


1931

Study on the utilization of xylose

Hsi Ch'ou Fang
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STUDY ON THE UTILIZATION OF XYLOSE.

By

Hsi Ch'ou Fang

1931
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A Thesis Submitted to the Graduate Faculty
for the Degree of
DOCTOR OF PHILOSOPHY.

Major Subject: Plant Chemistry.

Approved:

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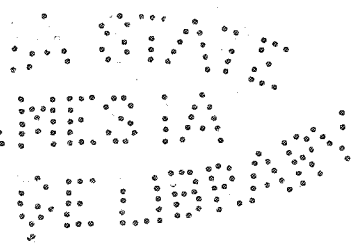
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1931

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STUDY ON THE UTILIZATION OF XYLOSE.

INTRODUCTION

Xylose has not been found free in nature but it occurs very widely distributed as a condensation product, namely xylan, which is a constituent of various hemicelluloses and gums. The commercial utilization of any of these natural products thus involves the possible utilizations of xylose.

As early as 1836, Koch (1) prepared xylose from wood gums and called it "holz-sucker" or wood sugar. In 1888 brewer's grain was used as another source for preparing xylose (2). One year after, Wheeler and Tollens (3) prepared xylose from beach wood. With the same method used by Wheeler and Tollens, Hebert (4) in 1890 obtained 16 grams of the sugar by hydrolyzing 50 grams of straw gum-xylan. A yield of 4% of xylose was obtained by Bertrand (5) from oat straw. Stone and Lotz (6) in 1891 showed that xylose could be prepared by hydrolysis of corn cobs. In 1892 Schulz and Tollens (7) showed also that a 5% yield of xylose could be obtained from wheat straw. Flax straw has likewise been used as a source of xylose (8). Tramp de Haas and Tollens (9) in 1895 made xylose from hard inner shells of coconuts. Xylose can also be obtained from the following materials: oat hulls (10), cotton seed hulls (11), and apricot seeds (12). It was reported that 12 to 13% of xylose could be obtained from apricot seeds (12).

and 10 to 12% from corn cobs (13). According to Sweeney (26), the xylose content of corn cobs probably runs about 35%.

In 1918 xylose like arabinose and rhamnose was sold around \$500 a pound because no efficient methods were available for commercial production. It was therefore regarded as a rare sugar and its usage was more or less confined to some chemical laboratory experiments. With the present method of preparation and with the annual production of 150,000,000 tons of corn stalks and 20,000,000 tons of corn cobs in the United States, not considering the other raw materials, cotton seed hulls and burrs, peanut shells, straw, etc., it is conceivable that xylose could be made as common and as cheap as glucose, if some profitable use were found for its utilization.

The investigations reported in this thesis for the utilization of xylose are limited to the three following phases of the general problem:

- I. Oxidation of xylose to xylonic acid;
- II. Preparation of pyrrole from ammonium xylonate;
- III. Fermentation of xylose to butyl alcohol. The present investigation is limited to the development of an analytical method for the determination of a mixture of butanol, acetone, and ethanol.

The taste of xylose and the similarity between the molecular structure of xylose and that of glucose, and above all, the abundant occurrences of xylose yielding materials in the plant tissues suggest a possible potential food value of xylose. On the other hand, there is some work which seems to indicate that xylose has no food value at all. Rockwood and Khoroizian (14) have shown that xylose as a food is not readily assimilated by man but that animals with long intestines could tolerate a somewhat larger amount. They based their work on the examination of the excreta for xylose. They found that the disappearance, or utilization, of xylose was greatest with the rabbit and was much less with the carnivorous cat and dog; while man had an intermediate position in the series.

PART I. OXIDATION OF XYLOSE TO XYLONIC ACID.

Introduction.

Like xylose, xylonic acid at present has very little use and there is practically no commercial demand for it. Its preparation and its usage have been of chemical interest only. This is more or less due to the cost of xylose. As the production of xylose is increased, there is reason to believe that xylonic acid can also be made cheaply.

The largest field for the use of xylonic acid would be found in the general field of organic acidifying agents. To compete in this field the price would need to be low. There is, however, always a demand for acids of particular properties and cost would not preclude such usage.

Xylonic acid may find some of its uses in medicine. For some time, a solution of calcium gluconate has been used to prevent milk fever in cattle by injecting the solution into some part of the body. With the similarity between the molecular structure of xylonic acid and that of gluconic acid, there is reason to believe that it is also possible to use calcium xylonate for the same purpose.

Since xylose has practically no food value, one would not expect that xylonic acid would be utilized by the body. It is possible that it might be used to advantage in diabetic food as an acidifying agent.

In order to prepare xylonic acid on a commercial scale the oxidizing agent must be carefully selected. It must have the following qualifications: First, the reagent must have a mild oxidizing power for with a stronger oxidizing agent like nitric acid, the oxidation of xylose gives rise to inactive xylotrihydroxyglutaric acid (15). Second, the reagent must be cheap. Third, the end products must be comparatively easy to separate. With these three requirements in mind, the investigation was directed mainly towards two reagents, hydrogen peroxide and chlorine, particular attention being devoted to the latter.

The experiments were mostly carried out in acidic solution for many workers have shown that in alkaline solutions most carbohydrates are so completely oxidized that they tend to be broken down into smaller molecules. For instance, by oxidation of 114 grams of glucose by means of copper sulfate and sodium hydroxide, Kef (16) obtained-

| | | |
|------------------------------|-------|------|
| Carbon dioxide | 5.83 | grs. |
| Formic acid | 14.71 | " |
| Glycollic acid | 22.00 | " |
| Glyceric acid | 14.00 | " |
| Trihydroxybutyric acid | 30.00 | " |
| Hexonic acids..... | 30.00 | " |
| (gluconic & mannonic) | | |

Literature Review.

The chemical preparation of xylonic acid has been mostly confined to the use of bromine, with relatively minor variations from the first preparation used by Allen and Tollens (17) in 1830 -- i.e. 4 years after the first isolation of xylose. They used 6 grams of xylose in 30 grams of water and then treated the solution with 12 grams of bromine at ordinary temperature. After all the halogen dissolved, the hydrogen bromide was removed with lead and silver carbonates. Having filtered the bromides, they passed hydrogen sulfide through the filtrate to remove lead and silver and then drove off the excess of hydrogen sulfide by boiling the solution. Calcium carbonate was added to the solution and then the excess of calcium carbonate was filtered out. The solution was concentrated down to syrup under vacuum, leaving the calcium salt as a syrup.

In 1891 Bertrand (18) discovered the characteristic cadmium double salt of xylonic acid - i.e. $(C_5H_9O_6)_2Cd \cdot CdBr_2 \cdot 2H_2O$; but his method of preparation of the acid was essentially that of Tollens. In fact, in seeking a method for the conversion of xylose to the corresponding monobasic acid a chemist still reverts to the classical principle used to such great advantage by Tollens but the yield of the acid has never been satisfactory for any commercial purpose.

Hudson and Isbell (19) in 1939 increased the yield of xylonic acid from bromine oxidation by using barium benzoate as a buffer. They claimed that a yield of 90% was obtained.

Experimental Procedure.

A. Hydrogen Peroxide Oxidation.

(a). For preliminary experiment, 10 grams of recrystallized xylose were weighed into a 100 cc. volumetric flask and water of known acidity was added to volume. The solution was transferred into a small three-neck flask, and was stirred with a mechanical stirrer while one mole equivalent of dilute hydrogen peroxide solution was added to the solution drop by drop from a small dropping funnel through one of the side arms of the flask. Increase of acidity was tested by titrating a few cc. of the solution with 0.1 N of sodium hydroxide once in several hours. The experiment was stopped, when the solution showed no apparent sign of acidity increase. The data are given in Table A. No attempt was made to isolate the products formed, on account of the small amount of acid present.

(b). After the failure to secure great increase of acidity in the preliminary experiments, an attempt was made to increase the production of acid by increasing the amount of hydrogen peroxide added and lowering the acidity during the reaction by adding zinc carbonate (excess) into the solution before the experiment. The experimental procedure was carried out exactly the same as that in Part a, except for the following: A solution of 100

Table A

Initial acidity and acidity increase with hydrogen peroxide oxidation of xylene at ordinary temperature

| Experiment Number | Hours | Concentration of xylene (%) | No. mole valents H ₂ O ₂ | Initial acidity (N) | Total acidity (N) | Acidity increase (N) | Theoretical acidity (N) |
|-------------------|-------|-----------------------------|--|---------------------|-------------------|----------------------|-------------------------|
| 1 | 115 | 10% | 1 | 0.125 | 0.379 | 0.256 | 0.045 |
| 2 | 115 | 10% | 1 | 0.150 | 0.339 | 0.231 | " |
| 3 | 115 | 10% | 1 | 0.236 | 0.41 | 0.176 | " |

grams of xylose in 1000 cc. was used. The solution was neutral to begin with. 150 grams of zinc carbonate were added to the solution. Six mole equivalents of dilute hydrogen peroxide were used. After the addition of all the hydrogen peroxide, the mixture was continuously stirred for about 24 hours before the experiment was stopped. The excess of zinc carbonate was filtered out, washed, dried and weighed. A sample of the insoluble material was taken for the analysis of zinc oxalate which might have been present. According to the analysis with the potassium permanganate method, 5% of oxalic acid was produced.

The filtrate was concentrated to about 600 cc. under reduced pressure. Powdered oxalic acid was carefully added to the solution bit by bit until zinc oxalate crystals no longer found as shown under the compound microscope. The zinc oxalate was filtered, and washed with about 200 cc. of distilled water. The wash water was added to the filtrate. The whole solution was transferred to a liter volumetric flask and water was added to volume. A sample of 10 cc. of the solution was titrated against standard sodium hydroxide for the total acid present. A total of about 65% acid (calculated as formic acid) was found.

