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A STUDY OF THE GLUCOFRUCTOFURANAN FROM THE NEW ZEALAND CABBAGE TREE *Cordyline australis*

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ABSTRACT

Extraction of the roots of the New Zealand cabbage tree *Cordyline australis* with water gave a glucofructofuranan in 60% yield (dry-weight basis). Viscosity measurements on aqueous solutions of the polysaccharide, and vapor pressure osmometry of the polysaccharide peracetate, showed the number average molecular weight of the glucofructofuranan to be ~3000. Complete hydrolysis with dilute acid gave only D-fructose and D-glucose, in the ratio of 16:1. The polysaccharide was methylated by using dimethyl sulfoxide–sodium hydroxide–methyl iodide, and the methylated polymer was hydrolyzed to give 1,3,4,6-tetra-*O*-methylfructose (5.6 mol), 2,3,4,6-tetra-*O*-methylglucose (1 mol), 1,3,4-tri-*O*-methylfructose (8.4 mol), 2,3,4-tri-*O*-methylglucose (0.1 mol), and 3,4-di-*O*-methylfructose (2.7 mol). These results, supported by ^{13}C-n.m.r. analyses, showed that the polymer is a highly branched glucofructofuranan containing mainly (1→2)-linked β-D-fructofuranosyl residues, with branching at O-6 of 15% of the D-fructosyl residues.

INTRODUCTION

Cordyline is a genus currently classified by Dahlgren *et al.*[1] in the family Asteliaceae, order Asparagales, and superorder Liliiflorae. There are about twenty species of *Cordyline* occurring in Africa, Indo-Malaysia, Australia, New Zealand, and Polynesia. One species has been reported in South America. Five species are endemic to New Zealand[2], but the most widespread there is *Cordyline australis*, now commonly called cabbage tree, which occurs in open places and at forest margins on the three main islands.

The present work began mainly because of the interest of one of us (B.L.F.) in the ethnobotany of *Cordyline* species throughout the Pacific area. The occurrence of large amounts of readily hydrolyzable glucofructofuranan in *Cordyline australis* explains not only why the early South Island Maori used the cooked roots and stems of the species as a food, but also the cooking methods used by them which hydrolyzed the polysaccharide to D-fructose. Large earth ovens,

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used for steaming *Cordyline australis,* have been the sites of many archeological excavations in southern New Zealand.

The finding that the roots of *Cordyline australis* may contain >60% of fructofuranan[3] suggested that the species could be come a commercial source of D-fructose, and prompted the present study.

RESULTS AND DISCUSSION

The meal prepared from the roots of *Cordyline australis* was extracted with ethanol; water washing then yielded a polysaccharide, along with small proportions of D-glucose, D-fructose, and oligosaccharides. The polysaccharide could be separated as a white, water-soluble powder by precipitation from the aqueous extract with acetone–ethanol, followed by drying, but, because it was very hygroscopic, it was more conveniently obtained by freeze-drying.

The polysaccharide was nonreducing to Fehling solution, and had $[\alpha]_D$ −40.4°, which is in the range observed for polysacharides that contain mainly β-D-fructofuranosyl residues, such as inulin from dahlia tubers[4] and bacterial levans[5]. The ease with which the polysaccharide underwent hydrolysis suggested that the D-fructosyl residues were present in the furanose form. For example, complete hydrolysis was achieved with aqueous trifluoroacetic acid (5mM) in 20 min at 100°, and by hydrochloric acid (0.05M) in 2 h at 55°. Examination by h.p.l.c. of the hydrolyzates from both procedures showed that the fructose to glucose ratio in the original polysaccharide was ~16:1.

Attempts to separate the polysaccharide into fractions exhibiting different monosaccharide analyses were unsuccessful, even though a wide range of methods was used, including some techniques previously found useful for the fractionation of glucofructofuranans[6], such as chromatography on DEAE-cellulose and DEAE-Sephadex, and such novel techniques as foam fractionation[7], a method successfully applied to the partial fractionation of various water-soluble polysaccharide mixtures[8]. Attempts to fractionate the peracetate of the glucofructofuranan were also unsuccessful. It was therefore concluded that the polysaccharide, as isolated, was homogeneous.

