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The development of a micro technique for qualitative organic analysis

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BOSTON UNIVERSITY
GRADUATE SCHOOL

Thesis

THE DEVELOPMENT OF A MICRO TECHNIQUE
FOR QUALITATIVE ORGANIC ANALYSIS

by

Arthur Rapport
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A.E.P.

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INTRODUCTION

During recent years, the micro method, as a method of instruction in the chemistry laboratory, has received the interest and approval of an increasing number of chemists all over the world. In many university laboratories, the micro method has been adapted to courses in quantitative organic and inorganic analysis. Much work has also been done in general organic and inorganic chemistry. The many values and advantages of the micro method in the chemical laboratory such as economy of time, economy of space, economy of materials, increased safety, and the development of an additional technique in handling small amounts of materials, made it seem desirable to attempt the adaptation of the laboratory work in qualitative organic analysis to the micro scale. The degree to which this attempt has been realized is indicated in the report of the research contained in the following pages.

The term "micro" as used in this thesis means reduced or very small amounts of the reagents employed in the experiments. The amount of liquid used varied from one to ten drops. In exceptional cases, such as the hydrolysis of an ester, one cubic centimeter of the organic liquid was used. The amounts of solids used varied from a few crystals to one tenth of a gram. The use of truly micro amounts, a few

hundredths of a milligram of liquids and solids, did not seem feasible on account of the very special and costly apparatus required, and also on account of the great amount of time that must be devoted by the student in order to master the technique demanded in the handling of such minute amounts of materials.

The experimental manipulations employed in this work, therefore, have been developed quite independently of those advocated in the literature dealing with micro technique. Such procedures as micro filtration on a microscope slide, the fractional distillation of a few drops of a liquid, crystallization of minute amounts of solids in capillary tubes, did not fit into the scheme of analysis so as to be consistent with the chief aim of this research -- the economy of time. The boundaries of qualitative organic analysis are so wide, the experimental procedures so numerous and varied, that the practical and rigid adaptation of the entire system of qualitative organic analysis to a strictly micro scale seems extremely difficult indeed, if not impossible.

LITERATURE

The most extensive literature in the field of micro methods in chemistry deals with general laboratory processes and qualitative inorganic analysis. There is very little literature on the micro method applied specifically to the procedures of qualitative organic analysis. A few micro qualitative organic experiments are described by Emich in his Microchemical Laboratory Manual. Nevertheless, works on general microchemical procedure contain many suggestions of value for the problem of micro qualitative organic analysis and for this reason, they have been included in the bibliography.

APPARATUS

The apparatus required for work in micro qualitative organic analysis differed from that required by the macro course primarily in its being of reduced size. Aside from the difference in size, certain differences in arrangement, such as will be discussed below, facilitated the carrying out of the various reactions.

The test tubes used exclusively were "micro" test tubes, three inches in length and one-quarter inch in internal diameter. The white spot plate and the microscope slide were used in reactions which involved the addition of a few drops of reagent to a few drops of substance, especially where a marked color change or precipitation occurred during the course of the reaction.

The reagents frequently employed in qualitative organic analysis were not kept in stock bottles on the shelves. They were kept in micro test tubes, each tube being fitted with a pipette that served as a dropper when the reagent was being used. The pipettes were made by drawing out ordinary glass tubing (four millimeters in diameter) over a Bunsen flame. The pipettes were cut so that they released about thirty-five drops to a cubic centimeter and reached almost to the bottom of the test tube into which they were inserted.

A short piece of rubber tubing which contained a glass plug at one end served efficiently as a one-holed stopper between the micro test tube and the pipette to which it was attached. This arrangement was found to be efficient in preventing contamination of the reagents by dust or foreign particles in the atmosphere.

A box for these modified reagent bottles described above was constructed from an ordinary small wooden box eight by twelve by two inches. The top of this box was covered with cardboard which contained forty-five evenly spaced holes whose diameter equalled the outside diameter of the micro test tubes. Then the micro test tubes, each containing reagent and pipette, were inserted into the holes so that they protruded one inch above the surface of the cardboard. Each hole was numbered on the cardboard. These numbers, together with the name of the corresponding reagents which were inserted into the box in alphabetical order, were rewritten on a separate piece of cardboard which served as a key to the identity of each micro reagent bottle.

This device was found to be most efficient in reducing to a minimum the time required to bring together reactant and reagent.