The solution was then steam distilled. About 15% of formic acid was determined by the mercuric chlo-

ride method.

The liquid left after the distillation was diluted and neutralized with zinc carbonate. The solution was concentrated under reduced pressure to a syrup and about 400 cc. of 95% alcohol was added. The mixture was placed in the ice box for about three weeks with frequent stirring. About 42 grams of zinc tartronate were isolated. M.P. 137°.

Identification: Dimethyl-tartronate, M.P. 54-5°.

| | | | |
|------------------------|--------|-------|--------|
| Analysis : Zn - Calcd. | 33.96% | Found | 33.89% |
| | | | 33.88% |

Unfortunately, the method could not be sufficiently standardized to recommend it as a method of preparation for tartronic acid. The failure is probably due to lack of control of the acidity during the reaction.

No zinc xyloate was isolated from this oxidation procedure.

B. Oxidation of xylose With Chlorine in Acid Solution.

The apparatus used in the study of the chlorine oxidation is shown in Fig. A.

A liter of 10% xylose solution was placed in the three-neck flask and stirred vigorously with the centrifugal stirrer C. While stirring, chlorine gas was forced into the flask F which had been calibrated, until a certain desired volume was reached; and then the stop

cock (three-way) was opened and the measured volume of gas transferred into the three-neck flask where the sugar was to be oxidized. It took about one hour for a liter of chlorine to be absorbed. At the end of each hour, after a liter of chlorine gas had been absorbed, the solution was neutralized carefully with ammonium hydroxide from the burette through the inner tube t, until the solution turned blue litmus paper slightly red. Testing the acidity of the solution was done by drawing out one or two drops of the solution with pipette. Now and then the connection between the safety tube S' and the side arm of the three-neck flask was loosened to release some pressure developed in the flask due to increase of solution volume by adding the aqueous ammonia on the one hand and partial decomposition of ammonium hydroxide by chlorine on the other. When the pressure was not so released, it had the tendency to stop the entrance of the chlorine gas from the flask F. For every volume of gas, room temperature and barometer reading were recorded and the volume was corrected to that under standard conditions.

At the end of the reaction, the solution was transferred into a distilling flask and concentrated under reduced pressure until ammonium chloride crystallized. It was then cooled, the ammonium chloride was

filtered with suction, and washed with a small volume of cold water. This process of concentrating was repeated three or four times until no ammonium chloride crystals were shown when the solution was syrupy.

The thin syrup was poured from the flask into a 500 cc. beaker, and about 300 cc. absolute alcohol was added. It was seeded with a small crystal of ammonium xylonate which was prepared with Tollens' method for other salts of sugar acid. (Allen and Tollens, Ann., 250: 306. 1890). With constant stirring, crystallization began to take place in about 8 days. At the end of about 3 weeks (usually longer), about 60 grams of crude xylonate were obtained. After three fractional recrystallizations from alcoholic solution, about 40 grams of pure ammonium xylonate were obtained - i.e. 30.6 percent yield of the theoretical. M.P. 120-122°. $(\alpha)_D^{27} = +6.8$

| | | | | | |
|--------|---|--------|--------|-------|--------|
| Anal.: | N | Calcd. | 7.617% | Found | 7.618% |
| | | | | | 7.616% |

The ammonium salt of xylonic acid has not been previously reported.

An attempt was made to prepare potassium xylonate with the same procedure for preparing ammonium xylonate, but no crystals of potassium xylonate were obtained even though the solution was left in the ice box for more than two months. Special effort was made to prepare the salt by using Tollens' method of preparing other salts of sugar

acids, but no crystallization took place.

C. Oxidation of Xylose With Chlorine in Basic Solution.

The experimental method of oxidizing the sugar with chlorine in basic solution was exactly the same as that just described except the solution was kept basic to litmus paper all the time. The yield of ammonium xylenate was very unsatisfactory.

- R = Reservoir
- B = Burette
- S + S' = Safety Traps
- t = Inner Tube
- C = Centrifugal Stirrer
- F = Flask

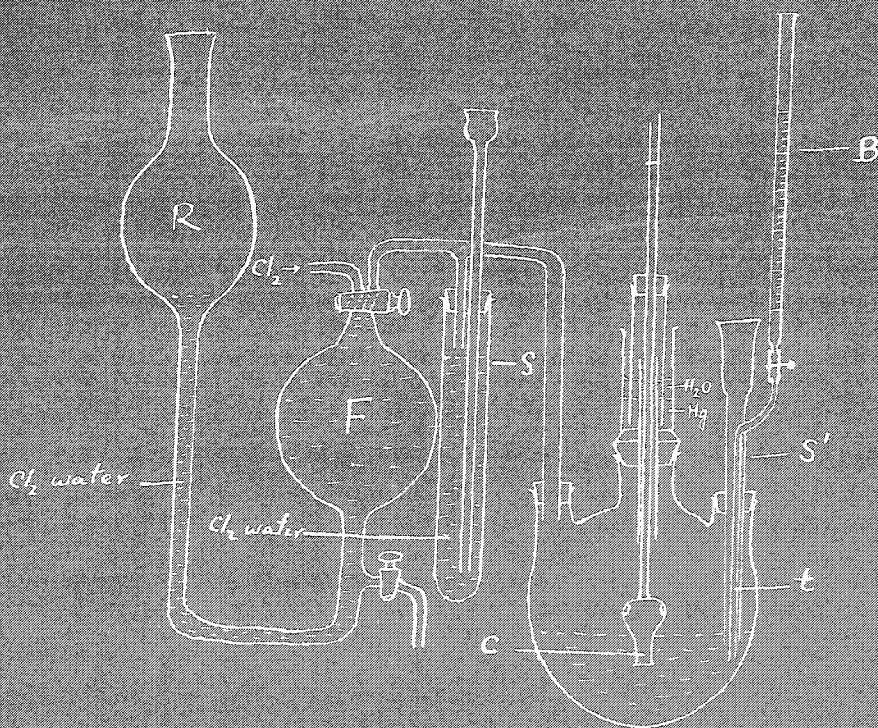


Fig. A

Discussion.

The oxidation of xylose and, as a matter of fact, most of the carbohydrate materials, to a desired product is not so simple a process as might be thought. Practically every experimental condition has a profound influence on the products formed. Of these conditions, probably hydrogen ion concentration is the most important. The necessity of adjusting the acidity of the sugar solution to be oxidized is emphasized in one of the statements made by Kline and Acree (20): "The regulation of the pH is therefore of prime importance in both the analytical method and in the practical application of these reactions in the production of sugar acids from the sugars by the use of chlorine and bromine".

The increase of hydrogen ion concentration retards the rate of oxidation. Stieglitz (21) has emphasized the fact that the oxidation of glucose is enormously accelerated in the presence of alkali and it is retarded by the presence of neutralizing acids. The same is true with other carbohydrates. From his study on the mechanism of carbohydrate oxidation, Evans (22) concludes that the tendency of the increased hydrogen ion concentration is to suppress the progress of oxidation. The data on Table A well illustrate this fact.

When the hydrogen ion concentration is not well regulated, basicity not only hastens the rate of oxidation, but also the products formed by this phase of oxidation are various. As mentioned in the introduction glucose is oxidized by copper sulfate and sodium hydroxide (16) into 6 or 7 products instead of gluconic acid alone as we ordinarily assume. Spohr (23) found that glucose when oxidized by hydrogen peroxide in alkaline solution gave formic acid, carbon dioxide, glycollic acid, and *d*-hydroxymethyl-*d*-arabonic acid.

On the other hand, when hydrogen ion concentration was carefully regulated to even slightly basic, Goebel (24) oxidized 9 grams of glucose with barium hypiodite, and was able to obtain 91% of crude calcium gluconate. Likewise Honig and Tempus (25) obtained quantitative yield of barium gluconate by using barium hypobromite as an oxidizing agent.

Oxidation of sugars on the acid side does not tend to form numerous products but has the disadvantage that the rate of oxidation is comparatively slow. In Tollens' method of preparing sugar acids, for instance, hardly any other product has ever been isolated other than the sugar acid and unoxidized sugar. When the hydrogen ion concentration was well regulated on the acid side by using barium benzoate as a buffer, Hudson and

Isbell (19) were able to prepare barium xylenate as well as barium gluconate quantitatively.

From the results as obtained by Goebel, Honig and Hudson, it is safe to say that the right hydrogen ion concentration suitable for sugar oxidation is somewhere between pH of 6 and 9. But for a practical purpose, it seems that it is much easier and safer to oxidize the sugars on the acid side than on the basic side. The difficulty in controlling the rate of oxidation on the basic side explains the fact that in the investigation no satisfactory yield of ammonium xylenate resulted from the chlorine oxidation on the basic solution.

On the other hand, the yield of sugar acid from direct chlorine oxidation is not very satisfactory either. This is probably not due to wrong adjustment of hydrogen ion concentration, but rather due to the difficulty in separating the acid from the unchanged sugar and to the difficulty in separation of the reaction product from the inorganic constituents.

On the whole, the method of preparing xylenic acid with chlorine is not sufficiently satisfactory to be recommended on a commercial scale. It is possible that the selection of a suitable Buffer for control of acidity during the reaction might completely remove these difficulties. The method reported in detail can

be recommended for the laboratory preparation of ammonium xylonate.

PART II. PREPARATION OF PYRROLE FROM
AMMONIUM XYLONATE.

Introduction.