It is well known that a homologous series of nonreducing oligofructosides is widely distributed in the roots and tubers of some higher plants, such as Jerusalem artichoke (*Helianthus tuberosus L.*), *Arnica montana L.*, and *Artemisia absinthium L.* (see refs. 9–11). When the polysaccharide isolated from the roots of *Cordyline australis via* acetone–ethanol precipitation was chromatographed, using methods known to separate these oligofructosides[11], none were detected.

In view of the generally accepted[12] role of sucrose-terminated β-D-fructosides in carbohydrate metabolism, in which sucrose is the primer onto which successive D-fructosyl groups are linked, it seemed likely that each molecule of the *Cordyline australis* polysaccharide contained a single, nonreducing α-D-glucopyranosyl end-group. Proof that the D-glucose unit was not a reducing terminal followed from the

facts that the polysaccharide did not reduce Fehling solution, and that bromine oxidation of the polysaccharide and subsequent hydrolysis gave both glucose and fructose. Were the D-glucose present as reducing end-groups, it would have been oxidized to D-gluconic acid. The reaction of the glucofructofuranan with aqueous sodium periodate was consistent with these observations, as it showed the presence of one set of three vicinal hydroxyl groups for each fifteen to eighteen hexosyl units. The D-glucosyl groups must have been responsible for the formic acid released during the periodate oxidation (if all of the D-fructosyl residues were present in the furanosyl form).

On the assumption of a single α-D-glucosyl group per molecule, it follows from the fructose:glucose ratio that the number-average molecular weight of the polysaccharide would be 2750. Vapor-pressure osmometry of the fully acetylated polymer in chloroform gave a number-average molecular weight of 5133, which corresponds to a degree of polymerization of 17.3 and a number-average molecular weight of 2800 for the original glucofructofuranan. The intrinsic viscosity, $[\eta]$, of the polysaccharide in water was 3.72, which represents a number-average molecular weight, M_n, of 3200 and a degree of polymerization of 19.8 (using the relationship $[\eta] = 0.33\ M_n^{0.3}$). This equation was obtained by us from a regression analysis of the data reported[13] for a similar, low-molecular-weight, highly branched glucofructofuranan. The assumption of no more than one α-D-glucosyl group per molecule is therefore consistent with the molecular-weight studies, in view of possible errors in determinations of both the viscosity and the vapor pressure.

Complete methylation of the glucofructofuranan was achieved by using a single Hakomori methylation[14], and the permethylated polysaccharide was completely hydrolyzed by heating in 1.0M aqueous trifluoroacetic acid for 2 h at 100°. However, g.l.c. analysis of the alditol acetates formed from the hydrolyzate showed the presence of many non-carbohydrate peaks having low retention-times, and an unusually high ratio of tetra- to di-*O*-methylfructosyl derivatives. For these reasons, the methylation procedure of Ciucanu and Kerek[15] was preferred for methylation analysis. Using this method, complete methylation was obtained in one hour in a single step, and no formation of non-carbohydrate products was observed on g.l.c. analysis of the mixture of partially methylated alditol acetates prepared from the hydrolyzate of the methylated polymer.

The conditions used for the hydrolysis of the methylated polysaccharide were based on those used by others for the hydrolysis of methylated fructofuranans. Some[16] used weaker acids and much longer hydrolysis times than we, whereas others[17] used much stronger acid for a shorter time. The absence of non-carbohydrate products in the g.l.c. trace from the Ciucanu–Kerek[15] methylation suggested that the hydrolysis conditions used for this work were not excessive. Furthermore, in the g.l.c. analysis, there was no evidence of incomplete hydrolysis, nor of any other oligosaccharides which could possibly be formed from partially methylated fructoses if excessively vigorous conditions of hydrolysis were used.

