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Oligosaccharide Structures Formed During the Hydrolysis of Lactose by *Aspergillus oryzae* β-Galactosidase

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ABSTRACT

Di-, tri-, tetra-, penta- and hexasaccharides were formed during the hydrolysis of lactose by transgalactosylation reaction of Aspergillus oryzae *β-galactosidase. In this study the isolation and characterization of the major constituents of tri-, tetra- and pentasaccharides are described. The structure elucidation of 3 tri-, 2 tetra- and 1 pentasaccharides was carried out by methylation analysis, mass spectrometry and* ^{13}C*-nmr spectrometry. The trisaccharides are* O-β-D-*galactopyranosyl-(1→3)-*O-β-D-*galactopyranosyl-(1→4)-*D-*glucose (3'-galactosyl-lactose),* O-β-D-*galactopyranosyl-(1→6)-*β-D-*galactopyranosyl-(1→4)-*D-*glucose (6'-galactosyl-lactose) and* O-β-D-*galactopyranosyl-(1→4)-*O-[β-D-*galactopyranosyl-(1→6)]-*D-*glucose (4,6-digalactosyl-glucose). Tetrasaccharides are* O-β-D-*galactopyranosyl-(1→6)-*O-β-D-*galactopyranosyl-(1→6)-*O-β-D-*galactopyranosyl-(1→4)-*D-*glucose and* O-β-D-*galactopyranosyl-(1→6)-*O-β-D-*galactopyranosyl-(1→3) [or* O-β-D-*galactopyranosyl-(1→3)-*O-β-D-*galactopyranosyl-(1→6)]-*O-β-D-*galactopyranosyl-(1→4)-*D-*glucose. Pentasaccharide is* O-β-D-*galactopyranosyl-(1→6)-*O-β-D-*galactopyranosyl-(1→6)-*O-β-D-*galactopyranosyl-(1→6)-*O-β-D-*galactopyranosyl-(1→4)-*D-*glucose.*

INTRODUCTION

β-Galactosidases (β-D-galactoside galactohydrolase, E.C. 3.2.1.23) are known to catalyze transgalactosylation reactions as well as hydrolysis.

The structures of 5 di-, 4 tri- and 1 tetrasaccharides formed during the hydrolysis of lactose with *Aspergillus niger* (Toba & Adachi, 1978), *Escherichia coli* (Huber *et al.*, 1976), *Kluyveromyces fragilis* (Pazur, 1953, 1954; Pazur *et al.*, 1958; Toba & Adachi, 1978), *K. lactis* (Asp *et al.*, 1980), *Pencillium chrysogenum* (Ballio & Russi, 1960), *Sporobolomyces singularis* (Gorin *et al.*, 1964) and *Streptococcus thermophilus* (Greenberg & Mahoney, 1983) β-galactosidase were elucidated.

Preliminary experiments showed that *A. oryzae* β-galactosidase (Galantase,® Tokyo Tanabe Co. Ltd, Tokyo, Japan) has better transfer ability than *A. niger* (Kyowa Hakko Co. Ltd, Tokyo, Japan), bovine liver (Sigma Chemical Co.), *Escherichia coli* ML 309 (Kaigai Seiyaku Co. Ltd, Tokyo, Japan) or *K. fragilis* (Kyowa Hakko Co. Ltd) with tri- and higher oligosaccharide production.

The present paper describes the structure elucidation of tri-, tetra- and pentasaccharides formed by transgalactosylation reaction of *A. oryzae* β-galactosidase by methylation analysis, mass spectrometry and ^{13}C-nmr spectrometry.

EXPERIMENTAL

Isolation of oligosaccharides

One-hundred-and-twenty-five millilitres of 30 w/v% lactose (reagent grade, Wako Pure Chemical Industries Co. Ltd, Osaka, Japan) in pH 4·8, 0·05M citrate–sodium phosphate buffer was incubated for 8 h at 37 °C with 1500 units of *A. oryzae* β-galactosidase (Galantase,® Tokyo Tanabe Co. Ltd, Tokyo, Japan). One unit is the quantity of enzyme which will form 1 μmole of *O*-nitrophenol per minute at 30 °C from *O*-nitrophenyl-β-D-galactoside. Small amounts of toluene were added. The reaction was stopped by heating the reaction mixture in a boiling water bath for 10 min.

