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Note
Assignment of ^{1}H and ^{13}C NMR chemical shifts of a D-mannan composed of α-(1 → 2) and α-(1 → 6) linkages obtained from *Candida kefyr* IFO 0586 strain

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Recently, Kanbe and Cutler [1] reported that the α-linked oligo-D-mannosyl side-chains of a cell-wall D-mannan of the pathogenic yeast *Candida albicans* is in large part responsible for the binding of yeast cells to the marginal zone of mouse spleen. Stratford [2] also noted the importance of α-linked oligo-D-mannosyl side-chains of cell wall D-mannan in the mechanism of several types of yeast flocculation. Furthermore, Nelson and co-workers [3,4] reported that the alkali-released α-linked D-manno-oligosaccharides obtained from a *C. albicans* cell-wall D-mannan were potent inhibitors of lymphoproliferation induced by the parent D-mannan. These facts seem of interest from the viewpoints of both host–parasite interactions and the biological roles of carbohydrates.

The outer chain moiety of many yeast D-mannans has a long backbone consisting solely of α-(1 → 6)-linked D-mannopyranose units to which are attached many side-chains containing various kind of linkages attached to O-2 of D-mannopyranose residues

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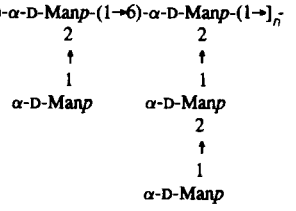
of the backbone in a comb-like structure [5–7]. In previous papers [8–12], it was shown that the assignments of the ^{1}H and ^{13}C chemical shifts for various oligosaccharides corresponding to the side-chains obtained from such types of yeast D-mannans could be utilized for the structural analysis of the parent D-mannans using such 2D-NMR techniques as $^{1}H-^{13}C$ COSY and/or 2D-HOHAHA.

Tsai et al. [13] reported the assignment of H-1 chemical shift of an α-(1 → 6)-linked oligo-D-mannosyl backbone substituted by one D-mannopyranose through O-2 in the α configuration, by the analysis of an inner-core moiety of D-mannan obtained from *Saccharomyces cerevisiae mnn* 2 mutant strain. Recently, Shibata et al. [11] made assignments of H-1 and H-2 chemical shifts of the same unit substituted by α-(1 → 2)-linked oligo-D-mannosyl side chains based on the findings of Tsai et al. [13] and Hernandez et al. [14]. The assignment of these ^{1}H and ^{13}C chemical shifts may be useful for the structural determination of the outer chain moiety of D-mannans using NMR analyses, without recourse to such destructive procedures as methylation analysis, acid hydrolysis, acetolysis, and so on.

Because of the structural simplicity of the D-mannan of the pathogenic yeast *Candida kefyr* IFO 0586, this D-mannan may be utilized to elucidate the cell–cell interaction mechanisms in host–parasite interactions of the genus *Candida*. Therefore, we assigned the chemical shifts in the ^{1}H and ^{13}C NMR spectra of this D-mannan (Fr. K) which has a long α-(1 → 6)-linked D-mannopyranosyl backbone and many short α-(1 → 2)-linked D-mannopyranosyl side-chains in a comb-like structure as follows [15].



In order to obtain oligosaccharides retaining the α-(1 → 6)-linked D-mannopyranose unit of the backbone, Fr. K was subjected to mild acetolysis [16]. Fig. 1A shows the elution profile of the acetolysates of Fr. K containing D-manno-oligosaccharides of dp 2–5 (M_2–M_5, respectively) on Bio-Gel P-2. The $^{1}H-^{13}C$ COSY spectra of M_2 and M_3 (Figs. 2A and B) indicated that these oligosaccharides have the structures **1** and **2**, respectively. However, in the spectra of M_4 and M_5 (Figs. 2C and D), the presence of a cross-peak (H-1, 5.082 or 5.083 ppm; C-1, 99.06 ppm) suggest that these oligosaccharides contain α-(1 → 6)-linked D-mannopyranose residues based on previous findings concerning the D-mannan of *S. cerevisiae* wild-type strain [17].

