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Clearing and deleading of plant extracts for reducing sugar determination

Egil Frode Lind
Iowa State College

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CLEARING AND DELEADING OF PLANT EXTRACTS
FOR REDUCING SUGAR DETERMINATION

by

Egil Frode Lind

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Plant Physiology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State College

1949
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INTRODUCTION

A number of compounds present in plant materials show reducing power when tested with copper or ferricyanide reagents. The non-sugars which show reducing power may be classified as impurities when we are concerned with sugar determination, and the object of clearing or clarification of plant extracts is the removal of such impurities without affecting the sugars present. Most of the impurities can be precipitated by ions of heavy metals. Since an excess of the heavy metals left in the cleared solution affects the reduction of cupric and ferric ions, the excess must be removed. When lead is the heavy metal used, the removal of excess lead is known as deleading.

Neutral and basic lead acetates were used in clearing plant extracts for sugar determination before 1880, and since then a number of workers have investigated the process. These workers agree that clearing is necessary for most plant materials. When clearing is found necessary, it is generally agreed upon that basic lead acetate as a clearing agent causes a loss of reducing sugars, particularly of fructose. The explanations of the cause of the loss are controversial, as could be expected, since most of the workers in the field have been more concerned with the practical than with the theo-
retical aspects of clearing.

In our research the effects of (1) no clearing, (2) neutral lead acetate clearing and (3) basic lead acetate clearing were studied. Pure glucose and fructose solutions were used, as well as alcoholic extracts of plant material with and without the addition of known quantities of pure reducing sugars and of representatives of the impurities. These comparisons were made to obtain information on the necessity of clearing.

Since it was concluded from this work that clearing is generally necessary, experiments were carried out to determine the conditions under which reducing sugars are lost in the clearing process. The effects on sugar loss of hydrogen ion activity and of the time between clearing and deleading were investigated. Further evidence is presented which permits a possible explanation of the cause of the sugar loss when basic lead acetate is used as a clearing agent. In conclusion a method is outlined for clearing and deleading of plant extracts for reducing sugar determination.
LITERATURE REVIEW

The sugar chemists have used basic lead acetate as a clearing agent since early in the latter part of the last century. When deleading was found necessary, different deleading agents were recommended. Plant chemists and plant physiologists adopted the method with modifications for clearing of plant sugar extracts around 1890. These groups of workers use essentially the same method today and, since the method was introduced, they have contributed a considerable number of papers on the subject.

Gill (9) examined solutions of invert sugar with and without addition of basic lead acetate and found a marked change in rotatory power when basic lead acetate was present. Further investigation showed that the fructose fraction of the invert sugar was changed, whereas the glucose fraction was not affected. Gill thought it probable that a soluble, dextrorotatory lead-fructose compound was formed when basic lead acetate is added to a fructose solution. In other experiments Gill showed for the first time that deleading was necessary after clearing with basic lead acetate. If deleading was omitted, low reducing sugar values were obtained, an effect which he attributed to the reduction of excess lead by a part of the sugars when the mixture was tested with Fehling's
solution. A "strong solution of sulphur dioxide" was recommended as a deleading agent.

Edson (5) repeated the experiments of Gill (9) using an invert sugar solution, and confirmed his conclusion that basic lead acetate changes the specific rotation of invert sugar. When acetic acid was added to the cleared solution, however, nearly normal rotatory power was restored, and when neutral lead acetate was used instead of basic lead acetate, no change in the rotatory power of an invert sugar solution was observed. Therefore neutral lead acetate was recommended as a clearing agent.

Bryan (1) and Prinsen Geerligs (8) showed that a loss of reducing sugars accompanied the use of basic lead acetate as a clearing agent, and that fructose was lost to a larger extent than glucose. In a more thorough study Watts and Tempany (23) confirmed the findings of Gill (9) in showing that addition of increasing amounts of basic lead acetate to an invert sugar solution caused the solution to become increasingly more dextro-rotatory. The change in rotatory power was attributed to a change in the fructose fraction of the invert sugar. It was also found that addition of acetic acid could restore the rotatory power to normal. The quantitative relations were such that normal rotatory power was obtained with a quantity of acetic acid which would change the applied basic lead acetate to neutral lead acetate. The effect of an excess of
acetic acid was not investigated.

Watts and Tempany raised the question whether clearing was at all necessary. In their work with cane juice they found the difference in reducing sugar content between raw and cleared juice to be as small as 0.015 per cent when the sugars were determined with Fehling's solution. Although the cleared juice gave a consistently lower reducing sugar value, clearing was not considered necessary, and so much more so because it was found that a small excess of lead left in the cleared solution could account for the loss. No deleading agent was used, however, and therefore the "small excess" of lead may have been considerably higher than the lead content of a corresponding delead solution. Even if clearing was unnecessary in the case reported, where only one type of plant material was tested, it can not be concluded that clearing is generally not necessary.

Parkin (16) confirmed the results of Gill (9) and Watts and Tempany (23) that basic lead acetate changed the rotation of a fructose solution to a more dextro-rotatory value, and that glucose was not affected. In further work with pure sugar solutions Parkin showed also that sucrose was not affected by basic lead acetate, and that added tannic acid could be removed by clearing with basic lead acetate. Clearing was found necessary for the plant material used because uncleared extracts gave considerably higher reducing sugar values with
Fehling's solution than did cleared extracts. No deleading agent was usually employed although one experiment was carried out to test the validity of this procedure. Hydrogen sulfide was used as a deleading agent and the conclusion was reached that it is permissible not to delead in a comparative study because the relative amounts of glucose, fructose and sucrose were found to be approximately the same with and without deleading.

Kynon (7) found an increase in precipitated lead when increasing quantities of basic lead acetate was used in clearing sugar solutions and, at the same time, a decrease in reducing sugar content. From these facts it was concluded that basic lead acetate precipitated reducing sugars. The experiments were not designed so that the quantitative relations between precipitated lead and the loss of reducing sugars could be established, but they did indicate such a loss. Unfortunately only little attention has been paid to this paper.

The loss of reducing sugars when basic lead acetate was used as a clearing agent, as reported by these early workers, has later been verified by most of the workers in the field. Three explanations have been advanced to account for the loss: (1) The reducing sugars are entrained in the lead precipitate, either during clearing or during deleading. (2) Fructose is transformed to glucose in the presence of basic lead acetate. (3) The reducing sugars react with basic lead acetate to form
insoluble or slightly soluble lead–sugar compounds which do not reduce cupric or ferric ions in alkaline solution.

