4-AMINOPYRIDINE AS A STANDARD IN ACIDIMETRY

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By

Clayton E. Van Hall

AN ABSTRACT

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

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THESIS ABSTRACT

The known properties of 4-aminopyridine make it attractive as a standard in acidimetry. This thesis includes an investigation of synthetic methods for the preparation of 4-aminopyridine and an evaluation of its properties as a standard for acidimetry.

The Lossen and Schmidt reactions are not applicable for the synthesis of 4-aminopyridine. The cleavage of 4-pyridylpyridinium dichloride with solutions containing potassium hydroxide, sodium hydroxide, ammonium hydroxide, or sodium carbonate results in very poor yields. 4-Aminopyridine can be separated from mixtures by steam distillation from an alkaline solution. A very pure product is obtained.

4-Aminopyridine satisfied many of the requirements of a standard for use in acidimetry. The neutralization of 4-aminopyridine with a strong acid is stoichiometric and methyl red indicator changes color at the equivalence point. The indicator blank is small and easily determined. Results agree to within one part per thousand with results obtained using two other standards, potassium acid phthalate and sodium carbonate. 4-Aminopyridine is not hygroscopic, and carbon dioxide has no effect on the titration. 4-Aminopyridine has a definite vapor pressure which causes substantial losses on prolonged heating. This property also enables purification by sublimation. 4-Aminopyridine may be recovered easily and economically.

The one major disadvantage of 4-aminopyridine is the fact that it is not commercially available.

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I. INTRODUCTION

A. Discussion of Primary Standards

Standard solutions assume an important role in all fields of chemistry, and because of this importance, much attention has been focused upon the development of primary standard materials for the preparation of solutions. Investigators are continually searching for new materials which might possess the desired properties, and they are developing refinements in the manufacture of known materials in order to make available substances in the high state of purity necessary for their use as standards.

An excellent review of the many aspects of standards appears in the papers presented at the Fourth Annual Summer Symposium sponsored by the Division of Analytical Chemistry in 1951 and published in Analytical Chemistry. An excellent definition of a primary standard which appears in one of these papers is as follows: Primary standards are chemical substances which by virtue of their purity can be weighed directly, either for the purpose of assaying a volumetric solution of unknown strength or for the preparation of a determinate solution of the substance itself (8).

To be considered for such use a chemical substance should meet a number of requirements.

1. It should be easy to obtain.
2. It should be easy to purify and dry.
3. It should be preservable in a pure state.
4. It should have a high equivalent weight.

5. It should react stoichiometrically.
6. It should be capable of being tested for impurities by qualitative tests of known sensitivity.
7. It should not be so hygroscopic as to take up moisture during weighing.
8. The indicator error should be negligible or easily determined.

There are approximately a dozen substances which have been recommended for the standardization of acids. These include such materials as sodium carbonate, sodium oxalate, thallous carbonate, potassium bicarbonate, sodium bicarbonate, potassium iodate, mercuric oxide, borax, diphenylguanidine, and tris(hydroxymethyl)aminomethane. In addition to these standards, other methods are available for obtaining standard acids. The use of constant boiling hydrochloric acid, sulfuric acid, and perchloric acid has been recommended by various authors for the preparation of standard solutions of these acids.

None of the recommended substances, however, possesses all of the properties desired of a primary standard. For each substance mentioned previously, some definite disadvantage can be noted. For example, sodium carbonate is hygroscopic and has a low equivalent weight. All carbonates and bicarbonates must be ignited to assure definite composition. Borax is a hydrate and care must be used in its preparation and storage. Diphenylguanidine requires an alcohol solvent and tris-(hydroxymethyl)aminomethane is unstable on heating and lacks a suitable indicator. In addition, both of the latter two substances are weak bases as compared to inorganic bases.

Because all standards now in use and those that have been recommended for use do not fill all of the requirements for standards, there

is still a definite need for a good standard for use in acidimetry.

A search for new substances which might serve as standards could lead naturally to organic compounds and nitrogen bases in particular.

Two properties which are of first consideration in a search are the melting point and the basicity of the substance. Most of the known nitrogen bases do not have desirable properties as far as standards are concerned. The common amines which are strong enough as bases are usually liquids or gases of low molecular weight which automatically excludes them from consideration, and solid amines of higher molecular weight usually are too weak as bases which tends to eliminate them unless some solvent is used to enhance their basicity.

B. 4-Aminopyridine as a Standard

An organic base which may possess the properties desired of a standard is 4-aminopyridine. 4-Aminopyridine is a weak monoacidic base with a molecular weight of 94.12. Two values for the ionization constant have been reported in the literature. Tropsch, in 1934, determined a value of 1.3×10^{-8} at 25° C. by conductivity measurements (20). In 1952, Albert reported a value for the pK_a of 9.2 which corresponds to a value of 1.6×10^{-8} for K_b (2). In comparison, diphenylguanidine has a K_b of 6.1×10^{-2} and tris(hydroxymethyl)aminomethane has a K_b of 1.2×10^{-6}.

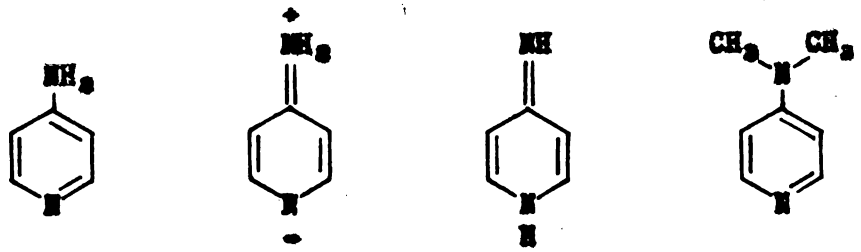
4-Aminopyridine is soluble in water and alcohol and moderately soluble in benzene and chloroform. Definite solubilities have been reported

in only one instance; Albert states that its solubility in water at 20° C. is 1:12 and in chloroform 1:40 at 20° and 1:20 at 61° C. (2).

4-Aminopyridine has a melting point of 159° C. which is quite high for an amine of low molecular weight. In contrast, 2-aminopyridine has a melting point of 58° C., and 3-aminopyridine has a melting point of 64° C.

A variance of melting points among isomers of a substance is not uncommon, and at times it can be taken as an indication of structural differences between the isomers. The difference of almost 100° between the melting point of 4-aminopyridine and the melting points of 2-, and 3-aminopyridine indicates that 4-aminopyridine must exist in some tautomeric form that is not characteristic of the 2- and 3-isomers.

A number of structures have been proposed for 4-aminopyridine, but there is still a question as to which is correct.



I II III IV

Lois and Curran, in their investigation of the dipole moments of several pyridine compounds, determined a dipole moment of 4.36 debye units for 4-aminopyridine in a dioxane solvent (16). The authors proposed that this high dipole moment resulted from a strong contribution of structure II. Previously Sidgwick had proposed that 4-aminopyridine could exist in

a tautomeric form, XXI (18). As this form would have a low dipole moment, Leis and Curran ruled out the presence of this form in any appreciable amount. Anderson and Seeger found that 4-aminopyridine possessed only one ultraviolet absorption band which corresponds to the same absorption band possessed by 4-dimethylaminopyridine, IV, which is unable to tautomerize (3). From this evidence they concluded that only the pyridine ring structure, I, can be assigned to 4-aminopyridine.

C. Statement of Problem

The purpose of this work was to investigate the properties of 4-aminopyridine to determine if the substance would fill the requirements of a standard. New methods of preparation of 4-aminopyridine were also investigated as the known methods were inadequate due to lengthy procedures and low yields.

XI. EXPERIMENTAL

A. The Preparation of 4-Aminopyridine

1. Previous methods of preparation.

The first reported synthesis of 4-aminopyridine was by Camps in 1902, who prepared the compound from isonicotinamide by the Hofmann synthesis (4). In that same year Kirpal also reported the preparation from isoquinolinic acid by the same procedure (11). More recent investigators have also recommended the Hofmann synthesis. In 1914, Chichibabin and Zeide reported 4-aminopyridine as a minor product in the amination of pyridine with sodamide (5). This same method is the basis for a German patent issued in 1923. In 1915, Rument and Dorn reported a preparation from 4-chloropyridine. The chlorine atom is replaced by an amino group by heating 4-chloropyridine in an ammoniacal zinc chloride solution for five hours in an autoclave at 230° C. (7). In 1924, Koenigs, Kisme, and Weiss prepared 4-aminopyridine from 4-chlorodipicolinic acid. On heating this compound in an autoclave for 12 hours with concentrated ammonium hydroxide at 150° C., the 4-chlorodipicolinic acid is converted to 4-aminodipicolinic acid which is then decarboxylated by heating with calcium oxide (12). The most widely used method of preparation was introduced by Koenigs and Greiner in 1931 (13). They prepared 4-aminopyridine from 4-pyridylpyridinium dichloride by cleaving this compound by heating with concentrated ammonium hydroxide in an autoclave for eight hours at 150° C. Yields of 60% were reported by this method. Koenigs and Greiner also found that some 4-aminopyridine

was produced when the cleavage was carried out using concentrated potassium hydroxide at normal pressure and reflux temperatures. In an attempt to reproduce the results of Koenigs and Greiner, more recent investigators found that the method was very erratic and seldom produced the yields originally claimed. As a result, Albert in 1950 developed a new method for cleaving the 4-pyridylpyridinium dichloride (2). Ammonia gas was bubbled through a boiling phenol solution of the salt, which resulted in an 80% yield of 4-aminopyridine. In the same year Hauser and Reynolds were able to obtain a maximum yield of 40% by refluxing 4-pyridylpyridinium dichloride with concentrated ammonium hydroxide for eight hours (9). Yields of 70% were obtained by these workers using the Hofmann synthesis. A very promising method for the preparation of 4-aminopyridine was developed in 1950 by den Hertog and Overhoff (6) and also Ochiai (17). 4-Nitropyridine-N-oxide was reduced with iron in glacial acetic acid resulting in a 90% yield of 4-aminopyridine. The 4-nitropyridine-N-oxide was prepared from pyridine-N-oxide which in turn was prepared from pyridine and perphthalic acid. The most recent paper on the preparation of 4-aminopyridine appeared in 1954. Wibaut, Hersberg, and Schlatmann investigated the original preparation of Koenigs and Greiner and were able to reproduce the results originally claimed by these workers (21).

