^{13}C Nuclear Magnetic Resonance Spectra of Glucobioses, Glucotrioses, and Glucans

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Natural-abundance carbon-13 n.m.r. spectra of all the glucobioses and of four selected glucotrioses in aqueous solution have been measured and are discussed. Peak assignments were made on the basis of comparison with the spectra of methyl glucopyranosides, four mono-*O*-methylglucoses and five methyl glucobiosides. Carbon-13 n.m.r. spectroscopy proved to be a useful tool for stereochemical characterisation of these oligosaccharides. In addition, carbon-13 n.m.r. spectra of the β-limit dextrins from glycogen and amylopectin have been measured and the differences between them are discussed.

SPECTROMETRIC methods (i.r.,[1] o.r.d.,[2] and ^1H n.m.r.[3,4]) have been used for establishing the configuration of glycosidic linkages of oligosaccharides and polysaccharides. The Karplus equation in ^1H n.m.r. has been applied extensively in the structural studies of monosaccharide pyranose and furanose derivatives.[5] Positions of linkage in oligosaccharides have been determined by chemical methods and by biochemical degradation.[6-10] Recently, the ^{13}C n.m.r. spectra of monosaccharides [11,12] and some common oligosaccharides [13,14] have been published and interpreted. These reports show that ^{13}C chemical shift differences in sugars could be explained in terms of steric hindrance and proximity effects

We have already reported that ^{13}C n.m.r. spectroscopy can be used for the determination of the anomeric configurations of glucobioses.[15] We now describe the application of ^{13}C n.m.r. to the configurational and conformational analysis of glucose oligomers and polymers, as well as to the determination of their linkage positions. Some tentative assignments [15] are corrected on the basis of further data reported here.

RESULTS AND DISCUSSION

In ^{13}C n.m.r. spectroscopy, it is difficult and laborious to give a full peak assignment of sugar carbon atoms, although information from previous work has made it considerably simpler.[11-14] Roberts and his co-workers [11] showed that (*a*) methylation of a hydroxy-group effects an 8—11 p.p.m. downfield shift in the resonance of the directly attached (*i.e.* α) carbon atom: (*b*) when both the methoxy- and the adjacent hydroxy-groups are equatorial, the shift of the β-carbon atom resonance on methylation is less than 1 p.p.m. (β-effect): (*c*) resonances of more remote carbon nuclei are generally shifted less than 0·3 p.p.m. We have found that the shift due to glycoside formation is of almost the same magnitude as the methylation shift. In the light of the broad generalisation recognized in earlier studies of monosaccharides,[11,12] resonances near 91—105 p.p.m. in spectra of glucobioses and glucotrioses could be attributable to anomeric carbon atoms, and resonances of the carbon nuclei involved in glycosidic linkages (except for 1,6-linkages) were found to occur in the range 79—88 p.p.m., downfield of the usual range for pyranose carbon atoms other than those at the anomeric positions.

Anomeric Carbon Nuclei.—As shown in Tables 1 and 2, the C-1 chemical shifts for most of α- and β-anomeric forms of each reducing sugar in aqueous solution are similar in magnitude to those of C-1 in α- and β-D-glucopyranose (1α and β),[16] and the C-1 resonance of the β-form is shifted downfield by about 3·9 p.p.m. from that of the α-form except in the case of 1,2-linked glucobioses. The C-1 resonances usually appeared at 93·2 p.p.m. for α-anomers and at 97·1 for β-anomers, but the C-1 signals of α-kojibiose (2-*O*-α-D-glucopyranosyl-α-D-glucopyranose) and β-sophorose (2-*O*-β-D-glucopyranosyl-β-D-glucopyranose) appeared at slightly higher field than those of the other reducing sugars. Moreover, isomaltose (6-*O*-α-D-glucopyranosyl-D-glucopyranose) showed the C-1 resonances at 93·8 p.p.m. for the α-anomer and 97·7

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for the β-anomer. Similar shifts also appeared in the case of isopanose [*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→6)-D-glucopyranose] which has an isomaltose unit on the reducing side.

The anomeric C-1 resonances of methylated D-glucopyranosides appeared in the same region as those of the corresponding anomeric carbon nuclei (C-1′, C-1″) of reducing glucobioses and glucotrioses. It is notable that the chemical shifts of the anomeric carbon nuclei of the inter-sugar α-linkage, except in the case of αα-trehalose, are in the region 97·5—101 p.p.m. and that those of the corresponding β-linkage, except in the case of ββ-trehalose, are downfield in the region 104—105 p.p.m. Throughout the series of reducing glucobioses, the resonances of the glycosidic carbon atoms appeared at lowest field among those of the remaining carbon nuclei and were observed as overlapped peaks of an anomeric pair except in the case of 1,2-linked glucobioses. As shown in Table 1, the C-1′ resonances of 1,2-linked glucobioses as well as the C-1 resonances were different from those of the other reducing disaccharides.