Entrainment of reducing sugars in the insoluble lead compounds formed during clearing is supported by the works of Deerr (4) and Pellet (19) who found that nearly the total quantity of sugars lost could be recovered by decomposing the clearing precipitate with sulfuric acid. An analogous entrainment of reducing sugars during deleading was also considered a possible explanation of the sugar loss. Pellet (20) lists the following factors affecting the quantity of sugar carried down: (1) The quantity and nature of the impurities. (2) The quantity of basic lead acetate added to a sugar solution of given concentration. (3) The concentration of the sugar solution. It was found that an increase in the concentration of basic lead acetate, or of reducing sugars, or of both resulted in an increased loss of reducing sugars. These findings support the theory of a reaction between basic lead acetate and reducing sugars as the cause of sugar loss as well as or better than they support the theory of entrainment.

Meade and Harris (17) worked with sugar cane products and obtained satisfactory clearing with neutral lead acetate. Different deleading agents were tested, and potassium oxalate was found to give slightly higher reducing sugar values than sodium carbonate and sodium sulfate. The ionic concentrations of the deleaded solutions were not the same, however, and the
findings of Weintraub and Price (24), who investigated the influence of ionic concentrations on alkaline oxidation of reducing sugars, can account for the differences found by Meade and Harris. In further investigations these latter workers tested phosphoric acid and disodium phosphate as deleading agents. Both gave consistent results and removed the lead so completely that no additional precipitate could be obtained with hydrogen sulfide.

If an excess of lead was left in a sugar solution, the reducing sugar value of this solution was found to be considerably lower than that of a control solution. Therefore deleading was deemed necessary. Meade and Harris (17, p. 508) suggested as a possible explanation that "... lead salts of reducing sugars are formed, which the oxalate breaks up, but which are not broken up by the boiling with Fehling's solution..."

Davis (3) found that no precipitate was formed when 5 or 10 ml. of saturated basic lead acetate was added to 50 ml. of a dilute solution of fructose, and that no loss of fructose occurred unless the lead salt was allowed to act upon the sugar for some length of time. If the basic lead acetate was left in the fructose solution for increasing lengths of time, increasing amounts of the sugar disappeared. As the time between clearing and deleading increased, the solution became increasingly yellow, but still no precipitate could be detected.
In fructose solutions which were delead with sodium carbonate 23 to 72 hours after clearing, the reducing sugar values determined with alkaline copper reagent were found to be considerably higher than the corresponding reducing sugar values obtained by determination of the rotatory power. From these results Davis concluded that fructose was transformed to glucose, a monosaccharide obtained by de Bruyn and van Ekenstein (2) on heating a 20 per cent solution of fructose with aqueous lead hydroxide to 70-100 degrees centigrade. The reasons for this conclusion were: (1) basic lead acetate was assumed to act upon fructose the same way as lead hydroxide, and (2) glucose was found to have approximately half the reducing power of fructose and to be nearly optically inactive. These facts could account for the different results when fructose was determined by reducing power and by rotatory power.

Because of the effect of time between clearing and de-leading on the loss of fructose, Davis pointed out the importance of making this time interval as short as possible.

Englis and Tsang (6) worked with pure reducing sugar solutions using basic lead acetate as a clearing agent and different deleading agents. They obtained varied recoveries, particularly of fructose. The loss of fructose varied from 1 to 35 per cent while the loss of glucose varied from 1 to 10 per cent. Tannic acid as a deleading agent gave practically full recovery. Disodium acid phosphate was a close second,
and was recommended as a deleading agent because: (1) A clear solution was nearly always obtained. (2) Disodium phosphate gave almost 100 per cent recovery of the reducing sugars.

The loss of reducing sugars was explained as being primarily due to entrainment of the reducing sugars in the lead precipitate during deleading, because a much smaller loss was obtained when the precipitate was washed. Still the loss of fructose was higher than that of glucose, and no explanation was offered to account for this selective loss. Their conclusion also ignores the losses which will occur in the clearing precipitate when plant extracts are used instead of pure sugar solutions.

Loomis (14) worked with extracts from a variety of plant material. From a comparison between the weight of the lead precipitate and the loss of the reducing sugars, it was found improbable that co-precipitation alone could account for the loss. It was concluded therefore that at least part of the loss was due to the formation of an insoluble or slightly soluble lead oxide-sugar compound. The only deleading agent tested that would give full recovery of reducing sugars after basic clearing was hydrogen sulfide.

Loomis (15) recommends sodium oxalate as a deleading agent because it leaves the deleadced solutions close to neutral and an excess of sodium oxalate had no effect on the re-
ducing power of the solution. No loss caused by co-precipitation was found with neutral lead acetate, even when this compound and sodium oxalate were applied in excess.

Puleston (21) confirmed the results of previous workers that basic lead acetate as a clearing agent causes a loss of reducing sugars in alcoholic plant sugar extracts, especially of fructose. The loss could not be controlled by the choice of deleading agent and it was concluded that the loss occurred during clearing rather than during deleading.

Hassid (10) used neutral lead acetate as a clearing agent and disodium phosphate as a deleading agent, and pointed out that colorless solutions of plant extracts were nearly always obtained when a small amount of charcoal (carboraffin) was added after deleading and the solution filtered under suction through talc on a Büchner funnel. When ceric sulfate is used in the reducing sugar determination according to Hassid (11), oxalate should be avoided as a deleading agent because of its apparent oxidation by the ceric ion.

Von Lippmann (12) dealt with sugar chemistry in detail. Lead-glucose compounds are described on pp. 552-553 and were called lead glucosates. According to this author glucose is not precipitated by basic lead acetate unless salts (ions) are present, and the precipitation is more complete in dilute than in concentrated solutions. Several formulas were suggested for lead glucosates, of which Soubeyran's formula $C_6H_8Pb_2O_6$
is in best agreement with the lead glucosate described in this thesis (p. 37). It was further pointed out that when different concentrations of glucose or of basic lead acetate were used, different lead glucosates would be formed. All lead glucosates were said to be insoluble in water, slightly soluble in a solution of lead acetate in water, and precipitable from such solution by the addition of ethyl alcohol. The lead glucosates that had been tested could be decomposed by carbon dioxide, the sugar being set free.

