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Note

Assignment of carbon-13 signals in nuclear magnetic resonance spectra of D-galactopyrano-α-D-mannopyranans from yeasts*

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Certain yeasts have cell walls that contain D-galacto-D-mannans having (1→6)-linked α-D-mannopyranosyl main- and side-chains with terminal D-galactopyranosyl units. Many different structures exist, according to the results of methylation analysis and partial acetolysis[1,2]. The latter technique was useful for galactomannans containing an α-D-galactopyranosyl units, as oligosaccharides were obtained, in high yields, with α-D-galactopyranosyl group linked to D-mannose-containing structures. For example, the D-galacto-D-mannan (**1**) of *Schizosaccharomyces octosporus* gave rise to 2-*O*-α-D-galactopyranosyl-D-mannose, and *O*-α-D-galactopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→2)-D-mannose was obtained from D-galacto-D-mannans of *Trichosporon fermentans*[2,3] (**2**) and *Candida lipolytica*[2] (**3**), respectively. D-Galacto-D-mannans having lower specific rotations were isolated from *Torulopsis magnoliae*, *Torulopsis gropengiesseri*, and *Torulopsis lactis-condensi*, but partial acetolysis did not apparently give rise to oligosaccharides containing the inferred β-D-galactopyranosyl residues, as the β-D-glycosidic linkage was comparatively unstable[2].

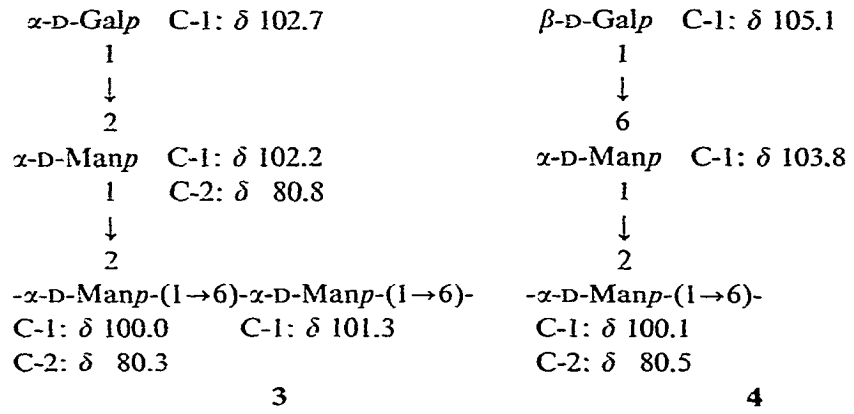
α-D-Gal*p* C-1: δ 102.8
1
↓
2
-α-D-Man*p*-(1→6)-α-D-Man*p*-(1→6)-
C-1: δ 100.0 C-1: δ 101.2
C-2: δ 81.2

1

α-D-Gal*p* C-1: δ 102.7, T_1 0.18 s
1
↓
2
α-D-Man*p* C-1: δ 102.2, T_1 0.10 s
1 C-2: δ 80.8, T_1 0.14 s
↓
2
-α-D-Man*p*-(1→6)-
C-1: δ 100.0, T_1 0.08 s
C-2: δ 80.2, T_1 0.08 s

2

N.R.C.C. No. 20128.



Examination of ^{13}C-n.m.r. spectra (Fig. 1; A,B,C,D,E,F) of the aforementioned six D-galacto-D-mannans showed that structures having β-D-galactopyranosyl groups gave C-1 signals at δ 105.1–104.9, at a field lower[4] than those of D-galacto-D-mannans having α-D-galactopyranosyl groups. A C-5 signal at δ 76.8 was also typical[5,6] of β-D-galactopyranosyl residues.

Assignments were made for other low-field ^{13}C-n.m.r. signals (Fig. 1,A) of the β-D-galacto-α-D-mannan (**4**) of *T. lactis-condensi*. For most D-mannose-containing polysaccharides of yeasts, the C-1 signal at δ 103.8 could be attributed to an α-D-mannopyranosyl, nonreducing end-group linked (1→2) to an adjacent α-D-mannopyranosyl residue[7]. However, previous[2] and present, more-accurate methylation analyses show that only 5% of the terminal D-mannopyranosyl residues are present, together with 32% of 6-*O*-substituted residues, so that the signal arises principally from the latter structure. Signals at δ 100.1 and 80.5 can be attributed, by a process of elimination and by analogy with previous studies[7], to C-1 and C-2, respectively, of the 2,6-di-*O*-substituted α-D-mannopyranosyl residues of the main chain. These data indicate that the galactomannan of *T. lactis-condensi* has a preponderant repeating unit **4**, which differs from the previously proposed structure that had a terminal α-D-galactopyranosyl group. This structure had been assigned on the basis of formation, in 3% yield, of *O*-α-D-galactopyranosyl-(1→6)-*O*-α-D-mannopyranosyl-(1→2)-D-mannose following partial acetolysis. The specific rotation of +99° corresponded to the presence of an α-D-galactopyranosyl group, by analogy with the similar value of *O*-α-D-galactopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→2)-D-mannose[2]. Thus, the galactomannan contains terminal α-D-galactopyranosyl groups, but their proportion is insufficient to give rise to detectable ^{13}C-n.m.r. signals.

