THE SYNTHESIS OF CERTAIN AMINOTETRAZOLE DERIVATIVES AND A STUDY OF THEIR BACTERIOCIDAL PROPERTIES

Thesis for the Degree of M. S.

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Charles Francis Froberger

1950

This is to certify that the

thesis entitled

THE SYNTHESIS OF CERTAIN AMINO TETRAZOLE DERIVATIVES AND A STUDY OF THEIR BACTERIOCIDAL PROPERTIES

presented by

Charles F. Froberger

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M.S. degree in Chemistry

Robert M. Herbst

Major professor

Date April 27, 1950

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THE SYNTHESIS OF CERTAIN AMINOTETRAZOLE DERIVATIVES AND A STUDY OF THEIR BACTERIOCIDAL PROPERTIES

By

Charles Francis Froberger

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I wish to thank Doctor Robert M. Herbst for the assistance and encouragement he has given me during this work.

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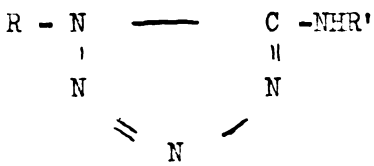
INTRODUCTION

The feasibility of preparing 1-alkyl-5-alkylaminotetrazole derivatives by the alkylation of the corresponding 1-alkyl-5-aminotetrazoles has recently been demonstrated (1).



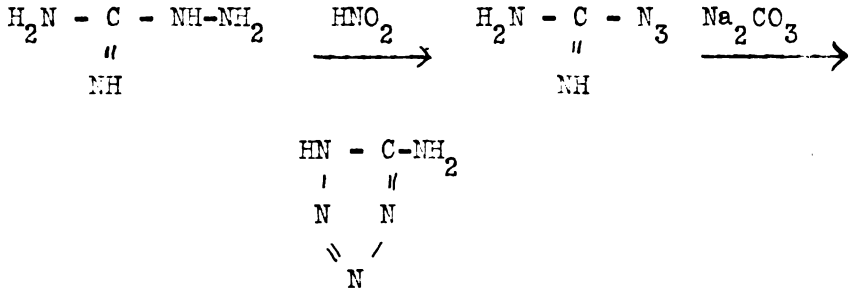
From an investigation of some of the pharmocological actions of an extensive series of these compounds, Gross and Featherstone (2) concluded that central nervous stimulatory action was common to many compounds of this group. Their observations indicated that maximum stimulatory action was exhibited by compounds in which the alkyl group in the 1-position contained five or six carbon atoms, and that the activity decreased markedly when either larger or smaller alkyl groups were substituted in the same position. No other investigations of the biological effect of compounds of this type have been reported.

Of particular interest was the observation that compounds in which a n-heptyl group was substituted in the 1-position exhibited definite soaplike characteristics, and appeared to be surface active substances. Such characteristics suggested the possibility that compounds of this type might have interesting bacteriostatic and bacteriocidal properties. Although the bacteriological properties of quaternary ammonium compounds, in which one of the substituents on the nitrogen is usually a large alkyl group, have been extensively investigated (3,4,5), such action has not been associated with secondary amines or their salts.

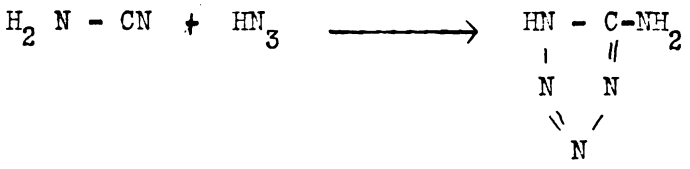
The synthesis of a number of 1-alkyl-5-alkylaminotetrazoles with large alkyl groups as substituents either in the 1-position or on the amino group, and a preliminary study of their bacteriostatic and bacteriocidal properties was therefore undertaken. The object of the synthetic portion of the investigation was to prepare a series of such tetrazole derivatives in which a large alkyl group was substituted in the 1-position while both large and small groups were introduced as substituents on the amino group. Conversely, a number of compounds with small alkyl groups in the 1-position and large substituents on the amino group were prepared. The object was to learn which type of compound would exhibit the most favorable bacteriological properties. The bacteriostatic titer and the phenol coefficient were determined for each of the new compounds prepared during the course of the work.

HISTORICAL

The preparation of 5-aminotetrazole was first reported by Thiele (6), who diazotized aminoguanidine. The resulting imide azide, guanyl azide, was heated with sodium carbonate or sodium acetate, to bring about rearrangement to the 5-aminotetrazole.

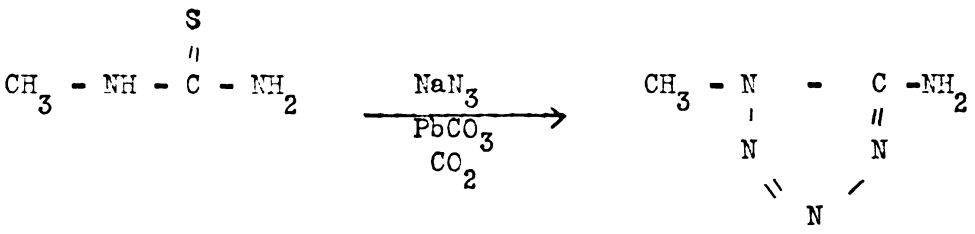


Cyanamide (7), or dicyandiamide (8) may also be treated with hydrazoic acid to form 5-aminotetrazole. The latter dissociates to cyanamide during the reaction.



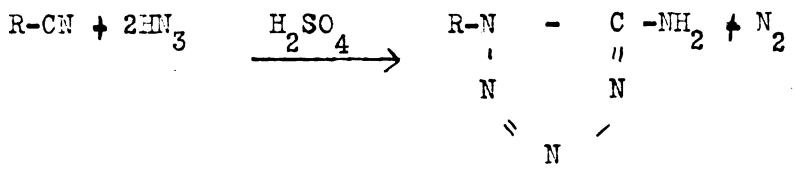
Direct alkylation of 5-aminotetrazole has resulted in the formation of a complex mixture of alkylated products. Thiele and Ingle (9) have described a variety of products resulting from methylation, ethylation, and benzylation of 5-aminotetrazole. After benzylation of 5-aminotetrazole with benzyl chloride in alkaline medium, at least four products could be isolated. 1-Methyl-5-aminotetrazole was one of the alkylated products isolated after the methylation of 5-aminotetrazole with methyl iodide.