These oligosaccharides (M_4 and M_5) were therefore reduced with $NaBH_4$ and then conventionally acetolyzed. Figs. 1B and C show the elution profiles of the products obtained from reduced M_4 and M_5 after acetolysis. Each oligosaccharide gave two major products, M_4-I and M_4-II, and M_5-I and M_5-II, respectively. The identical

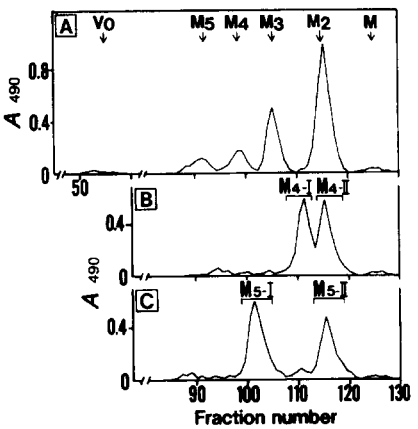


Fig. 1. Gel-filtration of the products obtained from Fr. K by mild acetolysis (A), and those obtained from the reduced tetraose (B) and pentaose (C) by conventional acetolysis. M_5–M_2 and M indicate D-manno-oligosaccharides, pentaose, tetraose, triose, and biose, and D-mannopyranose, respectively.

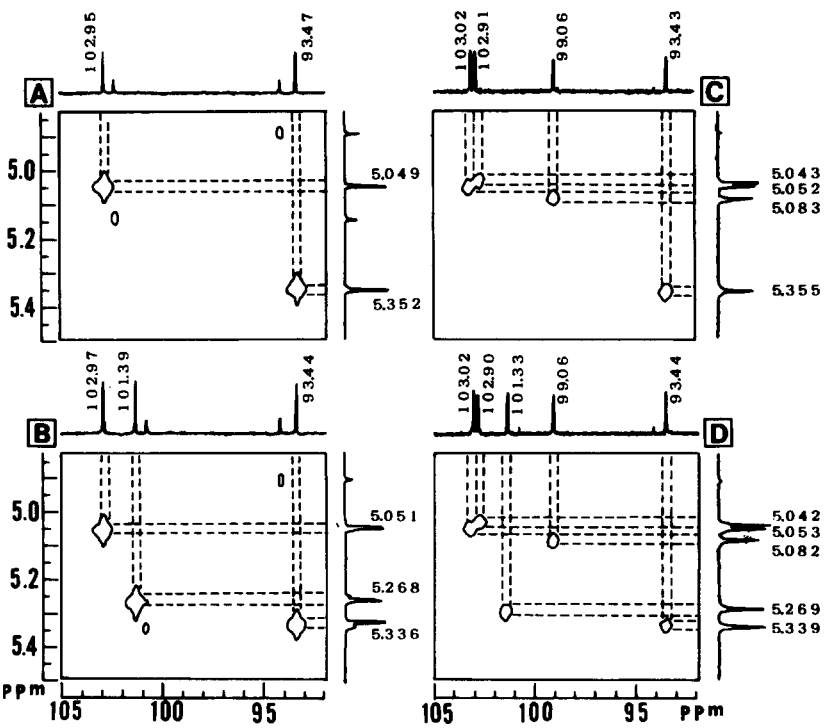


Fig. 2. ^{1}H–^{13}C COSY (anomeric region) spectra of D-manno-oligosaccharides, M_2 (A), M_3 (B), M_4 (C), and M_5 (D).

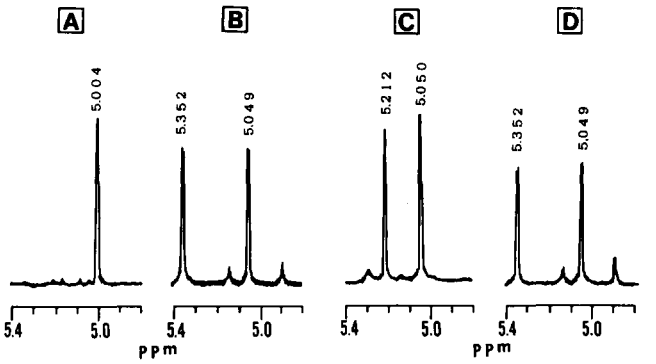


Fig. 3. ^{1}H NMR (H-1 region) spectra of the acetolysates, M_4-I (A), M_4-II (B), M_5-I (C), and M_5-II (D), obtained from the reduced D-mannotetraose and D-mannopentaose, respectively.