The carbohydrates in the reaction mixture were adsorbed on 200 g of charcoal–100 g of Celite on a Buchner funnel and were desorbed by a discontinuous gradient of water (3000 ml) and ethanol in water (15, 30 and 50%, respectively, each 3000 ml). The eluent was then demineralized by Amberlite IR-120B (H^+) and Amberlite IRA-45 (OH^-). The tri- and higher oligosaccharides were desorbed by 30% ethanol in water and the

oligosaccharides in the eluent were further purified by preparative paper chromatography.

Paper chromatography

Paper chromatography was performed on Whatman No. 3MM paper by the descending method for 8 days or 4–15 ascents using butan-1-ol–pyridine–water (6:4:3). Sugars were stained with silver nitrate reagent (Trevelyan *et al.*, 1950).

Methylation analysis as intact compound

The oligosaccharide (500 μg) was reduced with 0·5 ml of 1% sodium borodeuteride for 18 h at 5 °C. Thereafter, the solution was treated with Bio-Rad AG50W-X8(H^+) resin (100–200 mesh), filtered and concentrated to dryness *in vacuo*. To remove boric acid, methanol was evaporated from the residue several times. The resulting oligosaacharide alditol was methylated according to the method of Hakomori (1964). The permethylated oligosaccharide alditol was purified as suggested by Yamashita *et al.* (1978) and injected into a Hitachi M-52 gas chromatograph–mass spectrometer with a 3% SE-30 on Uniport B, 80–100 mesh, 1000 × 3 mm, glass column. Carrier gas: He, 0·9 kg/cm^2; injector: 240 °C; column temperature: 230 °C; chamber voltage: 25 eV; total emission: 100 μA; chamber temperature: 180 °C. The total mass-range covered was from m/z 0 to 750. Spectra were also taken on a JEOL JMS-01 SG-2 mass spectrometer using an ionization voltage of 20 eV with a direct inlet system.

Methylation analysis as alditol acetates

The methylated oligosaccharide alditol (500 μg) was hydrolyzed (2N trifluoroacetic acid 1 ml, 105 °C, 6 h), the acid being removed by evaporation under the nitrogen atmosphere and dried over KOH overnight. 0·5 ml of 1% sodium borodeuteride was added to the dry residue and the mixture kept for 18 h at 5 °C. Thereafter, the solution was treated with acetic acid and concentrated in a nitrogen atmosphere. To remove boric acid, methanol was evaporated from the residue several times. Acetic anhydride (0·5 ml) was added to the dry residue and the mixture kept at 100 °C for 8 h. After the solvents were removed, toluene

was evaporated from the residue several times and the alditol acetates, extracted into chloroform, were injected into a Hitachi M-52 gas chromatograph–mass spectrometer with a 3% SE-30 on Uniport B, 80–100 mesh, 1000 × 3 mm glass column. Carrier gas: He, 0·9 kg/cm^2; injector: 240 °C; column temperature: 230 °C; chamber voltage: 25 eV; total emission: 100 μA; chamber temperature: 180 °C. The total mass range covered was from m/z 0 to 750.

Carbohydrate analysis

Sugar composition and identification of reducing end residue were determined by gas–liquid chromatography. The oligosaccharide (500 μg) was hydrolyzed with 2N trifluoroacetic acid (1 ml) at 105 °C for 6 h, the acid being removed by evaporation under a nitrogen atmosphere and dried over KOH overnight. The monosaccharides were converted to the trimethylsilyl (TMS) derivatives by means of hexamethyldisilazane and trifluoroacetic acid in pyridine (Brobst & Lott, 1966). The TMS derivatives were injected into a Hitachi 163 gas chromatograph equipped with a 2% Dexsil 300GC on Uniport HP, 60–80 mesh, 500 × 3 mm stainless steel column. Carrier gas: N_2, 40 ml/min; injector and detector temperature: 350 °C; column temperature: 150–350 °C, 10 °C/min.

The oligosaccharide was reduced with sodium borohydride and hydrolyzed with 2N trifluoroacetic acid for 6 h at 105 °C. After removal of the acid by evaporation, ethyl acetate (50 μl) and trifluoroacetic anhydride (30 μl) were added to the dry residue. The mixture was kept at room temperature for 2 h (Imanari *et al.*, 1969). The trifluoroacetates were injected into a Hitachi 163 gas chromatograph equipped with a 10% SE-30 on Chromosorb W AM-DMCS, 80–100 mesh, 1000 × 3 mm stainless steel column. Carrier gas: N_2, 30 ml/min; injector and detector temperature: 250 °C; column temperature: 90 or 110 °C.