Pyrrrole has been known for a long time. The methods of formation are numerous but practically none of these can be even used for laboratory preparation. Crude mixtures of pyrrrole with other bases are obtained from coal tar. It also occurs in bone oil. The preparation of pure pyrrrole from the two sources mentioned above is not at all satisfactory for any practical purpose. Bone oil for instance contains several pyridine bases and fatty acid nitriles in addition to pyrrrole. Some of these products are of little value and all tend to complicate the extraction and the recovery of the more valuable products.

According to Michelman (27), in 1860 Weidel and Ciamician obtained pyrrrole by distilling gelatin. It is very apparent that gelatin is too costly for the preparation of pyrrrole. In 1925 Michelman (27) proposed to prepare pyrrrole by destructive distillation of leather scrap but the yield obtainable was not given.

Since the first publication of the method by Schwanert in 1860 (28) pyrrrole has usually been prepared by the dry distillation of ammonium mucate. It may be prepared from ammonium saccharate in good yield but

saccharic acid is comparatively more difficult to obtain and hence is more expensive. McKilvain and Holliger (29) recommended a method of preparation by adding glycerol to the ammonium mucate, yields of 37 to 40% of pyrrole being claimed from 630 grams of mucic acid.

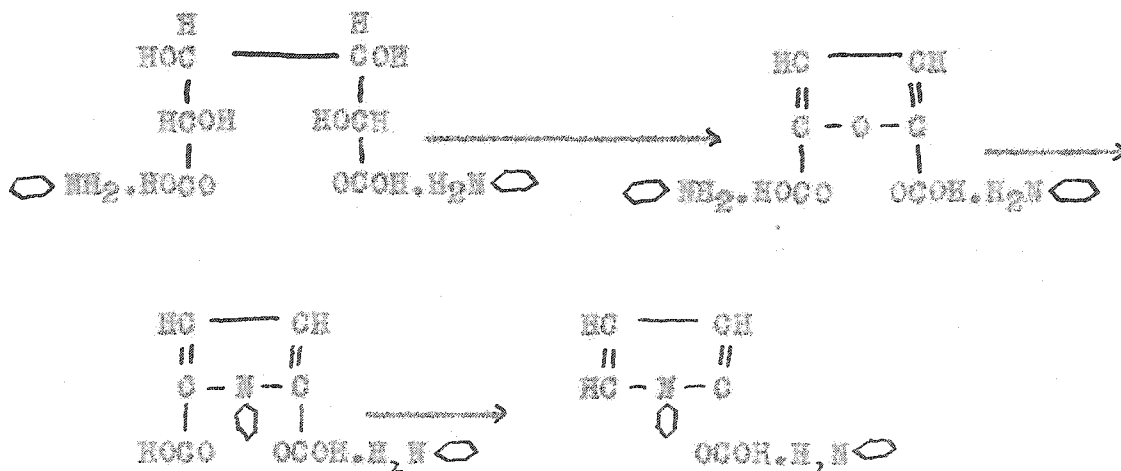
At present, pyrrole has very little use. Since its derivatives are widely related to many important substances, such as nicotine, hemoglobin, chlorophyll, indol, etc., there is every reason to believe that pyrrole would be important not only to the chemist himself but the biologist as well, if it could be produced at moderate prices.

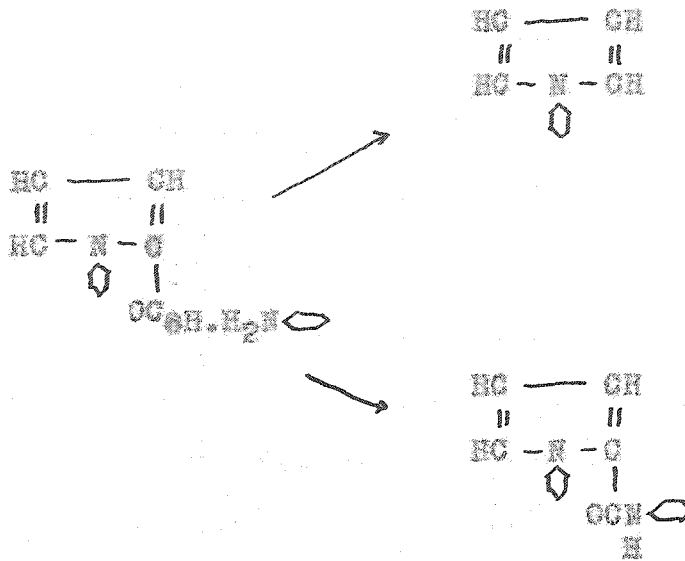
The possible use of ammonium xylostate as a source for the production of pyrrole has been investigated as a possible method of utilizing xylose. The experiment is supplemented with the pyrolysis of ammonium arabinate, ammonium gluconate, and ammonium galactonate.

The mechanism of pyrolysis of ammonium xylostate for the present remains unknown. In order to attempt any explanation for such reaction, it seems best to find support in some work on the dry distillation of mucic acid, as well as its salts, especially the ammonium salt. Heinzelmann in 1876 (30) heated mucic acid and hydrogen bromide in a closed tube for several

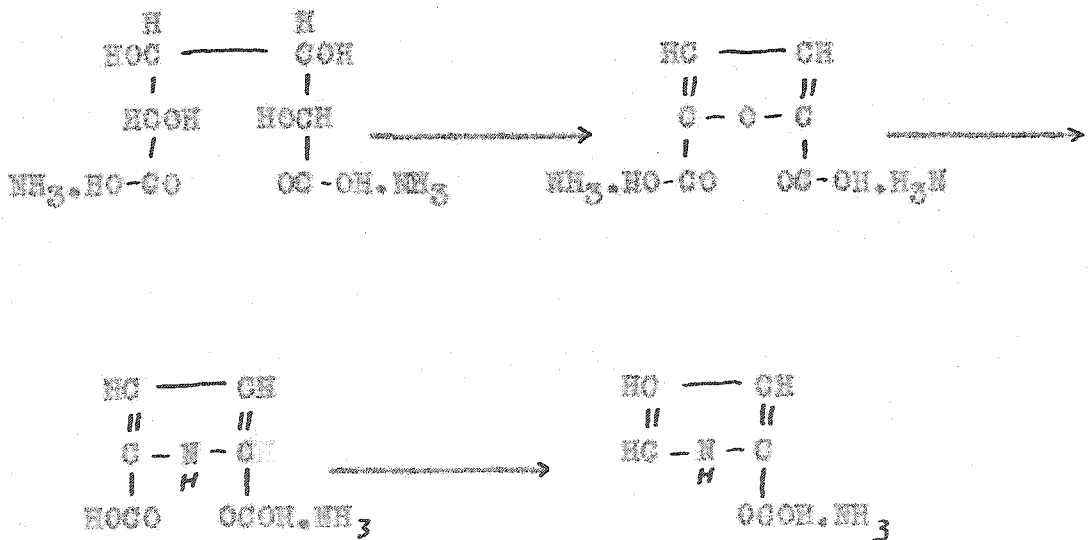
days on a water bath. Instead of obtaining bromocadipic acid, he obtained dehydromucic acid which on further heating yielded pyromucic acid. Schmitt and Cobenzl (31) reported that potassium dehydromucate was formed in large yields when neutral potassium mucate was distilled in the presence of an atmosphere of hydrogen; but Hill (32) claimed that this experiment could not be duplicated.

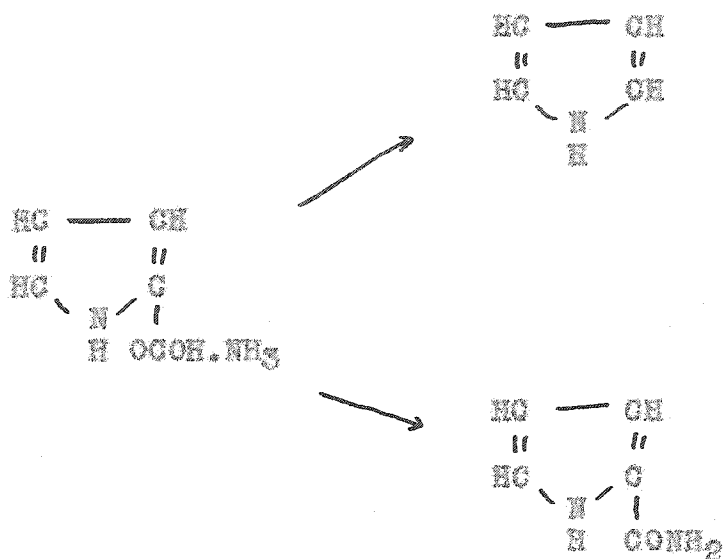
By distillation of ammonium mucate, Schwabert (33) obtained both pyrrole and pyrrolecarboxylamide. Distillation of mucic acid with primary amines gives in the same way N-substituted pyrroles. For instance, methyl, ethyl, and amyl pyrroles were so obtained by Bell (34); and phenylpyrrole by Pictet and Steinmann (35). The mechanism of the synthesis of phenylpyrrole was considered by Pictet and Steinmann to be as follows:



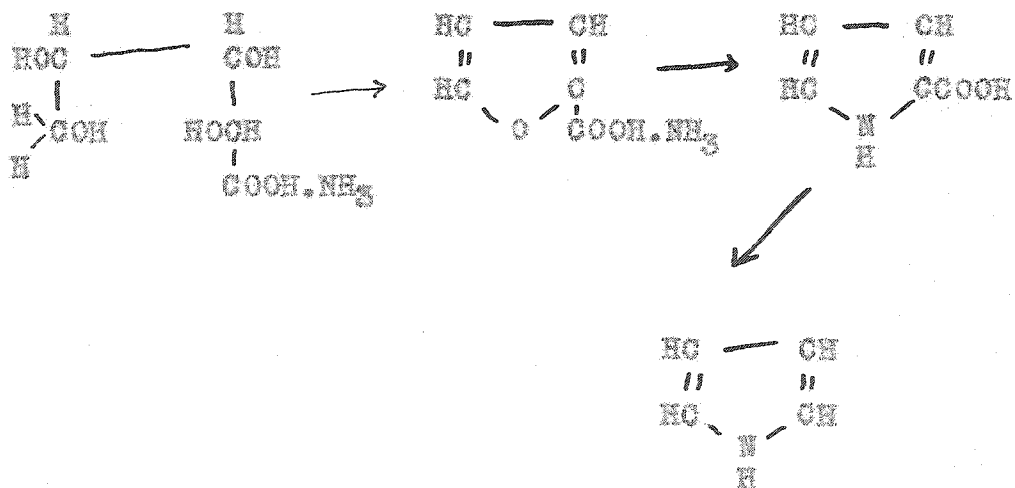


The same mechanism was applied to the pyrolysis of ammonium mucate (36):





Like the mechanism for the ammonium mucate distillation, it seems very probable that ammonium xylonate upon heating yields first ammonium pyromucate, then pyrrole- α -carboxylic acid and pyrrole:



The fact that the ammonium salts of the mono-basic hexonic acid upon heating also yielded pyrrole is rather difficult to explain. Whether it is due to further

oxidation by the air in the flask during heating to the dibasic acid remains to be investigated. One would expect pyridine to be formed in analogy to the above reactions for the formation of pyrrole.

