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# HIGH-FIELD, $^{13}$C-N.M.R. SPECTROSCOPY OF β-D-GLUCANS, AMYLOPECTIN, AND GLYCOGEN

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## ABSTRACT

$^{13}$C-N.m.r. spectra of several D-glucans, recorded at 100 MHz, have afforded information about structural detail not previously accessible at lower frequencies. Spectra of (1→4)- and (1→3)-linked β-D-glucans of oats, barley, and lichenan of Iceland moss demonstrate the presence, in each, of three, non-equivalent, 4-*O*-substituted residues, that the ratio of these to 3-*O*-substituted residues averages 2.4–2.5, and hence that the patterns of repeating sequences in the three polymers are essentially the same. A comparison of wheat amylopectin with a minor, amylopectin-like fraction of wheat starch indicates that they are strictly analogous in basic structure, and differ only in that the average length of branches in the minor fraction is 20–25% shorter. By combining the advantages of high-field operation with the use of dimethyl sulfoxide as solvent, a large number of distinctive resonances have been observed, representing end-units, branch-points, and residues adjacent to branch-points. Accordingly, these signals are even more prominent in the spectrum of glycogen, reflecting the higher incidence of branching in this polymer. At 100 MHz, the excellent resolution and sensitivity afforded constitute a potent basis for assessing the purity of polysaccharide preparations, as illustrated with wheat amylose and barley β-D-glucans.

## INTRODUCTION

Since the initial study of the $^{13}$C-n.m.r. spectrum of amylose by Dorman and Roberts[1], many glucans[2,3], as well as a host of other polysaccharides[4,5], have been examined by this technique. We now report on observations made on (1→4)- and (1→3)-linked β-D-glucans, and also on amylopectin and glycogen, as a supplement to earlier studies of these materials by chemical and enzymic methods.

The β-D-glucans examined are prominent constituents of oats and barley[6–8], and include "lichenan", the main polysaccharide[9] of Iceland moss. Chemical studies[10–15] have shown that the proportion of (1→4)-linkages in these molecules exceeds that of (1→3)-linkages by a factor of at least two. Based on the structure of tri- and tetra-saccharides formed from each polymer by degradation with a cellulase, and also a laminaranase, it was found[13–15] that 4-*O*-substituted residues occur in

sequences of two and, less frequently, three, whereas the 3-*O*-substituted residues occur singly. Support for the latter arrangement in lichenan was furnished[16] by $^{13}$C-n.m.r. spectroscopy, in that the 62.8-MHz spectrum of the polysaccharide in dimethyl sulfoxide exhibited sharp singlets for the C-1 and C-3 resonances of 3-*O*-substituted residues. A 25.2-MHz, $^{13}$C-n.m.r. spectrum of barley glucan[17] in deuterium oxide was not sufficiently resolved for a comparable analysis. It has been reported[18], on the basis of enzymolysis data, however, that some of the 3-*O*-substituted residues in barley glucan are in pairs, and sequences of three. Another possible structural variant among the glucans[15] is a slightly higher ratio of (1→4)- to (1→3)-linkages in the cereal glucans than in lichenan. These are among the items examined in the present study, by comparing the $^{13}$C-n.m.r. spectra of all three β-D-glucans, under the excellent conditions afforded at an operating frequency of 100 MHz.

In an analogous manner, 100-MHz spectra were used to compare the structures of two fractions of wheat amylopectin that, previously[19], were differentiated mainly on the basis of biosynthetic experiments with carbon-14. This aspect of the study also is extended to an examination of related features in the corresponding spectra of glycogen.

## RESULTS AND DISCUSSION

*β-D-Glucans.* — $^{13}$C-N.m.r. spectra of oat and barley β-D-glucans and lichenan, in $(CD_3)_2SO$ at 80°, were recorded initially at 22.6 MHz. As the three spectra were virtually superimposable, that (Fig. 1B) of the oat glucan is representative of the group. The corresponding spectrum taken at 100 MHz exhibits a striking improvement in resolution (Fig. 1C), such that 18 individual signals are observed as compared with 11 in Fig. 1B. Assignments for the signals are listed in Table I, with reference to a polymer structure (Fig. 2)[13–15] consisting of a major sequence (**1**) in which there are two consecutive (1→4)-linkages, and a minor sequence (**2**) containing three, per (1→3)-linkage. Most of these assignments appear to be straightforward, because of close analogies[16] with the spectra of cellulose and laminaran, although one intriguing uncertainty concerns the anomeric carbon peaks. As the chemical shifts for C-1 in laminaran and cellulose are δ 103.2 and 102.5, respectively[16], the peak at δ 103.4–103.5 (Table I) might be regarded as due to C-1 of the 3-*O*-substituted residue (*b*), and that at δ 102.5–102.6 as due to C-1 of 4-*O*-substituted residues. In the β-D-glucans, however, the anomeric carbon of *b* is bonded to position 4 of adjacent residue *a*, whereas, in laminaran, all linkages are (1→3). Analogously, in contrast to the arrangement in cellulose, although *c* is a 4-*O*-substituted residue, it is linked to position 3 of its neighbor (*b*). Some information bearing on these matters was furnished by spectra of two tetrasaccharides obtained[13–15] from all three glucans by enzymic hydrolysis.