A more clear cut preparation of 1-methyl-5-aminotetrazole, described by Stollé (10), was achieved by the interaction of methylthiourea and sodium azide in an atmosphere of carbon dioxide in the presence of lead carbonate.

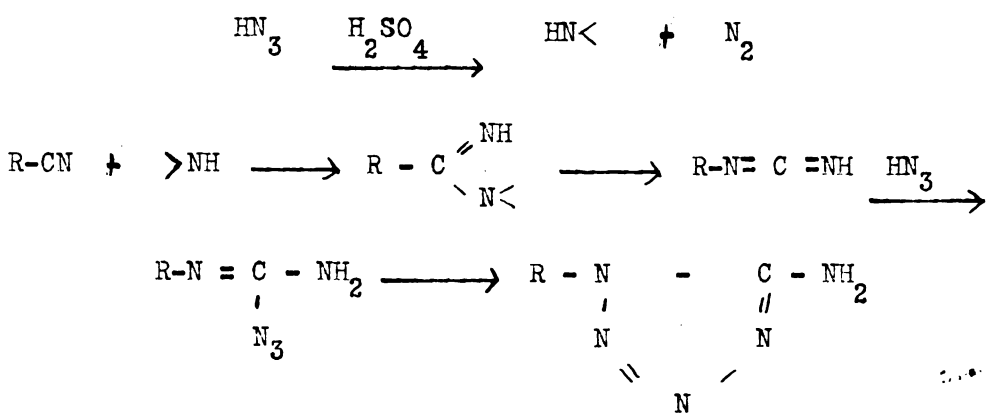


By application of this reaction to a variety of thiourea derivatives Stollé (11,12) prepared 1-methyl-5-aminotetrazole and a number of 1-aryl-5-aminotetrazoles. Stollé also correctly identified the compound prepared by Oliveri-Mandalà and Noto (13) by the interaction of hydrazoic acid and phenyl isothiocyanate in ethereal solution as 1-phenyl 5-aminotetrazole. Oliveri-Mandalà and Noto had considered the compound as an addition product of hydrazoic acid and phenyl isothiocyanate but Stollé showed that the sulfur had been eliminated during the reaction. The product was identical with the 1-phenyl-5-aminotetrazole formed by interaction of phenylthiourea and sodium azide.

A more attractive procedure that eliminates the use of lead carbonate in the presence of sodium azide or hydrazoic acid was developed by von Braun and Keller (14). The procedure involves the interaction of nitriles and hydrazoic acid in the presence of concentrated sulfuric acid. From the appropriate nitriles, 1-phenyl, 1-p-tolyl and 1-benzyl-5-aminotetrazole were prepared by these authors according to the following equation:

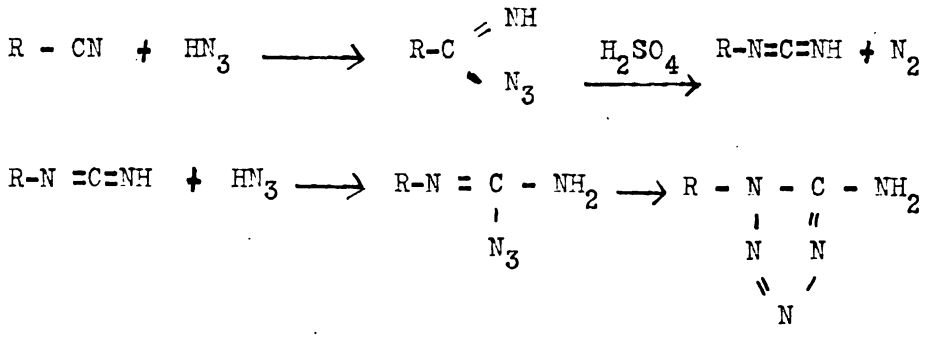


The mechanism postulated by von Braun and Keller (14) assumed the formation of a free imine radical (HN=) by the evolution of nitrogen from hydrazoic acid upon contact with sulfuric acid, as postulated by Schmidt (15). The imine radical was assumed to add to the cyanide group, followed by rearrangement of the addition product to a structure analagous to the isocyanate grouping. A second molecule of hydrazoic acid then added to the rearrangement product, and the resulting intermadiate then underwent cyclization.



The existence of a radical of the imine type seems rather doubtful, and a more logical explanation is the mechanism suggested by Herbst, Roberts, and Harvill (1). This involves the addition of hydrazoic acid directly to the cyanide linkage, followed by an acid catalyzed rearrangement, similar to the Curtius rearrangement of acid azides, accompanied by the elimination of nitrogen. The same rearrangement product, postulated by von Braun, is assumed to result and, after addition of another

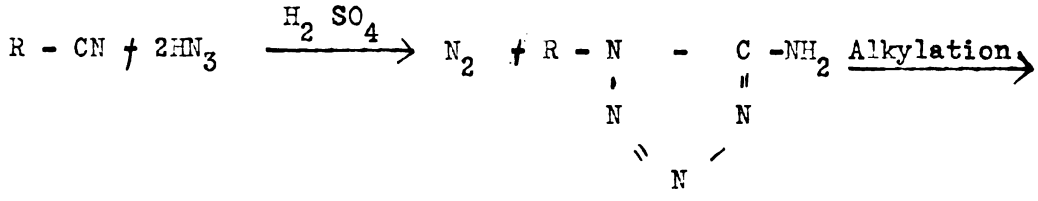
mole of hydrazoic acid, cyclizes to the 1-alkyl-5-aminotetrazole.

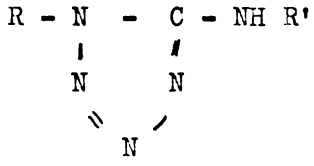


By this reaction, Herbst, Roberts, and Harvill have prepared an extensive series of 1-alkyl-5-aminotetrazoles (1).