2. Preparation by the Lossen reaction.

Several methods for the preparation of 4-aminopyridine were investigated in this work. The first method attempted was an application of

the Lossen rearrangement, and came as a result of the work of Snyder, Elston, and Kellom, who prepared various amines from the corresponding acids by this method (19). The procedure followed by these workers was to heat the acid with hydroxylamine hydrochloride in the presence of polyphosphoric acid. The resulting hydroxamic acid rearranged to an isocyanate which then decomposed to an amine and carbon dioxide.

$$RCOOH \xrightarrow[NH_2OH.HCl]{\text{Polyphosphoric Acid}} RCONHOH \longrightarrow RNCO \longrightarrow RNH_2 + CO_2$$

R = phenyl group, substituted phenyl group or naphthyl groups

Excellent yields of amines were obtained by this method from a wide variety of acids. It was felt that this synthesis might apply to the preparation of 4-aminopyridine from isonicotinic acid which is commercially available. The original method was varied in only one respect which was the recovery of the amine. This was due to the solubility of 4-aminopyridine in water.

Reagents:

Isonicotinic acid, obtained from Reilley Tar and Chemical Corp.

Hydroxylamine hydrochloride, Eastman White Label.

Polyphosphoric acid, obtained from Victor Chemical Works, 83% P_2O_5.

Sodium hydroxide, Merck Reagent Pellets.

Benzene, Merck Reagent Grade.

Sulfuric acid, DuPont C.P. Reagent, 95%.

Apparatus:

Soxhlet Extractor

Procedure:

Five grams (0.041 mole) of isonicotinic acid and 3.1 grams (0.045 mole) of hydroxylamine hydrochloride were added to 25 grams of polyphosphoric acid in an open 500 ml. tallform beaker. The mixture was stirred manually and heated slowly in an oil bath up to 180° C. At approximately 100° C., the evolution of hydrogen chloride was evident. No further reaction was noticed and the evolution of carbon dioxide did not take place on further heating. The hot mixture was poured onto crushed ice and the resulting solution made strongly alkaline by the addition of sodium hydroxide pellets. This solution was then evaporated to dryness on a steam bath. The solid residue was then pulverized and extracted with benzene in a soxhlet extractor. After 10 hours of extraction, the benzene was removed and evaporated to a small volume. On cooling, a white solid precipitated. This material had a melting point of 155-160° C. and was soluble in water, yielding an alkaline solution. Forty milligrams or 1% of the theoretical yield was recovered.

The same procedure as above was followed using 95% sulfuric acid in place of polyphosphoric acid. No trace of 4-aminopyridine was detected.

3. Preparation by the Schmidt reaction.

Another general method for the preparation of amines from the corresponding acids is the Schmidt reaction. A literature survey indicated that this method had never been tried for the preparation of 4-aminopyridine, and so it was felt that this method might be a practical synthesis.

$$RCOOH + HN_3 \xrightarrow{H_2SO_4} RNH_2 + CO_2 + N_2$$

Reagents:

Isonicotinic acid, Reilley Tar and Chemical Corp.

Sodium azide, Eastman Practical.

Sulfuric acid, Du Pont C.P. Reagent, 95%.

Chloroform, Merck Reagent Grade.

Sodium hydroxide, Merck Reagent Grade Pellets.

Apparatus:

A one liter 4-neck round bottom flask, equipped with a variable speed stirrer, a reflux condenser, a thermometer well, and a heating mantle.

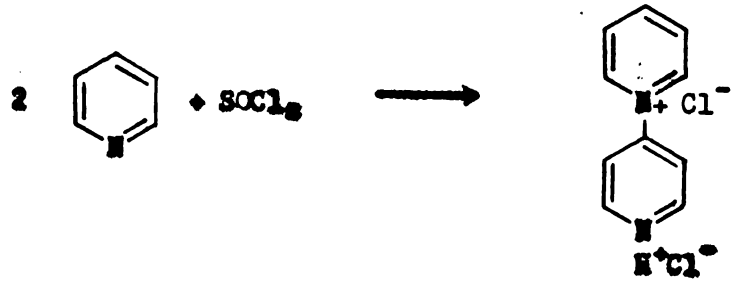
Procedure:

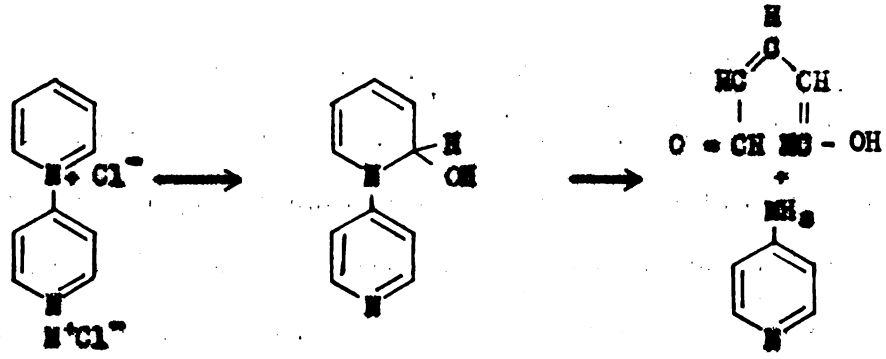
A mixture of 2.95 grams (0.024 mole) of isonicotinic acid, 41 ml. of concentrated sulfuric acid, and 80 ml. of chloroform was heated to 50° C. To this hot mixture was added 1.82 grams (0.024 mole) of sodium azide in small portions with constant stirring. No evolution of nitrogen

was noticed during the addition. After all the sodium amide was added, the mixture was kept at 50° C. for two hours. The mixture was allowed to cool and then poured onto crushed ice in a beaker. The resulting solution was made strongly alkaline by the addition of 100 grams of sodium hydroxide pellets. After the chloroform was removed by evaporation on a steam bath, the solution was transferred to a flask and steam distilled. After two liters of distillate were collected, the process was discontinued as the distillate was neutral. On evaporation of the distillate to dryness on a steam bath, no residue was left. During this procedure there were two indications that the desired reaction failed to take place. There was no evolution of nitrogen and the steam distillate was neutral.

4. Preparation from 4-pyridylpyridinium dichloride.

As both the Lossen and Schmidt reactions failed to produce any 4-aminopyridine it was decided that one of the existing methods for the preparation of the substance should be used. Of these methods, that employed by Koenigs and Greiner seemed the most promising as only one intermediate is involved and this can be prepared easily and in good yields. The preparation of the intermediate, 4-pyridylpyridinium dichloride, and its subsequent cleavage to 4-aminopyridine and glutaconic dialdehyde are summarized by the following equations:





The glutaconic dialdehyde resinifies under very strong alkaline conditions during the cleavage. The cleavage of 4-pyridylpyridinium dichloride to 4-aminopyridine can be effected by various alkaline reagents. Concentrated ammonium hydroxide has been used at reflux temperatures and atmospheric pressure, and also at elevated temperatures in an autoclave. Concentrated potassium hydroxide will also effect cleavage at reflux temperatures and normal pressure.

The best yields are obtained when the cleavage is run at elevated temperatures in an autoclave. However, this procedure may not be convenient, as in this investigation. Therefore, attention was centered on the preparation using inorganic bases at reflux temperatures. Several reactions were run using concentrated potassium hydroxide (50%), concentrated ammonium hydroxide (29%), concentrated sodium hydroxide (30%), and concentrated sodium carbonate (22%).

Various methods were tried for recovery of the 4-aminopyridine formed in the reactions. Steam distillation directly from the alkaline reaction mixture was used in most instances. However, solvent extraction with chloroform and benzene was also attempted.

4-a. Preparation of 4-pyridylpyridinium dichloride.

The 4-pyridylpyridinium dichloride used in this investigation was prepared by the method of Koenigs and Greiner (13).

Reagents:

Pyridine, Baker C.P. Analyzed, dried over potassium hydroxide.

Thionyl chloride, Eastman Practical.

Absolute ethyl alcohol.

Norite A.

Dilute hydrochloric acid (approx. 0.1 N)

Procedure:

One hundred grams (1.76 moles) of dried pyridine and 300 grams (2.54 moles) of thionyl chloride were mixed thoroughly in a 500 ml. filter flask. The flask was stoppered and left standing at room temperature for three days. The flask containing the mixture, which had thickened and turned brown, was then placed in a hot water bath and evaporated to dryness under reduced pressure using a water aspirator. One hundred ml. of absolute alcohol was added to the flask and the solid broken up with a glass rod. The flask and contents were then cooled to 0° C. in an ice bath and then filtered on a buchner funnel. The brown solid was washed once with 100 ml. of cold absolute alcohol and air-dried. Four preparations of 4-pyridylpyridinium dichloride were made by this procedure with yields of 100 grams (69%), 105 grams (72%), 103 grams (71%), and 117 grams (81%) respectively. M.p. 171-174° C.