One of these, released by a cellulase, is *O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→4)-D-glucose[13,15], which is represented in Fig. 2 by the four residues of segment **3**. Freshly dissolved in $(CD_3)_2SO$,

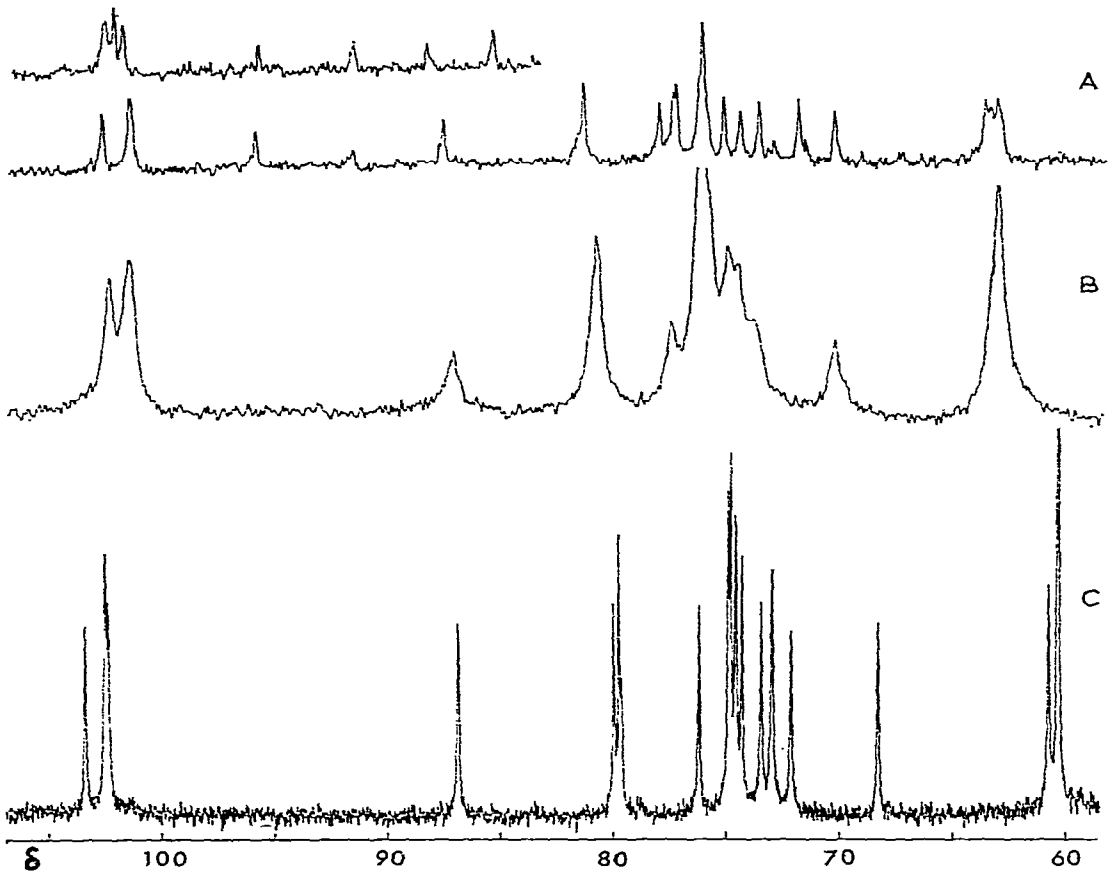


Fig. 1. $^{13}$C-N.m.r. spectra in $(CD_3)_2SO$ of A, tetrasaccharide **3** and (inset) tetrasaccharide **4** (downfield portion) at 22.6 MHz and 34°; B, β-D-glucan of oats at 22.6 MHz and 80°; and C, β-D-glucan of oats at 100 MHz and 90°.

compound **3** was preponderantly in the β-anomeric form, as shown (Fig. 1A) by the relative intensities of the C-1 signals (δ 96.8 and 92.2) of its β- and α-reducing end-units, respectively. Not surprisingly, therefore, the spectrum of **3** is closely analogous to that of the parent polymer, allowing for differences in line width, and chemical-shift changes due to the presence in **3** of a non-reducing end-unit that, originally, had been a 4-*O*-substituted residue (*d*). Accordingly, the peaks at δ 77.1 and 70.3 are attributed to C-5 and C-4, respectively, of the end-unit; this corresponds to a downfield shift (β-effect) of 9.5 p.p.m. for C-4, and an upfield shift (γ-effect) for C-5 of 0.7 p.p.m., associated with the glycosidic bond attached to C-4 of residue *d*.

As the relative disposition of C-1 signals is analogous to that in the glucan, the problem of assignment is the same. However, the C-1 region of the spectrum (inset, Fig. 1A) of the second tetrasaccharide, which was produced by a laminaranase, shows that the anomeric carbons of all of the 4-*O*-substituted residues need not resonate at essentially the same frequency. That is, C-1 of the 3-*O*-substituted residues

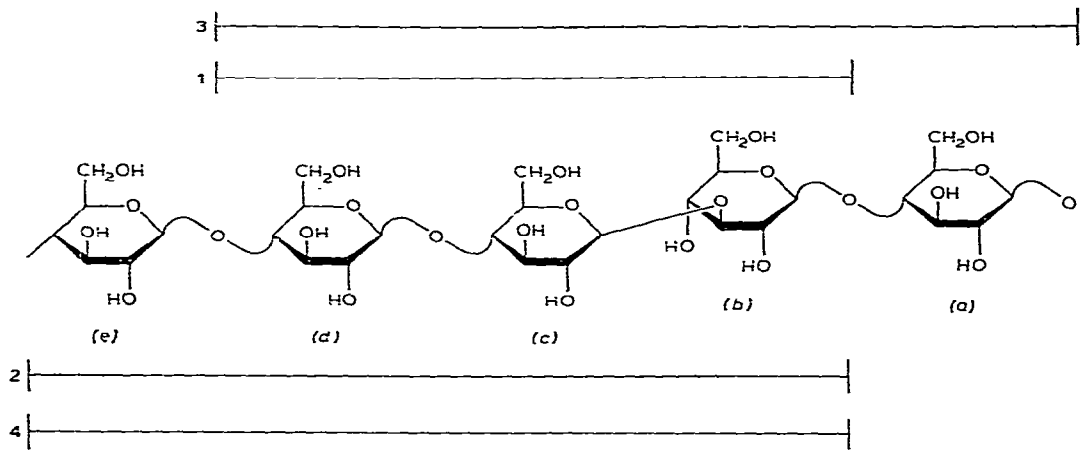


Fig. 2. Representation of a portion of β-D-glucan polymer: **1**, corresponds to a segment consisting of one 3-*O*-substituted and two 4-*O*-substituted residues (*b*, *c*, and *d*, respectively); **2**, corresponds to a segment consisting of one 3-*O*-substituted and three 4-*O*-substituted residues (*b*, *c*, *d*, and *e*, respectively); **3**, corresponds to a tetrasaccharide formed from the polymer by cellulase, in which *a* is the reducing end-unit, *d* is the non-reducing end-group, and *b* and *c* are internal residues; **4**, corresponds to a tetrasaccharide formed from the polymer by laminaranase, in which *b* is the reducing end-unit, *e* is the non-reducing end-group, and *c* and *d* are internal residues.

