THE DETERMINATION OF 5-SUBSTITUTED TETRAZOLES BY REACTION WITH ACID CHLORIDES

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Coe Durland Suydam, Junior

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By

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ABSTRACT

5-Substituted tetrazoles were acylated by p-nitrobenzoyl chloride followed by the formation of 2-substituted-5-p-nitrophenyl-1,3,4-oxadiazoles. The method used involved the refluxing of a sample containing a weighed amount of tetrazole and an aliquot of a p-nitrobenzoyl chloride solution in pyridine, and a blank containing the same aliquot of reagent solution. It was found that a four to one molar ratio of acid chloride to tetrazole should be present in the reaction mixture and that a reflux time from two to fifteen minutes was necessary for maximum acylation.

The acylation of 5-alkyl and aryl tetrazoles was quantitative within an [illegible] error of ±1.0 per cent. However, with the exception of 5-diethylaminotetrazole the 5-substituted aminotetrazoles were not quantitatively acylated. This was due to the rearrangement of the 5-substituted aminotetrazole to a 1-substituted-5-aminotetrazole which consumed no acid chloride.

The presence of 1-substituted-5-aminotetrazoles did not constitute an interference in the determination of tetrazoles, but it was found that 1-substituted tetrazoles did interfere in the determination.

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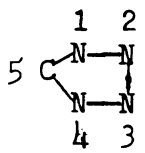
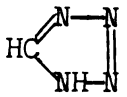
I. INTRODUCTION

The synthetic chemistry of tetrazoles has been extensively investigated since 1885 when the first tetrazole synthesis was reported by Bladin (1), a Swedish chemist.

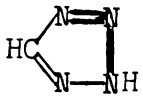
In a review article by Benson (2) tetrazoles are described as a "five membered, doubly unsaturated ring consisting of one carbon and four nitrogen atoms." According to the presently accepted system for numbering the atoms, the ring is drawn as follows:



The parent compound, tetrazole, itself, can exist in the two tautomeric forms I and II. This is true, also of all 5-monosubstituted tetrazoles.



I



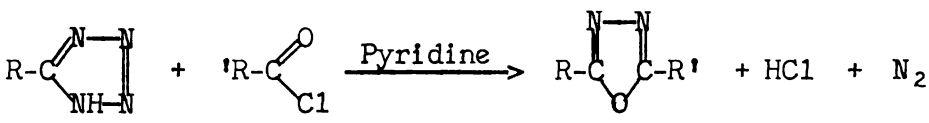
II

One of the most important chemical properties of tetrazoles is the acidity of those derivatives possessing a hydrogen in the one position. The dissociation constant for this hydrogen in a number of monosubstituted tetrazoles has been reported recently by Mihina (3) who showed that these dissociation constants are about a power of ten

lower than the dissociation constants for the corresponding carboxylic acids.

During all these investigations there has been no specific determination for the tetrazole ring reported in the literature. The one possible exception to this is a determination of tetrazoles based on their acidity. However, it should be mentioned that this method would be of little value when the tetrazole is in the presence of other acids. For this reason most investigators have relied on a Dumas elemental nitrogen analysis. The obvious disadvantage to this method is that it cannot differentiate between a ring nitrogen and a substituent nitrogen.

It was the purpose of this investigation to develop a method for the determination of 5-substituted tetrazoles. The particular method which is presented is based on an article by Huisgen et. al. (4) which describes the acylation of 5-substituted tetrazoles followed by the formation of 2,5-disubstituted-1,3,4-oxadiazoles. In this communication Huisgen reported that a number of 5-substituted tetrazoles were 100 per cent acylated on a preparative scale by six common acid chlorides in a pyridine reaction medium. The over-all reaction proceeds as follows:



Assuming the reaction proceeds quantitatively, there are two possible methods for determining the amount of tetrazole which has reacted. The first method involves a gasometric measurement of the

nitrogen gas evolved. The second method, on which this investigation is based, depends on the acidimetric determination of the acid chloride which reacts with the tetrazole.

II. EXPERIMENTAL

A. Chemicals

The chemicals in this investigation were not repurified unless otherwise stated. Repurification of the tetrazole samples was not considered necessary since the purity of the compounds could be determined by an acidimetric titration using standard base. The tetrazole samples used in this investigation were supplied by Dr. R. M. Herbst, Professor of Organic Chemistry at Michigan State University.

Chemicals used were:

3,5-Dinitrobenzoyl chloride, Eastman, C.P.

Benzoyl chloride, Eastman, C.P.

Acetyl chloride, Eastman, C.P.

p-Nitrobenzoyl chloride, Eastman, C.P.

Sodium hydroxide, Merck, C.P.

Primary standard used was:

Potassium acid phthalate, Bakers analyzed primary standard, oven-dried for two hours at 105°.

Solvents used were:

Pyridine, Baker's analyzed and Mallinckrodt analytical reagent

Ethyl acetate, Eastman, C.P.

Dioxane, Eastman, C.P.

B. Solutions

A 0.2N solution of sodium hydroxide was prepared carbonate-free by dissolving 18 g. of sodium hydroxide in a liter of water, treating with barium chloride, filtering off the barium carbonate, and making the solution up to two liters. The solution was protected from carbon dioxide by a calcium chloride tube filled with ascarite. The solution was standardized by titrating weighed portions of potassium acid phthalate with this solution to a visual end-point using phenolphthalein indicator (three drops of a 1 per cent solution in aqueous ethanol).

An approximately 0.2M solution of p-nitrobenzoyl chloride in pyridine was prepared by dissolving 37.2 g. of p-nitrobenzoyl chloride in one liter of pyridine. It was found necessary to heat the solution gently in order to completely dissolve the solid. The solution was protected from water vapor.

C. Apparatus

A Beckman model H2 pH meter, equipped with a glass electrode and a saturated calomel electrode, was used for the pH titrations.

For the measurement of nitrogen gas the particular arrangement used resembles, with modifications, the apparatus designed and constructed by Nicolas and Mansel (5). The modifications include the use of one gas buret instead of two, cylinder carbon dioxide, and a three-neck, round bottom flask.

D. Acylation Procedure

With only two variations the acylation of tetrazole samples was accomplished by refluxing five previously weighed samples and one blank

for two to fifteen minutes. Each sample contained approximately one millimole of tetrazole and a 20 ml. aliquot 0.2M solution of p-nitrobenzoyl chloride in pyridine. The blank contained a 20 ml. aliquot of the 0.2M solution of p-nitrobenzoyl chloride in pyridine. In the acylation of the monohydrate samples a 25 ml. aliquot of the 0.2M solution of p-nitrobenzoyl chloride in pyridine was used in all samples and blanks. Where sample solutions were more suitable, approximately ten millimoles of tetrazole were weighed out and dissolved in pyridine. This solution was transferred to a 100 ml. volumetric flask, and 10 ml. aliquots were used as samples.