retention times of M_4-II and M_5-II agree with that of M_2 already described and the product was identified as D-mannobiose (**1**) by ^{1}H NMR analysis (Figs. 3B and D). The ^{1}H NMR spectra of M_4-I and M_5-I (Figs. 3A and C) were in agreement with the structures of the reduced biose and triose (**3** and **4**, respectively). Therefore, the two oligosaccharides, M_4 and M_5, obtained from Fr. K by mild acetolysis were identified as **5** and **6**, respectively. The D-mannopentaose corresponding to **6** was obtained by Stewart et al. [18] from *S. cerevisiae* wild type D-mannan by short-term acetolysis. However, the D-mannotetraose corresponding to **5** has not hitherto been obtained, and therefore the assignment of chemical shifts of M_4 (**5**) together with M_5 (**6**) had not yet been made. ^{1}H–^{13}C COSY spectrometry of M_2–M_5 (**1**, **2**, **5**, and **6**, respectively) resulted in the assignments of the ^{1}H (H-1) and ^{13}C (C-1) signals as shown in Table 1. The result of assignment of the O-2 substituted α-(1 → 6)-linked D-mannopyranose unit (residue 1‴ corresponding to the cross-peak (H-1, 5.082 ppm; C-1, 99.06 ppm) in the spectra of M_4 and M_5 (Figs. 2C and D) is similar to that of the assignment for the same position of the

Table 1
^{1}H and ^{13}C NMr chemical shifts of D-manno-oligosaccharides (α anomer) obtained from Fr. K

Structure	Chemical shift (ppm)					
	H-1			C-1		
	Residue			Residue		
	1″	1′ 1⁗	1 1‴	1″	1′ 1⁗	1 1‴
1		5.049	5.352		102.95	93.47
2	5.051	5.268	5.336	102.97	101.39	93.44
5		5.052	5.355		103.02	93.43
		5.043	5.083		102.91	99.06
6	5.053	5.269	5.339	103.02	101.33	93.44
		5.042	5.082		102.90	99.06

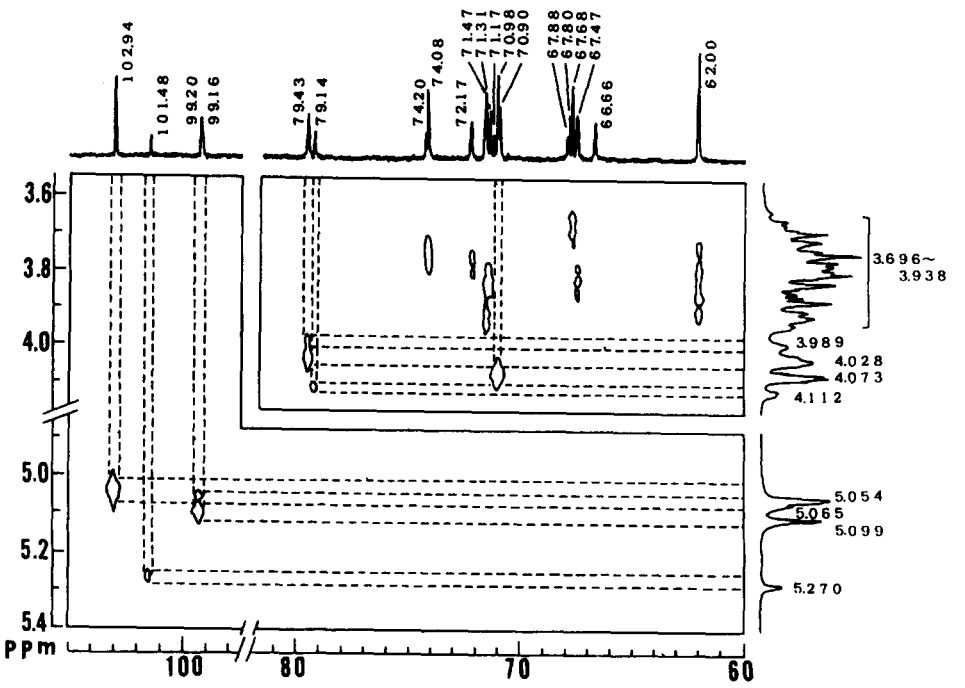


Fig. 4. $^{1}H-^{13}C$ COSY spectrum of Fr. K.

D-mannopyranose unit in D-mannopentaose (7) obtained from *S. cerevisiae* wild type D-mannan [17].

α-D-Man*p*-(1→2)-[α-D-Man*p*-(1→2)]$_n$-D-Man
1 n=0
2 n=1

α-D-Man*p*-(1→2)-[α-D-Man*p*-(1→2)]$_n$-D-Man-ol
3 n=0
4 n=1

α-D-Man*p*-(1→2)-[α-D-Man*p*-(1→2)]$_n$-D-Man
6
↑
1
α-D-Man*p*-(1→2)-α-D-Man*p*
5 n=0
6 n=1

α-D-Man*p*-(1→3)-α-D-Man*p*-(1→2)-[α-D-Man*p*-(1→2)]$_2$-D-Man
6
↑
1
α-D-Man*p*-(1→2)-α-D-Man*p*

