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Water-Soluble Carbohydrates and Fructan Structure Patterns from *Agave* and *Dasylirion* Species

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Fructans, storage carbohydrates with β-fructofuranosyl linkages, are found in ~15% of higher plants. The metabolic flexibility of those molecules allows them easily to polymerize and depolymerize to soluble carbohydrates according to plant development stage and environmental conditions. In this work, water-soluble carbohydrates, including fructan structure patterns, were compared among *Agave* and *Dasylirion* species grown in different environmental regions in Mexico. Fructans were the main storage carbohydrate present in *Agave* stems, in addition to other carbohydrates related to its metabolism, whereas *Dasylirion* spp. presented a different carbohydrate distribution. A good correlation of water-soluble carbohydrate content with climatic conditions was observed. Fructans in *Agave* and *Dasylirion* genera were found in the form of polydisperse molecules, where structural heterogeneity in the same plant was evidenced by methylation linkage analysis and chromatographic methods. Fructans from the studied species were classified into three groups depending on DP and linkage-type abundance. These storage carbohydrates share structural characteristics with fructans in plants that belong to the Asparagales members. *Agave* and *Dasylirion* fructans can be categorized as graminans and branched neo-fructans, which we have termed agavins.

KEYWORDS: *Agave*; *Dasylirion*; fructans; branching; partially methylated alditol acetates; gas chromatography coupled to mass spectrometry

INTRODUCTION

In plants, ~15% of higher species contain fructans, which in some species constitute the only reserved carbohydrate. Fructans are oligomers or polymers with β-fructofuranosyl residues, commonly water-soluble and synthesized from sucrose accumulation in the vacuole (*1*). Since soluble sugars, such as sucrose, have been thought to influence some events during plant development and gene expression (*2*) and because fructans act as an extension of sucrose metabolism (*3*), many physiological implications and advantages with respect to the presence of fructans in plants have been suggested and demonstrated (*4*−*7*). Among many studies, it has been shown that fructan's functions are not limited to storage, since they are implicated in vegetative developmental processes and osmoregulation aspects (*8*); in addition, their cryoprotective role has been demonstrated in cereals like oat and wheat (*6*), and tolerance to drought has also been demonstrated mainly in grasses (*9*, *10*) and in transgenic plants of tobacco (*5*) and sugar beet (*11*).

According to the way that β-fructofuranosyl units are linked, five major types of fructans can be identified: (i) linear inulin with β(2-1)-fructofuranosyl linkages, widely described in Asteraceae, (ii) levan (or phlein) with β(2-6) linkages found in grasses like *Phleum pratense*, (iii) graminans, which are mixed fructans containing type i and ii linkages (generally, they are branched fructans like those found in wheat and some members of the order Asparagales), (iv) inulin neoserie, which contains a glucose moiety between two fructofuranosyl units extended by β(2-1) linkages, characterized in onion and asparagus, and (v) levan neoserie, formed by β(2-1)- and β(2-6)-linked fructofuranosyl units on either end of a central sucrose molecule, which has been reported in oat (*1*). Fructans are usually present in plants as a heterogeneous mixture with different degrees of polymerization (DP) and structures. The type of fructans found in plants, as either oligomeric or polymeric molecules, and the presence of a specific type of fructan have been found to be species specific and highly influenced by the environmental conditions and developmental stage of the plant (*12*, *13*).

Through linkage analysis, Sims et al. (*12*) and Sims (*13*) showed a relationship among fructan structures present in species belonging to the fructan-rich Asparagales order, which includes the Agavaceae and Nolinaceae families, with eight and four genera, respectively. The presence of fructans in *Agave* has been reported since 1888 (*14*), *Agave vera cruz* and *Agave americana* being the most studied species (*15*, *16*). Oligofructans were reported in these species, indicating the presence of inulin, graminan, and inulin neoseries fructan types. More recently, the molecular structure of *Agave tequilana* was reported, showing a complex and highly branched molecule with both β(2-1) and β(2-6) linkages in which the presence of both internal

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Table 1. Geoclimatic Characteristics of Sampling Regions Where *Agave* and *Dasylirion* Species Were Collected

region	Los Altos, Jalisco	Pénjamo, Guanajuato	Ures, Sonora	Matatlán, Oaxaca	SolaVega, Oaxaca	Mérida, Yucatán	Pegüis, Chihuahua
species	*A. tequilana*	*A. tequilana*	*A. angustifolia*	*A. angustifolia*	*A. potatorum* and *A. cantala*	*A. fourcroydes*	*Dasylirion* spp.
abbreviation	At-J	At-G	Aa-S	Aa-O	Ap-O, Ac-O	Af-Y	Dsp-C
north latitude	20° 32′	20° 26′	29° 26′	16° 52′	16° 30′	20° 58′	29° 30′
west longitude	103° 40′	101° 43′	110° 23′	96° 23′	97° 59′	89° 37′	104° 30′
meters above level sea	2000	1780	380	1740	1440	10	800
annual temperature (°C)	8–22	18–24	maximum of 43, minimum of 12	26–28	12–18	24–28	maximum of 43, minimum of –23
pluvial precipitation (mm)	705–870	700–800	<400	800–2000	600–1500	700–1110	100–300
climate	temperate, subtropic, rainy, summer	semiwarm, subhumid, rainy, summer	dry, very warm	warm, subhumid, rainy, summer	temperate, subhumid, rainy, summer	warm, subhumid, rainy, summer	very dry, semiwarm

