Short Communication

Gas Chromatography of Carbohydrates in Alfalfa Nectar

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Techniques used for the qualitative and quantitative analysis of carbohydrates in plant tissue are time consuming and tedious (5). Analysis of individual sugars such as glucose, fructose, and sucrose has been accomplished primarily by paper or thin layer chromatography plus elution and colorimetric or titrimetric techniques for final determination (2,9). Various combinations of techniques are usually employed when making both quantitative and qualitative determinations but considerable time and effort per sample is inevitable.

This paper describes a technique whereby gas chromatography has been utilized for rapid quantitative and qualitative determination of carbohydrates in alfalfa (Medicago sativa var. Sonora) nectar.

The development of methods to analyze carbohydrates using gas-liquid chromatography has been hindered by the lack of volatility of polyhydroxy compounds (11). However, since the discovery of procedures to prepare volatile carbohydrate derivatives much progress has been made in the preparation of these derivatives. Recent investigators have prepared several different volatile carbohydrate derivatives (1,4,6). Sweeley et al. (7) were able to separate 20 different trimethylsilyl sugar derivatives by employing a SE-52 column and by using a temperature programming range from 125° to 275° at the rate of 2.3° increase per minute. Wells (11) summarized the relative retention times of trimethylsilyl derivatives in which he used 4 different columns (20% SE-52, 25% SE-30, 30% XE-60, and 15% EGS). His data showed that although the separation of anomeric pairs varied somewhat with the phase, the general order of elution remained the same.

Although work has been done on solving the problems of gas-liquid chromatographic analysis of carbohydrates standards, application of this technique in solving biological problems which deal with carbohydrates has been limited. Therefore, in this study it has been found that the ease of preparation of the trimethylsilyl derivative for the analysis of sugars from alfalfa nectar appears to be the most suitable one.

Nectar was collected from the inflorescences of alfalfa plants in 3 replicate samples grown in the greenhouse at the University of Wyoming. Collection was made by placing the racemes in an inverted position with their peduncles between a split cork, wedging the cork in a 15 ml glass centrifuge tube and centrifuging for 4 minutes at 500 × g. This procedure was repeated until sufficient amounts of nectar were collected. A 10 μl Hamilton syringe was used to transfer 10 μl of nectar into a 15 ml graduated sedimentation tube. In a procedure similar to that used by Sweeley et al. (8), an ampoule containing 1 ml of pyridine, hexamethyldisilazane, trimethylchlorosilane (9:3:1) (Applied Science Laboratories) was emptied into the sedimentation tube to form the trimethylsilyl sugar derivatives. The volume was checked so that exactly 1 ml was present. The samples were sealed with cork stoppers and allowed to stand overnight at room temperature. The following day 1 μl of sample was analyzed on a gas chromatograph (Aerograph Hy-Fi Model 600D) equipped with a hydrogen flame ionization detector which had a hydrogen flow rate of 37 ml/minute (Aerograph Model 650 Hydrogen Generator). The oven temperature was programmed from 120° to 275° at a rate of 5° per minute (Aerograph Model 128 Programmer). Sugar derivatives were separated on a column (5 ft by one-eighth in) which was packed with 5% SE-30 on acid washed Chromosorb W which had a flow rate of 30 ml of nitrogen per minute as carrier gas. The electrometer range was set at 1 and the attenuator set at 32. The signal was recorded on a 1 mv Leeds and Northrup Model H recorder. A mixture of known sugars at a concentration of 1 μg/μl of each sugar was mutarotated and subsequently their trimethylsilyl derivatives were formed. A standard curve was

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prepared by plotting the sample concentration versus peak area on log-log paper (fig 1). This curve was prepared from the average of 3 replicate injections for each determination and of 3 different amounts of the standard sugar solution.

Total percent sugar was also measured by diluting the nectar 1:3 with 80% ethanol (v/v) and transferring a small drop to a refractometer which was equipped for direct readings of percent sugar.

The top chromatograph line in figure 2 illustrates the peaks of a standard mixture of α-D-fructose, β-D-fructose, α-D-glucose, β-D-glucose and sucrose. Determination of α and β anomers was based on elution patterns as compared with the work of Sweeley et al. (7). The lower chromatograph line reveals the quantity of sugars from a sample of alfalfa nectar. The peaks closely correspond on each chromatogram.

Fraction 1, α-D-fructose, had the shortest retention time of 4 minutes. This fraction was followed closely by β-D-fructose with a retention time of 4.5 minutes. The fraction for β-D-fructose was very small and is shown between fractions 1 and 2. Fraction 2, α-D-glucose, and fraction 3, β-D-glucose, had retention times of 4.75 and 5.5 minutes respectively. Fraction 4 with a retention time of 12.5 minutes represents sucrose. When the standard solutions were allowed to equilibrate in pyridine only trace amounts of β-D-fructose were present while α and β-D-glucose were present in approximately equal amounts (fig 2, top chromatogram). The nectar sample lacks β-D-fructose while α-D-glucose was present in lower amounts than β-D-glucose.

Table I. Variation of Sugars in the Nectar of Alfalfa

<table>
<thead>
<tr>
<th>Sugar</th>
<th>No. 1</th>
<th>No. 2</th>
<th>No. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose</td>
<td>250</td>
<td>247</td>
<td>291</td>
</tr>
<tr>
<td>Glucose</td>
<td>228</td>
<td>218</td>
<td>250</td>
</tr>
<tr>
<td>Sucrose</td>
<td>269</td>
<td>252</td>
<td>270</td>
</tr>
<tr>
<td>Total sugar</td>
<td>747</td>
<td>717</td>
<td>811</td>
</tr>
</tbody>
</table>

Table I presents the net μg of each sugar present per μl of nectar and also the total sugar as measured by gas chromatography. The quantity in μg for each sugar per μl of nectar was calculated from a standard curve as referred to previously. The percent total sugar (g sugar/100 ml nectar) was found to vary from 71.7% to 81.1% as measured by gas chromatography. Using a refractometer, which was calibrated for sucrose as a standard, the percent total sugar (w/w) was found to vary from 37.6% to 64%. Such a high concentration of sugar was somewhat surprising. Yet this high concentration was probably a function of the very low relative humidity under which the alfalfa was grown. Feininger and Sackett (3) and Vansell (10) have reported wide variations in the percent total sugar in alfalfa nectar, which they attribute to fluctuations in relative humidity under which the plants were grown.

Gas-liquid chromatography of the trimethylsilyl derivatives of mono and disaccharides appears to be a very promising technique for rapid quantitative and qualitative analysis.

Fig. 1. Standard curves for glucose, fructose, and sucrose.

Fig. 2. Chromatograms showing α-D-fructose (1), β-D-fructose (between 1 and 2), α-D-glucose (2), β-D-glucose (3), and sucrose (4) standards (top), and alfalfa nectar (bottom).
Literature Cited


