Soluble carbohydrates in soybean

D. V. PHILLIPS AND A. E. SMITH
Department of Plant Pathology and Department of Agronomy, University of Georgia College of Agriculture
Experiment Stations, Georgia Station, Experiment, Georgia 30212

Received April 30, 1974


Gas-liquid chromatographic analysis of the trimethylsilyl ethers of the ethanol (90%)-extracted carbohydrates (soluble carbohydrates) from soybean (Glycine max) plants demonstrated the presence of major quantities of O-methylinositol, glucose, fructose, and sucrose, minor quantities of myo-inositol, and two unidentified components. In leaf blades and petioles of 6-week-old plants, O-methylinositol accounted for more than 50% of the total soluble carbohydrates present.


L'analyse par chromatographie en phase gazeuse des éthers triméthylsilyl des hydrates de carbone (séquelles) extraits du soja (Glycine max) par l'éthanol (90%) a révélé la présence de grandes quantités d'O-méthylérythritol, glucose, fructose, sucrose, et de petites quantités de myo-inositol ainsi que deux corps non identifiés. Dans les feuilles et les pétais de plantes âgées de 6 semaines, l'O-méthylérythritol représentait plus de 50% du total des hydrates de carbone solubles en présence.

Introduction

Soluble carbohydrates in soybean plants have been extensively studied by researchers concerned with photosynthesis and translocation of organic materials (3, 4, 5, 8, 12, 13, 14, 23). Most of these studies indicate that most of the translocated material is carbohydrate and that sucrose is the predominant carbohydrate with, in some cases, significant amounts of glucose and fructose present. However, Quillet and Bourdon (20) reported that maltose was more prevalent than sucrose or the hexoses in petioles and stems of soybean.

Since all of these studies involved the use of paper chromatography for the separation and identification of carbohydrates, we decided to examine soybean extracts by a newly developed gas-liquid chromatographic method (16). Our preliminary results indicated the presence of significant quantities of glucose, fructose, and sucrose but no maltose. We also detected other carbohydrates, one of which was the most prevalent compound in the 90% ethanol extracts. This paper describes the identification of the unknown components and the quantification of the soluble carbohydrates in soybean plants.

Material and Methods

Plant Materials

All quantitative data were obtained from soybean (Glycine max (L.) Merr., cv. Jackson) plants grown in flats containing methyl bromide fumigated soil in a greenhouse. The plants were sampled 4 and 6 weeks after planting. Soybean plants cv. Lee and cv. Semmes grown in solution culture (15) were used for plant extracts used in some mass spectral and nuclear magnetic resonance (nmr) analyses.

To eliminate the effects of diurnal variations all soybean plant parts were collected between 1:30 and 2:00 P.M. The soil was washed from the roots, the plant was divided into leaf blades, petioles, stems, and roots, and the parts were immediately shaken with finely divided dry ice. By this procedure all plant parts were solidly frozen (−78.5°C) within 1 min of the time the plant was removed from the soil. The frozen plant parts were broken into small pieces and lyophilized.

Stem exudates were obtained from 6-week-old plants between 1:30 and 2:30 P.M. as follows. All cotyledons, leaves, buds, and branches were removed from the main stem with a sharp razor blade, the flat of soil containing the plants was immersed to the top of the soil in warm water, and as the drops of exudate appeared on the cut surfaces, they were removed with a hypodermic syringe and transferred to a test tube immersed in dry ice. The exudates were cleared and lyophilized aliquots were handled the same as other samples.

Additional plants including the legumes white clover (Trifolium repens L.), alfalfa (Medicago falcata L.), cowpea (Vigna unguiculata (L.) Walp), peanut (Arachis hypogaea L.), and bean (Phaseolus vulgaris L.), as well as Bermudagrass (Cynodon dactylon (L.) Pers.), crabgrass (Digitaria sanguinalis L.), nutseed (Cyperus rotundus L.), onion (Allium sp.), turnip (Brassica rapa L.), corn (Zea mays L.), cucumber (Cucumis sativus L.), Petunia sp., and tomato (Lycopersicon esculentum Mill.) were collected from the greenhouse or field plots near midday.

Extraction and Analysis

Lyophilized plant materials were ground in a Wiley
mill and extracted and cleared as previously described (16). Aliquots of the cleared extract were frozen, stored at -20°C, and lyophilized before preparation for gas-liquid chromatographic (GLC) analysis. All GLC gas flow rates, temperatures, and program conditions were as described previously (16). Methyl nonadecanoate was used as the internal standard because of the presence of some myo-inositol in the samples.