^{13}C-Nmr spectrometry

Proton decoupled spectra were obtained with a JEOL model JNM-FX100 (25 MHz) spectrometer in D_2O (Merck, 99·7%) at 30 °C, operating in the Fourier transform mode, using a sweep width of 5 kHz, 8192 data points, a pulse width of 7 μs and a pulse angle of 45°. Chemical shifts are given relative to 3-(trimethylsilyl) propionic acid sodium salt (TSP-d_4).

RESULTS

Fractionation and purification of oligosaccharides

Figure 1 shows the paper chromatogram of oligosaccharides in the lactose hydrolyzates with *A. oryzae* β-galactosidase and oligosaccharides eluted from charcoal–celite with water, 15, 30 and 50% ethanol. The chromatogram shows that the 30% ethanol fraction contains only tri- and higher oligosaccharides. Thirty per cent of the ethanol fraction contains at least fifteen different oligosaccharides (OS1–OS15), as shown in Fig. 2. As Fig. 1 shows, five disaccharides are formed; twenty di- and higher oligosaccharides are formed during the enzymatic hydrolysis of lactose by *A. oryzae* β-galactosidase.

By preparative chromatography, ten oligosaccharide (OS2, OS3, OS4, OS6, OS10, OS11, OS12, OS13, OS14 and OS15) were isolated from the 30% ethanol fraction. Details of chromatoraphic mobilities, sugar composition, sugars at the reducing end and partial mass spectra of permethylated alditol of these ten oligosaccharides are given in Table 1. All ten contain glucose at the reducing end. The molar ratio of galactose and glucose and the fragments in the mass spectra indicate that OS2 is a hexasaccharide, OS3 and OS4 are pentasaccharides, OS6, OS10, OS11 and OS12 are tetrasaccharides and OS13, OS14 and OS15, trisaccharides.

Table 2 lists ^{13}C-nmr chemical shifts of the anomeric carbons of OS4, OS6, OS10, OS13, OS14 and OS15. All the anomeric carbons except C-1 resonate in the region 105·7–107·2 ppm and hence the galactose residues at the internal and non-reducing positions are linked via a β-like linkage (Bradbury & Jenkins, 1984).

Structure of oligosaccharides

OS4

The fragments *m/z* 848 (M-219-16), 644, 440, 236, 831(M-236-16), 627, 423 and 219 of the permethylated reduced oligosaccharide (Table 1) prove it to be a linear pentasaccharide. Gas chromatographic–mass spectrometric analysis of the reduced pentasaccharide as the permethylated alditol acetates indicates the presence of a 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylhexitol residue accompanying a 4-*O*-acetyl-1,2,3,5,6-penta-*O*-methylhexitol residue located at the reducing terminus and a 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol residue located at the non-reducing