Lead fructosates are described on pp. 883-884. The author stated that the lead fructosate \( \text{C}_6\text{H}_{12}\text{O}_6 \cdot \text{2Pb(OH)}_2 \) is formed upon addition of basic lead acetate to a concentrated solution of fructose. In dilute fructose solutions another fructosate of unknown composition (or several others?) is formed. The lead that has reacted with fructose can not be recovered by addition of an excess of sodium carbonate or sodium sulfate. If neutral lead acetate is used instead of basic lead acetate, the lead can be recovered with sodium sulfate.

Vogel and Georg (22) list the sugars and their derivatives in tabular form, giving the physical and chemical properties of each compound as well as references from which the data were compiled. Lloyd-Evans (13) gives a comprehensive review of the research on the alkaline oxidation of sugars.
MATERIALS AND METHODS

Materials

Basic lead acetate: Lead acetate, basic reagent, General Chemical Co., code no. BA 1823.
Ceric sulfate: Cerium sulfate, anhydrous-\(\text{Ce(SO}_4\text{)}_2\), Fisher Scientific Co., code no. C 258 and anhydrous-\(\text{Ce(HSO}_4\text{)}_4\), G. Frederick Smith Chemical Co.
Dipotassium phosphate: Reagent.
Fructose: Levulose, cryst., Fordomes Trading Co.
Glucose: Dextrose, anhydrous, Pfanstiehl Chemical Co.
Neutral lead acetate: Reagent.
Phenylhydrazine: Phenylhydrazine, Eastman Kodak Co., code no. 329.
Phenylhydrazine hydrochloride: Prepared according to Loomis and Shull (16).
Plant extracts: 80 per cent alcoholic extracts of corn leaves and sheaths, and of composite plant material were prepared according to Loomis and Shull (16).
Potassium acid phthalate: Reagent (cryst.), General Chemical Co., code no. 2083.
Potassium ferricyanide: Reagent.
Quercetin: Quercetin, Eastman Kodak Co., code no. 1635.
Setopaline C: Setopaline C (indicator), Eimer and Amend.
Sodium carbonate: Reagent.
Tannic acid: Acid Tannic Merck (U.S.P. powder).

Methods

Clearing and deleading plant extracts:

1. 200 ml. of alcoholic plant extract was evaporated down to 5-10 ml. in a 400 ml. beaker on a boiling water bath.
2. The residue was broken up with a rubber policeman and dissolved in 50 ml. of warm water.
3. After cooling, 2 or 3 ml. of saturated lead acetate was added.
4. The solution was then filtered into a 250 ml. volumetric flask containing 10 ml. of dipotassium phosphate solution, concentration 125.0 grams per liter, the precipitate washed and the solution made to volume.
5. After the lead phosphate precipitate had settled, 5 ml. of the solution was pipetted from the top of the flask, and the quantity of reducing sugars determined by the method described by Hassid (11). Dipotassium phosphate was chosen as the deleading agent because of the low solubility product of lead phosphate, and was used in solution to facilitate the
addition of the same quantity to all samples and to obtain a rapid deleading.

Clearing and deleading pure sugar solutions:

1. 200 ml. of distilled water was run into a 250 ml. volumetric flask.
2. 20 ml. of sugar solution, concentration 5.000 grams per liter, was added.
3. 3 ml. of saturated lead acetate was added.
4. The solution was delead with 10 ml. of dipotassium phosphate solution, concentration 125 grams per liter, and made to volume.
5. 5 ml. samples for reducing sugar determinations were pipetted from the top of the flask after the lead phosphate precipitate had settled.

The cleared solutions were adjusted to the desired pH value with sodium hydroxide when the effect of hydrogen ion activity on the recovery of reducing sugars was studied.

Reducing sugar determination:

The ceric sulfate method described by Hassid (11) was used. This method was chosen in preference to the Hanson-Walker-Bertrand method because: (1) the ceric sulfate method was less time-consuming. (2) titration with ceric sulfate, using Setopamine C as an indicator, gave a more distinct end-point than the corresponding titration with potassium permanganate. (3) the bottom meniscus could easily be read when
titrating with ceric sulfate. Ceric sulfate is stable in concentrations of 0.01 N or greater.

**pH determination:**

The pH values were measured with a Leeds and Northrup pH-meter no. 7663. Standard buffer, pH 3.97, was prepared by diluting 0.2 M potassium acid phthalate solution with distilled water 1:3.

**Phosphorous determination:**

(1) 50 ml. of the cleared and leaded solution were pipetted into a 250 ml. Erlenmeyer flask.

(2) Phosphorous was determined as magnesium pyrophosphate by the method described by Willard and Diehl (25) p. 204, avoiding the molybdenum reactions.

**Determination of lead in lead-sugar compounds:**

Lead was determined gravimetrically as lead sulfate.

The lead sugar compounds were decomposed with concentrated sulfuric acid, and the sulfuric acid evaporated off on a sand bath at 300-310 degrees centigrade. The residue was treated with sulfuric acid and the evaporation repeated. Finally the crucibles containing the lead sulfate were heated in a bunsen flame until constant weight was obtained.

**Osazone formation:**

(1) 2 g. of phenylhydrazine hydrochloride and 3 g. of sodium acetate were thoroughly mixed in a mortar.

(2) Approximately 0.5 g. of the mixture was added to each
test tube containing approximately 0.1 g. of lead-sugar compound in 5 ml. of water.

(3) The test tubes were heated on a boiling water bath for 10 min.

(4) The content of each test tube was filtered through a dry filter into a clean, dry test tube.

(5) The test tubes were heated on a boiling water bath for 1 hour and cooled slowly.

The crystals were examined under the microscope and photomicrographs were taken.
THE CLEARING AND DELEADING OF PLANT EXTRACTS

The Necessity of Clearing

Two reasons for clearing plant sugar extracts present themselves: (a) The removal of interfering impurities by clearing, and (b) the more convenient handling of cleared extracts, especially in the filtering process.

