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Cyclitols in Soybean

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The free cyclitols present in soybean plants were pinitol (1D-3-*O*-methyl-*chiro*-inositol), D-*chiro*-inositol, *myo*-inositol, and sequoyitol (5-*O*-methyl-*myo*-inositol). Pinitol was a major component of all plant parts and was found in large quantities in all soybean cultivars examined. D-*chiro*-Inositol, *myo*-inositol, and sequoyitol were minor components. Root nodules contained the same cyclitols found in other plant parts but the relative abundance differed considerably. In soybean seed pinitol was the only monosaccharide of significance. The use of a simple procedure to separate sugars from cyclitols is discussed.

Previously Phillips and Smith (1974) reported that an *O*-methylinositol, now identified as pinitol, was a major ethanol-soluble component in the vegetative portion of soybean plants and in some cases was more prevalent than glucose, fructose, and sucrose combined. They also found *myo*-inositol and an unidentified sugar alcohol which was subsequently found to be a cyclitol.

Delente and Ladenburg (1972) identified the one major monosaccharide in soybean seeds as galactose, but Hymowitz and Collins (1974) identified it as fructose. Pinitol has been identified in soybean flakes by Honig et al. (1971) and Nielsen (1960) and in "milk" made from seeds by Schweizer et al. (1978). Schweizer et al. (1978) also reported a new disaccharide, galactopinitol, but found only traces of fructose or galactose. Streeter and Bosler (1976) and Streeter (1980) reported on cyclitols in root nodules of soybean. Ruis and Hoffmann-Ostenhof (1969) reported that pinitol was synthesized from *myo*-inositol via sequoyitol in crimson clover. Previously Phillips and Smith (1974) were not able to confirm the presence of sequoyitol in soybean plants.

Biologists frequently attempt to relate the soluble or nonstructural carbohydrate levels to various biological functions. Since cyclitols are abundant in soybean plants (Phillips and Smith, 1974) and other legumes (Smith and Phillips, 1980) and some analytical methods fail to distinguish between cyclitols and other carbohydrates or do not detect cyclitols, it was important to identify the cyclitols in soybean plants. This paper reports the identification of the cyclitols in soyben plants, including seeds and root nodules.

MATERIALS AND METHODS

Soybean (*Glycine max* L. Merr.) plants from the growth chamber, greenhouse, or field were uprooted, the soil was washed from the roots, and the plants were divided into various parts depending on the analysis desired. The plant parts were frozen as soon as possible (always within 0.25 h) by placing in a freezer at −30 °C or by shaking with finely divided dry ice. The frozen parts were lyophilized and ground in a Wiley mill to pass a 40-mesh screen and then extracted with 80% ethanol. In some cases fresh or frozen plant parts were placed directly in a blender with sufficient 95% ethanol to give a final concentration of about 80% ethanol after blending.

Clearing, ion-exchange separations, and ^{13}C NMR spectroscopy methods were as previously described (Phillips and Smith, 1974). Gas chromatography was done as described by Loewus and Shah (1972), Phillips and Smith (1973), and Lee and Ballou (1965). Mass spectrometery methods were as described previously (Phillips and Smith, 1974; Smith and Phillips, 1981). Some samples were silylated with deuterated reagents (Sherman et al., 1970) prior to obtaining mass spectra.

When necessary, sugars were removed from cyclitol solutions by a modification of the method described by Roseman et al. (1952). A column of Dowex 21-K was regenerated with 1.0 N NaOH saturated with BaOH, and a column of Dowex 50W was regenerated with 2.0 N HCl. After the columns were rinsed with CO_2-free water, they were connected with the Dowex 21-K on top. The sample was added to the Dowex 21-K column and eluted with CO_2-free water. The cyclitols and the acyclic polyols passed rapidly through the columns, and the sugars were retained.

Solutions of cyclitols deionized by the above procedure were lyophilized and recrystallized from ethanol or methanol. Inositol methyl ethers were demethylated with boiling hydroiodic acid. Infrared spectra were obtained by the KBr wafer method on a Beckman spectrophotometer. Optical rotations were measured on a Perkin-Elmer 141 polarimeter.

