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OCCURRENCE OF SORBITOL IN *ZEA MAYS**

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Key Word Index—*Zea mays*; Gramineae; sorbitol; D-glucitol; seed; endosperm.

Abstract—The identity of sorbitol (D-glucitol) from maize seeds was confirmed by GC/MS of the TMSi-ether and by co-chromatography with authentic sorbitol. Sorbitol was found in seeds and silks but not in pollen or leaves. Both endosperm and embryo contained sorbitol, but endosperm accounted for most of the sorbitol recovered from intact seeds.

INTRODUCTION

Earlier reports in this series were concerned with levels of maize seed sugars as affected by genotype and stage of development [1–3]. One inbred, IL677a, was of special interest because its seeds contained more sugars (particularly sucrose and maltose) than other sugary (*su*) varieties included in the study. Gas chromatograph traces of IL677a seed extracts revealed a prominent compound that eluted near fructose when TMSi-oxime derivatives were chromatographed on 3% OV-17. The present study is concerned with this compound and its occurrence in various parts of the maize plant.

RESULTS

Sorbitol was identified as a constituent of mature, dry IL677a seeds. The GC retention time on OV-17 of the compound that eluted near fructose was about the same as that of authentic sorbitol, galactitol and L-iditol and was only slightly different from allitol and D-mannitol. This corn seed compound co-chromatographed with authentic TMSi-sorbitol when they were mixed together; the combined corn compound and standard produced a single sharp peak with a retention time of 6.21 min. The 150° temperature program was used, and the phenyl-β-D-glucopyranoside int. standard had a retention time of 15.2 min. The corn compound was purified by treatment with monobed ion-exchange resin, with 78% recovery under conditions that removed all detectable fructose and glucose with only 0.6% recovery of sucrose and 6% recovery of maltose. Combined GC/MS of the TMSi-derivatives gave a mass spectrum for this purified compound that was quite similar to that of the sorbitol standard. The results, expressed as *m/z* (rel. int. of seed compound followed by rel. int. of sorbitol standard), were 524 $[M-TMSiOH]^+$ (0.6, 0.5), 421 $[M-103-TMSiOH]^+$ (3.9, 4.8), 409 $[M-205]^+$ (1.6, 1.4), 319 $[M-205-TMSiOH]^+$ (71, 91), 307 $[M-307]^+$ (28, 32), 217 $[M-307-TMSiOH]^+$ (80, 72), 205 $[M-409]^+$ (100, 100). A computer program provided by the Varian Co. gave a good match (88%) between the two spectra. These results are due to cleavage among the various C–C bonds of sorbitol and loss of TMSiOH (*m/z* 90) [4, 5]. No fragments larger than *m/z* 524 were observed, in agreement with Petersson [4] who did not detect $[M]^+$ (*m/z* 614) or $[M-15]^+$. Unfortunately, various TMSi-hexitols have quite similar fragmentation patterns [4], so further characterization of the seed hexitol was needed.

The acetate of the seed compound co-chromatographed with authentic sorbitol acetate when the two were mixed and was completely separated from the other hexitol acetates tested (Table 1) when mixed with them. Hence, L-mannitol, D-iditol and L-talitol were also ruled out. Sorbitol dehydrogenase (EC 1.1.1.14) reacted with the purified seed hexitol and produced the expected amount of NADH (data not

Table 1. $R_{arabinitol}$ values of acetate derivatives of various hexitols*

Compound	$R_{arabinitol}$
Allitol	1.72
D-Mannitol	1.93
D-Talitol	1.97
Galactitol	2.15
Sorbitol	2.41
Corn hexitol	2.41
L-Iditol	2.90

*The derivatives were characterized by GC on 3% SP-2340 and retention times of the arabinitol acetate int. standard ranged from 5.81 to 5.94 min.

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shown), ruling out the possibility that L-glucitol could be present. Hence, nine of the 10 possible stereoisomeric hexitols [6] were eliminated, and the seed compound must be sorbitol (D-glucitol).

Information concerning the distribution of sorbitol in maize plants should aid in understanding its metabolic function, so various plant parts were analyzed. Sorbitol was found in endosperm, embryo and silk tissues but not in pollen or leaves, despite the substantial amounts of sugars present in extracts of the latter two tissues (Table 2). Sorbitol content increases several-fold during seed maturation if embryo and endosperm values of Table 2 are expressed on a per organ basis. The endosperm is so much larger than the embryo that the latter would contribute an insignificant proportion of the sorbitol when whole seeds are analysed.

Sorbitol was determined on mature, dry seeds of an *su* hybrid (Silver Queen) and 3 *su* inbreds in addition to IL677a. Sorbitol was detected in every seed sample analyzed. The results, with sorbitol content expressed as a percentage of the dry weight, were IL11a (0.16), IL14h (0.02), IL451b (0.03), Silver Queen (0.04) and IL677a (0.18). These results reveal great variation among the seed samples analyzed. Genetic differences among varieties are not necessarily responsible for all of the variation observed; environmental conditions during seed development may affect the sorbitol levels in mature seeds.

DISCUSSION

The presence of sorbitol in maize seeds is of interest, since important functions are ascribed to this compound in other plant species. Sorbitol is an early product of photosynthetic carbon fixation by green leaves of certain rosaceous species and is translocated to the developing fruits [7–10]. Additional roles as an osmoregulating agent [11] and protector against freezing damage [12] have been suggested and should not be overlooked in relation to corn seed enlargement and maturation.