The results of the methylation analysis, including retention times relative to

TABLE I

ANALYSIS OF THE ALDITOL ACETATES FROM THE HYDROLYZATE OF PERMETHYLATED GLUCOFRUCTOFURANAN (BY G.L.C./M.S.)

Number	*Component separated*	*Relative amount (moles)*	*Relative retention time*
1	2,4-Di-*O*-acetyl-1,3,4,6-tetra-*O*-methylmannitol	3.2	0.93
2	2,5-Di-*O*-acetyl-1,3,4,6-tetra-*O*-methylglucitol	2.4	0.94
3	1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol	**1.0**	**1.00**
4	2,5,6-Tri-*O*-acetyl-1,3,4-tri-*O*-methylglucitol	0.7	1.13
5	1,2,5-Tri-*O*-acetyl-3,4,6-tri-*O*-methylglucitol	9.1	1.14
6	1,2,5-Tri-*O*-acetyl-3,4,6-tri-*O*-methylmannitol		
7	1,5,6-Tri-*O*-acetyl-2,3,4-tri-*O*-methylglucitol	0.1	1.18
8	1,2,5,6-Tetra-*O*-acetyl-3,4-di-*O*-methylglucitol	2.7	1.33
9	1,2,5,6-Tetra-*O*-acetyl-3,4-di-*O*-methylmannitol		

1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol as unity, are given in Table I. The mole ratios reported are the means of three separate methylation analyses. All the partially methylated alditol acetates listed were identified by mass spectrometry[18]. The formation of both D-glucitol and D-mannitol derivatives by reduction of partially methylated D-fructoses makes the exact interpretation of these g.l.c. analyses difficult, especially because 1,3,4- and 3,4,6-tri-*O*-methyl-D-fructose give the same partially methylated mannitol acetates, in addition to partially methylated glucitol acetates, on reduction with sodium borohydride and acetylation. However, reduction of the hydrolyzate of the methylated polysaccharide with sodium borodeuteride[18] permitted identification by mass spectrometry of 2,3,6-tri-*O*-acetyl-1,3,4-tri-*O*-methylmannitol-*2-d* from the reduction and acetylation of 1,3,4-tri-*O*-methylfructose, and of 2,5,6-tri-*O*-acetyl-1,3,4-tri-*O*-methylmannitol-*5-d* from the reduction and acetylation of 3,4,6-tri-*O*-methylfructose.

The complete separation of 1,2,5,6-tetra-*O*-acetyl-3,4-di-*O*-methylglucitol from 1,2,5,6-tetra-*O*-acetyl-3,4-di-*O*-methylmannitol was not achieved in the capillary g.l.c. columns available.

If it is assumed that the ratio of mannitol derivative to glucitol derivative produced from the borohydride reduction of 1,3,4-tri-*O*-methylfructose is unity, the data presented in Table I lead to the conclusion that, on acid hydrolysis, the methylated glucofructofuranan gave, per mol of 2,3,4,6-tetra-*O*-methylglucose, 1,3,4,6-tetra-*O*-methylfructose (5.6 mol), 1,3,4-tri-*O*-methylfructose (1.4 mol), 3,4,6-tri-*O*-methylfructose (8.4 mol), 2,3,4-tri-*O*-methylglucose (0.1 mol), and 3,4-di-*O*-methylfructose (2.7 mol). These results showed that the original polymer is highly branched, and contains (1→2)-linked and a small number of (2→6)-linked β-D-fructofuranosyl residues. Branching occurs through O-6 of 15% of the fructosyl residues.

