

## Molecular Structures of Fructans from *Agave tequilana* Weber var. *azul*

MERCEDES G. LOPEZ,<sup>\*,†</sup> NORMA A. MANCILLA-MARGALLI,<sup>†</sup> AND  
 GUILLERMO MENDOZA-DÍAZ<sup>‡</sup>

Departamento de Biotecnología y Bioquímica, Centro de Investigación y de Estudios Avanzados del IPN, Unidad Irapuato, Apartado Postal 629, Irapuato, Gto., 36500 México, and Facultad de Química, Universidad de Guanajuato, Noria Alta s/n, Guanajuato, Gto., 36050 México

Agave plants utilize crassulacean acid metabolism (CAM) for CO<sub>2</sub> fixation. Fructans are the principal photosynthetic products generated by agave plants. These carbohydrates are fructose-bound polymers frequently with a single glucose moiety. *Agave tequilana* Weber var. *azul* is an economically important CAM species not only because it is the sole plant allowed for tequila production but because it is a potential source of prebiotics. Because of the large amounts of carbohydrates in *A. tequilana*, in this study the molecular structures of its fructans were determined by fructan derivatization for linkage analysis coupled with gas chromatography–mass spectrometry (GC–MS), nuclear magnetic resonance (NMR), and matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF-MS). Fructans were extracted from 8-year-old *A. tequilana* plants. The linkage types present in fructans from *A. tequilana* were determined by permethylation followed by reductive cleavage, acetylation, and finally GC-MS analysis. Analysis of the degree of polymerization (DP) estimated by <sup>1</sup>H NMR integration and <sup>13</sup>C NMR and confirmed by MALDI-TOF-MS showed a wide DP ranging from 3 to 29 units. All of the analyses performed demonstrated that fructans from *A. tequilana* consist of a complex mixture of fructooligosaccharides containing principally β(2 → 1) linkages, but also β(2 → 6) and branch moieties were observed. Finally, it can be stated that fructans from *A. tequilana* Weber var. *azul* are not an inulin type as previously thought.

**KEYWORDS:** *Agave tequilana*; fructans; linkage; permethylated alditol acetates; degree of polymerization (DP); matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF-MS)

### INTRODUCTION

Mexico has been considered the center of origin and biodiversity of the *Agave* genus due to the taxonomic diversity within its territory. Of the 310 species reported, about 272 can be found in this country (1). Members of the Agavaceae family are distributed within and well adapted to arid and semiarid regions. They have undergone some morphological and physiological adaptations to survive in these adverse conditions. A physiological plant adaptation is the use of crassulacean acid metabolism (CAM), which involves reduced transpirational water loss (2) by opening the stomata at night when the temperature is cooler and, hence, the evaporative demand decreases (3). The principal photosynthetic product of CAM in Agave plants has been reported to be fructan, a soluble fructose polymer with generally one glucose moiety per molecule (4, 5). Fructans in *Agave* species are synthesized and stored in the stems, and their main function in such CAM plants is as storage. They may also act as osmoprotectants during drought (6),

constituting another possible physiological adaptation to arid environments.

Fructan structures seem to be species dependent; in fact, Bonnett et al. (7) proposed the elucidation of fructan structures as a potential taxonomic marker in Poaceae. More recently, Sims et al. (8) reported the presence of a similar fructan structure in members of the Asparagales order, in which the Agavaceae family is included. However, in *Agave* species more than one fructan structure has been reported. Sánchez-Marroquín and Hope (4) and Bathia and Nandra (9) reported inulin, a fructan type with a β(2 → 1) linkage, as the principal storage carbohydrate in *Agave tequilana* and *Agave americana*, respectively. Meanwhile, reports (10–13) on *Agave vera cruz* showed the presence of a complex mixture of highly branched fructans with an internal glucose and containing both β(2 → 1) and β(2 → 6) linkages. More recently, Wang and Nobel (6) reported the presence of a DP5 in *Agave deserti*, primarily in the vascular tissue, where neokestose (DP3), a fructan with an internal glucose moiety, was the principal fructooligosaccharide.

Nuclear magnetic resonance (NMR) spectroscopy has been used extensively to identify fructan structures of plants (14). This analytical technique constitutes a tool that can provide

\* Corresponding author [telephone +52 (462) 623 9644; fax +52 (462) 624 5996; e-mail mlopez@ira.cinvestav.mx].

<sup>†</sup> Unidad Irapuato.

<sup>‡</sup> Universidad de Guanajuato.

insights into the degree of polymerization (DP) as well as the conformation of many molecules including macromolecules such as oligo- and polysaccharides.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of many oligosaccharides have previously been reported, including some fructans (14–19). Besides NMR, short-chain fructan structures also can be determined using gas chromatography coupled to mass spectrometry (GC-MS) upon derivatization of polar groups (20). Another excellent analytical tool is the use of matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF-MS) (21, 22).