The following reagents were kept in the micro reagent box:

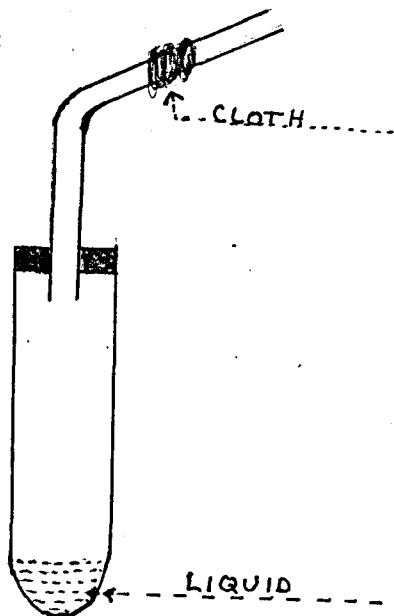
- | | |
|-----------------------|---------------------------|
| 1. alcohol (absolute) | 4. acetic acid (30% sol.) |
| 2. alcohol (95%) | 5. acetic acid (glacial) |
| 3. alcohol (75%) | 6. acetone |

7. acetophenone
8. acetyl chloride
9. allyl alcohol
10. aniline
11. benzene
12. benzenesulphonylchloride
13. benzoyl chloride
14. Bromine in carbon tetrachloride
15. bromine water
16. carbon tetrachloride
17. chlorine water
18. dimethyl sulphate
19. ether
20. Fehling's solution
21. ferric chloride (1% solution in dil. hydrochloric acid)
22. ferric chloride (10% solution)
23. ferrous sulphate (crystals)
24. formaldehyde
25. formic acid
26. hydrochloric acid (15% solution)
27. hydrochloric acid (concentrated)
28. ligroin
29. nitric acid
30. nitrobenzene
31. p-aminodimethylanilinehydrochloride
32. phenol

33. phenylhydrazine
34. potassium permanganate
35. potassium hydroxide
36. Schiff's reagent
37. sodium mercury amalgam (3%)
38. sodium carbonate (5% solution)
39. sulphuric acid
40. silver nitrate
41. toluene
42. water
43. zinc chloride in hydrochloric acid
44. pentanol-2
45. 2-methylbutanol-2

Another device which saved much time was a modified micro reflux distillation apparatus. The body of this apparatus was an ordinary six inch test tube. A cork stopper through which was inserted a piece of glass tubing eight inches in length and bent at a slightly obtuse angle in the middle, was placed at the open end to the test tube. A piece of cloth, previously soaked in ice water, was wrapped around the farther end of the glass tube to condense any vapor that might have reached there during the course of the distillation. This precaution was often found to be unnecessary as the vapor, arising from the relatively small amount of liquid at the bottom of the tube, condensed upon striking the cooler upper

part of the tube. This apparatus was used extensively in hydrolyzing esters. One cubic centimeter of ester and three cc. of 20% sodium hydroxide solution were found to be effective quantities in many cases. The following diagram illustrates the apparatus described above:



This apparatus was heated by means of a micro burner to which was attached, by means of rubber tubing, a small piece of ordinary glass tubing about three inches long which tapered rapidly to a small point at its upper end. In this way a micro flame was obtained which was not too hot to employ even in reactions involving only a few drops.

Distillation of small quantities was carried out in a small distillation flask of about fifteen cc. capacity. The side arm of this flask was passed through a hole in a long piece of rubber tubing which was then attached to the faucet. Small pieces of cloth, previously soaked in ice water, were wrapped around the side arm on either side of the rubber tubing.

This arrangement constituted a very satisfactory cooling device. The distillate was collected in a micro test tube. The source of heat was an oil bath heated over a Bunsen burner.

A micro suction filtration apparatus was arranged using a micro porcelain funnel and a four inch test tube with a side arm. The results were very satisfactory.

The determination of the specific gravity of liquid compounds was carried out using the apparatus described in Kamm's Qualitative Organic Analysis, page 129. This method is micro in nature.

The boiling points of the liquids were determined by distilling a 5 cc. sample of the compound using the distillation apparatus described above. The highest temperature recorded by the thermometer was taken as the boiling point.

EXPERIMENTS

The following pages contain a report of experiments in qualitative organic analysis which have been performed according to the micro method. The directions for most of these experiments have been taken from Kamm's Qualitative Organic Analysis (John Wiley & Sons - 1932.) Not all of the experiments contained in this book have been performed by the micro method. Some of these experiments, such as the determination of melting points, and the technique for the sodium decomposition method in the qualitative analysis for the elements have not been included in this work for the reason that these experiments are themselves micro in character. Other experiments, such as the sodium nitroprusside test for ketones and the iodoform tests for ethanol, have been omitted because they are not specific in character. Some experiments included in the differential analysis of the common sugars have been taken from Dr. Lyman C. Newell's manual, Experiments in Organic Chemistry.