Koenigs and Greiner recommended that the unpurified 4-pyridylpyridinium dichloride be used for the preparation of 4-aminopyridine. However, it can be purified by recrystallization from dilute hydrochloric acid. Both unpurified and purified 4-pyridylpyridinium dichloride were used in the preparations of 4-aminopyridine.

Procedure:

Thirty grams of unpurified 4-pyridylpyridinium dichloride was dissolved in 120 ml. of boiling dilute hydrochloric acid. Two grams of Norite A was added and the mixture boiled for a few minutes. The mixture was then filtered and the filtrate evaporated down on a hot plate until it became thick. The hot solution was then diluted with an equal volume of absolute alcohol and stored in a refrigerator for 24 hours. The solid that separated was collected on a filter and then dried for 24 hours in a desiccator over calcium chloride. Sixteen grams (55%) of a pale yellow flaky solid was recovered. A second batch of 40 grams of unpurified material was recrystallized yielding 32 grams (83%) of purified material.

4-b. Preparation by cleavage with 50% potassium hydroxide.

Five preparations of 4-aminopyridine were made using 50% potassium hydroxide as an alkaline cleaving agent. The conditions, such as time of reaction and recovery of amine, were varied from one procedure to another. Yields of 4-aminopyridine were based on the 4-pyridylpyridinium dichloride taken.

Reagents:

4-Pyridylpyridinium dichloride, unpurified and purified.

Potassium hydroxide, Merck Reagent Grade Pellets.

Benzene, Merck Reagent Grade.

Norite A.

Sodium hydroxide, Merck Reagent Grade.

Hydrochloric acid, Baker Analyzed Reagent, 36%.

Chloroform, Merck Reagent Grade.

Apparatus:

Soxhlet extractor.

Steam distillation apparatus.

A 500 ml. flask equipped with a condenser and a heating mantle.

Procedure:

Twenty-five grams (0.11 mole) of unpurified 4-pyridylpyridinium dichloride was added to 125 ml. of a 50% potassium hydroxide solution in a 500 ml. flask equipped with a condenser and heating mantle. Some heat was evolved on the addition of the salt to the base. The mixture was refluxed for 16 hours and during this time a hard black mass was formed. The mixture was then steam distilled with the flask heated to 170° C. in an oil bath. Seven hundred ml. of basic distillate were collected and evaporated to dryness on a steam bath. The distillation was discontinued due to failure of the apparatus. The dry residue was then extracted several times with 100 ml. portions of boiling benzene.

These extractions were combined, one gram of Norite A added, and then boiled for a few minutes. The solution was filtered and evaporated to a small volume on a steam bath. On cooling, white needle-like crystals of 4-aminopyridine precipitated. The crystals were collected on a filter and air-dried. A yield of 1.1 grams (11%) was obtained. M.p. 159° C.

The second procedure using 50% potassium hydroxide was similar to the first procedure. Twenty-five grams (0.11 mole) of unpurified 4-pyridylpyridinium dichloride was added to 125 ml. of a 50% potassium hydroxide solution and the mixture refluxed for four hours. The mixture was steam distilled at a normal temperature and after 2.2 liters of distillate were collected the distillation was stopped although the distillate was still basic to litmus paper. The distillate was treated identically as in the first procedure. A yield of 2.1 grams (19%) was recovered.

The same procedure was repeated using 84 grams (0.37 mole) of starting material and 200 ml. of a 50% potassium hydroxide solution. Fourteen liters of distillate were collected yielding 6.2 grams (18%) of 4-aminopyridine.

The fourth procedure used differed from the previous methods in that the reaction time and the method of recovery were changed. Thirty-five grams (0.15 mole) of unpurified 4-pyridylpyridinium dichloride was added to 130 ml. of a 50% potassium hydroxide solution and the mixture refluxed for 10 hours. The hot mixture was then transferred to a beaker, neutralized with concentrated hydrochloric acid, filtered, and evaporated

to dryness on a steam bath. The dry solid was extracted with three 200 ml. portions of boiling chloroform. The chloroform extractions were combined and evaporated to dryness on a steam bath. The residue was then dissolved in boiling benzene, one gram of Norite A added, and the solution boiled for a few minutes. The solution was filtered and evaporated to a small volume on steam bath. On cooling and filtration, 1.1 grams (7.6%) of 4-aminopyridine was obtained. The solid used in the chloroform extraction was dried, pulverized, and extracted with benzene in a soxhlet extractor for eight hours. The benzene was removed from the extractor, boiled a few minutes with one gram of Norite A, filtered, and evaporated to a small volume on a steam bath. On cooling and filtration, 0.4 grams (2.8%) of 4-aminopyridine was obtained raising the over-all yield to 1.5 grams (10%). The solid was removed from the extractor, dried, and dissolved in water. Enough potassium hydroxide was added to raise the pH of the solution to 14. This solution was then evaporated to dryness on a steam bath, and the solid residue was pulverized and extracted with benzene in a soxhlet extractor for 10 hours. The benzene was removed from the extractor and treated as before. An additional 0.7 grams (5%) of 4-aminopyridine was obtained raising the yield to 2.2 grams (15%).

Purified 4-pyridylpyridinium dichloride was used in the fifth procedure. Thirty-two grams (0.14 mole) of the purified salt was added to 130 ml. of a 50% potassium hydroxide solution and the mixture refluxed for 10 hours. The mixture was steam distilled until the distillate was

no longer basic. A total of 4.5 liters of distillate was collected and this was evaporated to dryness on a steam bath. The solid residue was then extracted several times with boiling benzene to which two grams of Norite A had been added. The benzene extractions were combined, filtered to remove the Norite, and evaporated to a small volume on a steam bath. The 4-aminopyridine that precipitated on cooling was collected on a filter and air-dried. A yield of 3.6 grams (27%) was recovered.

4-e. Preparation by cleavage with 30% sodium hydroxide.

This procedure was similar to one of the procedures using 50% potassium hydroxide.

Reagents:

4-Pyridylpyridinium dichloride, unpurified.

Sodium hydroxide, Merck Reagent Grade Pellets.

Benzene, Merck Reagent Grade.

Norite A.

Apparatus:

A 500 ml. flask equipped with a condenser and a heating mantle.

Steam distillation apparatus.

Procedure:

Fifty grams (0.22 mole) of unpurified 4-pyridylpyridinium dichloride was added to 220 ml. of 30% sodium hydroxide, and the mixture refluxed

for four hours. A hard black mass separated soon after mixing. The mixture was then steam distilled until the distillate was no longer basic. The 13 liters of distillate that were collected were evaporated to dryness on a steam bath. The residue was extracted several times with 100 ml. portions of boiling benzene. These extractions were combined, one gram of Norite A added, and then boiled for a few minutes. The solution was then filtered and evaporated to a small volume on a steam bath. The 4-aminopyridine that precipitated on cooling was collected on a filter and air-dried. A yield of 3.2 grams (16%) was obtained.

4-d. Preparation by cleavage with 29% ammonium hydroxide.

The cleavage by concentrated ammonium hydroxide has been used to obtain yields up to 40% of the theoretical. This procedure was quite similar to previous procedures, but the method of recovery of 4-aminopyridine was altered.

Reagents:

4-Pyridylpyridinium dichloride, unpurified.

Ammonium hydroxide, Du Pont, C.P. Analyzed, 29%.

Chloroform, Merck Reagent Grade.

Benzene, Merck Reagent Grade.

Sodium hydroxide, Merck Reagent Grade.

Norite A.

Apparatus:

A 500 ml. flask equipped with a condenser and a heating mantle.

Steam distillation apparatus.

Procedure:

Ten grams of unpurified 4-pyridylpyridinium dichloride (0.044 mole) was added to 100 ml. of 29% ammonium hydroxide solution and the mixture refluxed for four hours. The mixture was cooled, another 50 ml. of concentrated ammonium hydroxide added, and then refluxed for another six hours. The mixture was then cooled, filtered, and the filtrate evaporated to a paste on a steam bath. This paste was then extracted several times with 50 ml. portions of boiling chloroform. The chloroform extractions were combined and evaporated to dryness on a steam bath. No residue was left.

The cleavage with ammonium hydroxide was repeated using the same reflux time and steam distillation to separate the 4-aminopyridine formed.

Procedure:

Fifty grams of unpurified 4-pyridylpyridinium dichloride (0.22 mole) was added to 250 ml. of 29% ammonium hydroxide solution, and the mixture refluxed for 10 hours. Forty grams of sodium hydroxide was then added and the mixture steam distilled until the distillate was no longer basic, nine liters of distillate being collected. The distillate was evaporated to dryness on a steam bath, and then the solid residue was

extracted several times with 100 ml. portions of boiling benzene. The benzene extractions were combined, boiled with a gram of Norite A, filtered, and evaporated to a small volume. On cooling and filtration, 3.6 grams (18%) of 4-aminopyridine was obtained.

4-e. Preparation by cleavage with 22% sodium carbonate.