Inositol ^{13}C n.m.r. data[11] suggest that the C-1α resonance of 2-*O*-methyl-D-glucopyranose (2) may be 2—3 p.p.m. upfield from that of α-D-glucopyranose, owing to (1α) interaction between C-1 axial OH and C-2 equatorial OMe. Hence the resonance at 90·8 p.p.m. was directly assigned to C-1α. The same phenomenon was observed for the C-1α resonance of kojibiose, which carries a 2-*O*-glycosyl group, but in the case of sophorose the C-1β peak shifted in the reverse direction (1·5 p.p.m. upfield). The assignment of these peaks will be discussed later. The methoxy-proton resonances of the α- and β-anomeric forms of 2-*O*-methyl-D-glucopyranose (2) appeared separately at δ 3·46 and 3·60, respectively.[17] Similarly in the ^{13}C n.m.r. spectrum of (2), the methoxy-carbon chemical shifts of the α- and β-anomers were observed separately at 59·3 and 61·7 p.p.m., respectively. Kojibiose showed a similar tendency with the C-1′ shifts, but again sophorose showed the reverse behaviour. The assignments of 1,2-linked glucobioses were made as follows. In the case of kojibiose, the peaks at 99·0, 97·5, and 90·8 p.p.m. can apparently be attributed to C-1 and C-1′. In the light of the assignment for 2-*O*-methyl-D-glucopyranose (2), the peak at 90·8 p.p.m. must be due to C-1α. Also, methyl β-kojibioside [methyl α-D-glucopyranosyl-(1→2)-β-D-glucopyranoside] showed

TABLE 1

^{13}C N.m.r. spectra * of glucobioses and methyl glucobiosides

	C-1	C-2	C-3	C-4	C-5	C-6	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	OMe
1,1-Linkage													
α,α-Trehalose	94·8	73·9	74·4	71·5	77·3	62·5							
β,β-Trehalose	100·7	74·2	77·3	71·1	77·3	62·5							
α,β-Trehalose	101·3	74·0	74·6 or 74·3	70·9	72·9	62·0	104·0	74·3 or 74·6	77·4	70·9	76·8	62·3	
1,2-Linkage													
α-Kojibiose	90·8	77·1	73·1	71·1	73·1	62·0	97·5	73·1	74·4	71·1	73·1	62·0	
β-Kojibiose	97·5	79·9	75·8	71·1	77·1	62·0	99·0	73·1	74·4	71·1	73·1	62·0	
Methyl β-kojibioside	105·0	79·0	75·8	70·8	77·1	62·5	99·0	73·0	74·2	71·3	73·0	61·9	58·9
α-Sophorose	93·1	82·1	73·2	71·1	72·5	62·4	105·1	74·9	77·2	71·1	77·2	62·4	
β-Sophorose	95·8	82·8	77·2	71·1	77·2	62·4	103·9	74·9	77·2	71·1	77·2	62·4	
Methyl α-sophoroside	100·0	81·7	73·3	71·3	72·5	62·2	105·0	74·4	77·1	71·3	77·1	62·2	56·2
1,3-Linkage													
α-Nigerose	93·1	71·3	80·8	70·6	72·2	61·8	99·8	72·8	74·1	71·3	72·8	61·8	
β-Nigerose	97·0	74·1	83·2	70·6	76·6	61·8	99·8	72·8	74·1	71·3	72·8	61·8	
α-Laminaribiose	93·4	72·2	84·2	69·6	72·4	62·4	103·9	74·8	77·1	71·2	77·1	62·4	
β-Laminaribiose	97·2	74·8	86·7	69·6	77·1	62·4	103·9	74·8	77·1	71·2	77·1	62·4	
1,4-Linkage													
α-Maltose	93·2	73·0	74·6	78·5	71·6	62·5	101·0	74·3	74·6	71·0	73·4	62·5	
β-Maltose	97·1	75·7	77·8	78·5	76·1	62·5	101·0	74·3	74·6	71·0	73·4	62·5	
Methyl β-maltoside	104·4	74·6	77·8	78·7	76·1	62·3	101·1	74·3	74·6	70·9	73·4	62·3	58·7
α-Cellobiose	93·2	72·9	72·9	80·1	71·6	61·8	103·9	74·7	77·2 or 77·4	71·1	77·2 or 77·4	62·4	
β-Cellobiose	97·1	75·7	76·1	80·1	75·7	61·8	103·9	74·7	77·2 or 77·4	71·1	77·2 or 77·4	62·4	
Methyl β-cellobioside	104·5	74·2	76·4	80·3	75·9	61·8	103·9	74·6	77·5	71·2	77·2	62·4	58·9
1,6-Linkage													
α-Isomaltose	93·8	73·3	75·0	71·3	71·3	67·4	99·4	73·3	75·0	71·3	73·8	62·5	
β-Isomaltose	97·7	75·9	77·7	71·3	75·9	67·4	99·4	73·3	75·0	71·3	73·8	62·5	
α-Gentiobiose	93·3	72·9	74·5	71·1	71·8	70·2	103·8	74·5	77·1	71·1	77·1	62·5	
β-Gentiobiose	97·2	75·5	77·1	71·1	76·1	70·2	103·8	74·5	77·1	71·1	77·1	62·5	
Methyl β-gentiobioside	104·5	74·0	71·0 or 71·2	71·2	76·1	70·0	104·0	74·0	77·2	71·0 or 71·2	77·2	62·5	58·8

* Chemical shifts in p.p.m. downfield from external tetramethylsilane.

