A proposed chemical structure for fructans from blue agave plant(*Tequilana Weber var. azul)*
Una estructura quimica propuesta para las fructanas de la planta agave azul (*Tequilana Weber var. Azul)*

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Recibido: diciembre 3, 2007 / Aceptado: diciembre 6, 2004 / Publicado: diciembre 13, 2007

ABSTRACT. A chemical structure for agave Tequilana fructans is proposed based upon the combined results of permethylation and reductive cleavage techniques coupled to gas-liquid chromatography (GLC), as well as size exclusión chromatography /lightscattering.

Key words: Inulin, fructans, agave Tequilana, Chemical Structure, Permethylation and Reductive Cleavage, SEC/LS.

RESUMEN. Se propone una estructura química para las fructanas de agave *Tequilana* con base en los resultados combinados de las técnicas de permetilación y ruptura reductiva acopladas a cromatografía gas-líquido asi como también a cromatografía de exclusión de tamaños/dispersión de luz.

Palabras clave: Inulin/Fructanas, Agave Tequilana, Estructura química, Permetilación y Ruptura reductiva, SEC/LS.

FRUCTANS are plant reserve carbohydrate polymers with fructose as the repeat unit and a single glucose moiety. Fructans can be found both in monocots and dicotyledons, and are known as inulin owing to the fact that they were first isolated from *Inula Helenium.* Inulin is a b (2→1) linear fructan which is built from 1-kestose (Figure 1a). However, other type of fructans such as those found in grasses are b (2→6) macromolecules with building units based on 6-kestose (Figure 1b). Neokestose (Figure 1c) has been found in garlic and red squill. Only a few plants contain fructans in large amounts such as the root of chicory, and the tubers of Jerusalem artichoke. Another source of fructans is the blue agave plant (*Tequilana Weber var. azul*). Blue agave is traditionally used to prepare the worldwide famous Mexican spirit tequila. More than 80 wt % of the carbohydrate content in blue agave originates from fructans. Fructans from blue agave have an enormous potential as dietary supplements, a source of fructose and as drug excipients. These fructans are wrongly known, commercialy, as inulin since that denomination implies that the polysaccharide is a linear fructan. In this study, agave fructans have been isolated according to a method published elsewhere[1], omitting the ultrafiltration step, and have been subjected to reductive cleavage, size exclusion chromatography/light scattering (SEC/LS) and ^{13}C nuclear magnetic resonance (NMR) analyses.

The reductive-cleavage method allows one to simultaneously determine the ring form and the position of the linkage of each monosaccharide residue in a polysaccharide[2]. Permethylation of the polysaccharide, with methylene iodide under alkaline conditions, followed by the reductive cleavage with trimethylsilyl/triflate/ triethylsilane and acetylation with acetic anhydride was evaluated by gas-liquid chromatography/mass spectrometry. The products that could be obtained by the reductive cleavage method[3] were 1-O-acetyl-2,5-anhydro-3,4,6-tri-O-methyl-D-mannitol and 1-O-acetyl-2,5-anhydro-3,4,6-tri-O-methyl-D-glucitol which originated from (2→1)-linked ḅD fructofuranose. 1-O-acetyl-2,5-anhydro-3,4,6-tri-O-methyl-D-mannitol and 6-O-acetyl-2,5-anhydro-1,3,4-tri-O-methyl-D-glucitol from (2→6)-linked b-D-fructofuranose. 1,6-di-O-acetyl-2,5-anhydro- 3,4-di- O-methyl- D-mannitol and 1, 6 disubstitued b-D-fructofuranoses yielded 1,6-di-O-acetyl-2,5-anhydro-3,4-di-O-methyl-D- glucitol. Finally, from terminal a-D-glucopiranose it was obtained 1,5-anhydro-2,3,4,6,-tetra-O-methyl-D-glucitol and from a-D-glucopiranose linked at C6 it is expected to obtain 6-O-acetyl-1,5-anhydro-2,3,4-tri-O-methyl-D-glucitol.

Table 1 shows agave-fructan structural features obtained from the reductive-cleavage method. It can be seen that for each glucose moiety there are 15 fructose units with a variety of possible linkages. It is worthy to note that it was possible to detect 2→1 and 2→6 and even 1,6 linked fructofuranose which suggested that agave fructans are mixed inulin-levan type fructans. On the other hand, Figure 2 shows the molecular weight distribution obtained by SEC/LS[3]. Light scattering results showed that low molecular weight fructans (range 3-5) account for almost 10% of the total and that basically the molecular wight distribution was centered in a D.P. of 16, which agrees with the results obtained by the reductive cleavage method.

Table 2 shows the agave fructan NMR (in D_2O) signals found and literature assignements for chicory inulin[4] and sinistrin[5]. The data altogether suggest as well that agave fructans are a mixed type of fructans with 1,6 branches. Based upon these results we propose the chemical structure of agave fructan (Figure 3).

Economically important fructans, such as those from chicory and Jerusalem artichoke, are linear fructans and they are of the type inulin. Nowadays, agave fructans are only used as the source for producing tequila. However, these unique carbohydrate polymers could be used as functional foods and drug excipients. Therefore, it is extremely important to have an acurate description of the chemical structure of these compounds, since their structure dictate the properties of materials derived from them. This study showed that agave fructans are branched inulin-levan type fructans and should not be regarded as inulin type.