Experimental Procedure.

A. Pyrolysis of Ammonium Xylonate.

47 grams of powdered ammonium xylonate from two different sources, one from Tollens' bromine method for other salts of sugar acids and the other from chlorine oxidation as developed, were placed in a 250 cc. distilling flask and subjected to dry distillation. A total of about 50 cc. liquid was distilled over. The distillate was then extracted with 30 cc. of anhydrous ether, and the extract was fractionately distilled.

The fraction collected from 100° to 131° was distilled again. About 4 cc. of distillate was collected at 131°; and it was perfectly colorless at first, but soon turned dark brown. It had a strong odor of pyrrole; it polymerized when treated with concentrated hydrochloric acid. Yield 24.5% of the theoretical.

B. Pyrolysis of Ammonium Arabonate.

Arabinose was prepared from mesquite gum by using the method of Anderson and Sands (37). It was then oxidized with bromine as described by Allen and Tollens for preparation of other salts than ammonium salt of sugar acids (17), except with some slight modification. The method may be described as follows:

40 grams of arabinose were dissolved in 360 cc.

of water. The solution was placed in a three-neck flask and vigorously stirred with a mechanical stirrer. 50 grams of bromine was added to the solution slowly from a separatory funnel drop by drop. The other side arm of the flask was tightly corked. While the bromine was being added, the temperature of the solution was never allowed to rise above 31°. After 48 hours of stirring the excess of bromine was driven off by heating the solution on a water bath. The hydrobromic acid was removed with lead and silver carbonates. After the lead and silver bromides had been filtered out, a stream of hydrogen sulfide was bubbled through the filtrate until no more precipitate appeared. The sulfide was then filtered off and the hydrogen sulfide gas was driven from the solution by boiling. The solution was neutralized with ammonium hydroxide, and concentrated to syrup under vacuum. About 100 cc. of 95% alcohol was added to the syrup and the solution was placed in the ice box. With constant stirring crystallization began to take place in 4 days; and after 7 days about 25 grams of ammonium arabonate were isolated. According to Tollens (38) ammonium arabonate has been previously reported but no other reference could be found. It melted at 155 - 156° with decomposition. $(\alpha)_D^{34} = +10.0$

Anal. N; Calcd. 7.62%

Found. 7.55%
7.64%

55 grams of ammonium ararbonate were dry distilled according to the manner described for the pyrolysis of ammonium xylonate. A little more than 1 cc. of pyrrole was isolated. Yield about 5 or 6 percent of the theoretical.

C. Pyrolysis of Ammonium Gluconate.

Ammonium gluconate was prepared by neutralizing 100 grams of commercial gluconic acid with ammonium hydroxide.

100 grams of ammonium gluconate were dry distilled. The distillate was made acid with acetic acid, and then extracted with 30 cc. ether. When the regular procedure for isolating pyrrole was followed, about 2 cc. of pyrrole was isolated from the ether extract.

The mother liquor was then made strongly acidic with hydrochloric acid and concentrated to about one-fifth of the original volume. The solution was then saturated with potassium hydroxide and let stand over night. The solution gave a strong odor of pyridine but not enough to be separated.

D. Pyrolysis of Ammonium Galactonate.

Ammonium galactonate was prepared by the modified method as described for the preparation of ammonium ararbonate. A quantitative yield was obtained. The salt turned yellowish at 110° (106° according to Tollens), and decomposed at 142°.

100 grams of ammonium galactonate were distilled. The distillate was made acid with acetic acid. The method of isolating pyrrole was the same as that for isolating of pyrrole from ammonium gluconate. About $1\frac{1}{2}$ cc. of pyrrole was obtained. No pyridine save its odor was detected.

Discussion.

In the present experiment, the yield of pyrrole is rather low in comparison with that from the method recommended by McElvain and Bolliger (29). However, it is comparable with that obtained from direct dry distillation of ammonium succate (39), when the amount of material and the kind of apparatus used are considered. Undoubtedly the yield could be considerably increased, if a larger amount of the ammonium salt were distilled in an iron retort with the addition of some glycerol. For practical purposes, direct distillation is to be preferred, for according to Craig and Hixon (39), addition of glycerol causes more trouble in addition to its relative expensiveness. On the whole, it is very evident that ammonium xylonate can be a very promising source from which to prepare pyrrole, when some better method for preparing the salt is well developed.

The fact that with exactly the same experimental conditions, a quantitative yield of ammonium galactonate was obtained is very astonishing. Whether it is due to easy separation of the ammonium salt from the other constituents or due to right conditions for the oxidation of this particular hexose, remains to be investigated.

Under the experimental conditions, all the ammonium salts of the hexonic acids gave pyrrole instead of

pyridine. The fact is rather unexpected. It is probably due to easier formation of dibasic acid during heating than the pyridine produced.

PART III. A METHOD OF ANALYSIS FOR TERNARY
MIXTURES OF BUTANOL, ETHANOL,
AND ACETONE.

Introduction.

The butyl alcohol fermentation has been one of the most important chemical industries developed by the war. It is considered to be one of the most feasible ways by which products either grain or waste materials may be utilized. Qualitative experiments have shown that relatively high ratios of xylose may be included in this fermentation.

In fermenting either grain or sugars with the butyl organism, it has been found that acetone, butyl alcohol, and ethyl alcohol are produced in a fairly definite ratio (40). A quick and simple method of analysis with a fair degree of accuracy for such a mixture is essential for a study of the conditions of the fermentation as well as for industrial control. The following method was developed for the purpose of studying the effect of xylose on the solvent ratios in this fermentation.

Chemical methods for analyzing such a mixture are not only tedious but also confronted usually with many difficulties. The literature has failed to reveal any simple and at the same time quick physical method by which the percentage of each constituent in this particular mixture can be easily determined.

Reilly and his co-worker (41) determined the densities of a series of acetone, butyl alcohol and water mixtures. These data were represented by means of both triangular and rectangular coordinates, from which, together with a knowledge of the percentage by weight of one of the constituents, the composition of any mixture was obtained. But this method is limited to the binary system only.

Werkman and Osburn (42) have recently developed a method for determining butyl and ethyl alcohols in fermentation mixtures. The method is based upon the oxidation of the alcohols in the neutral volatile distillate by potassium dichromate and phosphoric acid. According to the authors, ethyl alcohol is oxidized to acetic acid and butyl alcohol to a mixture of butyric and acetic acids quantitatively. The acids are quantitatively determined by the partition method (43). The experimental data are not given.

Mixtures of two liquids, of which one is more soluble in water than the other have been determined by Begin (44) by measuring the volume of water required to produce turbidity. This turbidity method is claimed to be applicable to this ternary mixture if one of the constituents can be determined independently. However, the simplicity and the accuracy of the turbidity method are not

definitely shown.

The investigation to be described was undertaken to develop a simple as well as accurate method of analysis for this particular mixture of solvents as obtained in fermentation experiments. Such a method is essential for the study of the utilization of xylose for the production of these solvents.

The method as originally outlined was based on: first, the additive property of refractive indices of the constituents; second, the specific gravity of the total solvent being the summation of specific gravities of all its constituents; and third, the partition coefficient of the total solvent between carbon tetrachloride and water. For partition instead of using carbon tetrachloride, other organic solvents such as xylene may be conveniently used. With the three constituents as three variables and an equation for each separate method of determination, it was expected that one could simply solve for the percentage of each constituent in the mixture simultaneously without involving any chemical analysis.

As it shall be shown later, the partition method is rather complicated and its equation, as developed, is long. For practical purposes, the partition method would be omitted but it is very useful as a confirmation of the results obtained by the following method.

Acetone may be determined by the standard method of iodine titration which is easily carried out. This would then leave two unknown factors requiring two equations for the analysis of the mixture which can be obtained from the specific gravity and refraction measurements. Notwithstanding that the method involves one chemical analysis, it is still very simple and easily done. Besides its simplicity, it has other distinctive features; 1. Its results are satisfactory and reliable. Its possible error is within plus or minus three-tenths of a percent. 2. It is very economical both as to chemical and time.

As stated before, in the fermentation of grain or sugars including xylose under the conditions used in this laboratory, the solvents are produced in approximately a constant ratio. Since the method of analysis was designed for our own particular solutions, special attention is devoted to the ratio of solvents obtained under our experimental conditions which are about 6 parts of butyl alcohol, 3 parts of acetone, and 1 part of ethyl alcohol.