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peaks at 105·0 and 99·0 p.p.m. in the anomeric region, so that the peak at 99·0 p.p.m. could be assigned to C-1′β. The peak at 97·5 p.p.m. in the spectrum of kojibiose was of about twice the intensity of those at 99·0 and 90·8 p.p.m. and could be assigned to the overlapped resonances of C-1β and C-1′α. In the case of sophorose, four peaks (93·1, 95·8, 103·9, and 105·1 p.p.m.) were observed in the region of anomeric resonance. Those at 93·1 and 105·1 p.p.m. were very strong and had the same intensity, and those at 95·8 and 103·9 p.p.m. were weak, so that the former pair must be due to the anomeric C-1 and C-1′ of either the α- or the β-form, and the latter to C-1, and C-1′ of the other form. Since methyl α-sophoroside [methyl β-D-glucopyranosyl-(1→2)-α-D-glucopyranoside] showed two peaks at 100·0 and 105·0 p.p.m., a pair at 93·1 and 105·1 p.p.m. in the spectrum of sophorose should therefore be assigned to C-1 and C-1′ of the α-anomer, respectively. The weak pair of peaks at 95·8 and 103·9 p.p.m. should then be due to C-1 and C-1′ of the β-anomer, respectively. This reversal in chemical shift between kojibiose and sophorose could be interpreted in terms of a steric or a proximity effect. In kojibiose, which has an α-glycosidic linkage, the angle between the planes of the two glucose unit should be much smaller than that in sophorose, which has a β-glycosidic linkage. As a result, the non-reducing unit of kojibiose interferes more with the C-1α hydroxy-group than that at C-1β; the reverse is the case with sophorose.

TABLE 2

^{13}C N.m.r. spectra of some glucotrioses

Reducing end unit	(r)	C-1	C-2	C-3	C-4	C-5	C-6
Middle unit	(m)	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′
Nonreducing end unit	(n)	C-1″	C-2″	C-3″	C-4″	C-5″	C-6″
α-Maltotriose	(r)	93·4	73·4	74·7	78·5	71·6	62·4
β-Maltotriose	(r)	97·1	75·9	77·8	78·9	76·3	62·4
	(m)	101·1	73·1	74·7	78·5	72·7	62·4
	(n)	101·1	74·3	75·1	71·2	73·4	62·4
α-Panose	(r)	93·3	73·1	74·6	78·5	71·6	62·4
β-Panose	(r)	97·2	75·4	77·7	78·5	75·8	62·4
	(m)	101·1	74·3	74·6	71·2	71·2	67·4
	(n)	99·3	73·1	74·6	71·2	73·1	62·4
α-Isopanose	(r)	93·8	73·3	74·6	71·0	71·3	67·5
β-Isopanose	(r)	97·7	75·8	77·7	71·0	75·8	67·5
	(m)	99·3	73·3	74·6	78·8	71·6	62·3
	(n)	101·1	74·1	75·0	71·0	73·0	62·3
α-Gentiotriose	(r)	93·4	73·1	74·3	71·2	71·2	70·0 or 70·2
β-Gentiotriose	(r)	97·3	75·5	76·8	71·2	76·1	70·0 or 70·2
	(m)	103·7	74·6	77·1	71·2	76·1	70·0 or 70·2
	(n)	103·7	74·6	77·1	71·0	77·1	62·4

In spite of the sterically unfavourable axial substituents in structure (1), the α-anomer of (2) predominates in its equilibrium mixture. This fact could be due to *cis* hydrogen bonding between 2-OMe and 1α-OH. This interpretation could be applied to the equilibria of kojibiose and of sophorose.[18] α-Kojibiose is much more sterically hindered than the β-anomer, so that there is 50% β-anomer in equilibrium. However, β-sophorose is more sterically hindered than the α-anomer, which thus predominates. Also, the interpretation mentioned above explains the ^{13}C chemical shifts of these two sugars. In kojibiose the α-anomer resonances due to C-1 and C-1′ are shifted to much higher field.

In the nonreducing glucobioses, namely, αα-, ββ-, and αβ-trehalose (α-D-glucopyranosyl-α-D-glucopyranoside, β-D-glucopyranosyl-β-D-glucopyranoside, and α-D-glucopyranosyl-β-D-glucopyranoside), glucose units are linked to each other at the anomeric position. The spectrum of αα-trehalose showed only six absorptions and that of ββ-trehalose had only five. This simplicity suggests that these compounds have a very symmetrical structure. On the basis of general principles of sugar ^{13}C n.m.r., the lowest field resonances, at 94·8 p.p.m. for αα-trehalose and at 100·7 for ββ-trehalose, are assigned to their anomeric carbon nuclei. Comparison of identified anomeric carbon resonances of methyl α-D-glucopyranoside (1′α) and methyl β-D-glucopyranoside (1′β) with the spectrum of αβ-trehalose, which shows eleven resonances, leads to assignment of the peaks at 101·3 and 104·0 p.p.m. to C-1α and C-1β, respectively. Molecular models of αα- and ββ-trehalose suggest that hydrogen bonding between the ring oxygen and 2-OH should

