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Note

Epimerization of reducing terminal groups of (1 → 2)-linked D-gluco- and D-manno-disaccharides in aqueous sodium hydroxide

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Podzorski et al.[1] reported that a mixture of parent and epimerized disaccharides, obtained by β-elimination in aqueous 0.1 M NaOH at 25° from an α-D-Man*p*-(1 → 2)-D-Man group *O*-glycosidically linked to the peptide moiety of the cell-wall D-mannan of a pathogenic yeast (*Candida albicans* strain ATCC 44806), exhibited a significant suppressive effect on the growth of human peripheral blood lymphocytes in vitro. In consequence, we have studied the epimerization of α-D-Man*p*-(1 → 2)-D-Man, along with other (1 → 2)-linked D-gluco- and D-manno-disaccharides, namely α-D-Glc*p*-(1 → 2)-D-Glc, β-D-Glc*p*-(1 → 2)-D-Glc, and β-D-Man*p*-(1 → 2)-D-Man.

Fig. 1 shows the epimerization kinetics for the four (1 → 2)-linked bioses, depicted by plotting the ratio of amounts of each pair of the starting and resultant bioses by ^{1}H-NMR spectroscopy according to the previous description by Tsai et al.[2]. The assignment of anomeric-proton chemical shifts for each disaccharide is shown in Table I. The four starting bioses epimerized under first-order conditions from 0 to 9 h, giving rise to the reaction velocity-constants, 5.11×10^4, 7.04×10^4, 8.62×10^4, and 4.69×10^4 for the epimerization of α-D-Glc*p*-(1 → 2)-D-Glc, β-D-Glc*p*-(1 → 2)-D-Glc, α-D-Man*p*-(1 → 2)-D-Man, and β-D-Man*p*-(1 → 2)-D-Man, respectively. The ratios of the four C-2 epimers of the reducing terminal groups, α-D-Glc*p*-(1 → 2)-D-Man, β-D-Glc*p*-(1 → 2)-D-Man, α-D-Man*p*-(1 → 2)-D-Glc, and β-D-Man*p*-(1 → 2)-D-Glc, to the amounts of the corresponding starting bioses on a weight basis were 30.3, 47.0, 54.5, and 28.6%, respectively.

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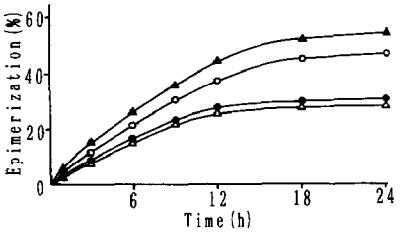

Fig. 1. Time courses of epimerization of the disaccharides: α-D-Glc*p*-(1 → 2)-D-Glc (●); β-D-Glc*p*-(1 → 2)-D-Glc (○); α-D-Man*p*-(1 → 2)-D-Man (▲); and β-D-Man*p*-(1 → 2)-D-Man (△). Each disaccharide was dissolved in 0.1 M NaOH at 25°. The molar ratio of starting biose and the corresponding epimer was determined by ^{1}H-NMR spectroscopy[2].

One of the four reaction mixtures containing an epimerization product, α-D-Man*p*-(1 → 2)-D-Glc, and the starting biose, α-D-Man*p*-(1 → 2)-D-Man, was fractionated to isolate the former biose. The biose mixture was treated with *Arthrobacter* GJM-1 exo-α-D-mannosidase followed by gel chromatography on a column of Bio-Gel P-2. The elution profile of the enzymolysis products is shown in Fig. 2. This treatment degraded the remaining α-D-Man*p*-(1 → 2)-D-Man to D-mannose, which was then separated from α-D-Man*p*-(1 → 2)-D-Glc by gel chromatography. The yield of α-D-Man*p*-(1 → 2)-D-Glc was 52% on a weight basis from the parent biose, and was thus in agreement with the epimer ratio determined by ^{1}H-NMR spectrometry (Fig. 1). The results of chemical analyses of α-D-Man*p*-(1 → 2)-D-Glc in comparison with those for α-D-Man*p*-(1 → 2)-D-Man are summarized in Table II. These data also indicate that the epimerization product of α-D-Man*p*-(1 → 2)-D-Man is α-D-Man*p*-(1 → 2)-D-Glc. The higher dextrorotation of α-D-Man*p*-(1 → 2)-D-Glc (+98.0°) than that (+43.8°) of α-D-Man*p*-(1 → 2)-D-Man, also substantiates the finding that the 2-hydroxy group epimerizes from the *S* to the *R* configuration in a sugar residue of the D-series.