After refluxing for the prescribed length of time, the samples and blank are treated with about 25 ml. of distilled water and removed from the hot plate on which they were heated.

When the flasks have cooled to room temperature, the solutions are transferred to 400 ml. beakers. In the process of transferring, the small amount of precipitate which clings to the walls of the flask is dissolved with a small amount of pyridine and also transferred to the beakers.

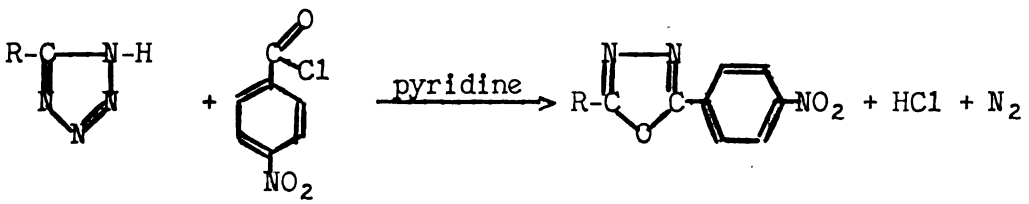
E. Titration Procedure

It was realized early in this investigation that a visual end-point indication would be impractical in the acidimetric titration of the refluxed solutions since these solutions were usually highly colored. As a result it became necessary to use a pH meter and titrate to a predetermined apparent pH.

The standardization control of the pH meter was adjusted to a pH of seven with a buffered aqueous solution. During the process of the titration, the samples and blanks were stirred continuously with a magnetic stirrer. The blank and all samples were titrated with standard sodium hydroxide to an apparent pH of 9.5.

F. Calculation of Results

The over-all acylation reaction between p-nitrobenzoyl chloride and any 5-substituted alkyl or aryl tetrazole, as described by Huisgen et al, (4) is as follows:



If one considers only the acidity of the acid chloride in the reaction mixture, there is an over-all loss of one equivalent of acidity for every mole of tetrazole which reacts. It also follows from this that the value obtained by subtracting the milliequivalents of acidity of the sample from the milliequivalents of acidity of the blank is a measure of the millimoles of tetrazole in the original sample if the acylation reaction is quantitative. Therefore, the quantitative nature of the acylation reaction can be expressed by the value, #Meq/Mm, when it is understood that this represents the over-all decrease in the number of milliequivalents of acidity per millimole of tetrazole in the sample. Table I shows the relation between per cent reaction and #Meq./Mm.

TABLE I

RELATION BETWEEN PERCENT REACTION AND #MEQ./MM.

Percent Reaction	#Meq./Mm.
0	-1.0
25	-0.5
50	0.0
75	0.5
100	1.0

G. Initial Work

1. Determination of Most Effective Solvent

Pyridine was chosen as the solvent for the acylation reaction because this is the solvent used by Huisgen et al (4). The possibility of using another solvent was not investigated in this work since pyridine proved to be effective.

2. Determination of the Most Effective Acylating Agent

The original work in this investigation was carried out using 3,5-dinitrobenzoyl chloride as the acylating agent. The solubility of this compound in pyridine is high enough to prepare a 0.365M solution; however, the solution must be heated in order to completely dissolve the solid. Once the solid is dissolved, the solution becomes intensely colored red within two hours. The intense color of this solution was considered a disadvantage since it was impossible to read the meniscus of the solution in the process of pipeting aliquots. While early work with 3,5-dinitrobenzoyl chloride indicated that it could be effectively used as an acylating agent, it was discarded because of its color.

The next two compounds to be considered were benzoyl and acetyl chlorides. While these two compounds did not give colored solutions

in pyridine, they formed relatively insoluble white precipitates. In the case of benzoyl chloride, the white precipitate can be dissolved by heating the solution strongly. However, both benzoyl and acetyl chlorides were discarded because of the insoluble precipitates which these two form in pyridine.

The most effective acylating agent was found to be p-nitrobenzoyl chloride. This compound is easily soluble in pyridine and can be dissolved in that solvent with only gentle heating. On the other hand, this compound resembles 3,5-dinitrobenzoyl chloride in that it also eventually forms a colored solution. The color, however, forms more slowly and is not as intense.

It was found that the increase in color intensity was accompanied by a decrease in acid strength as measured by an acidimetric titration with standard base using a pH meter. Figure 1 shows a typical apparent pH titration curve of p-nitrobenzoyl chloride in a 25 per cent aqueous pyridine solution. It can be seen that the end-point occurs at an apparent pH value of 9.5. Table II shows the variation of the acidic strength of this solution with time standing. As a result of this variation, it was considered necessary to run a blank containing the same aliquot of acylating solution as in each sample. Also, Table II depicts the variation in the acidic strength of the acylating reagent with the length of time refluxed. Again, for this reason it is necessary to reflux the blank for the same length of time that the samples are refluxed.