TABLE 3

^{13}C N.m.r. spectra of glucose and mono-*O*-methylglucoses

	C-1	C-2	C-3	C-4	C-5	C-6	OMe
α-D-Glucopyranose (1α)	93·3	73·1	74·4	71·2	72·9	62·4	
β-D-Glucopyranose (1β)	97·1	75·6	77·3	71·2	77·3	62·4	
Methyl α-D-glucopyranoside (1′α)	100·5	73·1	74·8	71·4	72·8	62·3	56·8
Methyl β-D-glucopyranoside (1′β)	104·5	74·6	77·3	71·2	77·3	62·4	58·8
2-*O*-Methyl-α-D-glucopyranose (2α)	90·7	81·9	73·5	71·3	72·8	62·4	59·3
2-*O*-Methyl-β-D-glucopyranose (2β)	97·1	85·2	76·8	71·3	77·3	62·4	61·7
3-*O*-Methyl-α-D-glucopyranose (3α)	93·4	72·6	84·1	70·6	72·8	62·3	61·3
3-*O*-Methyl-β-D-glucopyranose (3β)	97·2	75·1	86·7	70·4	77·3	62·3	61·3
4-*O*-Methyl-α-D-glucopyranose (4α)	93·2	73·0	73·9	80·5	71·7	62·1	61·6
4-*O*-Methyl-β-D-glucopyranose (4β)	97·1	75·8	76·7	80·5	76·1	62·1	61·6
6-*O*-Methyl-α-D-glucopyranose (5α)	93·3	73·0	74·3	71·4	71·4	72·6	60·3
6-*O*-Methyl-β-D-glucopyranose (5β)	97·3	75·8	77·2	71·4	75·8	72·6	60·3

[18] T. Usui, M. Yokoyama, N. Yamaoka, K. Matsuda, K. Tuzimura, H. Sugiyama, and S. Seto, submitted to *Carbohydrate Res.*

occur for symmetry acquisition, and that the anomeric protons of these monomeric units sterically interfere with each other. This stereochemical requirement migh cause these anomeric carbon resonances to move upfield.

In the case of glucotrioses, we selected maltotriose [*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-glucopyranose], panose [*O*-α-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranose-(1→4)-D-glucopyranose], isopanose, and gentiotriose [*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→6)-D-glucopyranose] as models for structural analysis of dextrins and starches. As shown in Table 1, maltose (4-*O*-α-D-glucopyranosyl-D-glucopyranose) has its C-1′ resonance shifted to slightly lower field than usual for α-linked glucobioses. This was also the case with maltotriose, panose, and isopanose. The C-1′ and C-1″ resonances of maltotriose appeared at 101·1 p.p.m. (see Table 2). The resonances of the glucosidic carbon atoms linked to the C-4 nuclei of panose and isopanose appeared at 101·1 p.p.m. and that of the carbon atom linked to the C-6 nucleus appeared at 99·3 p.p.m. Because of these regularities, the C-1, C-1′, and C-1″ nuclei of these sugars showed their resonances at the same chemical shifts as the corresponding glucobioses.

Carbon Nuclei attached to a Glycosidic Linkage.—For the determination of the position of attachment in the reducing units of glucobioses, spectral analysis of mono-*O*-methylglucose derivatives was expected to be useful. Since methylation of a hydroxy-group effected an 8—11 p.p.m. downfield shift in the position of the resonance of the directly attached carbon atom, comparison of *O*-methylglucopyranose spectra with the resonances of D-glucopyranose (1) easily gave the full assignment as shown in Table 3. Also, when the parent monosaccharide is known, such data may be helpful in establishing the position of methoxy-groups in partially methylated glucopyranoses. The C-2, -3, and -4 resonances of 2-, 3-, and 4-*O*-methyl-D-glucopyranoses [(2)—(4)] were readily assigned. They appeared between 80 and 87 p.p.m., distinct from other carbon resonances. In the case of 6-*O*-methyl-D-glucopyranose (5), either the peak at 71·4 or that at 72·6 p.p.m. could be assigned to C-6, in view of the expected 8—10 p.p.m. downfield shift. The C-1, -2, -3, and -4 resonances were expected to be unchanged by 6-*O*-methylation and were directly assigned from the spectrum of (1). The C-4 peak could thus be identified as that at 71·4 p.p.m. The C-5 resonance might overlap with this 71·4 p.p.m. peak in view of the expected 0·5—1·0 p.p.m. upfield shift due to the β-effect on 6-*O*-methylation.[11] Consequently, the peak at 72·6 p.p.m. was attributed to the C-6 signal, on the basis of relative peak heights. This assignment was confirmed when the peak at 72·6 p.p.m. was split into a triplet on proton off-resonance decoupling.