Fig. 3 shows the H–C COSY spectra of α-D-Man*p*-(1 → 2)-D-Man (A) and α-D-Man*p*-(1 → 2)-D-Glc (B). The chemical shifts of the ^{1}H- and ^{13}C-NMR signals assigned according to Allerhand and Berman[3] for α-D-Man*p*-(1 → 2)-D-Man and

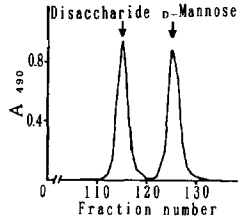

Fig. 2. Elution pattern of the *Arthrobacter* GJM-1 exo-α-D-mannosidase digestion-product of the α-D-Man*p*-(1 → 2)-D-Man and α-D-Man*p*-(1 → 2)-D-Glc mixture. This was applied to a column of Bio-Gel P-2 (2.5 × 100 cm), which was then developed with water (0.25 mL/min). Carbohydrate was determined by the phenol–H_2SO_4 method[13] on 50-μL aliquots.

TABLE I

Chemical shifts of the H-1 protons in the NMR spectra of (1 → 2)-linked D-gluco- and D-manno-disaccharides and their C-2 epimers

N [a] R	Chemical shift of H-1 proton (ppm) N	R
Starting disaccharide		
α-D-Glc*p*-(1 → 2)-α-D-Glc	5.077, 5.087 (3.92)	5.410, 5.419 (3.92)
α-D-Glc*p*-(1 → 2)-β-D-Glc	5.352, 5.3621 (3.92)	4.777, 4.796 (7.80)
β-D-Glc*p*-(1 → 2)-α-D-Glc	4.618, 4.638 (7.80)	5.403, 5.413 (3.92)
β-D-Glc*p*-(1 → 2)-β-D-Glc	4.705, 4.724 (7.80)	4.750, 4.770 (7.80)
α-D-Man*p*-(1 → 2)-α-D-Man	5.046, 5.051 (1.96)	5.350, 5.353 (1.48)
α-D-Man*p*-(1 → 2)-β-D-Man	5.138, 5.142 (1.44)	4.892
β-D-Man*p*-(1 → 2)-α-D-Man	4.757	5.280, 5.284 (1.48)
β-D-Man*p*-(1 → 2)-β-D-Man	4.813	4.968
Epimerized disaccharide		
α-D-Glc*p*-(1 → 2)-α-D-Man	5.112, 5.121 (3.92)	5.383, 5.386 (1.48)
α-D-Glc*p*-(1 → 2)-β-D-Man	5.160, 5.170 (3.92)	4.899
β-D-Glc*p*-(1 → 2)-α-D-Man	4.618, 4.638 (7.80)	5.289, 5.292 (1.48)
β-D-Glc*p*-(1 → 2)-β-D-Man	4.489, 4.508 (7.80)	4.956
α-D-Man*p*-(1 → 2)-α-D-Glc	5.020, 5.025 (1.96)	5.427, 5.435 (3.40)
α-D-Man*p*-(1 → 2)-β-D-Glc	5.250, 5.253 (1.44)	4.715, 4.734 (7.80)
β-D-Man*p*-(1 → 2)-α-D-Glc	4.823	5.397, 5.389 (3.40)
β-D-Man*p*-(1 → 2)-β-D-Glc	4.938	4.720, 4.739 (7.80)

[a] N; nonreducing terminal group, R; reducing terminal group, ( ); $J_{1,2}$ (Hz).

by Usui et al.[4] for α-D-Glc*p*-(1 → 2)-D-Glc are listed in Table III. These results confirm the structural change of the reducing terminal group of α-D-Man*p*-(1 → 2)-D-Man to α-D-Man*p*-(1 → 2)-D-Glc.

