THE USE OF CATION-EXCHANGE RESINS FOR THE HYDROLYSIS OF SUCROSE IN PLANT EXTRACTS

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There are a number of reports in the literature regarding the use of ion-exchange materials for the catalytic hydrolysis of esters, proteins, and sucrose (1, 2, 3, 6, 7). The work of Bodamer and Kunin (1) on the hydrolysis of sucrose by several readily available synthetic cation exchangers suggested that it might be possible to use this method for the determination of the total sugars in plant samples. The present paper is concerned with some comparative studies on the hydrolysis of sucrose in plant extracts by a cation-exchange resin, acid, and invertase.

Methods

In preliminary experiments, a number of synthetic cation-exchange resins were tested regarding their ability to hydrolyze sucrose. Of these, Dowex-50, Amberlite IR-100, Amberlite IR-120, Amberlite IRC-50, and Duolite C-3, only the Amberlite IRC-50 proved unsatisfactory. In the work that is reported here the Dowex-50 resin was used. It was received in the sodium form (200–400 mesh size) and was converted to the hydrogen form with 5% HCl after which it was exhaustively washed with distilled water. The resin was air dried, using a sintered-glass funnel, and known amounts weighed out for the hydrolysis experiments.

The plant samples were extracted with boiling 80% ethanol for 30 minutes. The alcohol was decanted off and the plant material was extracted in a Waring Blendor with hot 80% ethanol and filtered while hot. The alcohol filtrates were combined and concentrated under reduced pressure to remove the alcohol.

The alcohol-free extracts were filtered through Celite Analytical Filter-Aid (8) and then clarified with the aid of mixed anion- and cation-exchange resins (9). The resin was a Monobed preparation of Amberlite IR-120 and Amberlite IR-4B. Reducing sugars were determined by the

1 Work performed under contract no. W-7405-eng-26 for the Atomic Energy Commission.
2 Present address: Southern Research Institute, 917 S. 20th Street, Birmingham 5, Alabama.
3 The Dowex-50 resin was obtained from the Dow Chemical Company, Midland, Michigan, the Amberlite resins from the Rohm & Haas Company, Philadelphia, Pennsylvania and the Duolite C-3 resin from the Chemical Process Company, Redwood City, California.
Use of the modified Somogyi reagent (5). Total sugars were determined by the Somogyi method following either invertase or HCl hydrolysis (4). A 10-ml. aliquot of the cleared extract was heated with 10 ml. of HCl (sp. gr. 1.1) for 11 minutes in a water bath at 70°C. After cooling for 30 min., the solution was neutralized with 20% sodium hydroxide, made to volume, and analyzed for reducing sugar. For the invertase hydrolysis, 5 ml. of the cleared extract were added to 5 ml. of 0.2 M acetate buffer (pH 4.5) and 1 ml. of 0.2% invertase solution (Nutritional Biochemicals Corp.). The hydrolysis was carried out overnight, after which the mixture was neutralized with 0.1 N NaOH, made to volume, and analyzed for reducing sugar. Pure sucrose was also carried through the above procedures.

**Results**

**Pure sugars and polysaccharides.**—Preliminary experiments indicated that sucrose could be completely hydrolyzed by the following procedure: The sucrose, 5 to 250 mg. dissolved in 5 ml. of water, was mixed with 1 gm. (moist weight) of Dowex-50 in a test tube that was immersed in a boiling water bath. After 40 minutes of continuous stirring the solution was cooled and filtered through a sintered-glass filter. The effect of varying the temperature of the water bath or of the time of the hydrolysis is shown in figures 1 and 2.

A series of oligosaccharides was treated with Dowex-50 under the conditions that completely hydrolyzed sucrose. The sugar solutions were paper chromatographed using the techniques followed by Williams and
TABLE I

PERCENTAGES OF REDUCING SUGAR AND TOTAL SUGAR (EACH AS
GLUCOSE) AFTER ACID HYDROLYSIS OR DOWEX-50 HYDROLYSIS
OF EXTRACTS OF TOMATO, TOBACCO, AND BARLEY LEAVES.
BASED ON FRESH WEIGHT OF LEAVES.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Reducing sugar after HCl hydrolysis</th>
<th>Reducing sugar after Dowex-50 hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Tobacco</td>
<td>0.027</td>
<td>0.080</td>
</tr>
<tr>
<td>Tomato</td>
<td>0.078</td>
<td>0.13</td>
</tr>
<tr>
<td>Barley</td>
<td>0.046</td>
<td>0.33</td>
</tr>
</tbody>
</table>

BEVENUE (8). Melibiose, maltose, cellobiose, and turanose were not hydrolyzed, while raffinose and melezitose were partially hydrolyzed.

