Anal. Calcd. for $C_{11}H_{15}N_5O_5$: C, 44.5; H, 5.08; N, 23.6. Found: C, 44.5; H, 5.17; N, 23.4.

9-β-D-Fructopyranosyladenine (III). A solution of 8.2 g. of 1,3,4,5-tetra-O-benzoyl-D-fructopyranosyl bromide (VII)[14] in dry xylene was treated with 8 g. of chloromercuri-6-benzamidopurine and the nucleoside was isolated through the picrate as described for 9-α-D-fructofuranosyladenine (II) to yield 1.7 g. (46%) of a pale yellow foam which showed one spot at R_{Ad} 0.20 in solvent A and R_{Ad} 1.63 in solvent B; $[\alpha]D$ $-75 \pm 3°$ (1% in methanol).

Anal. Calcd. for $C_{11}H_{15}N_5O_5$: C, 44.5; H, 5.08; N, 23.6. Found: C, 43.8; H, 5.63; N, 21.6.

Treatment of 1.4 g. of this material with 20 ml. of hot ethanol caused crystallization to take place. Recrystallization from absolute ethanol gave 0.6 g. (16%) of material, m.p. 227–228° (dec.); $[\alpha]D$ $-171 \pm 4°$ (1% in water).

Anal. Calcd. for $C_{11}H_{15}N_5O_5$: C, 44.5; H, 5.08; N, 23.6. Found: C, 44.6; H, 5.12; N, 23.7.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MICHIGAN STATE UNIVERSITY]

Tetrazole Analogs of Amino Acids[1]

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The synthesis of analogs of several amino acids in which the carboxyl group is replaced by the acidic 5-tetrazolyl group is described. Tetrazole analogs of glycine, D,L-alanine, β-alanine, D,L-phenylalanine and D,L-tryptophan have been prepared. With the exception of the tryptophan analog each was prepared by at least two independent methods. Apparent dissociation constants of the tetrazole analogs were determined and are comparable to those of the respective amino acids. The tetrazole analogs were further characterized as phenylureas and as acetyl and benzoyl derivatives.

Numerous examples of metabolite antagonism have been noted for compounds that bear various relationships to the naturally occurring α-amino acids. One of the most thoroughly investigated is phenylalanine. Various changes in its structure have transformed phenylalanine into an inhibitor of bacterial growth. Among the changes sufficient to interfere with the nutritional effect of this amino acid are introduction of an amino group[4] or a fluorine atom[5] in the *para* position of the benzene ring. Substitution of certain heterocyclic rings for the phenyl group, such as 2-pyridyl,[6] 2-thienyl,[7] 2-furyl,[8] and 2-pyrryl,[9] has also resulted in analogs which exhibit specific antagonism for phenylalanine. 5-Methyltryptophan[10] and β-3-indolylacrylic acid[11] act as antimetabolites for tryptophan. The changes necessary to develop antimetabolite activity are not restricted to any one portion of the amino acid structure. Analogs of glycine, alanine, valine, and leucine with the sulfonic acid residue replacing the carboxyl group have shown specific inhibition of the utilization of these amino acids as measured by interference with bacterial growth.[12]

In view of the acidic character of the 5-substituted tetrazoles it has been suggested that analogs of biologically active carboxylic acids in which the carboxyl group is replaced by a 5-tetrazolyl group might interfere with the normal utilization of the respective carboxylic acids.[13] Tetrazole analogs of 3-indolylacetic acid and 2,4-dichlorophenoxyacetic acid antagonize the plant growth regulatory effects of these compounds,[14,15] and there are indications that the tetrazole analog of nicotinic acid will prevent growth of certain bacteria.[16]

These observations have encouraged us to prepare analogs of several amino acids in which the 5-tetrazolyl group replaces the carboxyl group. Analogs of glycine, D,L-alanine, β-alanine, D,L-phenylalanine and D,L-tryptophan are described in the following. The synthesis of each 5-amino-

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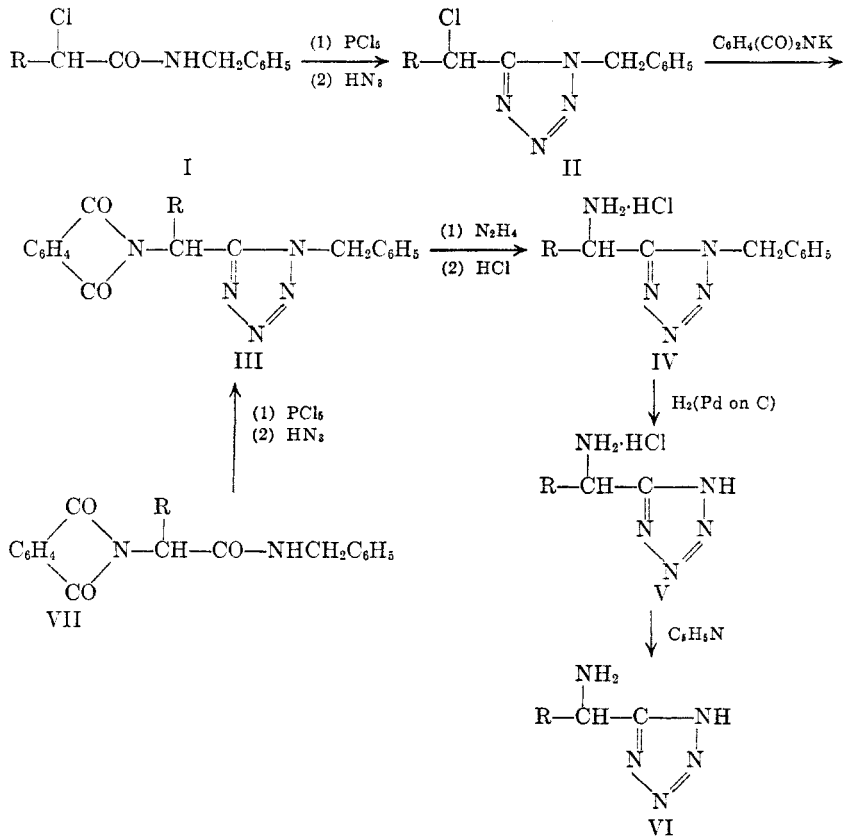
alkyltetrazole, with the exception of the tryptophan analog, was accomplished by two different methods so as to corroborate structures by independent syntheses. Three general synthetic approaches have been developed.