From a comparison of the peaks between 78 and 87 p.p.m. in the spectra of glucobioses with those of the corresponding *O*-methylglucoses, the assignments of *O*-methyl-glucoses could be extended to glucobioses. The C-2 and C-3 resonances of 1,2- and 1,3-linked glucobioses should depend on the configuration of the C-1 hydroxy-group as well as those of the 2- and 3-*O*-methylglucoses, and could be easily assigned from their relative intensities. The glycosidation shift of β-linked glucobioses was found to be very similar to the methylation shift of methylated glucoses. However, the shifts of α-linked glucobioses were 1·5—5 p.p.m. to higher field effect than the methylation shift (Tables 1—3).

In the case of gentiobiose (6-*O*-β-D-glucopyranosyl-D-glucopyranose), the C-6 resonance would be expected to occur in the range 70—74 p.p.m., having undergone the glycosidation shift of 8—10 p.p.m. downfield from its position in (1). Hence, either of the resonances at 70·2 and 71·0 p.p.m. could be due to this nucleus. By proton off-resonance decoupling the peak at 70·2 p.p.m. was split into a triplet; thus this peak was assigned to C-6. In the case of gentiotriose, C-6 and C-6′ could be assigned to the peaks at 70·0 or 70·2 p.p.m. by comparison with gentiobiose, but it could not be decided which resonance was due to which nucleus. The C-6 resonance of isomaltose, which has a 1,6α-linkage, might be expected to shift 2—4 p.p.m. downfield (glycosidation shift) from that of (1). Therefore the peak at 67·4 p.p.m. was assigned to C-6. Thus, the signals near 67·5 p.p.m. in the spectra of panose and isopanose were assigned to C-6′ and C-6, respectively.

Roberts *et al.*[14] reported that C-4 of maltose has two resonance peaks due to the two C-1 anomeric configurations. Unfortunately, our system did not give such a splitting of the maltose C-4 resonance, but the C-4 peak at 78·5 p.p.m. was a little broader than other resonances, and this could arise from the presence of two lines when separation was too small for full resolution. In the case of maltotriose, the peaks at 78·3 and 78·9 p.p.m. could be apparently assigned to the linkage carbon atoms (C-4 and C-4′). The relative peak heights were used to assign these peaks specifically; the C-4α resonance would be expected to show a slight upfield shift due to the steric interference of the C-1 OH with the α-linked non-reducing unit (C-3 and C-5 axial hydrogen atoms). For these reasons, the peak at 78·3 p.p.m. was assigned to C-4α and C-4′ (α and β), and the peak at 78·9 p.p.m. to C-4β. The same phenomenon was observed in the case of panose, which has a maltose unit on the reducing side; the C-4α and C-4β resonances appeared at 78·5 and 78·8 p.p.m., respectively.

Other Carbon Nuclei.—Comparisons of the ^{13}C n.m.r. spectra of the four kinds of mono-*O*-methyl-D-glucopyranose (2)—(5) with the resonances of (1) readily gave the full assignment of these derivatives. In the case of (2), the C-1α peak showed a 2·6 p.p.m. upfield shift due to hydrogen bonding between 1α-OH and 2-OMe. From the same reason, the 2α-OMe resonance was considered to be that at 59·3 p.p.m. and the 2β-OMe resonance to be that at 61·7 p.p.m. Our results for (3) were consistent with Roberts' assignment.[14] In the case of (4), peaks at 73·9 and 71·7 p.p.m. could be directly assigned to the C-3α and C-5α nuclei, respectively. In the α-anomer the C-3 resonance showed a 0·5 p.p.m.

upfield shift from (1α), but the C-5 resonance showed a 1·2 p.p.m. upfield shift. From this fact the peak at 76·1 p.p.m., which was 1·2 p.p.m. to higher field than in (1β), was assigned to C-5β, and that at 76·7 p.p.m. to C-3β. This C-5 upfield shift might attributed to steric perturbation due to hydrogen-bonding between 4-OMe and 6-OH. The C-5 1·5 p.p.m. upfield shift of (5) can probably be interpreted similarly.

The assignment of glucobiose and of glucotriose spectra was relatively easy, but there were some problems. In the case of 1,1-linked non-reducing glucobioses, chemical shifts of αα- and ββ-trehalose (except for the anomeric carbon nuclei) were identical with those of the glucopyranosides (1′α) and (1′β), respectively. Similarly, in αβ-trehalose the spectrum of the α-linked unit could be well correlated with the data for (1′α), and the β-linked unit with the data for (1′β). However, the C-3β and C-5β resonances of αβ-trehalose cannot be distinguished. The peak due to C-6 would be expected to appear at slightly higher field than that of C-6′, because the glucopyranoside (1′α), kojibiose, and nigerose (3-*O*-α-D-glucopyranosyl-D-glucopyranose), which have α-glycosidic linkages, show slight upfield shifts for the C-6 resonance.