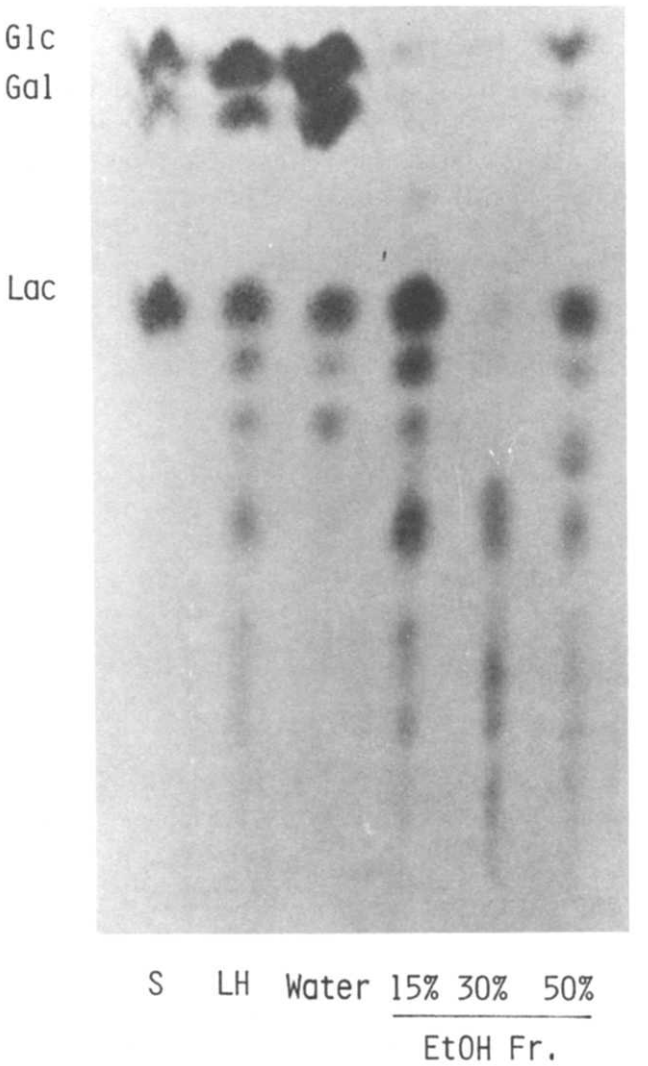

Fig. 1. Paper chromatography of the oligosaccharides eluted from a charcoal–Celite layer with water, 15, 30 and 50% ethanol. S, standard compounds; LH, lactose hydrolyzate; solvent, butan-1-ol–pyridine–water (6:4:3), four ascents.

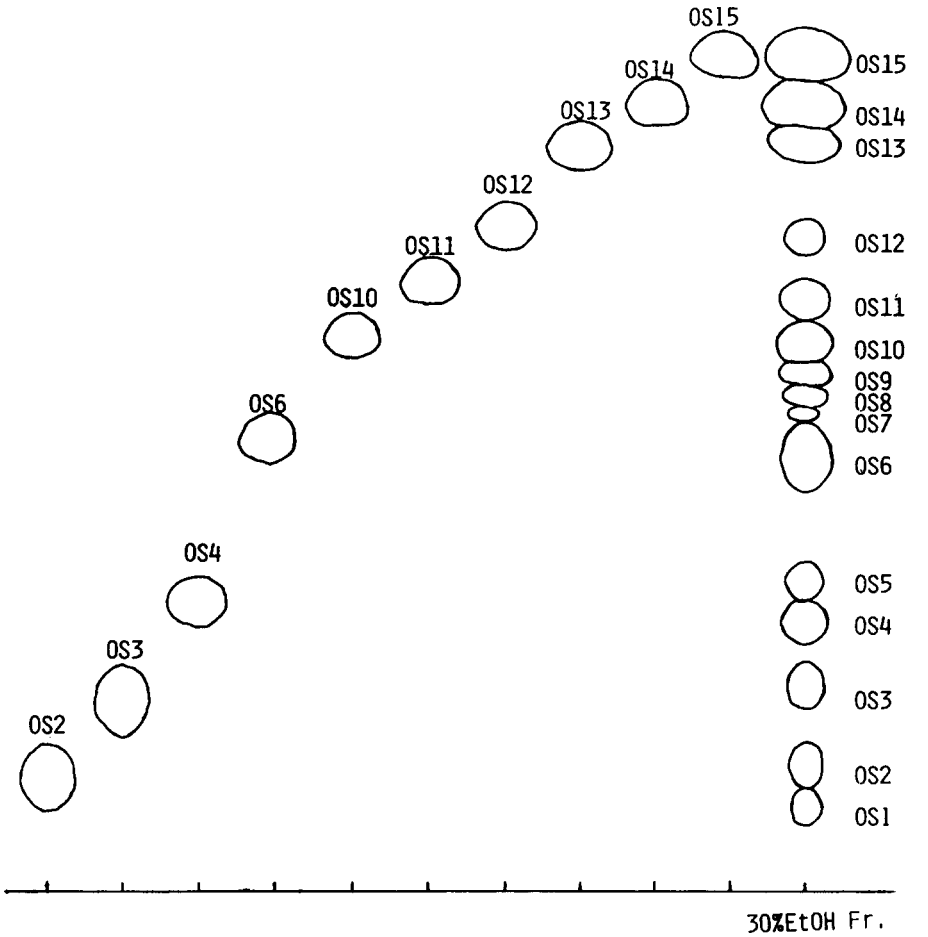

Fig. 2. Paper chromatography of the oligosaccharides isolated from the lactose hydrolyzate. Solvent, butan-1-ol–pyridine–water (6:4:3), fifteen ascents.

terminus (Table 3). It can be concluded that all the internal hexose residues are substituted at position 6 and the penultimate hexose residue must be linked 1→4 to the reducing terminus.