This procedure using sodium carbonate was very similar to previous procedures. A very short reflux time was used and any 4-aminopyridine formed was separated by extraction methods.

Reagents:

4-Pyridylpyridinium dichloride, unpurified.

Sodium Carbonate Monohydrate, Mallinckrodt Analytical Reagent.

Chloroform, Merck Reagent Grade.

Sodium hydroxide, Merck Reagent Grade.

Benzene, Merck Reagent Grade.

Norite A.

Apparatus:

Five hundred ml. and 1000 ml. flasks equipped with condensers and heating mantles.

Procedure:

Ten grams (0.044 mole) of unpurified 4-pyridylpyridinium dichloride was added to 100 ml. of a 22% sodium carbonate solution that had been heated to boiling. A hard black solid separated soon after mixing.

The mixture was refluxed for one hour, allowed to cool, and then filtered. The filtrate was extracted three times with 50 ml. portions of chloroform in a separatory funnel. The chloroform extractions were combined and evaporated to dryness on a steam bath. No trace of 4-aminopyridine was found. The aqueous solution from the extraction was evaporated to dryness on a steam bath and the solid residue extracted three times with 50 ml. portions of boiling chloroform. These extractions were combined and evaporated to dryness on a steam bath. No residue was left.

The cleavage with sodium carbonate was repeated using a longer reflux time and steam distillation to seperate any 4-aminopyridine formed.

Procedure:

Fifty grams (0.22 mole) of unpurified 4-pyridylpyridinium dichloride was added to 400 ml. of a 22% sodium carbonate solution and the mixture refluxed for eight hours. A hard black mass formed soon after mixing. Forty grams of sodium hydroxide was then added and the mixture steam distilled until the distillate was no longer basic, nine liters of distillate being collected. The distillate was evaporated to dryness on a steam bath, and the solid residue extracted several times with 100 ml. portions of boiling benzene. The benzene extractions were combined, boiled with a gram of Norite A, filtered, and evaporated to a small volume. On cooling and filtration, 1.3 grams (6.3%) of 4-aminopyridine was obtained.

5. Summary of methods of preparation.

The Leuckart reaction produced 4-aminopyridine in very small amounts and the Schmidt reaction failed completely. Yields of 4-aminopyridine obtained by cleavage of 4-pyridylpyridinium dichloride were very low. The results obtained by the latter method are summarized in Table I.

TABLE I

SUMMARY OF ALKALINE CLEAVAGE OF 4-PYRIDYLPYRIDINIUM DICHLORIDE

Starting Material	Alkaline Reagent	Reflux Time (Hrs.)	Separation Process	Yield
25 g.	50% KOH	18	Steam distillation at 170° C.	1.1 g. 11%
25 g.	50% KOH	4	Steam distillation at 100° C.	2.1 g. 19%
84 g.	50% KOH	4	Steam distillation at 100° C.	6.2 g. 18%
32 g.[a]	50% KOH	10	Steam distillation at 100° C.	3.6 g. 27%
32 g.	50% KOH	10	Extraction by Chloroform and Benzene	2.1 g. 15%
50 g.	30% NaOH	4	Steam distillation at 100° C.	3.2 g. 16%
10 g.	29% NH_4OH	10	Extraction by Chloroform	No yield
50 g.	29% NH_4OH	10	Steam distillation at 100° C.	3.6 g. 18%
10 g.	22% Na_2CO_3	1	Extraction by Chloroform	No yield
50 g.	22% Na_2CO_3	8	Steam distillation at 100° C.	1.3 g. 6.3%

[a] Purified

The procedures followed in the cleavage of 4-pyridylpyridinium dichloride were not varied with the intention of finding specific changes in yields caused by altering conditions. However, some general conclusions may be made from the results obtained using the various procedures. Strong bases such as potassium hydroxide and sodium hydroxide are no more efficient than a weak base such as ammonium hydroxide. Sodium carbonate is much less efficient, however. The use of purified 4-pyridylpyridinium dichloride increased the yield about 10% but this increase in yield does not warrant the purification process due to the low recovery of material. The time of refluxing did not have any evident effect on the yield, and steam distillation, although more tedious, was more effective than solvent extraction in removing 4-aminopyridine from the reaction mixture.

B. The Properties of 4-Aminopyridine

1. Preparation and standardization of 0.1 N hydrochloric acid.

In the determination of various properties of a substance it is desirable that some means be available by which the properties of that substance may be evaluated in terms of the purity of the substance itself. With 4-aminopyridine this was accomplished conveniently and accurately by titration of samples of 4-aminopyridine. This procedure served a twofold purpose, as it also provided a comparison of 4-aminopyridine with two other standards, potassium acid phthalate and sodium carbonate, which were used in the standardization of the acid also.

Six liters of 0.1 N hydrochloric acid solution were prepared from reagent grade hydrochloric acid and stored in a carboy equipped with a siphon delivery tube. The carboy was covered to protect it from direct sunlight.

The acid was standardized by two methods: titration with sodium hydroxide solution which had been standardized against potassium acid phthalate, and titration of sodium carbonate. Two different lots of potassium acid phthalate were used, one a Bureau of Standards Sample, and the other a sample from a commercial manufacturer. The sodium carbonate used was also a commercial product. A buret calibrated at 25° C. was used for the titrations. As the titrations were carried out in the temperature range from 25° C. to 27° C., temperature corrections were not applied. A few calculations in instances where a temperature correction might apply, indicated that the correction as given by Kolthoff and Stenger would have no effect in the parts per 1000 range (15). Buret corrections and blank corrections were applied however.

Two liters of a 0.1 N sodium hydroxide solution were prepared by dissolving the proper amount of reagent grade pellets in two liters of distilled water. The sodium hydroxide was freed from carbonate by addition of barium chloride to the hot solution. The barium carbonate was removed by filtration under suction through a cotton pad into a polyethylene storage bottle. This storage bottle was equipped with a siphon delivery tube and a soda lime tube.

The procedure of Hillebrand, Lundell, Hoffman, and Bright was followed in the standardization of the sodium hydroxide solution against potassium acid phthalate (10).

Reagents:

Potassium Acid Phthalate, Bureau of Standards, Sample No. 84, Assay 99.97%.

Potassium Acid Phthalate, Baker Analyzed Reagent, Lot No. 3381, Assay 100.03%.

Phenolphthalein Indicator, 0.1%.

Apparatus:

Calibrated Buret.

Procedure:

Approximately 0.9 gram samples of potassium acid phthalate that had been pulverized and dried two hours at 120° C. were accurately weighed into 250 ml. flasks which had been swept free of carbon dioxide with nitrogen. Fifty ml. of recently boiled distilled water and three drops of 0.1% phenolphthalein indicator were added and the sample titrated with sodium hydroxide. The endpoint was determined by a color comparison with the same volume of a buffered solution with a pH of 8.6 containing the same amount of indicator. An indicator blank was determined by titrating the approximate volume of distilled water as that at the equivalence point in the titration and containing the same amount of indicator. All titrations were performed using a nitrogen atmosphere. A blank correction of 0.01 ml. and buret corrections were applied to the volumes of acid used in the titrations. The results of the standardization are tabulated in Table II.

TABLE II

STANDARDIZATION OF SODIUM HYDROXIDE WITH POTASSIUM ACID PHTHALATE

G. KAP	Ml. NaOH	N.
0.8157[a]	34.95	0.1143
0.7177[a]	30.73	0.1144
0.8123[a]	34.79	0.1144
0.8133[a]	34.83	0.1144
0.9319[b]	39.86	0.1144
0.9293[b]	39.79	0.1143
0.9323[b]	39.90	0.1144
0.9319[b]	39.90	0.1143
0.9309[b]	39.84	0.1144
0.9297[b]	39.80	0.1144
	Average	0.1144

[a] Baker's Analyzed Reagent.
[b] Bureau of Standards Sample.

The acid was standardized with the standard sodium hydroxide by measuring out 40 ml. of acid into 250 ml. flasks from the calibrated buret and then titrating the acid samples with the standard sodium hydroxide solution using the same buret. The endpoint was determined in the same manner as in the standardization of the sodium hydroxide solution. An indicator blank correction was determined also in the same manner. Both buret corrections and an indicator blank correction were

applied to the volumes of acid and base used in the titrations. The values for the normality of the hydrochloric acid solution are contained in Table III.

TABLE III

STANDARDIZATION OF HYDROCHLORIC ACID WITH 0.1144 N SODIUM HYDROXIDE

Ml. HCl	Ml. NaOH		N. HCl
40.07	35.77		0.1021
40.07	35.77		0.1021
40.07	35.78		0.1021
40.07	35.77		0.1021
40.07	35.77		0.1021
40.07	35.77		0.1021
40.07	35.79		0.1022
40.07	35.77		0.1021
40.07	35.77		0.1021
40.07	35.79		0.1022
		Average	0.1021

Primary standard grade sodium carbonate was the second substance with which the acid was standardized, and the procedure of Kolthoff and Sandell was followed (14).

Reagents:

Sodium Carbonate Anhydrous, Mallinckrodt Primary Standard, ignited for 2 hours at 285° C., assay 99.95-100.05%.

Phenolphthalein Indicator, 0.1%.

Bromcresol Green Indicator, 0.04%.