Methyl glucobiosides were helpful in the interpretation of the spectra of kojibiose and sophorose. The spectrum of methyl β-kojibioside showed 13 peaks, which could be directly assigned except for four (61·9, 62·5, 70·8, and 71·3 p.p.m.) by comparison of data for *O*-methylated glucoses with data for kojibiose itself. The peaks at 71·3 and 70·8 p.p.m. were attributed to C-4 and C-4′, but they cannot be distinguished. The peaks at 62·5 and 61·9 p.p.m. are assigned to the C-6 and C-6′ resonances. If the C-6 upfield shift of expected for an α-linked glucose can be applied in this case, the peak at 61·9 p.p.m. should be identified as the C-6′ resonance. The spectra of methyl α-sophoriside and the related sugars allowed the straightforward assignment of data for sophorose.

In the spectra of nigerose and laminaribiose (3-*O*-β-D-glucopyranosyl-D-glucopyranose), the C-2α and C-5α resonances were very close to each other (71·3 and 72·2 p.p.m. in nigerose and 72·2 and 72·4 p.p.m. in laminaribiose). In both cases the peaks at higher field should be assigned to C-2α, because the C-2 nuclei would be more affected than the C-5 nuclei by C-3 glycosidation.

In the case of maltose the assignment of the C-3 and C-5 resonances was different from that of Roberts.[14] As mentioned in the case of (4), the peaks at 71·6 and 76·1 p.p.m. could be assigned to C-5α and -5β, respectively. The peaks at 74·6 and 77·8 p.p.m. could be assigned to C-3α and -3β, respectively. In cellobiose (4-*O*-β-D-glucopyranosyl-D-glucopyranose) our assignment was identical with that of Roberts.[14] Direct assignment of 1,6-linked glucobioses was possible because of their regularity. In the spectrum of methyl β-gentiobioside [methyl *O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside] the C-4 and C-4′ resonances at 71·2 and 71·0 p.p.m. could not be distinguished.

We used the same procedure as for the assignment of glucobioses for the glucotriose assignments. The middle unit C-5′ resonance of maltotriose appeared at 72·7 p.p.m.; soluble starch [14] and the β-limit dextrins had the same chemical shift for C-5 (72·5—73·0 p.p.m.).

The regularity observed for the correlation of the chemical shifts with the configurations of glucobioses and glucotrioses might allow the relationship between the conformation of glucose and its ^{13}C chemical shifts to be used for the conformational analysis of glucobioses and glucotrioses. The resonances of C-1, -3, and -5 (1,3-diaxial) for compounds with C-1 α-substituents (hydroxy- and *O*-glucosyl group) were observed to be about 3—5 p.p.m. upfield from those for compounds with the β-configuration; this was also observed in analysis of the ^{13}C spectra (C-1, -3, and -5) of the glucopyranoses (1α) and (1β).[19] This uniform variation might be due to varying 1—3 steric interaction of the anomeric centre distorting the six-membered ring.[20,21] For this reason, these compounds should exist in a *C*1 chair conformation as anticipated from conformational analysis.

All the assignments of glucobioses and (especially) of glucotrioses, for which absorption peaks are very close to each other, are tentative and can be confirmed only by the isotope-labelling method.

Glucans.—The extension of our study to the biologically important and naturally occurring glucose polymers was restricted by excessive widths of resonance peaks and low solubilities of polysaccharides. The present research was limited to β-limit dextrins, which are soluble products of the degradation of amylopectin or glycogen by β-amylase. Amylopectin and glycogen are highly branched polysaccharides containing only D-glucose units with α-1,4- and α-1,6-linkages. The difference between them is the degree of 1,6-branching.[22] β-Amylase hydrolyses α-1,4-linkages of glucans to maltose from the reducing end of the chain and cannot cleave 1,6-glycosidic linkages. We have measured the spectra of the β-limit dextrins of animal glycogens (rabbit liver and oyster), plant glycogens of sweet corn and *Lentinus edodes* (' shitake ' mushroom), and maylopectins (waxy maize and potato). We have also examined isomaltodextrin, which has only α-1,6-linkages, and cyclohexa-amylose, which has only α-1,4-linkages.

Cyclohexa-amylose showed six peaks. The C-1, -3, -4, and -6 resonances could be easily assigned to the peaks at 102·9, 75·4, 82·8, and 62·3 p.p.m., respectively. The C-2 and -5 resonances should be those at 73·7 and 73·5 p.p.m. It was difficult to decide which of them was due to C-5, although it was possible that this was the peak at 73·5 p.p.m., deduced by superimposition on the spectrum of glucobiose. The C-1 and -4 resonances of cyclohexa-amylose were 2 and 4 p.p.m. to lower field than

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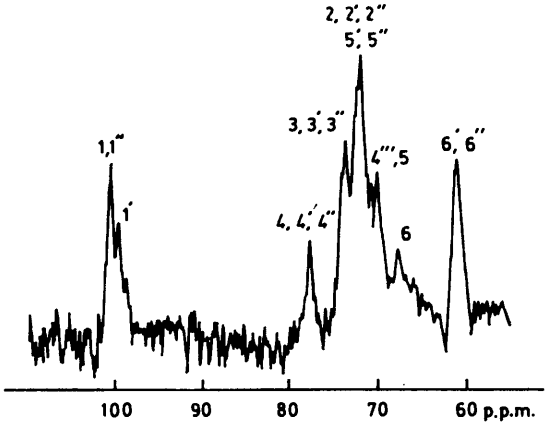
those of other α-1,4-linked sugars for an unknown reason. The spectrum of isomaltodextrin showed five peaks, which were quite close to those of isomaltose and could be assigned as shown in Table 4. The spectra of the β-limit dextrins were almost identical with each other. The spectrum of the β-limit dextrin of rabbit liver will be described as an example (Table 4 and Figure). Com-