RESULTS AND DISCUSSION

The major cyclitol in soybean plants was previously identified as an *O*-methylinositol (Phillips and Smith,

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Table I. Pinitol Content of Foliage from 24 Cultivars Representing Four Maturity Groups (V-VIII) at Three Weeks and Near Maturity

cultivar	maturity group	% pinitol in the 80% ethanol soluble carbohydrate[a]	
		3 weeks	maturity
Dare	V	66	81
Essex	V	78	62
Coker 136	VI	82	
Davis	VI		
Lee 68	VI		57
Lee 74	VI	80	
McNair 600	VI	73	53
Pickett	VI		47
Pickett 71	VI	82	
Tracy	VI	84	
Yelredo	VI		53
Bossier	VII	81	57
Bragg	VII	80	78
Brooks	VII	77	
GaSoy 17	VII	72	
Jackson	VII		68
McNair 800	VII	76	59
Ransom	VII	70	60
Semmes	VII	80	40
Bienville	VIII		67
Cobb	VIII	83	38
Hampton 266A	VIII	79	66
Hutton	VIII	80	
Stuart	VIII	79	

[a] Percent pinitol = (milligrams of pinitol per milligram of total carbohydrate) × 100. Total carbohydrate = sum of pinitol, sucrose, glucose, and fructose. Small quantities or traces of D-*chiro*-inositol, *myo*-inositol, and an unidentified component were not included in the total. The range of concentrations were as follows: pinitol, 6.6-17.7 mg/g, sucrose, 1.8-3.5 mg/g, glucose, 0.5-6.1 mg/g, and fructose, 0.3-2.0 mg/g dry weight of tissue extracted. Each entry is the mean of duplicate analyses of duplicate samples at each sampling time.

1974). This has now been identified as pinitol (1D-3-*O*-methyl-*chiro*-inositol). The mass spectrum of the persilylated material indicates that it is an *O*-methylinositol, and the ^{13}C NMR chemical shifts are nearly identical with those reported by Dorman et al. (1970) for pinitol. After recrystallization from ethanol, the melting point was 185–186 °C (lit. 186 °C), and the optical rotation was $[\alpha]^{30}_D$ +64° (lit. $[\alpha]^{24}_D$ +65°). The infrared spectrum of pinitol was identical with that in the Sadtler Index. Demethylation of pinitol with boiling hydroiodic acid gave D-*chiro*-inositol which was identified by GC–MS and optical rotation.

The pinitol concentration in shoots of examined cultivars ranged from 6.6 to 17.7 mg/g dry weight of tissue and accounted for between 38% and 84% of the total soluble carbohydrate (Table I). The apparent differences in pinitol content between cultivars harvested near maturity may have, in part, been due to differences in the degrees of maturity since these cultivars represent different maturity groups and were planted and grown together. Differences may also have been due to relative amounts of leaf and stem tissue included in the extracted sample. These influences may be greater in mature shoots than the genetic differences in pinitol levels among cultivars. In plants harvested at 3 weeks, the pinitol levels were more uniform among cultivars. The presence of pinitol at relatively high levels in every soybean cultivar examined was not surprising, since pinitol has been a major carbohydrate in all legumes examined (Smith and Phillips, 1980).

Delente and Ladenburg (1972) reported the only major monosaccharide in soybean seeds to be galactose, but Hymowitz and Collins (1974) identified it as fructose. Cochromatography by the procedure of Phillips and Smith (1973) indicated this component was neither galactose nor fructose but pinitol. This confirms the work of Honig et al. (1971), who identified pinitol in soybean flakes at about the same concentration, and Schweizer et al. (1978), who crystallized pinitol from soybean "milk" made from seeds but found only traces of galactose or fructose. This leaves no doubt that the major monosaccharide in soybean seed is pinitol.

The second most prevalent cyclitol in most preparations of soybean plant tissue is D-*chiro*-inositol (1D-*chiro*-inositol). It was identified by the mass spectrum of its persilylated derivative which indicated it to be an inositol (Sherman et al., 1970) and by cochromatography with D-*chiro*-inositol and L-*chiro*-inositol under conditions which separate all of the inositols except D-*chiro*- from L-*chiro*-inositol (Loewus and Shah, 1972).

It was confirmed to be D rather than L by the optical rotation of a preparation from soybean stems and leaves. After removal of all sugars and crystallization of part of the pinitol from solution, a preparation was obtained which contained 76.3% pinitol, 7.1% *myo*-inositol, 16.6% *chiro*-inositol, and a trace of sequoyitol. Using $[\alpha]$ +65° for the pinitol and no rotation for *myo*-inositol and sequoyitol, the *chiro*-inositol was calculated to have a rotation of $[\alpha]$ +60°. This is close to the literature value (+65°) and confirmed D-*chiro*-inositol since no other inositol has a strong (+) rotation. This component was previously labeled unknown no. 2 (Phillips and Smith, 1974). *myo*-Inositol was identified by cochromatography and by the mass spectrum of its trimethylsilyl derivative. The concentration of these two cyclitols vary somewhat with age and the plant part extracted but are generally in the range previously indicated (Phillips and Smith, 1974).