We conclude that, in 0.1 M NaOH solution during 24 h at 25°, (1 → 2)-linked D-gluco- and D-manno-disaccharides are epimerized to give respectively the isomers containing the corresponding epimeric monosaccharide residues, Man and Glc, as the reducing-terminal residues. One of the epimerization products, α-D-

TABLE II

Sugar component, methylation, and specific rotation analyses of disaccharides

		Disaccharide	
		α-D-Man*p*-(1 → 2)-D-Man	α-D-Man*p*-(1 → 2)-D-Glc
Molar ratio of sugar components			
D-Mannose (1.00) [a]		99.9	49.7
D-Glucose (2.82)		ND [b]	50.1
Methylation analysis (acetylated alditol derivatives)			
2,3,4,6-tetra-*O*-methyl-D-glucitol	(1.00)	ND	ND
2,3,4,6-tetra-*O*-methyl-D-mannitol	(1.00)	1.00	1.00
3,4,6-tri-*O*-methyl-D-glucitol	(1.84)	ND	0.93
3,4,6-tri-*O*-methyl-D-mannitol	(1.93)	0.88	ND
Specific rotation [a]			
$[\alpha]_D^{20}$ (*c* 1.0, water)		+43.8°	+98.0°

[a] ( ); retention time in GLC analysis as described by Lindberg[13]. [b] ND; not detected.

Man*p*-(1 → 2)-D-Glc, was isolated from the epimerization mixture of α-D-Man*p*-(1 → 2)-D-Man by treatment with exo-α-D-mannosidase followed by gel chromatography.

TABLE III

Anomeric proton and carbon signals (chemical shift, ppm) for α-D-Manp-(1 → 2)-D-Man and α-D-Manp-(1 → 2)-D-Glc

	α-D-Man*p*-(1 → 2)-α-D-Man		α-D-Man*p*-(1 → 2)-β-D-Man	
H-1	5.046 5.051 (1.96) [a]	5.350 5.354 (1.48)	5.138 5.142 (1.44)	4.895 5.025 (1.96)
C-1	102.95	93.47	102.31	94.30
C-2	70.90	79.87	71.02	79.31
C-3	71.28	70.90	71.20	74.32
C-4	67.79	68.04	67.72	67.64
C-5	74.08	73.40	73.82	77.54
C-6	61.93	61.96	62.01	61.79
	α-D-Man*p*-(1 → 2)-α-D-Glc		α-D-Man*p*-(1 → 2)-β-D-Glc	
H-1	5.020 5.435 (3.40)	5.427 5.253 (1.44)	5.250 4.734 (7.80)	4.715
C-1	98.31	90.12	100.67	97.02
C-2	71.13	76.71	71.15	80.15
C-3	71.31	72.16	71.23	75.36
C-4	67.59	70.54	67.57	70.68
C-5	73.72	72.23	73.68	76.04
C-6	61.58	61.77	61.74	61.79

[a] ( ), $J_{1,2}$ (Hz).