of this tetrasaccharide, *O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→3)-D-glucose[13,15] (corresponding to segment **4**, Fig. 2), is not represented in the region downfield of δ 100, because it is at the reducing-end. Nevertheless, the peak at δ 103.9 corresponds to those furthest downfield in the other two spectra, suggesting the possibility that, in all three instances, the least-shielded carbon is C-1 of 4-*O*-substituted residue *c*, engaged in the (1→3)-linkage. Of course, this may require that linkage conformations remain the same.

An analogous problem pertains[20] to the proton n.m.r. signals of these glucans. The 400-MHz, $^1$H-n.m.r. spectrum of lichenan contains one doublet at δ 4.47 and (at least) two overlapping doublets at δ 4.37 and 4.34 (Fig. 3). Although H-1′ of β-laminaribiose resonates at lower field than H-1′ of β-cellobiose (δ 4.63 *vs* 4.51 in $D_2O$)[21], it is not certain[20] as to which anomeric proton in lichenan produces the signal at δ 4.47. Thus, it has not yet been feasible to use selective $^1$H-decoupling to confirm C-1 assignments in the β-D-glucan spectra.

As the signals in Fig. 1B for C-4 (δ 79.9) and C-3 (δ 87.0) engaged in the inter-residue linkages are well separated, their integrals were used to estimate the ratios of (1→4)- to (1→3)-linkages. To ensure that the results for the three glucans were directly comparable, uniform experimental conditions were employed, including repetition times that were several-fold longer than the approximate $T_1$ values (<0.5 sec.). The proportions of (1→4)- to (1→3)-linkages were found (Table II) to vary from 2.2 to 2.7.

In all, 18 individual signals are observed in the 100-MHz spectrum of oat

TABLE I

$^{13}$C-N.M.R. RESONANCES IN SPECTRA OF β-D-GLUCANS[a] AND TETRASACCHARIDES 3 AND 4[b]

| *Oat glucan* | | | *Compound* 3 | | *Compound* 4 | |
|---|---|---|---|---|---|---|
| *Chemical shift (δ) at* 22.6 MHz | *100 MHz* | *Carbon assignment* | *Chemical shift (δ)*[c] | *Carbon assignment* | *Chemical shift (δ)*[c] | *Carbon assignment* |
| 103.5 | 103.36 | 1*c* | 104.2 | 1*c* | 103.9 | 1*c* |
| 102.6 | 102.45 } 102.33 } | 1*a,b,d* | 102.8 | 1*b,d* | 103.5 | 1*d,e* |
| | | | | | 103.0 | |
| | | | 96.8 | 1*a*(β) | 96.6 | 1*b*(β) |
| | | | 92.2 | 1*a*(α) | 92.0 | 1*b*(α) |
| 87.0 | 86.87 | 3*b* | 87.8 | 3*b* | 88.2 | 3*b*(β) |
| | | | | | 85.2 | 3*b*(α) |
| 79.9 | 79.96, 79.73 | 4*a,c* | 80.8 | 4*a,c* | 80.7 | 4*c,d* |
| 79.9 | 79.63 | 4*d* | 77.1 | 5*d* | 77.0 | 5*e* |
| 76.4 | 76.20 | 5*b* | 76.6 } | 5*b* | 76.7 } | 5*b*(α,β) |
| | | | 76.3 } | 3*d* | 76.4 } | 3*e* |
| 75.1 | 74.92–74.82 | 3*a,c,d* | 75.1 } | 3*a,c* | 75.0 } | 3*c,d* |
| 74.8 | 74.59–74.31 | 5*a,c,d* | 74.9 } | 5*a,c* | 74.6 } | 5*c,d* |
| 73.4–73.1 | 73.45–72.97 | 2*a,c,d* | 73.9 | 2*c* | 73.8 } | 2*c* |
| | | | 73.2 | 2*a*(β) | 73.5 } | 2*b*(β) |
| | | | | | 73.2 } | 2*d* |
| 72.4 | 72.12 | 2*b* | 72.2 | 2*b* | 72.1 | |
| | | | 71.5 | 2*a*(α) | 71.2 | 2*b*(α) |
| | | | 70.3 | 4*d* | 70.2 | 4*e* |
| 68.4 | 68.27 | 4*b* | 68.6 | 4*b* | 68.8 | 4*b* |
| 60.8 | 60.74 | 6*b* | 61.2 | 6*b* | 61.8 | 6*b* |
| 60.5 | 60.34 | 6*a,c,d* | 61.0–60.6 | 6*a,c,d* | 61.0–60.5 | 6*c,d,e* |

[a]Data listed are for the β-D-glucan from oats, although barley glucan and lichenan gave closely similar sets of data. [b]Chemical shifts in p.p.m. relative to tetramethylsilane. [c]Recorded at 22.6 MHz.