**Mass Spectrometry and Nuclear Magnetic Resonance Spectroscopy**

Mass spectra were recorded with a DuPont 22-492 mass spectrometer and a Finnigan 1015-C mass spectrometer. All samples were trimethylsilyl derivatives (16) dissolved in dimethylformamide and were admitted through a gas chromatograph equipped with a column packed with chromosorb W coated with 5% SE-30. All spectra were obtained at 70-eV ionizing potential.

Proton-decoupled 13C nmr spectra were obtained at 25.15 mHz on a JEOL PS 100/PFT 100 spectrometer. The samples were examined as about 0.3 M solutions in D2O at 27°C. Accumulation time was 15 min. The chemical shifts were referenced to external tetramethylsilane and the values reported are corrected for diamagnetic susceptibility.

**Results**

**Identification of Carbohydrates**

Chromatography with derivatives of known carbohydrates indicated the presence of glucose, fructose, myo-inositol, sucrose, and three unidentified components in extracts from soybean plants. The unidentified components had retention times of 0.37, 0.90, and 1.14 relative to α-glucose (to be referred to as 0.37 Rg, 0.90 Rg, and 1.14 Rg, respectively). A typical chromatogram of a soybean petiole extract is shown in Fig. 1.

The component 0.90 Rg (C in Fig. 1) was always present and was usually the most abundant component in the extract. Since there was apparently no other GLC peak associated with 0.90 Rg at mutarotation equilibrium (16), it appeared that this component might be a sugar alcohol.

The 70-eV mass spectrum of this component (Fig. 2) indicated a molecular ion at mass: charge ratio (M:E) 554 and several fragments common to the trimethylsilyl derivatives of the inositols (22). The molecular ion at 554 and several ions 58 units below major fragments of the inositols indicated the 0.90 Rg GLC peak to be an inositol monomethyl ether. The 70-eV mass spectrum of (−) quebrachitol was identical with that of 0.90 Rg except for relative intensities of some ions. Cochromatography indicated these two compounds were not identical, but the retention times were similar.

Repeated passage of stem and petiole extracts through columns of Dowex 21K (OH−) (21) produced a fraction containing about 95% 0.90 Rg with 1.14 Rg and myo-inositol as the only contaminates detectable by GLC. The 13C nmr spectrum of this fraction (Table 1) compared with that of (−) quebrachitol and the spectra published by Dorman et al. (7) confirmed that 0.90 Rg was an O-methylinositol. Plouvier (18, 19) has isolated both (−) bornesitol and (+) ononitol from legumes. However, from present data we have not been able to determine which of the several possible O-methylinositols (1, 2) this is. Hereafter this

![Fig. 1. Gas-liquid chromatographic separation of trimethylsilyl ethers of the 90% ethanol soluble carbohydrates from soybean petioles. Components: (A) unknown 1, (B) fructose, (C) O-methylinositol, (D) α-glucose, (E) β-glucose and unknown 2, and (F) sucrose. All conditions for gas-liquid chromatography are as described by Phillips and Smith (16).](image-url)
component will be referred to as O-methylinositol.

The 0.37 Rg component appeared, on the basis of retention time, to be a pentose or a deoxyhexose. However, cochromatography indicated that it was not arabinose, ribose, xylose, lyxose, ribulose, deoxyglucose, or deoxygalactose. The 70-eV mass spectrum of this component (Fig. 3) indicated an ion at M:E 423. Since the molecular ion is very weak and may not be detected but the M+15 ion is usually prominent in the 70-eV spectra of silylated sugars (6, 17), the absence of a peak at M:E 408 indicates that M:E 423 is probably the M+15 ion. This would indicate a molecular ion at M:E 438. Any persilylated pentose would have

![Fig. 2. Mass spectrum (70 eV) of the trimethylsilyl ether of O-methylinositol from soybean. Ions present at mass:charge ratios (M:E) 45, 59, 73, 75, and 89 are not shown.](image)

![Fig. 3. Mass spectrum (70 eV) of the trimethylsilyl ether of unknown I from soybean. Ions present at mass:charge ratios (M:E) 45, 59, 73, 75, and 89 are not shown.](image)
a molecular ion at 438 as would an inositol trimethyl ether. The fragmentation pattern, the retention on Dowex 21K (OH-) columns, and the presence of two small GLC peaks apparently associated with this peak in concentrated samples silylated at mutarotation equilibrium (16) indicate this component may be a sugar and not an O-methyl alcohol. Hereafter this component will be referred to as unknown 1.