SOLUBILITY BEHAVIOR OF LIQUIDS

The solubility behavior of the compounds listed below was determined by adding one drop of the organic compound to fifteen drops of the solvent contained in a micro test tube. All the solubility tests were very conclusive except the determination of solubility in cold conc. sulphuric acid. It is recommended that this test be performed according to the directions given in Kamm's Qualitative Organic Analysis in order to eliminate the inconclusiveness of the micro solubility test.

<u>Compound</u>	<u>Solvent</u>		Dilute HCl	Dilute KOH	Conc. H ₂ SO ₄	Ether
	Water					
1. Ethyl Bromide	-		-	-	-	+
2. Ethyl Benzoate	-		-	-	+	+
3. Acetophenone	-		-	-	+	+
4. Aniline	-		+	-	+	+
5. Nitrobenzene	-		-	-	+	+

The solubility behavior of solids was determined exactly as is described in Kamm's Qualitative Organic Analysis, pages 138-139. One tenth of a gram of the solid substance and three cubic centimeters of solvent were the quantities used.

PRELIMINARY TESTS FOR THE ELEMENTS

1. For halogen.

Three drops of the alkaline solution* were acidified with five drops of nitric acid. The solution was boiled for one minute. A few drops of dilute silver nitrate solution were then added. A whitish precipitate of silver bromide was deposited. The organic compound used was ethylbromide.

2. For bromine.

Five drops of nitric acid were added to three drops of the stock solution* in a micro test tube. The solution was boiled for one minute. Two drops of carbon tetrachloride were added. Then a few drops of freshly prepared chlorine water were introduced. The carbon tetrachloride layer was colored brown due to the liberated bromine.

3. For sulphur.

A. To ten drops of the stock solution were added five drops of dilute hydrochloric acid and one drop of conc. hydrochloric acid. To this solution one drop of ferric chloride and one drop of a solution of p-aminodimethylanilinehydrochloride were added. The solution was colored bluish green. This constituted a positive test for sulphur. Benzenesulphonylchloride was the organic compound used.

B. Lead acetate test.

To ten drops of the stock solution five drops of

*The terms alkaline solution and stock solution refer to the solution obtained after 10 cc. of water are added to the sodium decomposition product. Cf. Kamm, Qualitative Organic Analysis

glacial acetic acid were added. A small piece of lead acetate paper was introduced into the solution. The paper turned black. This was a positive lead acetate test for sulphur.

TESTS FOR UNSATURATION

- A. Decolorization of a solution of bromine in carbon tetrachloride.

On a white spot plate one drop of amylene was dissolved in ~~two~~ drops of carbon tetrachloride. To this solution several drops of a dilute solution of bromine in carbon tetrachloride were added. Each drop of the bromine solution was decolorized upon addition to the amylene. This constituted a positive test for unsaturation in the amylene linkages.

This test was repeated using in place of amylene one drop of each of the following compounds: phenol solution, allyl alcohol, ethyl alcohol, and acetophenone. The results were as follows: phenol, allyl and alcohol decolorized the solution of bromine in carbon tetrachloride. Ethyl alcohol and acetophenone did not decolorize the bromine solution.

A few crystals of cinnamic acid were dissolved in two drops of carbon tetrachloride and the bromine solution was added drop by drop. No decolorization of the bromine solution took place.

The entire experiment was also performed using a micro test tube in place of the spot plate. The results obtained with the micro test tube were equally conclusive.

- B. Decolorization of a potassium permanganate solution.

Two drops of a 5% solution of sodium carbonate and one drop of amylene were mixed on a spot plate. To this

mixture, one drop of a 2% solution of potassium permanganate was added. The permanganate solution was decolorized, that is, the color changed to brown immediately. Several more drops of the permanganate solution were then added and each drop was decolorized immediately. This constituted a positive potassium permanganate test for unsaturation in the amylenic linkages.

This test was repeated, using in place of the amylenic a few crystals of cinnamic acid, one drop of a 1% solution of phenol and one drop of formic acid. All of these compounds decolorized the potassium permanganate solution. No decolorization of the permanganate solution was obtained when one drop of toluene and one drop of a 1% solution of benzoic acid were used in place of the compounds listed above.

The same results were obtained when the above experiments were performed using the micro test tube in place of the spot plate.

DIFFERENTIATION BETWEEN AROMATIC AND ALIPHATIC
HYDROCARBONS BY MEANS OF DIMETHYL SULPHATE

Ten drops of ligroin were added to ten drops of dimethyl sulphate. Two layers formed. The test tube was shaken and then allowed to stand. The two layers persisted. This test indicated an aliphatic hydrocarbon which is insoluble in the dimethyl sulphate reagent. This experiment was repeated using ten drops of benzene in place of ligroin. The benzene was completely soluble in the dimethyl sulphate. This indicated an aromatic compound which is completely soluble in the dimethyl sulphate reagent. The use of ten drops of reagent and reactant was required in order to render the two layers formed in the first case visible. When less than ten drops were used, the two layers could not be distinguished.