*A. tequilana* Weber var. *azul* is an economically important crop because it is the only plant allowed for the production of tequila, which is at the present time one of the most consumed alcoholic beverages worldwide. *A. tequilana* is cultivated mainly in the state of Jalisco in Mexico and some other restricted regions of the country established as protected territories by the origin denomination of tequila (Official Norm NOM006SCF1 1993) (23, 24). Because of extensive evidence of the presence of fructans in *A. tequilana* and other *Agave* species and their relevance as the principal carbohydrate source for tequila production, it is highly important to be precise about their identity, DP, and concentration. Therefore, the principal aim of this work was to determine those parameters by powerful techniques such as GC-MS, NMR, and MALDI-TOF-MS.

## MATERIALS AND METHODS

**Plant Material.** Eight-year-old *A. tequilana* Weber var. *azul* plants were harvested in the zone of Los Altos, Jalisco, Mexico, in the summer of 2000. The leaves were cut off, keeping the stems and base leaves, a part usually called the “head” or “pine” due to its similarity to a pineapple fruit. The pines were milled and the pulp solid fraction was stored at  $-20\text{ }^\circ\text{C}$  until their analysis.

**Extraction and Purification of Agave Fructans.** Thirty grams of agave pine was extracted with 100 mL of 80% w/v ethanol and shaken for 1 h at  $75\text{ }^\circ\text{C}$ . The sample was filtered and re-extracted twice with 50 mL of water at  $70\text{ }^\circ\text{C}$  for 30 min. The supernatants were combined, reduced to 10 mL, and desalted by passing through an AG 50W-X4, 200–400 mesh, ion exchange column (Bio-Rad, Hercules, CA) adjusting to neutral pH. The eluent was passed through an activated charcoal column and concentrated by rotatory evaporation under reduced pressure. Precipitation of high-DP fructans was achieved by adding 30 mL of absolute ethanol. Samples were freeze-dried and stored in a humidity-free container. Separation of fructans by their DP was made with  $20 \times 20$  cm preparative silica gel TLC plates (Aldrich, Milwaukee, WI). Plates were developed with a solvent system of  $\text{PrOH}/\text{EtOAc}/\text{H}_2\text{O}$  (40:40:20 v/v) as the mobile phase. Plates were sprayed with diphenylamine-aniline-phosphoric acid (DPA) reagent (25) to distinguish between aldoses and ketoses. Long-chain fructans were recovered from the TLC, re-extracted with water, and freeze-dried.

**Fructan Derivatization.** One hundred micrograms of fructans from an agave pine was dissolved in 100  $\mu\text{L}$  of dry  $\text{Me}_2\text{SO}$ , purged with dry nitrogen, and stirred overnight. After this time, 100  $\mu\text{L}$  of 1 M solution of KH in DMSO and 50  $\mu\text{L}$  of  $\text{CH}_3\text{I}$  were added to the sample and stirred again for 8 h. The permethylated sample was hydrolyzed first by mild acid hydrolysis with 0.5 M TFA at  $60\text{ }^\circ\text{C}$  for 1 h (26). The sample was passed through a C18 Sep-Pak cartridge and recovered with 8 mL of acetonitrile. One hundred microliters of TFA was added and heated for 2 h at  $120\text{ }^\circ\text{C}$ . A reductive cleavage of the partially methylated carbohydrates was performed by mild alkaline borodeuteride treatment in 1 M ammonium hydroxide followed by acetylation with 250  $\mu\text{L}$  of acetic anhydride.

**GC-MS of Permethylated Alditol Acetates (PMAA).** The derivatized carbohydrates were separated and identified by GC-MS. Samples were dissolved in 100  $\mu\text{L}$  of  $\text{CH}_2\text{Cl}_2$ , and 1  $\mu\text{L}$  was injected into the GC-MS. Derivatized monosaccharides were separated on a  $30\text{ m} \times 0.25\text{ mm i.d.} \times 0.25\text{ }\mu\text{m}$  SP-2330 column (Supelco, Bellefonte, PA), using helium as the carrier gas at 2.5 mL/min. The oven temperature was  $80\text{ }^\circ\text{C}$  for 2 min and then ramped at a rate of  $30\text{ }^\circ\text{C}/\text{min}$  to  $170\text{ }^\circ\text{C}$

and then at  $4\text{ }^\circ\text{C}/\text{min}$  to  $240\text{ }^\circ\text{C}$  and held for 10 min. Injector and detector temperatures were  $300\text{ }^\circ\text{C}$ , and column head pressure was kept at 5 psi.

