Carbohydrate Research, 72 (1979) 229–234

Note

Carbon-13 spin–lattice relaxation studies for resonance assignment to specific, carbon positions of dextrans*

FRED R. SEYMOUR,

Fleming Department of Rehabilitation, Baylor College of Medicine, Texas Medical Center, Houston, Texas 77030 (U.S.A.)

ROGER D. KNAPP,

Division of Atherosclerosis and Lipoprotein Research, Baylor College of Medicine and Methodist Hospital, Texas Medical Center, Houston, Texas 77030 (U.S.A.)

AND STEPHEN H. BISHOP**

Marrs McLean Department of Biochemistry, Baylor College of Medicine, Texas Medical Center, Houston, Texas 77030 (U.S.A.)

(Received September 15th, 1978; accepted for publication, October 14th, 1978)

Assignment of ^{13}C-n.m.r. resonances to carbon positions of D-glucan has been made by comparison with spectra of analogous monosaccharides, and by use of the fact that *O*-alkylation results in a down-field, chemical-shift displacement ($\Delta\delta$) of ~10 p.p.m. for the corresponding resonance[2]. Resonances can then be grouped into general classes, but further differentiation is difficult. We now describe an additional method of assignment based on measurements of spin–lattice relaxation times (T_1).

Linear dextran has a simple spectrum (67.8, 71.5, 72.0, 73.2, 75.2, and 99.6 p.p.m.) closely approximating the saccharide resonances of a 1,6-di-*O*-substituted α-D-glucopyranoside monomer, encouraging the analysis of polysaccharide spectra by considering the individual residues as an assembly of specifically *O*-alkylated monosaccharides. Structural variants of linear dextran occur that are due to branched α-D-glucopyranosyl residues; branched dextrans exhibit resonances identical to those of linear dextran, but contain additional resonances proportional in intensity to the degree of branching. The chemical shifts of these branching resonances are dependent on the type, but not on the degree, of branching of a dextran[1–3].

Dextrans contain a terminal, nonreducing group corresponding to each branch-point residue. A dextran branching through C-2 contains equal proportions of 1,2,6-tri- and 1-mono-*O*-substituted α-D-glucopyranoside residues. These two new residues,

*Unusual Dextrans, Part VI. For Part V, see ref. 1.

**Present address: Department of Zoology, Iowa State University, Ames, Iowa 50011, U.S.A.

if considered as isolated assemblies of *O*-alkyl-substituted monosaccharides, give twelve resonances. If these twelve resonances could be resolved, and assigned to specific carbon positions in the polymer sub-unit, it would then be possible to measure the $\Delta\delta$ value for each carbon position of the D-glucopyranoside residue resulting from the formation of an *O*-alkyl (inter-saccharide) linkage at a specific position (*e.g.*, C-2). This information concerning the $\Delta\delta$ value could allow the prediction of the ^{13}C-n.m.r. spectra of other glucans. Such assignments would be greatly simpli-