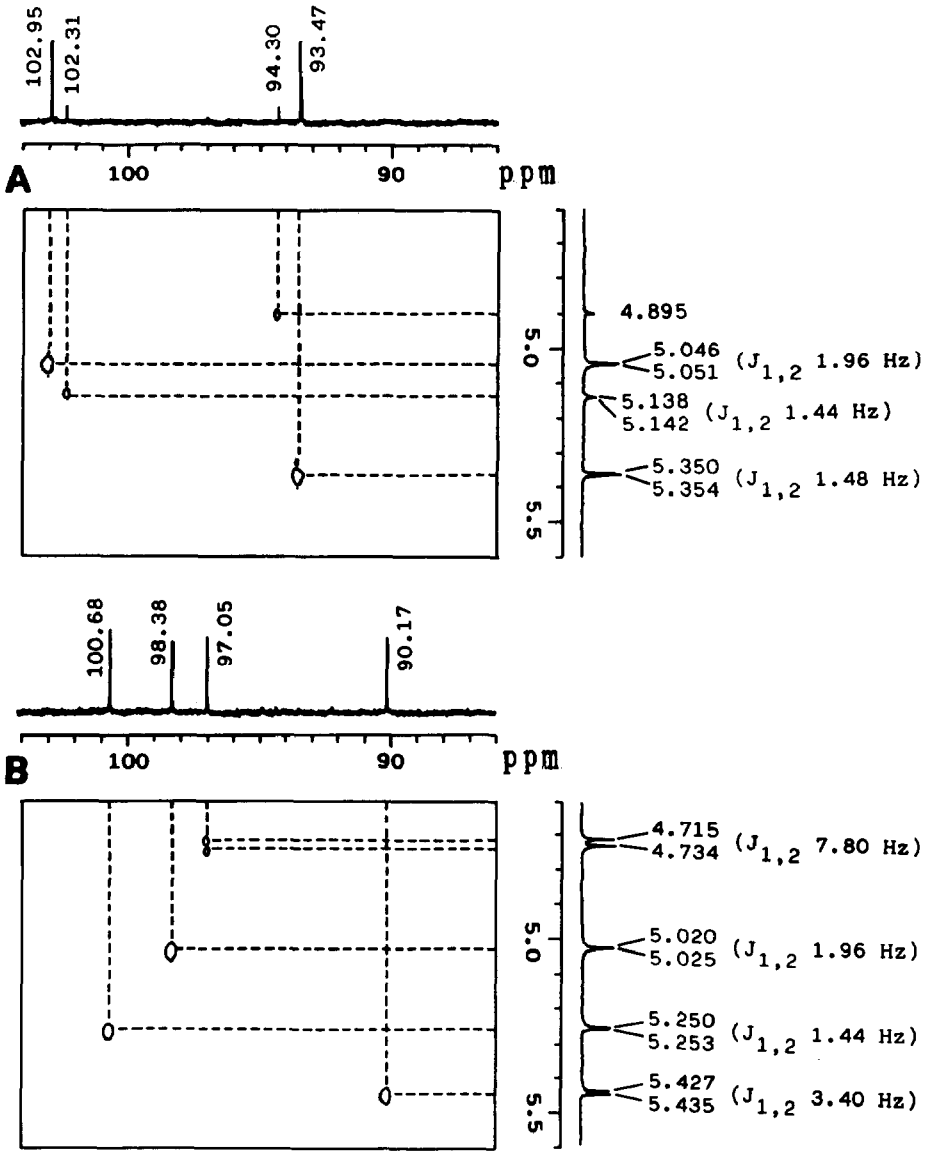

Fig. 3. H–C COSY spectra of α-D-Man*p*-(1 → 2)-D-Man (**A**) and α-D-Man*p*-(1 → 2)-D-Glc (**B**). Each biose (1%, w/v) was dissolved in D_2O and the determination was conducted at 55° using acetone (2.217 ppm) and CD_3OD (49.00 ppm) as the internal standards in accordance with the description by Kobayashi *et al.* [6].

EXPERIMENTAL PROCEDURES

General. — ^{1}H-NMR spectra were measured as described by Kobayashi et al. [5] by use of a Jeol JNM-GSX 400 spectrometer. ^{1}H–^{13}C correlation (H–C COSY) spectra were measured with the same spectrometer as described by Kobayashi et

al.[6]. Specific rotations were determined by means of a JAS DIP-360 digital polarimeter ($c = 1.0$, $l = 1.0$) The sample was dissolved in water, and measurement made 3 h after dissolution in water at 20°.

