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Note

Isolation and identification of *O*-β-D-fructofuranosyl-(2 → 1)-*O*-β-D-fructofuranosyl-(2 → 1)-D-fructose, a product of the enzymic hydrolysis of the inulin from *Cichorium intybus*

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There is a growing demand for sweeteners, alternative to sucrose, that have additional nutritional properties, for example, a low calorific value, a dietary fibre effect, or a selective bifidogenic (for certain bacteria from the Bifidobacterium family)-promoting effect.

Non-reducing glucofructo-oligosaccharides[1–4] GF_n (**1**) with n usually 1–5, and obtained by the action of fructosyltransferases[5] on saccharose, are typical examples of sweeteners with the above-mentioned properties[6]. Hydrolysis of inulin by a specifical endo-type inulinase[7] yields not only GF_n but also the reducing fructo-oligosaccharides F_n (**2**). The source of these oligofructoses is inulin as, for example, found in chicory roots. Inulin is a linear glucofructan GF_n with n in the range[8] 2 to > 50.

We now report on the title trisaccharide F_3 (**2**, $n = 1$), isolated from the mixture obtained by the hydrolysis of inulin extracted from the roots of chicory (*Cichorium intybus*) with an endo-inulinase[7]. Although the ^{13}C NMR data for the disaccharide F_2 (**2**, $n = 0$) have been reported[9], for F_3 only partial data have been published hitherto[10].

Inulin was partially hydrolysed by an endo-inulinase[7] to give a mixture of products that contained ~ 85% of oligosaccharides. This proportion was increased to ~ 95% by chromatography on a cation-exchange (K^+) resin. The poor mixture was fractionated according to dp on octadecyl silica gel (Fig. 1), and fraction DP_3 was fractionated further on Aminex HPX-87K (Fig. 2A). HPLC chromatography (Fig. 2B) of the major fraction shown in Fig. 2A gave pure F_3, the structure of which was established by NMR spectroscopy as *O*-β-D-fructofuranosyl-(2 → 1)-*O*-

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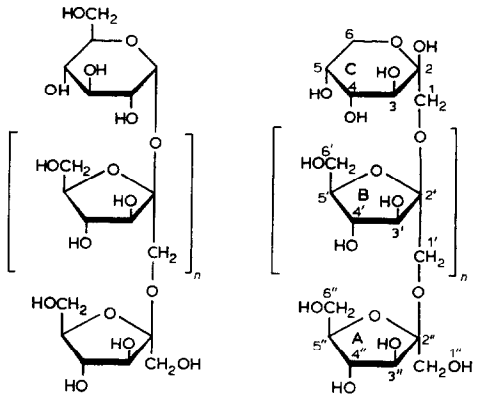

β-D-fructofuranosyl-(2 → 1)-D-fructose (**2**, $n = 1$). A non-reducing anhydro analogue of this compound has been reported[9].

The ^{1}H NMR data of F_3 are given in Table I; there were no resonances typical of H-1 of D-glucopyranosyl moieties, which indicates the absence of nystose (GF_3) as a contaminant.

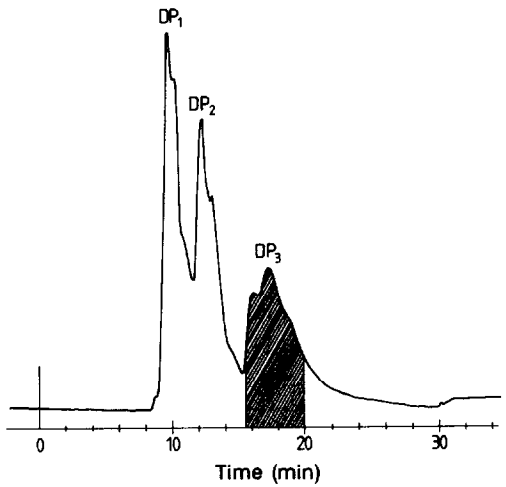

Fig. 1. HPLC of enriched oligosaccharide fraction on octadecyl silica gel (see Experimental for details).

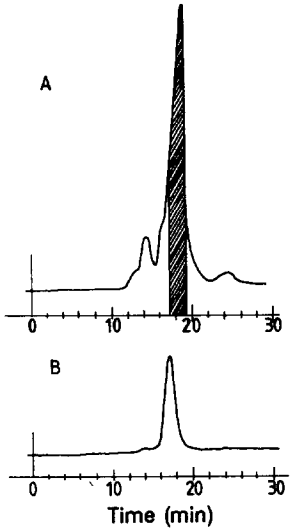

Fig. 2. A, HPLC of fraction DP_3 from Fig. 1 on Aminex HPX-87K (see Experimental for details); B, rechromatography of marked fraction in A.