7

Table 2
^{1}H and ^{13}C NMR chemical shifts of D-mannan, Fr. K

Structure	Chemical shift (ppm)			
	H-1 (H-2)		C-1 (C-2)	
[→6)-α-D-Man*p*-(1→6)-α-D-Man*p*-(1→]$_n$	5.099	5.065	99.20	99.16
2↑ 2↑	(4.027)	(3.989)	(79.43)	(79.43)
1 1				
α-D-Man*p* α-D-Man*p*	5.054	5.270	102.94	101.48
2↑	(4.073)	(4.112)	(70.98)	(79.14)
1				
α-D-Man*p*		5.054		102.94
		(4.073)		(70.98)

Fig. 4 shows the $^{1}H-^{13}C$ COSY spectrum of Fr. K. The strong cross-peak (H-1, 5.054 ppm; C-1, 102.94 ppm) and the weak one (H-1, 5.270 ppm; C-1, 101.48 ppm) correspond to the nonreducing terminal and internal α-(1 → 2)-linked D-mannopyranose units, respectively. Therefore, the other two cross-peaks, (H-1, 5.099 ppm; C-1, 99.20 ppm) and (H-1, 5.065 ppm; C-1, 99.16 ppm), were identified as being from the 2,6-branched D-mannopyranose units of the backbone, that is, the former strong and the latter weak cross-peaks correspond to the D-mannopyranose units in the α-(1 → 6)-linked D-mannan backbone substituted at O-2 by single D-mannopyranosyl and α-(1 → 2)-linked D-mannobiosyl units in the α configuration. This finding indicates that the H-1 signal of the O-2 substituted D-mannopyranose units in the α-(1 → 6)-linked D-mannan backbone bearing oligo-D-mannosyl side-chains is shifted upfield in comparison with that of a D-mannopyranose substituted one. Further, this interpretation is supported by the result of acetolysis of Fr. K, which gave a large amount of M_2 and relatively small amounts of M_3 (Fig. 2A). Therefore, both H-1 signals of two kinds of O-2 substituted D-mannopyranose units in the α-(1 → 6)-linked D-mannan backbone in Fr. K are confirmed to appear downfield of the unsubstituted one, 4.905 ppm [15,17]. The H-2 chemical shifts were assigned by 2D-HOHAHA analysis of Fr. K (spectrum not shown, see ref. [15]). Therefore, the ^{1}H (H-1 and H-2) and ^{13}C (C-1 and C-2) chemical shifts in the spectrum of Fr. K were assigned as in Table 2.

The present study achieves the assignments of ^{1}H (H-1 and H-2) and ^{13}C (C-1 and C-2) chemical shifts of 2,6-branched D-mannopyranose residue constituting the connecting point between the backbone and the side-chain of a D-mannan having a comb-like structure. These assignments should prove useful for the structural determination of various yeast D-mannans by $^{1}H-^{13}C$ COSY and/or 2D-HOHAHA NMR analyses, without any chemical degradation procedures.

1. Experimental

Materials.—The mannan of *C. kefyr* IFO 0586 strain, Fr. K, was the specimen used in a previous study [15]. Bio-Gel P-2 (400 mesh), fractionation range 100–1800 Da, was purchased from Bio-Rad (Richmond, CA, USA).

Mild acetolysis of Fr. K.—This procedure was as described by Kobayashi et al. [16], using 100:100:1 Ac_2O–AcOH–H_2SO_4 for 36 h at 40°C. After *O*-deacetylation, the mixture of oligosaccharides was fractionated on a column (2.5 × 100 cm) of Bio-Gel P-2.

Reduction of oligosaccharides M4 and M5.—This was done as previously described [17].

Acetolysis of reduced M4 and M5.—This was performed as previously described [19]; a modification of the method of Kocourek and Ballou [20], with 10:10:1 Ac_2O–AcOH–H_2SO_4 for 13 h at 40°C. After *O*-deacetylation, the mixture of oligosaccharides was fractionated on a column (2.5 × 100 cm) of Bio-Gel P-2.

NMR spectroscopy.—^1H NMR spectra (internal acetone, 2.217 ppm) were measured with a Jeol JNM-GSX 400 spectrometer on solutions (3–10 mg sample/0.7 mL) in D_2O at 70°C. ^{13}C NMR spectra (internal CD_3OD, 49.00 ppm) were measured with the same spectrometer on solutions (15–25 mg sample/0.7 mL) in D_2O at 55°C. ^1H–^{13}C COSY spectra were also recorded under the same conditions as for the ^1H and ^{13}C NMR spectra. The 2D-HOHAHA spectrum was recorded for a solution (10 mg sample/0.7 mL) in D_2O at 45°C.

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