On the basis of chemical, mass spectral and nmr data, oligosaccharide OS4 is established to be β-Gal*-(1→6)-β-Gal-(1→6)-β-Gal-(1→6)-β-Gal-(1→4)-Glc.†

* Gal = D-galactose.
† Glc = D-glucose.

TABLE 1
Characteristics of Ten Oligosaccharides Isolated from the Lactose Hydrolyzate

Oligosaccharide	R_{OS15}[a]	*Molar ratios of*		*Sugars in reducing position*	*Fragments (m/z) in mass spectra of permethylated alditol*[b]
		Gal	*Glc*		
OS2	0·17	4·97	1	Glc	1053, 1052 (M-219-16), 1035, 848, 831, 644, 627, 440, 423, 236, 219 (base), 187
OS3	0·26	4·00	1	Glc	848 (M-219-16), 847, 831, 830, 802, 644, 627, 440, 423, 296, 236, 219 (base), 187
OS4	0·35	4·05	1	Glc	849, 848 (M-219-16), 831, 644, 627, 440, 423, 236 (base), 219, 187, 134, 133, 101
OS6	0·53	3·05	1	Glc	644 (M-219-16), 627, 598, 440, 429, 423, 281, 236, 219, 207, 187, 111, 101, 88 (base)
OS10	0·68	3·03	1	Glc	
OS11	0·74	2·92	1	Glc	
OS12	0·81	3·08	1	Glc	
OS13	0·90	2·13	1	Glc	541 (M-134), 440 (M-219-16), 382, 249, 219, 204, 187, 155, 134, 133, 111, 101, 88 (base)
OS14	0·94	1·98	1	Glc	441, 440 (M-219-16), 296, 236, 219, 187, 172, 134, 115, 111, 101, 89, 88 (base)
OS15	1·00	2·08	1	Glc	585 (M-90), 542, 541 (M-134), 440 (M-219-16), 423 (M-236-16), 236, 219, 187 (base), 172, 159, 155, 134, 127, 111, 101, 89, 88, 75, 71

[a] Mobility relative to that of OS15 in solvent system of butan-1-ol–pyridine–water (6:4:3) for fifteen ascents. Relative mobility of OS15 to lactose was 0·61 in the same solvent system for a 7-day descent.
[b] Deuterated derivatives.

TABLE 2

^{13}C-nmr Chemical Shifts[a] of the Anomeric Carbons[b] for the Oligosaccharides Isolated from the Lactose Hydrolyzate

Oligosaccharide	*C''''-1*	*C'''-1*	*C''-1*	*C'-1*	*C-1*	
					β	*α*
OS4	107·12		106·20	105·86	98·50	94·60
OS6		106·49		105·91	98·55	94·65
OS10		107·13	106·10	105·86	98·55	94·65
OS13			106·00	105·70	98·70	94·60
OS14			106·15	105·96	98·55	94·65
OS15			107·17	105·42	98·60	93·58

[a] Chemical shifts (ppm) from TSP-d_4.

[b] Unprimed numbers refer to reducing residue, primed numbers to next residue, etc.

TABLE 3

Alditol Acetates Present in the Hydrolyzate of the Permethylated Oligosaccharide Alditols Isolated from the Lactose Hydrolyzate

Permethylated hexitol acetate[a]	*Oligosaccharide*					*Prominent peaks (m/z)*
	OS4	*OS6*	*OS10*	*OS14*	*OS15*	
4-Ac-1,2,3,5,6-Me_5-[b]	+	+	+	+	+	205, 173, 131, 128, 113, 101 (base), 90, 59, 46, 43
1,5-Ac_2-2,3,4,6-Me_4-	+	+	+	+	+	205, 162, 161, 145, 129, 118, 113, 102, 101, 89, 88, 87, 75, 71, 43 (base)
1,5,6-Ac_3-2,3,4-Me_3-	+	+	+	+		233, 189, 173, 162, 159, 129, 118, 113, 102, 99, 87, 43 (base)
1,3,5-Ac_3-2,4,6-Me_3-			+		+	234, 233, 202, 161, 129, 118, 102, 88, 71, 59, 45, 43 (base)

[a] Deuterated derivatives.