Scheme A employs the initial formation of a 1-benzyl-5-α-haloalkyltetrazole (II) from an *N*-benzyl-α-haloamide (I) using the von Braun procedure.[17] Interaction of II with potassium phthalimide gave a 1-benzyl-5-α-phthalimidoalkyltetrazole (III) which upon removal of the phthalyl group by treatment with hydrazine[18,19] gave the hydrochloride of a 1-benzyl-5-α-aminoalkyltetrazole (IV). Removal of the benzyl group by hydrogenolysis[20] gave the hydrochloride of a 5-α-aminoalkyltetrazole (V) from which the free amino acid analog (VI) was obtained by treatment with pyridine in absolute ethanol. In cases where the basicity of the amino group was too great to permit the latter type of exchange, silver oxide in aqueous suspension was used to liberate the free amino acid analog. Because of the severe irritation of mucous membranes caused by the α-haloalkyltetrazole (II), it was advantageous to use an alternate sequence of reactions; the *N*-benzyl-α-phthalimidoamide (VII) was formed first and converted into the tetrazole (III) by treatment successively with phosphorus pentachloride and hydrazoic acid.

Scheme A permits the formation of the tetrazole ring by an unequivocal procedure from an *N*-substituted amide and formation of the final product by a series of unambiguous reactions.

Scheme B provides a method for converting an amino acid into its tetrazole analog. The phthalyl derivative of the amino acid is converted successively into the acid chloride and amide. Dehydration of the latter gave the α-phthalimidonitrile (VIII). Using the general procedure of Behringer and Kohl[21] for the preparation of 5-substituted tetrazoles, VIII was converted into the 5-α-phthalimidoalkyltetrazole (IX) by treatment in refluxing tetrahydrofuran with aluminum azide formed *in situ* from aluminum chloride and sodium azide. The procedure of Behringer and Kohl for isolation of the tetrazoles was modified. Tetrahydrofuran was displaced from the reaction mixture by distillation while the volume was kept constant by addition of water. The aluminum salt of the tetrazole separated from the aqueous



medium and was decomposed, after resuspension in fresh water, by warming with dilute hydrochloric acid. Removal of the phthalyl moiety gave the α-aminoalkyltetrazole hydrochloride (V)

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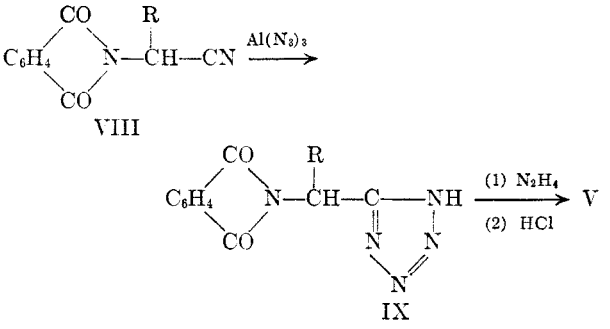
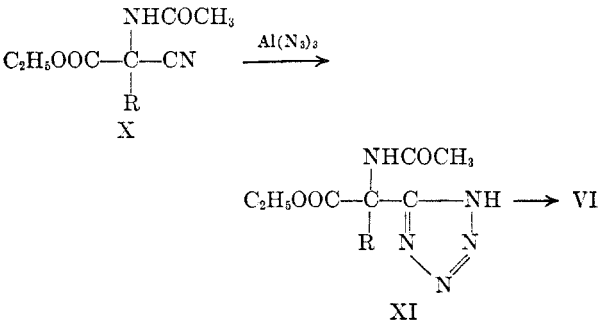
from which the free amino acid analog (VI) was liberated with pyridine or silver oxide. This route opens the possibility of starting with an optically active amino acid and preparing the optically active tetrazole analog of the same configuration.



After completion of this work a procedure for the conversion of nitriles into 5-substituted tetrazoles by interaction with lithium azide or ammonium azides in dimethylformamide was described.[22]

Scheme C is an adaptation of a procedure used for the preparation of a number of amino acids. Ethyl acetamidocyanoacetate was alkylated and the resulting nitrile (X) treated with aluminum azide in tetrahydrofuran to form the 5-substituted tetrazole (XI). The latter was converted into the amino acid analog (VI) either by stepwise or by a single step hydrolysis and decarboxylation. The former procedure was used to provide a logical sequence of intermediates after which the latter technique was employed for preparative purposes. This route offers a process closely analogous to that used for the synthesis of many amino acids differing only in the conversion of the cyano group to the 5-tetrazolyl rather than to the carboxyl group.



5-α-Aminomethyltetrazole (VI, R=H), the glycine analog, was prepared following both *Schemes A* and *B*. The sequence of reactions involved in *Scheme B* was initiated with phthalimidoacetonitrile (VIII, R=H) prepared from potassium phthalimide and chloroacetonitrile. As glycine is optically inactive, the pursuit of *Scheme B* from the amino acid offered no advantage. The hydrochloride (V, R=H) of this compound has been described by Behringer and Kohl[21] but its behavior as a glycine analog apparently was not recognized.

The D,L-alanine anlog, D,L-5-α-aminoethyltetrazole (VI, R=CH_3) was synthesized from α-bromopropionyl bromide using *Scheme A* and from D,L-alanine using *Scheme B*. So as to avoid handling the α-halotetrazole, the modified sequence involving *N*-benzyl-α-phthalimidopropionamide (VII, R=CH_3) was followed.