**$^1\text{H}$  NMR Spectroscopy.** Five milligrams of agave fructan was dissolved in 500  $\mu\text{L}$  of deuterated water ( $\text{D}_2\text{O}$ ).  $^1\text{H}$  NMR spectra were generated with a Varian 300 MHz apparatus in a Fourier transformed mode, using 32 scans. The sample was analyzed at  $30\text{ }^\circ\text{C}$  with an acquisition time of 3.5 s with a  $90^\circ$  pulse.

**$^{13}\text{C}$  NMR Spectroscopy.** Five milligrams of agave fructan was dissolved in 500  $\mu\text{L}$  of deuterated water ( $\text{D}_2\text{O}$ ).  $^{13}\text{C}$  NMR spectra were recorded with a Varian 300 MHz, using 32 scans. The sample was analyzed at  $30\text{ }^\circ\text{C}$  with an acquisition time of 3.5 s with a  $90^\circ$  pulse. Spectra were analyzed for determination of primary, secondary, and tertiary carbons in the structure. Carbon signals were assigned by comparison with the chemical shifts of sucrose (DP2), 1-kestotriose (DP3), 1,1-kestotetraose (DP4), and 1,1,1-kestopentaose (DP5) used as reference materials. All of them are fructan-type inulin with  $\beta(2 \rightarrow 1)$ -linkages; all materials were purchased from Megazyme International Ireland except for sucrose (Sigma).  $\text{Me}_4\text{Si}$  was used as external reference for resonance measurements.

**MALDI-TOF-MS Analysis.** MALDI-TOF-MS measurement was performed using a Hewlett-Packard (Cupertino, CA) LDI AOOXP MS in the positive ion mode. The instrument was operated at an accelerating voltage of 30 kV and an extractor voltage of 9 kV. The pressure was  $\sim 2.1 \times 10^{-6}$  Torr. The sample was ionized with a nitrogen laser ( $\lambda = 337\text{ nm}$ ) with a pulse width of 3 ns and a 4–7.5 J pulse. The sample was dissolved in water, and the matrix was 2,5-dihydroxybenzoic acid; sample mixtures from 1 to 2  $\mu\text{L}$  were applied into the probe and quickly dried under vacuum. The sample solution was serially dried with matrix to obtain optimal sensitivity. A mixture of maltooligosaccharides was used as the calibration standard.

## RESULTS AND DISCUSSION

**Linkage Types.** The results of the permethylation analysis of agave fructans provided highly valuable information on the real molecular structure present in *A. tequilana* Weber var. *azul*. **Figure 1** shows a typical GC run of the reductive cleavage of agave fructan; a good separation of all permethylated alditol acetates was obtained from the agave fructan. **Table 1** lists all of the PMAA found. 2,5-Anhydro-1,3,4,6-tetra-*O*-methyl-D-mannitol (**1**) and 2,5-anhydro-1,3,4,6-tetra-*O*-methyl-D-glucitol (**2**) are the products of a terminal  $\beta$ -D-Fruf. 1,5-Anhydro-2,3,4,6-tetra-*O*-methyl-D-glucitol (**3**) resulted from the presence of a terminal  $\alpha$ -D-Glcp. The existence of both (2  $\rightarrow$  1) linked  $\beta$ -D-Fruf and (2  $\rightarrow$  6) linked  $\beta$ -D-Fruf in the fructan molecule is justified by the presence of 1-*O*-acetyl-2,5-anhydro-3,4,6-tri-*O*-methyl-D-mannitol (**4**), 6-*O*-acetyl-2,5-anhydro-1,3,4-tri-*O*-methyl-D-mannitol (**5**), and 1-*O*-acetyl-2,5-anhydro-3,4,6-tri-*O*-methyl-D-glucitol (**6**). The presence of internal glucose is confirmed by 6-*O*-acetyl-1,5-anhydro-2,3,4-tri-*O*-methyl-D-glucitol (**7**), and finally 1,6-di-*O*-acetyl-2,5-anhydro-3,4-di-*O*-methyl-D-mannitol (**8**) and 1,6-di-*O*-acetyl-2,5-anhydro-3,4-di-*O*-methyl-D-glucitol (**9**) compounds are well-known as 1,6-di- $\beta$ -D-Fruf. Quantitative data were not obtained from this determination; however, the characterization of all of these carbohydrates helped in the elucidation of the linkage types present in the fructans. Therefore, the fructose/glucose ratio was not established. Nevertheless, an estimated ratio can be determined by the NMR information. Spies et al. (27) reported the structure of the fructan sinistrin from *Urginea maritima*, the molecular structure of which was 30 units long with both (2  $\rightarrow$  1)  $\beta$ -D-Fruf and (2  $\rightarrow$  6)  $\beta$ -D-Fruf linkages as well as a  $\beta$ -D-Fruf residue linked to an  $\alpha$ -D-Glcp as an internal moiety. Praznik (personal communication) has found that preliminary studies on agave fructan showed a structure similar to that of sinistrin.