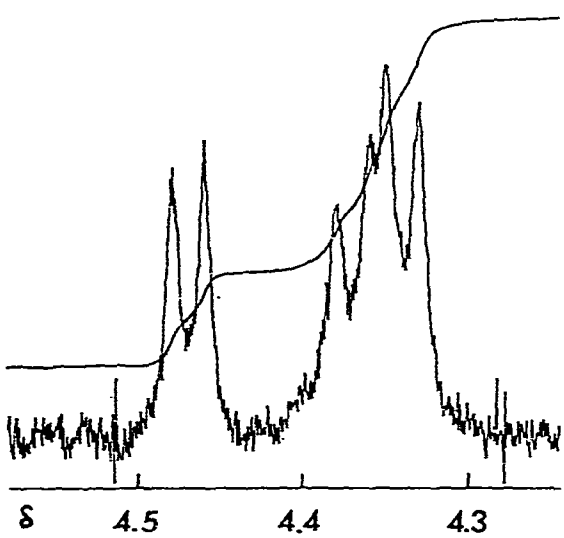


Fig. 3. Partial, 400-MHz, $^1$H-n.m.r. spectrum of lichenan in 95:5 $(CD_3)_2SO$–$D_2O$ at 90°.

TABLE II

RATIOS OF (1→4)- TO (1→3)-LINKAGES IN β-D-GLUCANS

| | *Relative areas of $^{13}C$-n.m.r. resonances* | | |
|---|---|---|---|
| | *22.6 MHz* | *100 MHz* | |
| | *C-4/C-3* | *C-4/C-3* | *C-$I_{103}$/C-$I_{102}$*[a] |
| Oat glucan | 2.2 | 2.6 | 2.3 |
| Barley glucan | 2.7 | 2.6 | 2.3 |
| Lichenan | 2.4 | 2.2 | 2.4[b] |

[a]C-1 peak at δ 103.4, compared with those at δ ~ 102.4. [b]The ratio of integrals for H-1 peaks at δ 4.4–4.3 to that at δ 4.47 (Fig. 3) was 2.6.

glucan. However, as some obviously represent more than one carbon, the glucan cannot be constituted solely of sequence **1**. The fact that signals in Fig. 1C attributed (Table I) to C-2 to C-6 of 3-*O*-substituted residues are all relatively sharp singlets adds weight to the earlier evidence[13–16] that most of these residues occupy isolated positions within the linear polymer.

Of particular significance is the distinct presence of *three* resonances (at δ 79.9, 79.7 and 79.6) attributable to C-4 nuclei located in glycosidic bonds. This observation is in accord with the reported[13,15,15a] occurrence of three different types of 4-*O*-substituted residues in sequences **1** and **2** and also, as one of these resonances is relatively minor, with a lower incidence of sequence **2** in the polymer. The minor signal at δ 79.6 must be that of C-4 of the central 4-*O*-substituted residue (*d*) in **2**; from the appearance of the signals at δ 102.5 and 102.3, C-1 of this residue probably resonates at the more downfield position, thereby contributing to the increased height of the signal at that position. Analogously, C-2 of the same residue probably augments the relatively strong signal at δ 73.5, while C-3 and C-5 augment the tallest signals at δ 74.9 and 74.5.

In the proton-coupled, $^{13}C$-n.m.r. spectrum of the oat glucan, splitting of the various anomeric carbon signals due to direct coupling with anomeric protons, although partially masked for the signals at δ 102.5 by overlap, was uniformly close to 160 Hz, as anticipated[4,22] for the β-D-*gluco* configuration throughout.

In the 100-MHz spectrum of barley glucan (Fig. 4), there are 18 signals that correspond in chemical shift and relative intensity to those described for the oat glucan. However, one also finds many minor signals, including weak resonances at δ 96.5 and 92.0 attributable to aldoses, none of which were evident in the 22.6-MHz spectrum. There is no apparent relationship between this group of signals and the spectrum of the glucan. Presumably, they are due to carbohydrate contaminants, because their chemical shifts are not characteristic of such other possibilities as protein.

The 100-MHz, $^{13}C$-n.m.r. spectrum of lichenan is a close counterpart of the spectrum of oat glucan, and hence is of comparably better quality than that ob-

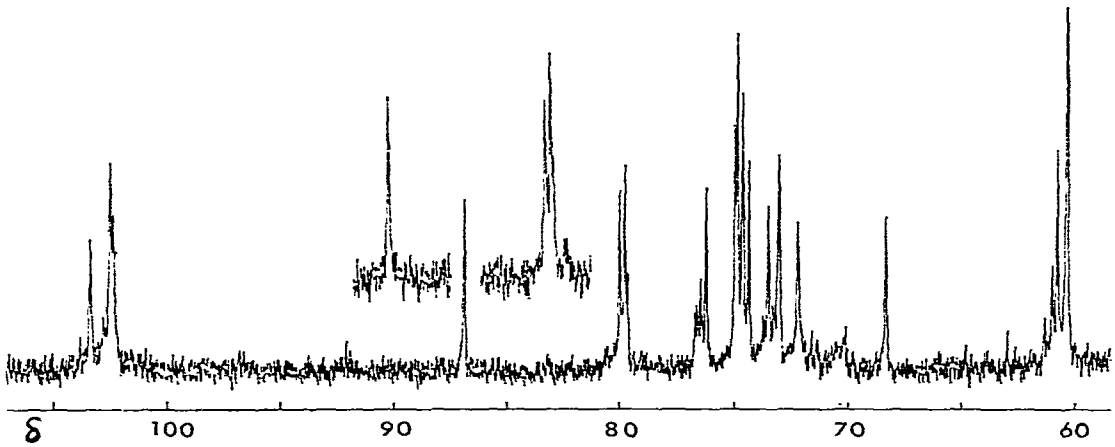


Fig. 4. $^{13}$C-N.m.r. spectrum at 100 MHz of β-D-glucan of barley in $(CD_3)_2SO$ at 90°. Inset, δ 80–88 region of the corresponding spectrum of lichenan.

tained[16] at 62.8 MHz. Seen in the inset of Fig. 4, which illustrates the spectral region of δ 80–88 for lichenan, is the same distinctive pattern of C-4 and C-3 signals as observed in the spectra of the oat and barley glucans.