5-β-Aminoethyltetrazole, the β-alanine analog, was prepared according to *Schemes A* and *B*. As potassium phthalimide caused dehydrohalogenation of *N*-benzyl-β-bromopropionamide, the sequence of steps in *Scheme A* was modified to the extent of preparing *N*-benzyl-β-phthalimidopropionamide by interaction of β-phthalimidopropionyl chloride and benzylamine Subsequent steps of *Scheme A* were followed without change. *Scheme B* was shortened by preparation of β-phthalimidopropionitrile from phthalimide and acrylonitrile[23] rather than from the amino acid. The hydrochloride of the β-alanine analog has been described as a potential histamine antagonist[21,24]; however, its amphoteric character was not noted.

The D,L,-phenylalanine analog, 5-α-amino-β-phenylethyltetrazole (VI, R=$C_6H_5CH_2$), was prepared following *Schemes B* and *C*. The intermediate, ethyl α-acetamido-α-5-tetrazolyl-β-phenylpropionate (XI, R=$C_6H_5CH_2$) obtained in *Scheme C* was converted into the amino acid analog both by stepwise degradation and by a single step hydrolysis and decarboxylation.

The analog of D,L-tryptophan, 5-α-amino-β-3-indolylethyltetrazole (VI, R=3-indolylmethyl), was prepared only by *Scheme C*. The intermediate ethyl α-acetamido-α-5-tetrazolyl-β-3-indolyl propionate (XI, R=3-indolylmethyl) was converted into the amino acid analog by stepwise hydrolysis and decarboxylation.

The tetrazole analogs are very similar to the corresponding amino acids in both physical and chemical properties. The glycine, D,L-alanine, and β-alanine analogs are soluble in water, aqueous acids and alkalies. The phenylalanine and tryptophan analogs are only slightly soluble in water but readily soluble in dilute, aqueous acids and alkalies. All of the analogs are insoluble in acetone, ethanol and non-polar solvents. All have high melting points and all decompose at the melting point which may vary with the rate of heating. Using methods applicable to the characterization of amino acids permitted the preparation of phenylureas and of acetyl and benzoyl derivatives. The benzoyl derivatives and phenylureas melted with gas evolution. The benzoyl derivative of D,L-alanine exhibited a double melting point; after

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(23) A. Galat, *J. Am. Chem. Soc.*, **67**, 1414 (1945).

(24) C. Ainsworth, *J. Am. Chem. Soc.*, **75**, 5728 (1953).

melting at 176–177° it solidified and on continued heating remelted at 199–200°. Although this behavior has not been investigated, the possibility that bicyclic compounds such as tetrazoloimidazoles are formed on melting, analogous to the formation of azlactones and hydantoins from acylamino- and phenylureido acids, respectively, is not without merit.

Potentiometric determination of the dissociation constants of the 5-aminoalkyltetrazoles served to emphasize the analogy with amino acids. Apparent pK_1 values were obtained by titrating the aminoalkyltetrazoles with standard hydrochloric acid; pK_2 values were taken from titration curves with standard alkali. Inspection of the pK_1 values given in Table I shows that the tetrazolyl group is slightly weaker as an acid than the carboxyl group of the corresponding amino acid. This result could be anticipated from comparison of the apparent acidic dissociation constants of 5-alkyltetrazoles and the corresponding carboxylic acids.[25] Examination of the pK_2 values of the alkylaminotetrazoles indicates that the basicity of the amino group is lower than that of the amino group in the corresponding amino acids. Both pK_1 and pK_2 values decrease in the same order observed for the corresponding amino acids.[26]

TABLE I

APPARENT DISSOCIATION CONSTANTS OF SOME 5-AMINOALKYLTETRAZOLES AND CORRESPONDING AMINO ACIDS IN AQUEOUS SOLUTION AT 25°

Tetrazole	Apparent pK_1	Apparent pK_2	Amino Acid[a]	Apparent pK_1	Apparent 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-Phenylalanine	1.83	9.13

[a] The pK values for the amino acids were taken from reference 26.

EXPERIMENTAL[27]

Preparation of the Glycine Analog. 1-Benzyl-5-chloromethyltetrazole (II, R = H) was prepared from *N*-benzylchloroacetamide[28] in benzene solution by interaction successively with phosphorus pentachloride and hydrazoic acid.[17] The compound is a rather severe irritant of mucous membranes and must be handled with considerable care.

1-Benzyl-5-phthalimidomethyltetrazole (III, R = H). A mixture of 21 g. (0.1 mole) of 1-benzyl-5-chloromethyltetrazole and 21 g. (0.114 mole) of potassium phthalimide was refluxed in 250 ml. of dry xylene for 5 hr. The hot suspension was filtered; the product crystallized from the filtrate on cooling, yield 26.4 g. (83%), m.p. 132–133° after recrystallization from toluene.

Anal. Calcd. for $C_{17}H_{13}N_5O$: C, 63.9; H, 4.1; N, 21.9. Found: C, 64.1; H, 4.2; N, 22.2.

1-Benzyl-5-aminomethyltetrazole hydrochloride (IV, R = H). The technique of Ing and Manske[18] as modified by Sheehan and Frank[19] was adapted to this case. A suspension of 10.6 g. of 1-benzyl-5-phthalimidomethyltetrazole in 120 ml. of absolute ethanol was treated with 33 ml. of 1*M* hydrazine hydrate in ethanol and the mixture stirred at reflux temperature for 3 hr. After evaporation of the solvent the residue was heated with 75 ml. of 2*N* hydrochloric acid for 10 minutes at 50°. The suspension was filtered and the filtrate evaporated to dryness. The residue was recrystallized from aqueous isopropyl alcohol, yield 4 g. (53%), m.p. 228–229°.

Anal. Calcd. for $C_9H_{12}ClN_5$: C, 47.9; H, 5.4; Cl, 15.7; N, 31.0. Found: C, 48.1; H, 5.3; Cl, 15.7; N, 31.2.