As was the case with the Hakomori methylation[14], the Ciucanu–Kerek procedure[15] gave more tetra-*O*-methylfructose than expected from the amount of

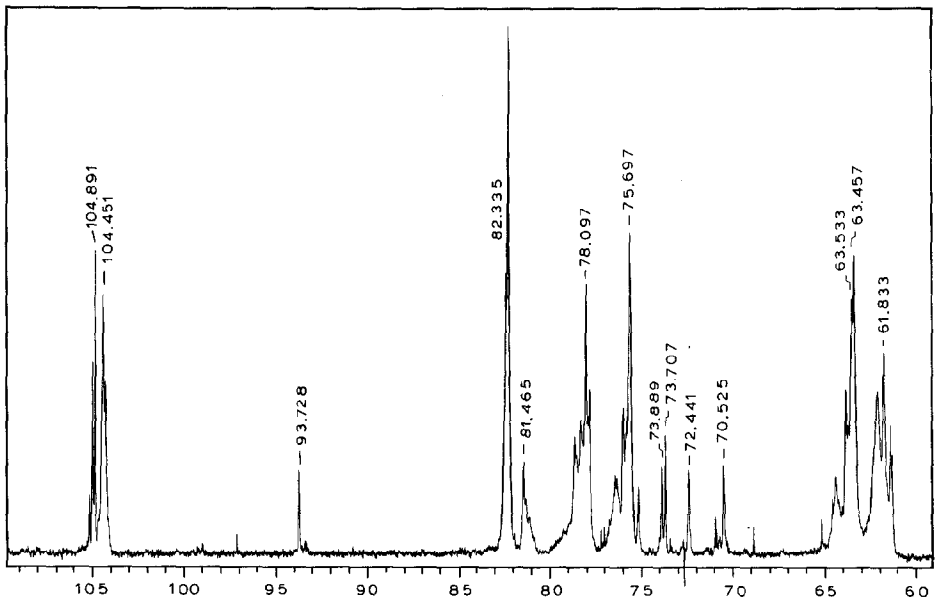

Fig. 1. Proton-decoupled, natural-abundance, Fourier-transform, ^{13}C-n.m.r. spectrum of the water-soluble glucofructofuranan of *Cordyline australis* in D_2O (100 mg/mL) at 30°, recorded at 75 MHz. Chemical shifts are relative to external tetramethylsilane.

di-*O*-methylfructose obtained. Inconsistencies of this kind have also been found in the methylation analysis of several other β-fructofuranosyl polysaccharides[16,19,20].

The methylation analysis also showed that two kinds of glucosyl group form part of the polysaccharide, namely, a nonreducing-terminal glucosyl and a small proportion of a nonreducing, (1→6-linked glucosyl group. Because, from the molecular-weight determinations, it is likely that no more than one glucosyl group occurs per molecule, some molecules have a terminal glucosyl, while others possibly have a glucosyl group linked at O-6 to a terminal β-D-fructofuranosyl residue, as had been observed in various oligosaccharides isolated by Bacon[21]. The glucofructofuranan isolated from *Cordyline terminalis*[22] also has 6-*O*-substituted α-D-glucosyl groups.

The ^{13}C-n.m.r. spectrum of the polysaccharide (see Fig. 1 and Table II) shows six groups of intense signals due to the fructosyl carbon atoms. Each group contains 4–5 multiplets, as expected for a polymer having ~16 fructosyl residues with five different substitution-patterns.

Signals at 104.4 and 104.5 p.p.m. are typical [6,23–25] of C-2 of β-D-fructofuranosyl residues substituted at C-1. The former, being less intense, may be that of C-2 of the fructosyl residue adjacent to the α-D-glucopyranosyl residue. The signal at 104.9 p.p.m. is assigned to C-2 of a terminal β-D-fructofuranosyl residue[23–30], whereas the 105.0-p.p.m. signal originates from C-2 of a 1,2,6-tri-*O*-substituted residue, the upfield shift expected as a consequence of 1-*O*-substitution

TABLE II

CHEMICAL-SHIFT ASSIGNMENTS (P.P.M.) FOR THE ^{13}C-N.M.R. SPECTRUM OF THE GLUCOFRUCTOFURANAN FROM *Cordyline australis* ROOTS

Residue	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
β-D-Fructosyl	61.4	104.4	77.9	75.7	81.1	63.5
	61.5	104.5	78.1	76.0	81.3	63.5
	61.8	104.9	78.4	76.5	81.5	64.0
	62.1	105.0	78.6	76.7	82.4	64.4
		105.3			82.5	
α-D-Glucosyl	93.4	72.4	73.9	70.5	73.7	68.9
	93.7			71.0		

being offset by the effect of 6-*O*-substitution. The signal at 105.3 p.p.m. may be that of C-2 of 2,6-di-*O*-substituted furanosyl residues.