The region δ 3.5–3.8 was too complex for analysis, but the resonances in the region δ 4.3–3.9 indicate that there were two β-D-fructofuranosyl moieties and a D-fructose moiety that was ~80% β pyranose and ~20% β furanose.

The assignments were based on the results of De Bruyn and co-workers[11,12]. The presence of a doublet and a triplet ($J_{3,4} \approx J_{4,5} \approx 8.3$ Hz) at δ 4.05–4.25 is typical for H-3 and H-4, respectively, of a β-D-fructofuranosyl moiety. The pattern for the resonances at δ 3.95–4.0 is typical for the resonances of H-5,6A of a β-D-fructopyranose residue and the degenerate doublet at δ 4.08 is typical for H-4 of a β-D-fructofuranose residue. The integration of these signals indicates the

TABLE I

^{1}H NMR data for a solution of F_3 (2, $n = 1$) in D_2O

	Chemical shifts (δ in ppm vs. Me_4Si)						
	H-1a	H-1b	H-3	H-4	H-5	H-6a	H-6b
β-D-Fru*f* A			4.21	4.13			
β-D-Fru*f* B			4.14	4.08			
β-D-Fru*p* C					3.96	3.97	
β-D-Fru*f* C			4.08	4.08			
	Coupling constants (Hz)						
	$J_{1a,1b}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$	
β-D-Fru*f* A		8.3	8.3				
β-D-Fru*f* B		8.3	8.3				
β-D-Fru*p* C			3.2	1	1.6	−13.3	

TABLE II

^{13}C NMR data for a solution of F_3 (**2**, $n = 1$) in D_2O

	C-1	C-2	C-3	C-4	C-5	C-6
β-D-Fru*f* A	61.1 [a]	104.4	77.6	74.9 [a]	81.9 [a]	63.0
β-D-Fru*f* B	61.2 [a]	103.7	78.5	75.1 [a]	81.6 [a]	63.0
β-D-Fru*p* C	64.2	98.5	68.9	69.8	70.2	62.6
β-D-Fru*f* C	62.7	104.6	75.0	75.2	82.0	62.9

[a] Assignments for rings A and B may be reversed.

approximate ratios 1:1:0.8:0.2 for β-D-Fru*f* A, β-D-Fru*f* B, β-D-Fru*p* C, and β-D-Fru*f* C. The proportions of the last two moieties are close to those found[13,14] for a solution of D-fructose in D_2O.

The ^{13}C NMR data for F_3 (Table II) were assigned by comparison with known data[15,16]. The resonance at δ 104.4 is assigned to C-2″ of β-D-Fru*f* A. The other resonance in this region at δ 103.7 (with a deviation of 0.7 ppm from the reference data) was assigned to C-2′ of β-D-Fru*f* B, the linkage of which to β-D-Fru*p* C may cause such a deviation[16,17]. The presence of a D-fructopyranose ring is confirmed by its C-2 resonance at δ 98.5. The resonance at δ 104.4 shows a shoulder that probably originates from the resonance for C-2 of the β-D-Fru*f* modification of residue C.

For the two β-D-Fru*f* rings A and B, the resonances for C-5′,5″, C-3′,3″, and C-4′,4″ are expected at δ 82, 77.6, and 74.7, respectively. The resonance for C-3′ has a rather high frequency (δ 78.5) which reflects the effect of the 2′-linked β-D-Fru*p* residue.

There are five resonances in the region δ 60–64, among which that at δ 63.0 is for 2 C. The resonances for C-6′,6″ of the β-D-fructofuranosyl residues are expected at δ 63.2 and those for C-1′,1″ at δ 61.1. The resonance at δ 98.5 indicates that a β-D-Fru*p* residue is present[15]. When the reference data for β-D-fructopyranose[15] are used, and taking into consideration differences up to 2.5 ppm in the β effects of glycosylation[15], good agreement is found with the data for F_3.

For the β-D-Fru*p* C residue, the following resonances were found: δ 64.2 (C-1, expected δ 64.7), 62.6 (C-6, expected δ 64.1), 68.9 (C-3, expected δ 68.9), 69.8 (C-4, expected δ 70.5), and 70.2 (C-5, expected δ 70.0). Although the resonances for β-D-Fru*f* C are of low intensity, they can be assigned by comparison with those[15] of β-D-fructofuranoses, namely, δ 62.7 (C-1, δ 63.6), 104.6 (C-2, δ 102.2), 75.0 (C-3, δ 76.4), 75.2 (C-4, δ 75.4), 82.0 (C-5, δ 81.6), and 62.9 (C-6, δ 63.2).