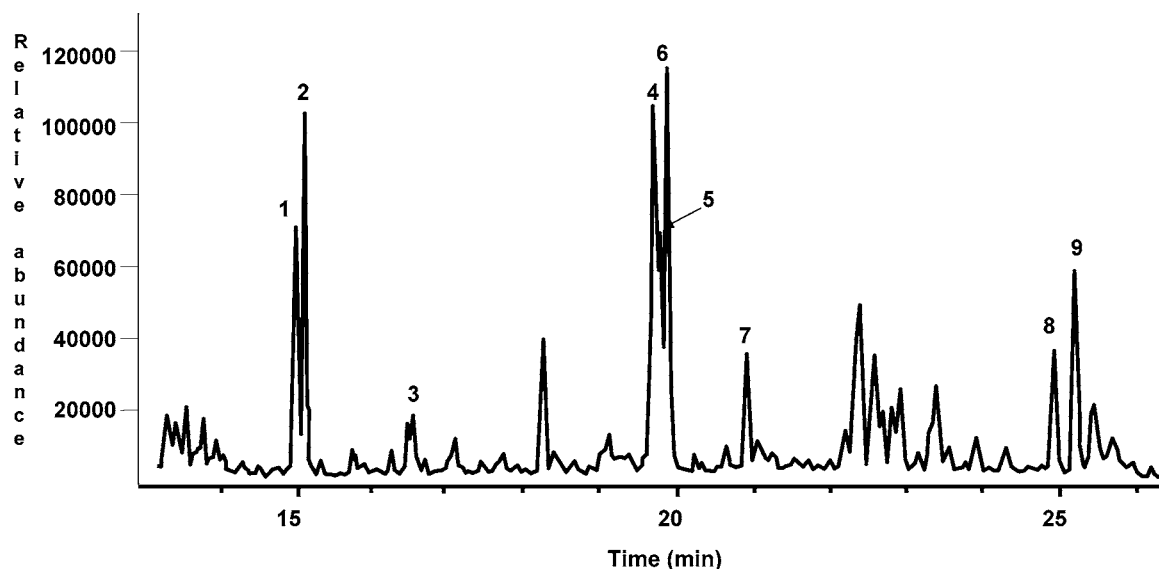


Figure 1. GC profile of the derivatization of fructan from *A. tequilana* Weber var. *azul*. See corresponding numbers in text and Table 1.

Table 1. Identified Permethyated Alditol Acetates<sup>a</sup> from the Derivatization of Fructan from *A. tequilana* Weber var. *azul*

<i>t<sub>R</sub></i> <sup>b</sup>	compound	linkage type
14.88	(1) 2,5-anhydro-1,3,4,6-tetra- <i>O</i> -methyl- <i>D</i> -mannitol	<i>l</i> - $\beta$ - <i>D</i> -Fruf
15.03	(2) 2,5-anhydro-1,3,4,6-tetra- <i>O</i> -methyl- <i>D</i> -glucitol	<i>t</i> - $\beta$ - <i>D</i> -Fruf
16.45	(3) 1,5-anhydro-2,3,4,6-tetra- <i>O</i> -methyl- <i>D</i> -glucitol	<i>t</i> - $\alpha$ - <i>D</i> -Glc <sub>p</sub>
19.62	(4) 1- <i>O</i> -acetyl-2,5-anhydro-3,4,6-tri- <i>O</i> -methyl- <i>D</i> -mannitol	(2 $\rightarrow$ 1) $\beta$ - <i>D</i> -Fruf
19.69	(5) 6- <i>O</i> -acetyl-2,5-anhydro-1,3,4-tri- <i>O</i> -methyl- <i>D</i> -mannitol	(2 $\rightarrow$ 6) $\beta$ - <i>D</i> -Fruf
19.80	(6) 1- <i>O</i> -acetyl-2,5-anhydro-3,4,6-tri- <i>O</i> -methyl- <i>D</i> -glucitol	(2 $\rightarrow$ 1) $\beta$ - <i>D</i> -Fruf
20.83	(7) 6- <i>O</i> -acetyl-1,5-anhydro-2,3,4-tri- <i>O</i> -methyl- <i>D</i> -glucitol	$\beta$ - $\alpha$ - <i>D</i> -Glc <sub>p</sub>
24.87	(8) 1,6-di- <i>O</i> -acetyl-2,5-anhydro-3,4-di- <i>O</i> -methyl- <i>D</i> -mannitol	1,6-di- $\beta$ - <i>D</i> -Fruf
25.13	(9) 1,6-di- <i>O</i> -acetyl-2,5-anhydro-3,4-di- <i>O</i> -methyl- <i>D</i> -glucitol	1,6-di- $\beta$ - <i>D</i> -Fruf

<sup>a</sup> GC elution order. <sup>b</sup> Retention time. <sup>c</sup> *t*, terminal. <sup>d</sup> *i*, internal.