The ^{13}C-n.m.r. spectrum of the α-D-galacto-α-D-mannan (**2**) of *Tr. fermentans* contains C-1 signals at δ 102.7 and 102.2, and C-2 signals of 2-*O*-substituted units at δ 80.8 and 80.2 (Fig. 1,D). Such resonances could be assigned by measurement of T_1 values, since, in branched D-mannans, T_1 values of nuclei increase with the increased segmental motion of the hexosyl residues, on going progressively from the main

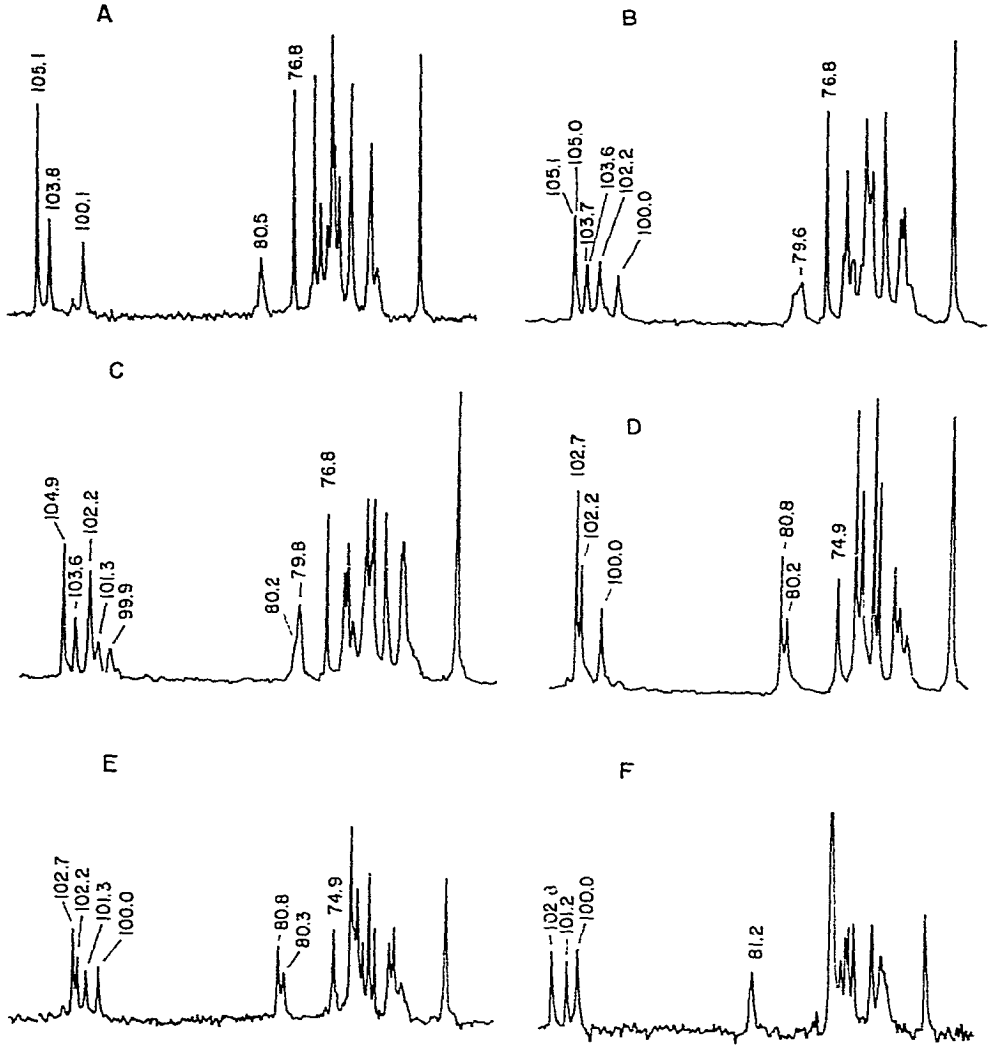


Fig. 1. ^{13}C-N.m.r. spectra of yeast D-galacto-D-mannans for solutions in deuterium oxide at 70°. Shift values are expressed in δ relative to external Me_4Si. The β-D-galacto-D-mannans were extracted from the following yeasts: (A) *Torulopsis lactis-condensi*, (B) *Torulopsis gropengiesseri*, and (C) *Torulopsis magnoliae*; and the α-D-galacto-D-mannans from: (D) *Trichosporon fermentans*, (E) *Candida lipolytica*, and (F) *Schizosaccharomyces octosporus*.

chain to the nonreducing ends[8]. The C-1 signals were assigned according to the increasing T_1 values of 0.08 s (δ 100.0; 2,6-di-*O*-substituted α-D-mannopyranosyl main-chain residues), 0.10 s (δ 102.2; 2-*O*-substituted α-D-mannopyranosyl side-chain residues), and 0.18 s (δ 102.7; nonreducing α-D-galactopyranosyl end-group). Similarly, the signal at δ 80.2 arises from C-2 of 2,6-di-*O*-substituted α-D-mannopyranosyl units of the main chain, since its T_1 value is 0.08 s, which is lower than the

value of 0.14 s for the C-2 resonance at δ 80.8 of the 2-*O*-substituted α-D-mannopyranosyl side-chain residues.