Procedure:

Samples of sodium carbonate, 0.2 to 0.25 gram, were accurately weighed into 250 ml. flasks and dissolved in 25 ml. of distilled water. One drop of phenolphthalein solution was added and the solution titrated with acid until colorless. Two drops of bromcresol green indicator were added and the titration continued until the indicator began to change to green. The solution was then boiled a few minutes, cooled to room temperature, and the titration continued until the color changed to green again. A blank was determined by taking an equal volume of water containing approximately the same amounts of sodium chloride and indicator as were present at the equivalence point and titrating with acid to the same color. A blank correction of 0.03 ml. and buret corrections were applied to the volumes of acid used in the titrations. The results are in Table IV.

TABLE IV

STANDARDIZATION OF HYDROCHLORIC ACID WITH SODIUM CARBONATE

G. Na_2CO_3	Ml. HCl		N. HCl
0.2396	44.28		0.1021
0.2194	40.54		0.1021
0.2233	41.25		0.1021
0.2260	41.76		0.1021
0.2182	40.31		0.1021
0.2388	44.11		0.1021
0.2669	49.35		0.1020
0.2212	40.88		0.1021
0.2116	39.14		0.1021
0.2320	42.51		0.1022
		Average	0.1021

2. Elemental analysis of 4-aminopyridine.

The 4-aminopyridine used for the various tests was obtained from the various preparative procedures. Ten grams of the material was recrystallized once from benzene. A melting point of 161° C. was found using a Fisher melting point block with a 2-3° temperature rise per minute. The material had the following elemental analysis as reported by Micro-Tech Laboratories.

TABLE V

ELEMENTAL ANALYSIS OF 4-AMINOPYRIDINE

	Calculated	Found		Average
		I	II	
Carbon	63.80	63.89	63.66	63.78
Hydrogen	6.43	6.39	6.28	6.33
Nitrogen	29.77	29.96	29.74	29.85
Total	100.00	100.24	99.68	99.96

An attempt was made to determine the nitrogen content of the 4-aminopyridine by the Kjeldahl, Wilfarth, and Cumming method (1). Ordinarily this method is not successful with heterocyclic type organic compounds. However, if 4-aminopyridine exists primarily in a tautomeric form, then this method would be expected to give reasonable results. Several determinations were made and the results are shown in Table VI. The digestion time was varied also, but this had no appreciable effect on the results.

TABLE VI

ANALYSIS BY KJELDAHL METHOD

Digestion Time	Percent Nitrogen Found[a]
2	29.16
4	29.10
4	29.25
8	28.58

[a] Calc. 29.77%

This method did not give satisfactory results with the analysis of 4-aminopyridine. This recrystallized 4-aminopyridine was also tested for the presence of chloride ion. As the 4-aminopyridine was prepared from a substance containing a considerable amount of chloride ion, its presence might be suspected.

Procedure:

A 0.5000 gram sample of 4-aminopyridine was dissolved in 10 ml. of 2 N nitric acid and 5 ml. of 0.1 N silver nitrate added. This solution was compared to a solution of 10 ml. of 1 N nitric acid containing five mg. of sodium chloride per liter and treated with five ml. of 0.1 N silver nitrate. A blank was also run which consisted of 10 ml. of 1 N nitric acid to which was added five ml. of 0.1 N silver nitrate.

No opalescence was observed with either the blank or the sample containing 4-aminopyridine. Considerable opalescence was observed with the standard solution of sodium chloride. The sensitivity of this test is 0.01% NaCl.

3. Selection of an indicator.

Several experiments were necessary to establish the conditions under which 4-aminopyridine could be titrated successfully. The first experiment was the selection of an indicator to be used in the titrations.

The proper indicator for the titration of 4-aminopyridine must have a color change in the pH range at the equivalence point. To determine the pH of the equivalence point, preliminary calculations using

approximate concentrations to be encountered in actual titrations and a K_b of 1.3×10^{-9}, were made with the following equations:

$$[H^+] = \sqrt{\frac{K_w}{K_b} \cdot c}$$

where c is the concentration of the salt formed at the equivalence point. The results calculated for various concentrations are contained in Table VII.

TABLE VII

CALCULATED pH AT EQUIVALENCE POINT

c	$[H^+]$	pH
2.1×10^{-2}	4.0×10^{-6}	5.4
4.0×10^{-2}	5.6×10^{-6}	5.3
6.2×10^{-2}	6.9×10^{-6}	5.2

The calculated pH values at the equivalence point are in the pH range of methyl red which is 4.4 to 6.0. To definitely establish that methyl red was the proper indicator, two samples of 4-aminopyridine were titrated with hydrochloric acid and the titration followed with a pH meter. Two drops of 0.1% methyl red indicator were also added to the solution and the color change of the indicator correlated with the change in pH.

Apparatus:

Beckman pH Meter, Model H-2, equipped with a glass indicator electrode and a calomel reference electrode.

Magnetic stirrer.

Calibrated buret.

Reagents:

Hydrochloric acid, 0.1 N.

Hydrochloric acid, 0.5 N.

Methyl red indicator, 0.1%

Procedure:

A 0.2693 gram sample of recrystallized 4-aminopyridine was placed in a 250 ml. beaker and 100 ml. of recently boiled distilled water and two drops of 0.1% methyl red indicator added. The electrodes of the pH meter were then dipped in the solution. To this solution were added small increments of 0.1 N acid from the buret. The solution was stirred thoroughly after each addition but not during the reading of the pH. This same procedure was followed in the titration of 1.1602 grams of 4-aminopyridine with 0.5 N acid.

Figure 1 shows the titration curves obtained by plotting ml. of acid added vs. the pH. The data for these curves are contained in the Appendix. As the inflection points in both curves occurred in the methyl red pH range, this indicator would be suitable for the titration of 4-aminopyridine with hydrochloric acid.

Approximate values for the ionization constant of 4-aminopyridine were calculated using information obtained from the titration curves. The ionization constant for amines is usually expressed by the following equations:

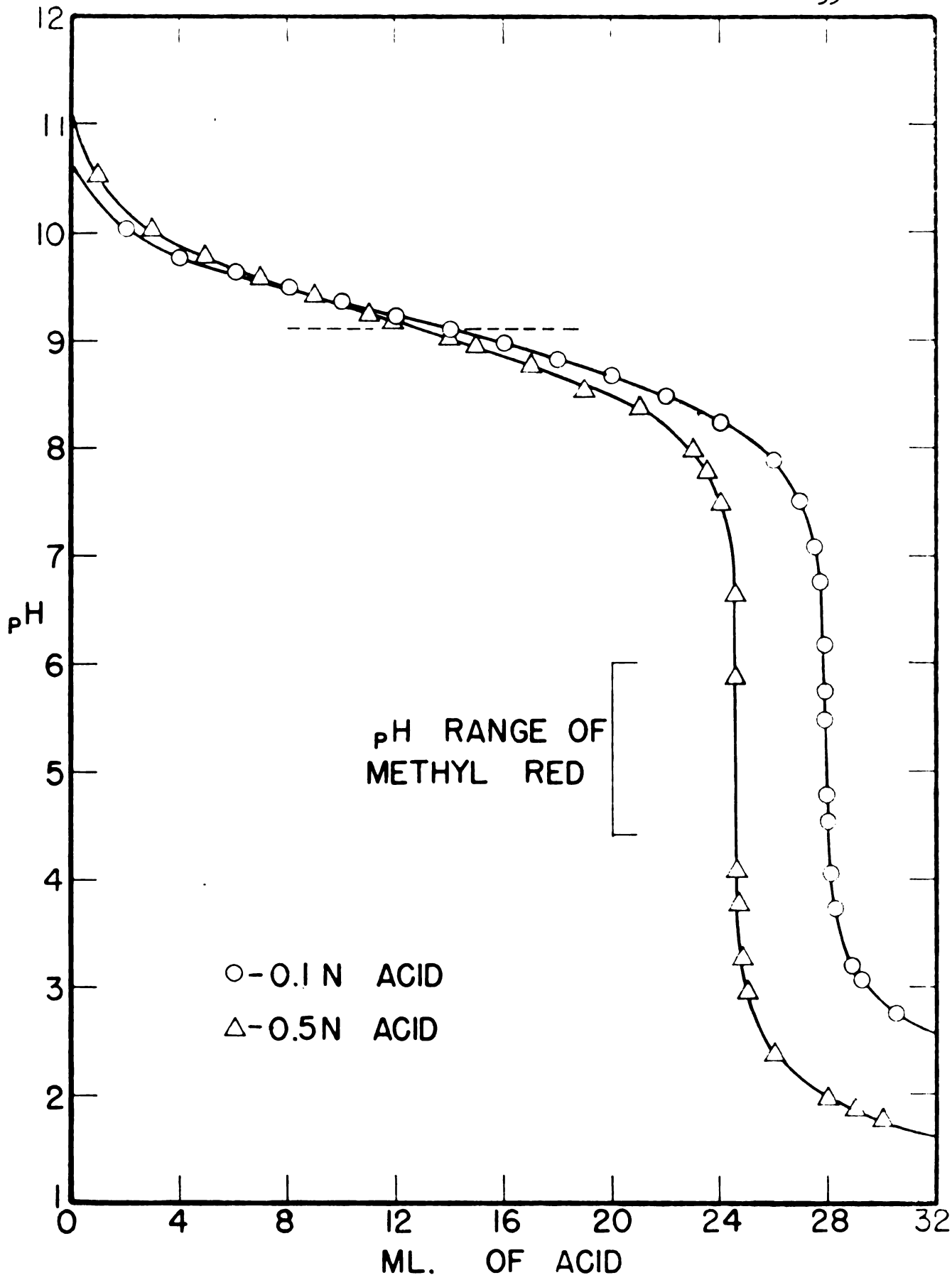


FIGURE 1. TITRATION OF 4-AMINOPYRIDINE WITH HYDROCHLORIC ACID.

$$K_b = \frac{[RNH_3^+][OH^-]}{[RNH_3OH]}$$

At the midway point in the titration of an amine with an acid, $[RNH_3^+] = [RNH_3OH]$; and then $K_b = [OH^-]$. The $[OH^-]$ can be determined from the pH at the midpoint in the titration. The data in Table VIII were obtained in this manner.