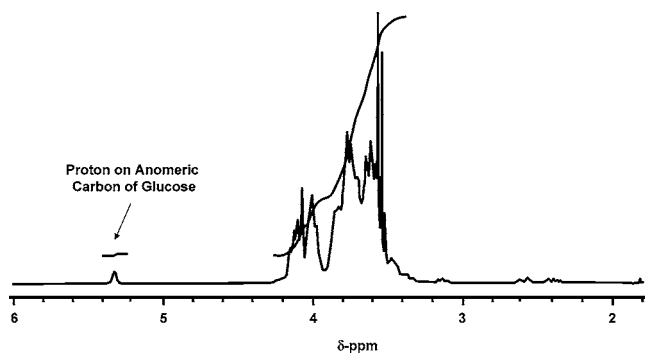


Figure 2. Proton NMR spectra of fructan from *A. tequilana*.

**<sup>1</sup>H NMR.** Proton NMR of fructans has been less studied than <sup>13</sup>C NMR, resulting in very few reports on oligofructans being available (14, 19, 29). Figure 2 presents the <sup>1</sup>H NMR spectrum of agave fructan. Reference compounds of the kestose and nystose type show chemical shifts for H-1 of the  $\alpha$ -*D*-Glc<sub>p</sub> residue ranging from  $\delta$  5.15 to 5.44 (14, 19, 29). The agave fructan had a shift of  $\delta$  5.23. According to these data, the resonance of H-1 from agave fructan seems to agree very well with the chemical shift of an internal glucose. The rest of the proton signals have been reported to appear in a narrow region between  $\delta$  3.25 and 4.28. Proton assignments are very complex; however, their integration provides relevant information on the length of a polymer. On the basis of the proton integration of agave fructan (Figure 2), it can be established that *A. tequilana*

fructan is constituted of at least 16 residues; therefore, the glucose/fructose ratio is 1–15 at least.

**<sup>13</sup>C NMR.** The <sup>13</sup>C NMR spectrum of *A. tequilana* fructan is shown in Figure 3. The spectra of sucrose (DP2), 1-kestose (DP3), nystose (DP4), and 1,1,1-kestopentaose (DP5) are shown in the same figure. These molecules were used as reference materials, all of which contain  $\beta$ (2  $\rightarrow$  1) linkages and are considered to be the smallest possible fructans and the structural building blocks of larger inulins (oligo- and polyfructans). The agave fructan carbon signals were assigned by comparison to reference materials and chemical shifts of other fructans previously reported (8, 14–19, 27–29).

The signals from 104.0 to 104.9 ppm correspond to the anomeric region of the C-2 of  $\beta$ -*D*-Fruf residues. The agave fructan spectrum shows four signals in this region ( $\delta$  104.87, 104.68, 104.54, and 104.06), indicating the presence of at least four different  $\beta$ -*D*-Fruf units. Spies et al. (27) reported a chemical shift at 104.88 ppm due to a C-2 of an internal  $\beta$ -*D*-Fruf unit with (2  $\rightarrow$  6) linkage for sinistrin, a special fructan type present in *U. maritima*. In another study, Sims et al. (8) reported chemical shifts of 104.7 and 104.9 ppm for C-2 of  $\beta$ -*D*-Fruf with (2  $\rightarrow$  1) linkage from two *Phormium* species; therefore, the chemical shift at 104.87 ppm in agave fructan could be assigned to either of these two linkage types. However, the existence of (2  $\rightarrow$  6) linkage is confirmed with a signal at 81.12 ppm due to C-5 of a  $\beta$ -*D*-Fruf unit substituted at O-6 (15) and another at  $\delta$  64.11 (C-6) characteristic of a  $\beta$ (2  $\rightarrow$  6) link of *D*-Fruf moieties (8); this same signal is observed in the agave fructan spectrum. The resonance at 104.51 ppm has been assigned to an internal C-2 of  $\beta$ -*D*-Fruf of 1-kestose, the elongation of this type of inulin, and has been related to an upfield shift of  $\sim$ 0.1 ppm (19).

