0	
	8.10	8.0	
	8.27	10.0	
	8.38	12.0	
	8.50	14.0	
	8.63	16.0	
	8.73	18.0	
	8.85	20.0	
	8.99	22.0	
	9.05	23.0	
	9.13	24.0	
	9.20	25.0	
	9.29	26.0	
	9.34	26.5	
	9.39	27.0	
	9.45	27.5	
	9.50	28.0	
	9.58	28.5	
	9.65	29.0	
	9.85	30.0	
	9.97	30.5	
	10.13	31.0	
	10.31	31.5	
	10.51	32.0	
	10.68	32.5	
	10.84	33.0	
	10.95	33.5	
	11.11	34.5	
	11.25	36.0	
	11.45	39.0	

APPENDIX I (Continued)

Compound	pH	Ml. of Aq. NaOH 0.09688 N	Apparent pK_2
5-(α-Aminoethyl)tetrazole	5.80	0.0	8.77
0.3183 g./125 ml.	7.60	2.0	
aqueous solution	7.95	4.0	
	8.15	6.0	
	8.30	8.0	
	8.45	10.0	
	8.55	12.0	
	8.70	14.0	
	8.82	16.0	
	8.95	18.0	
	9.08	20.0	
	9.22	22.0	
	9.40	24.0	
	9.45	24.5	
	9.50	25.0	
	9.59	25.5	
	9.65	26.0	
	9.80	27.0	
	10.09	28.0	
	10.45	29.0	
	10.84	30.0	
	11.09	31.0	
	11.30	33.0	
	11.50	35.0	
	11.65	38.0	
	11.76	42.0	
	11.86	46.0	

APPENDIX I (Continued)

Compound	pH	Ml. of Aq. NaOH 0.09688 N	Apparent pK_2
5-(β-Aminoethyl)tetrazole	6.65	0.0	9.58
0.3106 g./125 ml.	8.85	6.0	
aqueous solution	8.95	7.0	
	9.13	9.0	
	9.20	10.0	
	9.35	12.0	
	9.40	13.0	
	9.50	15.0	
	9.65	17.0	
	9.70	18.0	
	9.85	20.0	
	9.90	21.0	
	9.99	22.0	
	10.05	23.0	
	10.15	24.0	
	10.25	25.0	
	10.39	26.0	
	10.45	26.5	
	10.50	27.0	
	10.64	28.0	
	10.79	29.0	
	10.90	30.0	
	11.02	31.0	
	11.10	32.0	
	11.21	33.5	
	11.25	34.5	
	11.35	36.0	
	11.49	40.0	

APPENDIX I (Continued)

Compound	pH	Ml. of Aq. NaOH 0.09688 N	Apparent pK_2
5-(α-Amino-β-phenyl-ethyl-tetrazole ..... 0.1796 g./125 ml. aqueous solution	5.27	0.0	8.18
	7.15	1.0	
	7.50	2.0	
	7.75	3.0	
	7.95	4.0	
	8.15	5.0	
	8.28	6.0	
	8.40	6.5	
	8.45	6.7	
	8.50	7.0	
	8.60	7.5	
	8.75	8.0	
	8.85	8.3	
	8.93	8.4	
	8.97	8.5	
	9.10	8.8	
	9.30	9.0	
	9.35	9.1	
	9.55	9.3	
	9.70	9.4	
	10.00	9.6	
	10.25	9.8	
	10.40	10.0	
	10.65	10.5	
	10.85	11.0	
	11.05	12.0	

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