TABLE VIII

THE IONIZATION CONSTANT OF 4-AMINOPYRIDINE

Normality of HCl	Ml. of HCl To Midpoint	pH at Midpoint	pOH	K_b
0.1	14.00	9.1	4.9	1.3×10^{-5}
0.5	12.32	9.1	4.9	1.3×10^{-5}

The values for the K_b of 4-aminopyridine determined in this manner agree with the value of 1.3×10^{-5} as reported by Tropsch (20).

4. Hygroscopicity.

The hygroscopicity of 4-aminopyridine was determined by noting the change in weight of a sample of 4-aminopyridine that was exposed to the air over a period of time under normal changes in humidity and temperature. The results in Table IX are cumulative, the percent change in weight being the total change for any number of days.

Procedure:

A 1.0040 gram sample of recrystallized 4-aminopyridine was placed in a previously dried weighing bottle and the open bottle placed in a beaker with a second empty weighing bottle to be used as a tare. The sample was not pulverized or dried. The beaker was covered with a ribbed watch glass to permit free access of air. The bottle containing the 4-aminopyridine and the tare were weighed daily for several days and then at longer intervals.

TABLE IX

HYGROSCOPICITY OF 4-AMINOPYRIDINE

Days	Change in Weight Of Sample	Change in Weight of Tare	Total Change	Percent Change
1	-0.0001	-0.0001	0.0000	0.00
2	-0.0001	-0.0001	0.0000	0.00
3	0.0000	0.0000	0.0000	0.00
4	+0.0001	+0.0001	0.0000	0.00
5	0.0000	0.0000	0.0000	0.00
6	0.0000	0.0000	0.0000	0.00
7	-0.0001	-0.0001	0.0000	0.00
8	-0.0001	-0.0001	0.0000	0.00
9	0.0000	0.0000	0.0000	0.00
10	0.0000	0.0000	0.0000	0.00
14	-0.0002	0.0000	-0.0002	-0.02
17	-0.0001	+0.0001	-0.0002	-0.02
20	-0.0004	-0.0003	-0.0001	-0.01
25	-0.0001	+0.0003	-0.0004	-0.04
28	-0.0001	+0.0003	-0.0004	-0.04
32	-0.0003	+0.0003	-0.0006	-0.06
37	-0.0004	+0.0001	-0.0005	-0.05
46	-0.0008	-0.0002	-0.0006	-0.06
53	-0.0007	0.0000	-0.0007	-0.07
60	-0.0009	-0.0001	-0.0008	-0.08
95	-0.0011	+0.0004	-0.0015	-0.15

4-Aminopyridine shows no hygroscopic tendency but actually loses weight steadily under varying humidity and temperature conditions. This loss in weight is caused by the vapor pressure of 4-aminopyridine.

5. Loss on heating at 105° C.

4-Aminopyridine has a definite vapor pressure as is evidenced by the loss in weight on standing, sublimation during preparation, and its being capable of steam distillation. As the extent of that effect was of interest, it was studied also.

Procedure:

A 1.0080 gram sample of recrystallized 4-aminopyridine was placed in a previously dried weighing bottle and heated in a drying oven at 105° C. for varying periods of time, from 1/4 hour to 10 hours. The bottle containing the 4-aminopyridine was cooled and weighed after the various periods of heating. The results of this procedure are contained in Table I.

The data in Table I indicates that a considerable loss of 4-aminopyridine results on prolonged heating at 105° C. The loss per hour remains fairly constant however.

TABLE X

LOSS ON HEATING AT 105° C.

Heating Time (Hrs.)	4-Aminopyridine			Percent Loss		
	G.	Loss on Heating	Total Loss	For Heating	Total	Per Hour
0	1.0080					
1/4	1.0067	0.0013	0.0013	0.13	0.13	0.52
1/2	1.0061	0.0006	0.0019	0.06	0.19	0.12
3/4	1.0045	0.0016	0.0035	0.16	0.35	0.21
1	1.0035	0.0010	0.0045	0.10	0.45	0.10
2	1.0009	0.0026	0.0071	0.26	0.70	0.13
3	0.9965	0.0044	0.0115	0.44	1.14	0.15
4	0.9911	0.0054	0.0169	0.54	1.68	0.14
5	0.9841	0.0074	0.0239	0.71	2.37	0.14
6	0.9737	0.0104	0.0343	1.04	3.40	0.17
7	0.9588	0.0149	0.0492	1.53	4.88	0.22
8	0.9424	0.0164	0.0656	1.71	6.51	0.21
9	0.9254	0.0170	0.0826	1.80	8.19	0.20
10	0.9070	0.0184	0.1010	1.99	10.02	0.80

Total heating time = 56.5 hours
Total loss in weight = 0.1010 grm.
Total percent loss = 10.02 percent
Average loss per hour = 0.18 percent

6. Effect of pulverization.

It is a normal procedure to pulverize all materials used as standards prior to drying. This is important to facilitate removal of adsorbed moisture or occluded solvent. To determine if pulverization had any definite effect on the results obtained with 4-aminopyridine, samples of unpulverized crystalline 4-aminopyridine were titrated and the results compared with those obtained on titration of samples that had been pulverized.

Procedure:

Samples of unpulverized 4-aminopyridine of approximately 0.39 gram which had been dried two hours at 105° C. were accurately weighed into 250 ml. flasks. Fifty ml. of recently boiled distilled water and two drops of 0.1% methyl red indicator were added and the solution titrated with 0.1 N acid to a definite pink color. An indicator blank was determined by titrating approximately the same volume of recently boiled distilled water as at the equivalence point and containing 2 drops of methyl red indicator to the same shade of pink. Three samples of 4-aminopyridine that had been pulverized to approximately 100 mesh and dried for 2 hours at 105° C. were titrated by the same procedure. Buret corrections and a blank correction of 0.02 ml. were applied to the volumes of acid used in the titrations. The values for the weight of 4-aminopyridine found and the percent purity in Table XI were calculated using 0.1021 as the normality of the acid. The values given for the normality were calculated assuming 100.0% purity.

TABLE XI

EFFECT OF PULVERIZATION

4-Aminopyridine G. taken	G. found	Ml. HCl	Percent Purity	N
		Unpulverized		
0.3869	0.3864	40.21	99.87	0.1022
0.3858	0.3856	40.13	99.96	0.1021
0.3854	0.3850	40.06	99.89	0.1022
		Pulverized		
0.3743	0.3738	38.90	99.87	0.1022
0.3686	0.3683	38.31	99.93	0.1022
0.3958	0.3956	41.17	99.96	0.1022

It was concluded from these data that pulverization has no effect on the results obtained. However, all subsequent samples of 4-aminopyridine were pulverized prior to drying with the exception of a few cases where it is mentioned.

7. Effect of carbon dioxide.

Alkaline aqueous solutions are subject to absorption of carbon dioxide from the air, and as a water solution of 4-aminopyridine is alkaline, the possibility of this effect was investigated. Samples of 4-aminopyridine that had been pulverized and dried for two hours at 105°C. were titrated using a nitrogen atmosphere. Except for the use of the

nitrogen atmosphere, the procedure used in these titrations was identical with that used in previous titrations. Corrections and calculations were made in the same manner also. The results obtained are tabulated in Table XII.

TABLE XII

THE EFFECT OF CARBON DIOXIDE

4-Aminopyridine			Percent	
G. taken	G. Found	Ml. HCl	Purity	N.
0.3688	0.3686	40.44	99.95	0.1022
0.3731	0.3727	38.78	99.88	0.1022
0.3720	0.3717	38.68	99.92	0.1022

From the data, it is evident that any absorption of carbon dioxide from the air by an alkaline solution of 4-aminopyridine had no effect as the titration results obtained show no change from previous results. Therefore, all subsequent titrations were made using a normal atmosphere.

8. Effect of heating at 105° C.

The effect of prolonged heating was determined by titrating two samples of the 4-aminopyridine that had been used in the loss on heating tests. This material which had already been heated for 55.5 hours was heated for an additional 10 hours at 105° C. Two samples of original material were also heated for 10 hours at 105° C. and then titrated. The material was not pulverized.

TABLE XIII

THE EFFECT OF HEATING AT 105° C.

Hours of Heating	4-Aminopyridine G. taken	G. found	Ml. HCl	Percent Purity	N.
10	0.3564	0.3567	37.12	100.1	0.1020
10	0.3857	0.3861	40.18	100.1	0.1020
65.5	0.3877	0.3882	40.40	100.1	0.1020
65.5	0.3910	0.3916	40.75	100.2	0.1020

The results obtained show a distinct effect on both the purity of the samples and the calculated normalities. As there is no difference between the results obtained with the material heated 10 hours and that which was heated for 65.5 hours, it was concluded that the change that had taken place, was complete in 10 hours and the additional heating had no further effect. This change in the material could be attributed to the formation of some decomposition products of lower equivalent weight or the removal of volatile impurities such as benzene or pyridine which were not removed by the customary two hour drying time. During the heating process, no visual change in the material was detected.