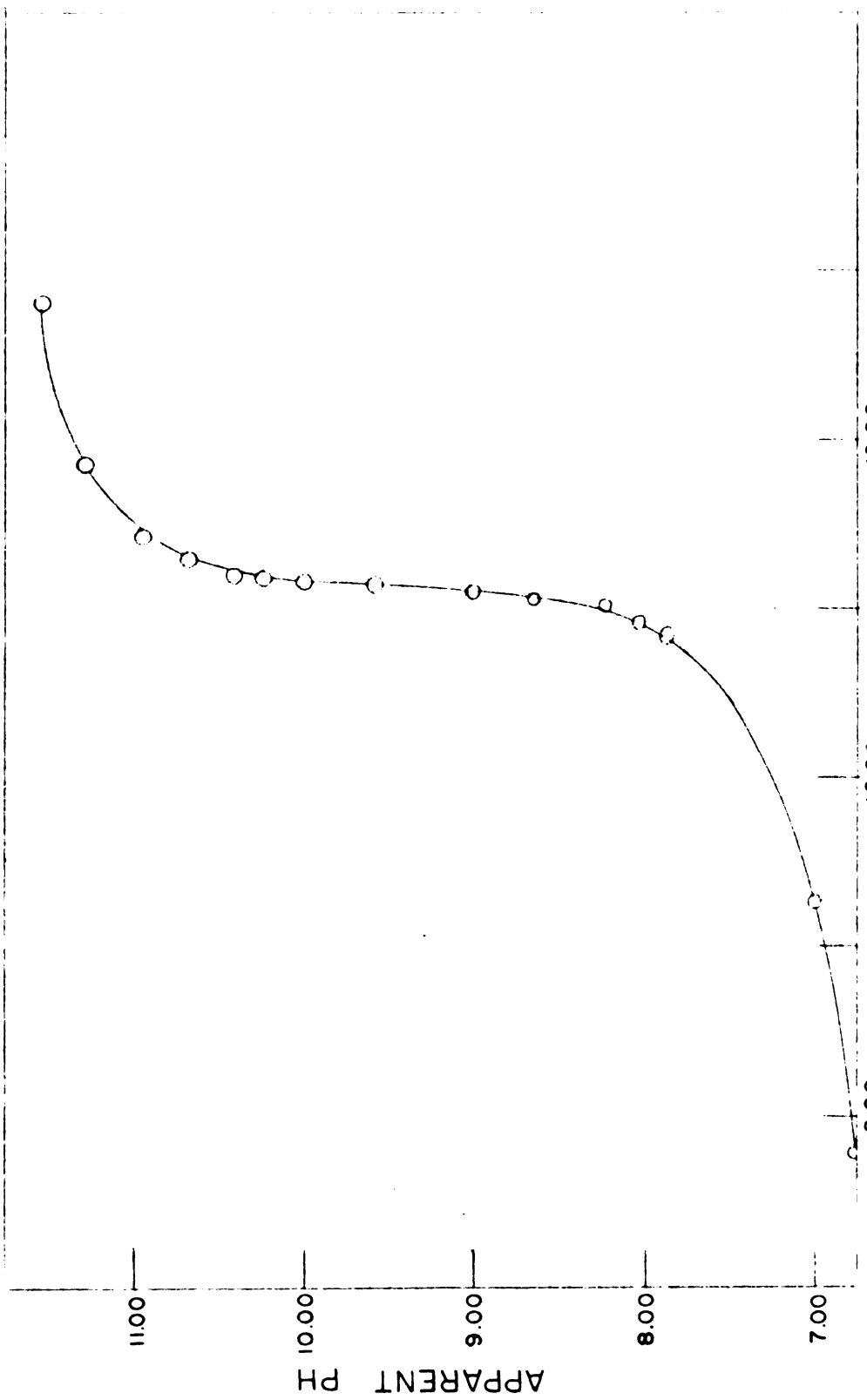


FIGURE I TITRATION CURVE OF P-NITROBENZOYL CHLORIDE IN A 3:1 WATER-PYRIDINE SOLUTION

TABLE II

VARIATION OF THE ACID STRENGTH OF THE ACYLATING REAGENT WITH TIME STANDING AND REFLUX TIME

Time Standing Days	Meq. Acid Found*			
	Reflux Time, Min.			
	0	2	15	30
0	3.879	3.933	3.933	3.925
1	3.906	3.912	3.912	3.912
2	3.871	3.901	3.900	3.887
3	3.801	3.858	3.855	3.855
4	3.774	3.839	3.837	---

*20 ml. aliquots were titrated to an apparent pH of 9.5 with 0.2694N NaOH and the strength of the reagent is reported in terms of milliequivalents of acidity per 20 ml. aliquot.

3. Determination of the Most Effective Molar Ratio of Acid Chloride to Tetrazole in the Reaction Mixture

Table III shows the results of varying the ratio of acid chloride to tetrazole in the reaction mixture. It shows that a molar ratio of at least 3:1 is necessary in order to make the acylation reaction proceed quantitatively. The disadvantage of using a large excess of reagent is that there is a change of only one milliequivalent in the presence of six milliequivalents. The experimental conditions used in this investigation include the use of a 4:1 molar ratio in order to insure quantitative acylation in every case. Furthermore, for monohydrate samples it appears necessary to use a 5:1 molar ratio of acid chloride to tetrazole in order to assure the presence of a four molar excess of acid chloride for acylation.

TABLE III

EFFECT OF MOLAR RATIO OF ACID CHLORIDE TO TETRAZOLE ON THE QUANTITATIVE NATURE OF THE ACYLATION REACTION

Molar Ratio	Mg. Tetrazole*	Ml. of 0.2M Acid chloride Solution	NaOH		Mmole Unreacted Tetrazole
			Ml.	N	
5:0	0	25	35.04	0.3009	0
1:1	354.7	12.50	18.19	0.3009	0.55
2:1	148.3	10.00	11.32	0.3009	0.10
3:1	205.2	21.00	24.78	0.3009	0.00
4:1	181.4	24.00	28.90	0.3009	0.00

*5-Phenyltetrazole was refluxed with p-nitrobenzoyl chloride for fifteen minutes.

4. Determination of the Most Effective Reflux Time

Table IV shows the results of varying the reflux time and its effect on the quantitative nature of the acylation reaction. As indicated previously, the value of #Meq./Mm. describes the quantitative nature of the reaction. It can be seen from Table IV that a reflux time between two and fifteen minutes is necessary in order to obtain quantitative acylation.

TABLE IV

EFFECT OF VARYING REFLUX TIME ON THE QUANTITATIVE NATURE OF THE ACYLATION REACTION

Reflux Time Min.	Mg. Tetrazole*	Ml. of 0.2M Acid chloride Solution	Ml. of NaOH for blank - Ml. of NaOH for sample**	N NaOH	#Meq./Mm.
360	146.2	20	2.50	0.2694	0.662
120	146.2	20	3.41	0.2694	0.917
60	146.2	20	3.60	0.2694	0.970
30	146.2	20	3.62	0.2694	0.975
15	146.2	20	3.65	0.2694	0.983
2	146.2	20	3.72	0.2694	1.002

*5-Phenyltetrazole was refluxed with p-nitrobenzoyl chloride.

**A blank, containing 20 ml. of reagent solution, was run concurrently with each sample.

III. DISCUSSION OF RESULTS

A. Determination of 5-Alkyl and Aryl Substituted Tetrazoles

A number of 5-alkyl and aryl substituted tetrazoles were run in order to assure that acylation of these compounds was quantitative. Previous to the determination of these samples, the purity of the tetrazoles used was checked according to the method described in Experimental Procedures. Table V gives the purity of these samples titrated as monoprotic acids.

Furthermore, in order to make certain that the acylation procedure being used was following the same reaction reported by Huisgen *et al*, (4) the precipitate obtained in the acylation of 5-phenyltetrazole with p-nitrobenzoyl chloride was purified by recrystallization in ethyl acetate. The product of this particular acylation should be 2-phenyl-5-p-nitrophenyl-1,3,4-oxadiazole. Stolle' and Leverkus (7) have reported that this compound has a melting point of 209°C. The melting point of the substance obtained was 207°C. On this basis, it was assumed that the acylation processes involved in this investigation and those reported by Huisgen *et al* (4) are the same.