DISCUSSION

In this work, a number of 1-alkyl-5-aminotetrazoles were prepared by the method of von Braun and Keller (14). These 1-alkyl-5-aminotetrazoles were methylated, ethylated, and benzylated to give the corresponding 1-alkyl-5-alkylaminotetrazoles, which, for convenience, were separated as the hydrochlorides.



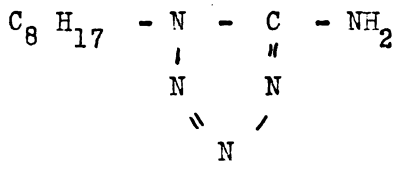


All of the 1-alkyl-5-alkylaminotetrazoles were characterized as phenylthiourea, p-nitrobenzamide, or 3, 5-dinitrobenzamide derivatives.

Of the 1-alkyl-5-aminotetrazoles prepared, 1-n-octyl-5-aminotetrazole was the only one which had not been previously reported. It was synthesized by the action of hydrazoic acid on nonanenitrile, according to the method of von Braun and Keller (14). Although the 1-alkyl-5-aminotetrazoles are represented as primary amines, the basic properties of the amino group are masked, possibly due to the character of the heterocyclic ring (1). They are relatively high melting solids, moderately soluble in hot water. Their aqueous solutions are usually neutral or slightly acid. They can be acylated and alkylated, but only under considerably more drastic conditions than are usually required

for primary amines. For example, the alkylation of 1-n-octyl-5-aminotetrazole with dimethylsulfate is accomplished only after heating to about 100°C, when an exothermic reaction is initiated and the temperature continues to rise to 160°C. On the other hand, the alkylation of n-octylamine occurs almost immediately upon the addition of dimethylsulfate to the amine at room temperature.

The method of synthesis of 1-n-octyl-5-aminotetrazole, which was completely analogous to that used for the preparation of 1-n-heptyl, 1-n-nonyl and 1-n-undecyl-5-aminotetrazole (1), makes it reasonable to assume that the structure may be written as follows:



This assumption is further supported by the formation of secondary amines upon alkylation and the formation of typical derivatives of the secondary amines, all of which gave results in conformity with the calculated values upon quantitative determination of the nitrogen content.

Alkylation of the 1-alkyl-5-aminotetrazole may conceivably follow two distinct courses. In addition to the formation of secondary amines, the possibility of interaction of the alkylating agent with the ring nitrogen to form a quaternary salt must be considered. The formation of quaternary compounds has been observed with several pentamethylene tetrazole derivatives (16). If a quaternary salt had been formed by alkylation of the 5-aminotetrazole derivative, it would be anticipated

that the quaternary base liberated, upon the addition of alkali, would be extremely soluble in water and difficult to extract with the common immiscible organic solvents. Furthermore, distillation of quaternary bases usually is accompanied by extensive decomposition. However, if alkylation occurred on the amino group of the 1-alkyl-5-aminotetrazole, the base liberated, upon addition of alkali would be relatively insoluble in water, capable of being extracted with ether or benzene, and distillable without decomposition. The alkylated product should also form derivatives characteristic of secondary amines, such as substituted thioureas and amides. Since the bases liberated on treatment of the alkylated products with alkali were insoluble in water, soluble in ether or benzene, and distillable under reduced pressure without decomposition, it is reasonable to assume the products are the 5-alkylamino derivatives. This assumption is further substantiated by the formation of a phenylthiourea or nitrobenzamide derivative in each instance. Nitrogen determinations upon all products and derivatives were in conformity with these conclusions.

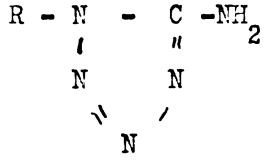
In alkylating the various 1-alkyl-5-aminotetrazoles, several procedures were employed. The methylation, ethylation, and benzylation were accomplished by heating a mixture of equimolecular quantities of the amine and dimethylsulfate, diethylsulfate, or benzyl chloride, to a sufficiently high temperature to initiate an exothermic reaction. From the resulting homogeneous melt, the 1-alkyl-5-alkylaminotetrazoles were isolated. Alkylation with n-octyl chloride was brought about by reaction in a sealed tube.

Phenol coefficients and bacteriostatic titers were determined for each of the secondary amines by the usual standard procedures, using 24-hour cultures of Staphylococcus aureus and Eberthella typhosa, which had been transferred for three consecutive days prior to use. A blank, using distilled water, was run along with each series of tests. Organic matter interfered with the bacteriocidal action of the compounds. From the data recorded in Table IV, it will be noted that the 1-alkyl-5-alkylaminotetrazoles are more effective against Gram negative organisms than against Gram positive organisms. It is noteworthy that this effect is the reverse of that usually exhibited by the quaternary salts. Although the data which has been assembled are not sufficient to determine the optimum structure for maximum bacteriological activity, it does appear to be essential that one of the alkyl groups should be rather large. Furthermore, whether the large alkyl group is present as a substituent in the 1-position or on the amino group seems to be immaterial.

Since the introduction of small alkyl groups as substituents on the amino group during the alkylation process could be accomplished more easily and with better yields than the introduction of large alkyl groups, it would be more profitable in any extension of this investigation, to direct efforts toward the preparation of 1-alkyl-5-alkylaminotetrazoles with the large group in the 1-position.

TABLE I

1-Alkyl-5-Aminotetrazoles

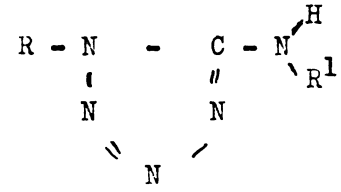


1-alkyl-5-aminotetrazole	R	Formula	M.P.* °C.	Yield	Analysis % N** Calc.	Found	Ref.
methyl	CH_3	$C_2H_5N_5$	227.5-228°C.	34.5%	---	---	9,10
ethyl	C_2H_5	$C_3H_7N_5$	148.5-149°C.	40%	---	---	1
n-octyl	$n\text{-}C_8H_{17}$	$C_9H_{19}N_5$	164-164.5°C.	67%	35.5	35.6	
benzyl	$C_6H_5CH_2$	$C_8H_9N_5$	188.5-189.5°C.	30%	---	---	14

* All melting points are corrected.
** Nitrogen determined by micro-Dumas technique.