Monomer	Linkage	Mean (%)[§]
β D fructofuranose	Terminal	22.0
	1- linked	30.8
	6- linked	21.2
	1, 6 linked	19.8
α D- glucopiranose	Terminal	7.3
	6- linked	0.0

[§] Mean of 10 measures, adjusted for sucrose; with these values one can determine the degree of polymerization (mean number) as DP= Σ(Fructose+Glucose)/Glucose = 16

Table 1. Linkage determination in blue agave fructan by the reductive cleavage method.

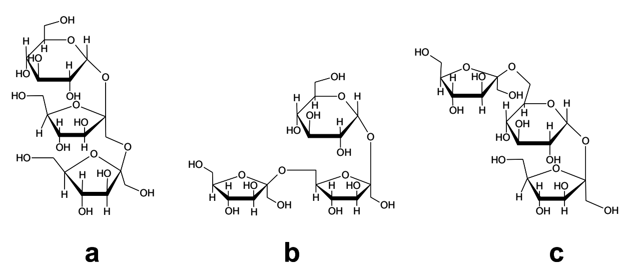


Figure 1. Fructans building units a) 1 –kestose (inulin type fructans), b) 6 -kestose (levan type fructans) and neokestose.

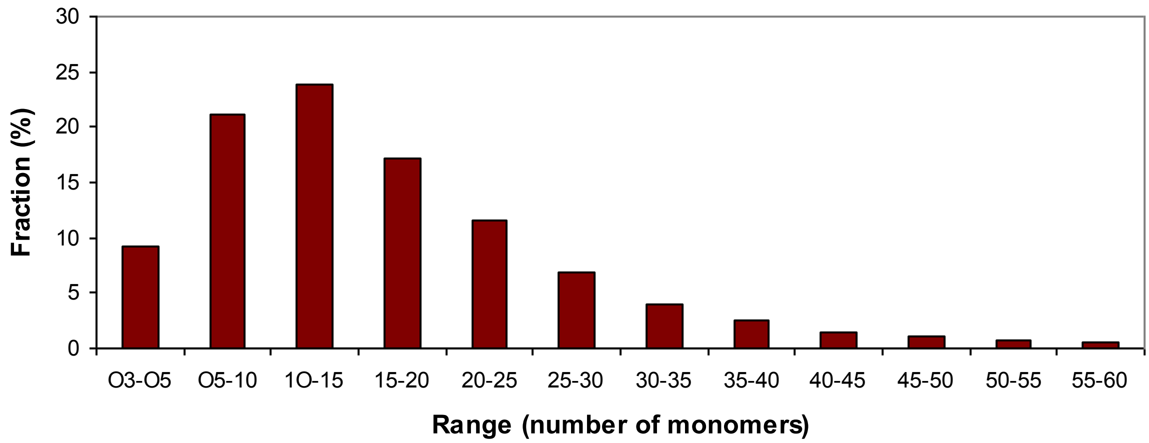
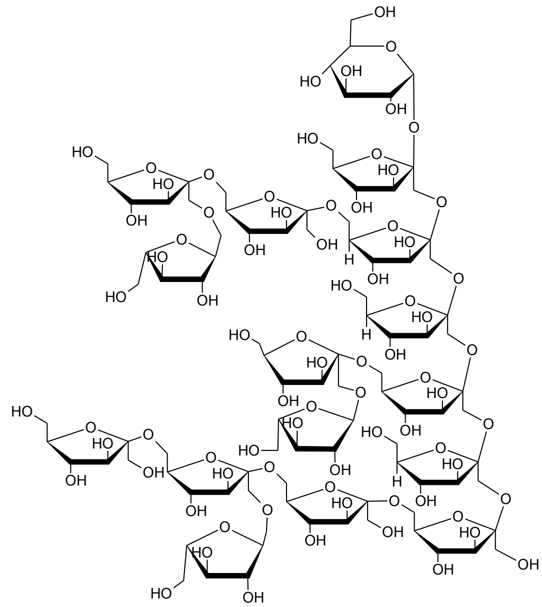


Figure 2. Molecular weight distribution of agave fructans. Mn = 2690 g/mol D.P. = 16

	Chicory[§]		Sinistrin[+]				Agave fructan			
	Fruf 2-1	α-D Glcp 1	Fruf 2-6	Fruf 2-1	Fruf-2	1,6 Fruf-2	Fruf 2-1	Fruf -2	1,6 Fruf-2	α-D Glcp 1
C1	60.6	92.2	60.7	61.2	60.8	60.8	60.2	62.0		92.2
C2	103.5	69.8	104.9	104.0	104.5	104.6	103.0	103.5	103.6	69.0
C3	76.8	72.2	77.3	77.5	77.5	77.5	76.3	76.3	76.3	72.2
C4	74.0	69.0	76.0	75.2	75.4				74.1	69.0
C5	80.9	71.4	81.0	81.9	81.9	81.0	80.9	80.9	80.0	70.8
C6	61.9	60.0	64.0	63.1	63.4	63.0	63.0			60.2

[§] After [4], + After [5]

Table 2. NMR signals for chicory inulin, fructan sinistrin and fructans from agave (ppm)



Aknowledgment: Dr. W. Praznik from Institut für Chemie, Universität für Bodenkultur, Wien Austria is graefully aknowledged for conducting reductive-cleavage analysis.

Figure 3. Proposed chemical structure for agave fructans

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