In our extracts of root nodules, the concentration of *myo*-inositol and D-*chiro*-inositol was often severalfold higher than in other plant parts. The concentration of pinitol in root nodules (4.0–14.2 mg/g dry weight) was similar to that reported in other plant organs (Phillips and Smith, 1974). Sequoyitol was present in trace amounts in root nodules. These results agree with the relative concentrations in nodules reported by Streeter and Bosler (1976) and Streeter (1980).

Ruis and Hoffmann-Ostenhof (1969) described the synthesis of pinitol via sequoyitol in crimson clover. We have analyzed many soybean preparations under conditions which separate sequoyitol from other cyclitols known to be present in soybeans and have found only trace amounts of sequoyitol. We have, however, confirmed the presence of sequoyitol by GC–MS. Apparently sequoyitol is present in most soybean tissues in extremely low quantities.

The mass spectrum of a component previously labeled unknown no. 1 (Phillips and Smith, 1974) was determined by GC–MS of several samples on three different instruments. Data from gas chromatography, ion-exchange separations, HPLC, and GC–MS using deuterium labeling indicate that this component is probably not a cyclitol nor is it any of the common sugars. The estimated concentration of this unidentified component in the 80% ethanol soluble fraction from soybean plants varies from a trace to 2 or 3 times the concentration of *myo*-inositol (D. V. Phillips and A. E. Smith, unpublished experiments).

In soybean preparations which have had all of the sugars removed, one additional component was detected. This

component was indistinguishable from sorbitol or mannitol by our GC–MS procedures and was present in only trace amounts in most soybean extracts.

Cyclitols are known to occur in a number of plant families (Plouvier, 1958; Angyal and Anderson, 1959), however, in soybean, and in all legumes examined, the cyclitols consitute a major portion of the nonstructural carbohydrates (Smith and Phillips, 1980). In recent years there have been numerous studies published which attempted to relate nonstructural carbohydrates to some biological function in leguminous plants. Almost all of those have used methods which either failed to distinguish between th cyclitols and sugars and other carbohydrates or failed to even detect cyclitols. Thus many of these studies have attempted to relate some biological function to only a part, in some cases a small part, of the carbohydrate present.

Cyclitols may be equivalent to sugars in some biological processes but in others they are not (Dreyer et al., 1979; Talbot and Seidler, 1979) and in many biological processes the effects of cyclitols, other than *myo*-inositol, are unknown.

While there is no substitute for identification by GC–MS, NMR, etc., in species where this has not been done, there is a useful procedure for those who may not have ready access to analytical equipment and are working with species where cyclitols are known to occur. The sugars and most carbohydrates other than cyclitols can be completely removed from solution by the modification of the procedure of Roseman et al. (1952) described under Materials and Methods. The cyclitols, other sugar alcohols, and methyl glucosides remain in solution. We have used this method extensively on soybeans and other legumes and have found only small amounts of other sugar alcohols. Methyl glucosides have been found only in white clover (*Trifolium repens* L.) (Smith and Phillips, 1981). Thus, in most cases, the contribution of the cyclitols to the nonstructural carbohydrate pool can be determined directly—after removal of the sugars from the sample—by any analytical procedure which detects cyclitols.

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Properties of a Chromium Complex from Higher Plants

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A biologically formed chromium complex has been isolated from alfalfa. It appears to be distinct from the glucose tolerance factor isolated from brewer's yeast. The in vivo and in vitro formation is described along with analysis of constituents and stability parameters affecting the complex. The chromium complex is identical regardless of the valence or mode of incorporation of the chromium. The complex is specific for chromium insertion and is extremely stable once formed. The complex is anionic and contains no peptides or deoxyribose units.

The trace element chromium has been widely studied since its definition as a micronutrient in 1959 (Schwarz and Mertz, 1959). Deficiency conditions have been described in pregnancy (Davidson and Burt, 1973), diabetes (Gurson and Saner, 1978), senescence (Levine et al., 1968), and protein-calorie malnutrition (Gurson and Saner, 1973). The form of chromium which is best absorbed in the gut has also been sought. Since chromium oxide is unusually inert, chromate and amino acids have been examined as the active gut complex. Schwarz and Mertz (1959) have proposed a glucose tolerance factor (GTF) as the biologically active complex that protects the chromium from olation, which inactivates it.

This work describes another biologically formed chromium complex distinct from GTF. This chromium complex is found in alfalfa, crested wheatgrass, beans, and wheat (Blincoe, 1974; Huffman and Allaway, 1973). Pre-

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