TABLE II

1-Alkyl-5-Alkylaminotetrazoles



1-alkyl-5-alkylaminotetrazole	Formula	B.P. °C.	M.P. °C.* of Hydrochloride	Yield of Hydrochloride	Analysis of % N*** in Hydrochlorides Calc.	Found
1-methyl-5-ethylamino-	$C_4H_9N_5$	115-119°/23mm	203-4°**	45%	---	---
1-methyl-5-n-octylamino-	$C_{10}H_{20}N_5$	---	197-197.5°	22%	28.3	28.5
1-n-octyl-5-methylamino-	$C_{10}H_{20}N_5$	147-151°/4mm	200.5-201°	38%	28.3	28.2
1-ethyl-5-n-octylamino-	$C_{11}H_{23}N_5$	---	162.5-163.5°	29%	26.8	27.0
1-n-octyl-5-ethylamino-	$C_{11}H_{23}N_5$	160-164°/8mm	165.5-166°	40%	26.8	27.1
1-benzyl-5-n-octylamino-	$C_{17}H_{25}N_5$	---	161-162.5°	---	21.6	20.5
1-n-octyl-5-benzylamino-	$C_{17}H_{25}N_5$	---	167-167.5°	25%	21.6	21.7

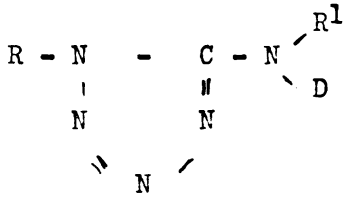
* All melting points are corrected.

** Identical with an authentic sample of 1-methyl-5-ethylaminotetrazole hydrochloride (1).

*** Nitrogen determined by micro-Dumas technique.

TABLE III

Derivatives of 1-Alkyl-5-Alkylaminotetrazoles

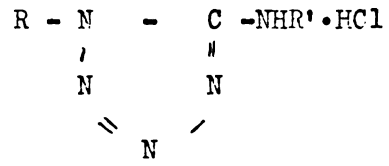


Derivative	D	Formula	M.P. °C. *	Analysis of % N *** Calc.	Found
Phenylthiourea of: a) 1-methyl-5-ethylamino-	-CS-NHC$_6$H$_5$	$C_{11}H_{14}N_6S$	149-149.5° **	----	----
b) 1-n-octyl-5-methylamino-	-CS-NHC$_6$H$_5$	$C_{17}H_{26}N_6S$	83.5-84°	24.3	24.4
p-Nitrobenzamide of: a) 1-n-octyl-5-ethylamino-	-CO- NO_2	$C_{18}H_{26}N_6O_3$	56.5-57°	22.5	22.7
3,5-dinitrobenzamide of: a) 1-methyl-5-n-octylamino-	-CO- NO_2, NO_2	$C_{17}H_{23}N_7O_5$	70.5-71°	24.2	24.1
b) 1-ethyl-5-n-octylamino	-CO- NO_2, NO_2	$C_{18}H_{25}N_7O_5$	57-57.5°	23.4	23.5
c) 1-n-octyl-5-benzylamino	-CO- NO_2, NO_2	$C_{23}H_{27}N_7O_5$	58-59°	20.4	20.3

* All melting points are corrected.
** Identical with an authentic sample of 1-methyl-5-ethylaminotetrazole hydrochloride (1).
*** Nitrogen determined by micro-Dumas technique.

TABLE IV

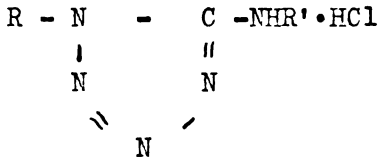
Bacteriostatic Titer of 1-Alkyl-5-Alkylaminotetrazole Hydrochlorides



1-alkyl-5-alkylaminotetrazole hydrochloride	Bacteriostatic titer	
	Staphylococcus aureus	Eberthella typhosa
1-methyl-5-ethylamino-	none	none
1-methyl-5-n-octylamino-	1-1000	1-2000
1-n-octyl-5-methylamino-	1-5000	1-10,000
1-ethyl-5-n-octylamino-	1-1000	1-10,000
1-n-octyl-5-ethylamino	1-5000	1-10,000
1-n-octyl-5-benzylamino	1-20,000	1-20,000

TABLE V

Phenol Coefficient of 1-Alkyl-5-Alkylaminotetrazole Hydrochlorides

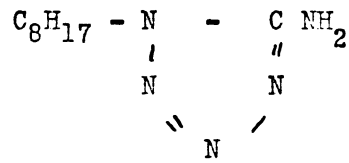


1-alkyl-5-alkylaminotetrazole hydrochloride	Phenol Coefficient	
	Staphylococcus aureus	Eberthella typhosa
1-methyl-5-n-octylamino-	13	17
1-n-octyl-5-methylamino-	10	12
1-ethyl-5-n-octylamino-	15	17
1-n-octyl-5-ethylamino-	15	17
1-n-octyl-5-benzylamino-	below 100	below 100

EXPERIMENTAL

All of the 1-alkyl-5-aminotetrazoles were prepared following the procedure of von Braun and Keller (14). The 1-methyl, 1-ethyl, and 1-benzyl-5-aminotetrazoles have been previously described (1,9,10,14). The melting points of these compounds compared favorably with those recorded in the literature. Since 1-n-octyl-5-aminotetrazole has not been previously described, the procedure employed for its preparation will be given in detail. The other 1-alkyl-5-aminotetrazoles were prepared in a completely comparable manner.

Preparation of 1-n-Octyl-5-Aminotetrazole

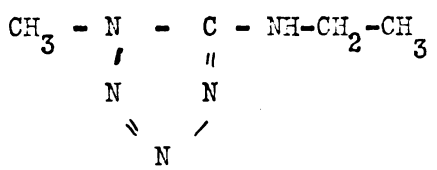


Concentrated sulfuric acid (300 ml.) was added dropwise to a mechanically stirred solution of 69.6 g. (0.5 mole) of nonanenitrile and 55 g. (1.25 mole) of hydrazoic acid in about 500 ml. of benzene. After addition of about 75 ml. of sulfuric acid, the solution turned chalky white, and the temperature rose to 40° C. The solution was cooled and kept between the temperature range of 30-35° C. Addition of the sulfuric acid was accompanied by vigorous evolution of nitrogen. After about two hours, the addition of the sulfuric acid was completed, and the resulting solution was cautiously warmed to 40° C. The stirring was continued for 6-7 hours after complete addition of the sulfuric acid. After separation from benzene, the acid layer was poured onto about 300 g. of ice and neutralized with about 600 g. of sodium carbonate. The resulting

solution was evaporated to dryness on a steam bath, with the aid of a stream of air blowing across the top of the beaker.