HALOGEN COMPOUNDS

A. One drop of benzyl chloride was added to two drops of alc. silver nitrate in a micro test tube. The solution turned cloudy at once. The test tube was heated over a micro flame and a white precipitate of silver chloride was formed. The experiment was repeated with one drop of benzoyl chloride. A flocculent precipitate of silver chloride formed at once. The test tube was then heated over a micro flame and a solid mass of silver chloride was deposited. The experiment was again repeated using one drop of ethyl bromide in place of benzoyl chloride. No precipitate formed in the cold but on heating, a precipitate of silver bromide was deposited.

The same reaction was carried out using bromobenzene and carbon tetrachloride in place of the compounds mentioned above. In both cases the alc. silver nitrate solution remained clear both before and after heating.

B. A few drops of acetyl chloride were added to ten drops of water in a micro test tube. A violent reaction took place, much heat was evolved and the acetyl chloride disappeared completely. This experiment was repeated using a few drops of benzoyl chloride in place of acetyl chloride. Two layers formed. The test tube was shaken vigorously for a few minutes and some of the benzoyl chloride disappeared, which indicated that a slight hydrolysis of the benzoyl chloride took place.

Six drops of acetyl chloride were added to ten drops of aniline in a micro test tube. Heat was liberated, white fumes were evolved, and a solid white mass was formed. After a few moments a few drops of water were added, and the acetyl derivative of aniline, acetanilide, separated out.

ALCOHOLS AND PHENOLS

A. In a micro test tube small pieces of sodium were added to ten drops of iso-amyl alcohol. The sodium dissolved readily, a small amount of heat was evolved and gas bubbles (hydrogen) were given off.

The experiment was repeated using acetone in place of the alcohol. The sodium dissolved readily in the acetone.

The sodium test was repeated with toluene. No reaction was obtained.

B. Three drops of acetyl chloride were added to a few crystals of phenol in a micro test tube. After one minute, ten drops of water were added. A milky white solution resulted which, upon standing, separated into two layers. The water layer (top) was withdrawn by means of a pipette and ten drops of dilute potassium hydroxide were added to the liquid remaining in the bottom of the tube. The liquid was not soluble in the dilute potassium hydroxide solution which indicated that phenylacetate was formed as the product of the above reaction.

Three drops of acetyl chloride were added to six drops of ethanol in a micro test tube. A reaction immediately ensued, evidenced by the evolution of much heat. The test tube was allowed to cool and a few drops of a 5% solution of sodium carbonate were added. The characteristically fruity odor of ethyl acetate developed immediately.

DIFFERENTIATION BETWEEN PRIMARY,
SECONDARY, and TERTIARY ALCOHOLS

The reagent used in the following experiment was concentrated hydrochloric acid which contained one mole of fused zinc chloride to one mole of the acid. The reagent was kept at 26 - 27°C.

One drop of a primary alcohol, pentanol-1, was added to six drops of the hydrochloric acid-zinc chloride reagent. The pentanol was completely soluble in the reagent.

This experiment was repeated using a secondary alcohol, pentanol-2, in place of pentanol-1. No reaction was at first observed, but within five minutes the solution turned cloudy.

The experiment was again repeated using a tertiary alcohol, 2 methylbutanol-2. This alcohol reacted with the reagent at once to give two phases which was observed by the cloudy appearance of the mixture.

FERRIC CHLORIDE TEST FOR PHENOLS

One drop of ferric chloride was added to three drops of a one-tenth percent aqueous solution of phenol on a white spot plate. The ferric chloride was decolorized to purple at once. The same result was obtained when dilute solutions (1/10%) of resorcinol, acetoacetic ester, salicylic

acid were used in place of phenol. Negative results were obtained with dilute solutions of benzoic acid and p-hydroxybenzoic acid.

Several drops of bromine water were added to two drops of a dilute aqueous solution of phenol on a microscope slide. The bromine water was decolorized immediately upon addition to the phenol solution. A white precipitate of tribromophenol formed. This experiment was repeated using two drops of aniline in place of phenol. The bromine water was decolorized and a whitish precipitate of tribromoaniline resulted.

One drop of bromine water was added to two drops of a dilute solution of salicylic acid. A white precipitate formed. Another drop of bromine water imparted a faint yellow color to the solution. This experiment was repeated using two drops of a solution of resorcinol in place of the salicylic acid. The bromine water was decolorized to a light yellow and a yellowish white precipitate formed. Another drop of bromine water imparted a yellow bromine color to the solution.