From the aforementioned assignments, use of gated decoupled spectra in which the nuclear Overhauser enhancement was eliminated, followed by integration, enabled the ratio of 1,2-di-*O*-substituted β-D-fructofuranosyl residues to total D-fructosyl residues in the molecule to be calculated as 0.51, which compares reasonably with the value of 0.46 calculated from the methylation data presented in Table I.

The ^{13}C-n.m.r. spectrum of the *Cordyline australis* glucofructofuranan also showed two groups of signals at positions expected for C-5 of β-D-fructofuranosyl residues (see Fig. 1). The presence of these two groups reflects the occurrence in the polysaccharide of some β-D-fructofuranosyl residues that carry substituents at O-6 and some that do not. These signals also occurred in gated decoupled spectra in which nuclear Overhauser enhancement was eliminated, and integration showed that the ratio of the area under the 82.3-p.p.m. group to that under the 81.5-p.p.m. group was ~3:1. From the methylation data, the ratio of β-D-fructosyl residues not carrying a substituent at O-6 to those with a substituent at O-6 is 3.4:1. It therefore follows that the former group is that of C-5 of β-D-fructofuranosyl residues in the polymer that are not linked through O-6, and the latter are those of C-5 of β-D-fructofuranosyl residues that are linked through O-6.

We have found that no signals near 81.5 p.p.m. occur in the ^{13}C-n.m.r. spectra of inulin, sucrose, fructose, stachyose, and raffinose. This is also true of the spectra of the first three of these compounds, published by Seymour *et al.*[29,31]. Also, in the ^{13}C-n.m.r. spectra of levans and various levan fractions of different molecular weights, published by Seymour *et al.*[29,31], signals at both 81.5 and 82.4 p.p.m. (or thereabouts) occur, as would be expected from the foregoing assignments. It is of interest that the levan isolated from rye grass (*Lolium perenne*)[6] also showed ^{13}C-n.m.r. signals at ~81.5 and 82.4 p.p.m., even though all O-6 atoms of the β-D-fructofuranosyl residues were considered to be substituted, and the polysaccharide was assumed to be free from (1→2)-linked β-D-fructofuranosyl residues. The signal at ~82.4 p.p.m. was assigned[5] to C-5 of a β-D-fructosyl residue which was also

substituted at O-2 by a terminal α-D-glucopyranosyl group. This may be the correct assignment for the levan from *Lolium perrene*, but that assignment cannot completely account for the 81.5- and 82.4-p.p.m. groups of signals in the spectrum of *Cordyline australis* polysaccharide, because of the relative areas of the two signals in the gated decoupled spectrum. Likewise, the signal at 82.4 p.p.m. in the spectra of the levan fractions reported by Seymour *et al.*[29,31] cannot be assigned to the C-5 atom of a fructosyl residue adjacent to a terminal glucosyl group, because of the high molecular weight of these fractions.

The assignment of the C-1 and C-6 groups of signals follows from those of inulin and various levans[25,28,29]. The other two major groups of signals are those of the C-3 and C-4 atoms of fructosyl residues, and their presence confirms that the polysaccharide is unsubstituted at these two positions. Signals diagnostic of 1-*O*-linked α-D-glucopyranosyl groups are also present in the spectrum (see Table II). The 68.9-p.p.m. signal supports the conclusion that some C-6 atoms of the glucosyl group are substituted[27,32].