9. Stability.

Two samples of the original lot of recrystallized 4-aminopyridine which had been stored for six months in a closed weighing bottle on a shelf in the laboratory were titrated to determine if any change had

taken place. These samples were pulverized and dried for two hours at 105° C. and then titrated. The data are tabulated in Table XIV.

TABLE XIV

STABILITY

4-Aminopyridine			Percent	
G. taken	G. found	Ml. HCl	Purity	N.
0.3820	0.3824	39.79	100.1	0.1020
0.3892	0.3893	40.51	100.0	0.1021

A definite change in the material had taken place on standing. This could be explained also by the formation of decomposition products of lower equivalent weight or by the gradual loss of volatile impurities such as benzene or pyridine.

10. Recovery of 4-aminopyridine.

4-Aminopyridine can be recovered readily from aqueous solutions by evaporation of an alkaline solution of the base and extraction of the dry residue with benzene.

Procedure:

The aqueous solutions containing 4-aminopyridine were made alkaline to a pH of approximately 14 and then evaporated to dryness on a steam bath. The dry residue was then pulverized, placed in a soxhlet extractor and extracted with benzene until the extraction is complete.

The time of extraction varied with the amount of 4-aminopyridine present, three to four grams taking about 24 hours. The benzene solution was then removed from the extractor, boiled for a few minutes with one gram of Norite A, and then filtered. The filtrate was evaporated to a small volume and allowed to cool. The needles of 4-aminopyridine that separated were collected on a filter and air-dried. Several recoveries were made in this manner. From a total of 11.2 grams, 9.5 grams (85%) were recovered.

The effect of recrystallization was investigated using this recovered material. Samples of material that were recrystallized 1, 2, and 3 times from benzene were titrated with acid. The material that was recrystallized once would correspond to the original lot of 4-aminopyridine, each lot having one more recrystallization than involved in the original separation. The results are tabulated in Table XV.

There was no significant change in either the purity or the normality on recrystallization of this recovered material. However, titration results obtained with the recovered material varied by two parts per 1000 from results obtained with material used in previous tests.

A sample of this recovered material was tested for residue after sublimation and ignition.

Procedure:

A 0.5000 gram sample of recovered 4-aminopyridine was weighed into a tared platinum dish. The platinum dish was then inserted into the sublimation apparatus used in the preparation of the sublimed material

TABLE XV

EFFECT OF RECRYSTALLIZATION

4-Aminopyridine G. taken	4-Aminopyridine G. found	Ml. HCl	Percent Purity	N
		One Recrystallization		
0.3942	0.3948	41.08	100.1	0.1020
0.3792	0.3798	39.52	100.2	0.1020
0.3885	0.3890	40.48	100.1	0.1020
		Two Recrystallizations		
0.3802	0.3806	39.61	100.1	0.1020
0.3824	0.3829	39.85	100.1	0.1020
0.3885	0.3891	40.49	100.2	0.1019
		Three Recrystallizations		
0.3916	0.3922	40.81	100.2	0.1020
0.3892	0.3898	40.56	100.2	0.1020

used in later titrations. The 4-aminopyridine was sublimed completely and the platinum dish reweighed. The platinum dish was then ignited in the flame of a Meeker burner for a few minutes and reweighed again. After sublimation alone, the sample had a residue of 0.2 mg. (0.04%). After ignition there was no weighable residue.

11. Effect of sublimation.

The sublimation of 4-aminopyridine was evident in several instances in this work. The loss on heating was due to sublimation, as well as

the loss on standing exposed to air. During the preparation of 4-aminopyridine, this sublimation was noticed. However, only Wibaut, Haayberg, and Schlatmann have recommended sublimation as a means of purification of 4-aminopyridine (21).

To see what effect sublimation would have on the titration results, some of the original material, some recovered material, and some of the material obtained from later preparations were sublimed at 55-60° and a pressure of approximately 10 mm. of mercury. Samples of this material were then dried for two hours at 105° C. and titrated with acid. The results of these titrations are collected in Table XVI.

Some of the sublimed material obtained from later preparations was resublimed and titrated. These results are also included in Table XVI.

Sublimation caused a definite change in the original material, increasing the percent purity and decreasing the calculated normality of the acid. No changes were observed in the recovered material, however, and the sublimed material obtained from a later preparation also gave the same results. Double sublimation also caused no changes. There was no visible change in the appearance of the 4-aminopyridine on sublimation and the melting point remained at 161° C.

Several properties of sublimed 4-aminopyridine were studied to see if they varied from those of the 4-aminopyridine that was recrystallized from benzene and used in the prior determination of properties.

The hygroscopicity of sublimed 4-aminopyridine was determined by a procedure similar to that used with the recrystallized 4-aminopyridine.

TABLE XVI

EFFECT OF SUBLIMATION

4-Aminopyridine G. taken	4-Aminopyridine G. Found	Ml. HCl	Percent Purity	N.
		Original 4-Aminopyridine		
0.3650	0.3654	38.02	100.1	0.1020
		Recovered 4-Aminopyridine		
0.3808	0.3815	39.70	100.2	0.1019
0.3799	0.3804	39.58	100.1	0.1020
0.3822	0.3825	39.80	100.1	0.1020
		4-Aminopyridine from NaOH Preparation		
0.3698	0.3697	38.47	100.1	0.1020
0.3713	0.3718	38.69	100.1	0.1020
0.3753	0.3758	39.11	100.1	0.1020
0.3717	0.3722	38.73	100.1	0.1020
0.3845	0.3851	40.07	100.2	0.1020
0.3797	0.3802	39.56	100.1	0.1020
		4-Aminopyridine Sublimed Twice		
0.3826	0.3831	39.87	100.1	0.1020
0.3961	0.3966	41.27	100.1	0.1020

A 0.1986 gram sample of sublimed material was dried for two hours at 105° C., cooled in a desiccator, and then weighed. The open weighing bottle containing the 4-aminopyridine was then placed in an open beaker which was covered with a ribbed watch glass to allow free access of air. A tare was treated similarly. Both were weighed daily for several days and then at longer intervals.

The data in Table XVII show the same tendency with sublimed 4-aminopyridine as with recrystallized 4-aminopyridine, but here it was slightly greater.

The loss on heating at 105° C. was also studied using sublimed 4-aminopyridine. The procedure followed was identical with that used with the recrystallized material. The results are contained in Table XVIII.

A sample of this sublimed material was tested for residue after sublimation and ignition, following the procedure used with recovered material. On sublimation alone, the sample had a residue of 0.3 mg. (0.06%). After ignition there was no weighable residue.

TABLE XVII

HYGROSCOPICITY OF SUBLIMED 4-AMINOPYRIDINE

Days	Change in Weight of Sample	Change in Weight of Tare	Total Change	Percent Change
1	+0.0007	+0.0007	0.0000	0.00
2	+0.0009	+0.0009	0.0000	0.00
3	+0.0005	+0.0007	-0.0002	-0.04
4	+0.0006	+0.0008	-0.0002	-0.04
5	+0.0007	+0.0008	-0.0001	-0.02
6	-0.0001	+0.0004	-0.0005	-0.10
7	+0.0004	+0.0007	-0.0003	-0.06
8	+0.0006	+0.0008	-0.0002	-0.04
9	+0.0006	+0.0008	-0.0002	-0.04
10	+0.0006	+0.0008	-0.0002	-0.04
14	+0.0005	+0.0007	-0.0002	-0.04
17	+0.0007	+0.0009	-0.0002	-0.04
20	+0.0007	+0.0010	-0.0003	-0.06
25	+0.0005	+0.0009	-0.0004	-0.08
28	+0.0004	+0.0008	-0.0004	-0.08
32	+0.0002	+0.0009	-0.0007	-0.14
37	+0.0001	+0.0010	-0.0009	-0.18
44	-0.0007	+0.0008	-0.0015	-0.30
50	-0.0011	+0.0010	-0.0021	-0.42
60	-0.0018	+0.0012	-0.0030	-0.60
76	-0.0029	+0.0013	-0.0042	-0.84

TABLE XVIII

LOSS ON HEATING AT 105° C.

Heating Time (Hrs.)	4-Aminopyridine			Percent Loss		
	G.	Loss on Heating	Total Loss	Per Heating	Total	Per Hour
0	0.4948					
1/4	0.4938	0.0010	0.0010	0.20	0.20	0.80
1/2	0.4935	0.0003	0.0013	0.06	0.26	0.12
3/4	0.4926	0.0009	0.0022	0.18	0.44	0.24
1	0.4917	0.0009	0.0031	0.18	0.63	0.18
2	0.4894	0.0023	0.0054	0.47	1.09	0.24
3	0.4858	0.0036	0.0090	0.74	1.82	0.25
4	0.4804	0.0054	0.0144	1.11	2.91	0.28
5	0.4738	0.0066	0.0210	1.37	4.24	0.27
6	0.4642	0.0096	0.0306	2.03	6.10	0.34
7	0.4496	0.0146	0.0452	3.15	9.12	0.45
8	0.4355	0.0141	0.0593	3.14	11.98	0.39
9	0.4180	0.0175	0.0768	4.02	15.52	0.45
10	0.3994	0.0186	0.0954	4.45	19.28	0.45

Total heating time = 56.5 hours
Total loss in weight-0.0954 gram
Total percent loss = 19.28 percent
Average loss per hour = 0.34 percent

12. Purity of 4-Aminopyridine.

Table XIX contains the percents of purity calculated for all samples of 4-aminopyridine titrated. The results are based on an acid normality of 0.1021.

