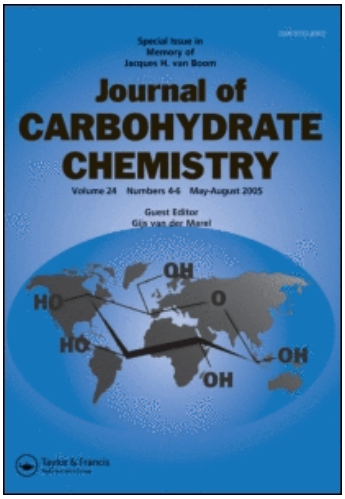

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Sucrosyl-(1→2)-β-Isomaltulose: Enzymatic Synthesis and Structure Determination

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ABSTRACT

By enzymatic reaction of sucrose (**1**) and isomelezitose (**2**) with the enzyme inulinase (NOVO, SP230) a novel tetrasaccharide (**3**) was synthesised,.the molecular weight of which was confirmed by electrospray-ionisation mass spectrometry and gel permeation chromatography. Its structure was established by acid hydrolysis as well as ^{1}H and ^{13}C NMR spectroscopy to be sucrosyl-(1→2)-β-isomaltulose **3** (α-D-glucopyranosyl-(1→2)-β-D-fructofuranosyl-(1→2)-β-D-fructofuranosyl-(6→1)-α-D-glucopyranoside).

Key Words: Tetrasaccharide; Inulinase; Sucrose; Isomelezitose.

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INTRODUCTION

As generally known honey constitutes a food with a high nutritive value and healthful properties. Among others the quality of honey is correlated to both content and distribution of mono- and oligosaccharides, respectively.[1–3] Some of the oligosaccharides show interesting properties such as stability against digestive enzymes and this qualifies them for application in functional foods.[4]

Industrially processed oligosaccharides are produced by enzymatic reactions of available renewable carbohydrates such as starch, inulin, and sucrose. For instance, fructooligosaccharides are obtained by enzymatic cleavage of inulin[5] or by transfer of fructose to sucrose.[6] These facts prompted us to investigate possibilities of synthesising oligosaccharides by enzymatic reaction in the presence of isomelezitose **2** (α-D-glucopyranosyl-(1→2)-β-D-fructofuranosyl-(6→1)-α-D-glucopyranoside), a trisaccharide which is found in honey.[7] Thus, the interaction of isomelezitose **2** as acceptor molecule with sucrose **1** as donor molecule was tested with commercially available enzymes. In the case of inulinase (NOVO, SP230), a new oligosaccharide was isolated which proved to be a tetrasaccharide by positive ion electrospray ionisation mass spectrometry (ESI-MS m/z 689.3 $[M + Na]^+$) and gel permeation chromatography (GPC). Since the test for reducing sugars with 2,2′-bicinchoninate,[8] was negative, this tetrasaccharide **3** was shown to be a non-reducing sugar.

The structure of the tetrasaccharide **3** could not be identified by comparison with reference substances by high performance anion exchange (HPAEC) or gas chromatography (GC). Acid hydrolysis and NMR spectroscopic analysis surprisingly revealed the tetrasaccharide to be sucrosyl-(1→2)-β-isomaltulose (**3**), an oligosaccharide which to our knowledge was not described previously in the literature.

Mild hydrolysis of the unknown oligosaccharide with oxalic acid afforded glucose, fructose, sucrose, and isomaltulose. Glucose and fructose were formed in an equimolar ratio of 1 : 1 and the amount of sucrose, showed a maximum after 20 min hydrolysis. No sucrose was present after 150 min hydrolysis and only isomaltulose, glucose, and fructose were detectable. The sum of glucose and fructose, respectively, and sucrose was equimolar to isomaltulose; isomelezitose (**2**) could not be detected (Table 1).

The tetrasaccharide **3** was analysed by one-dimensional (1D) and two-dimensional (2D) homonuclear and heteronuclear NMR spectroscopic techniques. These NMR data, together with comparisons of the ^{13}C NMR data of the reference compounds isomaltulose and sucrose, showed the tetrasaccharide consist of isomaltulose and sucrose subunits.

Table 1. Hydrolysis of sucrosyl-(1 → 2)-β-isomaltulose (**3**).

Carbohydrate (mmol/mL)	0 min	5 min	10 min	20 min	40 min	80 min	150 min
Glucose	0	0.8	1.3	4.3	8.6	12.1	12.8
Fructose	0	0.8	1.2	4.4	8.8	12.3	13.0
Sucrose	0	3.3	4.1	6.3	4.2	0.8	0.0
Isomaltulose	0	4.0	4.7	10.8	13.0	13.3	13.4
Sucrosyl-(1 → 2)-β-isomaltulose (**3**)	14.5	6.8	5.7	3.4	0.8	0.0	0.0

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Table 2. Selected ^{13}C and ^{1}H NMR data of tetrasaccharide **3**.