Tannins, flavones and plant pigments are typical impurities present in most plant material. These compounds reduce both ferric and cupric ions in alkaline solution and the presence of any one of them necessitates its removal before reliable reducing sugar determinations can be made. The errors possible from the inclusion of naturally occurring or added non-sugar reducing substances are shown in tables 1, 2 and 3. Each of the figures in these three tables was calculated from the mean of four replicates which checked within 0.10 ml. in the titration with ceric sulfate. With the concentrations of ceric sulfate used, 2.60 ml. of ceric sulfate was equivalent to 1.00 mg. of glucose for the results reported in tables 1 and 2, while the corresponding figure for the data in table 3 was 2.90 ml. of ceric sulfate. These figures fix the standard error of estimate in the tables at 1 per cent or less.

Pure solutions of glucose and fructose with and without the addition of tannic acid were used (table 1) and the effects
Table 1. Effect of clearing with neutral lead acetate on pure solutions of glucose and fructose, and on these solutions when tannic acid was added to make the solutions 0.02 per cent tannic acid.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Not cleared</th>
<th></th>
<th>Cleared</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mg. as glucose</td>
<td>Percentage recovery</td>
<td>Mg. as glucose</td>
<td>Percentage recovery</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.00</td>
<td>100</td>
<td>2.00</td>
<td>100</td>
</tr>
<tr>
<td>Fructose</td>
<td>1.98</td>
<td>99</td>
<td>1.97</td>
<td>98.5</td>
</tr>
<tr>
<td>Glucose plus tann. acid</td>
<td>2.12</td>
<td>106</td>
<td>2.01</td>
<td>100.5</td>
</tr>
<tr>
<td>Fructose plus tann. acid</td>
<td>2.09</td>
<td>104.5</td>
<td>1.98</td>
<td>99</td>
</tr>
</tbody>
</table>
on reducing sugar determinations of no clearing and of clearing with neutral lead acetate were compared. Dipotassium phosphate was the deleading agent.

From the data in Table 1 it may be concluded:

1. Tannic acid showed reducing power and may therefore be classified as an impurity in reducing sugar determinations.
2. The effect of the tannic acid was eliminated by clearing with neutral lead acetate followed by deleading with dipotassium phosphate.
3. Fructose showed a slightly lower reducing power than glucose when calculated as glucose.

The results presented in Table 2 were obtained by adding known quantities of tannic acid, glucose and fructose to aliquots of 80 per cent alcoholic extract of corn leaves before clearing. Comparisons were made of the effects on the recovery of reducing sugars of: (a) no clearing, (b) neutral lead acetate clearing with dipotassium phosphate deleading, and (c) basic lead acetate clearing with dipotassium phosphate deleading. The results of an analogous set of experiments are reported in Table 3. Eighty per cent alcoholic extract of composite plant material was used instead of corn leaf extract, and quercetin was chosen as a representative of the impurities instead of tannic acid. Both tannic acid and quercetin are considered valid representatives of the interfering
Table 2. Effect of clearing with neutral and basic lead acetate on alcoholic extract of corn leaves and upon extract plus known quantities of glucose, fructose and tannic acid added before clearing.

The sugars were added to give 0.50 mg. per sample to be determined; tannic acid to make the solution 0.03 per cent tannic acid.

<table>
<thead>
<tr>
<th>Sample</th>
<th>No clearing</th>
<th></th>
<th>N-lead clearing</th>
<th></th>
<th>B-lead clearing</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mg. as glucose</td>
<td>Per cent recovery</td>
<td>Mg. as glucose</td>
<td>Per cent recovery</td>
<td>Mg. as glucose</td>
<td>Per cent recovery</td>
</tr>
<tr>
<td>Pure extract</td>
<td>1.87</td>
<td>110.5*</td>
<td>1.69</td>
<td>100.0</td>
<td>1.38</td>
<td>81.5</td>
</tr>
<tr>
<td>Extract plus tann. ac.</td>
<td>2.07</td>
<td>123.5</td>
<td>1.70</td>
<td>100.5</td>
<td>1.38</td>
<td>81.5</td>
</tr>
<tr>
<td>Extract plus glucose</td>
<td>2.36</td>
<td>98.0+</td>
<td>2.17</td>
<td>96.0</td>
<td>1.88</td>
<td>100.0</td>
</tr>
<tr>
<td>Extract plus glucose and tann. ac.</td>
<td>2.56</td>
<td>98.0</td>
<td>2.17</td>
<td>94.0</td>
<td>1.88</td>
<td>100.0</td>
</tr>
<tr>
<td>Extract plus fructose</td>
<td>2.31</td>
<td>88.0</td>
<td>2.13</td>
<td>88.0</td>
<td>1.66</td>
<td>56.0</td>
</tr>
<tr>
<td>Extract plus fructose and tann. ac.</td>
<td>2.51</td>
<td>88.0</td>
<td>2.14</td>
<td>88.0</td>
<td>1.66</td>
<td>56.0</td>
</tr>
</tbody>
</table>

* Based on neutral clearing as 100 per cent.

+ All percentages below this point are recoveries of added sugars. Because of the smaller base, 0.5 mg. instead of 1.7, differences of more than 3 per cent are required for significance.
Table 3. Effect of clearing with neutral and basic lead acetates on alcoholic extracts of composite plant material and upon extracts of the same plant material to which had been added known quantities of glucose, fructose and quercetin before clearing.

The sugars were added to give 0.50 mg. reducing sugar per sample to be determined; quercetin to make the solution 0.05 per cent quercetin.

<table>
<thead>
<tr>
<th>Sample</th>
<th>No clearing</th>
<th>N-lead clearing</th>
<th>B-lead clearing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mg. as glucose</td>
<td>Per cent recovery</td>
<td>Mg. as glucose</td>
</tr>
<tr>
<td>Pure extract</td>
<td>2.68</td>
<td>115.0*</td>
<td>2.33</td>
</tr>
<tr>
<td>Extract plus quercetin</td>
<td>3.01</td>
<td>129.0</td>
<td>2.35</td>
</tr>
<tr>
<td>Extract plus glucose</td>
<td>3.17</td>
<td>98.0†</td>
<td>2.84</td>
</tr>
<tr>
<td>Extract plus glucose and quercetin</td>
<td>3.50</td>
<td>98.0</td>
<td>2.84</td>
</tr>
<tr>
<td>Extract plus fructose</td>
<td>3.15</td>
<td>96.0</td>
<td>2.82</td>
</tr>
<tr>
<td>Extract plus fructose and quercetin</td>
<td>3.48</td>
<td>96.0</td>
<td>2.81</td>
</tr>
</tbody>
</table>

* Based on neutral clearing as 100 per cent.