PREPARATION OF THE PHTHALEIN COLORS

A small amount of phenol was placed in a micro test tube. Approximately twice as much phthalic anhydride was added. This mixture was moistened with three drops of conc. sulphuric acid. The test tube was placed in an oil bath and heated to 180°C for three minutes. The tube was removed from the bath and allowed to cool. Ten drops of water and ten drops of a 10% solution of sodium hydroxide were added. The wine-red color of phenolphthalein in alkaline solution developed.

The above experiment was repeated using equal amounts of resorcinol and alpha-naphthol in place of phenol. As a result fluorescein and alpha-naphtholphthalein were produced. The fluorescein was colored orange-green by reflected light and red by transmitted light. The alpha-naphtholphthalein was colored greenish-gray in alkaline solution.

ALDEHYDES, KETONES, and ESTERS

A micro test tube was first cleaned by boiling in hot sodium hydroxide solution. Then two drops of a 2% solution of acetaldehyde were added to five drops of an ammoniacal solution of silver oxide in the micro test tube. A silver mirror was deposited on the sides of the tube. This experiment was repeated using two drops of benzaldehyde in place of acetaldehyde. A silver mirror was obtained on warming the test tube over a micro burner. The same result was obtained with two drops of a 2% solution of glucose. When the experiment was repeated using two drops of acetone in place of acetaldehyde, no silver mirror was obtained even after heating.

One drop of acetaldehyde solution was added to one drop of fuchsin-aldehyde reagent on a white spot plate. A wine-red color developed immediately. The same result was obtained when one drop of benzaldehyde, dissolved in one drop of absolute alcohol, was added to the fuchsin-aldehyde reagent. The result was duplicated with formaldehyde. Acetone and acetophenone gave negative results, no color changes, with the reagent.

The phenylhydrazine solution used in the following experiment was prepared by dissolving five drops of phenylhydrazine in fifteen drops of a 30% acetic acid.

A. Water soluble aldehydes and ketones.

Two drops of acetone were added to two drops of water in a micro test tube. To this solution one drop of the phenylhydrazine solution was added. A white precipitate of acetone phenylhydrazone formed. This experiment was repeated using one drop of formaldehyde in place of acetone. A white precipitate of formaldehyde phenylhydrazone formed immediately.

B. Water insoluble aldehydes and ketones.

Two drops of benzaldehyde were dissolved in five drops of alcohol in a micro test tube. A few drops of water were added. A slight precipitate formed which was redissolved by adding a drop of alcohol. To the clear solution two drops of the phenylhydrazine were added. A yellowish precipitate of benzaldehyde phenylhydrazone formed immediately. The same procedure was repeated using two drops of acetophenone in place of the benzaldehyde. The solution remained clear for several minutes. One drop of acetic acid was then added and a yellowish precipitate of acetophenone phenylhydrazone formed.

The Hydrolysis of Ethyl Benzoate.

One cc. of ethyl benzoate and three cc. of a 20% solution of sodium hydroxide were placed in a micro reflux apparatus. A few chips of porous porcelain plate were added and the mixture was boiled over a micro flame. In twenty minutes the ester layer disappeared, which indicated that hydrolysis was complete. The mixture was transferred to a micro distilling

apparatus, and about 1 cc. of the alkaline solution was distilled over into a micro test tube. The distillate was saturated with potassium carbonate and a layer of ethanol separated out from the solution. The residue in the distilling flask was cooled, diluted with water and acidified with dilute sulphuric acid. A white precipitate of benzoic acid formed immediately. The benzoic acid was removed by suction filtration, dried and its melting point was determined. It was found to be 120°C.

A derivative of the ethanol was prepared as follows: Two drops from the ethanol layer were added to 0.1 grams of 3-nitrophthalic anhydride in a micro test tube. The test tube was then placed in a small beaker of boiling water for ten minutes. Then the tube was removed from the beaker, 1 cc. of water was added, and the test tube heated over a micro flame until the solid dissolved. The solution was filtered using an ordinary funnel and the filtrate was cooled under tap water with frequent shaking. A white crystalline solid, 3-nitroethylphthalate separated out. This solid was separated from the solution by micro suction filtration, dried, and a melting point determination was made. The 3-nitroethylphthalate melted at 156°C. -- the melting point given in the literature.

CARBOHYDRATES

A. Molisch test

Five drops of a 2% solution of fructose were added to five drops of conc. sulphuric acid in a micro test tube. Two drops of an alpha-naphthol solution were added. The two layers did not mix. At the contact zone, a violent red ring appeared. This constituted a positive Molisch test for a carbohydrate. This test was repeated using 2% solution of sucrose, dextrose, lactose, maltose, xylose, arabinose, and galactose. All these sugars gave positive Molisch tests.

B. Fehling's solution test

One drop of Fehling's solution was added to two drops of a 2% solution of glucose in a micro test tube. The test tube was heated over a micro flame and an orange-red precipitate of cuprous oxide formed. This was a positive Fehling's test.