C-no.	^{13}C (ppm)	^{1}H (ppm)	Direct $^{13}C-^{1}H$ cross-peak	Long-range $^{13}C-^{1}H$ cross-peak
1	92.43	5.45	X	H-1/C-8
2	71.08	3.56	X	
4	69.14	3.49	X	H-4/C-6
6	60.08			H-4/C-6
8	103.21			H-1/C-8
9	76.62	4.28	X	
10	73.83	4.07	X	
15	76.42	4.21	X	
16	74.83	4.14	X	
17	79.14	4.04	X	
18	68.81			H-19/C-18
19	98.48	5.01	X	H-19/C-18
20	71.33	3.59	X	
22	69.38	3.47	X	

Note: Glucose C1–C6 (sucrose component), fructose C7–C12 (sucrose component), fructose C13–C18 (isomaltulose component), glucose C19–C24 (isomaltulose component).

Selected NMR data are summarised in (Table 2), while the complete data are reported in Experimental section.

The overlap of the ^{1}H NMR signals complicated the identification of the linkage between the two units. The assignment was possible by comparison of the ^{13}C NMR data of the tetrasaccharide with the reference data for sucrose and isomaltulose. The ^{13}C NMR data of two carbons from the fructose units differ significantly in their chemical shifts. The signal of the free anomeric C-atom of the isomaltulose moiety in the tetrasaccharide was shifted downfield by 1.59 ppm compared to that in the disaccharide isomaltulose. Further, the signal of the C1-atom of the fructose of the isomaltulose moiety in the tetrasaccharide was shifted by 2.65 ppm upfield compared to that in the disaccharide isomaltulose. The signal of the C1-atom of the fructose unit of the sucrose moiety was shifted upfield by 1.71 ppm compared to that in sucrose (Table 3). The changes of the other chemical shifts are in the range of less than 1 ppm.

Table 3. Selected ^{13}C NMR data for the tetrasaccharide **3**, sucrose (**1**) and isomaltulose.

C-no.	Tetrasaccharide (**3**)	Sucrose (**2**)	Isomaltulose	Difference between **3** and sucrose/ isomaltulose
7	61.08	62.79		−1.71
8	103.21	104.12		−0.91
13	60.54		63.19	−2.65
14	103.76		102.17	1.59

Note: Glucose C1–C6 (sucrose component), fructose C7–C12 (sucrose component), fructose C13–C18 (isomaltulose component), glucose C19–C24 (isomaltulose component).

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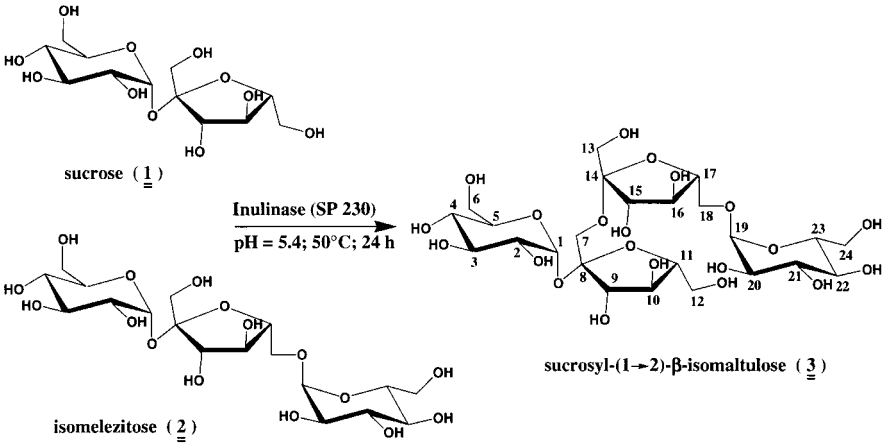

Scheme 1. Synthesis of sucrosyl-(1→2)-β-isomaltulose (**3**).

Based on these results the linkage of the two components is formed by the bond between the two fructose moieties, that is, a linkage between C7 (the primary C1 in the fructose of the sucrose component) and C14 (the anomeric C-atom in the fructose of the isomaltulose component). These findings were unambiguously substantiated by the long range $^{13}C-^{1}H$ correlation data.

The results show that a novel tetrasaccharide **3** was obtained by incubating sucrose and isomelezitose with inulinase (Sch. 1) having the structure sucrosyl-(1→2)-β-isomaltulose (**3**,α-D-glucopyranosyl-(1→2)-β-D-fructofuranosyl-(1→2)-β-D-fructofuranosyl-(6→1)-α-D-glucopyranoside).