The determination values for the 5-alkyl and aryl tetrazoles can be found in Table VI. Also, Table VII compares the acidimetric determination. Much of the error involved in the deviation between these two methods of determination can be accounted for by the relatively high expected error of at least four parts per thousand for the acylation determination. This error is due to the necessity of taking four buret readings, each of which includes an error of approximately one

part per thousand. The average error for this determination, based on the comparisons in Table VII, is ±0.010 for the #Meq./Mm. value. This would correspond to a ten part per thousand error for this value

TABLE V

PURITY OF 5-ALKYL AND ARYL SUBSTITUTED TETRAZOLES

Mg. Sample	NaOH		#Meq./Mm.
	Ml.	Normality	
5-Phenyltetrazole			
152.9	5.24	0.2009	1.007
144.6	4.95	0.2009	1.006
5-Methyltetrazole			
184.5	12.25	0.1758	0.982
174.6	11.65	0.1758	0.986
5-Benzyltetrazole			
357.3	12.72	0.1758	1.000
347.4	12.34	0.1758	1.002
5-γ-Phenylpropyltetrazole			
407.4	12.24	0.1758	0.997
389.2	11.76	0.1758	0.999
5-(3'-Cyclohexylpropyl)tetrazole			
544.4	9.33	0.3009	1.002
635.0	10.90	0.3009	1.003

TABLE VI
DETERMINATION OF 5-ALKYL AND ARYL SUBSTITUTED TETRAZOLES BY ACYLATION WITH p-NITROBENZOYL CHLORIDE

Sample	Mg. Tetrazole	Ml. of 0.2M p-Nitrobenzoyl Chloride Solution	Ml. of NaOH		N NaOH	Reflux Time Min.	#Meq./Mm.
			Blank	Sample			
5-Phenyltetrazole							
1	154.4	20	38.90	33.65	0.2009	2	0.998
2	141.7	20	38.90	34.11	0.2009	2	0.993
3	123.6	20	38.90	34.74	0.2009	2	0.996
4	154.3	20	36.75	31.53	0.2009	2	0.993
5	151.9	20	36.75	31.94	0.2009	2	0.994
5-Methyltetrazole							
1	85.9	20	38.81	33.89	0.2009	2	0.967
2	77.3	20	38.81	34.34	0.2009	2	0.976
3	109.0	20	38.81	32.55	0.2009	2	0.970
4	117.5	20	38.81	32.04	0.2009	2	0.972
5	97.5	20	38.81	33.20	0.2009	2	0.971
5-Benzyltetrazole							
1	223.8	20	26.48	21.74	0.3009	2	1.020
2	155.7	20	26.48	23.11	0.3009	2	1.043
3	188.0	20	26.48	22.50	0.3009	2	1.020
4	179.7	20	26.48	23.18	0.3009	2	1.032
5	154.0	20	26.48	22.70	0.3009	2	1.013
5-γ-Phenylpropyltetrazole							
1	86.4	20	39.50	37.26	0.2010	2	0.981
2	149.1	20	39.50	34.18	0.2010	2	1.010
3	184.0	20	39.50	34.61	0.2010	2	1.006
4	180.4	20	39.50	34.65	0.2010	2	1.017
5	204.0	20	39.50	34.00	0.2010	2	1.020
5-(3'-cyclohexylpropyl)tetrazole							
1	177.8	20	39.40	34.88	0.2010	2	0.993
2	208.7	20	39.40	34.01	0.2010	2	1.008
3	214.1	20	39.40	33.87	0.2010	2	1.009
4	196.4	20	39.40	34.35	0.2010	2	1.004
5	226.2	20	39.40	33.58	0.2010	2	1.005

TABLE VII

COMPARISON OF ACIDIMETRIC AND ACYLATION DETERMINATION OF 5-SUBSTITUTED TETRAZOLES

Average Purity	Acylation Value		Deviations
5-Phenyltetrazole			
1.007	0.999		-0.008
	0.993		-0.014
	0.997		-0.010
	0.994		-0.013
	0.994		-0.013
		Avg.	0.012
5-Methyltetrazole			
0.983	0.967		-0.015
	0.977		-0.006
	0.970		-0.012
	0.972		-0.010
	0.972		-0.011
		Avg.	0.011
5-Benzyltetrazole			
1.0014	1.020		+0.0198
	1.043		+0.0422
	1.020		+0.0193
	1.032		+0.0319
	1.013		+0.0128
		Avg.	0.021
5-γ-Phenylpropyltetrazole			
0.999	0.981		-0.018
	1.010		+0.010
	1.006		+0.010
	1.017		+0.009
	1.020		+0.009
		Avg.	0.011
5-(3'-Cyclohexylpropyl)tetrazole			
1.003	0.993		-0.010
	1.008		+0.001
	1.009		+0.001
	1.004		+0.000
	1.005		+0.000
		Avg.	0.002

B. Investigation of the Possible Acylation of 1-Substituted Tetrazoles and 1,5-Disubstituted Tetrazoles.

According to the reaction reported by Huisgen et al (4), 1-substituted tetrazoles and 1,5-disubstituted tetrazoles should not be acylated by the procedure used in this investigation.

It was found, however, that the 1-p-tolyltetrazole consumed acylating reagent to the extent that it appeared to be about 75 per cent acylated. It is not known for certain, though, whether this compound was acylated or underwent some other reaction. Unfortunately, little work has been done on the reactions of 1-substituted tetrazoles, and there is no information in the literature concerning this problem.

On the other hand, the 1,5-disubstituted tetrazoles used in this investigation did not appear to consume acylating reagent. In the attempted acylation of 1-phenyl-5-aminotetrazole there was a small apparent consumption of acylating reagent (see Table VIII). However, previous to the attempted acylation of both of the 1,5-disubstituted tetrazoles used, a purity check was performed according to the method described in Experimental Procedures. While the amount of standard base consumed in these titrations was so small as to preclude an accurate measure of the acidity of these compounds, the samples required about 0.02 milliequivalents of standard base per millimole of sample. It is this 0.02 milliequivalents of inherent acidity which accounts for the #Meq./Mm. value obtained for 1-phenyl-5-aminotetrazole. Therefore, it would appear from the evidence obtained that neither the 1-phenyl-5-aminotetrazole nor the 1-benzyl-5-aminotetrazole consumes acylating reagent. Moreover, it is interesting to note that there is