This test was repeated using two drops of a 2% solution of sucrose instead of glucose. A negative result was obtained.

The above experiment was also successfully performed on a microscope slide using the same quantities of test solutions and reagent.

C. Osazone formation

One drop of phenylhydrozine and two drops of glacial acetic acid were added to ten drops of a 1% solution of glucose

in a micro test tube. The test tube was plugged loosely with cotton and immersed in a 30 cc. beaker containing boiling water. In about five minutes the yellowish brown glucosazone crystals appeared. The tube was removed from the boiling bath, and allowed to cool in the air for a few minutes. The crystals were then removed from the tube by means of a suction pipette, placed on a slide and examined under the microscope. They were found to be typical glucosazone crystals. This experiment was repeated using ten drops of a 2% solution of sucrose instead of glucose. Sucrosazone crystals formed in thirty minutes.

D. Cobaltous nitrate test for sucrose.

Six drops of a 2% solution of sucrose and three drops of cobaltous nitrate solution were placed in a micro test tube. One drop of a 50% solution of sodium hydroxide was then added. A delicate amythest color was produced. The test tube was heated over a micro burner for a few minutes and the amythest color did not fade. This constituted a positive cobaltous nitrate test for sucrose.

E. Differentiation between arabinose and xylose by means of the lead acetate test.

Three drops of a 5% solution of lead acetate were added to three drops of a 2% solution of arabinose in a micro test tube. The tube was heated over a micro flame and one drop of conc. ammonium hydroxide was added. A whitish precipitate formed. This precipitate was heated and its color changed to salmon pink. This was a positive test for arabinose.

When this experiment was repeated with xylose the color of the precipitate after heating was yellow.

AMINES

1. Five drops of acetylchloride were added to five drops of aniline in a micro test tube. A white solid mass formed. Ten drops of water were then added and acetanilide, the product of the reaction, separated out. This experiment was repeated using dimethylaniline in place of aniline but no reaction occurred.

2. Nine drops of conc. hydrochloric acid and fifteen drops of water were added to three drops of aniline in a micro test tube. The solution was cooled to 6°C . Then ten drops of a solution of 0.8 gms. of sodium nitrite in 3 cc. of water were added. The mixture was cooled to 10°C .

Five drops of the cold diazonium solution prepared as above were placed in a micro test tube and warmed over a micro flame. A gas (nitrogen) was evolved, and the color of the solution changed to dark brown. The solution had a distinctly phenolic odor.

One drop of the cold diazonium solution was added to one drop of a 1% solution of beta-naphthol in sodium hydroxide. An orange red precipitate formed immediately. This experiment was performed on the microscope slide.

Differentiation between primary, secondary, and tertiary amines by means of benzenesulphonylchloride.

1. One drop of benzenesulphonylchloride was added to ten drops of a 5% solution of sodium hydroxide in a micro test tube. The mixture was first shaken and then warmed gently over a micro flame. No hydrolysis of the benzenesulphonylchloride took place. The solution was then acidified with dilute hydrochloric acid. The benzenesulphonylchloride was not soluble in the dilute hydrochloric acid solution.

2. The first part of the above experiment was repeated but one drop of aniline was added to the alkaline solution before the addition of the acid chloride. A reaction took place and the salt of the sulphonyl derivative of the aniline dissolved in the dilute sodium hydroxide solution. The solution was then acidified with dilute hydrochloric acid and the sulphonyl derivative of the aniline was precipitated as a white solid.

3. This procedure was repeated using monamethylaniline in place of aniline. The sulphonyl derivative of the monomethylaniline was not soluble either in the alkaline solution or in the dilute hydrochloric acid.

4. Test #3 was repeated using one drop of pure dimethylaniline in place of monomethylaniline. As the reaction progressed the oily insoluble layer of the dimethylaniline

sank to the bottom of the test tube. This was due to the formation of an additional product between the tertiary anine and the benzenesulphonylchloride reagent. When the solution was acidified with dilute hydrochloric acid, this addition complex was broken up and the dimethylaniline dissolved completely in the acid solution.

INDIFFERENT GROUPS CONTAINING NITROGEN

A. Amides.

1. A few crystals of ammonium benzoate were placed on a watch glass and moistened with a few drops of a 20% solution of sodium hydroxide. The strong odor of ammonium was detected. This experiment was repeated with urea and benzamide but no odor of ammonia could be detected in either case.

Twenty drops of a 20% solution of sodium hydroxide were added to a small amount of urea in a micro test tube. The solution was heated to boiling over a micro flame. The odor of ammonia was readily detected. This experiment was repeated using a few crystals of benzamide dissolved in two drops of absolute alcohol. The odor of ammonia was detected.