and external glucose was demonstrated (*17*). In Mexico, the origin center of the *Agave* genus and endemic region for Nolinaceae as *Dasylirion* and *Nolina*, the majority of *Agave* species grow well in different and sometimes contrasting environmental atmospheres, whereas *Dasylirion* is confined mainly to the northern region where extreme climate prevails. The presence of fructans in these species is probably a decisive contributing factor for their ability to grow in dry environments. In an attempt to correlate possible different fructan structures with environmental characteristics, in this paper we report a structural comparison of fructans from a number of *Agave* and *Dasylirion* species grown in different regions of Mexico, in addition to the quantification of other water-soluble carbohydrates (WSC) related to fructan metabolism in their stems (or pines), which is the main storage carbohydrate organ in these plants.

MATERIALS AND METHODS

Standard Material. Sucrose was supplied by Sigma; 1-kestotriose, 1,1-kestotetrose, and 1,1,1-kestotetrose standards (inulin DP3, DP4, and DP5, respectively) were from Megazyme. Fructans from onion and dahlia bulbs were extracted and derivatized to PAAMs as described below.

Biological Material. Table 1 describes the different regions from which *Agave* and *Dasylirion* plants were collected as well as many geographic and climatic characteristics. Five different *Agave* species growing in diverse geoclimatic conditions were harvested from different cultivated plantations, whereas *Dasylirion* spp. was harvested in the wild. All *Agave* plants were 6 years old; at this age, most *Agave* plants have reached their maturity and their inflorescences start to emerge. On the other hand, the age of *Dasylirion* was unknown, although the presence of inflorescence indicated that plants were mature. All plants were collected during the spring season (2002). *Agave* and *Dasylirion* stems were pulverized with liquid nitrogen, freeze-dried, and stored in a desiccator until they were analyzed.

Determination of Water-Soluble Carbohydrate Amounts. One hundred milligrams of freeze-dried material was used to extract soluble carbohydrates with hot water by stirring for 15 min at 80 °C. Suspensions were filtered and diluted. Total soluble carbohydrates were determined by the phenol/sulfuric acid method (*18*) using fructose as a standard. Determination of the amounts of sucrose, D-fructose, and D-glucose were made by enzymatic analysis employing a commercial kit according to the supplier's instructions (Boehringer Mannheim, Mannheim, Germany). The presence and quantitation of fructans were assessed by the fructan assay procedure kit (Megazyme) following the manufacturer's instructions.

Fructan Extraction. *Agave* and *Dasylirion* fructans were extracted using the method of López et al. (*17*). In brief, 30 g of freeze-dried stem was treated with an 80% ethanolic solution followed by aqueous extractions. Soluble carbohydrates were deionized, and fructans were precipitated by addition of absolute ethanol. Fructan samples were freeze-dried and stored in a humidity-free container.

Thin-Layer Chromatography. One microliter of 10% fructan solutions was applied to silica gel TLC plates with aluminum support (10 cm × 10 cm, Aldrich). TLC plates were developed three times in a butanol/propanol/water system (3:12:4, v/v/v), and carbohydrate spots were visualized with aniline/diphenylamine/phosphoric acid reagent in acetone base using the method of Anderson et al. (*19*).

Glycosyl Linkage Analysis. Ten milligrams of *Agave* and *Dasylirion* fructans were dissolved in 500 μL of DMSO, stirred, and sonicated overnight or until complete dissolution. Derivatization to PAAMs was carried out using the method of Ciucanu and Kerek with some modifications (*20*). Methylation was carried out by subsequent additions of pulverized NaOH and CH_3I. Permethylated carbohydrates were extracted three times with chloroform, washed with water, and dried under a stream of nitrogen. Those derivatives were hydrolyzed under mild acid conditions with 0.5 M TFA at 90 °C for 1 h. Reduction was carried out with $NaBD_4$ dissolved in 1 M NH_4OH at 60 °C for 1 h. Excessive borate was destroyed with acetic acid, and the products were taken to complete dryness with repeated addition of 15% acetic acid in a methanolic solution. Acetylation was performed at 90 °C for 2 h using 500 μL of acetic anhydride and 250 μL of pyridine as a catalyst. The products were extracted with CH_2Cl_2; the organic phases were washed with water and dried under a stream of N_2. The derivatized fructans were dissolved in 4 mL of CH_2Cl_2. One microliter was injected in a split-less mode on a gas chromatograph (Hewlett-Packard 5890 series) and separated on a 30 m × 0.25 mm (inside diameter) × 0.25 μm HP5 column (Hewlett-Packard) with a GC initial temperature of 60 °C for 3 min followed by a temperature program: 4 °C/min until 160 °C for 1 min, 0.5 °C/min until 180 °C, and then 20 °C/min until 300 °C held for 10 min. The injector and detector temperatures were 300 °C. He was used as the carrier gas (2 mL/min), and the pressure was held at 5 psi. A mass spectrometer (Hewlett-Packard 5972 series) was used for the identification of compounds in the electron ionization mode. The ionization spectra of all compounds were compared with those from derivatized standards prepared in this work. The quantitation of derivatized monosaccharides was accomplished with a flame ionization detector using effective carbon response (ecr) by area correction (*21*). Data are the average of at least three independent determinations.