The synthesis can only be rationalised by the enzymatic transfer of an isomaltulose moiety, originating from the isomelezitose (**2**) as donor molecule, to sucrose (**1**) as acceptor molecule with the enzyme inulinase catalysing the formation of the 1,2-β-linkage. Furthermore, the energy-rich linkage of the sucrosyl moiety in the isomelezitose molecule ($\Delta G^{\circ} = -24.5\,\mathrm{kJ/mol}$)[9] maybe considered to be the reason for this transfer of an isomaltulose moiety from the isomelezitose (**2**).

The enzymatic transfer of a saccharide-moiety with a DP > 1 is rare. For example, the branching enzyme (EC 2.4.1.18), responsible for the glycogen synthesis, produces α-1,6-branching linkages by a transglycosylation reaction of α-1,4-glucan. This enzyme first cleaves α-1,4-glucosidic linkages of α-glucan and then transfers the chain fragment at a non-reducing end of α-1,4-glucan to create α-1,6-branching linkages.[10]

The enzymatic transfer of a disaccharide-moiety originating in the sucrose moiety of a trisaccharide donor molecule has not been described previously to our knowledge.

EXPERIMENTAL

General Methods

1D (^{1}H, ^{13}C, and DEPT-135) and 2D homonuclear (COSY and TOCSY with a mixing time of 110 msec) and heteronuclear (one-bond and multiple-bond $^{13}C-^{1}H$ shift

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correlations) NMR spectra were recorded of a D_2O solution of the tetrasaccharide (40 mg in 0.7 mL) at 300 K on a Bruker AVANCE DMX-600 spectrometer locked to the deuterium signal of the solvent. Chemical shifts are given in parts per million (ppm) downfield from tetramethylsilane. The mass spectrum was recorded on a MAT-95-XLT Thermo Finnigan (Bremen/Germany) mass spectrometer using the electrospray-ionisation mode. The GPC was performed on Fractogel HW 40S (Toso Haas) with a refractive index detector at a pressure of 5 bar and a temperature of 60°C. The mobile phase was deionised water at a flow rate of 540 mL/hr.

The carbohydrates were detected by HPAEC (column: Carbopac PA 1.4 mm × 250 mm, anion exchanger; pre-column: Carbopac PA1, 4 mm × 50 mm, anion exchanger; eluent: 0.22 M NaOH with a 0–20% gradient of 0.5 M sodium acetate in 15 min; flow rate: 1 mL/min; sample ($c = 50\,\mu g/mL$): 0.02 mL; detection: pulsed amperometric detector: gold electrode, potentials (E) and pulse time settings (t) of the detector $E = 0.05$ V and $t = 480$ msec, $E = 0.60$ V and $t = 300$ msec, and $E = -0.60$ V and $t = 240$ msec).

The novel tetrasaccharide **3** was obtained by reacting sucrose **1** (10 g, 29 mmol) and isomelezitose **2** (10 g, 20 mmol) in sodium acetate buffer (30 mL, 0.05 M), pH 5.4 at 50°C for 7 hr in the presence of inulinase (0.1 mL; NOVO SP 230, 1800 INU/mL). The reaction was stopped by heating the solution to 90°C for 15 min. The product was isolated from the reaction solution by GPC on Fractogel HW 40S.

Rechromatography on the same column gave the tetrasaccharide **3** with a minimum purity of 93% and a yield of 500 mg (5%).

The hydrolysis experiments were performed in 0.5% oxalic acid at 65°C. Samples were taken between 5 and 150 min. The cooled and diluted samples were analysed by HPAEC.

^{1}H NMR (600 MHz, D_2O): $\delta = 3.47$ (t, H-22), 3.49 (t, H-4), 3.56 (q, H-2), 3.59 (q, H-20), 3.68–3.97 (m, H-3, H-4, H-5, H-6, H-6′, H-7, H-7′, H-11, H-12, H-12′, H-13, H-13′, H-18, H-18′, H-21, H-23, H-24, H-24′), 4.04 (m, H-17), 4.07 (m, H-10), 4.14 (t, H-16), 4.21 (d, H-15), 4.28 (d, H-9), 5.01 (d, H-19), 5.45 (d, H-1).

^{13}C NMR (150 MHz, D_2O): $\delta = 60.08$ (t, C-6), 60.42 (t, C-24), 60.54 (t, C-13), 61.08 (t, C-7), 62.15 (t, C-12), 68.81 (t, C-18), 69.14 (d, C-4), 69.38 (d, C-22), 71.08 (d, C-2), 71.33 (d, C-20), 71.95 (d, C-23), 72.43 (d, C-5), 72.56 (d, C-3), 72.91 (d, C-21), 73.83 (d, C-10), 74.83 (d, C-16), 76.42 (d, C-15), 76.62 (d, C-9), 79.14 (d, C-17), 81.21 (d, C-11), 92.43 (d, C-1), 98.48 (d, C-19), 103.21 (s, C-8), 103.76 (s, C-14).

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