[b] Ac = acetyl. Me = methyl.

OS6

The fragments *m/z* 644 (M-219-16), 440, 236, 627(M-236-16), 423 and 219 in the mass spectrum of the permethylated reduced oligosaccharide (Table 1) prove it to be a linear tetrasaccharide. Gas chromatographic–mass spectrometric analysis of the reduced tetrasaccharide as the permethylated alditol acetates indicates the presence of a 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylhexitol residue accompanying a 4-*O*-acetyl-1,2,3,5,6-penta-*O*-methylhexitol residue located at the reducing terminus and of a 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol residue located at the non-reducing terminus (Table 3). It can be concluded that all the internal hexose residues are substituted at position 6 and the penultimate hexose residue must be linked 1→4 to the reducing terminus.

Based on chemical, mass spectral and nmr data, oligosaccharide OS6 is established to be β-Gal-(1→6)-β-Gal-(1→6)-β-Gal-(1→4)-Glc.

OS10

The fragments *m/z* 644 (M-219-16), 440, 236, 627(M-236-16), 423 and 219 in the mass spectrum of the permethylated reduced oligosaccharide (Table 1) prove it to be a linear tetrasaccharide. Gas chromatographic–mass spectrometric analysis of the reduced tetrasaccharide as permethylated alditol acetate indicates the presence of a 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylhexitol and a 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylhexitol residue accompanying a 4-*O*-acetyl-1,2,3,5,6-penta-*O*-methylhexitol residue located at the reducing terminus and of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol residue located at the non-reducing terminus (Table 3). It can be concluded that the internal hexose residues were substituted at positions 3 and 6, while the penultimate hexose residue must be linked 1→4 to the reducing terminus.

On the basis of chemical, mass spectral and nmr data, oligosaccharide OS10 is established to be β-Gal-(1→6)-β-Gal-(1→3)-β-Gal-(1→4)-Glc or β-Gal-(1→3)-β-Gal-(1→6)-β-Gal-(1→4)-Glc.

OS13

The absence of the fragment *m/z* 236 and the presence of *m/z* 440 with a high intensity in the mass spectrum of the permethylated deuteride-reduced oligosaccharide (Fig. 3) indicate a double substitution of the reducing glucose residue. The presence of the fragments *m/z* 134 and 382 shows that galactose is attached to C-4 of glucose, while the presence of fragment *m/z* 249 shows that galactose is attached to C-6 of glucose.