TABLE VIII

INVESTIGATION OF THE POSSIBLE ACYLATION OF 1-SUBSTITUTED TETRAZOLES AND 1,5-DISUBSTITUTED TETRAZOLES

Sample	Mg. Tetrazole	Ml. of 0.2M p-Nitro-benzoyl Chloride	Ml. of NaOH		N NaOH	Reflux Time Min.	#Meq./Mm..
			Blank	Sample			
1-Tolyltetrazole							
1	152.7	20	40.10	37.92	0.2012	2	0.4630
2	156.8	20	40.10	37.46	0.2012	2	0.5461
3	191.1	20	40.10	36.90	0.2012	2	0.5428
4	175.2	20	40.10	37.18	0.2012	2	0.5405
5	173.3	20	40.10	37.28	0.2012	2	0.5278
6	162.1	20	26.38	25.25	0.3009	15	0.3380
7	150.6	20	26.38	25.53	0.3009	15	0.2737
8	164.6	20	26.38	25.58	0.3009	15	0.0884
9	156.7	20	26.38	25.00	0.3009	15	0.4502
10	199.4	20	26.38	24.83	0.3009	15	0.3770
1-Phenyl-5-aminotetrazole							
1	197.9	20	39.34	39.23	0.2045	2	0.0183
2	192.4	20	39.34	39.23	0.2045	2	0.0188
3	196.9	20	39.34	39.23	0.2045	2	0.0184
4	183.3	20	39.34	39.21	0.2045	2	0.0234
5	178.5	20	39.34	39.20	0.2045	2	0.0258
6	205.1	20	38.50	36.66	0.2052	15	0.0111
7	209.1	20	38.50	37.80	0.2052	15	0.0152
8	217.2	20	38.50	37.50	0.2052	15	0.0163
9	198.2	20	38.50	37.03	0.2052	15	0.0245
1-Benzyl-5-aminotetrazole							
1	240.7	20	26.42	26.38	0.3009	2	--
2	229.9	20	26.42	26.41	0.3009	2	--
3	258.6	20	26.42	26.40	0.3009	2	--
4	246.9	20	26.42	26.40	0.3009	2	--
5	250.1	20	26.42	26.42	0.3009	2	--
6	268.6	20	26.36	26.36	0.3009	15	--
7	250.3	20	26.36	26.33	0.3009	15	--
8	261.3	20	26.36	26.34	0.3009	15	--
9	252.8	20	26.36	26.35	0.3009	15	--
10	318.1	20	26.36	26.35	0.3009	15	--

no evidence for rearrangement of either of the 1-substituted-5-amino-tetrazole compounds, to the corresponding 5-substituted aminotetrazoles as reported by Garbrecht and Herbst (6). If a rearrangement of this type had occurred, this would have been reflected in a higher #Meq./Mm. value since the compounds formed would have been acylated to some degree.

C. Determination of 5-Substituted Aminotetrazoles

Of the four types of substituted tetrazoles which have been studied in this investigation, the aminotetrazoles were the only compounds which did not appear to be quantitatively acylated. Previous to the acylation of these 5-substituted aminotetrazoles a purity check was performed according to the method described in Experimental Procedures.(See Table IX). It can be seen from the values of #Meq./Mm. in Table X that these compounds appeared to be from 60 per cent to 100 per cent acylated; however, there can be noticed much variation in these values. There is one notable exception to this trend. The acylation of 5-diethylaminotetrazole is quantitative.

The results obtained from the acylation of the monosubstituted aminotetrazoles can be explained in light of the work done by Garbrecht and Herbst (6) in which the rearrangement of 5-benzylamino-tetrazole to 5-amino-1-benzyltetrazole was studied. It was found that this rearrangement occurred when the compound was held at its melting point for a few minutes. In a subsequent study using 1-phenyl-5-amino-tetrazole, it was found that this rearranged by an equilibrium process as it was possible to go from one isomer to the other.

TABLE IX

PURITY OF 5-SUBSTITUTED AMINOTETRAZOLES

Mg. Sample	NaOH		#Meq./Mm.
	Ml.	Normality	
5-Aminotetrazole monohydrate			
231.4	13.02	0.1758	1.012
247.6	13.90	0.1758	1.013
5-Acetylaminotetrazole			
270.4	12.11	0.1758	1.001
274.0	12.21	0.1758	0.996
5-Benzylaminotetrazole			
362.6	11.70	0.1758	0.981
354.7	11.44	0.1758	0.983
5-Diethylaminotetrazole			
404.9	9.51	0.3009	0.998
458.5	10.80	0.3009	1.001

Assuming that this rearrangement is taking place in the refluxing solution of tetrazole and acid chloride, it would be expected that the 1-subsituted-5-aminotetrazole formed would not be acylated. This could account for the low value for #Meq./Mm. for all of the monosubstituted aminotetrazoles.

On the other hand, a semiquantitative gasometric analysis using the apparatus described previously in Experimental Procedures, was made on 5-aminotetrazole in order to collect any nitrogen gas which might be liberated as a result of acylation. The volume of nitrogen gas received indicated that the 5-aminotetrazole was being quantitatively acylated. Again, assuming that this is true, the low value for #Meq./Mm.

TABLE X

DETERMINATION OF 5-SUBSTITUTED AMINOTETRAZOLES

Sample	Mg. Tetrazole	Ml. of 0.2M p-Nitro-benzoyl Chloride	Ml. of NaOH		N NaOH	Reflux Time Min.	#Meq./Mm.
			Blank	Sample			
5-Aminotetrazole							
1	110.5	25	34.99	32.77	0.3009	2	0.624
2	123.8	25	34.99	34.07	0.3009	2	0.231
3	103.2	25	34.99	32.90	0.3009	2	0.629
4	108.1	25	34.99	33.00	0.3009	2	0.571
5	115.9	25	34.99	33.80	0.3009	2	0.319
5-Acetylaminotetrazole							
1	128.9	20	40.08	36.47	0.2012	2	0.716
2	126.3	20	40.08	36.58	0.2012	2	0.709
3	143.2	20	40.08	36.09	0.2012	2	0.712
4	140.9	20	40.08	36.11	0.2012	2	0.720
5	142.4	20	40.08	35.89	0.2012	2	0.751
5-Diethylaminotetrazole							
1	176.4	20	40.04	33.80	0.2012	2	1.005
2	158.6	20	40.04	34.43	0.2012	2	1.005
3	160.4	20	40.04	34.28	0.2012	2	1.003
4	141.2	20	40.04	35.01	0.2012	2	1.011
5	141.1	20	40.04	35.05	0.2012	2	1.005
5-Phenylaminotetrazole							
1	157.6	20	40.02	38.63	0.2012	2	0.306
2	171.0	20	40.02	38.71	0.2012	2	0.248
3	145.4	20	40.02	38.78	0.2012	2	0.277
4	169.9	20	40.02	38.90	0.2012	2	0.214
5	175.6	20	40.02	38.93	0.2012	2	0.201
6	111.9	20	38.84	38.04	0.2045	15	0.236
7	111.9	20	38.84	38.25	0.2045	15	0.173
8	111.9	20	38.84	38.10	0.2045	15	0.218
9	111.9	20	38.84	38.24	0.2045	15	0.177
10	111.9	20	38.84	38.16	0.2045	15	0.194

TABLE X (Cont.)