Overall, therefore, these spectral data confirm the earlier evidence, obtained by enzymic degradation, that the three β-D-glucans are constituted basically of sequences of **1** and **2**. The ratios of (1→4)- to (1→3)-linkages, estimated from integrals for the C-3 singlet at δ 86.8 and the group of C-4 signals at δ ~79.8, are given in Table II. Also, as the C-1 singlet at δ 103.4 was almost fully resolved from the composite C-1 signal at δ ~102.4, an additional set of ratios was estimated from this pair (Table II). Values in Table II vary from 2.3 to 2.7, for an average of 2.4–2.5 (1→4)-linkages per (1→3)-linkage, or an ~2:1 ratio of sequences **1** and **2**. In our earlier studies, the isolated yields of enzymolysis products from the three polymers, and also periodate-oxidation data, indicated that lichenan contains a slightly lower proportion of (1→4)-linkages than do the cereal glucans. In conjunction with the periodate results[13,15], it is worth noting that barley glucan was more extensively overoxidized[15] than oat glucan, and even more so than lichenan. Hence, the evidence, from reducing-end C-1 signals in the spectrum of barley glucan (Fig. 4), of the presence of short-chain components helps to account for this difference observed in chemical properties. In comparing the enzymic data with the n.m.r. spectra, allowance must be made for the fact[13,15] that 15–25% of each glucan did not yield identifiable products, whereas the structural information given by the 100-MHz spectra presented here surely represents a higher percentage of each polysaccharide. Consequently, we see no basis for maintaining that there are significant differences between the three, in terms of linkage ratios or of linkage sequences.

*Amylopectins.* — In biosynthetic studies of the formation of wheat starch[19,23], separation of the amylose and amylopectin components[19] was accompanied by the isolation of a third fraction amounting to ~5% of the starch. Although this minor

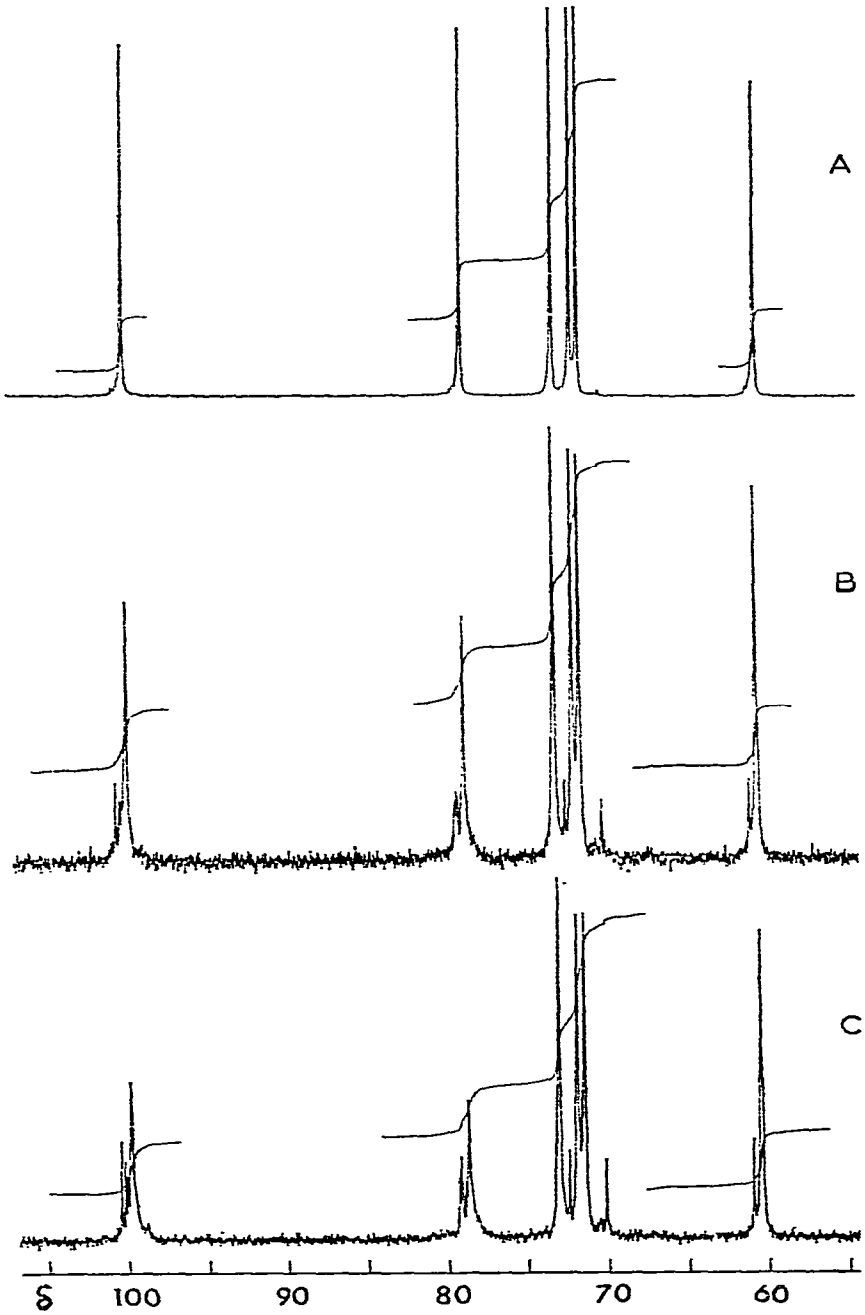


Fig. 5. $^{13}$C-N.m.r. spectra at 100 MHz and 90° in $(CD_3)_2SO$ of A, wheat amylose; B, main fraction of wheat amylopectin ("B"); and C, minor fraction of wheat amylopectin ("C").