RESULTS AND DISCUSSION

Influence of Environment on Water-Soluble Carbohydrates. The carbohydrate content in Agavaceae and Nolinaceae plants is one of the most appreciated attributes that influence their commercial uses as fiber, sweeteners, and supplement ingredients. **Figure 1** shows the soluble carbohydrate profiles

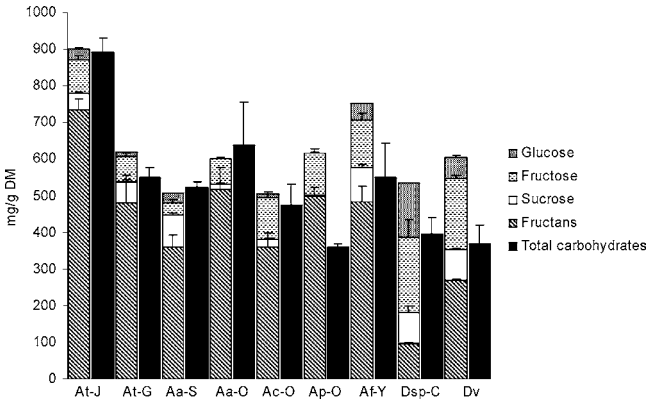

Figure 1. Soluble carbohydrate patterns of *Agave* and *Dasylirion* plants. Abbreviations are taken from **Table 1**. *Dahlia variabilis*, (Dv), was used as reference material. Bars represent the standard deviation of three determinations.

found in the studied *Agave* and *Dasylirion* species. The majority of these species exhibited a range of soluble carbohydrates in dry weight between 360 and 640 mg/g; these values indicate a high carbohydrate content compared to those of other fructan-rich crops such as dahlia (350 mg/g) determined in this study or those reported for chicory [240 mg/g (*22*)] or perennial ryegrass *Lolium perenne* [up to 370 mg/g (*23*)]. Discrepancies between total soluble carbohydrates and the sum total of glucose, fructose, and sucrose can be explained on the basis of the capability of each individual test to identify a specific analyte; therefore, this comparison can only be taken as a mere estimation. Although *A. tequilana* grown in Jalisco and Guanajuato belong to the same variety ("azul", blue variety), the WSC concentration differed significantly (900 and 550 mg/g, respectively). This behavior could be the result of environmental conditions, since plants in both locations are considered to be genetically identical due to their vegetative propagation (by rhizomes) (*24*). Both Jalisco and Guanajuato States are included in the origin denomination region for tequila elaboration, which is the main use for this kind of *Agave*. The high WSC concentration in *A. tequilana* from Jalisco agrees with reported conditions in Los Altos, Jalisco, where high sea level and fresh nocturnal temperatures favor uptake of CO_2 and, consequently, carbohydrate accumulation (*25*).

Fructans were the principal WSC in all *Agave* species, representing more than 60% of the total soluble carbohydrates. The highest fructan percent was found in *Agave angustifolia* var. Haw. from Oaxaca (85.81%) and the lowest percent in *Agave fourcroydes* (64.22%). The low value found for *A. fourcroydes* might be related to its popular use as a source for fiber production; however, recently, it has been used for alcoholic beverage elaboration, like other *Agaves*. Similarly, a low fructan concentration concomitant with a fibrous texture was also observed in *Dasylirion*, a plant that is used for both fiber and alcoholic beverage (sotol) production. *Dasylirion* presented the highest fructose (38.43%) and glucose (27.36%) levels of all studied species. Fructans and sucrose in this plant represent ∼18 and ∼16%, respectively.

The small amount of fructans found in *Dasylirion* might be further explained by the presence of the floral organ, since the fructan concentration is also affected by ontogenetic aspects (*26*). Thus, depolymerization and mobilization of fructans have been observed to cover energy-demanding activities such as regrowth in grasses after defoliation (*10*), during grain filling in cereals (*27*), sprouting in Asteraceae (*28*), and inflorescence development in alpine and daylily plants (*29*). The inflorescence