† All percentages below this point are recoveries of added sugars. Because of the smaller base, 0.5 mg. instead of 2.3, differences of more than 8 per cent are required for significance.
impurities because they occur naturally in plants, they show reducing power and they contain the functional groups of naturally occurring tannins and flavonoids respectively.

From the data reported in tables 2 and 3 it may be concluded that:

(1) The uncleared solutions showed a markedly higher reducing value than the cleared solutions, with basic lead lowering the reducing value of the extract twice as much as neutral lead. The plant extract used was known to be high in fructose.

(2) Tannic acid and quercetin showed reducing power.

(3) The reducing power due to tannic acid and to quercetin was eliminated by clearing with either neutral or basic lead acetate, with the neutral lead being fully as effective as the basic.

(4) When neutral lead acetate was used as a clearing agent, the added quantities of glucose and fructose were fully recovered.

(5) When basic lead acetate was the clearing agent, the added glucose was recovered while approximately 40 per cent of the added fructose was lost.

It appears necessary to clear plant extracts for sugar determination whenever impurities of the type tested are present. Neutral lead acetate was found to be an effective clearing agent.
In addition to the removal of reducing impurities, clearing facilitates the handling of the plant sugar extracts during the sugar determination. Proteinaceous material and substances like gums are precipitated by the lead and not allowed to precipitate during the alkaline oxidation of the reducing sugars, in which case the filtering in the Munson-Walker or similar methods is greatly retarded and a turbid solution is obtained with iodometric or ceric sulfate methods.

Plant pigments must be reduced to a low value in solutions to be tested for reducing sugars by the ceric sulfate or iodometric methods. Normally an excess of neutral lead gives satisfactory clearing. Where it does not, as in preparing solutions for measurements of specific rotation, Hassid (10) recommended filtering through finely ground charcoal after clearing with neutral lead acetate and deleading.

Loss of Reducing Sugars in the Clearing Process

The data of tables 2 and 3 corroborate the findings of Bryan (1), Prinsen Geerligs (8), Loomis (14), Puleston (21) and others in showing losses of reducing power with basic lead clearing beyond that obtained with neutral clearing. The neutral clearing used in our experiments was sufficient to remove all of the considerable quantities of materials specifically added as impurities, and basic clearing was of no advantage. Neither neutral nor basic lead clearing will
remove all types of reducing impurities, and it is not possible, with the information available, to determine whether basic lead was more effective in removing all of the impurities in the control extracts used here. The two compounds added here are representatives of the most important classes of impurities, however, and thus this work substantiates the experiments of Loomis (14) who found no gain in purification of plant extracts from the use of basic lead.

The data on the loss of added fructose, in contrast, are clear cut and highly significant for all basic lead clearings. The quantities of lead used were moderate and the deleading procedure was that recommended by Englis and Tsang (6) for best recovery of sugars from basic lead clearing. The conclusion seems inescapable that basic lead clearing is not safe for plant extracts containing fructose. The differences between our results and those of Englis and Tsang (6) are undoubtedly attributable to the action of the naturally occurring impurities which were absent in their experiments.

The 88 per cent recoveries of added fructose shown in table 2 show significant difference at the 5 per cent level, and may represent unknown reactions between natural impurities and fructose. The losses represented, however, are only 0.06 mg. and could have been due to chance.
Effects of Hydrogen Ion Activity and Time in Contact with Lead on the Loss of Reducing Sugars

Since it was found that loss of reducing sugars occurred during the clearing with basic lead acetate, experiments were carried out to study the loss of reducing sugars as a function of the hydrogen ion concentration of the solutions after the lead acetate had been added. Four pH values were selected for this purpose:

1. pH 5.0±0.05 was chosen as an acid solution, and was obtained by adding neutral lead acetate to a reducing sugar solution and adjusting to pH 5.0 with 0.2 M acetic acid.

2. pH 6.0±0.05 was the pH of our solutions when cleared with neutral lead acetate. No adjustment was necessary.

3. pH 8.0±0.05 corresponds to the pH of a solution cleared with basic lead acetate, and was obtained either by adding basic lead acetate to the reducing sugar solution or by adding neutral lead acetate followed by adjustment to pH 8.0 with ammonium hydroxide or sodium hydroxide. The results were not affected by the way in which the pH was obtained.

4. pH 10.0±0.10 was chosen as an extremely alkaline solution, and was obtained by adding neutral lead acetate and adjusting to pH 10.0 with ammonium or sodium hydroxide.

Solutions of glucose and fructose were studied separately,
both being of concentration 0.400±0.0002 g. per liter, and
3 ml. of saturated neutral (or basic) lead acetate being
added per 250 ml. of cleared sugar solution.

Davis (3) reported that the loss of reducing sugars from
solutions cleared with basic lead acetate increased with in­
creasing time intervals between clearing and deleading. To
obtain more information on this point, especially with respect
to the rate of the postulated reaction between basic lead ace­
tate and reducing sugars, the loss of reducing sugars was
studied as a function of the time between adding and removing
lead at each of the four pH values, 5.0, 6.0, 8.0 and 10.0.

The results are presented in table 4 and figures 1 to 3.
All figures are calculated from the mean of four replicates
which checked within 0.10 ml. in the ceric sulfate titration,
2.60 ml. of ceric sulfate were equivalent to 1.00 mg. of glu­
cose, and each sample contained 2.00 mg. of glucose or fruc­
tose at the start of the experiment.

It may be concluded that:
(1) Glucose was almost completely recovered even after 24 hours
    between treating and deleading with pH's up to 8.0.
(2) Glucose was lost rapidly at pH 10.0.
(3) Fructose was almost completely recovered only after imme­
    diate deleading with pH's up to 8.0.
(4) Fructose was lost in increasing amounts with increasing
time intervals between treating and deleading at all pH
Table 4. The effect of hydrogen ion activity and time on the recovery of reducing sugars when lead acetate was added. Recoveries are given in per cent.