The measurements used in the analysis were first studied on dilute solution of each constituent, then on binary mixtures, and finally on the ternary mixture in the 6:3:1 ratio.

Experimental Procedure.

In purification of the solvents for preparation of the known mixtures, the constituents were subjected to the following purification as well as fractionating: Ethyl alcohol was first refluxed with air slaked lime; carbon tetrachloride and xylene were both dried over calcium chloride; the n-butyl alcohol and acetone were both dehydrated with anhydrous sodium carbonate. Each distillate was then tightly stoppered and held in an air thermostat for at least twelve hours before its specific gravity was determined. All experiments were conducted at 25°.

Solutions were made up by accurately weighing each constituent and then mixing and diluting to volume in the same flask. As in the case of each distillate mentioned above, they were placed in the thermostat for not less than twelve hours to attain constant temperature.

The specific gravity determinations were made with a Chainometric Westphal balance. It was found that specific gravity was an additive property under the experimental conditions. This is clearly shown in Table III.

The refractive index of each solution was determined by means of an immersion refractometer. The solutions to be examined were transferred to small beakers which had been kept in the constant-temperature compartment at 25°.

Before carrying out the determinations, the water bath of the refractometer was regulated to the same temperature as the thermostat. In order that there be no great change of temperature of the solution due to conduction of heat by the prism of the instrument, the instrument should also be kept in the thermostat. With all parts at the same temperature, five readings for each solution were taken immediately, and their average reported as the final measurement. Preliminary experiments revealed that satisfactory partition measurements could be obtained, when 15 cc. of each solution was extracted with 30 cc. of carbon tetrachloride or 30 cc. xylene. Amounts of less than 30 cc. of immiscible solvent showed a tendency to emulsify with some of the solutions. The partition was made by shaking the immiscible liquids in a separatory funnel for 2 or 3 minutes and then placing in the thermostat for about two hours. The mixtures were separated carefully and the refractive indices of the water solutions were measured as described above.

Results

The experimental data for the unitary solution are presented in Table I, and represented graphically in Fig. I and II.

As may be noticed from Figs. I, III, IV, and V, grams solvent per hundred cubic centimeters is plotted against the scale readings of the immersion refractometer instead of refractive indices. This was done to avoid the long figures of the corresponding values of the refractive indices. The readings as presented in the tables may be converted into the corresponding refractive indices by tables such as those given in Leach (45).

With the experimental data from Table I, the following equations were developed:

S = Sp. gravity before extraction.

C = Concentration or grams per 100 cc. solution.

R = Scale reading of immersion refractometer before extraction.

R' = Scale reading of immersion refractometer after extraction.

B = Butyl alcohol

A = Acetone.

E = Ethyl alcohol.

$$S_B = -0.00140C_B + 1 \dots\dots\dots \text{Eq. 1}$$

$$S_A = -0.0015C_A + 1 \dots\dots\dots \text{Eq. 2}$$

$$S_B = -0.0017C_B + 1 \dots\dots\dots \text{Eq. 3}$$

$$S_{B+A+E} = -0.0014C_B - 0.0015C_A - 0.0017C_E + 1 \text{ Eq. 4}$$

$$R_B = 2.80C_B + 13.26 \dots\dots\dots \text{Eq. 5}$$

$$R_A = 1.98C_A + 13.26 \dots\dots\dots \text{Eq. 6}$$

$$R_E = 1.73C_E + 13.26 \dots\dots\dots \text{Eq. 7}$$

$$R_{B+A+E} = 2.80C_B + 1.98C_A + 1.73C_E + 13.26 \text{ Eq. 8}$$

$$R'_B = \frac{14.80C_B - (C_B)^2}{8.85} + 14.10 \dots\dots\dots \text{Eq. 9}$$

$$R'_A = \frac{32.60C_A - (C_A)^2}{25.6} + 14.10 \dots\dots\dots \text{Eq. 10}$$

$$R'_E = 1.60C_E + 14.10 \dots\dots\dots \text{Eq. 11}$$

$$R'_{B+A+E} = \frac{14.80C_B - (C_B)^2}{8.85} + \frac{32.60C_A - (C_A)^2}{25.6} + 1.60C_E + 14.10 \dots\dots\dots \text{Eq. 12}$$

Table II contains the experimental results and calculated values of the binary solutions. The experimental results

Table I

Specific gravity and scale readings of immersion refractometer for unitary solutions of butenol, ethanol and acetone at 25°C.

| Concentration gas/100 cc. of solution | Specific Gravity | Scale reading of Immersion Refrac- tometer | Scale Reading of Immersion Refrac- tometer after extraction. |
|---|---|--|---|
| | Experim. : Calcd. by: Experim. : Calcd. by: | Experim. : Calcd. by: | Experim. : Calcd. by: |
| | : 30.1, 2.3 : | : 29.5, 6.7 : | : 29.9, 10.1 : |
| Butenol | | | |
| 2 | 0.9973 | 18.82 | 17.00 |
| 4 | 0.9943 | 24.50 | 19.00 |
| 6 | 0.9914 | 30.15 | 20.10 |
| pure | 0.8076 | | |
| Acetone | | | |
| 2 | 0.9971 | 17.25 | 16.40 |
| 4 | 0.9940 | 21.20 | 18.40 |
| 6 | 0.9910 | 25.18 | 20.20 |
| 8 | 0.9882 | 29.09 | |
| pure | 0.7880 | | |
| Ethanol | | | |
| 2 | 0.9969 | 16.72 | 17.25 |
| 4 | 0.9922 | 20.34 | 20.39 |
| 6 | 0.9896 | 23.65 | 23.72 |
| 8 | 0.9860 | 27.40 | |
| pure | 0.7875 | | |
| water | 1.0000 | 15.26 | 14.10 |

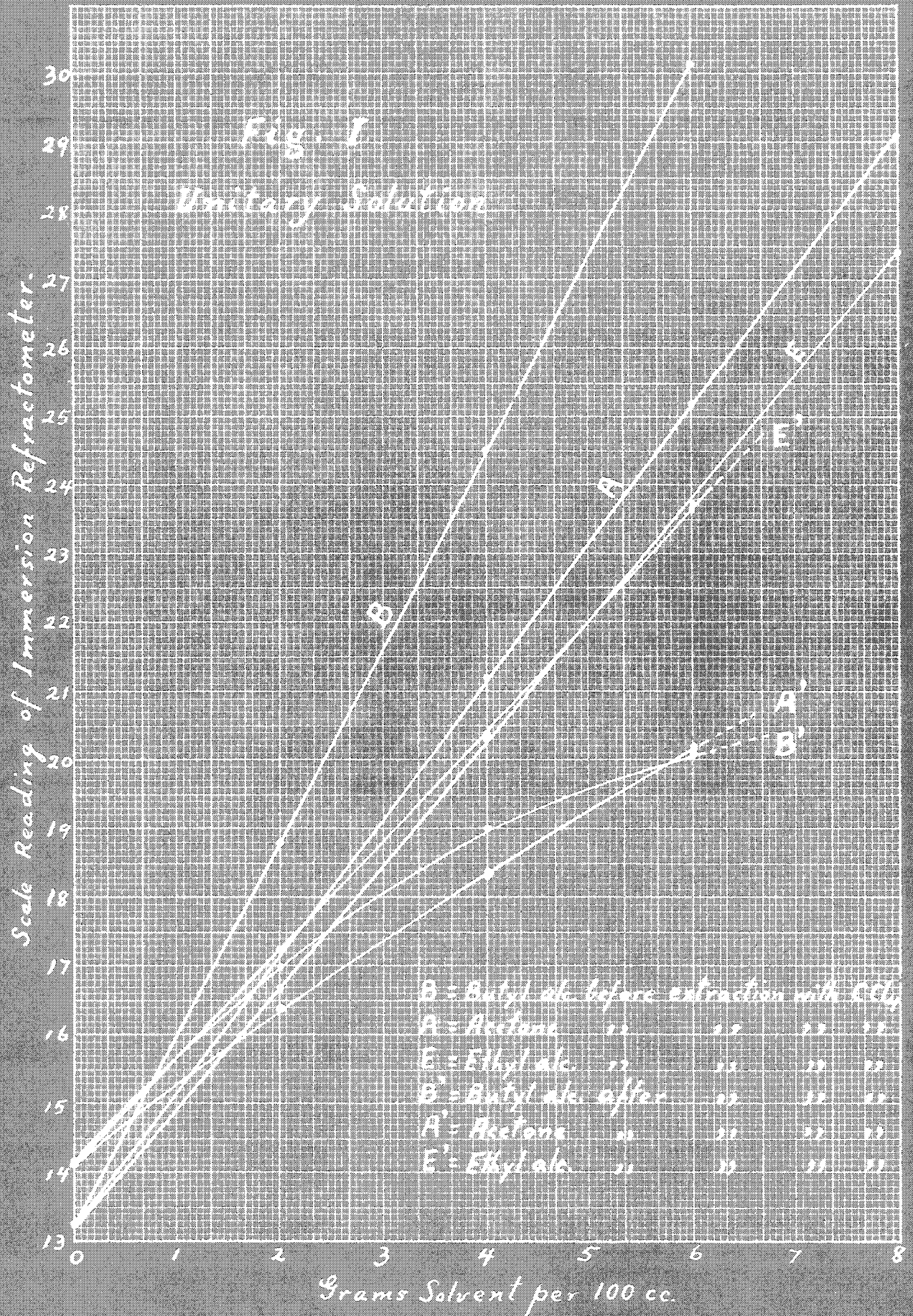
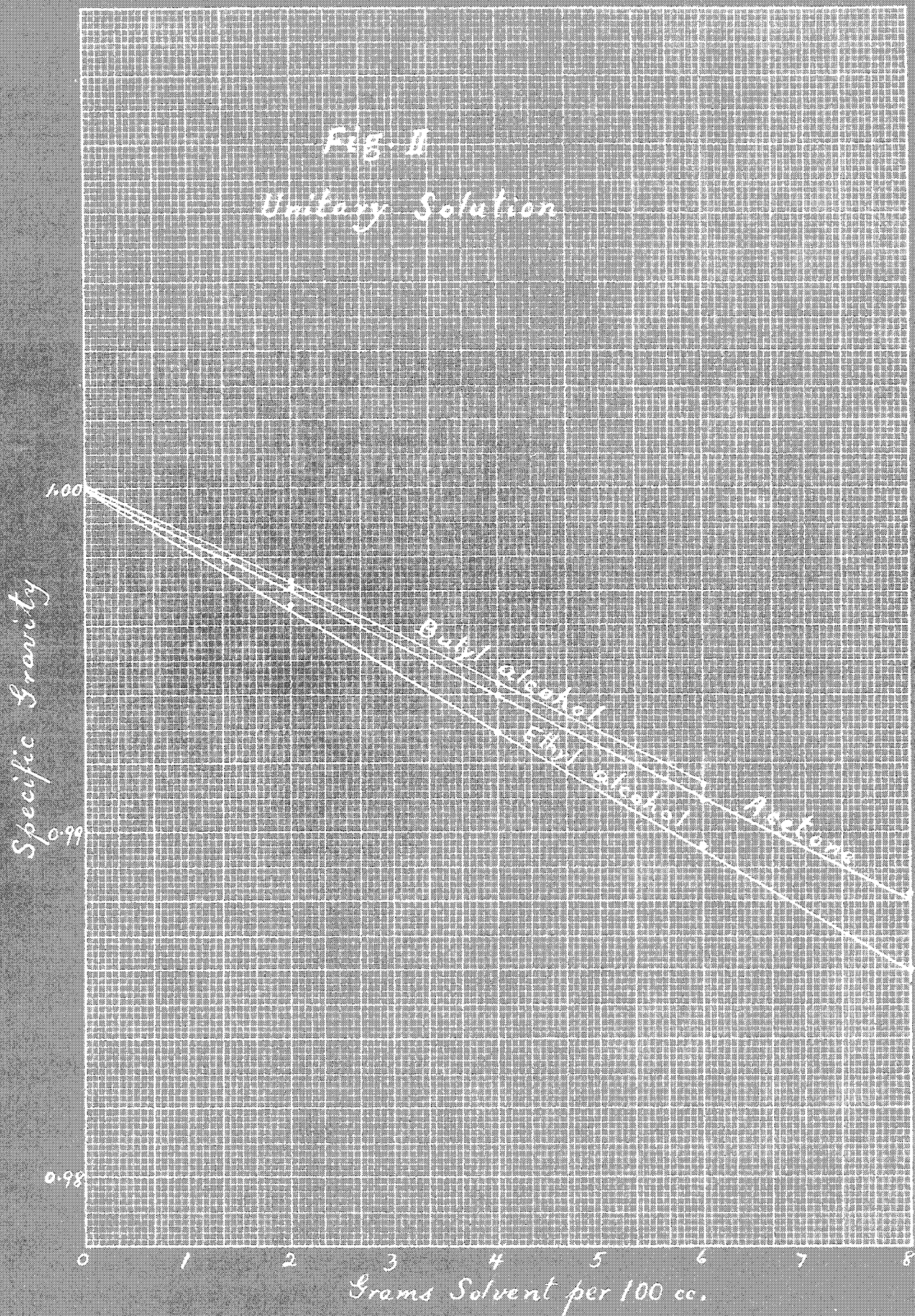