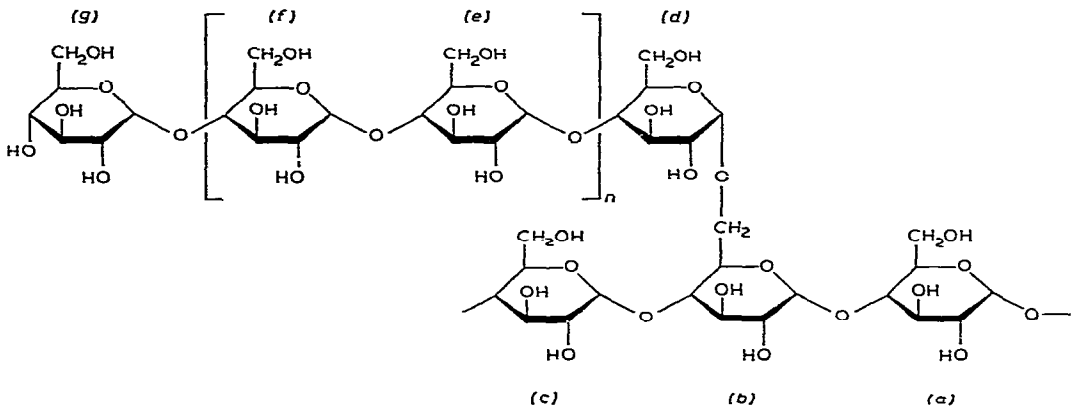


Fig. 6. Representation of a portion of the amylopectin or glycogen molecule; *n* corresponds to the number of repeating sequences of residues *e* and *f* commensurate with the length of the branch.

TABLE III

$^{13}$C-N.M.R. RESONANCES IN 100-MHz SPECTRA OF AMYLOPECTINS AND GLYCOGEN

| *Carbon assignment* | *Chemical shift (δ)*[a] | | | |
|---|---|---|---|---|
| | *Amylose* | *Amylopectin "B"* | *Amylopectin "C"* | *Glycogen* |
| C-1 (*g*) | | 100.43 | 100.44 | 100.54 |
| C-1 (*a,c,d*) | | 100.20–100.06 | 100.20–99.90 | 100.34–100.06 |
| C-1 | 99.86 | 99.82 | 99.82 | 99.86–99.47 |
| | | 99.69–99.65 | | 98.88 |
| C-4 (*a,c,d*) | | 79.29 | 79.28 | 79.40 |
| | | 79.17–78.88 | 79.17–78.87 | 79.28 |
| C-4 | 78.77 | 78.71 | 78.72 | 78.94 |
| | | | 78.59 | 78.71–78.29 |
| | | 73.24–73.16 | 73.25–73.16 | 73.79 |
| C-3 | 73.07 | 73.04 | 73.04 | 73.24 |
| | | 72.41–72.00 | 72.41–72.01 | 72.52 |
| C-2 | 71.93 | 71.89 | 71.89 | 72.05 |
| | | 71.73–71.60 | 71.71–71.54 | |
| C-5 | 71.48 | 71.45 | 71.45 | 71.70 |
| | | 71.29 | | 71.20–70.47 |
| C-4 (*g*) | | 70.13 | 70.14 | 70.27 |
| C-6 (*g*) | | 60.95 | 60.94 | 61.13 |
| C-6 | 60.50 | 60.47 | 60.47 | 60.60 |
| | | 60.34 | 60.35 | |

[a]In p.p.m. relative to tetramethylsilane.

fraction incorporated administered carbon-14 tracer at a notably different rate than the amylopectin[23], it was "amylopectin-like" in terms of its beta-amylolysis and periodate-oxidation characteristics[19]. We have re-examined the structure of the minor component (designated amylopectin "C"), by comparing its $^{13}$C-n.m.r. spectrum with that of the main amylopectin material ("B").

Isolation of the amylose fraction entailed[19] its crystallization in the form of a complex with 1-pentanol. Although this procedure effectively separated it from amylopectin, as shown by iodine absorption measurements[19], the 100-MHz, $^{13}C$-n.m.r. spectrum of the amylose in $(CD_3)_2SO$ at 90° (Fig. 5A) suggests that a small proportion of amylopectin had been retained. Thus, the very weak signals at $\delta$ 100.4, 79.3, 72.5, 70.2, and 60.9 are much more prominent in the spectrum of amylopectin B (Fig. 5B), and even relatively stronger in that of amylopectin C (Fig. 5C). Nevertheless, the latter two spectra are dominated by six major signals attributable to multiples ($n$) of residues $e$ and $f$ (represented in Fig. 6) that comprise the main segments of branches in amylopectin. As $e$ and $f$ are structurally analogous to the residues in amylose, the chemical shifts of these six signals coincide with those in Fig. 5A for amylose (Table III).

The minor resonances just cited appear to form a distinctive group in the spectra in Figs. 5B and 5C. A probable origin of these signals is the reducing end-unit of branches ($g$, Fig. 6). That is, the most reasonable assignment (Table III) for the peak at $\delta$ 70.1 is an unsubstituted C-4, which occurs only in an end-unit. Hence, the other three peaks would be C-1, C-2 (or C-3), and C-6, respectively, of the same unit. In addition, there are two clusters of minor C-1 and C-4 signals at $\delta \sim 100$ and $\sim 79$ in Figs. 5B and 5C. It is apparent from expansions of these regions (Figs. 7B and 7C) that the numbers of peaks resolved, and their chemical shifts, are the same for both amylopectins. The most likely source of so many non-equivalent C-1 and C-4 peaks (Table III) is the group of residues contiguous with a branch-point ($b$), *i.e.*, $a$, $c$, and $d$ in Fig. 6.

In view of the fact that each signal in the spectrum of amylopectin C has a close counterpart in that of amylopectin B, the minor component of the starch appears to contain only structures that are typical of an amylopectin, and the basic arrangement of branching within the two polymers must be closely analogous. Evidently, however, the difference between them in relative signal intensities is a reflection of differences in average chain-lengths. An estimate, based on the ratio of integrals for the C-6 signals of end-units (at $\delta$ 60.9) and those of the other residues ($\delta$ 60.5), indicates that chains of amylopectin B are $\sim 25\%$ longer than those of amylopectin C. That is, the latter would contain proportionately fewer multiples ($n$, Fig. 6) of residues $e$ and $f$. This result is in reasonable agreement with previous measurements[19] of a 10–15% difference, based on the release of maltose by beta-amylolysis, although, by contrast, periodate end-group analysis indicated no significant difference between them.