The experiment was again repeated using acetanilide in place of benzamide. No odor of ammonia was detected but an amber colored ring appeared (aniline) which floated on top of the alkaline solution.

2. 1.5 cc. of a mixture of equal volumes of sulphuric acid and water were added to .1 gm. of p-bromoacetanilide in a micro reflux apparatus. The mixture was refluxed for a half hour. Then several drops of water were added and no precipitate of p-bromoacetanilide formed. 5 cc. of water were then added and the solution was made alkaline with sodium

hydroxide. A white precipitate of p-bromoaniline formed.

B. Nitro compounds

1. To 0.2 cc. of nitrobenzene in a micro test tube were added a few pieces of mossy tin and five drops of conc. hydrochloric acid. Hydrogen was evolved and a definite reaction took place. The color of the solution changed from pale yellow to amber -- an indication that the nitrobenzene was reduced to aniline. The reduction was hastened by warming the tube gently over a micro burner. The tube was allowed to cool and 1 cc. of a 50% solution of potassium hydroxide was added. An amber ring (aniline) appeared at the top of the potassium hydroxide solution. The aniline was extracted with 0.5 cc. of ether and the ether layer removed by means of a pipette into a micro test tube. The ether was then evaporated. Ten drops of water were added to the aniline which remained and a few drops of bromine water were also added. A white precipitate of tribromoaniline formed immediately.

2. One drop of nitrobenzene was dissolved in ten drops of 75% alcohol in a micro test tube. A colorless solution resulted. One drop of a 10% solution of sodium hydroxide was then added. The solution still remained colorless. A small fragment of a 3% sodium amalgam was then introduced. The color of the solution became pale yellow at first, then deeper yellow and finally yellowish green. The sodium amalgam liquefied readily. The amalgam liquefied more readily than in a blank

portion which contained no nitro compound.

The same color changes were observed when p-nitrobenzoic acid was used in place of nitrobenzene.

IDENTIFICATION OF "UNKNOWNNS"

In order to ascertain the value of the micro method in qualitative organic analysis six "unknown" compounds were identified according to the technique developed in the preceding pages. The result was very gratifying.

The time required for the analysis and identification of all six compounds was fourteen hours -- an average of two hours and twenty minutes for each compound. This comparatively short time was possible because of the availability of the reagents contained in the micro reagent box, and the speed and facility with which the micro reactions were carried out. The tests applied to each compound were carried out using the same quantities of reagent and reactant stated in the preceding pages.

The six "unknowns" were identified as: benzamide, dimethylaniline, ethyl malonate, m-nitrobenzaldehyde, acetanilide, and glucose. A detailed report of the method employed in the identification of each compound is contained in the following pages.

The scheme of analysis which was followed in the identification of these "unknowns" is the one proposed by Kamm in his Qualitative Organic Analysis.

A. The Identification of Benzamide

1. The melting point of this compound was determined to be 126°C .
2. The preliminary tests for the elements which were applied after a few fragments of the compound were subjected to decomposition with metallic sodium, determined the presence of nitrogen and the absence of sulphur and the halogens.
3. The solubility behavior test placed the compound in group #7, since it was found to be soluble in cold conc. sulphuric acid and contained nitrogen.

4. A few fragments of the compound were boiled with ten drops of 20% sodium hydroxide solution. The strong odor of ammonia was detected.

5. The preceding test indicated the compound to be an amide. The literature was then consulted and benzamide was found to possess a melting point of 128°C -- very close to the melting point already determined.

6. Benzoic acid was chosen as the derivative that was prepared from the compound. This derivative was prepared in the following manner:

A small amount of the compound (benzamide) was placed at the bottom of a micro test tube. Twenty drops of a 20% solution of sodium hydroxide were added and the solution was heated to boiling. The benzamide was converted to benzoic acid and ammonia, the benzoic acid forming a soluble sodium benzoate. The solution was allowed to cool, acidified with

dilute hydrochloric acid and the benzoic acid was precipitated. The benzoic acid was separated from the solution by micro suction filtration, dried, and a melting point determination was made. The melting point of the benzoic acid was 121°C .

B. The Identification of Dimethylaniline

1. The boiling point of this compound was found to be 195°C .

2. The specific gravity of the compound was determined to be $0.950\overline{20}$.

3. The preliminary tests for the elements indicated the presence of nitrogen and the absence of sulphur and the halogens.

4. The solubility test placed the compound in group #3, since it was soluble in dilute hydrochloric acid. The compound was thought to be an amine because it contained nitrogen.

5. The compound was found to be a tertiary amine by the application of the benzenesulphonylchloride test.

6. The literature was consulted and the physical constants given for dimethylaniline (B. P. 198° ; Sp. Gr. $0.958\overline{20}$) were found to be in close agreement with those already determined for this compound.