Sample	Mg. Tetrazole	Ml. of 0.2M p-Nitro-benzoyl Chloride	Ml. of NaOH		N NaOH	Reflux Time Min.	#Meq./Mm.
			Blank	Sample			
5-Benzylaminotetrazole							
1	184.9	20	27.63	23.66	0.3009	2	1.144
2	219.6	20	27.63	23.60	0.3009	2	0.973
3	205.5	20	27.63	24.26	0.3009	2	0.869
4	214.3	20	27.63	23.90	0.3009	2	0.923
5	213.6	20	27.63	23.91	0.3009	2	0.923
6	284.2	20	27.68	21.80	0.3009	15	1.097
7	205.5	20	27.68	23.63	0.3009	15	1.045
8	195.0	20	27.68	24.20	0.3009	15	0.946
9	173.9	20	27.68	24.50	0.3009	15	0.969
10	215.3	20	27.68	24.18	0.3009	15	0.862

obtained for 5-aminotetrazole must be explained by some process other than incomplete acylation.

The precipitate formed on acylating 5-aminotetrazole was purified by recrystallization from dioxane. Figure 2 shows the acidimetric titration in 25 per cent aqueous pyridine with standard sodium hydroxide using a pH meter. It is apparent that the substance acts as a monoprotic acid. The equivalent weight of this substance, when calculated from the data presented in Figure 2 is 267.9 g. This value is between the equivalent weights for 2-amino-5-p-nitrophenyl-1,3,4-oxadiazole and the p-nitrobenzoylated derivative of this compound, 2-p-nitrobenzamido-5-p-nitrophenyl-1,3,4-oxadiazole. This would indicate that a mixture of these two compounds is formed when 5-aminotetrazole is acylated with p-nitrobenzoyl chloride. That the substance isolated acts as a monoprotic acid in the acylating medium is shown in Figure 3.