5-Aminomethyltetrazole (VI, R = H). A solution of 10.5 g. of 1-benzyl-5-aminomethyltetrazole hydrochloride in a mixture of 200 ml. of absolute ethanol and 30 ml. of water was shaken with 2.5 g. of 5% palladium on charcoal at an initial pressure of 47 p.s.i. The temperature of the reaction mixture was kept at 60° during the hydrogenolysis. After separation of the catalyst, evaporation of the solvent left 6.2 g. (97%) of crude hydrochloride which was dissolved in 100 ml. of absolute ethanol and treated with 3.6 g. of pyridine. On chilling the glycine analog separated slowly from the solution, yield 3.8 g. (85%). The product was recrystallized by dissolution in a small amount of water and addition of sufficient absolute ethanol to give a 95% ethanol solution; the glycine analog crystallized slowly on standing overnight in a refrigerator, m.p. 267° with decomposition.

Anal. Calcd. for $C_2H_5N_5$: C, 24.2; H, 5.1; N, 70.7. Found: C, 24.5; H, 5.2; N, 70.6.

The hydrochloride of this product has been described by Behringer and Kohl.[21]

Phthalimidoacetonitrile (VIII, R = H) was prepared from potassium phthalimide and chloroacetonitrile in dimethylformamide, yield 64%, m.p. 127.5–128.5°. Sonn and Falkenheim[29] report m.p. 124–125° for this compound prepared from the same reagents in xylene.

Anal. Calcd. for $C_{10}H_6N_2O_2$: N, 15.1. Found: N, 15.3.

5-Phthalimidomethyltetrazole (IX, R = H). A suspension of 46.5 g. of phthalimidoacetonitrile and 50 g. of sodium azide in 100 ml. of dry tetrahydrofuran was treated at room temperature with 35 g. of anhydrous aluminum chloride dissolved in 300 ml. of the same solvent. After the mixture was refluxed for 24 hr. with continuous stirring, tetrahydrofuran was removed by distillation while the volume was kept constant by the gradual addition of water. After the aqueous suspension cooled, the aluminum salt was filtered off, resuspended in 450 ml. of water and 50 ml. of concentrated hydrochloric acid, and stirred at room temperature for 1 hr. The crude product was filtered from the chilled suspension, yield 50.6 g. (89%), m.p. 233.5–235° with decomposition. The pure product was obtained by recrystallization from ethanol–ethyl acetate mixture, m.p. 234–235° with decomposition.

Anal. Calcd. for $C_{11}H_9N_5O_2$: C, 52.4; H, 3.1; N, 30.6. Found: C, 52.4; H, 3.0; N, 30.8.

5-Aminomethyltetrazole (VI, R = H). A suspension of 45.8 g. of 5-phthalimidomethyltetrazole in 300 ml. of absolute ethanol was treated with 200 ml. of 1*M* hydrazine hydrate in ethanol. The mixture was stirred at reflux temperature for 3 hr., chilled overnight, and filtered. The solid was suspended in 450 ml. of 2*N* hydrochloric acid and warmed at 50–55° for 15 minutes. The precipitate was filtered from the cooled solution and the filtrate evaporated to dryness under reduced pressure. The residue of 5-aminomethyltetrazole

(25) J. S. Mihina and R. M. Herbst, *J. Org. Chem.*, **15**, 1082 (1950).

(26) E. J. Cohn and J. T. Edsall, Proteins, Amino Acids and Peptides, Reinhold Publishing Corp., New York, 1943, p. 84.

(27) Microanalyses were done on all compounds by Micro-Tech Laboratories, Skokie, Illinois. Melting points were done in open capillaries and are not corrected.

(28) W. A. Jacobs and M. Heidelberger, *J. Biol. Chem.*, **20**, 685 (1915).

(29) A. Sonn and S. Falkenheim, *Ber.*, **55**, 2975 (1922).

hydrochloride (23.6 g.) was treated with pyridine in absolute ethanol and the product recrystallized as in the previous example to give 14.6 g. (74%) of the free glycine analog, m.p. 268.5° with decomposition. The product was identical with the material prepared according to *Scheme A*.

5-Acetamidomethyltetrazole, aceturic acid analog, was prepared from VI (R = H) by heating in glacial acetic acid with acetic anhydride. The residue left upon evaporation of the solvent under reduced pressure was recrystallized from amyl acetate, m.p. 159.5–161°.

Anal. Calcd. for $C_4H_7N_5O$: C, 34.0; H, 5.0; N, 49.6. Found: C, 34.2; H, 4.9; N, 49.6.

5-Benzamidomethyltetrazole, hippuric acid analog, was prepared from VI (R = H) in aqueous alkaline solution by treatment with benzoyl chloride. The product was recrystallized from water, m.p. 229.5–230° with decomposition.

Anal. Calcd. for $C_9H_9N_5O$: C, 53.2; H, 4.5; N, 34.5. Found: C, 53.4; H, 4.6; N, 34.3.

N-Phenyl-N'-(5-tetrazolylmethyl)urea was prepared by shaking an aqueous, alkaline solution of VI (R = H) with phenyl isocyanate and isolating the product in the manner usual for phenylureido acids. Recrystallization from water gave the pure product, m.p. 194.5–195° with decomposition.

Anal. Calcd. for $C_9H_{11}N_6O$: C, 49.5; H, 4.6; N, 38.5. Found: C, 49.6; H, 4.8; N, 38.5.

Preparation of the D,L-*Alanine Analog. N-Benzyl-α-bromopropionamide* was prepared from α-bromopropionyl bromide and benzylamine in benzene solution in 52% yield, m.p. 91.5–92.5°, previously reported m.p. 93.5–94.5°.[30]

Anal. Calcd. for $C_{10}H_{12}BrNO$: Br, 33.0; N, 5.8. Found: Br, 33.1; N, 6.0.

N-Benzyl-α-phthalimidopropionamide (VII, R = CH_3). A mixture of 22.2 g. of potassium phthalimide and 24.2 g. of *N*-benzyl-α-bromopropionamide in 75 ml. of dimethylformamide was heated on a steam bath for 1 hr. with continuous stirring. The mixture was diluted with 100 ml. of chloroform and 250 ml. of water. The organic layer was separated and the aqueous layer washed with 50 ml. of chloroform. The combined chloroform solutions were washed with 0.2*N* sodium hydroxide and water, the solvent removed by evaporation and the residue recrystallized from toluene, yield 18 g. (59%), m.p. 141–142°.