The carbon signal of agave fructan at 104.54 ppm was assigned to an internal (2  $\rightarrow$  1)  $\beta$ -*D*-Fruf unit, which is the most intense signal in the anomeric region. This is an indication of the predominance of this type of linkage in the fructan molecule from *A. tequilana*. It is noteworthy that the signals are absent in the regions from 105.0 to 105.6 and from 103.24 to 103.35,

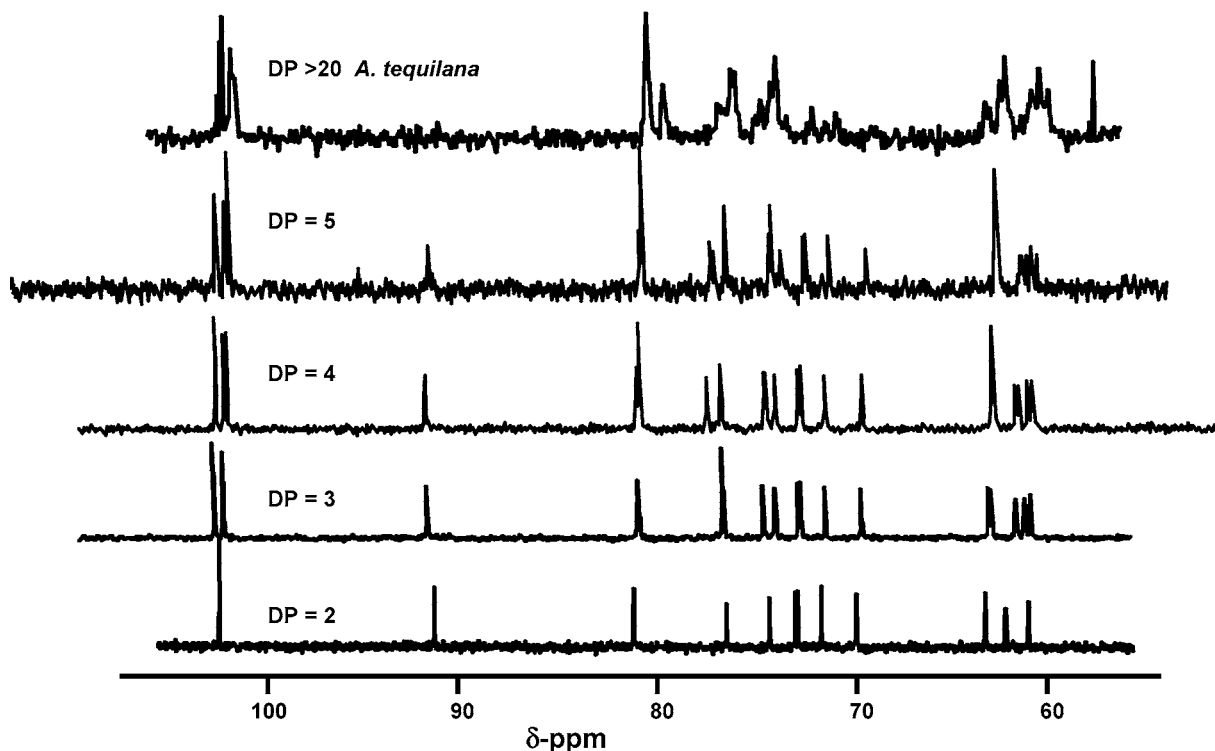


Figure 3. Carbon-13 NMR spectra of sucrose (DP2), 1-kestose (DP3), nystose (DP4), 1,1,1-kestopentaose (DP5), and fructan from *A. tequilana*.

which have been related to C-2 of a 1,2,6-tri- and 2,6-di-*O*-substituted Fruf unit, and C-2 of a  $\beta(2 \rightarrow 6)$  D-Fruf unit of a neokestose, respectively (13, 14). The lack of signals at  $\delta$  102.3 and 98.9 for C-2 indicates the absence of  $\beta$ -D-Fruf as a reducing moiety (28).