<table>
<thead>
<tr>
<th>Time (hrs.) before deleading</th>
<th>pH 5.0</th>
<th>6.0</th>
<th>8.0</th>
<th>10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>100</td>
<td>99.5</td>
<td>99.0</td>
<td>99.5</td>
</tr>
<tr>
<td>Fructose</td>
<td>99.5</td>
<td>98.5</td>
<td>97.5</td>
<td>95.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>100</td>
<td>99.5</td>
<td>99.5</td>
<td>95.0</td>
</tr>
<tr>
<td>Fructose</td>
<td>99.5</td>
<td>97.5</td>
<td>95.5</td>
<td>92.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>99.0</td>
<td>96.5</td>
<td>94.0</td>
<td>88.5</td>
</tr>
<tr>
<td>Fructose</td>
<td>99.0</td>
<td>95.5</td>
<td>93.0</td>
<td>83.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>99.0</td>
<td>95.5</td>
<td>93.0</td>
<td>83.0</td>
</tr>
<tr>
<td>Fructose</td>
<td>99.0</td>
<td>95.5</td>
<td>93.0</td>
<td>83.0</td>
</tr>
</tbody>
</table>
Fig. 1. Effect of hydrogen ion activity on recovery of reducing sugars with different time intervals (0-24 hours) before deleading.
Fig. 2. Per cent recovery of glucose and fructose from solutions containing neutral and basic lead acetate, and delead with dipotassium phosphate after different time intervals.
Fig. 3. Effect of time before deleading on recovery of reducing sugars at pH 10.0.
values, the loss increasing with increasing pH.

(5) Glucose and fructose were lost at approximately the same rate at pH 10.0, but the glucose loss showed an initial lag.

(6) The rate of the loss of fructose at all pH values and the rate of the loss of glucose at pH 10.0 showed a gradual decrease with increasing time, while the loss increased with time, indicating that a chemical reaction was involved.

Thus it seems very probable that the loss of reducing sugars occurs during clearing and is caused by a reaction between lead acetate and reducing sugars, mainly fructose. It should be added that no precipitate was formed in any of the solutions, even when left for 24 hours before deleading. Therefore further information was sought with respect to the formation of lead-sugar compounds during clearing.
LEAD-SUGAR COMPOUNDS

Formation of Lead-sugar Compounds During Clearing

Based on the hypothesis that lead-sugar compounds are formed when basic lead acetate is added to a dilute solution of reducing sugars, an attempt was made to obtain information on this point from indirect evidence. Neutral lead acetate was added quantitatively to dilute reducing sugar solutions and the pH value of the solutions adjusted to 10.0. After given time intervals an excess of dipotassium phosphate was added quantitatively and reducing sugars and phosphorous were determined.

If the basic lead acetate did not react with the sugars, the phosphorous content of the delead solutions should be constant, but if the basic lead acetate reacted with the sugars to form compounds which can not be decomposed by dipotassium phosphate, the phosphorous content of the delead solutions should increase as the loss of reducing sugars increased with time.

The results are presented in table 5. The phosphorous content was found to increase with increasing loss of reducing sugars. The lead corresponding to the increase in phosphorous content was calculated, assuming that lead is precipitated as
Table 5. Loss of reducing sugars and corresponding increase in phosphorous content in solutions of glucose and fructose treated with lead acetate at pH=10 and delead with dipotassium acid phosphate. All figures are calculated per 50 ml solution; each figure from three replicates.

<table>
<thead>
<tr>
<th>Time (hrs.) between treating and deleading</th>
<th>Loss of reducing sugars (mg.)</th>
<th>Increase in P-content (mg.)</th>
<th>Pb not accounted for (mg.)</th>
<th>Per cent Pb in the postulated lead-sugar compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose Fructose</td>
<td>Glucose Fructose</td>
<td>Glucose Fructose</td>
<td>Glucose Fructose</td>
</tr>
<tr>
<td>0</td>
<td>0.2</td>
<td>2.7</td>
<td>0.0</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>0.0</td>
<td>0.6</td>
<td>0.0</td>
<td>6.0</td>
</tr>
<tr>
<td>1</td>
<td>2.1</td>
<td>4.0</td>
<td>0.5</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>0.0</td>
<td>0.6</td>
<td>0.0</td>
<td>6.0</td>
</tr>
<tr>
<td>4</td>
<td>4.9</td>
<td>6.0</td>
<td>1.1</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>0.0</td>
<td>0.6</td>
<td>0.0</td>
<td>6.0</td>
</tr>
<tr>
<td>9</td>
<td>7.0</td>
<td>8.5</td>
<td>1.6</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>0.0</td>
<td>0.6</td>
<td>0.0</td>
<td>6.0</td>
</tr>
<tr>
<td>24</td>
<td>10.2</td>
<td>12.3</td>
<td>2.3</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>0.0</td>
<td>0.6</td>
<td>0.0</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>69.3±0.2</td>
<td>70.3±0.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Pb\(_3\)(PO\(_4\))\(_2\) by K\(_2\)HPO\(_4\), and tabulated as "Pb not accounted for". Finally, the lead content of the postulated lead-sugar compounds was calculated from the corresponding figures in the two columns: "Loss of reducing sugars" and "Pb not accounted for".

From the data in table 5 it may be concluded that:

1. The loss of reducing sugars increased with increasing time intervals between treating and deleading, in agreement with the corresponding data in table 4.

2. The phosphorous content of the deledaded solutions increased as the loss of reducing sugars increased with time, indicating that the basic lead acetate had reacted with the reducing sugars.

3. The relatively high standard errors for the two shortest time intervals between clearing and deleading were probably caused by the difficulty in weighing the small quantities involved.

4. There is probably no significant difference between the lead contents of the postulated lead-glucose compound and the lead fructose compound.

5. The lead content of the lead-sugar compounds indicates that 1 mole of reducing sugar reacted with 2 moles of lead.

Thus it seems very probable that basic lead acetate reacts with reducing sugars during clearing to form lead-sugar
compounds which do not reduce cupric or ferric ions in alkaline solution.

Synthesis of Lead-glucose Compounds and Lead-fructose Compounds Under Clearing Conditions

Following von Lippmann (12), who stated that lead-glucosates and lead-fructosates were formed when basic lead acetate was added to dilute reducing sugar solutions, that the lead-sugar compounds were slightly soluble in an excess lead acetate, and that the lead-sugar compounds could be precipitated from such solution by the addition of ethyl alcohol, six reaction flasks were set up. Each flask contained 2 liters of a dilute glucose or fructose solution, concentrations being 0.500 g. per liter, 0.250 g. per liter and 0.125 g. per liter. Thirty ml. of saturated neutral lead acetate was added to each 2 liters of sugar solution and the pH value adjusted to pH 10.0±0.10. After 24 hours 2 liters of 95 per cent ethyl alcohol was added to each flask. A yellowish white precipitate was formed. After thorough washing with warm water the precipitate was dried in a desiccator over calcium chloride.