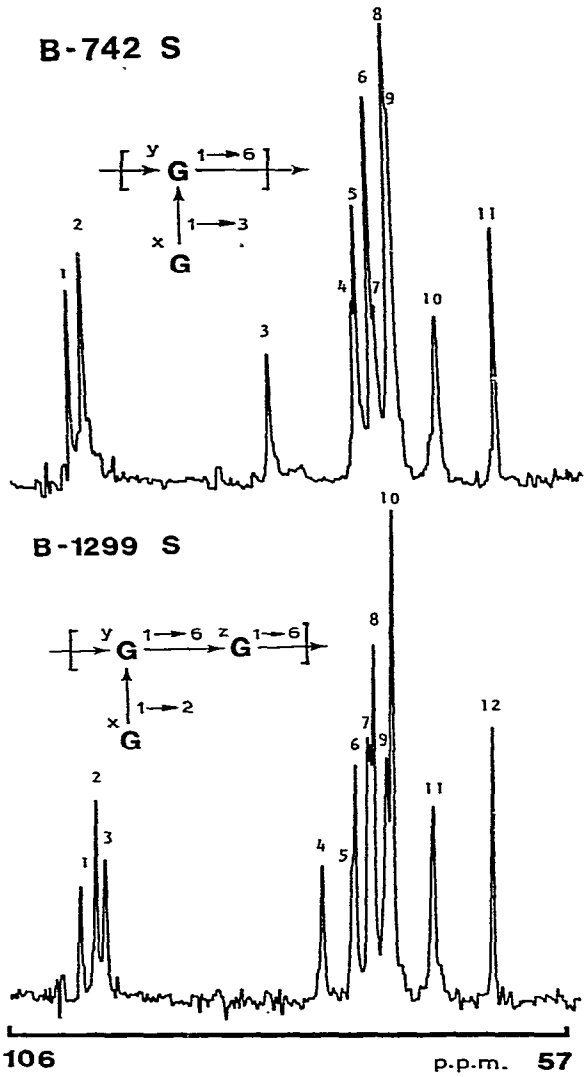

Fig. 1. ^{13}C-N.m.r. spectra at 90° of dextran B-742 fraction S and dextran B-1299 fraction S. [The numerical assignments of resonances correspond to those resonances listed in Table I. The average repeating-unit of the polymer (G) represents the α-D-glucopyranosyl residue. The plots show fully recovered resonance-intensities.]

TABLE I

^{13}C-N.M.R. CHEMICAL SHIFTS (δ) AND SPIN–LATTICE RELAXATION-TIMES (T_1) FOR THE S FRACTIONS OF DEXTRANS PRODUCED BY *Leuconostoc mesenteroides* (NRRL) B-742 AND B-1299

	Peak number[a]											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
B-742												
δ (p.p.m.)	100.81	99.56	82.89	75.09	74.97	73.57[b]	73.14	71.74	71.41	67.67	62.46	
T_1 (msec)[c]	250	150	110	140	180	180	140	110	170	63	208	
B-1299												
δ (p.p.m.)	99.57	98.22	97.37	77.83	75.20	74.84	73.76	73.18	72.04	71.51	67.83	62.49
T_1 (msec)[c]	203	241	140	130	215	150	169	190	126	156	83	223

[a]Resonances are numbered in Fig. 1. [b]Split resonance, with extra peak at 73.46. [c]Based on two determinations per polymer. Maximum probable error, ±12%.

fied if the branching resonances could be divided into resonances of branch points and terminal residues.

The conventional assumption that all (1→6)-linkages are located in the dextran backbone is employed; for these highly branched dextrans, this indicates "comb-like" branching. Permethylation g.l.c.–m.s. data[3,4] and the ^{13}C-n.m.r. spectra are consistent with the polymer sub-units (see Fig. 1). Terminal residues are expected to have greater freedom of motion, and larger T_1 values, than backbone-chain residues[6]; on this basis, the additional resonances are assigned either to the branch-point or the terminal residues.

Leuconostoc mesenteroides dextran B-742 fraction S (ref. 7) has each backbone residue *O*-substituted[5], the repeating disaccharide sub-unit predicting 12 resonances. Three classes of carbon position (primary alcohol, secondary alcohol, and hemiacetal) are represented, and although comparisons of the magnitude of T_1 can be made between carbon positions of any class (*e.g.*, the two C-6 resonances), such comparisons cannot be made directly between these classes. Based on magnitudes of T_1, resonance 10 represents *y*C-6 (where *y* refers to branch-point, and *x* to terminal-residue, resonances), in agreement with assignments based on the $\Delta\delta$ of substitution (see Fig. 1 and Table I). On the basis of substitution $\Delta\delta$, resonance 3 has been assigned to *y*C-3, and this is now supported by a small T_1 value. The remaining, secondary-hydroxyl resonances (4–9, representing C-2 through C-5) may be grouped according to the magnitude of T_1. Resonances 4 and 7 (as for 3), having small T_1 values (110–140 msec), are assigned to the remaining *y*-residue positions (C-2, C-4, and C-5). Resonances 5, 6, and 9, having large T_1 values (170–180 msec) are assigned to the *x*-residue (C-2 through C-5). The anomeric resonance having the larger T_1 value (resonance 1) is assigned to the *x*C-1, and resonance 2 to the *y*C-1. Relative to the (1→6)-linked α-D-glucopyranosyl residue, $\Delta\delta$ for *x*C-1 is −0.03 p.p.m., and for the 1,3,6-trisubstituted residue, $\Delta\delta$ for *y*C-1 is +1.22 p.p.m. In general, and with the exception of the previous anomeric-resonance set, the resonances having the smaller T_1 value are also less intense, providing a simple method for separating the resonance sets. For well resolved glycan spectra, resonances that are more intense than indicated by alternative structural data represent residues having increased mobility, which, for most polymers, are side-chain or terminal residues. Resonance 8 is intense, but has a small T_1 value, and it is assumed to be a composite *x*- and *y*-residue resonance. The slight splitting of resonance 6 (0.11 p.p.m.) is an as-yet-unaccountable, spectral feature. G.l.c.–m.s. data[8] for *L. mesenteroides* dextran B-1299 fraction S (ref. 7) indicate terminal (*x*), branch-point (*y*), and chain-extending (*z*) residues for each sub-unit, which could give an 18-resonance spectrum. A high signal-to-noise spectrum yields 13 resonances, but one (73.55 p.p.m.) was not resolved to yield T_1 data. Resonances 1, 5, and 8–11 are identical to those for linear dextran, and represent the *z*-residue. As fewer than 18 resonances are resolved, many, if not all, peaks in the 70–75-p.p.m. region are composite resonances. The unresolved, linked *y*C-6 and *z*C-6 resonances (resonance 12) would be expected to have less freedom of motion than the terminal, non-linked *x*C-6 (resonance 11), and agree with the large T_1 of