The  $^{13}\text{C}$  NMR spectrum of sucrose in Figure 3 shows an anomeric region with a signal at 93.03 ppm due to C-1 of an  $\alpha$ -D-Glcp moiety. This signal is weaker than the anomeric C-2 of  $\beta$ -D-Fruf ( $\delta$  104.53) in a relative ratio of 1:1. This ratio is observed in all reference materials, and it changes quite drastically once an overlapping of many ketoanomeric signals takes place. The region from  $\delta$  60.77 to 64.11 comprises signals due to C-1 and C-6 of  $\beta$ -D-Fruf residues that sometimes are overlapped. The C-1 signal from the agave fructan spectrum ( $\delta$  60.77) is attributed to the presence of a  $\beta(2 \rightarrow 6)$  linkage because a value of 60.88 ppm has been reported for  $\beta$ -D-Fruf in 6-kestose (14) and an upfield shift of 0.11 ppm can be related to a polymerization phenomenon. A resonance at  $\delta$  61.2 is typical of a levan molecule, a polyfructan with a  $\beta(2 \rightarrow 6)$  linkage (29), so that it might be due to internal  $\beta(2 \rightarrow 6)$ -D-Fruf residues. Similarly, the resonance at  $\delta$  61.67 has been assigned for a terminal Fruf residue. On the other hand, De Bruyn and Van Loo (28) reported that the resonance at  $\delta$  63.10 is due to a Fruf residue linked in a  $\beta(2 \rightarrow 6)$  form with an internal glucose in a neokestose-type molecule. Shiomi and Onodera (17) attributed this signal to an internal Fruf residue in an inulin molecule. Due to the strong signal in the agave fructan spectrum and the absence of an anomeric signal of Fruf linked to an internal glucose, the  $\delta$  63.10 resonance in the agave fructan might be attributed to the presence of an internal  $\beta(2 \rightarrow 1)$  Fruf residue. This assignment is confirmed by the presence of a second signal at  $\delta$  63.37 (17, 19). The resonance downfield at  $\delta$  64.00 is assigned to a  $\beta(2 \rightarrow 6)$  linkage or branched  $\beta$ -D-Fruf residues (18, 27); thus,  $\delta$  64.11 in the agave fructan spectrum could be due to either  $\beta$ -D-Fruf moiety. Another significant region for fructan structural elucidation is located

from  $\delta$  79.80 to 83.90, where C-5 signals are found. The agave fructan presents two strong signals, one at  $\delta$  83.90 and other at 81.12 ppm. According to Goto et al. (29) the former could be attributed to  $\beta(2 \rightarrow 1)$  linkages and the latter to a  $\beta(2 \rightarrow 6)$  linkage. However, these resonances could also be due to a C-5 of  $\beta$ -D-Fruf residues linked through O-6 and a residue lacking a substituent at O-6, respectively (8, 15). A difference of 0.9 ppm between both of them agrees with a previous assignment (8).

Sims et al. (8) demonstrated a structural similarity among *Phormium* species to that previously reported in *A. vera cruz* and *Cordyline australis*, all of them belonging to the Asparagales order. All fructans from these species are based on 1-kestose, neokestose, and a branched tetrasaccharide. This suggests that fructan from *A. tequilana* might be of a similar structure because structural variation of fructan seems to be species specific.

The MALDI-TOF-MS of the resolved sodium adduct peaks of *A. tequilana* fructans is shown in Figure 4. It can clearly be seen that the extract displayed a complex mixture of fructan molecules; this mixture presented a molecular weight distribution of 527–4739 Da, which corresponds to a range of DP from 3 to 29. In the same figure, it can also be observed that there is a decreasing trend in the amount of each fructan as the molecular weight increases. Among all of the different analytical measurements performed with agave fructans, MALDI-TOF-MS proved to be the best choice to establish the DP distribution of these types of carbohydrates. A molecule structure for the fructans in *A. tequilana* Weber var. *azul* is proposed here for the first time (Figure 5). This molecule presents the three well-known fructan-based types: inulin, levan, and neoinulin. The presence of different  $\beta$ -D-Fruf moieties, including (2  $\rightarrow$  1), (2  $\rightarrow$  6), and branched linkages, the former being the most abundant, as well as NMR data allowed the establishment of the fructan type