The 1.14 Rg component is assumed to be a sugar alcohol or methylated sugar alcohol since it was not retained on the column of Dowex 21K (21) and appears to have only one GLC peak at mutarotation equilibrium. Its GLC retention time is very close to, but not identical with, those of sorbitol and mannitol. Mass spectra of this component indicated it to be a polyhydroxy compound probably a sugar alcohol, but a molecular weight could not be determined, apparently because of contamination by β-glucose. Hereafter this component is referred to as unknown 2.

**Quantification in Soybean Plant Extracts**

The quantities of each carbohydrate in different organs of soybean plants (cv. Jackson) at 4 and 6 weeks after planting are shown in Table 2. No other carbohydrates were detected in the extracts from any plant organs at either age. These same carbohydrates were detected in extracts from cv. Lee and cv. Semmes.

The total carbohydrate concentration in all plant parts was lower at 6 weeks than at 4, although not significantly lower in the leaves and roots. The concentrations of fructose, glucose, myo-inositol, and sucrose generally decreased while the concentration of unknown 1, O-methylinositol, and unknown 2 increased or remained the same between the 4- and 6-week harvests. Thus, at the 6-week harvest, O-methylinositol was the predominant soluble carbohydrate in leaves, petioles, and stems, and the second most prevalent carbohydrate in roots.

**Comparisons of Stem Extracts and Stem Exudates**

Since O-methylinositol was present in large amounts in petioles and stems, it appeared that this carbohydrate might be translocated. Analysis of stem exudate (Table 3) indicated the same carbohydrates as in stem extracts, but the relative quantification was different.

**Table 2**

Quantities of 90%-ethanol-soluble carbohydrates in Jackson soybean

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Plant age, weeks</th>
<th>Leaf blade, mg/g*</th>
<th>Petiole, mg/g</th>
<th>Stem, mg/g</th>
<th>Root, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-methylinositol</td>
<td>4</td>
<td>6.18 d f</td>
<td>15.08 b</td>
<td>13.01 bc</td>
<td>2.11 e</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>12.07 c</td>
<td>20.29 a</td>
<td>14.17 b</td>
<td>5.02 d</td>
</tr>
<tr>
<td>Glucose</td>
<td>4</td>
<td>3.54 e</td>
<td>22.94 b</td>
<td>30.91 a</td>
<td>2.35 e</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.15 f</td>
<td>9.61 d</td>
<td>13.34 d</td>
<td>1.21 e</td>
</tr>
<tr>
<td>Fructose</td>
<td>4</td>
<td>4.54 cd</td>
<td>20.63 b</td>
<td>34.32 a</td>
<td>3.52 d</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Trace</td>
<td>4.92 cd</td>
<td>8.09 c</td>
<td>Trace</td>
</tr>
<tr>
<td>Sucrose</td>
<td>4</td>
<td>6.94 b</td>
<td>3.57 b</td>
<td>16.46 g</td>
<td>17.66 a</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5.14 b</td>
<td>2.68 b</td>
<td>5.71 b</td>
<td>13.51 a</td>
</tr>
<tr>
<td>myo-Inositol</td>
<td>4</td>
<td>2.39 a</td>
<td>0.20 cd</td>
<td>0.39 c</td>
<td>0.27 cd</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.84 b</td>
<td>Trace</td>
<td>Trace</td>
<td>0.11 d</td>
</tr>
<tr>
<td>Unknown 1</td>
<td>4</td>
<td>1.67 a</td>
<td>0.86 ab</td>
<td>0.45 b</td>
<td>0.29 b</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.20 a</td>
<td>1.15 ab</td>
<td>0.47 b</td>
<td>0.32 b</td>
</tr>
<tr>
<td>Unknown 2</td>
<td>4</td>
<td>3.08 a</td>
<td>0.27 b</td>
<td>0.77 b</td>
<td>1.06 b</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2.96 a</td>
<td>1.32 b</td>
<td>1.72 ab</td>
<td>1.60 ab</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>28.34 d</td>
<td>63.55 b</td>
<td>96.28 a</td>
<td>27.26 d</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>22.36 d</td>
<td>39.97 c</td>
<td>43.50 c</td>
<td>21.78 d</td>
</tr>
</tbody>
</table>

*Values for a given carbohydrate not followed by the same letter are significantly different (P = .01) as determined by Duncan's new multiple range test. Letters should be disregarded in all comparisons between different carbohydrates.
tive concentrations differed. Fructose and glucose accounted for a much lower percentage of the total carbohydrates in stem exudate than in stem extracts. All other components accounted for a higher percentage of the exudate than the extract.