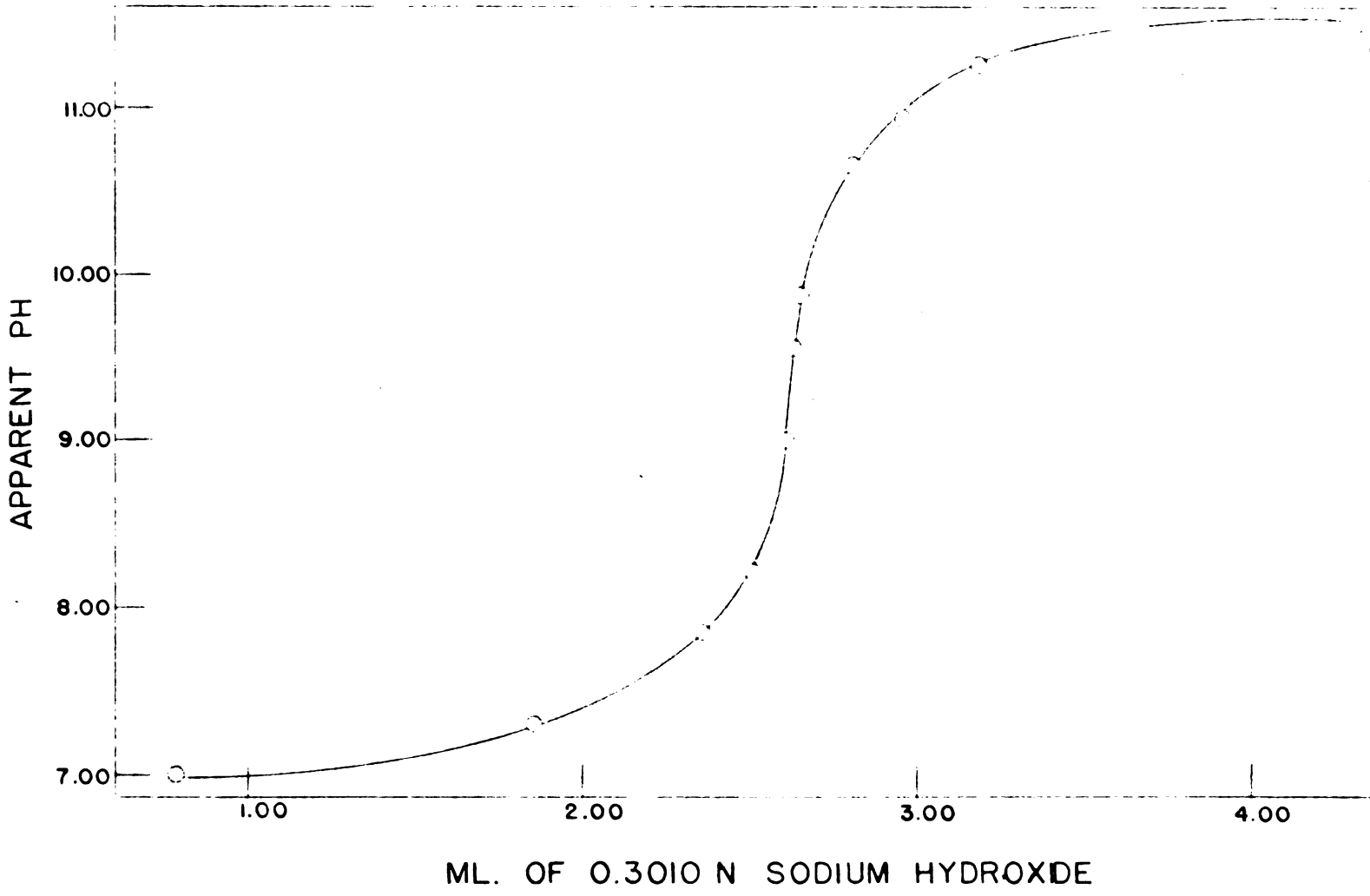


FIGURE 2. TITRATION CURVE OF PURIFIED PRODUCT FROM THE ACYLATION OF 5-AMINOTETRAZOLE IN A 3:1 WATER-PYRIDINE SOLUTION

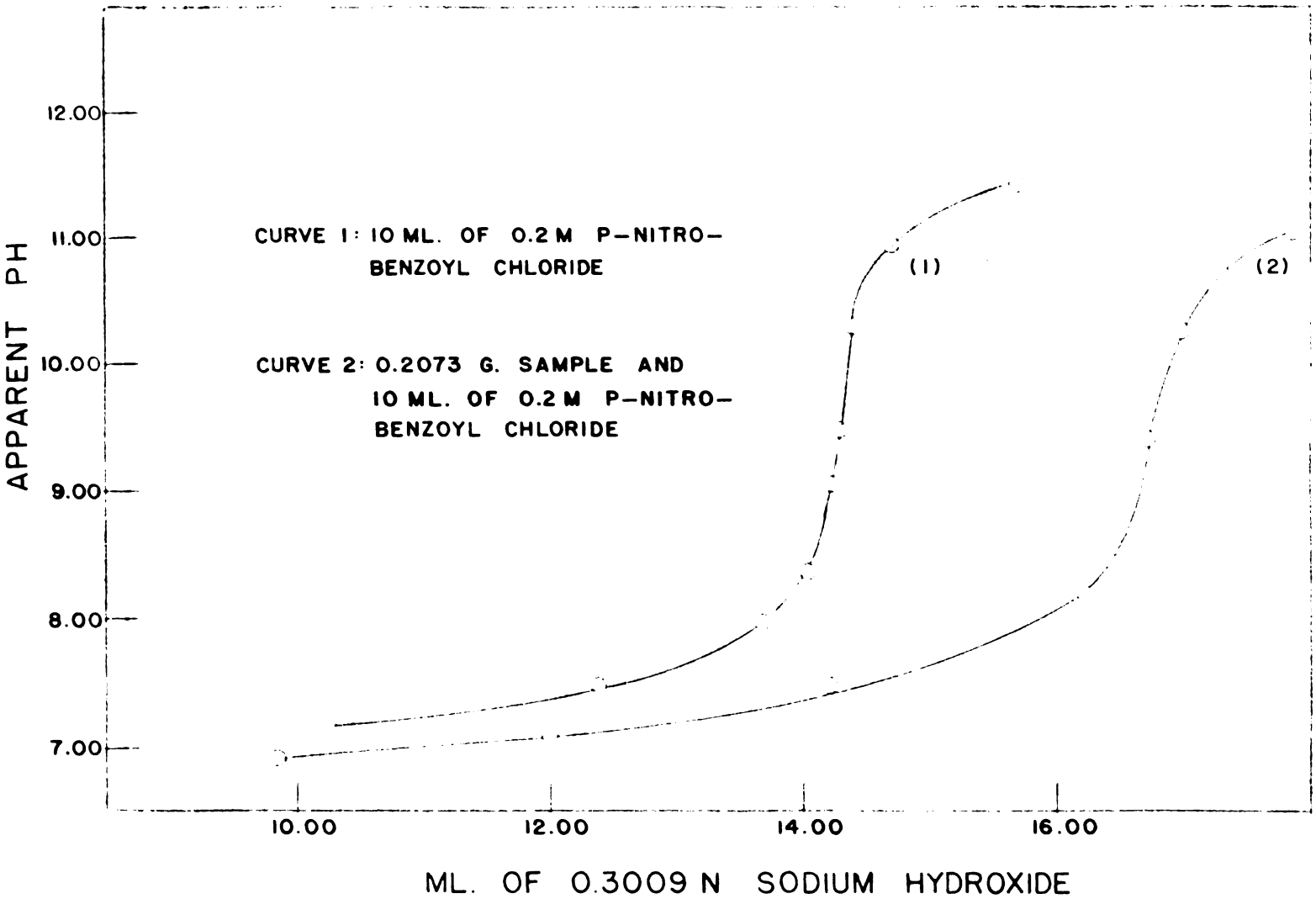


FIGURE 3. TITRATION CURVES OF PURIFIED PRODUCT FROM THE ACYLATION OF 5-AMINOTETRAZOLE

Curve 1 is an apparent pH titration of a blank containing a 10 ml. aliquot of 0.2M solution of p-nitrobenzoyl chloride in pyridine. Curve 2 represents the same aliquot of acylating reagent and a weighed sample of the substance isolated from the acylation of 5-aminotetrazole. The equivalent weight of the substance according to the data shown in this Figure is 261.7 g.

From a similar study in which pure-2-benzamido-5-phenyl-1,3,4-oxadiazole was titrated acidimetrically in a 25 per cent aqueous pyridine solution and in the acylation medium, it was found that this acts as a monoprotic acid in both cases (see Figure 4).

This evidence suggests that the acylation of 5-aminotetrazole is accompanied by one other reaction besides the oxadiazole formation. It is postulated that after the quantitative formation of the 2-amino-5-p-nitrophenyl-1,3,4-oxadiazole, this compound is partially benzoylated. The resulting mixture, composed of the 2-amino- and the 2-p-nitrobenzamido-1,3,4-oxadiazoles, is titrated as a monoprotic acid. However, to explain the low results obtained, in spite of apparent acylation, both the benzoylated and the unbenzoylated oxadiazoles must be acidic. The acidity of the benzoylated oxadiazole has been shown. The acidity of the 2-amino-1,3,4-oxadiazole can be postulated on the basis of the conjugated system between the amino group and the p-nitrophenyl group which would have a tendency to delocalize the electrons around the amino nitrogen toward the oxadiazole ring. The decrease in the #Meq./Mm. value must be attributed to the milliequivalents of the unbenzoylated 2-amino-1,3,4-oxadiazole present in the titration mixture since the milliequivalents of acidity lost due to consumption of reagent