The solid left, after evaporation of the water, was extracted repeatedly with 1000 ml. of boiling isopropyl alcohol, using the mother liquor for successive extractions. The resulting crude product was recrystallized from aqueous isopropyl alcohol. Yield 66 g.-67%. M. p. 164-164.5° C. (cor.).

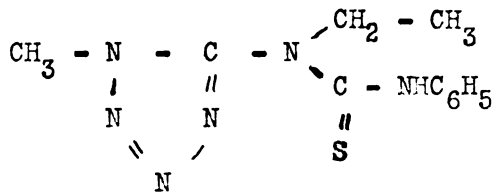
Preparation of 1-Methyl-5-Ethylaminotetrazole



A mixture of 34 g. (0.34 mole) of 1-methyl-5-aminotetrazole and 56.4 g. (0.34 mole) of diethylsulfate was heated on a steam bath with occasional stirring. When the temperature of the mixture approached 100° C., a rapid exothermic reaction took place, causing the temperature to rise to 170° C. After the reaction mixture had been heated on the steam bath for an additional 30 minutes, it was allowed to cool to room temperature. The base was isolated by adding a warm solution of the crude ethosulfate in 100 ml. of absolute ethanol to a warm solution of 10 g. of sodium in 100 ml. of absolute ethanol. After refluxing the resulting mixture for ten minutes, the ethanol was distilled off under reduced pressure. The residue was suspended in 100 ml. of water, and after addition of about 200 g. of anhydrous potassium carbonate, the base was extracted from the sludge with six 200 ml. portions of benzene. The benzene extracts were combined and the solvent removed by distillation. The residue remaining after removal of the solvent, was distilled

under reduced pressure. The yield of product distilling at 115-119° at 23 mm was 24 g. or 53%. The hydrochloride was prepared by dissolving the entire quantity of base in 75 ml. of absolute isopropyl alcohol, and adding dry hydrogen chloride in excess. After recrystallization from absolute isopropyl alcohol, 25.2 g. (45%) of pure hydrochloride was obtained. M. p. 203-204° C. (cor.).

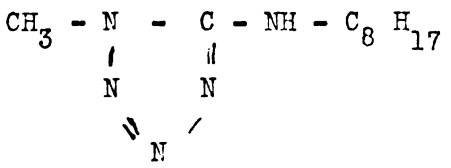
Preparation of the Phenylthiourea Derivative of 1-Methyl-5-Ethylaminotetrazole



About 1 g. of the 1-methyl-5-ethylaminotetrazole hydrochloride was dissolved in a minimum amount of water. The water solution was saturated with potassium carbonate and extracted with ether. After evaporation of the ether, one gram of phenyl isothiocyanate was added to the residue and the solution warmed on a steam bath. After cooling, the partially solid mixture was filtered. To the filtrate, a solution of equal volumes of ethanol and ligroin was added, and the resulting precipitate was filtered off by suction. The combined precipitates were recrystallized twice from 95% ethanol. M. p. 149-149.5° C. (cor.).

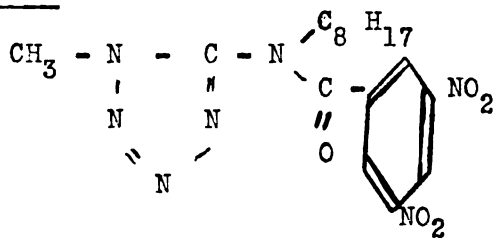
A mixed melting point of the phenylthiourea derivative with an authentic preparation (1) showed no depression of the melting point.

Preparation of 1-Methyl-5-n-Octylaminotetrazole



A mixture of 10 g. (0.1 mole) of 1-methyl-5-aminotetrazole, 15 g. (0.1 mole) of n-octyl chloride and 25 ml. of absolute ethanol was sealed into a long combustion tube. The sealed tube was placed in an oven for 48 hours at a temperature of 180° C. At the end of this time, the tube was opened and the contents evaporated to dryness on a steam bath. The residue was taken up in a minimum amount of water, a few drops of concentrated hydrochloric acid added, and the solution extracted with ether. The water solution was saturated with potassium carbonate, and the free base extracted with ether. Excess dry hydrogen chloride was bubbled into the ethereal solution. The precipitate of hydrochloride was filtered off, and recrystallized twice from absolute isopropyl alcohol. The yield of the hydrochloride was 5.5 g. (22%). M. p. 197-197.5° C. (cor.).

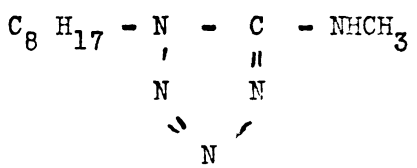
Preparation of the 3,5-Dinitrobenzamide Derivative of 1-Methyl-5-n-Octylaminotetrazole



About 1 g. of 1-methyl-5-n-octylaminotetrazole hydrochloride was dissolved in a minimum amount of water. The water solution was saturated with potassium carbonate and extracted with ether. After evaporation of the ether, the free amine was dissolved in 10 ml. of dry benzene and

added to a solution of 1 g. of 3,5-dinitrobenzoyl chloride in 5 ml. of benzene. The resulting solution was refluxed for 15 minutes, and then allowed to cool. The solution was filtered, and the precipitate washed with 10 ml. of warm benzene, the washings being added to the original filtrate. The benzene solution was then washed with 10 ml. of 2% sodium carbonate, followed by 10 ml. of 2% hydrochloric acid, and finally 10 ml. of distilled water. The benzene was evaporated and the residue recrystallized four times from dilute ethanol. M. p. 70.5-71° C. (cor.).