The D-galacto-D-mannan (3) of *C. lipolytica* has a structure similar to that of *Tr. fermentans*, except that many of the (1→6)-linked α-D-mannopyranosyl residues of the main chain are not substituted by side chains, and therefore it gives rise to the typical C-1 signal[7] at δ 101.3 (Fig. 1,E). A similar signal occurs in the ^{13}C-n.m.r. spectrum (Fig. 1,F) of the D-galacto-D-mannan of *S. octosporus*, in accord with its determined structure **1**. The other, low-field signals were assigned as follows: δ 102.8 to C-1 of nonreducing α-D-galactopyranosyl groups, 100.0 to C-1 of 2,6-di-*O*-substituted-α-D-mannopyranosyl main-chain residues, and 81.2 to C-2 of 2,6-di-*O*-substituted α-D-mannopyranosyl main-chain residues.

The C-1 shifts of α- and β-D-galactopyranosyl groups distinguish them from those of β-D-galactofuranosyl groups, which are from δ 109.5 to 106.5. Also, the latter groups give rise to C-2 and -4 signals at comparatively low field[9–13]. However, the C-1 signals of α-D-galactofuranosyl and α-D-galactopyranosyl groups should not be readily distinguishable[14], although those of C-2 and -4 of the former groups should be at low field, at approximately δ 77 and 82, respectively[6].

EXPERIMENTAL

Methods. — ^{13}C-N.m.r. spectra were obtained for solutions of D-galacto-D-mannans in deuterium oxide at 70°, under conditions previously described[15]. T_1 values were measured, for degassed, 20% solutions of D-galacto-D-mannan in deuterium oxide at 70°, by the Freeman–Hill modification[16] of the inversion-recovery, Fourier-transform method. Twenty τ-values were used to define the signals, the minimum accuracy of the method being ±5%.

The D-galacto-D-mannan of *T. lactis-condensi* was methylated successively by the methods of Haworth and Kuhn, and the product converted into partially *O*-methylated alditol acetates. These were identified by g.l.c.–m.s. (electron-impact mode) with ECNSS-M as the liquid phase[17]. The peaks were quantitatively determined by triangulation.

REFERENCES

1 P. A. J. Gorin, J. F. T. Spencer, and D. E. Eveleigh, *Carbohydr. Res.*, 11 (1969) 387–398.
2 P. A. J. Gorin, J. F. T. Spencer, and R. J. Magus, *Can. J. Chem.*, 47 (1969) 3569–3576.
3 P. A. J. Gorin and J. F. T. Spencer, *Can. J. Chem.*, 47 (1968) 2299–2304.
4 L. D. Hall and L. F. Johnson, *J. Chem. Soc., Chem. Commun.*, (1969) 509–510.
5 P. A. J. Gorin, *Can. J. Chem.*, 52 (1974) 458–461.
6 P. A. J. Gorin and M. Mazurek, *Can. J. Chem.*, 53 (1975) 1212–1223.
7 P. A. J. Gorin, *Can. J. Chem.*, 51 (1973) 2375–2883.
8 P. A. J. Gorin and M. Mazurek, *Carbohydr. Res.*, 72 (1979) c1–c5.
9 M. Ogura, T. Kohama, M. Fujimoto, A. Kuniaka, H. Yoshino, and H. Sugiyama, *Agric. Biol. Chem.*, 38 (1974) 2563–2564.
10 L. Mendonça, P. A. J. Gorin, K. O. Lloyd, and L. R. Travassos, *Biochemistry*, 15 (1976) 2423–2431.

11 E. M. BARRETO-BERGTER, L. R. TRAVASSOS, AND P. A. J. GORIN, *Carbohydr. Res.*, 86 (1980) 273–285.
12 L. MENDONÇA-PREVIATO, P. A. J. GORIN, AND L. R. TRAVASSOS, *Infect. Immun.*, 29 (1980) 934–939.
13 P. A. J. GORIN, E. M. BARRETO-BERGTER, AND F. S. DA CRUZ, *Carbohydr. Res.*, 88 (1981) 177–188.
14 P.-E. JANNSON AND B. LINDBERG, *Carbohydr. Res.*, 82 (1980) 97–102.
15 P. A. J. GORIN, R. H. HASKINS, L. R. TRAVASSOS, AND L. MENDONÇA-PREVIATO, *Carbohydr. Res.*, 55 (1977) 21–33.
16 R. FREEMAN AND H. D. W. HILL, *J. Chem. Phys.*, 51 (1969) 3140–3141.
17 P.-E. JANNSON, L. KENNE, H. LIEDGREN, B. LINDBERG, AND J. LÖNNGREN, *Chem. Commun. Univ. Stockholm*, (1976) No. 8.