resonance 12 (T_1 223 msec), thus rationalizing the single *x*C-6 resonance which is larger than the combined *y*C-6 and *z*C-6 resonances. Resonance 4 has been identified[2] as *y*C-2, and its relatively small T_1 value (130 msec) confirms this assignment. In the 95–105-p.p.m. region, resonance 1 corresponds to linear dextran, and is assigned to *z*C-1, resonance 2 (T_1 241 msec) is assigned to *x*C-1, and resonance 3 (T_1 = 140 msec) is assigned to *y*C-1. This assignment of anomeric resonances aids in reconciling the g.l.c.–m.s. data, which indicate approximately equal proportions of residue types, to the inequality of the ^{13}C-n.m.r., anomeric peak-heights, as the *x*-residue has greater freedom of motion and contributes a more intense resonance. Relative to the (1→6)-linked α-D-glucopyranosyl residue, $\Delta\delta$ for *x*C-1 is −2.21, and for the 1,2,6-tri-*O*-substituted residue, $\Delta\delta$ for *y*C-1 is −1.17 p.p.m. For both 1,2,6- and 1,3,6-branching of dextrans, the branch-point, anomeric resonances are downfield from the corresponding, terminal anomeric resonances.

In conclusion, the fortuitous existence of well-defined, highly branched dextrans allows the correlation, to carbon-atom positions, of several resonances associated with branching residues, permitting the $\Delta\delta$ value of *O*-substitution to be established for D-glucopyranosyl residues. This $\Delta\delta$ value can then aid in predicting the ^{13}C-n.m.r. spectra for specific D-glucans. Importantly, knowledge of the resonances associated with specific residues, combined with measurements of T_1, can then allow reversal of this reasoning process, establishing the position of residues in polysaccharides, or the order of saccharides in oligomers.

EXPERIMENTAL

The ^{13}C-n.m.r. conditions and methods of preparation of dextran samples have previously been described[2,3]. In general, a Varian XL-100-15 spectrometer equipped with a Nicolet TT-100 system was employed in the Fourier-transform mode. The dextran samples (~0.3 g/4 mL of D_2O) were maintained at 90°. Chemical shifts are expressed in p.p.m. relative to external Me_4Si, but were actually calculated by reference to the lock signal. Values of T_1 were established by the inversion recovery method, employing the 180°–τ–90°–T pulse sequence.

ACKNOWLEDGMENTS

This work was supported, in part, by a Robert A. Welch Foundation Grant (Q294), a National Science Foundation Grant (BMS-74-10433), and National Institutes of Health Grants (HL-17269 and HL-17372).

REFERENCES

1 F. R. Seymour, R. D. Knapp, E. C. M. Chen, A. Jeanes, and S. H. Bishop, *Carbohydr. Res.*, 71 (1979) 231–250.
2 F. R. Seymour, R. D. Knapp, and S. H. Bishop, *Carbohydr. Res.*, 51 (1976) 179–194.
3 F. R. Seymour, R. D. Knapp, S. H. Bishop, and A. Jeanes, *Carbohydr. Res.*, 68 (1979) 123–140.

4 F. R. SEYMOUR, M. E. SLODKI, R. L. PLATTNER, AND A. JEANES, *Carbohydr. Res.*, 53 (1077) 153–166.
5 F. R. SEYMOUR, E. C. M. CHEN, AND S. H. BISHOP, *Carbohydr. Res.*, 68 (1979) 113–121.
6 J. R. LYERLA AND G. C. LEVY, in G. C. LEVY (Ed.), *Topics in Carbon-13 NMR Spectroscopy*, Vol. 1, Wiley–Interscience, New York, 1974, Chapter 3.
7 A. JEANES, W. C. HAYNES, C. A. WILHAM, J. C. RANKIN, E. H. MELVIN, M. J. AUSTIN, J. E. CLUSKEY, B. E. FISCHER, H. M. TSUCHIYA, AND C. E. RIST, *J. Am. Chem. Soc.*, 76 (1954) 5041–5052.