The six flasks were numbered as follows:

"1G" containing 0.125 g. of glucose per liter
"3G" containing 0.250 g. of glucose per liter
"5G" containing 0.500 g. of glucose per liter
"2F" containing 0.125 g. of fructose per liter
"4F" containing 0.250 g. of fructose per liter
"6F" containing 0.500 g. of fructose per liter

Determination of Lead Content of the Lead-glucose
and the Lead-fructose Compounds

The lead contents were determined according to the outline on p. 16, and the results are given in tables 6 and 7.

It may be concluded that:

(1) There was a small but significant difference between the lead content of the lead-glucose compounds and that of the lead-fructose compounds.

(2) The lead contents of the lead-glucose compounds showed no significant difference, indicating that the same lead-glucose compound was formed at the three concentrations.

(3) The conclusions of (2) apply to the lead-fructose compounds as well.

(4) The lead contents of the synthesized lead-sugar compounds were in close agreement with the lead contents calculated from indirect data presented in table 5, indicating that lead-sugar compounds of these lead contents are formed during clearing, and verifying the finding that 2 moles of lead reacts with 1 mole of reducing sugar during clearing.
Table 6. Lead content of lead-glucose compound.

<table>
<thead>
<tr>
<th>Lead-glucose compound from flask</th>
<th>Per cent lead</th>
<th>Fiducial limits of per cent lead at 5% level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1G</td>
<td>68.5±0.1</td>
<td>68.2-68.8</td>
</tr>
<tr>
<td>3G</td>
<td>68.3±0.3</td>
<td>67.5-69.1</td>
</tr>
<tr>
<td>5G</td>
<td>68.5±0.1</td>
<td>68.2-68.8</td>
</tr>
</tbody>
</table>

Table 7. Lead content of lead-fructose compound.

<table>
<thead>
<tr>
<th>Lead-fructose compound from flask</th>
<th>Per cent lead</th>
<th>Fiducial limits of per cent lead at 5% level</th>
</tr>
</thead>
<tbody>
<tr>
<td>2F</td>
<td>70.3±0.1</td>
<td>70.0-70.6</td>
</tr>
<tr>
<td>4F</td>
<td>70.4±0.2</td>
<td>69.9-70.9</td>
</tr>
<tr>
<td>6F</td>
<td>70.6±0.4</td>
<td>69.6-71.6</td>
</tr>
</tbody>
</table>
Some Properties of the Lead-glucose Compound
and the Lead-fructose Compound

Both the lead-glucose compound and the lead-fructose compound showed the same properties as far as they were tested.

Appearance: The washed and dried precipitate was a white to yellowish white amorphous powder, the fructose compound being slightly more yellow than the glucose compound.

Solubility: The precipitate was insoluble in cold and hot water and bases, and in organic solvents, slightly soluble in dilute acids and decomposed in strong acids.

Reducing power: The precipitate had no reducing power when tested with alkaline copper or ferricyanide reagent, and only a very slight reducing power was shown upon treatment with dilute acids.

Osazone formation: Glucosazones were formed in abundance when the lead-sugar compounds were treated with phenylhydrazine hydrochloride as outlined on p. 16. Photomicrographs of the glucosazones formed by lead-sugar compounds "5G" and "6F" are presented in figures 4 and 5. These two were chosen arbitrarily since all six lead sugar compounds gave the same glucosazone.
Fig. 4. Glucosazone crystals from the synthesized lead-glucose compound ("5G").

Fig. 5. Glucosazone crystals from the synthesized lead-fructose compound ("6F").
DISCUSSION

Alcoholic plant extracts prepared for sugar determination contain a number of compounds which interfere with the reducing sugar determinations in various ways. One group of compounds show reducing power. Tannic acid and quercetin were chosen as representatives of this group for the experiments reported in tables 1, 2 and 3. Derivatives of these compounds are naturally occurring impurities in most plant material, and on the basis of the results obtained, it seems evident that these compounds must be removed before reliable reducing sugar determinations can be made. A second group of compounds, non-reducing pigments, were found to obscure the end point of titration when ceric sulfate or iodometric methods were used for reducing sugar determination, and gummy materials clogged the filters when the Munson-Walker method was used. These findings indicate strongly that clearing is generally necessary, and even if it can be shown that clearing is not necessary for a certain plant material, it is usually easier to clear than to establish the lack of necessity for clearing.

Our results agree with those of numerous earlier workers in showing a loss of fructose from alkaline solutions treated with lead acetates. Four explanations of this loss have been
offered by various workers:

(1) The loss is caused by occlusion of sugar molecules in the heavy lead precipitate during clearing. Deerr (4); Pellet (19).

(2) The loss is caused by occlusion during deleading. Englis and Tsang (6).

(3) The loss is caused by a transformation of fructose into glucose. Davis (3).

(4) The loss is caused by the formation of an insoluble or slightly soluble lead-sugar compound. Eynon (7); Loomis (14).

The first explanation, which is based on the fact that a significant increase in recovery was obtained when the precipitate was decomposed with dilute sulfuric acid, can account for neither the selective loss of fructose nor the fact that basic lead acetate causes a loss while neutral lead acetate does not. When an increase in recovery was obtained by a sulfuric acid treatment of the precipitate, it seems probable that this result was due to a decomposition of a lead sugar compound formed by a reaction between basic lead acetate and the reducing sugars. If this is the case the selective loss of fructose can be explained on the basis of the different functional groups of fructose and glucose, and the fact that no loss is found with neutral lead acetate as a clearing agent, can be accounted for since glucose and fructose are more re-
active in alkaline than in neutral solution (13).

The second explanation was advanced because it was found that washing the precipitate after deleading pure sugar solutions reduced clearing losses (6). Fuleston (21) repeated the Englis and Tsang experiments and found, as they did, that the substitution of phosphate for oxalate as a deleading agent prevented the loss of sugars when lead was added to pure sugar solutions. When plant extracts or plant extracts plus pure sugars were used, however, the phosphate deleading was no better than the oxalate. The sugars were apparently lost in the clearing precipitate, and could not be recovered without losing the benefits of clearing. Neutral lead avoided all difficulty.