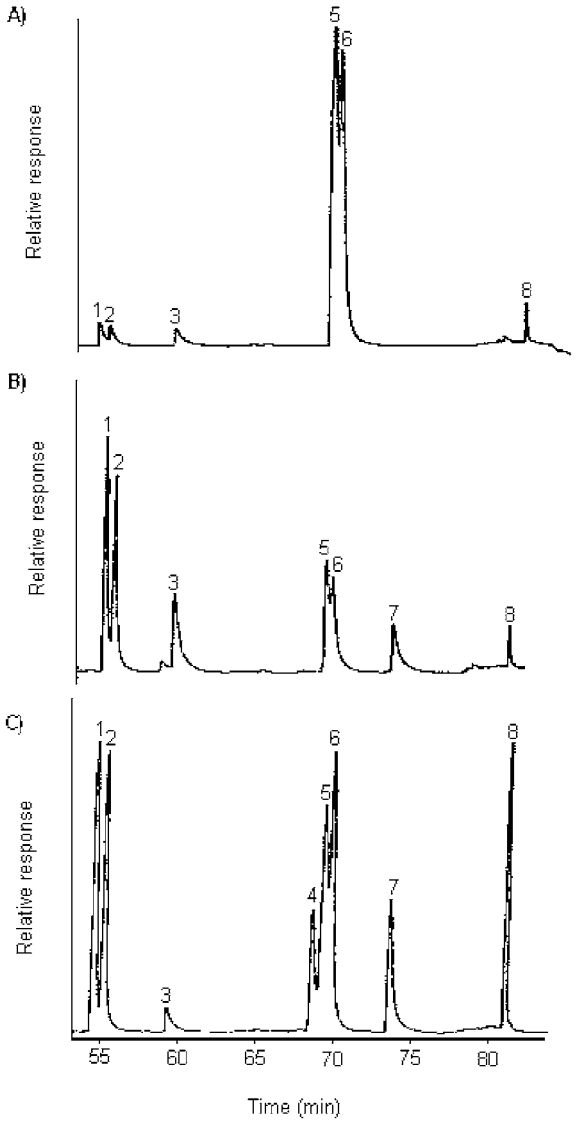

Figure 2. Chromatographic profile of derivatization products of fructans from (**A**) *D. variabilis* (dahlia), (**B**) *Allium cepa* (onion), and (**C**) *A. angustifolia* (from Sonora). Numbered peaks correspond to elution order, and they were identified as indicated in **Table 2**.

emergence in *Dasylirion* plants might have caused a drop in the fructan content to supply the energy required for this event, where high sucrose and monosaccharide concentrations might be important in keeping the osmotic potential necessary for turgor pressure.

Distribution of Water-Soluble Carbohydrates. Although all *Agave* species presented fructans as the most abundant WSC, an important difference among these species was the distribution of the others soluble carbohydrates: sucrose, fructose, and glucose. *Agave* species from Oaxaca (*A. angustifolia*, *Agave potatorum*, and *Agave cantala*), presented the same behavior: a very low sucrose concentration and a small glucose amount; these species also exhibited a high fructose content. The relation of high fructose concentration and an almost imperceptible glucose amount could reflect a physiologic state of active hydrolysis of fructans in the stems by fructan exohydrolase (FEH), a fructan-degradative enzyme that releases fructose moieties from the nonreducing ends. The highest fructose concentration in *Agaves* was observed in *A. fourcroydes*, but a considerable amount of glucose was also detected, indicating a possible physiologic difference between these species. Again,

Table 2. Partially Methylated Alditol Acetates Identified from Fructans from *Agave* and *Dasylirion* Species, *D. variabilis*, and *A. cepa*

peak[a]	t_R[b]	derivative compound	linkage type[c]	fragmentation pattern[d]
1	50.21	2,5-di-*O*-acetyl-(2-deuterio)-1,3,4,6-tetra-*O*-methyl-D-mannitol	*t*-β-D-Fru*f*	129 (100), 162 (46.6), 161 (30.0), 87 (25.0), 101 (15.8), 102 (15.0), 75 (11.7), 145 (8.3), 72 (8.3), 146 (6.8)
2	50.81	2,5-di-*O*-acetyl-(2-deuterio)-1,3,4,6-tetra-*O*-methyl-D-glucitol	*t*-β-D-Fru*f*	129 (100), 162 (38.9), 161 (34.7), 87 (24.5), 101 (15.2), 102 (14.4), 75 (10.1), 72 (10.1), 146 (5.8), 145 (5.08)
3	55.07	1,5-di-*O*-acetyl-(1-deuterio)-2,3,4,6-tetra-*O*-methylglucitol	*t*-α-D-Glc*p*	102 (100), 129 (62.0), 118 (55.7), 101 (52.4), 145 (40.0), 71, 72 (36.6), 87 (36.0), 162 (27.8), 161 (26.2), 205 (11.4)
4	63.71	2,5,6-tri-*O*-acetyl-(2-deuterio)-1,3,4-tri-*O*-methylmannitol	(2→6)-β-D-Fru*f*	129 (100), 162 (45.2), 87 (35.8), 99 (16.9), 189 (15.0), 71, 72, 102 (13.2), 75 (12.2), 60 (10.3)
5	64.36	1,2,5-tri-*O*-acetyl-(2-deuterio)-3,4,6-tri-*O*-methylmannitol	(2→1)-β-D-Fru*f*	129 (100), 87 (33.8), 161 (25.4), 190 (23.7), 101 (14.4), 100 (13.5), 71, 72 (10.1), 75 (8.47), 145 (6.7)
6	64.94	2,5,6-tri-*O*-acetyl-(2-deuterio)-1,3,4-tri-*O*-methylglucitol and 1,2,5-tri-*O*-acetyl-(2-deuterio)-3,4,6-tri-*O*-methylglucitol	(2→1)/(2→6)-β-D-Fru*f*	129 (100), 87 (30.3), 161 (29.4), 190 (14.1), 162 (11.6), 101 (10.7), 100 (8.9), 71, 72, 75, 118 (7.1), 189 (6.2),
7	68.67	1,5,6-tri-*O*-acetyl-(1-deuterio)-2,3,4-tri-*O*-methylglucitol	*i*-α-D-Glc*p*	102 (100), 118 (75.8), 129 (55.1), 87 (51.7), 101 (27.5), 162 (22.4), 71 (22), 189 (13.7), 145 (6.8), 233 (4.3)
8	78.14	1,2,5,6-tetra-*O*-acetyl-(2-deuterio)-3,4-di-*O*-methylhexitol	1,6-di-β-D-Fru*f*	129 (100), 87 (42.5), 190 (20.3), 189 (17.5), 100 (16.2), 99 (14.8), 60 (11.1), 71, 72 (7.4)

[a] Peak numbers correspond to the elution order shown in **Figure 2**. [b] Retention time (minutes) in the HP5 column. [c] *t*, terminal; *i*, internal. [d] Values in parentheses are the relative intensities of the fragments.

this might indicate an active FEH, in concert with an important invertase activity leading to hydrolysis of sucrose into glucose and fructose, and/or probably a decrease in the extent of glucose reincorporation in other metabolic pathways.