Anal. Calcd. for $C_{18}H_{16}N_2O_3$: C, 70.1; H, 5.2; N, 9.1. Found: C, 70.1; H, 5.3; N, 9.2.

1-Benzyl-5-α-phthalimidoethyltetrazole (III, R = CH_3). A suspension of 6.2 g. of *N*-benzyl-β-phthalimidopropionamide in 100 ml. of dry benzene was treated at room temperature with 4.2 g. of powdered phosphorus pentachloride. A clear solution formed after stirring the mixture for several minutes. Stirring was continued for 2 hr. when 20 ml. of a benzene solution containing 2.78 g. of hydrazoic acid was added. A colorless precipitate started to separate quickly, but stirring was continued for 2 hr. at room temperature and then for 2 hr. at reflux temperature. The precipitate that separated on chilling the solution was filtered off and washed with water, yield 5.9 g. (88%), m.p. 147°. The product was recrystallized from toluene, m.p. 146–147°.

Anal. Calcd. for $C_{18}H_{15}N_5O_2$: C, 64.9; H, 4.5; N, 21.0. Found: C, 65.0; H, 4.5; N, 20.9.

1-Benzyl-5-α-aminoethyltetrazole hydrochloride (IV, R = CH_3) was prepared from 1-benzyl-5-α-phthalimidoethyltetrazole by treatment with hydrazine in ethanol as described for the lower homolog (IV, R = H). The crude hydrochloride was recrystallized several times from isopropyl alcohol, yield 60%, m.p. 184–184.5°.

Anal. Calcd. for $C_{10}H_{14}ClN_5$: C, 50.1; H, 5.9; Cl, 14.8; N, 29.2. Found: C, 50.0; H, 5.9; Cl, 14.9; N, 29.2.

5-α-Aminoethyltetrazole (VI, R = CH_3). A solution of 0.5 g. of 1-benzyl-5-α-aminoethyltetrazole hydrochloride in a mixture of 65 ml. of absolute ethanol and 10 ml. of water was shaken with 0.5 g. of 5% palladium on charcoal at 50–55° and an initial hydrogen pressure of 40 p.s.i. Isolation of the tetrazole followed the procedure described for the glycine analog, yield 110 mg., m.p. 267–268° with decomposition. The product is identical with the material obtained according to *Scheme B* as described in the succeeding paragraphs.

α-Phthalimidopropionamide. A stirred suspension of 68.5 g. of phthalyl-D,L-alanine[31] in 500 ml. of benzene was treated with 61.3 g. of thionyl chloride. The mixture was heated with stirring on a steam bath until a clear solution formed. After cooling to 15° ammonia gas was bubbled into the solution until precipitation was complete. The solid was filtered off, dried, suspended in 1 l. of water, filtered and dried again, yield 46.5 g. (64%) of crude product, m.p. 209–210°. Radde[32] reported m.p. 211–212° for this compound. The crude material was used in the next step.

α-Phthalimidopropionitrile (VIII, R = CH_3) was prepared by dehydrating 44.5 g. of the crude amide by heating for 10 min. in 200 ml. of pyridine with 120 ml. of benzenesulfonyl chloride. The reaction mixture was cooled, poured into water and the solid that separated was recrystallized from methanol, m.p. 136–138°, yield almost quantitative. Radde[32] reported m.p. 139–140° for this product.

5-α-Phthalimidoethyltetrazole (IX, R = CH_3). From 49.3 g. of α-phthalimidopropionitrile by interaction with aluminum azide in tetrahydrofuran as described for the glycine analog (IX, R = H), 54.3 g. (91%) of crude product, m.p. 229–231° with decomposition, was obtained. The analytical sample was recrystallized from aqueous ethanol, m.p. 230–231° with decomposition.

Anal. Calcd. for $C_{11}H_9N_5O_2$: C, 54.3; H, 3.7; N, 28.8. Found: C, 54.3; H, 3.8; N, 28.8.

α-Aminoethyltetrazole (VI, R = CH_3). From 37.1 g. of 5-α-phthalimidoethyltetrazole by treatment with hydrazine in ethanol, 20 g. of crude hydrochloride was obtained in a manner analogous to that described for the glycine analog. Treatment of the crude hydrochloride with pyridine in ethanol, followed by recrystallization from water by addition of ethanol, gave the D,L-alanine analog in 40% over-all yield, m.p. 272–273° with decomposition. There was no depression of the mixture melting point with the material prepared following *Scheme A*.

Anal. Calcd. for $C_3H_7N_5$: C, 31.9; H, 6.2; N, 61.9. Found: C, 31.8; H, 6.1; N, 62.1.

5-α-Acetamidoethyltetrazole was prepared from 5-α-aminoethyltetrazole with acetic anhydride in glacial acetic acid. It was recrystallized from amyl acetate, m.p. 145–145.5°.

Anal. Calcd. for $C_5H_9N_5O$: C, 38.7; H, 5.9; N, 45.1. Found: C, 38.7; H, 5.7; N, 45.1.

5-α-Benzamidoethyltetrazole was obtained from the free alanine analog in aqueous, alkaline solution on treatment with benzoyl chloride. The product was recrystallized from water, m.p. 176–177° followed by solidification and remelting at 199–200° on continued heating.

Anal. Calcd. for $C_{10}H_{11}N_5O$: C, 55.3; H, 5.1; N, 32.2. Found: C, 55.5; H, 5.1; N, 32.4.

N-Phenyl-N'-(α-5-tetrazolylethyl)urea formed from the alanine analog in aqueous, alkaline solution on shaking with phenyl isocyanate. The derivative was recrystallized from water, m.p. 184–185° with decomposition.