Englis and Tsang (6) also used tannic acid as a deleading agent and obtained better recovery of reducing sugars than with a number of conventional deleading compounds. Tannic acid has a reducing power (tables 1 and 2) and if basic lead acetate reacted with the reducing sugars, some tannic acid would be left in the dealed solution since the tannic acid was added quantitatively to precipitate the basic lead acetate originally added. Thus the reducing power of the tannic acid which was left in the dealed solution replaced the reducing power lost in the reaction between basic lead acetate and the reducing sugars.

The third explanation is based on the facts that no pre-
Precipitation was observed when basic lead acetate was added to a dilute fructose solution, and that the mixture turned yellow upon standing while fructose disappeared. David (3) determined the loss of fructose, both by means of the rotatory power and by means of the reducing power of the solution, and found that the loss as determined by the reducing power was considerably less than the loss as determined by the rotatory power. According to Davis these findings agreed with de Bruyn and van Ekenstein's description (2) of the conversion of fructose to glutose. This conclusion seems questionable, however, because Davis used solutions of fructose far more dilute than those used by de Bruyn and van Ekenstein.

When the description by von Lippmann (12) of the formation of lead fructosates upon addition of basic lead acetate to a dilute solution of fructose is considered it is seen that the qualitative observations made by Davis agree closely with this description. Furthermore, Vogel and Georg (22) give the properties of the lead fructosates described by von Lippmann and state that these compounds do not reduce cupric ions when boiled with Fehling's solution, are slightly soluble in an excess of lead acetate and show dextro-rotation in such solution. These facts can account for Davis' quantitative observations if it is assumed that lead fructosates are formed when basic lead acetate is added to a dilute fructose solution.
Thus it appears that the experimental results upon which the first three explanations are based, do not contradict the fourth explanation offered, and that it is probable that the loss of reducing sugars is caused by a reaction between basic lead acetate and reducing sugars.

In our own research the data of table 4 and figures 2 and 3 are particularly pertinent. Pure sugar solutions were used and uniform deleading with the phosphate agent recommended by Englis and Tsang (6) to prevent the loss of sugars with basic lead. Fructose in figure 2 and both fructose and glucose in figure 3 show the decreasing rates of loss with time characteristic of mass action effects in chemical reactions. Under the conditions of these experiments, at least, there seems little doubt that lead-sugar compounds of fructose were formed at pH 8 and of both fructose and glucose at pH 10. Since the rate of formation of the compounds is slow, the use of minimum quantities of basic lead combined with rapid deleading would be expected to give the small losses characteristic of clearing.

If, as seems highly probable, lead-sugar or lead-fructose compounds are formed during clearing with basic lead, the properties of these compounds become important in the interpretation of clearing losses. The lead-sugar compounds do not reduce cupric or ferric ions in alkaline solution and they contain approximately 70 per cent lead, i.e. 2 moles of lead per
mole of monosaccharide. Lead-sugar compounds were synthesized (p. 33) under conditions closely approximating those of usual clearing with basic lead acetate. Their lead contents were approximately 70 per cent, the lead-glucose compound being slightly lower (tables 6 and 7). These compounds were shown to be lead-sugar compounds by the fact that they contained uniform percentages of lead and formed glucosazones upon treatment with phenylhydrazine hydrochloride (figures 4 and 5).

The observed properties of the synthesized lead-sugar compounds (p. 39) agreed with the properties listed by von Lippmann (12). In addition, Vogel and Georg (22) state that lead-fructose compounds, dissolved in a dilute solution of lead acetate, show a slight dextro-rotatory power. On the basis of these properties it seems possible not only to explain the loss of reducing sugars as caused by a reaction between basic lead acetate and reducing sugars during clearing, but also to explain the selective loss of fructose.

The lead-fructose compound formed during clearing would reduce the levo-rotatory power of an invert sugar solution as observed by Gill (9) and others. When the reducing sugars are determined by their reducing power, the fact that the lead-glucose compounds can be broken down by addition of a suitable deleading agent whereas lead-fructose compounds cannot (von Lippmann (12)), may explain the selective loss of fructose.
Glucose was lost rapidly when in contact with basic lead at pH 10 (figure 3), but not at pH 8 (figure 2). It seems possible that the loss of glucose at the higher pH is preceded by its conversion to fructose. The initial lag in the glucose curve of figure 3 would then be explained as due to the time required to build up an appreciable fructose concentration in the solution, and the later uniform rates of loss as due to a reaction between lead and fructose in both cases. At a pH of 8 the conversion was apparently so slow that the resulting loss of glucose was very small.
SUMMARY

1. Tannic acid and quercetin showed reducing power when added to plant extracts as representatives of naturally occurring impurities. The added impurities were removed quantitatively by neutral lead acetate clearing.

2. Cleared plant extracts gave a sharper end point of titration when Hassid's ceric sulfate method (11) was used for reducing sugar determination.

3. Basic lead acetate caused a loss of about 40 per cent of fructose which had been added to an alcoholic plant extract before clearing. This loss could not be avoided by the use of dipotassium phosphate as a deleading agent.

4. Fructose was lost slowly with increasing time in contact with lead acetate at pH 6, appreciably at pH 8 and rapidly at pH 10.

5. Glucose was recovered when in contact with lead up to 24 hours at pH 5, pH 6 and pH 8, but lost rapidly with increasing time at pH 10.

6. The glucose loss at pH 10 was possibly due to a conversion of glucose to fructose followed by a loss of the fructose formed.

7. A loss of reducing sugars, mainly of fructose, occurred during clearing. The formation of lead-sugar compounds, probably lead-fructose, appears to be the cause of the loss.
8. Lead-glucose and lead-fructose compounds were synthesized under conditions comparable to those of clearing. The lead-sugar compounds contained approximately 70 per cent lead, had no reducing power when tested with cupric and ferric ions in alkaline solution, and formed glucosazones when treated with phenylhydrazine hydrochloride.

9. Where entrainment is involved in sugar losses during clearing, it seems probable that it is the lead-sugar compounds rather than the pure sugars which are entrained.

10. Neutral lead acetate should be used instead of basic for the clearing of plant extracts. Large excesses should be avoided and the lead should be removed as fast as possible. Oxalate is a satisfactory deleading agent when the Munson-Walker method is used, but dipotassium or disodium phosphate should be used with the ceric sulfate method.
LITERATURE CITED


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