The high level of fructan accumulation in *Agave* stems contrasts with that of *Dasylirion*, which contains both fructans and sucrose in similar concentrations and in smaller proportions compared to fructose and glucose. These might suggest an adaptation of *Dasylirion* species to drier environmental conditions in addition to specific species and ontogenetic factors, since the accumulation of hexoses from fructan hydrolysis has also been observed in some grasses subjected to dry environments (*8*). On the other hand, differences found in WSC among *Agave* species suggest a metabolic flexibility that may enable the most suitable adaptation to varying environmental conditions, where water availability is one of the most limiting factors. The mechanism by which fructans confer protection to drought is not completely understood; different responses have been observed when plants are subjected to drought stress, but all of them implicate an adjustment either in fructan concentration or in its DP (*10*), indicating active participation of FEH and fructosyltransferases (FT), enzymes involved in fructan catabolism and anabolism, respectively.

TLC Fructan Profile. The ethanolic precipitation allowed the separation of fructans from monosaccharides, sucrose, and very short fructans that remained soluble. In general, low DP fructan fractions were poorly represented in the analyzed species; therefore, the main fructan fractions corresponded to molecules with higher DP. In all *Agave* species and *Dasylirion*, a spot was seen between sucrose and 1-kestotriose (DP3, inulin-type). In similar TLC systems, neofructan oligoseries (with an internal glucose moiety) have R_f values larger than that of the corresponding inulin series DP (*30*). Therefore, a spot observed with an R_f between those of sucrose and DP3 corresponds to 6G-kestotriose (DP3, neoinulin-type). In accordance with other Asparagales-like onion and asparagus, 6-kestotriose (DP3, levan-type) was not evident (*12*, *13*). Spots corresponding to DP4 and DP5 were also visualized, although they were less intense; it was difficult to establish if they either belong to inulin neoserie or represent a mixture of both types. The presence of at least two types of DP3 in *Agave* and *Dasylirion* plants might be indicative of two existing fructan types: inulin, as in Asteracae such as chicory, Jerusalem artichoke, and dahlia, and neoinulin, as in Asparagales-like onion, garlic, and asparagus.

A. tequilana (from Jalisco) exhibited the most different TLC pattern; this species contains almost exclusively monosaccharides (glucose and fructose), some sucrose (a very tenue spot), and only a light spot corresponding to 6G-kestotriose; fructooligosaccharides were absent, and fructan fractions consisted mainly of molecules with a high DP. In *Agave* species from Oaxaca and *A. fourcroydes* (from Yucatan), 1-kestotriose was detected in small amounts; this molecule was more evident in *A. angustifolia* (from Sonora) and very abundant in *A. tequilana* (from Guanajuato) and *Dasylirion* spp.

Identification of Glycosyl Derivatives. Precipitated *Agave* and *Dasylirion* fructan fractions were derivatized to establish the structural diversity among *Agave* and *Dasylirion* plants by methylation−acetylation analysis. **Figure 2** shows representative chromatograms for dahlia, onion, and *A. angustifolia* from Sonora. The derivatization products (PAAMs) of *A. angustifolia* were compared with those from well-studied dahlia (Asterales) and onion (Asparagales). Chromatographic profiles for all *Agave* and *Dasylirion* species indicated quantitative more than qualitative differences among them. The identity of each carbohydrate derivative was determined using criteria discussed by Carpita and Shea (*31*) and by comparison with standards and fragmentation patterns of spectra generated by electron-impact mass spectrometry reported in **Table 2**.

Dahlia contains fructan-type inulin, linear β(2-1) linkages with one glucose at the nonreducing end, and a very low percent of branched structures (**Figures 2A** and **3**). Reduced fructose, like other ketoses, produces mannitol and glucitol epimers. In the case of the terminal β-D-fructofuranose (*t*-β-D-Fru*f*), both epimers were resolved well in the column used and correspond

Table 3. Quantitative Contribution (percent molar) of Each Derivative in *Agave* and *Dasylirion* Fructans

	estimated DP[a]	α-D-Glc*p*	*i*-α-D-Glc*p*	*t*-β-D-Fru*f*	(2-6)-β-D-Fru*f*	(2–1)-β-D-Fru*f*	1,6-di-β-D-Fru*f*
group I							
At-J	18.12	0.20	0.79	4.70	3.46	5.53	3.42
Aa-S	13.07	0.18	0.82	4.51	1.90	3.92	1.74
Aa-O	31.75	0.21	0.79	10.51	6.01	9.64	4.59
Ap-O	15.34	0.17	0.83	5.19	2.12	4.84	2.19
group II							
Ac-O	11.17	0.33	0.67	4.27	0.95	3.71	1.24
Af-Y	6.66	0.31	0.69	2.81	0.49	1.82	0.55
Dsp-C	9.09	0.38	0.62	3.21	0.84	3.08	0.96
group III							
At-G	7.13	0.52	0.48	2.99	0.65	1.75	0.74
standard[b]							
Dv	37.43	1	nd	2.44	nd	33.17	0.82
Ac	4.79	0.66	0.34	2.38	nd	1.32	0.09

[a] The estimated DP of each species was based on the sum of the relative abundance of both terminal and internal α-D-glucopyranoside considered as a unit. [b] Derivatives were compared with those found in dahlia (Dv) and onion (Ac).