Fig. II
Unitary Solution



are also shown by graphs on Fig. III. It may be noticed that in the table, the calculated values for the refractometer scale readings after extraction are higher than the determined values. If one refers back to Fig. I, one will notice that the lines for butyl alcohol and acetone after extraction are curves, instead of straight lines. Theoretically if there is no chemical action, either polymerization or dissociation, the partition law takes the simple form:

$$\frac{C_1}{C_2} = \text{Constant.}$$

where C_1 is the concentration of the solute in solvent 1, and C_2 is the concentration of the solute in solvent 2. Then it follows that the lines for the two said solvents after extraction should be straight lines. Since they are curves, some chemical action, probably polymerization, must have taken place. This goes to explain the fact that the determined values for the refractometer scale readings after extraction in Table II are actually lower than the calculated ones. Since the calculated values are consistently higher by an approximate value of 0.95, for practical purposes, instead of using 14.10 in Equation 12, the empirical value 13.14 may be well used.

Table II

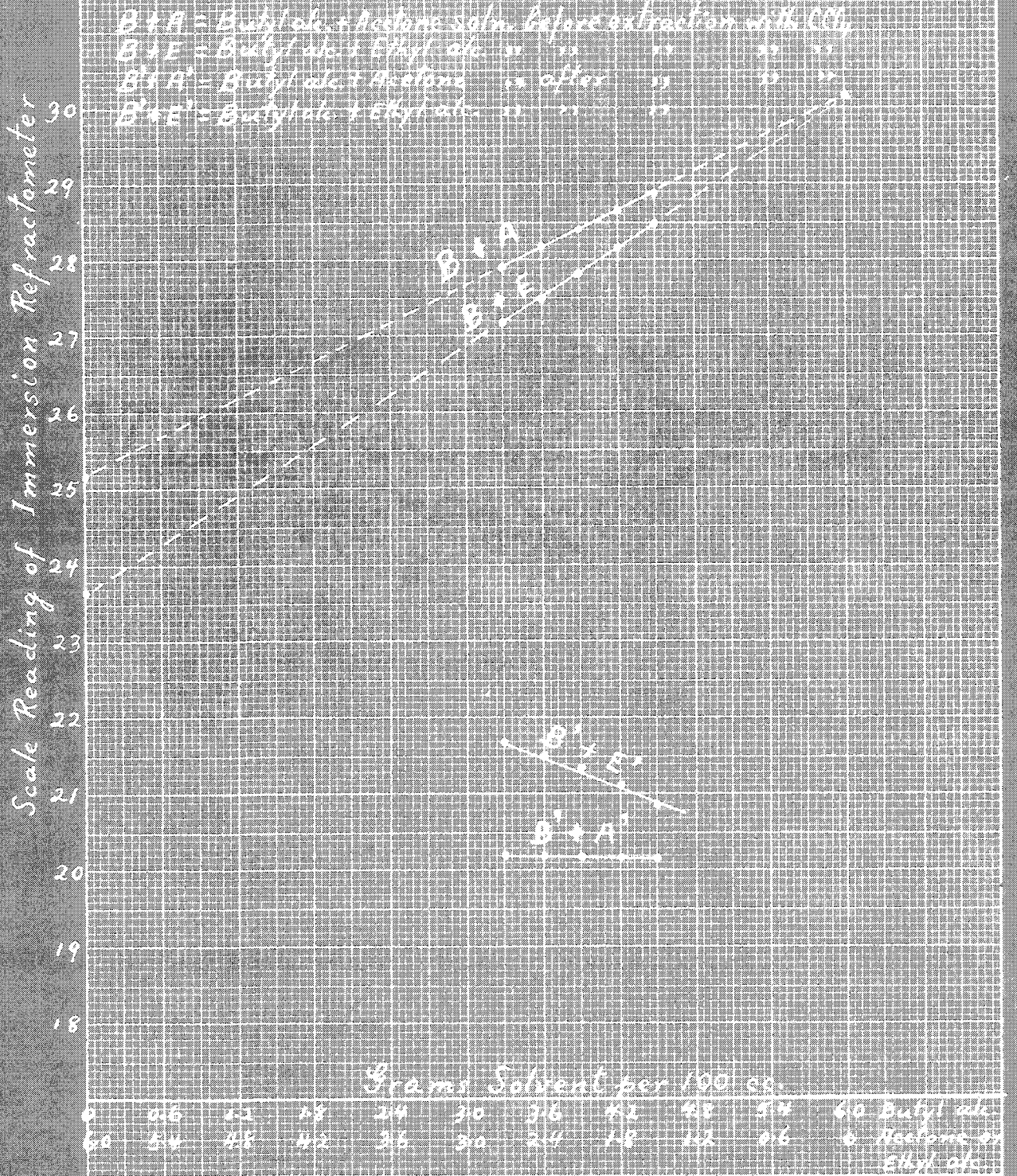
Specific gravity and scale readings of immersion refractometer
for binary solutions of butanol, acetone and ethanol at 25°C.

| Ratio of conc. GMS./100 cc. soln. | Specific Gravity | Scale Reading of Immersion Refractometer | Scale Reading of Immersion Refractometer after Refraction |
|--------------------------------------|--|--|--|
| | Experi.m.: Calc'd. by: Experi.m.: Calc'd. by: Experi.m.: Calc'd. by: Experi.m.: Calc'd. by: Experi.m.: Calc'd. by: | Eq. 4: Eq. 8: Eq. 12: - 0.96 | |
| 5.5/2.7 | 0.9910 | 27.19 | 21.72 |
| 3.6/2.4 | 0.9910 | 27.49 | 21.50 |
| 3.9/2.1 | 0.9909 | 27.22 | 21.36 |
| 4.2/1.8 | 0.9911 | 28.16 | 21.08 |
| 4.5/1.5 | 0.9906 | 28.48 | 20.80 |
| 3.5/2.7 | 0.9918 | 27.92 | 20.22 |
| 5.6/2.4 | 0.9918 | 26.13 | 20.20 |
| 3.9/2.1 | 0.9918 | 28.41 | 20.19 |
| 4.2/1.8 | 0.9918 | 28.65 | 20.17 |
| 4.5/1.5 | 0.9918 | 28.28 | 20.16 |

Butanol/Ethanol

Butanol/Acetone

Fig. II
Binary Solution



As stated before, other organic solvents such as xylene may be used to substitute for carbon tetrachloride in extraction. The actual experimental data by xylene extraction are given in Table III and graphed in Fig. IV.