The spectrum of the β-limit dextrin from rabbit liver

parison of the chemical shifts of the β-limit dextrin with those of maltotriose, isomaltodextrin, and cyclohexa-amylose brought out the similarities in this group as shown in Table 4. The peaks at 100·6, 78·1, and 62·1 p.p.m. could be easily assigned to C-1 and -1'', C-4, -4', and -4'', and C-6' and -6'', respectively. By comparison of the data for the β-limit dextrin with those for isomaltodextrin the peaks at 67·3, 71·0, and 99·5 p.p.m. could be apparently assigned to the carbon nuclei of the branching units (C-1', -5, and -6) as shown in the Scheme. The peak at 67·3 p.p.m. could be assigned to C-6; this peak was broader than the others. The peak at 99·5 p.p.m. could be identified as the C-1' resonance. The C-4''' resonance of a non-reducing end of a chain would be expected to have the same chemical shift in maltose or maltotriose, so that it seems likely that the C-5 and C-4''' peaks were overlapping at 71·0 p.p.m. The steric environments of C-2, -2', and -2'', C-3, -3', and -3'', and C-5' and -5'' would not differ much from those of maltotriose or panose. The peak at 72·6 was twice as intense as that at 74·3 p.p.m. From this result, the C-2, -2', and -2'' and C-5' and -5'' resonances appear to overlap at 72·6 p.p.m., and the C-3, -3', and -3'' resonances could be assigned to the peak at 74·3 p.p.m. on the basis of their lower chemical shifts and the relative peak height. Though this interpretation is tentative, it is supported by the study of soluble starch by Roberts *et al.*[14]

As already mentioned, a clear difference in chemical shifts between β-limit dextrin of glycogen and that of amylopectin was not detected, but the spectra differed in the relative peak heights. It has been reported,[23] on the basis of results from a chemical method, that the degree of branching in glycogen is roughly twice as much as that in amylopectin. The relative intensities of the 67·3, 71·0, 78·1 and 99·5 p.p.m. peaks should indicate the degree of branching. Throughout the entire spectra the β-limit dextrins of animal glycogen and plant glycogen had larger intensities in the peaks at 67·3, 71·0, and 99·5 p.p.m. than those of amylopectin, but for the peak at 78·1 p.p.m. the reverse tendency was shown. Hence, it may be concluded that ^{13}C n.m.r. spectra can be used not only to detect branching in these dextrins but also to make a reasonable estimate of the extent of branching.

EXPERIMENTAL

Measurement of ^{13}C *N.m.r. Spectra.*—Spectra were measured at 25·1 MHz with a JEOL PS-100 spectrometer. All protons were decoupled. The carbohydrates were examined as aqueous *ca.* 1—2M-solutions. Chemical shifts were measured by using methanol and benzene (128·5 and 49·8 p.p.m., respectively, downfield from internal Me_4Si) as external standards, and were referred to Me_4Si. Spectra were determined after multiple scanning by use of a time-averaging device.

Preparation of Samples.—In general, preparations were carried out by well known procedures.

(A) *Monosaccharides.* The anomeric methyl glucopyranosides were synthesized by the conventional method.[24] 2-, 4-, and 6-Mono-*O*-methyl-D-glucoses were prepared *via* methylation of the corresponding tetra-*O*-acetylglucose.[25] 3-*O*-Methyl-D-glucose was obtained *via* methylation of 1,2 : 5,6-di-*O*-isopropylidene-3-*O*-methyl-α-D-glucose.[26]

(B) *Oligosaccharides.* αβ-Trehalose and ββ-Trehalose were prepared by the method of Michael.[27,28] αα-Trehalose was prepared from Baheris yeast according to the procedure of Sato.[29] Kojibiose was prepared by the method of Matsuda.[30] Sophorose,[31] gentiobiose,[32] and gentiotriose [33] were synthesized by Konigs–Knorr condensations. Nigerose,[34] laminaribiose,[35] isomaltose,[36] panose,[37] and isopanose [38] were prepared by known procedures. Maltose, cellobiose, and cyclohexa-amylose were commercial products, used without further purification.

(C) *Methyl glucobiosides.* Methyl β-kojibioside and methyl α-sophoroside were synthesized.[18] Methyl β-maltoside, methyl β-cellobioside, and methyl β-gentiobioside were prepared by methylation of the corresponding glucobiosyl bromides, as for methyl β-glucopyranoside.