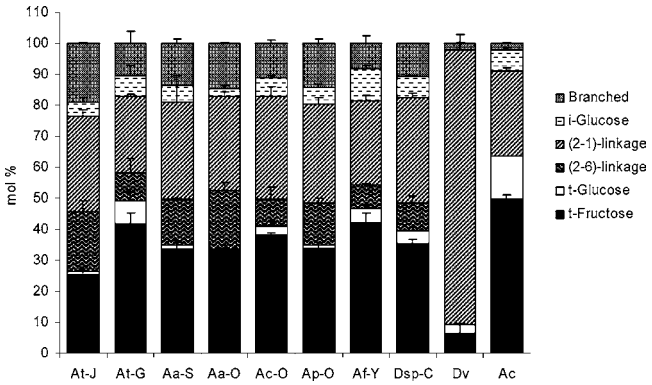

Figure 3. Glycosyl linkage composition in mole percentage of fructans from *Agave* and *Dasylirion* species, *D. variabilis* (Dv), and *A. cepa* (Ac). Abbreviations are taken from **Table 1**. Bars represent the standard deviation of three independent determinations.

to peaks 1 and 2 (**Figure 2**). These symmetrical molecules are characterized by the presence of a doublet at *m*/*z* 161 and 162 as primary fragments and doublets at *m*/*z* 205 and 206, *m*/*z* 145 and 146, and *m*/*z* 101 and 102 as secondary fragments. Peak 3 was assigned to the terminal α-D-glucopyranose (*t*-α-D-Glc*p*) with a base fragment at *m*/*z* 102, primary fragments at *m*/*z* 161 and 162 similar in intensity, and diagnostic fragments at *m*/*z* 118 and 205 from the less favored cleavage between C2 and C3 contiguous to methoxylated carbons. The major fructan contribution in dahlia and other Asterales corresponds to β(2-1)-fructofuranose (β2-1-D-Fru*f*), represented as peaks 5 and 6 in **Figure 2**, which correspond to mannitol and glucitol epimers, respectively. A significant amount of the 1,6-di-β-D-fructofuranose unit (1,6-di-β-D-Fru*f*) was identified in dahlia (peak 8); this derivative is produced from branched fructans reported previously in minor amounts in some Asterales such as chicory and dahlia (*32*). Mannitol and glucitol epimers derived from this moiety were not chromatographically resolved; therefore, the fragmentation pattern corresponds to the mixture of both configurations.

The chromatographic profile of PAAMs from onion (**Figure 2B**) shows a significant contribution of *t*-β-D-Fru*f* (peaks 1 and 2), indicating a shorter fructan chain [∼DP3−10 (*33*)]. The fragmentation pattern of an additional peak (7) indicates the presence of internal α-D-glucopyranose (*i*-α-D-Glc*p*), with a fragment at *m*/*z* 233, indicative of an additional acetyl group in the C6 position. This derivative was observed in all *Agave* species and *Dasylirion* spp. PAAM's chromatographic profile

Table 4. Ratio Correlation between Different Residues Present in Fructans from *Agave* and *Dasylirion* Species

	i-D-Glc/*t*-D-Glc	β(2-1)/β(2-6)	β(2-6)/1,6-di-Fru	*t*-Fru/1,6-di-Fru
group I				
At-J	3.95	1.60	1.01	1.37
Aa-S	4.56	2.06	1.09	2.59
Aa-O	3.76	1.60	1.31	2.29
Ap-O	4.88	2.28	0.97	2.37
group II				
Ac-O	2.03	3.91	0.77	3.44
Af-Y	2.23	3.71	0.89	5.11
Dsp-C	1.63	3.67	0.88	3.34
group III				
At-G	0.92	2.69	0.88	4.04

and indicates the presence of the neofructan type in these species, which has been reported as a characteristic of Alliaceae family members like onion, garlic, and asparagus.

A typical chromatogram for PAAMs from *Agave* and *Dasylirion* species is shown in **Figure 2C** (*A. angustifolia*, Sonora), and it is evident that fructans in these plants present a structural diversity compared to fructans in other crops. The peaks corresponding to both *t*- and *i*-α-D-Glc*p* are present, in addition to *t*-, β(2-1)-, and 1,6-di-β-D-Fru*f*, indicative of the presence of terminal-, β(2-1)-, and branched fructose linkages, respectively. However, an additional moiety was identified in the elution of peak 4. This corresponded to the mannitol configuration of 2-6-D-fructofuranose (β2-6-D-Fru*f*), characterized by a fragment at *m*/*z* 189 indicating that O6 must bear an acetyl substitution; therefore, in *Agave* and *Dasylirion* species, there are β(2-6) linkages. Reduction of methylated derivatives with deuterated borohydride introduces asymmetry into 2-1- and 2-6-linked fructofuranose that otherwise would yield identical fragments. In this way, the β(2-1)-fructofuranosyl linkage was differentiated with ions at *m*/*z* 190 and 161 as the major fragment, whereas the β(2-6) linkage generated fragments at *m*/*z* 189 and 162. Mannitol epimers of these compounds were chromatographically well-resolved in peaks 4 and 5; however, glucitol epimers were not (**Figure 2** and **Table 2**). Therefore, peak 6 in *Agave* chromatogram contains both glucitol configurations of β(2-1) and β(2-6) linkages (2,5,6-tri-*O*-acetyl-2-deuterio-1,3,4-tri-*O*-methylglucitol and 1,2,5-tri-*O*-acetyl-2-deuterio-3,4,6-tri-*O*-methylglucitol, respectively), and its fragmentation pattern resulted in all ions being present in both derivatives. For quantification, the amount of each derivative was determined as the *m*/*z* 189/*m*/*z* 190 ratio (resulting from the reduction with