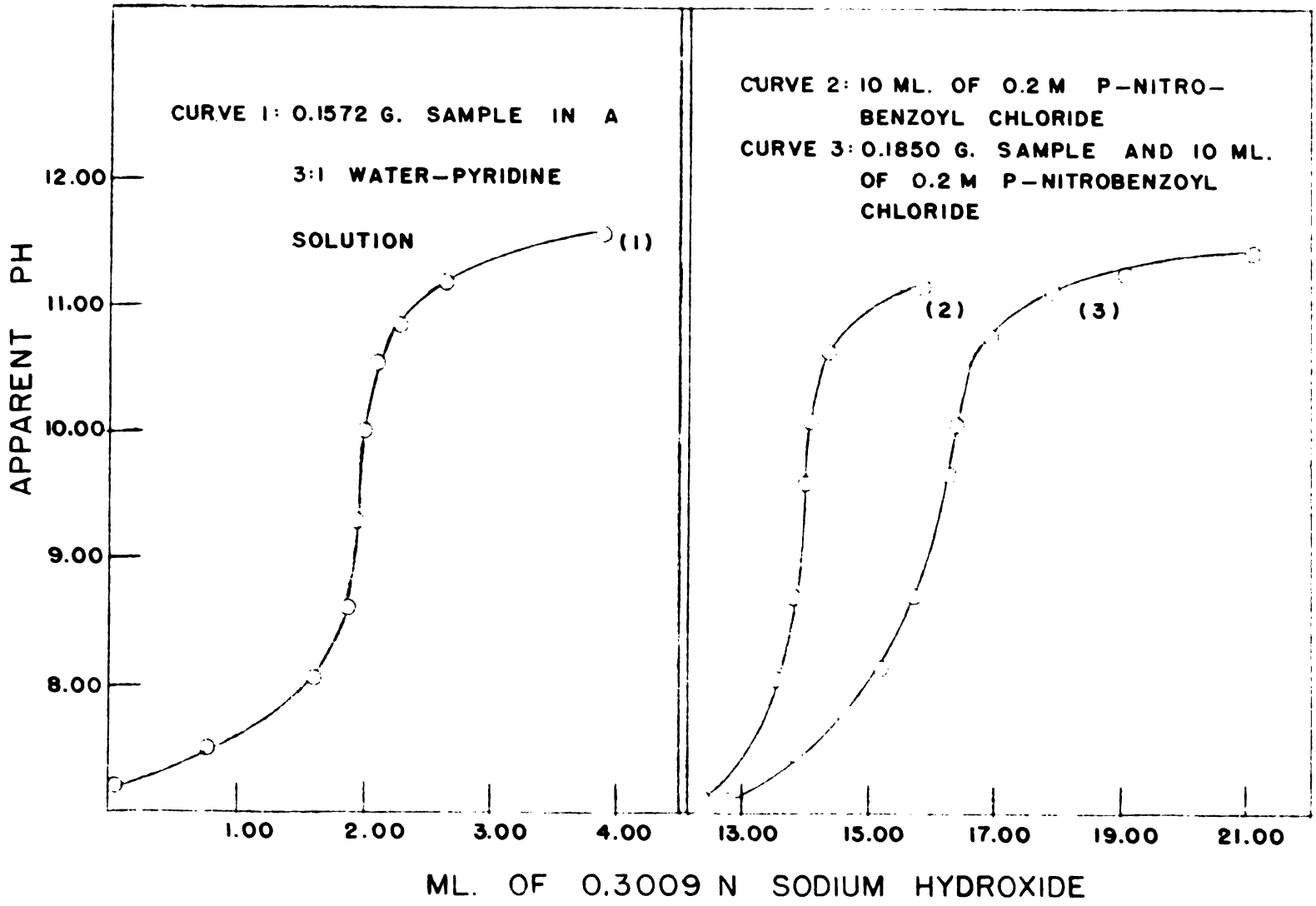


FIGURE 4. TITRATION CURVES OF 2-BENZAMIDO-5-PHENYL-1,3,4-OXADIAZOLE

in the benzoylation process are gained by the acidity of the benzoylated 2-amino-1,3,4-oxadiazole.

From the preceeding discussion it is evident that the determination of 5-aminotetrazole and 5-substituted aminotetrazoles by acylation procedures is complicated by at least two other processes--the first which involves the rearrangement of 5-substituted aminotetrazoles and the second which involves the formation of acidic substances.

IV. SUMMARY AND CONCLUSIONS

Table XI summarizes the results obtained from the acylation of the 5-substituted tetrazoles used in this investigation.

It is evident from this table that the acylation of 5-alkyl and aryl tetrazoles is, within an [illegible] error of ±1.0 per cent, quantitative by the procedures used. The acylation method used in this work involved the refluxing of a sample (containing a weighed amount of tetrazole and an aliquot of a p-nitrobenzoyl chloride solution in pyridine) and a blank (containing the same aliquot of reagent solution). It was found that a four to one molar ratio of acid chloride to tetrazole should be present in the reaction mixture and that a reflux time from two to fifteen minutes was necessary for maximum acylation.

Nevertheless, it was discovered that with one exception, 5-substituted aminotetrazoles were not quantitatively acylated. The one exception to this trend is the quantitative acylation of 5-diethylaminotetrazole.

A number of studies were conducted in order to explain the results obtained from the acylation of this type of tetrazole. The volume of nitrogen gas evolved in the acylation of 5-aminotetrazole indicated that this particular reaction was quantitative. It was also found that the oxadiazole formed in the reaction was a mixture of 2-amino- and 2-p-nitrobenzamido-5-p-nitrophenyl-1,3,4-oxadiazole. The benzoylated compound acts as a monoprotic acid in the titration medium, but this does not explain the results obtained for 5-aminotetrazole. The

TABLE XI

SUMMARY OF DETERMINATIONS

Acidimetric Purity	Reflux Time Min.	Acylation Determination Value
5-Phenyltetrazole		
1.007	2	0.995
5-Methyltetrazole		
0.9834	2	0.972
5-Benzyltetrazole		
1.0014	2	1.022
5-γ-Phenylpropyltetrazole		
0.9985	2	1.002
5-(3'-cyclohexylpropyl)tetrazole		
1.003	2	1.005
1-p-Tolyltetrazole		
--	2	0.5240
	15	0.3045
1-Phenyl-5-aminotetrazole		
--	2	0.0209
	15	0.0168
1-Benzyl-5-aminotetrazole		
--	2	0.000
	15	0.000
5-Aminotetrazole		
1.013	2	0.4746
5-Acetylaminotetrazole		
0.9983	2	0.7217
5-Diethylaminotetrazole		
0.9992	2	1.006
5-Phenylaminotetrazole		
--	2	0.2491
	15	0.1997
5-Benzylaminotetrazole		
0.9821	2	0.967
	15	0.984

acidity lost in benzoylation would be gained from the acidity of the benzoylated compound. However, the low results obtained for the 5-substituted aminotetrazole can be explained by the rearrangement of the 5-substituted aminotetrazole to a 1-substituted-5-aminotetrazole which has been shown to consume no acid chloride.

Furthermore, in order to determine the possible interference of the presence of 1-substitutedtetrazoles, a sample was run and it was found to consume acid chloride, but not quantitatively.

From the above discussion, the following conclusions can be drawn:

1. The method described in this investigation can be used to determine 5-alkyl and aryl substituted tetrazoles.
2. The method described in this investigation cannot be used to determine 5-substituted aminotetrazoles.
3. The presence of 1-substituted-5-aminotetrazoles does not constitute an interference in the determination of tetrazoles.
4. The presence of 1-substituted tetrazoles does constitute an interference in the determination of tetrazoles.

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