Materials. — Kojibiose, α-D-Glc*p*-(1 → 2)-D-Glc, was kindly donated by Dr. T. Nakajima, Tohoku University, Sendai, Japan. Sophorose [β-D-Glc*p*-(1 → 2)-D-Glc] was purchased from Extrasynthese Laboratories, Genay, France. α-D-Man*p*-(1 → 2)-D-Man was a stock specimen of our laboratory isolated from the D-mannans of *C. albicans* strains[7,8] by acetolysis[9,10]. β-D-Man*p*-(1 → 2)-D-Man was isolated from the same D-mannans by acid treatment [11] with hot 10 mM HCl for 1 h. The *Arthrobacter* GJM-1 exo-α-D-mannosidase was the same specimen as used in previous work[12]. The column packing for gel chromatography, Bio-Gel P-2 (−400 mesh), fractionation range 100–1800 daltons, was purchased from Bio-Rad Laboratories, Richmond, CA, USA.

Alkali treatment of disaccharides. — Each disaccharide (35 mg) was dissolved in 0.1 M NaOH (35 mL) and the solution was kept at 25°. Aliquots (5 mL) were taken at intervals of 1, 3, 6, 9, 12, 18, and 24 h. Each solution was neutralized with 0.5 M HCl, concentrated in vacuo, and deionized by the addition of a mixture of Amberlite IR-120 (H^+) and Amberlite IR-410 (OH^-) resins. The supernatant was collected by filtration, and the resin thoroughly washed with water. The combined filtrate and washings were concentrated in vacuo to dryness. To determine the molar ratio of the starting biose and its epimer, each reaction mixture was analyzed by ^{1}H-NMR spectroscopy[2]. The epimerization ratio of the starting bioses at each interval was computed by the following formula: epimerization ratio $(\%) = (A/A + B) \times 100$, where A represents the integration of H-1 signals of ^{1}H-NMR chemical shifts corresponding to epimerization products, and B indicates the integration of H-1 signals corresponding to the unchanged parent disaccharides. The results are summarized in Table I.

Digestion of the epimerization mixture from α-D-Manp-(1 → 2)-D-Man by exo-α-D-mannosidase. — Essentially the method of Kobayashi et al.[5] was used. The reaction product was applied to a column (2.5 × 100 cm) of Bio-Gel P-2 that was eluted with water (0.25 mL/min). Aliquots (20 μL) of the eluates, were assayed for carbohydrate content by the phenol–H_2SO_4 method[13]. Eluates corresponding to the elution position of biose were combined, concentrated in vacuo, and lyophilized.

Sugar-component analysis of the exo-α-D-mannosidase-indigestible epimerization product from α-D-Manp-(1 → 2)-D-Man. — The epimerization product (5 mg) dissolved in 0.5 M H_2SO_4 (20 mL) was heated for 5 h in a boiling-water bath and then the solution was deionized by using a mixture of Amberlite IR-120 (H^+) and Amberlite IR-410 (OH^-) resins. The supernatant was collected by filtration, and the resin was thoroughly washed with water. The combined filtrate and washings were concentrated in vacuo to dryness and the residue was dissolved in 0.5 M borate buffer (pH 8.7, 200 μL), and the solution was analyzed by high-performance liquid chromatography using a TOSOH CCPM liquid chromatograph

equipped with a column (4.5 × 150 mm) of TOSOH TSKgel Sugar AXG. Elution was conducted with the same buffer at 100 kg/cm^2, and the eluate was monitored for monosaccharides. Under these chromatographic conditions, the relative retention-times for D-glucose and D-mannose were 2.82 and 1.00, respectively. Conversion of peak dimensions into the molar ratio of monosaccharides was done by means of an SIC Chromatocorder II.

*Methylation analysis of epimerized disaccharide obtained from α-D-Man*p*-(1 → 2)-D-Man*. — Methylation of the disaccharide was conducted by the Hakomori method[14], and the resultant per-*O*-methylated disaccharide was converted into a mixture of *O*-methyl-*O*-acetyl-D-glycitols which were then analyzed by GLC according to Lindberg[15].

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