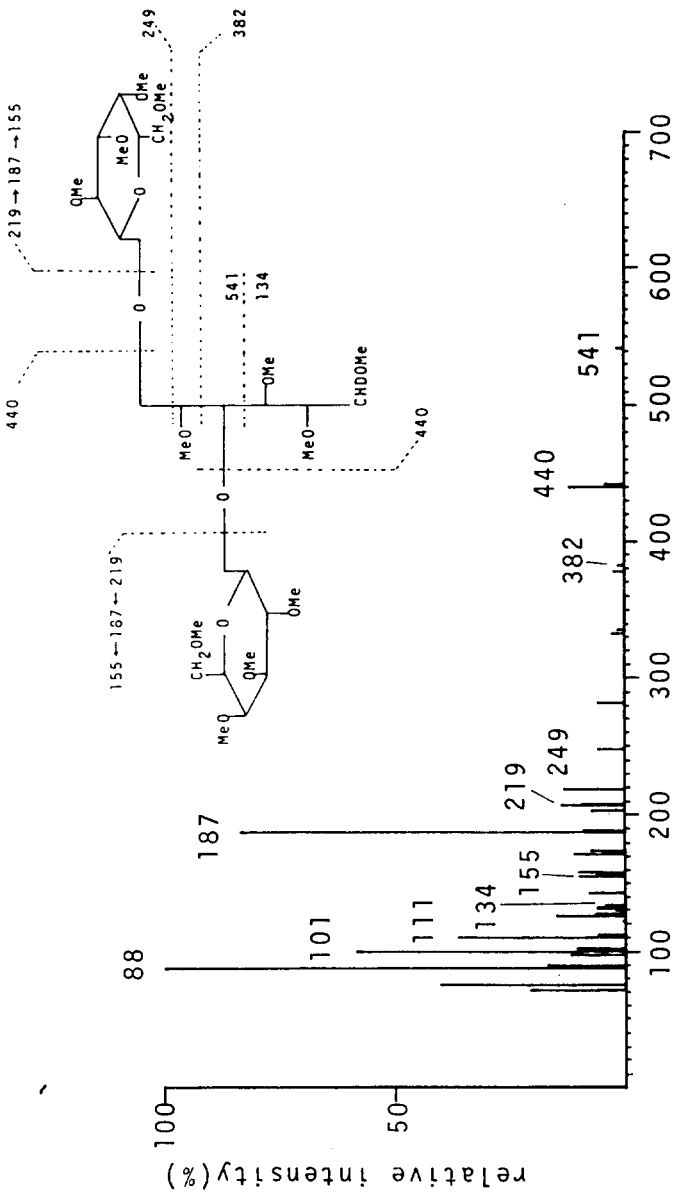

Fig. 3. Mass spectrum and fragmentation pattern of the permethylated oligosaccharide OS13 alditol.

Based on chemical, mass spectral and nmr data, the structure of oligosaccharide OS13 is β-Gal-(1→6)-[β-Gal-(1→4)] Glc (4,6-digalactosyl-glucose).

OS14

The fragments *m/z* 440, 236, 423 and 219 in the mass spectrum of the permethylated reduced oligosaccharide (Table 1) prove it to be a linear trisaccharide. Gas chromatographic–mass spectrometric analysis of the reduced trisaccharide as permethylated alditol acetate indicates the presence of a 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylhexitol residue accompanying a 4-*O*-acetyl-1,2,3,5,6-penta-*O*-methylhexitol residue located at the reducing terminus and of a 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol residue located at the non-reducing terminus (Table 3). It can be concluded that the internal hexose residue was substituted at position 6 and the penultimate hexose residue must be linked 1→4 to the reducing terminus.

On the basis of chemical, mass spectral and nmr data the structure of oligosaccharide OS14 is established to be β-Gal-(1→6)-β-Gal-(1→4)-Glc (6′-galactosyl-lactose).

OS15

The fragments *m/z* 440, 236, 423 and 219 in the mass spectrum of the permethylated reduced oligosaccharide (Table 1) prove it to be a linear

TABLE 4

Structures of the Oligosaccharides Isolated from the Lactose Hydrolyzate with *Aspergillus oryzae* β-Galactosidase

OS4	β-D-Gal-(1→6)-β-D-Gal-(1→6)-β-D-Gal-(1→6)-β-D-Gal-(1→4)-D-Glc[a]
OS6	β-D-Gal-(1→6)-β-D-Gal-(1→6)-β-D-Gal-(1→4)-D-Glc
OS10	β-D-Gal{(1→6)-β-D-Gal-(1→3) / (1→3)-β-D-Gal-(1→6)}β-D-Gal-(1→4)-D-Glc
OS13	β-D-Gal-(1→6) / β-D-Gal-(1→4) > D-Glc
OS14	β-D-Gal-(1→6)-β-D-Gal-(1→4)-D-Glc
OS15	β-D-Gal-(1→3)-β-D-Gal-(1→4)-D-Glc

[a] Gal = D-galactose. Glc = D-glucose.

trisaccharide. Gas chromatographic–mass spectrometric analysis of the reduced trisaccharide as permethylated alditol acetate indicates the presence of a 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylhexitol accompanying a 4-*O*-acetyl-1,2,3,5,6-penta-*O*-methylhexitol residue located at the reducing terminus and of a 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol residue located at the non-reducing terminus (Table 3). It can be concluded that the internal hexose residue was substituted at position 3 and the penultimate hexose residue must be linked $1 \rightarrow 4$ to the reducing terminus.

Based on chemical, mass spectral and nmr data, the structure of oligosaccharide OS15 is established to be β-Gal-$(1 \rightarrow 3)$-β-Gal-$(1 \rightarrow 4)$-Glc (3′-galactosyl-lactose).

The structures of the oligosaccharides OS4, OS6, OS10, OS13, OS14 and OS15 are summarised in Table 4.