7. The derivative for this compound, p-nitrosodimethylaniline, was prepared according to the directions given in Mulliken's The Identification of Pure Organic Compounds -- V

The melting point of this derivative was found to be 85°C -- the melting point given in the literature.

C. The Identification of Ethyl Malonate

1. The boiling point of this compound was determined to be 195°C .
2. The specific gravity determination gave a value of $1.051 \frac{20}{20}$.
3. The preliminary tests for the elements indicated the absence of nitrogen, sulphur, and the halogens.
4. The solubility tests placed the compound in group #5, since it was soluble only in cold conc. sulphuric acid and ether.
5. The bromine and potassium permanganate unsaturation tests both gave positive results.
6. The phenylhydrazine test for ketones and aldehydes was negative.
7. The compound was subjected to hydrolysis using the same technique and apparatus already described for the hydrolysis of ethyl benzoate. The compound was readily hydrolyzed.
8. Consultation of the literature showed that the physical constants given for ethyl malonate (B. P. 198°C ; Sp. Gr. $1.054 \frac{25}{25}$) agreed very closely with those already determined for this compound.
9. The ethanol obtained as a result of the above hydrolysis was converted into the 3-nitroethylphthalate derivative, by means of 3-nitrophthalic anhydride. The melting point of this derivati

was determined to be 156°C -- the value given in the literature.

The malonic acid obtained after acidification with dilute sulphuric acid was extracted with 1 cc. of ether. The ether layer was transferred to a small beaker by means of a pipette. The ether was evaporated, the remaining malonic acid crystals were dried, and a melting point determination was made: The melting point was found to be 132°C . The value given in the literature was 133°C .

D. The Identification of m-nitrobenzaldehyde

1. The melting point of this compound was found to be 58°C .

2. The preliminary tests for the elements indicated the presence of nitrogen and the absence of sulphur and the halogens.

3. The bromine and potassium permanganate unsaturation tests were both negative.

4. The solubility tests placed the compound in group #7.

5. The hydrolysis tests for amides was negative but the sodium amalgam test for the presence of nitro groups was positive.

6. Schiff's reagent's test and the ammoniacal silver oxide test for aldehydes were applied. They were both positive.

7. The literature was then consulted and m-nitrobenzaldehyde was found to possess a melting point of 58°C .

8. The derivative prepared was m-nitrobenzaldehyde phenylhydrazone according to the procedure already described for

the preparation of phenylhydrazones of water insoluble aldehydes and ketones. The melting point of the derivative was found to be 121°C -- in exact agreement with the value given in the literature.

E. The Identification of Acetanilide

1. The melting point of this compound was found to be 113°C .

2. The preliminary tests for the elements indicated the presence of nitrogen and the absence of sulphur and the halogens.

3. The bromine and potassium permanganate unsaturation tests were both negative.

4. The solubility tests placed the compound in group #7.

5. The test for amides and the sodium amalgam tests were both negative.

6. The literature was consulted and acetanilide was found to possess a melting point of 114°C .

7. The compound was subjected to hydrolysis using the same apparatus and procedure already described for the hydrolysis of p-bromoacetanilide. The aniline was extracted with a few cc. of ether, the ether layer transferred to a small beaker by means of a pipette and evaporated. Two small drops of aniline remained. Ten drops of water were added to the aniline in the beaker and then a few drops of bromine water were added. A white precipitate of tribromoaniline formed immediately.

Due to the very small amount of tribromaniline which formed, it was impossible to obtain enough for a melting point determination. The identification of this "unknown" was made after a consideration of the reactions described above and the melting point initially determined.

F. The Identification of Glucose.

1. This compound did not possess a definite melting point. The melting point range was from 90 to 115°C.
2. The preliminary tests for the elements indicated the absence of nitrogen, sulphur, and the halogens.
3. The solubility tests placed the compound in group #2, since it was soluble in water and insoluble in ether.
4. Fehling's solution test was applied and it was positive.
5. A micro aniline acetate paper test was applied and the result was negative.
6. Tests #4 and #5 indicated that the compound was glucose. As a confirmatory test the osazone test was applied. Crystals formed in five minutes, the time limit for the formation of glucosazone crystals. These crystals were examined under the microscope and were found to be typical glucosazone crystals.

CONCLUSION

A micro technique for qualitative organic analysis has been developed. This technique made possible the analysis and identification of organic compounds in a much shorter time than is required when a macro technique is used.

A great saving of materials was also accomplished by this method. This saving of time and materials was not effected at the expense of inaccuracy and in conclusiveness of the various tests used. All of these tests were as definite and conclusive as those performed on the macro scale.

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