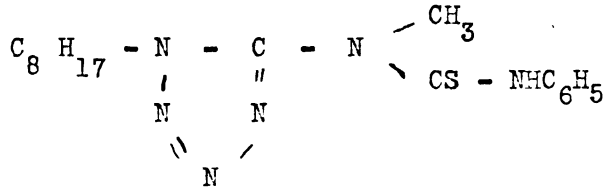
Preparation of 1-n-Octyl-5-Methylaminotetrazole



A mixture of 19.7 g. (0.1 mole) of 1-n-octyl-5-aminotetrazole and 12.6 g. (0.1 mole) of dimethylsulfate was heated in an oil bath to a temperature of 170° C. Heating was continued for one hour, after which the reaction mixture was allowed to cool to room temperature. The base was isolated by adding a warm solution of the crude methosulfate in 175 ml. of absolute ethanol to a warm solution of 2.5 g. of sodium in 100 ml. of absolute ethanol. After refluxing the resulting mixture for one hour, the ethanol was distilled off under reduced pressure. The residue was suspended in 150 ml. of water and after addition of about 300 g. of anhydrous potassium carbonate, the base was extracted from the sludge with six 200 ml. portions of benzene. The benzene extracts were combined and the solvent removed by distillation. The residue was distilled under reduced pressure and the fraction coming over

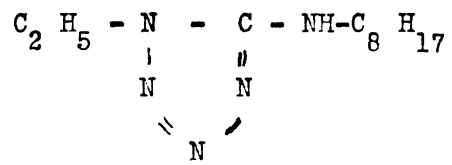
at 147-151° at 4 mm. was collected. The yield was 9.5 g. (45%). The hydrochloride was prepared by dissolving the entire quantity of base in 25 ml. of absolute isopropyl alcohol, and adding dry hydrogen chloride in excess. After recrystallization from absolute isopropyl alcohol, 8.8 gm. (38%) of pure hydrochloride was obtained. M. p. 200.5-201° C. (cor.).

Preparation of the Phenylthiourea Derivative of 1-n-Octyl-5-Methylaminotetrazole



About 0.5 g. of the 1-n-octyl-5-methylaminotetrazole hydrochloride was dissolved in a minimum amount of water. The water solution was saturated with potassium carbonate and extracted with ether. After evaporation of the ether, 0.5 g. phenyl isothiocyanate was added to the residue. When the reaction was complete, the resulting solution was cooled in an ice-salt bath and ligroin added. The solution was filtered and the precipitate was crushed and washed with ligroin. The precipitate was recrystallized twice from 95% ethanol. M. p. 83.5-84° C. (cor.).

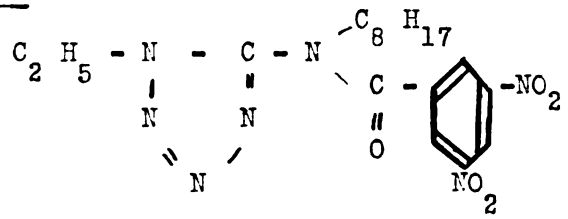
Preparation of 1-Ethyl 5-n-Octylaminotetrazole



A mixture of 5.6 g. (0.05 mole) of 1-ethyl-5-aminotetrazole and 7.4 g. (0.05 mole) of octyl chloride was heated together on an oil bath

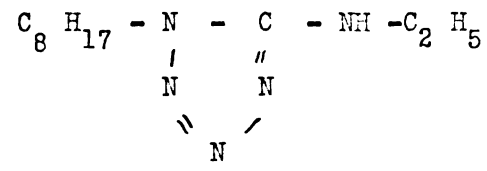
to a temperature of 180° C. Heating was continued for one hour, after which the reaction mixture was allowed to cool to room temperature. The solid mass was recrystallized four times from hot ethyl acetate. The weight of pure hydrochloride recovered was 3.6 g. (29%). M. p. 162.5-163.5° C. (cor.).

Preparation of the 3,5-Dinitrobenzamide Derivative of 1-Ethyl-5-n-Octylaminotetrazole



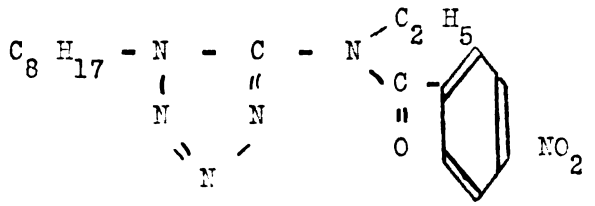
About 1 g. of 1-ethyl-5-n-octylaminotetrazole hydrochloride was dissolved in a minimum amount of water. The water solution was saturated with potassium carbonate and extracted with ether. After evaporation of the ether, the free amine was dissolved in 10 ml. of dry benzene and added to a solution of 1 g. of 3,5-dinitrobenzoyl chloride in 5 ml. of benzene. The resulting solution was refluxed for 15 minutes, and then allowed to cool. The solution was filtered, and the precipitate washed with 10 ml. of warm benzene, the washings being added to the original filtrate. The benzene solution was then washed with 10 ml. of 2% sodium carbonate, followed by 10 ml. of 2% hydrochloric acid, and finally 10 ml. of distilled water. The benzene was evaporated and the residue recrystallized four times from dilute ethanol. M. p. 57-57.5° C. (cor.).

Preparation of 1-n-Octyl-5-Ethylaminotetrazole



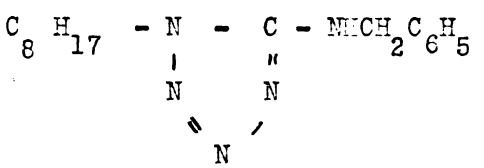
A mixture of 19.7 g. (0.1 mole) of 1-n-octyl-5-aminotetrazole and 15.4 g. (0.1 mole) of diethylsulfate was heated on a steam bath with occasional stirring. When the temperature of the mixture approached 95° C., an exothermic reaction took place, causing the temperature to rise to 120° C. Heating was continued for thirty minutes on the steam bath, after which the reaction mixture was allowed to cool to room temperature. The base was isolated by adding a warm solution of the crude ethosulfate in 100 ml. of absolute ethanol to a warm solution of 2.5 g. of sodium in 75 ml. of absolute ethanol. After refluxing the resulting mixture for one hour, the ethanol was distilled off under reduced pressure. The residue was suspended in 150 ml. of water, and after addition of about 300 g. of anhydrous potassium carbonate, the base was extracted from the sludge with six 200 ml. portions of benzene. The benzene extracts were combined and the solvent removed by distillation. The residue remaining after removal of the solvent, was distilled under reduced pressure. The yield of product distilling at 160-164° at 8 mm. was 18 g. or 69%. The hydrochloride was prepared by dissolving the entire quantity of base in 50 ml. of absolute isopropyl alcohol, and adding dry hydrogen chloride in excess. After recrystallization from absolute isopropyl alcohol, 12 g. (40%) of pure hydrochloride was obtained. M. p. 165.5-166° C. (cor.).