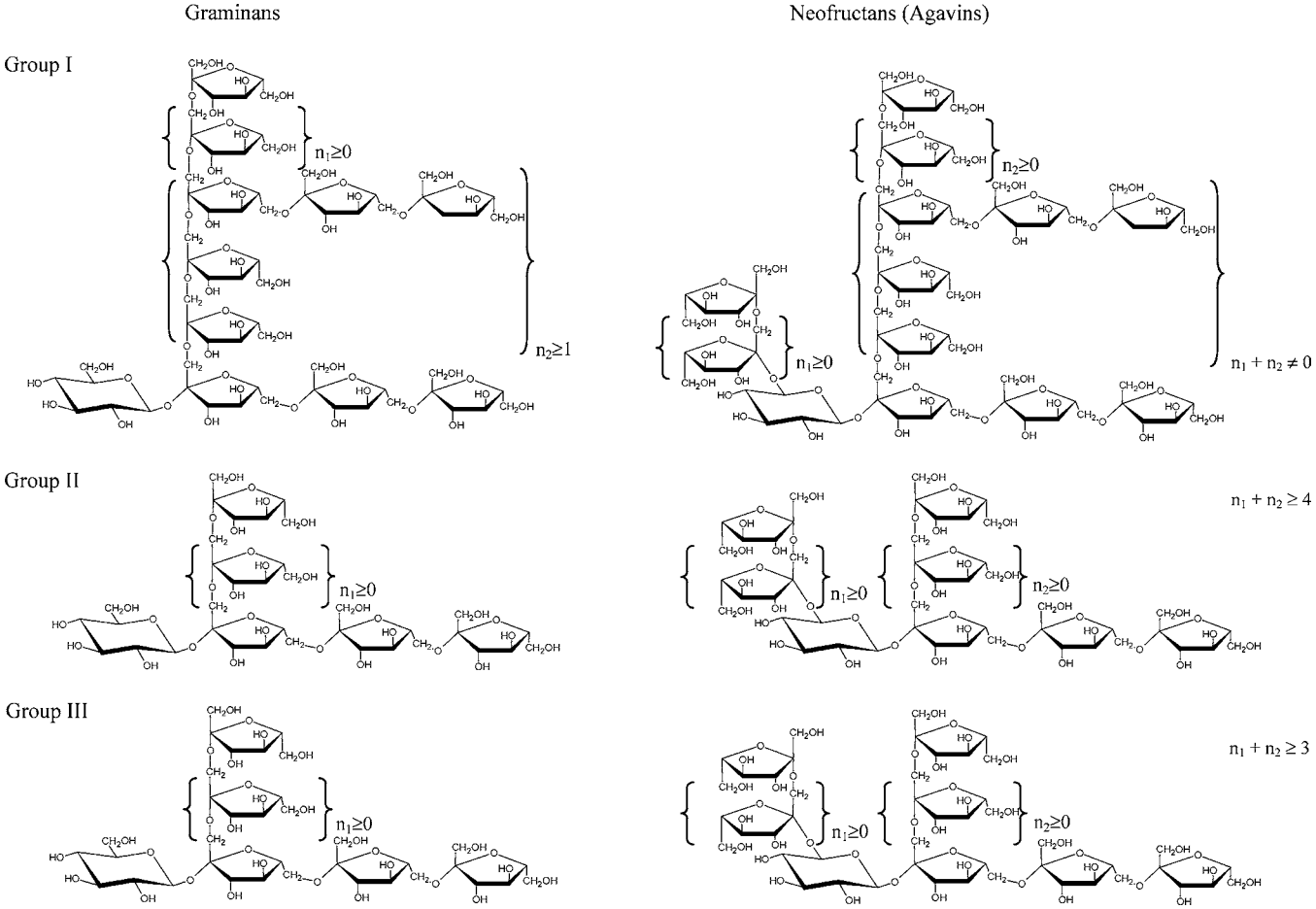

Figure 4. Proposed fructan structures for *Agave* and *Dasylirion* species. Molecular structures based on the three proposed groups, and two types of fructans within the groups (A for graminans and B for agavins).

$NaBD_4$), taking into account the natural abundance of ^{13}C (1.11%) (*32*).