The β configurations of the non-reducing moieties A and B are indicated by the chemical shifts for the C-3′ and C-3″ resonances (expected at δ 76.4 for β and δ 82.9 for α with ±2 ppm caused by the linkage[17]). For the reducing moiety, the α-D-Fru*f* form occurs to the extent of 4–10%, whereas the α-D-Fru*p* form is almost non-existent[13].

For sugar units 2-linked to the β-D-Fru*f* moieties, some generalities have been proposed[17]. Thus, the upfield shift of 2 ppm for the C-1 resonance and the downfield shift of 1.1–1.8 ppm for the C-2 resonance accord with the reported data. Also, a slight downfield shift for the C-3″ resonance was observed, but that for C-3′ was 1 ppm to lower field for which no explanation can be offered.

For the reducing terminal β-D-Fru*f* residue, the 1-linkage caused shifts in the following resonances: 0.9 ppm upfield for C-1, 2.4 ppm downfield for C-2, and 1.4 ppm upfield for C-3.

Thus, the NMR data indicate structure **2** ($n = 1$) for F_3.

EXPERIMENTAL

Isolation of the fructotrisaccharide (*inulotriose*) F_3.—Inulin, extracted from chicory roots (*Cichorium intybus*), was partially hydrolysed by an endo-inulinase[7]. The crude syrupy product, which contained 85% of oligosaccharides, was desalted by ion-exchange and further enriched by chromatography on a cation-exchange (K^+) resin (Illinois Water Treatment System). This commercial process gave an enriched fraction that contained 95% of fructo-oligosaccharides (mainly GF_n) and a poor fraction with 55% oligosaccharides ($GF_n + F_n$).

GLC (1 h, 50°) of the trimethylsilyl derivatives (pyridine–hexamethyldisilazane–chlorotrimethylsilane, 3:3:1) on a fused-silica OV1 capillary column revealed that the major component (25%) in this fraction had dp 3. The enriched fraction was fractionated as follows.

(*a*) *Preparative HPLC on octadecyl silica gel.* A column (250 × 22 mm) of RSiL C18 HL (10 μm) (RSL-Biorad, Eke, Belgium) was used with a Varian 5000 LC instrument equipped with a Waters R · 401 refractive index detector. An aqueous 20% solution of the poor fraction was injected into a 1-mL sample loop, and eluted with H_2O at 7 mL/min at room temperature. Fraction DP_3 has *T* 17.8 min (Fig. 1). The fractionation was repeated ten times and yielded ~ 100 mg of DP_3.

(*b*) *Preparative HPLC on Aminex HPX-87K.* A solution of DP_3 (100 mg) in water (10 mL) was injected (0.5-mL sample loop, 15 injections) onto 2 columns (300 × 78 mm) of Aminex HPX-87K (RSL-Biorad) in series and eluted with water (adjusted to pH 9.6 with KOH) at 0.6 mL/min at room temperature. The instrument and detector were as in (*a*). The appropriate fractions were combined and concentrated to give *O*-β-D-fructofuranosyl-(2 → 1)-*O*-β-D-fructofuranosyl-(2 → 1)-D-fructose (F_3, 75 mg), mp 120° (dec), $[\alpha]_D^{20}$ −35° (*c* 1, H_2O, 1 h).

Anal. Calcd for $C_{18}H_{32}O_{16}$: C, 42.83; H, 6.34; O, 50.83. Found: C, 43.02; H, 6.81.

Hydrolysis (0.1 M HCl, 1 h, 60°) of F_3 gave D-fructose, mp 102°, $[\alpha]_D^{20}$ −98° (*c* 2, H_2O, 48 h) (cf. ref. 18).

NMR spectra.—The ^1H NMR spectra (500.11 MHz) were recorded with a Bruker AM 500 spectrometer, at room temperature, using quadrature detection, a pulse angle of 19°, and a resolution of 0.33 Hz/point. The ^{13}C NMR spectra (90.55

MHz) were recorded with a Bruker AM 360 spectrometer, using a pulse angle of 18° and a resolution of 1.327 Hz/point.

Each sample was dissolved in D_2O. For the ^1H NMR spectra, the water resonance (δ 4.8) was used as a secondary reference; for the ^{13}C NMR spectra, dioxane (δ 67.4) was used as a secondary reference.

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