DISCUSSION

In the present paper, twenty di- and higher oligosaccharides are detected in the lactose hydrolyzate of *A. oryzae* β-galactosidase (Galantase®). The numbers of di- and higher oligosaccharides formed during enzymatic hydrolysis of lactose are 11–12, at most, with *K. fragilis* (Roberts & Pettinati, 1957; Toba & Adachi, 1978) and *A. niger* (Toba & Adachi, 1978) β-galactosidase. Thus, the number of oligosaccharides formed in our study was about twice as many as those reported by the above workers.

The quantitative and qualitative analyses of tri- and tetrasaccharides formed during the enzymatic lactose hydrolysis are reported by several researchers (Burvall *et al.*, 1979; Deya *et al.*, 1982; Huber *et al.*, 1976; Nakanishi *et al.*, 1983). At 20% lactose Burvall *et al.* (1979) obtained 13% oligosaccharides by *K. lactis* β-galactosidase and, at 4·65% lactose, Nakanishi *et al.* (1983) obtained about 13% trisaccharides and about 2% tetrasaccharides by the *Bacillus circulans* enzyme. Huber *et al.* (1976) obtained a maximum oligosaccharide production of 32% of the total sugar at a concentration of 17·1% lactose with *E. coli* β-galactosidase. Although they reported considerably more tri- and tetrasaccharide formation than others, Huber *et al.* (1976) mention that penta- or higher oligosaccharides are not detected in lactose hydrolyzate by the *E. coli* enzyme.

However, Deya *et al.* (1982) show the production of one pentasaccharide other than tri- and tetrasaccharides by *A. oryzae* β-galactosidase (Sumylact LL) from lactose. In the present study, one hexasaccharide (OS2), two pentasaccharides (OS3 and OS4), four tetrasaccharides (OS6, OS10, OS11 and OS12), and three trisaccharides (OS13, OS14 and OS15) are verified. By comparison of paper chromatographic mobilities with these oligosaccharides, OS1 is tentatively presumed to be a hexasaccharide, OS5 is a pentasaccharide and OS7, OS8 and OS9, tetrasaccharides. Therefore, two hexasaccharides, three pentasaccharides, seven tetrasaccharides and three trisaccharides are formed during the hydrolysis of 30% lactose with *A. oryzae* β-galactosidase (Galantase®). It appears that β-galactosidase from *A. oryzae* tends to form higher oligosaccharides compared with enzymes of other origins.

In this study the structures of six oligosaccharides of fifteen oligosaccharides formed have been elucidated. Oligosaccharide OS6 was previously found in lactose hydrolyzates with *K. lactis* β-galactosidase (Asp *et al.*, 1980). Oligosaccharide OS14 is also reported to be present in the hydrolyzate of lactose using β-galactosidase from *K. lactis* (Asp *et al.*, 1980), *P. chrysogenum* (Ballio & Russi, 1960) and yeast (Pazur, 1953; 1954). Deya *et al.* (1982) presume the oligosaccharides OS13, OS14 and OS15 are present in the hydrolyzate of lactose with *A. oryzae* β-galactosidase (Sumylact LL) by partial acid hydrolysis, whereas the oligosaccharides OS4 and OS10 have not, to our knowledge, previously been identified in the enzymatic lactose hydrolyzate.

Asp *et al.* (1980) reveal that 1→6 galactosidic linkages are formed preferentially during the hydrolysis of lactose by *K. lactis* β-galactosidase, where the results of Deya *et al.* (1982) and our own results show that 1→3 galactosidic linkages are formed, in addition to 1→6 linkages, by the *A. oryzae* enzyme. Each of the oligosaccharides determined in the lactose hydrolyzate of *A. oryzae* β-galactosidase characteristically has a lactose residue at the reducing end, whereas the oligosaccharides in the lactose hydrolyzate of the *K. lactis* enzyme also contain an allolactose residue at the reducing end (Asp *et al.*, 1980).

It can be expected that detailed study of other oligosaccharides presented in the lactose hydrolyzate by *A. oryzae* β-galactosidase will detect the oligosaccharide previously found with *K. lactis* (Asp *et al.*, 1980), *S. singularis* (Gorin *et al.*, 1964) and yeast (Pazur, 1953; 1954) enzymes and furnish more precise information about the specificity of the galactosidic linkages formed.

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