Glycosyl Linkage Constituents from Fructan Structure. **Table 3** lists the quantitative contribution in percent molar of each derivative in the *Agave* and *Dasylirion* studied species and compared to the proportions found in dahlia and onion. A graphic way to see the most relevant structural differences among *Agave* and *Dasylirion* fructans can be observed in **Figure 3**. Although *Agave* and *Dasylirion* fructans are structurally similar, important differences in the contribution of each derivative among all the assayed samples were established; besides, interesting relationships were found which allowed grouping of *Agave* and *Dasylirion* plants into three groups (**Tables 3** and **4**). Group I includes *A. tequilana* (Jalisco), *A. angustifolia* (Sonora and Oaxaca), and *A. potatorum* (Oaxaca); group II is constituted by *A. cantala* (Oaxaca), *A. fourcroydes* (Yucatan), and *Dasylirion* spp. (Chihuahua), and the less similar fructan, *A. tequilana* (Guanajuato), is the only member of group III. Theoretically, fructans should contain, if any, only one moiety of glucose per molecule. According to this, and from the data collected in this work, it was deduced that at least two types of fructans are present in *Agave* and *Dasylirion* species: fructans with terminal glucose and neofructan series. It is also relevant to mention that the cores of these two types of structures, 1-kestose and neokestose, were observed via TLC. Neofructans were more abundant in species clustered in group I, having approximately four neofructan structures by each molecule with terminal glucose; in group II, the relation was 2:1, and in addition, *A. tequilana* (Guanajuato) presented an equal amount of fructan and neofructan types. A major contribution to β(2-1) linkages in relation to β(2-6) linkages was observed for all species, with ratio values of 2, 4, and 3 for groups I−III, respectively. Interestingly, a ratio of ∼1 was observed between the β(2-6) linkage and branched moieties for group I, and it was not significantly different from the value of 0.8 found for the rest of the species. Another important piece of data was the ratio between terminal fructose and branched moieties, which is correlated with the length chain and branching points, since in molecules with a high DP or molecules that are highly branched this ratio should be ∼1. In this context, group I presented the lowest value, with a ratio of 2 for all members except *A. tequilana* (Jalisco), which had a ratio of 1, indicating the presence of highly branched fructans. *A. cantala* and *Dasylirion* spp. presented a ratio of 3, while less branched structures were found in *A. tequilana* (Guanajuato) and *A. fourcroydes* with ratios of 4 and 5, respectively. To obtain an estimation of the average DP present in each species, the percent of both terminal and internal glucose was considered as the unity and the percent of each remaining moiety was compared to this value (**Table 3**). The values obtained for dahlia and onion validated this method, since the DP values calculated in this way were in the range of their previously reported DP (*33*, *34*). In this respect, plant species within group I present a high DP (from 13 to 32); meanwhile, the rest of the fructans (groups II and III) have lower DPs in their stems (from 7 to 11). There are not many reports about the DP range for *Agave* species. For *A. vera cruz*, a DP range of 3−32 was determined (*35*), while for *A. tequilana*, it varies from 3 to 29 (*17*). Values for all *Agave* and *Dasylirion* fructans studied here are within

reported ranges; however, it is broad and suggests the presence of heterogeneous polydisperse fructans in these species.

Likely Fructan Structures in *Agave* and *Dasylirion* Plants. Although from these new data it is difficult to elucidate molecular structures for *Agave* and *Dasylirion*, useful information about the predominant structural characteristics of fructans in these plants can be deduced. **Figure 4** shows the proposed general structures for the three groups of fructans in *Agave* and *Dasylirion* species. It also shows two types of molecules within each group (A for graminans and B for agavins), where $n_1-n_4 \geq 0$; n varies according to plant species and environmental conditions. One possible structure places *Agave* and *Dasylirion* fructans in the fructan group named graminans, since both β-fructofuranosyl linkages are present, in addition to branched fructofuranosyl moieties. The basic component for branched fructans is bifurcose, a DP4 branched molecule; therefore, from this structure, FT enzymes should catalyze the fructosyltransference during polymer formation. On the other hand, the second molecule type found in *Agave* and *Dasylirion* is characterized by internal α-D-Glc*p* in addition to branched linkages. Although this fructan type has not been molecularly characterized, we are calling it agavins. The closest fructan type previously reported would be the one for *Urginea maritima* (*36*).

The results found for *Agave* species and *Dasylirion* spp. indicate that WSCs seem to follow a defined pattern according to the environmental characteristics prevailing in the regions where they grow. This is supported by the fact that the WSC distribution was similar in *Agave* species from the same region (from Oaxaca), whereas they differed in the same species (*A. tequilana* and *A. angustifolia*) grown in different environments.

The fructan structural characteristics determined for these species coincided with those reported for other Asparagales members. In general, they could be characterized by the low representation of inulin compared with Asterales; in addition, 6-kestotriose (DP3, levan-type) was not evident, and consequently, levans, if they are, could be present in only extremely small amounts. On the other hand, in the order Asparagales, it is possible to distinguish the *Allium* genera with its predominant neoserie and linear fructans from other less taxonomically related genera like *Phormium*, *Cordyline*, *Urginea*, and *Agave* characterized by branched and graminan structures (*12*, *13*). In this work, some Asparagales such as *Agave* and *Dasylirion* species are categorized as branched graminans and agavins. Differences in the contribution of each kind of structure and chain length found among those genera may reflect differences attributed not only to the species but also to the physiological state of the plants and their adaptation capacity in different geoclimatic conditions.

ABBREVIATIONS USED

DMSO, dimethyl sulfoxide; DP, degree of polymerization; FT, fructosyltransferase; FEH, fructan exohydrolase; GC, gas chromatograph; PAAMs, partially methylated alditol acetates; TFA, trifluoroacetic acid; TLC, thin-layer chromatography; WSC, water-soluble carbohydrates.

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