Because of the excellent quality of the present $^{13}C$-n.m.r. spectra of amylopectins B and C, and the facts that there are striking analogies between their chemical shift patterns and that differences are confined to the relative intensities of signals, we submit that fraction C is properly designated an amylopectin, characterized by unusually short branches. Hence, it is distinct from the main amylopectin component*

*Indeed, amylopectin B itself exhibits[19] heterogeneity on the basis of carbon-14 incorporation rates.

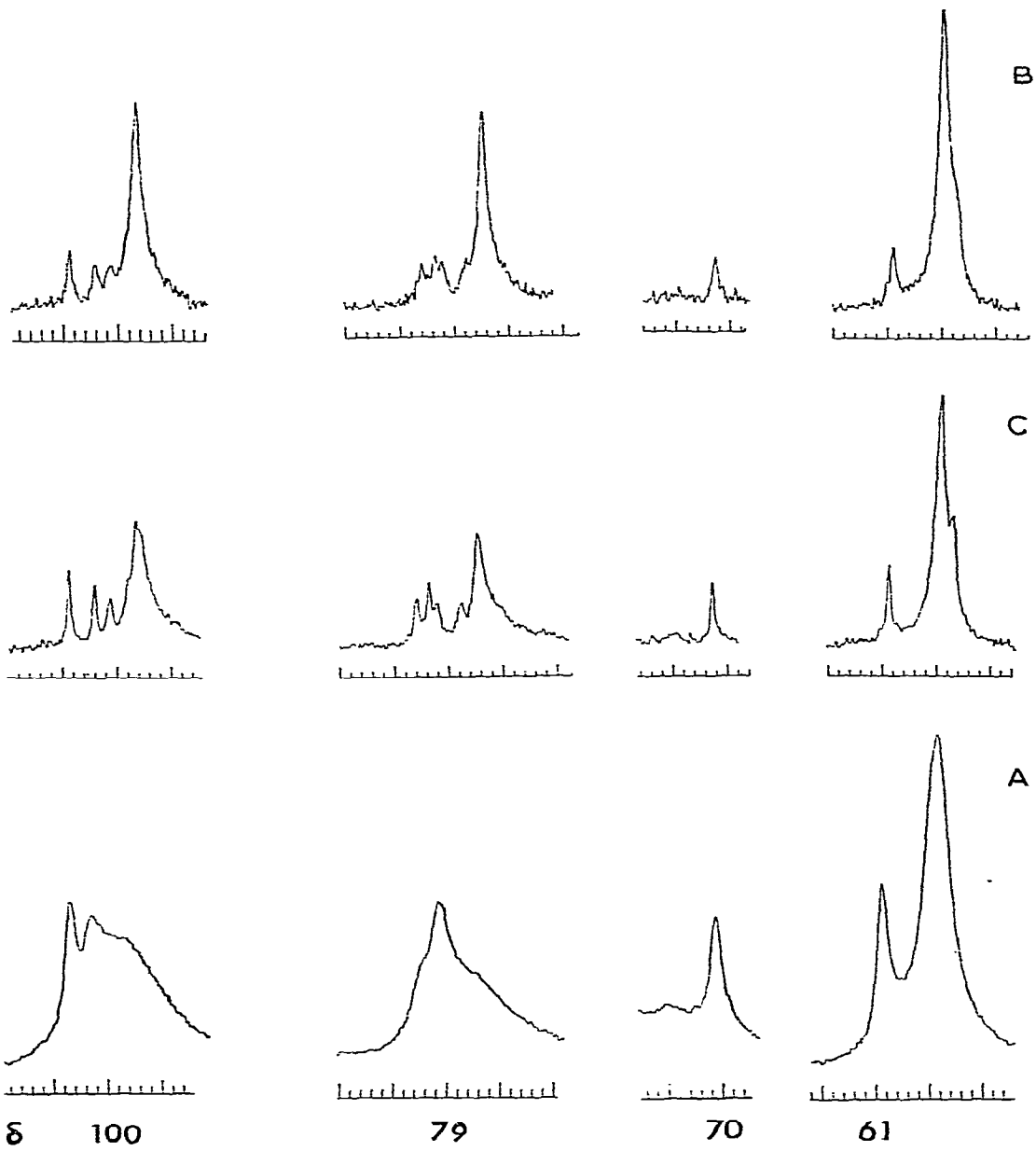


Fig. 7. Sections of 100-MHz spectra in $(CD_3)_2SO$ (90°) of A, glycogen (commercial); B, amylopectin B; and C, amylopectin C. The latter two are expanded sections of Figs. 5B and 5C, respectively.

of wheat starch in constitution, as well as in its biosynthetic and solubility characteristics.

*Glycogen.* — The average chain-length of branches in glycogen is much shorter than in amylopectin[24,25]—about one-half of the chain length of amylopectin B.

Accordingly, signals attributable to end-units (*g*) are found to be correspondingly more prominent in the spectrum of glycogen, as seen from expanded sections of the latter spectrum (Fig. 7A) in relation to corresponding sections of the two amylopectin spectra (Figs. 7B and 7C). For example, compared to the main C-6 signal ($\delta$ 60.5), the intensities of the C-4 signal at $\delta$ 70.1 and the C-6 signal at $\delta$ 60.9, attributed to end-unit *g*, increase in the order glycogen > amylopectin C > amylopectin B. Similarly, a consistent trend is found on comparing Figs. 7A, B, and C, in the progressively increasing importance of the groups of minor C-1 and C-4 signals ascribed to residues *a*, *c*, and *d* (Table III), all of which should become more populous with a decrease in average chain-length. Nevertheless, there appears to be a slight difference between glycogen and the amylopectins in the distribution of chemical shifts within these groups of C-1 and C-4 signals, which probably reflects variations in the arrangement of branching within these two classes of polymers[24,25].