Anal. Calcd. for $C_{10}H_{12}N_6O$: C, 51.7; H, 5.2; N, 36.2. Found: C, 51.9; H, 5.4; N, 36.2.

Preparation of the β-Alanine Analog. N-Benzyl-β-phthalimidopropionamide. Phthalyl-β-alanine was prepared in 91% yield from β-alanine and phthalic anhydride as described by Gabriel.[31] The acid chloride, m.p. 105–106.5°, was prepared in 91% yield from the acid on treatment with thionyl chloride in benzene solution. Gabriel[33] reported m.p. 107–108° for the acid chloride. β-Phthalimidopropionly chloride (38.6 g.) was added in small portions during 20

(30) S. Kushner, R. I. Cassell, J. Morton, and J. H. Williams, *J. Org. Chem.*, 16, 1283 (1951).

(31) S. Gabriel, *Ber.*, **38**, 630 (1905).

(32) E. Radde, *Ber.*, **55**, 3174 (1922).

(33) S. Gabriel, *Ber.*, **41**, 242 (1908).

minutes to a stirred and cooled solution of 34.9 g. of benzylamine in 500 ml. of dry benzene. When interaction was complete the solid was filtered off, dried and digested with 750 ml. of water for 2 hr. The insoluble amide was filtered off and dried, yield 50.9 g., m.p. 194–197°. Recrystallization from absolute ethanol gave 41 g. (82%) of pure product, m.p. 198–199.5°; the analytical sample was crystallized again from absolute ethanol, m.p. 198–198.5°.

Anal. Calcd. for $C_{18}H_{16}N_2O$: C, 70.1; H, 5.2; N, 9.1. Found: C, 70.0; H, 5.0; N, 9.2.

1-Benzyl-5-β-phthalimidoethyltetrazole. *N*-Benzyl-β-phthalimidopropionamide (35.1 g.) was treated with phosphorus pentachloride in dry benzene. It was necessary to warm and stir the mixture at 60° for 2 hr. to bring about complete interaction as evidenced by the formation of a homogeneous solution. The imidyl chloride solution was treated with hydrazoic acid as in previous examples. The tetrazole had an appreciable solubility in benzene; concentration of the benzene mother liquors was essential to insure maximum recovery. The product was recrystallized from toluene, yield 25.5 g. (67%), m.p. 156.5–158°. The analytical sample was recrystallized again from toluene, m.p. 159–159.5°.

Anal. Calcd. for $C_{18}H_{15}N_5O_2$: C, 64.9; H, 4.5; N, 21.0. Found: C, 65.0; H, 4.3; N, 21.2.

1-Benzyl-5-β-aminoethyltetrazole hydrochloride was prepared from 1-benzyl-5-β-phthalimidoethyltetrazole by treatment with hydrazine in ethanol solution by the same techniques employed for the other analogs. The yield of crude hydrochloride was 75%. Recrystallization from aqueous isopropyl alcohol gave the pure hydrochloride, m.p. 138.5–139.5°.

Anal. Calcd. for $C_{10}H_{14}ClN_5$: C, 50.1; H, 5.9; Cl, 14.8; N, 29.2. Found: C, 49.9; H, 6.0; Cl, 15.0; N, 29.0.

5-β-Aminoethyltetrazole hydrochloride was prepared by hydrogenolysis of 1-benzyl-5-β-aminoethyltetrazole hydrochloride as described for the other analogs. After crystallization from ethanol-ether the yield of hydrochloride was 37%, m.p. 127.5–129°.

Anal. Calcd. for $C_3H_8ClN_5$: C, 24.1; H, 5.4; Cl, 23.7; N, 46.8. Found: C, 24.1; H, 5.5; Cl, 23.5; N, 46.7.

This compound was prepared by Behringer and Kohl,[21] m.p. 132°, and by Ainsworth,[24] m.p. 128–129°. The same product was obtained following the sequence of *Scheme B* as described in the succeeding paragraphs.

5-β-Phthalimidoethyltetrazole. β-Phthalimidopropionitrile, prepared from acrylonitrile and phthalimide,[23] was treated with aluminum azide in tetrahydrofuran. The product was isolated from the reaction mixture by the modified technique described for the comparable derivative of the glycine analog (IX, R = H), yield 94%, m.p. 249.5–250.5° with decomposition after recrystallization from ethanol–ethyl acetate mixture.

Anal. Calcd. for $C_{11}H_9N_5O_2$: C, 54.3; H, 3.7; N, 28.8. Found: C, 54.3; H, 3.9; N, 29.0.

Behringer and Kohl[21] report m.p. 241° for this intermediate.

5-β-Aminoethyltetrazole hydrochloride was obtained from the phthalyl derivative by treatment with hydrazine in ethanol solution, yield 84%, m.p. 130–132°. The product is identical with the material obtained from 1-benzyl-5-β-aminoethyltetrazole hydrochloride.

5-β-Aminoethyltetrazole. A solution of 7.45 g. of the hydrochloride in 65 ml. of water was stirred in a dark place for 24 hr. with 6.1 g. of powdered silver oxide. The resulting suspension was filtered, the filtrate saturated with hydrogen sulfide and the silver sulfide removed by filtration. After treatment with Norite the clear filtrate was evaporated to a small volume. Dilution with acetone precipitated the amino acid analog which was further purified by dissolving in a small volume of water and again precipitating with acetone, yield 3.0 g. (58%), m.p. 223–224° with decomposition.

Anal. Calcd. for $C_3H_7N_5$: C, 31.9; H, 6.2; N, 61.9. Found: C, 32.1; H, 6.3; N, 62.0.

5-β-Acetamidoethyltetrazole was prepared from the β-alanine analog by treatment with acetic anhydride in glacial acetic acid. The product was crystallized from amyl acetate, m.p. 202–203°.

Anal. Calcd. for $C_5H_9N_5O$: C, 38.7; H, 5.9; N, 45.1. Found: C, 38.8; H, 6.1; N, 45.3.