Table IV contains both the calculated and the determined values of the ternary solution which is of 6:3:1 ratio throughout. As in the case of binary solution, the calculated values of scale reading after extraction are higher than the actual or determined ones. Furthermore, when these actual values are plotted against the concentrations of the solutions as shown in Fig. V, they form a curve. As in the case of the binary solutions, they are probably due to association of the molecules. Again, for practical purposes, the empirical value 13.14 may be used in calculation when the Equation 12 is used.

Table V shows the calculated percentage of both butyl alcohol and ethyl alcohol in some of the ternary solutions, together with the percentage of acetone which is determined independently by iodine titration method. The possible error of the calculated value in every case is within three-tenths of a percent.

Table III

Scale readings of immersion refractometer of binary soln. after extraction with CCl_4 and xylene at 25°C.

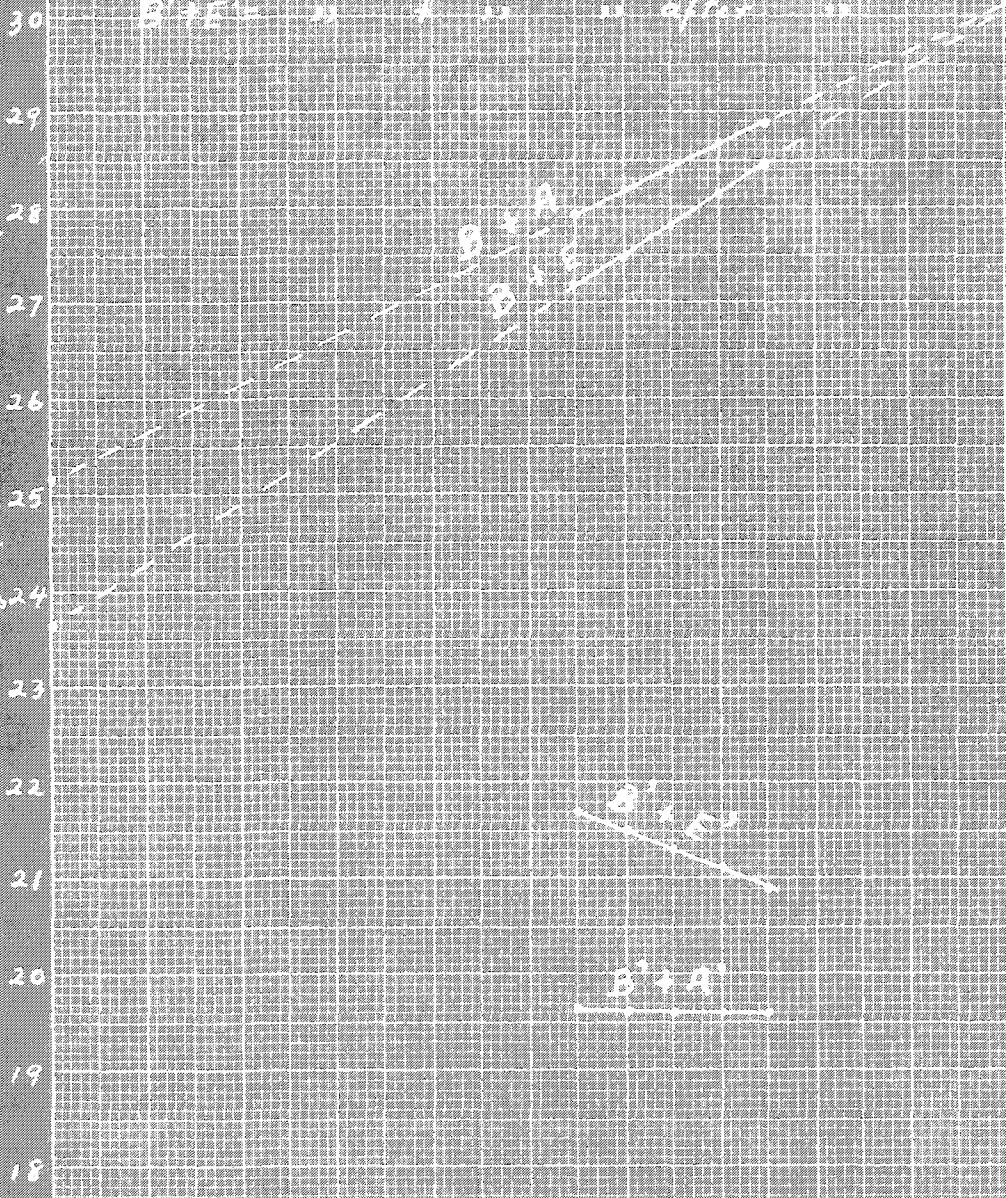
| Ratio of conc. gms/100 cc. solution | Butanol/Acetone | | Butanol/Ethanol | |
|-------------------------------------|---|---|---|---|
| | Scale Readings of Immersion Refractometer after | Scale Readings of Immersion Refractometer after | Scale Readings of Immersion Refractometer after | Scale Readings of Immersion Refractometer after |
| 1. 3.3/2.7 | 20.22 | 19.65 | 21.72 | 21.72 |
| 2. 3.6/2.4 | 20.20 | 19.60 | 21.50 | 21.52 |
| 3. 3.9/2.1 | 20.19 | 19.60 | 21.26 | 21.32 |
| 4. 4.2/1.8 | 20.17 | 19.60 | 21.08 | 21.12 |
| 5. 4.5/1.5 | 20.16 | 19.60 | 20.80 | 20.91 |

Fig. 10

Binary Solution

1. n_D^{20} of pure solvent
 2. n_D^{20} of pure solute
 3. n_D^{20} of solution
 4. n_D^{20} of other

Scale Reading of Immersion Refractometer.



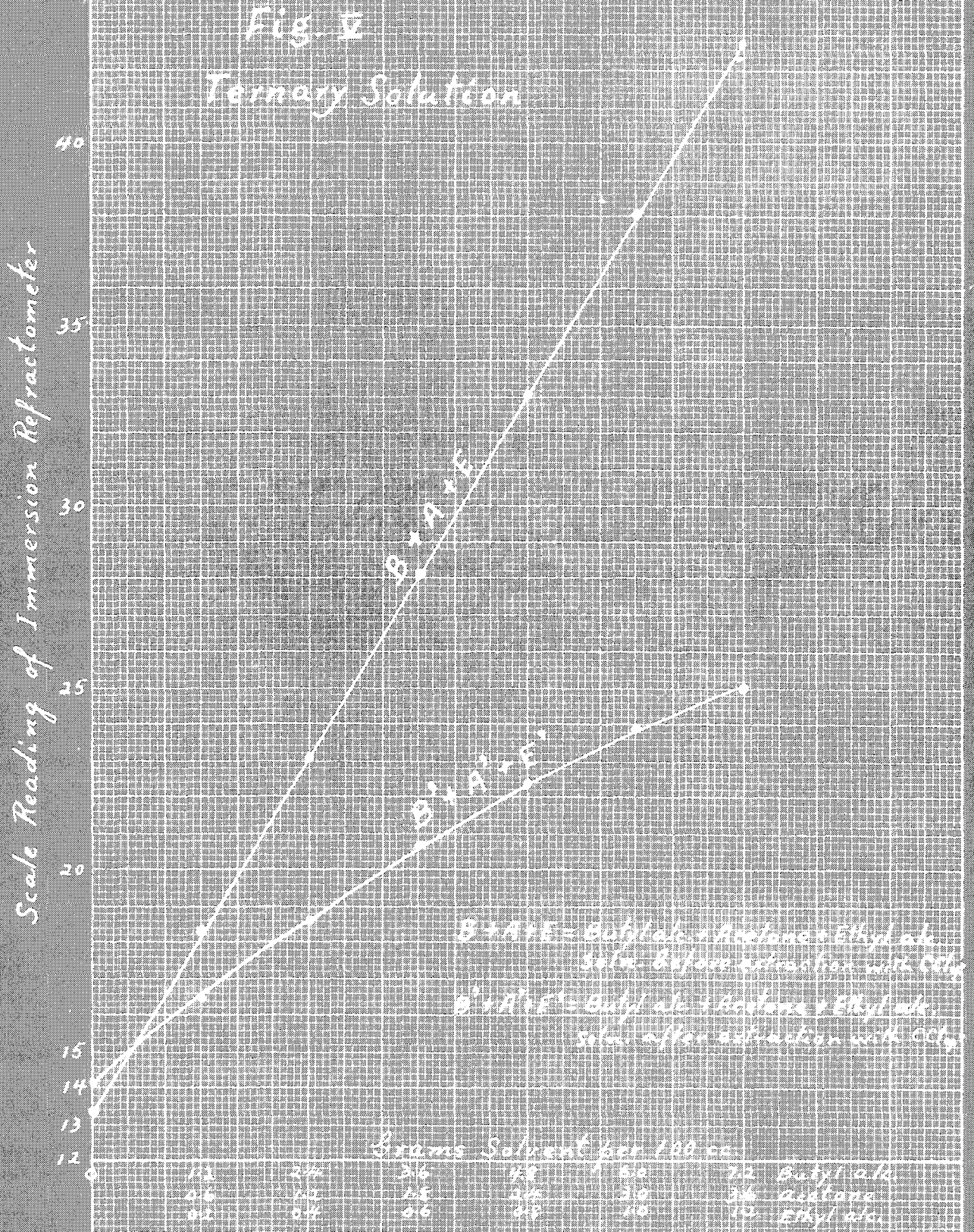
Grams Solute per 100 cc

| | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| 0 | 0.5 | 1.0 | 1.5 | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 | 7.0 | 8.0 | 9.0 | 10.0 |
| 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |

Table IV

Specific gravity and scale readings of immersion refractometers for ternary solutions of butanol, acetone and ethanol at 25°.