Preparation of the p-Nitrobenzamide Derivative of 1-n-Octyl-5-Ethylaminotetrazole



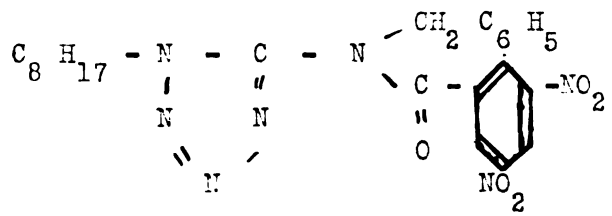
About 0.6 g. 1-n-octyl-5-ethylaminotetrazole hydrochloride was dissolved in a minimum amount of water. The water solution was saturated with potassium carbonate and extracted with ether. After evaporation of the ether, the free amine was dissolved in 5 ml. of dry benzene and added to a solution of 0.6 g. of p-nitrobenzoyl chloride in 10 ml. of benzene. The resulting solution was refluxed for 15 minutes, and then allowed to cool. The solution was filtered, and the precipitate recrystallized three times from dilute ethanol. M. p. 56.5-57° C. (cor.).

Preparation of 1-n-Octyl-5-Benzylaminotetrazole



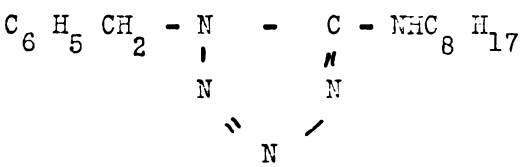
A mixture of 19.7 g. (0.1 mole) of 1-n-octyl 5-aminotetrazole and 12.6 g. (0.1 mole) of benzyl chloride was heated in an oil bath to a temperature of 170° C. Heating was continued for two hours, after which the reaction mixture was allowed to cool to room temperature. The solid mass was recrystallized twice from absolute isopropyl alcohol. The melting point of the solid was 119-120° C. The solid was recrystallized four times from boiling water. The weight of the pure hydrochloride recovered was 8 g. (25%). M. p. 167-167.5° C. (cor.).

Preparation of the 3,5-Dinitrobenzamide Derivative of 1-n-Octyl-5-Benzylaminotetrazole



About 1 g. of 1-n-octyl-5-benzylaminotetrazole hydrochloride was suspended in about 10 ml. of 50% potassium hydroxide solution. The aqueous layer was saturated with potassium carbonate and extracted with ether. After evaporation of the ether, the free amine was dissolved in 10 ml. of dry benzene and added to a solution of 1 g. of 3,5-dinitrobenzoyl chloride in 5 ml. of benzene. The resulting solution was refluxed for 15 minutes, and then allowed to cool. The solution was filtered, and the precipitate washed with 10 ml. of warm benzene, the washings being added to the original filtrate. The benzene solution was then washed with 10 ml. of 2% sodium carbonate followed by 10 ml. of 2% hydrochloric acid, and finally 10 ml. of distilled water. The benzene was evaporated and the residue recrystallized four times from dilute ethanol. M. p. 58-59° C. (cor.).

Preparation of 1-Benzyl-5-n-Octylaminotetrazole



A mixture of 4.4 g. (0.025 mole) of 1-benzyl-5-aminotetrazole and 4.8 g. (0.025 mole) of octyl bromide and 15 ml. of mesitylene was heated in an oil bath until the two layers disappeared. After cooling,

the mass was recrystallized repeatedly from water, isopropyl alcohol, and ethyl acetate. Weight of hydrobromide recovered was one gram. M. p. 148-151° C. The hydrobromide was dissolved in a minimum amount of water. The water solution was saturated with potassium carbonate and extracted with ether. To the ethereal solution, dry hydrogen chloride was added and a white precipitate was formed. The hydrochloride was recrystallized four times from hot water. The weight of hydrochloride recovered was about 0.2 g. M. p. 154-157° C. (cor.). Determination of nitrogen showed that the hydrochloride was still impure.

Alkylation by a sealed tube reaction was also unsuccessful.

Bacteriostatic Titers

Bacteriostatic titers were determined for each of the secondary amines in the following manner: Dilutions of the secondary amines were made up with distilled water. To each dilution, 0.5 ml. of a 24-hour culture of S. aureus or E. typhosa which had been transferred for three consecutive days prior to use, was added. The dilutions remained at room temperature for 3½-4 hours. After this interval, one loopful of each dilution was transferred to 10 ml. of FDA broth. The broth cultures were incubated at 37° C., and readings were taken at 24, 48, and 72 hours. The highest dilution showing no growth after 72 hours incubation was the bacteriostatic titer. Tables VI-XI show the bacteriostatic titers for the compounds tested.

TABLE VI

1-Methyl-5-Ethylaminotetrazole Hydrochloride

	S. aureus			E. typhosa		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
1-100	+	+ +	+ + +	+	+ +	+ + +
1-200	+	+ +	+ + +	+	+ +	+ + +
1-500	+	+ +	+ + +	+	+ +	+ + +
1-1000	+	+ +	+ + +	+	+ +	+ + +
Water	+	+ +	+ + +	+	+ +	+ + +

TABLE VII

1-Methyl-5-n-Octylaminotetrazole Hydrochloride

	S. aureus			E. typhosa		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
1-1000	—	—	—	—	—	—
1-2000	+	+	+ +	—	—	—
1-5000	—	+	+ +	—	+	+ +
1-10,000	+	+ +	+ + +	—	+	+ +
1-20,000	+	+ +	+ + +	+	+ +	+ + +
Water	+	+ +	+ + +	+	+ +	+ + +