The ^{13}C-n.m.r. spectrum of the glucofructofuranan from the roots of *Cordyline australis* was thus consistent with the methylation results, and supported a polysaccharide structure which contained mainly (2→1)-linked β-D-fructofuranosyl residues. Branching occurs at O-6 of many of the fructosyl residues, and a small proportion of fructosyl residues are linked through O-2 and O-6 only. The polysaccharide chain contains no reducing end-group, and an α-D-glucopyranosyl group is linked to O-2 of a β-D-fructofuranosyl residue at one end of the polymer. Of these α-D-glucopyranosyl groups, ~10% are substituted at O-6 with D-fructofuranosyl residues. Because the total amount of tetra-*O*-methylhexose obtained from the hydrolyzate of the permethylated polysaccharide was not in good agreement with the total di-*O*-methylhexose content, use of gated decoupled ^{13}C-n.m.r. spectra was the preferred method for providing quantitative information on the substitution patterns involved in the polysaccharide structure.

The extraction of wood meal prepared from the stems of *Cordyline australis* also gave a cold-water-soluble polysaccharide (yield 35%, oven-dry wood basis). However, whereas the main root polysaccharide was found to be a glucofructofuranan at all times of the year, the composition of the stem polysaccharide varied with the season. Only the polysaccharide extracted from summerwood of *Cordyline australis* was a glucofructofuranan, and it had a fructose:glucose ratio, ^{13}C-n.m.r. spectrum, and chemical properties similar to those of the root polysaccharide.

We conclude that the nonreducing polysaccharides extracted by water from the roots, and summerwood, of *Cordyline australis* can be placed in the "branched" (or mixed-linkage type) classification for glucofructofuranans isolated from monocotyledonous plants[11]. They resemble, for example, the low-molecular-weight fructan of wheat flour (*Triticum vulgare*)[33], the glucofructofuranan from *Cordyline terminalis*[22], and sinistrin, a fructan from the bulbs of red squill (*Urginea maritima*)[13].

EXPERIMENTAL

General methods. — A Perkin–Elmer 141 polarimeter was used for measurement of optical rotations. All evaporations were conducted at a bath temperature below 40°. Gas-liquid chromatography (g.l.c.) was conducted in a Varian model 3700 gas chromatograph equipped with a flame ionization detector and a Hewlett–Packard 3390 A integrator-recorder. Partially methylated alditol acetates were separated on SE-30 and BP-10 capillary columns operated in the split mode, with helium as the carrier gas. The oven temperature program was typically 100–280° at a rate of 6° per min. The injection port and detector were both kept at 290°.

Three systems were used for g.l.c.–mass spectrometry (g.l.c.–m.s.), namely, (*i*) a Shimadzu QP 1000 g.l.c.–mass spectrometer using electron impact m.s. at 70 eV, (*ii*) the same instrument, using chemical ionization with isobutane gas, and (*iii*) a Hewlett-Packard 5985 B g.l.c.-mass spectrometer, using electron-impact m.s. at 70 eV. The mass spectrometers were equipped with 12 m × 0.22 mm (i.d.) capillary columns coated with BP-10 (0.25 μm).

Water extracts of *Cordyline australis* meal, and polysaccharide hydrolyzates, were analyzed for monosaccharides and oligosaccharides by using a Varian model 500 liquid chromatograph equipped with a U6K (Waters Associates) injector and a Waters Sugar Pak 1 column, 30 cm by 6.5 mm i.d., maintained at 90°. Milli Q water was used as eluant at a flow rate of 0.5 mL per min. Detection was accomplished by using a differential refractive-index detector (R 401, Waters Associates). Peak areas were measured with a Hewlett–Packard 3390 A integrator-recorder.

Isolation of polysaccharide. — Specimens of *Cordyline australis* were collected from a stand in the Otago Coast Forest, in south-east Otago, New Zealand. Within 2 h of collection, samples of roots and stems were cut into sections and dried in a forced-air oven for 2 d at 30°, after which they were ground to meal in a Wiley mill. The meal (30 g) was extracted with water (600 mL) containing calcium carbonate (2.4 g) for 90 min at 70°. The mixture was then filtered, freeze-dried, the solid dissolved in water, and the solution added dropwise to ethanol. The precipitate (17.9 g) was separated by centrifugation, and twice further dissolved and precipitated with ethanol. From 30 g of dry root-meal, 14.3 g of a gluco-fructofuranan, $[\alpha]_D^{20}$ −40.4° (*c* 2.0, water) was obtained.