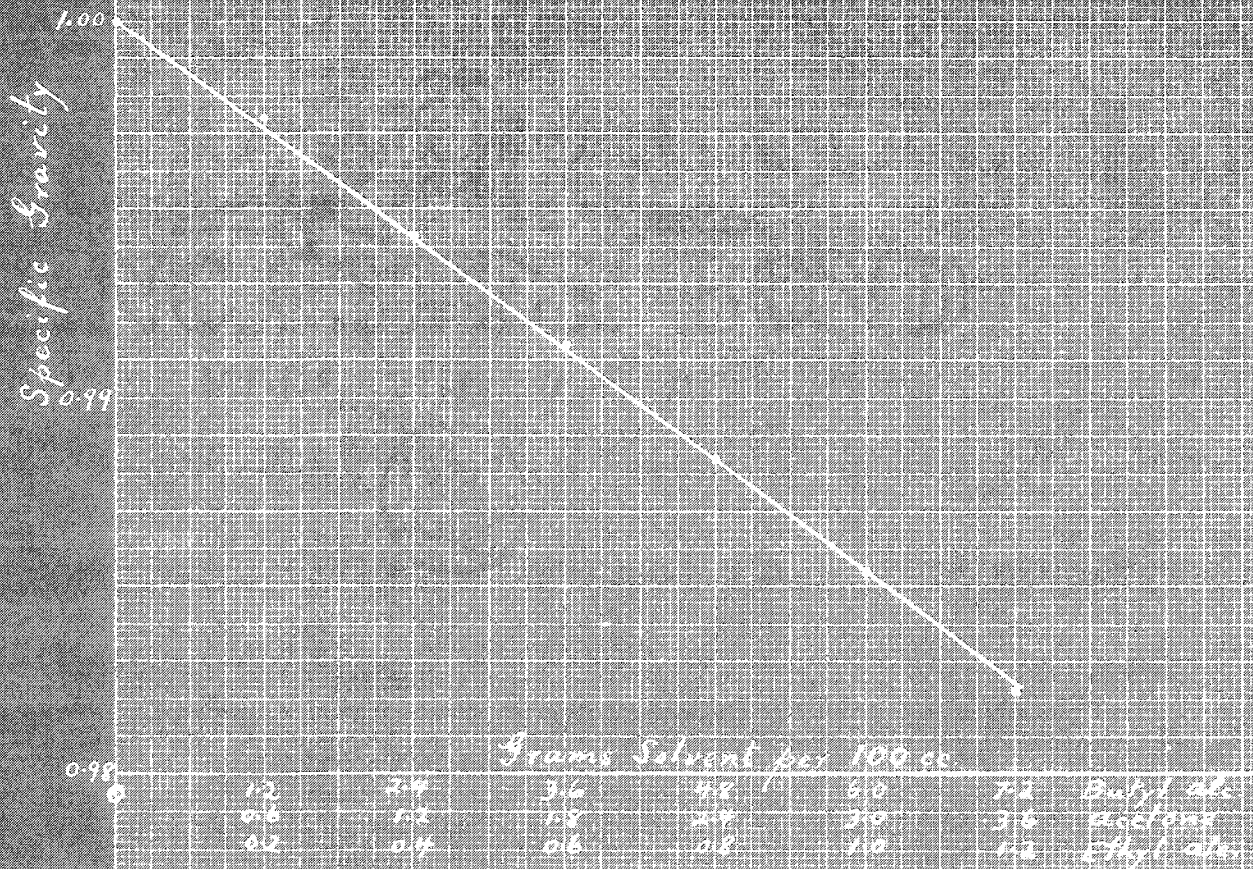
| Ratio of Conc. gas./100 cc. solution | Specific Gravity | | Scale Readings of Immersion Refractometer | | Scale Readings of Immersion Refractometer after extraction | | |
|--|-------------------------|---------------------|---|---------------------|--|-----------------|-------|
| | Butanol/Acetone/Ethanol | Experim. : Eq. 4 | Calcd. by: Eq. 5 | Experim. : Eq. 8 | Calcd. by: Eq. 12 | Calcd. -0.96 | |
| 1. 0.6/0.3/0.1 | 0.9965 | 0.9965 | 16.24 | 16.71 | 15.25 | 15.69 | 14.68 |
| 2. 1.2/0.6/0.2 | 0.9974 | 0.9971 | 16.64 | 18.13 | 16.45 | 16.86 | 15.60 |
| 3. 1.8/0.9/0.3 | 0.9958 | 0.9956 | 20.59 | 20.59 | 17.50 | 18.29 | 17.33 |
| 4. 2.4/1.2/0.4 | 0.9945 | 0.9942 | 23.22 | 23.05 | 18.59 | 19.57 | 18.60 |
| 5. 3.0/1.5/0.5 | 0.9930 | 0.9927 | 25.65 | 25.79 | 20.05 | 20.66 | 19.70 |
| 6. 3.6/1.8/0.6 | 0.9914 | 0.9913 | 28.12 | 27.94 | 20.65 | 21.71 | 20.75 |
| 7. 4.2/2.1/0.7 | 0.9898 | 0.9898 | 30.59 | 30.39 | 21.74 | 22.67 | 21.71 |
| 8. 4.8/2.4/0.8 | 0.9884 | 0.9883 | 32.96 | 32.64 | 22.33 | 23.42 | 22.46 |
| 9. 5.4/2.7/0.9 | 0.9868 | 0.9869 | 35.64 | 35.50 | 23.42 | 24.31 | 23.38 |
| 10. 6.0/3.0/1.0 | 0.9854 | 0.9856 | 38.03 | 37.75 | 25.76 | 25.02 | 24.06 |
| 11. 6.6/3.3/1.1 | 0.9839 | 0.9839 | 40.21 | 40.17 | 24.70 | 25.60 | 24.64 |
| 12. 7.2/3.6/1.2 | 0.9822 | 0.9825 | 42.76 | 42.62 | 24.92 | 26.12 | 25.16 |



D

Fig. 5

Ternary Solution



| Grams Solvent per 100 cc | | | | | | |
|--------------------------|-----|-----|-----|-----|-----|-----------|
| 12 | 24 | 36 | 48 | 60 | 72 | Buyl. alc |
| 84 | 96 | 108 | 120 | 132 | 144 | act. alc |
| 156 | 168 | 180 | 192 | 204 | 216 | Ethyl alc |

Table V

Accuracy obtained by analyzing for acetone and calculating butanol and ethanol concentrations by means of Eq. 4 and 8

| Solution Number | Specific Gravity | Reading of Refractometer | Grams per 100 cc. of solution | Acetone by iodine titration | Butanol | Ethanol | Calcd. Actual | Calcd. Actual |
|-----------------|------------------|--------------------------|-------------------------------|-----------------------------|---------|---------|---------------|---------------|
| 1 | 0.9943 | 25.22 | 1.2 | 2.6 | 3.4 | 0.19 | 0.4 | 0.4 |
| 2 | 0.9914 | 28.12 | 1.8 | 3.83 | 3.6 | 0.51 | 0.6 | 0.6 |
| 3 | 0.9884 | 32.96 | 2.4 | 5.05 | 4.8 | 0.80 | 0.8 | 0.8 |
| 4 | 0.9835 | 42.70 | 3.6 | 7.27 | 7.2 | 1.15 | 1.2 | 1.2 |

SUMMARY.

The investigations reported in this thesis for the utilization of xylose are limited to three phases of the general problem; I. Oxidation of xylose to xylonic acid; II. Preparation of pyrrole from ammonium xylonate; III. Development of an analytical method which is intended for the analysis of mixtures of butanol, acetone, and ethanol produced by fermentation of xylose.

A chlorine oxidation apparatus has been devised.

Oxidation of xylose on the acid side gave more favorable results than that on the basic side. Reasons for the preference of oxidation of the sugar on the acid side to that on the basic side are discussed.

A new salt, ammonium xylonate, was prepared from two sources, one from Tollens' bromine method for the preparation of salts of sugar acids other than the ammonium xylonate and the other from the chlorine method as developed.

Yields of pyrrole 24.5% of the theoretical were produced by dry distillation of 47 grams of ammonium xylonate. The yield is comparable to that from ammonium succinate. It is deemed that it can be considerably increased, when larger quantities of the salt are available and a more suitable apparatus is used. Other salts such as

ammonium arabonate, ammonium gluconate, and ammonium galactonate also yielded pyrrole when subjected to dry distillation, but the yield was rather low. A theoretical discussion on the mechanism of pyrolysis of ammonium xyloate is also presented.

A quantitative yield of ammonium galactonate was obtained by using Tollens' bromine method with slight modification.

A simple method for analyzing a ternary solution of butanol, acetone and ethanol has been developed. The method is based on: first, the additive property of refractive indices of the constituents under the experimental conditions; second, the specific gravity of the total solvent being the summation of specific gravities of all its constituents; and third, the partition coefficient of the total solvent between carbon tetrachloride and water. The possible error of the method is within three-tenths of a percent.

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