TABLE VIII

1-n-Octyl-5-Methylaminotetrazole Hydrochloride

	S. aureus			E. typhosa		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
1-5000	—	—	—	—	—	—
1-10,000	faint	+	+ +	—	—	—
1-20,000	+	+ +	+ + +	+	+ +	+ + +
Water	+	+ +	+ + +	+	+ +	+ + +

TABLE IX

1-Ethyl-5-n-Octylaminotetrazole Hydrochloride

	S. aureus			E. typhosa		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
1-1000	—	—		—	—	—
1-2000	—	faint	+	—	—	—
1-5000	+	+ +	+ + +	—	—	—
1-10,000	+	+ +	+ + +	—	—	—
1-20,000	+	+ +	+ + +	+	+ +	+ + +
Water	+	+ +	+ + +	+	+ +	+ + +

TABLE X

1-n-Octyl-5-Ethylaminotetrazole Hydrochloride

	S. aureus			E. typhosa		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
1-5000	—	—	—	—	—	—
1-10,000	—	+	+ +	—	—	—
1-20,000	+	+ +	+ + +	+	+ +	+ + +
Water	+	+ +	+ + +	+	+ +	+ + +

TABLE XI

1-n-Octyl-5-Benzylaminotetrazole Hydrochloride

	S. aureus			E. typhosa		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
1-10,000	—	—	—	—	—	—
1-20,000	—	—	—	—	—	—
1-50,000	—	+	+ +	+	+ +	+ + +
1-100,000	+	+ +	+ + +	+	+ +	+ + +
Water	+	+ +	+ + +	+	+ +	+ + +

Phenol Coefficients

Phenol coefficients were determined for each of the secondary amines by the method recommended by the United States Food and Drug Administration (17). This method was applied using S. aureus, strain 209. The following data was obtained.

TABLE XII

Phenol Coefficient of 1-Methyl-5-n-Octylaminotetrazole Hydrochloride

S. aureus				E. typhosa			
Phenol	5 min.	10 min.	15 min.	Phenol	5 min.	10 min.	15 min.
1-70	—	—	—	1-90	—	—	—
1-80	+	—	—	1-100	+	—	—
1-90	+	+	—	1-110	+	+	+
Tetrazole				Tetrazole			
1-600	—	—	—	1-1200	—	—	—
1-800	+	—	—	1-1500	—	—	—
1-1000	+	—	—	1-1700	+	—	—
1-1200	+	+	+	1-1800	+	+	+

TABLE XIII

Phenol Coefficient of 1-n-Octyl-5-Methylamino Hydrochloride

S. aureus				E. typhosa			
Phenol	5 min.	10 min.	15 min.	Phenol	5 min.	10 min.	15 min.
1-70	—	—	—	1-90	—	—	—
1-80	+	—	—	1-100	+	—	—
1-90	+	+	+	1-110	+	+	+
Tetrazole				Tetrazole			
1-600	—	—	—	1-1000	—	—	—
1-800	+	—	—	1-1200	+	—	—
1-1000	+	+	+	1-1500	+	+	+

TABLE XIV

Phenol Coefficient of 1-Ethyl-5-n-Octylaminotetrazole Hydrochloride

S. aureus				E. typhosa			
Phenol	5 min.	10 min.	15 min.	Phenol	5 min.	10 min.	15 min.
1-70	+	—	—	1-90	+	—	—
1-80	+	—	—	1-100	+	—	—
1-90	+	+	+	1-110	+	+	+
Tetrazole				Tetrazole			
1-1000	+	—	—	1-1500	—	—	—
1-1200	+	—	—	1-1700	+	—	—
1-1500	+	+	+	1-1800	+	+	+

TABLE XV

Phenol Coefficient of 1-n-Octyl-5-Ethylaminotetrazole Hydrochloride

S. aureus			
Phenol	5 min.	10 min.	15 min.
1-70	+	—	—
1-80	+	—	—
1-90	+	+	+
Tetrazole			
1-1000	+	—	—
1-1200	+	—	—
1-1500	+	+	+

E. typhosa			
Phenol	5 min.	10 min.	15 min.
1-90	+	—	—
1-100	+	—	—
1-110	+	+	+
Tetrazole			
1-1500	—	—	—
1-1700	+	+	+
1-1800	+	+	—

TABLE XVI

Phenol Coefficient of 1-n-Octyl-5-Benzylaminotetrazole Hydrochloride

S. aureus			
Phenol	5 min.	10 min.	15 min.
1-70	+	—	—
1-80	+	—	—
1-90	+	+	+
Tetrazole			
1-10,000	+	+	+
1-15,000	+	+	+
1-20,000	+	+	+

E. typhosa			
Phenol	5 min.	10 min.	15 min.
1-90	—	—	—
1-100	+	—	—
1-110	+	+	—
Tetrazole			
1-10,000	+	+	+
1-15,000	+	+	+
1-20,000	+	+	+

SUMMARY

1) The following 1-alkyl-5-aminotetrazoles were prepared:

a) 1-methyl-5-aminotetrazole

b) 1-ethyl-5-aminotetrazole

c) 1-n-octyl-5-aminotetrazole

d) 1-benzyl-5-aminotetrazole

Of these, 1-n-octyl-5-aminotetrazole is a new compound.

2) The following 1-alkyl-5-alkylaminotetrazoles were prepared and isolated as hydrochlorides:

a) 1-methyl-5-ethylaminotetrazole

b) 1-methyl-5-n-octylaminotetrazole

c) 1-n-octyl-5-methylaminotetrazole

d) 1-ethyl-5-n-octylaminotetrazole

e) 1-n-octyl-5-ethylaminotetrazole

f) 1-n-octyl-5-benzylaminotetrazole

Of these, only the 1-methyl-5-ethylaminotetrazole has been previously described.

3) Derivatives of all the 1-alkyl-5-alkylaminotetrazoles were prepared.

4) Phenol coefficients and bacteriostatic titers were determined for all of the secondary amines.

5) Pronounced bacteriostatic and bacteriocidal action was observed in all of the secondary amines which carried a large alkyl group as a substituent either in the ring or on the amino group.

6) The 1-alkyl-5-alkylaminotetrazoles were more effective against Gram negative organisms than against Gram positive organisms.

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