Characterization of the glucofructofuranan. — The polysaccharide (0.20 g) was hydrolyzed in hydrochloric acid (0.05M) for 4 h at 55°. The monosaccharide mixture was separated chromatographically. D-Glucose, $[\alpha]_D^{20}$ +51° (*c* 1.0, H_2O) was characterized as *N*-(*p*-nitrophenyl)-D-glucosylamine; m.p. and mixed m.p. 180–181°. D-Fructose, $[\alpha]_D^{20}$ −90° (*c* 1.0, H_2O) was obtained as crystals, m.p. and mixed m.p. 98°. The mole ratio of monosaccharides in the hydrolyzate was determined by h.p.l.c. to be fructose:glucose = 16.1:1.

Bromine oxidation. — The method of Boggs and Smith[22] was used, with similar results.

Sodium periodate oxidation. — Polysaccharide (0.100 g) was oxidized in the

dark with 0.14M sodium metaperiodate (100 mL) at 5°. Analyses for periodate consumption and formic acid formation were made[34]. The moles of periodate consumed and of formic acid produced per hexosyl residue or group were as follows: 0.75, 0.052 (3 d); 1.05, 0.057 (6 d); 1.15, 0.061 (9 d); 1.15, 0.062 (12 d); 1.20, 0.062 (14 d); and 1.22, 0.062 (16 d).

Molecular weight determinations. — Molecular weights were determined by vapor pressure osmometry of acetylated glucofructofuranans by means of a Hitachi Perkin–Elmer apparatus, model No. 115, using chloroform (1 mL) as solvent at 30.2° (main oven) and 26.0° (sub oven). The instrument was calibrated by using benzil. The polysaccharide acetates were prepared by the method[35] of Haworth and Streight.

Viscosity measurements on aqueous solutions of glucofructofuranan (0.03–0.14 $g.cm^{-3}$) were made at 25.0°, using an Ubbelohde viscometer (capillary length 90 mm, inside diameter 0.4 mm, bulb volume 4 mL). The viscometer gave an efflux time of 463 s when calibrated with water.

Methylation of the glucofructofuranan. — Two methylation procedures were used. In one of these, the dry polysaccharide (0.19 g) was methylated by the Hakomori method[14], to give fully methylated glucofructofuranan (0.35 g) which showed no hydroxyl absorption at 3600–3460 cm^{-1}. The polysaccharide (0.09 g) was also methylated by the Ciucanu–Kerek method[15], to give fully methylated glucofructofuranan (0.05 g). In both cases, methylated polymer (50 mg) was hydrolyzed with trifluoroacetic acid (1.0M, 10 mL) for 2 h at 100°, the neutralized hydrolyzate was converted into partially methylated alditol acetates, and these were examined by capillary g.l.c. and g.l.c.–m.s. as already described. In some cases, the hydrolyzate was reduced with sodium borodeuteride prior to acetylation, to assist the interpretation of g.l.c.–m.s. analyses, as already described.

^{13}C-N.m.r. spectroscopy. — ^{13}C-N.m.r. spectra were recorded, for solutions of polysaccharides (50–100 mg/mL) in deuterium oxide in 5-mm tubes at 30°, in a Varian XL-300 spectrometer operated in the pulsed Fourier-transform mode with broad-band noise-decoupling. ^{13}C-Chemical shifts were measured relative to internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate, and are reported in p.p.m. relative to external tetramethylsilane.

Gated decoupled spectra without nuclear Overhauser enhancement were recorded with a Varian VXR-300 spectrometer fitted with a 5-mm probe. The concentration of polysaccharide solutions was 100 mg/mL of 1:1 (v/v) dimethyl sulfoxide-d_6–deuterium oxide. Other experimental conditions were: temperature: 30°, pulse: 90° (8.4 μs), repetition time: 5 s, and transients: 9792. These conditions were based on relaxation times (T_1) reported for inulin[25]. Integrated values were determined from peak areas.

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