5-β-Benzamidoethyltetrazole was obtained by shaking an aqueous, alkaline solution of the β-aminoethyltetrazole with benzoyl chloride. The product was recrystallized from water, m.p. 200.5–201° with decomposition.

Anal. Calcd. for $C_{10}H_{11}N_5O$: C, 55.3; H, 5.1; N, 32.2. Found: C, 55.1; H, 5.2; N, 32.3.

Ainsworth[24] reports m.p. 206° for this derivative.

N-Phenyl-N'-(β-5-tetrazolylethyl)urea was prepared by shaking an aqueous, alkaline solution of the β-aminoethyltetrazole with phenyl isocyanate. The product separated on acidification of the clear, alkaline solution and was recrystallized from aqueous ethanol, m.p. 199–199.5° with decomposition.

Anal. Calcd. for $C_{10}H_{12}N_6O$: C, 51.7; H, 5.2; N, 36.2. Found: C, 51.9; H, 5.4; N, 36.3.

Preparation of the D,L-*Phenylalanine Analog. α-Phthalimido-β-phenylpropionyl chloride* was prepared from phthalyl-D,L-phenylalanine[19] by treatment with phosphorus pentachloride in benzene suspension, yield 97%, m.p. 134–136°. Sheehan and Frank[19] report m.p. 124–126° for the acid chloride.

α-Phthalimido-β-phenylpropionitrile (VIII, R = benzyl) was prepared by converting the acid chloride into amide with aqueous ammonia and treating the amide with benzenesulfonyl chloride in pyridine as recommended by Peterson and Niemann.[34]

5-(α-Phthalimido-β-phenylethyl)tetrazole (IX, R = benzyl) was prepared from 8.1 g. of α-phthalimido-β-phenylpropionitrile, 6.5 g. of sodium azide and 4.4 g. of anhydrous aluminum chloride in 75 ml. of dry tetrahydrofuran. The reaction and isolation followed the technique described for other examples. The yield of tetrazole was 9.2 g. (95%), m.p. 212.5–213° with decomposition after crystallization from ethyl acetate.

Anal. Calcd. for $C_{17}H_{13}N_5O_2$: C, 63.9; H, 4.1; N, 21.9. Found: C, 64.0; H, 4.4; N, 22.2.

5-(α-Amino-β-phenylethyl)tetrazole (VI, R = benzyl) was prepared from the phthalyl derivative (IX, R = benzyl) by treatment with hydrazine in ethanol solution. The free amino acid analog was obtained by adjusting its solution in dilute hydrochloric acid to *p*H 5, yield 82%, m.p. 271–272° with decomposition. Mixture melting point with the analytical sample prepared according to *Scheme C* as described in the following paragraphs was not depressed.

Ethyl α-acetamido-α-cyano-β-phenylpropionate (X, R = benzyl) was prepared from ethyl acetamidocyanoacetate and benzyl chloride as described by Albertson and Tullar.[35]

Ethyl α-acetamido-α-5-tetrazolyl-β-phenylpropionate (XI, R = benzyl) *and 5-(α-amino-β-phenylethyl)tetrazole* (VI, R = benzyl) were both prepared from 67.8 g. of the cyanopropionate (X, R = benzyl), 50 g. of sodium azide and 34.1 g. of anhydrous aluminum chloride in 550 ml. of tetrahydrofuran. After the stirred reaction mixture was refluxed for 24 hr., the tetrahydrofuran was displaced with water as previously described. The suspended aluminum salt was filtered off and dried. The filtrate was acidified to Congo red and cooled overnight (Caution: hydrazoic acid liberated) and the crystallizate separated by filtration, yield 8 g. of crude ester (XI, R = benzyl). Recrystallization from ethanol gave the pure ester, m.p. 147.5–148.5°.

Anal. Calcd. for $C_{14}H_{17}N_5O_3$: C, 55.4; H, 5.7; N, 23.1. Found: C, 55.4; H, 5.4; N, 23.1.

The aluminum salt was refluxed with 450 ml. of concentrated hydrochloric acid for 3 hr. and the mixture evaporated

(34) P. E. Peterson and C. Niemann, *J. Am. Chem. Soc.*, **79**, 1389 (1957).

(35) N. F. Albertson and B. F. Tullar, *J. Am. Chem. Soc.*, **67**, 502 (1945).

almost to dryness under reduced pressure. The residue was taken up in 250 ml. of 95% ethanol and treated with 30 g. of pyridine. The amino acid analog crystallized from the solution on chilling overnight, yield 26 g. Recrystallization from water gave pure 5-(α-amino-β-phenylethyl)tetrazole, m.p. 270.5–271.5° with decomposition.

Anal. Calcd. for $C_9H_{11}N_5$: C, 57.1; H, 5.9; N, 37.0. Found: C, 56.9; H, 6.0; N, 37.2.

The amino acid analog was also prepared by refluxing 2 g. of the tetrazolylpropionate (XI, R = benzyl) with 25 ml. of concentrated hydrochloric acid. The hydrolyzate was brought to *p*H 6 by addition of aqueous ammonia to precipitate the aminoalkyltetrazole. After recrystallization from water the product was identical with the material described in the preceding paragraph, yield 0.9 g. (72%), m.p. 276–277° with decomposition on rapid heating.

α-Acetamido-α-5-tetrazolyl-β-phenylpropionic acid. A suspension of 1.5 g. of the ester (XI, R = benzyl) in a solution of 0.8 g. of sodium hydroxide in 16 ml. of water was boiled for 1 hr. The solution was filtered and acidified (*p*H 2–3) with dilute hydrochloric acid. The product separated slowly on chilling after crystallization was initiated. It was recrystallized from tetrahydrofuran by addition of petroleum ether, m.p. 110° with gas evolution followed by solidification and remelting at 224–225° on continued heating.

Anal. Calcd. for $C_{12}H_{13}N_5O_3$: C, 52.4; H, 4.8; N, 25.5. Found: C, 52.7; H, 5.0; N, 25.6.