Starch and inulin were treated with Dowex-50 and separately with HCl under conditions that would completely hydrolyze sucrose. The filtrates, after removal of the resin or neutralization of the HCl, were analyzed for reducing sugars. No reducing sugars were found from either the resin- or HCl-hydrolyzed starch samples. The HCl-hydrolyzed inulin sample titrated completely as reducing sugar, while from the resin hydrolysis only a trace of reducing sugar could be found.

PLANT EXTRACTS.—Twenty-five gm. (fresh weight) of leaves from greenhouse-grown tomatoes, tobacco, and barley were extracted and clarified. Reducing and total sugars (each calculated as glucose) after either acid hydrolysis or Dowex-50 hydrolysis were determined on the plant extracts. The results are shown in table I, where it is apparent that the two methods gave very similar results.

Another plant sample was extracted with alcohol and then clarified. Reducing sugars after acid hydrolysis, invertase hydrolysis, and Dowex-50 hydrolysis were determined. The values were as follows (measured as mg. glucose per aliquot): acid hydrolysis, 70.5 mg.; invertase hydrolysis, 69.2 mg.; and Dowex-50 hydrolysis, 71.8 mg. The values agree very well and indicate the usefulness of the Dowex-50 hydrolysis method for determining total sugars in plant extracts. This is true provided that sucrose is the only oligosaccharide present. If raffinose is a major constituent, acid must be used, since the Dowex-50 treatment only partially hydrolyzes raffinose.

TABLE II

RECOVERY OF ADDED SUCROSE FROM A PLANT EXTRACT.

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>Added sucrose</th>
<th>Reducing sugar found</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml.</td>
<td>mg.</td>
<td>mg.</td>
<td>%</td>
</tr>
<tr>
<td>125</td>
<td>None</td>
<td>1.8</td>
<td>99</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>11.7</td>
<td>99</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>27.0</td>
<td>101</td>
</tr>
<tr>
<td>1</td>
<td>50</td>
<td>51.4</td>
<td>99</td>
</tr>
</tbody>
</table>
RECOVERY EXPERIMENTS.—A series of aliquots of a plant extract to which had been added variable amounts of sucrose were hydrolyzed by Dowex-50. The filtrates were analyzed for reducing sugar and the results as shown in table II indicate that the recoveries are essentially quantitative.

Discussion

Sucrose was readily hydrolyzed by the Dowex-50, a sulfonic acid cation-exchange resin. Bodamer and Kunin (1) similarly noted that the sulfonic acid resins caused rapid sucrose inversion as opposed to the carboxylic type resins. They also suggested that the rate of sucrose inversion was a function of the resin particle size and degree of porosity. In the present work a fine-mesh resin was used in connection with constant stirring, so that in all probability the maximum contact between sugar and resin was obtained.

The results from the hydrolysis of sucrose in plants indicate that the cation-exchange resin hydrolysis is as effective as either the acid hydrolysis or invertase hydrolysis. The method requires no special equipment: the resin is stable and can be used over again. When compared with the acid methods, the resin hydrolysis is considerably easier. There is no excess acid to neutralize and volumes can be kept small. It is very often found that the acid method hydrolyzes non-sugar materials, such as dextrines or inulin, which are subsequently determined as reducing sugar. With the resin method, the hydrolysis of such compounds is very small.

The resin hydrolysis is quicker than the invertase method, and since the resin is stable, there is no problem of maintaining a labile enzyme preparation on hand.

The combination of the batch-resin clarification technique of Williams and Bevene (9) with the resin hydrolysis of sucrose appears to offer a number of advantages in the analysis of the sugar in plant extracts. The resin clarification seems to remove non-sugar reducing materials from the plant extracts that are not removed by such conventional methods as lead phosphate or lead oxalate. It was noted in these experiments that plant extracts cleared by these latter procedures were yellow in color, whereas the batch-resin cleared extracts were water clear. Not only is it an advantage to have the solutions clear when measuring reducing sugar by titration method (8) but, according to Roberts (4), the coloring matter in conventionally cleared extracts may yield quite erroneous results in terms of reducing sugars and total sugars.

Summary

1. Sucrose was completely hydrolyzed by a sulfonic acid-type cation-exchange resin.
2. The resin hydrolysis method was compared with acid and invertase hydrolysis methods for determining the total sugar concentration of plant extracts. All three methods gave comparable results.
3. There are a number of advantages in the use of the cation-exchange resin hydrolysis method that may make it useful to the routine analysis of plant extracts.

I wish to thank Miss Eleanor Schumacher for technical assistance in these experiments.

LITERATURE CITED