Sorbitol probably is not a translocated form of carbon in maize, since it was not detected in green leaves (this study) and since labeled sucrose appears in pedicel tissue as a result of $^{14}CO_2$ fixation by the leaves, with labeled hexoses moving into cells of developing endosperm [13, 14]. Thus, sorbitol biosynthesis most likely occurs in certain non-photosynthetic tissues, the source of carbon being hexose units of translocated sucrose. Whether this hexitol is precursor for a significant proportion of seed starch and wall polysaccharide of seeds and silks remains to be established. Similar questions arise for the coconut

Table 2. Occurrence of sorbitol and sugars in various maize tissues

Tissue and inbred used	Sorbitol	Fructose	Glucose	Sucrose	Maltose
			(% fr. wt)		
1. Endosperm (IL677a)*					
21 days post-pollination	0.14	0.77	0.74	3.84	0.20
35 days post-pollination	0.23	0.44	0.53	1.85	1.08
2. Embryo(IL677a)*					
21 days post-pollination	0.03	0.24	0.23	1.26	0.02
35 days post-pollination	0.12	0.36	0.32	2.07	0.11
3. Pollen					
IL677a	ND†	0.35	0.35	0.67	0.05
IL14h	ND	0.98	0.91	2.02	0.36
4. Silk			(% dry wt)		
IL677a	0.23	6.43	9.99	2.33	ND
IL451b	1.12	7.95	13.93	2.99	ND
5. Leaf (harvested 9 a.m.)					
IL677a					
Inner whorl	ND	4.42	7.25	2.25	0.03
Outer leaves	ND	5.11	8.01	4.79	0.06
IL451b					
Inner whorl	ND	3.49	4.78	1.26	ND
Outer leaves	ND	2.56	3.49	2.74	0.02
6. Leaf (harvested 9 p.m.)					
IL677a					
Inner whorl	ND	3.13	4.91	10.48	0.10
Outer leaves	ND	3.70	8.00	7.71	0.07
IL451b					
Inner whorl	ND	2.01	2.59	7.68	0.09
Outer leaves	ND	2.55	4.51	7.39	0.09

*The mean fr. wt/endosperm was 178 mg at 21 days post-pollination and 328 mg at 35 days. The mean fr. wt/embryo was 9.1 mg at 21 days and 61 mg at 35 days.

†Not detectable.

where sorbitol has been found in the liquid endosperm [15].

Other compounds were not rigorously excluded in the several earlier reports of sorbitol in Gramineae: two concerned with immature barley seeds and one with corn, in addition to a reported trace of sorbitol in corn meal [16–19]. In particular, there was no mention of purification, determination of mass spectrum or chromatography using conditions known to separate the various hexitols. Hence, the present work establishes that this particular hexitol does occur in at least one species of Gramineae. Not all species of Rosaceae produce sorbitol [20], and similar variation may occur in Gramineae.

EXPERIMENTAL

Plant material. Mature seed of the various inbred maize varieties was produced at Urbana, IL, in 1977 and maintained in cold storage until use. Other plant materials were produced in the field in 1980 and 1981. Immature seed was harvested from self-pollinated plants and stored frozen as in earlier work [3]. Leaf samples from 7-week-old plants (harvested on a sunny day) were frozen and stored in the same manner, with the five outer leaves stored separately from the mostly non-photosynthetic inner whorl. Sugars were extracted from pollen and unpollinated silks immediately after these samples were collected and brought back from the field.

Chemicals. Most of the chemicals were from commercial sources. However, allitol was generously provided by Prof. T. Reichstein, University of Basel, and D-talitol was from Prof. H. G. Britton, University of London. L-Iditol was produced together with sorbitol in the laboratory by reduction of L-sorbose with $NaBH_4$.

Extraction and analysis of plant materials. Sugars were extracted with 80% EtOH, and the TMSi-oxime derivatives were determined by GC on 3% OV-17 as described earlier [3], except that dry seeds were ground to pass a 20-mesh screen prior to extraction. The standard temp. program consisted of 180° for 2 min, 10°/min to 275°. He carrier gas flow was 20 cm^3/min. A lower temp. program (150° for 2 min, 2°/min to 174°, then 10°/min to 275°; He carrier gas at 38 cm^3/min) was used when improved sepn of sorbitol from fructose was desired. Injector and FID heater temps. were 285° and 300°, respectively. Hexitol acetates were prepared by heating each hexitol sample in 25 μl Ac_2O according to ref. [21], except that *ca* 0.1 mg NaOAc catalyst was added. The coiled glass column (183 cm × 3 mm) contained 3% SP-2340 (Supelco, Inc., Bellefonte, PA). The He carrier gas was 20 cm^3/min, and the temp. program was 205° for 1 min, then 1°/min to 220°. Retention times of the various hexitol acetates were similar to those recently reported [22]. Combined GC/MS was carried out at an ionization potential of 70 eV and recorded with a Spectrosystem 100. The separator and source temps. were 200° and 250°, respectively.

A 1:1 (v/v) mixture of Dowex 50W-X8(H^+) and Dowex 1-X8(OH^-) was used to remove sugars from the corn seed hexitol [7]. The first preparation was purified at room temperature (*ca* 24°) by loading 0.5 ml of seed extract onto a glass column that contained 1 ml of the resin, eluting with 5 ml H_2O, evaporating to dryness at 40° under red. pres., and dissolving the sample in 0.5 ml H_2O. Similar procedures were used to purify a second sample, except that sugars were removed more thoroughly by incubating the sample with resin for 12 hr at 90°. Standard spectrophotometric procedures were used for the determination of sorbitol with sorbitol dehydrogenase [23], which was from Sigma.

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