5-(α-Acetamido-β-phenylethyl)tetrazole. The tetrazolylpropionic acid derivative (20 mg.) described in the preceding paragraph was heated at 170° for a few minutes then recrystallized from water, m.p. 226°.

The same compound was prepared by acetylation of the amino acid analog (VI, R = benzyl) with acetic anhydride in glacial acetic acid solution. After recrystallization from aqueous ethanol the acetyl derivative melted at 224.5–225.5°.

Anal. Calcd. for $C_{11}H_{13}N_5O$: C, 57.1; H, 5.7; N, 30.3. Found: C, 57.2; H, 5.8; N, 30.2.

5-(α-Benzamido-β-phenylethyl)tetrazole was prepared by treating an aqueous, alkaline solution of the amino acid analog (VI, R = benzyl) with benzoyl chloride and recrystallizing the product from aqueous ethanol, m.p. 234–235° with decomposition.

Anal. Calcd. for $C_{16}H_{15}N_5O$: C, 65.5; H, 5.2; N, 23.9. Found: C, 65.5; H, 5.0; N, 24.0.

N-Phenyl-N'-(α-5-tetrazolyl-β-phenylethyl)urea formed when an aqueous, alkaline solution of the amino acid analog (VI, R = benzyl) was shaken with phenyl isocyanate. The product was recrystallized from aqueous ethanol, m.p. 188.5–189.5° with decomposition.

Anal. Calcd. for $C_{16}H_{16}N_6O$: C, 62.3; H, 5.2; N, 27.3. Found: C, 62.4; H, 5.2; N, 27.4.

Preparation of the D,L-*Tryptophan Analog. Ethyl α-acetamido-α-cyano-β-3-indolylpropionate* was prepared by alkylation of ethyl acetamidocyanoacetate with gramine according to Albertson and Tullar.[35]

Ethyl α-acetamido-α-5-tetrazolyl-β-3-indolylpropionate (XI, R = 3-indolylmethyl). A solution of 13.3 g. of anhydrous aluminum chloride in 200 ml. of dry tetrahydrofuran was added to a stirred suspension of 19.5 g. of sodium azide in 50 ml. of the same solvent. After heating the stirred mixture under reflux for 1 hr. and then cooling to room temperature, 29.9 g. of ethyl α-acetamido-α-cyano-β-3-indolylpropionate was added. The temperature was slowly raised to the boiling point and maintained there for 24 hr. with stirring. The mixture was then diluted with 150 ml. of water, cooled to 5° and acidified by slow addition during 20 min. with cooling of 50 ml. of concentrated hydrochloric acid. The entire mixture was poured into 300 ml. of ether, the organic layer separated, and the aqueous portion extracted with 100 ml. of 1:1 ether–tetrahydrofuran. The combined organic layers were washed with 100 ml. of water and dried over sodium sulfate. Removal of the solvent under reduced pressure left a residue that was recrystallized from chloroform to give the product, yield 11 g. (32%). A second crystallization from chloroform gave the analytical sample, m.p. 183.5–185° with decomposition.

Anal. Calcd. for $C_{16}H_{18}N_6O_3$: C, 56.1; H, 5.3; N, 24.6. Found: C, 56.0; H, 5.3; N, 24.5.

α-Acetamido-α-5-tetrazolyl-β-3-indolylpropionic acid was obtained by hydrolysis of 6.8 g. of the ester (XI, R = 3-indolylmethyl) with a solution of 3.2 g. of sodium hydroxide in 32 ml. of water at reflux temperature for 3 hr. The hydrolyzate was treated with Norit and the clear solution was acidified with 8.4 ml. of concentrated hydrochloric acid. Crystallization of the product was initiated while cooling the solution. On recrystallization from water the product separated as a dihydrate, m.p. 153–155° with decomposition.

Anal. (Air dried) Calcd. for $C_{14}H_{14}N_6O_3 \cdot 2H_2O$: N, 24.0. Found: N, 24.1, 23.9. (Dried at 100° *in vacuo*) Calcd. for $C_{14}H_{14}N_6O_3$: N, 26.7. Found: N, 26.7.

5-(α-Acetamido-β-3-indolylethyl)tetrazole was formed when 7.4 g. of the propionic acid derivative just described was heated in 200 ml. of boiling water for 2.5 hr. The hot solution was treated with Norite. The product crystallized on cooling the filtrate, yield 5.8 g. (91%). Recrystallization from water gave the analytical sample, m.p. 223–223.5° with decomposition.

Anal. Calcd. for $C_{13}H_{14}N_6O$: C, 57.8; H, 5.2; N, 31.1. Found: C, 57.9; H, 5.3; N, 31.2.

5-(α-Amino-β-3-indolylethyl)tetrazole (VI, R = 3-indolylmethyl). A solution of 1.3 g. of the acetyl derivative in 16 ml. of water containing 1.3 g. of sodium hydroxide was boiled under reflux for 12 hr. Concentrated hydrochloric acid (2.7 ml.) was added to the hot solution followed immediately by aqueous ammonia sufficient to adjust the acidity to *p*H 5 rapidly. Crystallization was initiated as the solution cooled, yield 0.8 g. (70%). A single crystallization from water gave the analytical sample, m.p. 268.5–269° with decomposition.

Anal. Calcd. for $C_{11}H_{12}N_6$: C, 57.9; H, 5.3; N, 36.8. Found: C, 57.8; H, 5.5; N, 36.9.

Determination of Apparent pK Values. Potentiometric titrations of the 5-aminoalkyltetrazoles were done at 25 ± 1° using a Beckman *p*H Meter, Model H-2. Solutions of 0.2–0.3 g. of the tetrazoles in 100–125 ml. of water were titrated with 0.1 *N* sodium hydroxide and 0.1 *N* hydrochloric acid. *p*K values were taken from large scale plots of the region of half neutralization on each leg of the curves. In each case the titration curves exhibited the form typical for an amino acid. The pK_1 and pK_2 values for the aminoalkyltetrazoles and the comparable amino acids are recorded in Table I.

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