Occurrence of O-methylinositol and Unknowns in Other Plants

In a limited survey, a component with the same GLC retention time as the O-methylinositol from soybean was found to be a major component of white clover, alfalfa, and peanut and a minor component of bean and cowpea. No mass spectra were obtained. A component with the same retention time as the unknown 1 was a major component of alfalfa and peanut and a minor component of white clover, bean, and cowpea. A component with the retention time of the unknown 2 was a major component of cowpea and a minor component of white clover and alfalfa. No components with the retention time of O-methylinositol, unknown 1, or unknown 2 were found in bermudagrass, crabgrass, turnip, wild onion, petunia, corn, cucumber, nutsedge, or tomato.

Discussion

Inositol methyl ethers have been known to occur in plants for many years (1, 2). However, O-methylinositol apparently has not previously been recognized as a major soluble carbohydrate in soybean plants (3, 4, 5, 8, 13, 14, 20, 23). The reason that O-methylinositol was not detected in previous studies was probably due to the use of paper chromatography to identify the carbohydrates. In our limited attempts to separate the carbohydrates from soybean plants by paper and thin-layer chromatography, O-methylinositol always cochromatographed with one (usually fructose) of the sugars. In addition, most of the reagents used to detect carbohydrates do not react with O-methylinositol. Thus, it would seem difficult to detect O-methylinositol by paper chromatography, particularly when there was no reason to suspect its presence.

In most of these studies (4, 5, 8, 13, 14, 23), concerned primarily with translocation of organic solutes in soybean, the procedure has been to apply $^{14}$CO$_2$ to soybean leaves and after varying times determine the radioactive compounds in the petioles or stems by using paper chromatography. Since O-methylinositol was not identified in these previous studies, but we have found it to be the predominant carbohydrate in petioles, stems, and stem exudates, two explanations that seem possible are (a) that O-methylinositol is not translocated and, therefore, did not become labeled in the translocation experiments; or (b) that it is translocated and became labeled in translocation experiments but cochromatographed with one of the other sugars and was misidentified.

Quillet and Bourdon (20) reported maltose to be a major soluble carbohydrate of soybean petioles and stems during flowering and pod formation. Although their identification of maltose seems convincing, we have not found soluble maltose in soybean plants of any age or developmental stage. They reported maltose equivalent to 45, 38, and 14% of the total carbohydrates in petioles, stems, and roots, respectively. Since we have found O-methylinositol equivalent to 51, 33, and 23% of the total in petioles, stems, and roots, respectively, of plants in the pod-filling stage, it is tempting to speculate that they may have been measuring O-methylinositol.

Hamlen et al. (9, 10, 11) found several unidentified carbohydrates in root exudates from alfalfa. Component U$_1$, the predominant component in exudates from older plants had a retention time of 0.92 R$_g$ on OV-1. This is very close to our O-methylinositol with a retention time of 0.90 R$_g$ on SE-30. Although they did not show peaks above M:E 460, a comparison of the mass spectrum of their component U$_1$ (10) with our Fig. 1 indicates their U$_1$ to be O-methylinositol. Since we found a major component in alfalfa with an identical retention time as O-methylinositol from soybean, it appears that their U$_1$ is the same O-methylinositol present in soybean. They also reported a component, with a retention time of 1.09 R$_g$ (OV-1), which had a mass spectrum indistinguishable from U$_1$ and is, thus, apparently a second O-methylinositol. In our alfalfa extracts a component present at 1.09 R$_g$ (SE-30) was very small. They (11) also reported a component with a retention time of 1.13 R$_g$ (OV-1), which might be the same as our unknown 2 (1.14 R$_g$, SE-30). They did not report a component with a retention time similar to our unknown 1, although we detected this component in alfalfa extracts.
The differences in the carbohydrate levels in 4- and 6-week-old plants may not be due entirely to differences in age. Since these plants grew in a greenhouse under natural light, variations in light intensity before sampling possibly influenced the carbohydrate levels. In addition, the 6-week-old plants were flowering and had some small pods, whereas those at 4 weeks were vegetative, although they were under an inductive photoperiod. An examination of the influence of age and photoperiod is in progress.

Although our limited survey indicated an O-methylinositol to be present only in legumes, inositol methyl ethers are known to occur in plants of several other families (1, 2, 19). The important point is not that O-methylinositol may be widespread, but that in at least some of the legumes it is a major carbohydrate and under some conditions is more prevalent than glucose, fructose, and sucrose combined. Research is needed on the synthesis, metabolism, and function of O-methylinositol in plants.

Acknowledgments

We are indebted to H. C. Higman, W. K. Austin, Jr., and F. E. Barton, USDA-ARS Russell Research Center, and J. M. McGuire EPA Southeast Environmental Research Laboratory, Athens, Georgia, for mass spectra and 13C nuclear magnetic resonance spectra.