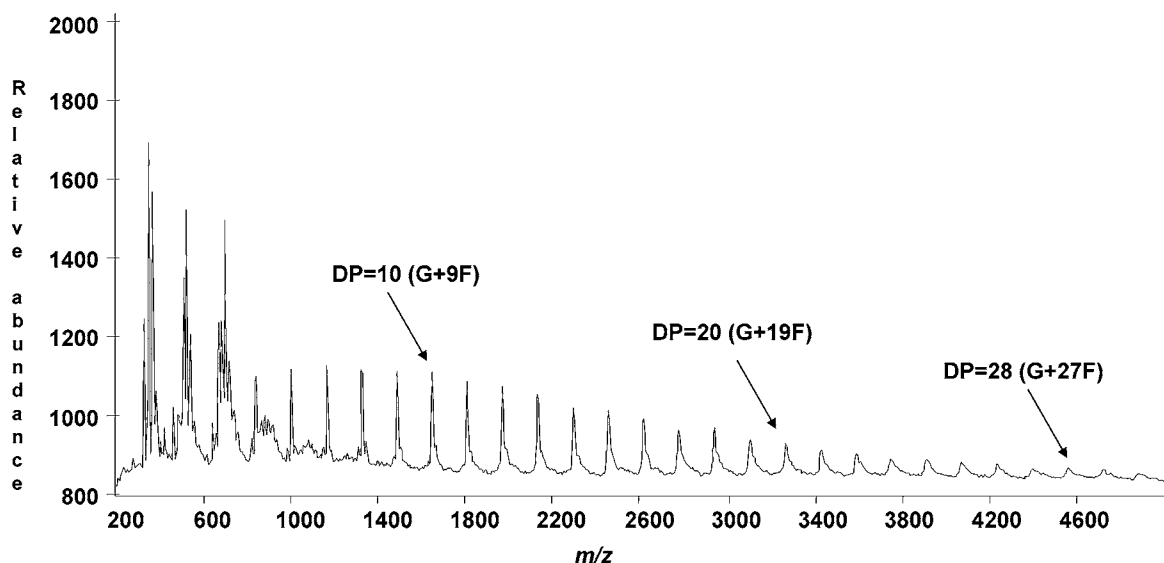


Figure 4. Positive ion MALDI-TOF-MS mass spectrum of fructan from *A. tequilana* Weber var. *azul* recorded with 2,5-dihydroxybenzoic acid as the matrix. Numbers in parentheses are the number of fructose units in each fructooligosaccharide.

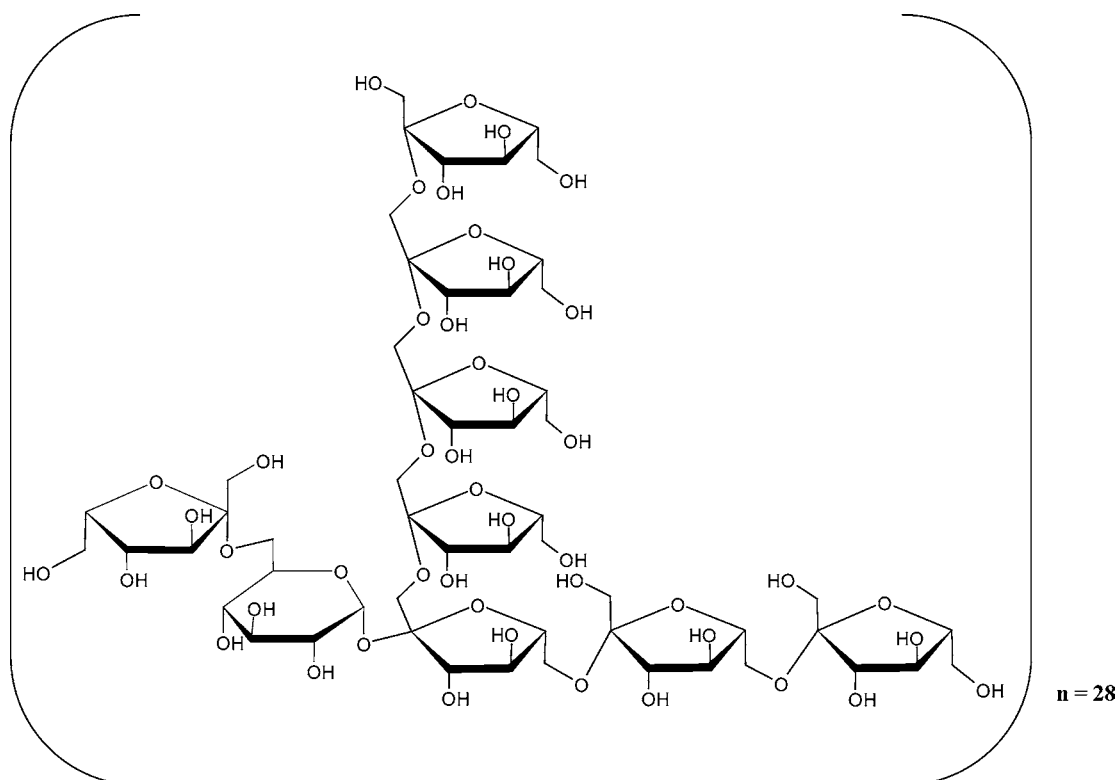


Figure 5. Proposed fructan structure for an 8-year-old *A. tequilana* Weber var. *azul*.

present in *A. tequilana*. These linkage types are characteristic of species included in the Asparagales order to which *A. tequilana* belongs. Finally, it is relevant to mention that the physiological implications of fructan metabolism in agave plants need to be studied carefully, because they could point to many other relevant roles such as drought resistance under the adverse condition where most agave plants grow.

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