Three broad resonances are apparent in the spectrum of intact glycogen shown in Fig. 8A, *i.e.*, at $\delta$ ~99, 71, and 67. As the chemical shift of the latter is close to that expected for *O*-substitution at C-6, the signal probably comes from branch-point *b*. A relatively low rate of segmental motion for this 4,6-di-*O*-substituted residue may account[5] for the breadth of its signals; by implication, the other two broad signals are designated C-1 and C-5 of residue *b*. In accord with these considerations, the spectrum of amylopectin C also contains corresponding resonances although, there, they are relatively weaker, and are barely discernible in the spectrum of amylopectin B (Fig. 5).

An additional fact concerning many of the minor peaks in these spectra is that they were not observed previously[5,26] with deuterium oxide solutions of amylopectin and glycogen at 25 MHz. Thus, neither the C-6 signal of end-unit *g*, nor the C-1 and C-4 signals of *a*, *c*, and *d* were detected, even with the aid of resolution enhancement[26]. This marked distinction between the earlier spectra and the present ones is due primarily to the difference in solvent used [$D_2O$ *vs* $(CD_3)_2SO$] rather than to the operating frequency of the spectrometer. Indeed, several of the minor signals were evident in the 22.6-MHz spectra as shoulders of major peaks.

The solvent effect on $^{13}C$-n.m.r. spectra of these polymers is amplified by a comparison of glycogen in $D_2O$ and in $(CD_3)_2SO$ (Fig. 8). Although line widths are narrower in the aqueous medium, far fewer individual peaks are observed than is commensurate with the large variety of non-equivalent types of residues in glycogen. The absence of a distinct resonance for C-6 of end-units, in particular, may be regarded as anomalous[26] in the light of extensive studies on several classes of α-D-glucans. It is not apparent why this signal appears when $(CD_3)_2SO$ is used, although the overall solvent effect is probably related to differential modes of solvation of the residues in main-chain segments (*f*) as compared with residues (*a*, *c*, and *d*) in the vicinity of branch-points, or with end-units (*g*). Therefore, because many water-soluble polysaccharides are also readily soluble in dimethyl sulfoxide, it may be beneficial to employ both solvents in $^{13}C$-n.m.r. studies of polysaccharides, especially those that are branched.

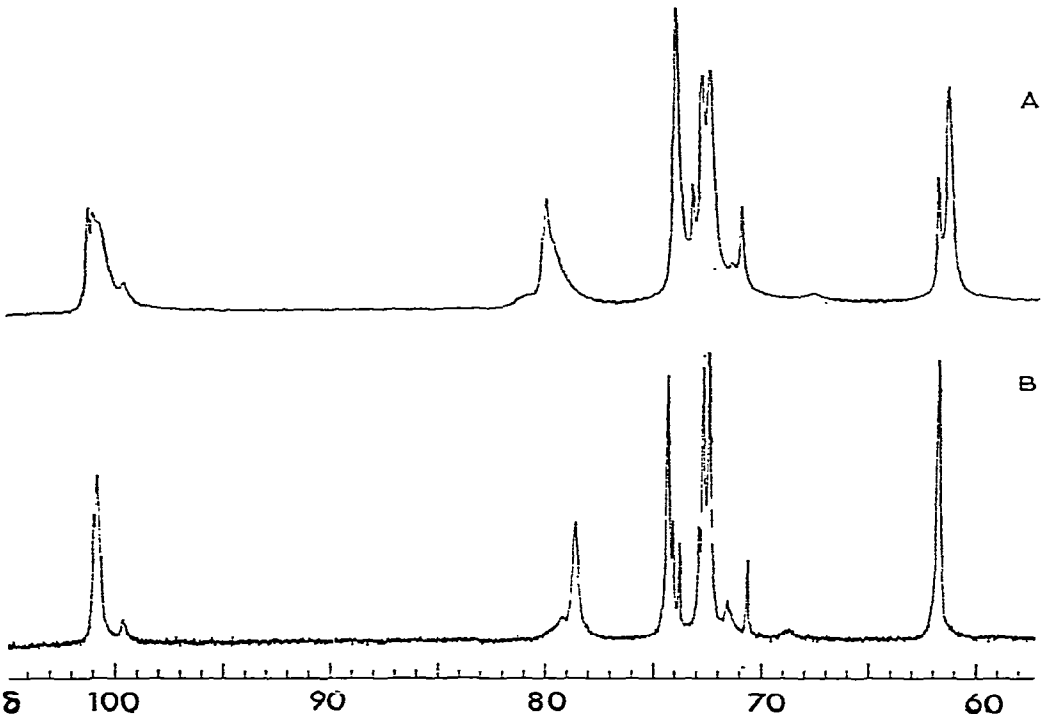


Fig. 8. $^{13}$C-N.m.r. spectra at 100 MHz and 90° of glycogen: A, in $(CD_3)_2SO$ (corresponding to Fig. 7A); and B, in $D_2O$.

## EXPERIMENTAL

Methods of preparation have already been described for oat and barley glucans[13], lichenan[15], and amylopectins B and C[19]. $^{13}$C-N.m.r. spectra were recorded with a Bruker WH-400 or WH-90 spectrometer operating at 100 MHz or 22.6 MHz, respectively. A spectral window of 5000–6000 Hz was used, and the cycling time was 2 s, for a flip angle about 75% of the 90° pulse. Sample concentrations for the β-D-glucans and the amylopectins were 90 mg/mL of $(CD_3)_2SO$ at 22.6 MHz, and 45 mg/mL at 100 MHz, whereas for glycogen the concentration was 150 mg/mL at either 22.6 MHz or 100 MHz. A total of ~50,000 scans was accumulated for the β-D-glucan spectra, and 10,000–20,000 scans for the spectra of the amylopectins and glycogen. Chemical shifts ($\delta$) are referenced to tetramethylsilane, using the $(CD_3)_2SO$ signal at $\delta$ 39.6.

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