TABLE XIX

PERCENT PURITY OF 4-AMINOPYRIDINE

Sublimed	Recrystallised 4-Aminopyridine		
	Original	Recovered	Later Prep.
100.1	99.87	100.1	100.1
100.1	99.96	100.2	100.1
100.1	99.89	100.1	100.1
100.1	99.95	100.1	100.1
100.1	100.1	100.1	
100.2	99.87	100.2	
100.2	99.88	100.2	
100.1	99.92	100.2	
100.1	99.93		
100.1	99.96		
Average 100.1	99.93	100.2	100.1

The 4-aminopyridine used for the determination of the various properties had an average purity of 99.93%. Prolonged heating at 105° C. and sublimation raised the purity to 100.1%. Storage also tended to

raise the purity of this material above 100.0%. The sublimed 4-aminopyridine, regardless of source, had a purity of 100.1% with resublimation having no effect. The recovered 4-aminopyridine had a purity of 100.1-100.2% with recrystallization and sublimation having no appreciable effect.

Several preparations of 4-aminopyridine made since the original lot, also had the same purity, 100.1%. The only difference between the first lot and later lots of 4-aminopyridine was that with the former, quantities of 4-aminopyridine that had been separated by extraction and that had been prepared from purified 4-pyridylpyridinium dichloride were introduced. In all later preparations the 4-aminopyridine was separated by steam distillation only. Sublimation and recrystallization had no effect upon the purity of later preparations. The results obtained on recrystallization of 4-aminopyridine obtained by a later preparation are in Table XX.

TABLE XX

EFFECT OF RECRYSTALLIZATION

4-Aminopyridine G. taken	4-Aminopyridine G. found	Ml. HCl	Percent Purity	N.
		One Recrystallization		
0.3848	0.3852	40.08	100.1	0.1020
0.3833	0.3836	39.92	100.1	0.1020
		Two Recrystallizations		
0.3797	0.3801	39.55	100.1	0.1020
0.3855	0.3858	40.15	100.1	0.1020

A sample of this new material was tested for residue after sublimation and ignition following the procedure used with recovered material. On sublimation alone, the sample had a residue of 0.4 mg. (0.08%). After ignition there was no weighable residue.

It was concluded that the sublimed 4-aminopyridine, the recovered 4-aminopyridine, and the later preparations of 4-aminopyridine, all of which had a purity of 100.1%, were the pure material, despite the fact that the effective purity was over 100.0%. The reason for this high purity is not known.

13. Comparison of Standards.

All the values for the normality of the acid obtained using the various substances are combined in Table XXI. The calculated results are based on a purity of 100.0%.

The normality results obtained by standardisation with sodium hydroxide and sodium carbonate show very good agreement. The normality results obtained with 4-aminopyridine show trends analogous to the purity results and as the purity increases, the normality decreases. The material recrystallised from benzene yields an average normality of 0.1022 which is one part per 1000 high. The sublimed material, recovered material and later preparations all show an average normality of 0.1020, which is one part per 1000 low.

TABLE XXI

COMPARISON OF STANDARDS

NaOH	Na_2CO_3	4-Aminopyridine		
		Recrystallized From Benzene[a]	Sublimed	Recrystallized From Benzene
0.1021	0.1021	0.1022	0.1020[a]	0.1020[b]
0.1021	0.1021	0.1021	0.1019[b]	0.1020[b]
0.1021	0.1021	0.1022	0.1020[b]	0.1020[b]
0.1021	0.1021	0.1022	0.1020[b]	0.1020[b]
0.1021	0.1021	0.1022	0.1020[c]	0.1020[b]
0.1021	0.1021	0.1022	0.1020[c]	0.1019[b]
0.1022	0.1020	0.1022	0.1020[c]	0.1020[b]
0.1021	0.1021	0.1022	0.1020[c]	0.1020[b]
0.1021	0.1021	0.1022	0.1020[d]	0.1020[c]
0.1022	0.1022	0.1020	0.1020[c]	0.1020[c]
			0.1020[d]	0.1020[c]
			0.1020[d]	0.1020[c]
Average 0.1021	0.1021	0.1022	0.1020	0.1020

a. Original recrystallized 4-aminopyridine.
b. Recovered 4-aminopyridine.
c. Later preparation of 4-aminopyridine.
d. Doubly sublimed.

III CONCLUSIONS

4-Aminopyridine does meet many of the requirements of a standard for use in acidimetry. The neutralization of 4-aminopyridine with a strong acid is stoichiometric and the substance may be titrated successfully using methyl red as an indicator. The indicator blank is small and easily determined. Results that agree to within one part per 1000 with results obtained using two other standards, potassium acid phthalate and sodium carbonate, can be obtained using 4-aminopyridine as a standard. 4-Aminopyridine is not hygroscopic and carbon dioxide has no effect on the titration results. 4-Aminopyridine has a definite vapor pressure which causes substantial losses on prolonged heating. This property also enables purification by sublimation. 4-Aminopyridine may be recovered easily and economically.

At the present time 4-Aminopyridine is not available commercially. However, there are a number of methods for its preparation. The Lossen and Schmidt reactions are not applicable, and the cleavage of 4-pyridylpyridinium dichloride results in very poor yields. The separation of 4-aminopyridine by steam distillation produces a very pure product.

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APPENDIX

TABLE XXII

TITRATION OF 4-AMINOPYRIDINE WITH 0.1 N HYDROCHLORIC ACID

Ml. HCl	pH	Ml. HCl	pH	Ml. HCl	pH	Ml. HCl	pH
0.00	10.68	20.08	8.66	27.92	5.96	32.10	2.57
1.05	10.32	21.08	8.57	27.97	5.74	32.60	2.53
2.13	10.04	22.11	8.48	28.00	5.47	33.10	2.48
3.05	9.92	23.10	8.37	28.04	4.78	34.10	2.40
4.08	9.77	24.09	8.24	28.07	4.53	35.11	2.35
5.08	9.76	24.59	8.18	28.13	4.26	36.11	2.28
6.08	9.64	25.11	8.11	28.20	4.06	37.11	2.24
7.09	9.56	25.43	8.03	28.22	3.96	38.11	2.20
8.08	9.49	25.60	8.00	28.29	3.83	39.11	2.17
9.10	9.44	25.90	7.93	28.35	3.72	40.11	2.14
10.08	9.36	26.10	7.89	28.40	3.63	41.12	2.10
11.10	9.31	26.40	7.81	28.50	3.53	42.13	2.07
12.08	9.23	26.61	7.73	28.63	3.43	43.14	2.05
13.09	9.17	26.87	7.63	28.80	3.33	44.14	2.03
14.09	9.11	27.09	7.50	28.99	3.20	45.15	2.00
15.09	9.03	27.35	7.33	29.33	3.07	46.17	1.97
16.08	8.98	27.55	7.08	29.89	2.90	47.17	1.96
17.09	8.90	27.71	6.76	30.60	2.76	48.16	1.95
18.09	8.83	27.84	6.38	31.10	2.68	49.16	1.93
19.09	8.75	27.89	6.18	31.60	2.63	50.16	1.92

TABLE XXIII

TITRATION OF 4-AMINOPYRIDINE WITH 0.5 N HYDROCHLORIC ACID

Ml. HCl	pH	Ml. HCl	pH	Ml. HCl	pH	Ml. HCl	pH
0.00	11.14	20.54	8.46	24.67	4.07	29.06	1.85
1.00	10.53	21.04	8.38	24.72	3.77	30.06	1.76
2.01	10.23	21.54	8.30	24.75	3.56	31.06	1.68
3.01	10.04	22.06	8.21	24.80	3.37	32.06	1.62
4.01	9.92	22.55	8.12	24.85	3.26	33.06	1.57
5.02	9.79	23.05	7.96	24.89	3.16	34.06	1.53
6.02	9.69	23.55	7.79	24.95	3.06	35.09	1.47
7.03	9.60	23.71	7.73	25.06	2.95	36.07	1.44
8.03	9.51	23.84	7.64	25.16	2.86	37.07	1.40
9.04	9.42	23.90	7.62	25.26	2.76	38.07	1.37
10.04	9.34	23.99	7.53	25.36	2.67	39.07	1.33
11.04	9.25	24.05	7.48	25.46	2.63	40.07	1.30
12.04	9.17	24.15	7.43	25.56	2.56	41.08	1.27
13.04	9.02	24.24	7.33	25.66	2.53	42.08	1.25
14.04	8.15	24.29	7.29	25.76	2.47	43.09	1.23
14.10	9.02	24.35	7.17	25.85	2.45	44.09	1.20
15.04	8.95	24.39	7.06	25.96	2.40	45.10	1.18
16.04	8.87	24.44	6.96	26.06	2.37	46.11	1.17
17.04	8.77	24.50	6.77	26.56	2.23	47.11	1.16
18.04	8.65	24.55	6.63	27.06	2.14	48.11	1.15
19.04	8.55	24.59	6.33	27.56	2.06	49.12	1.13
20.04	8.43	24.63	5.88	28.06	1.97	50.12	1.11

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