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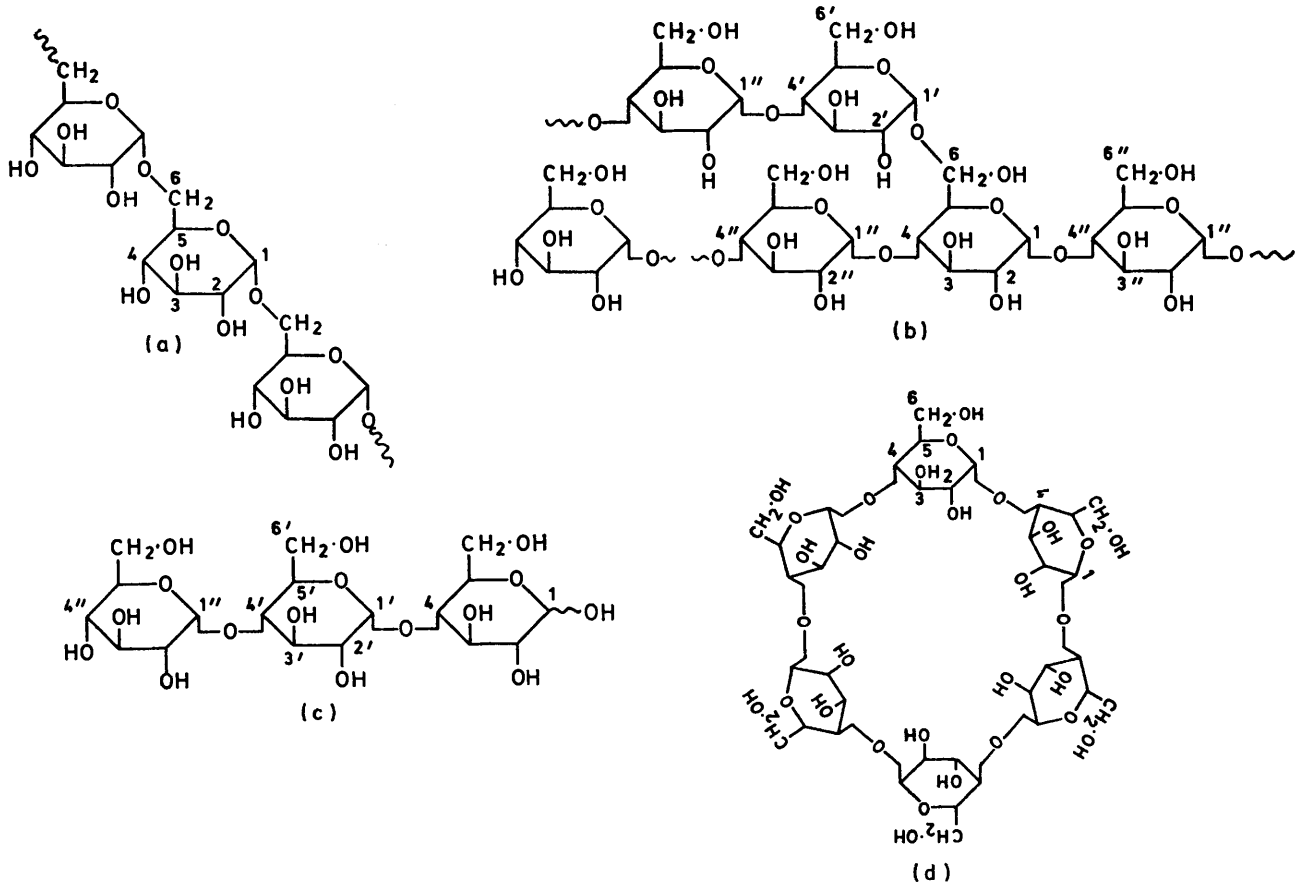

SCHEME

TABLE 4

Comparison of the ^{13}C n.m.r. spectra of β-limit dextrin and related compounds *

Peak no.	Isomaltodextrin	(b) Rabbit liver β-limit dextrin	(c) Maltotriose	(d) Cyclohexa-amylose
1	99·3(C-1)	100·6	101·1(C-1′)	102·9(C-1)
2	74·8(C-3)	99·5	78·5(C-4′)	82·8(C-4)
3	73·0(C-2)	78·1	74·7(C-3′)	75·4(C-3)
4	71·1(C-4, -5)	74·3	73·1(C-2′)	73·7(C-2)
5	67·1(C-6)	72·6	72·7(C-5′)	73·5(C-5)
6		71·0	62·4(C-6′)	62·3(C-6)
7		67·3		
8		62·1		

* Numbering systems are shown in the Scheme.

(D) *Polysaccharides.* Isomaltodextrin was a commercial product. β-Limit dextrins were prepared by use of β-amylase from waxy-maize amylopectin, potato starch, plant glycogens of sweet corn and *Lentinus edodes*, oyster glycogen, and rabbit liver glycogen.[39] The plant glycogens of sweet corn [40] and *Lentinus edoes* [41] were prepared by known procedures. Waxy-maize amylopectin, potato starch, oyster glycogen, and rabbit liver glycogen were commercial products, and were used after removal of fats with ether. Crystalline soy bean β-amylase was obtained from Nagase Sangyo Co., Ltd.

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