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PROTON MAGNETIC RESONANCE SPECTRA OF D-GLUCO-OLIGOSACCHARIDES AND D-GLUCANS

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ABSTRACT

The p.m.r. spectra of some D-gluco-oligosaccharides and D-glucans in deuterium oxide were studied with respect to the anomeric proton. In (1→2)-linked glucobioses, the effect of change in configuration of the hydroxyl group at C-1 on the chemical shifts of the glycosidic proton is noted. Equilibrium mixtures of (1→2)-linked glucobioses contained more α-anomer than did the other examples, despite the cis configuration of substituents at C-1 and C-2. Some D-glucans were investigated with regard to the degree of branching, although solubility was a limitation.

INTRODUCTION

Proton n.m.r. (p.m.r.) spectroscopy is of great value for establishing the configuration and conformation of carbohydrates in solution[1–3]. Van der Veen[4] noted that the glycosidic-linkage protons of oligosaccharides resonate downfield of the non-anomeric protons, although the published data showed only poor resolution.

The p.m.r. spectra of permethyl[5] and pertrimethylsilyl[6] ethers have been used for determining glycosidic configuration. This paper describes the p.m.r. spectra of unmodified glucobioses and glucotrioses with respect to the chemical shifts and coupling constants of the anomeric protons. The equilibrium proportion of α- and β-anomers of reducing sugars in deuterium oxide solution is readily determined by p.m.r.[1]. In addition, p.m.r. has been evaluated with some D-glucans[7]. The determination of degree of branching of β-limit dextrins and a water-soluble dextran were readily made on the basis of anomeric-proton signals. The ratio of (1→6) to (1→4) linkage of β-limit dextrins from glycogen and amylopectin agreed well with data determined by chemical methods[8]. Acetates and benzoates of polysaccharides have already been similarly studied[9].

RESULTS AND DISCUSSION

Anomeric proton at the reducing end. — In the p.m.r. spectra of reducing oligosaccharides, it is necessary[4] to differentiate the signals of the inter-sugar

TABLE I

CHEMICAL SHIFTS[a] AND COUPLING CONSTANTS ($J_{1,2}$, Hz) OF THE ANOMERIC PROTONS[b]

Compound	*Anomeric proton*		*Glycosidic proton*			*O-CH₃*
	H-1α	*H-1β*	*H-1*	*H-1'*	*H-1''*	
D-Glucopyranose	5.26 (3.0)	4.66 (7.5)				
α-Linkage						
Methyl α-D-glucopyranoside			4.79 (3.3)			3.40
α,α-Trehalose (α, α)-(1→1)			5.20 (3.0)			
α,β-Trehalose (α, β)-(1→1)			5.24 (3.0)			
Kojibiose α-(1→2)	5.47 (3.0)	4.83 (7.3)		α 5.13 (3.0)		
				β 5.39 (3.0)		
Methyl β-kojibioside			4.54 (7.5)	5.32 (3.0)		3.60
Nigerose α-(1→3)	5.27 (3.0)	4.69 (7.5)		5.38 (3.0)		
Maltose α-(1→4)	5.26 (3.0)	4.67 (7.5)		5.40 (3.0)		
Methyl β-maltoside			4.40 (7.5)	5.39 (3.0)		3.59
Isomaltose α-(1→6)	5.28 (3.0)	4.68 (7.0)		4.99 (3.0)		
Maltotriose α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-D-Glc	5.27 (3.0)	4.66 (7.5)		5.40 (3.0)	5.40 (3.0)	
Panose α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→4)-D-Glc	5.27 (3.2)	4.67 (7.7)		5.41 (3.0)	5.00 (3.0)	
Isopanose α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→6)-D-Glc	5.29 (3.0)	4.68 (7.5)		4.99 (3.0)	5.40 (3.0)	
β-Linkage						
Methyl β-D-glucopyranoside			4.39 (7.0)			3.56
β,β-Trehalose (β, β)-(1→1)			4.74 (7.5)			
α,β-Trehalose (α, β)-(1→1)			4.67 (8.0)			
Sophorose β-(1→2)	5.46 (3.5)	4.74		α 4.65 (7.5)		
		or 4.77 (7.5)		β 4.74		
				or 4.77 (7.5)		
Methyl α-sophoroside			4.95 (3.0)	4.61 (7.0)		3.42
Methyl β-sophoroside			4.59 (7.5)	4.88 (7.5)		3.64
Laminarabiose β-(1→3)	5.28 (3.0)	4.71 (7.5)		α 4.74 (7.5)		
				β 4.75 (7.5)		
Methyl β-laminarabioside			4.48 (7.5)	4.79 (7.5)		3.64
Cellobiose β-(1→4)	5.26 (3.0)	4.68 (7.5)		4.52 (7.5)		
Methyl β-cellobioside			4.39 (7.5)	4.52 (7.0)		3.59
Gentiobiose β-(1→6)	5.25 (3.0)	4.67 (7.0)		4.56 (7.5)		
Methyl β-gentiobioside			4.38 (7.0)	4.54 (7.5)		3.59
Gentriotriose β-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→6)-D-Glc	5.26 (3.0)	4.68 (7.5)		4.54 (7.5)	4.54 (7.5)	

[a]In p.p.m. downfield from DSS. [b]In D_2O at room temperature.

anomeric protons (H-1′ and H-1″) from those (H-1) of the reducing end. It was generally found necessary to heat the solution, to shift the interfering HOD signal upfield, in order to observe the signal of anomeric proton. The temperature dependence of the chemical shifts and the coupling constants of the anomeric-proton signals was negligible (about 0.03 p.p.m. downfield shift). The anomeric-proton signal from the reducing end can readily be distinguished from signals of the other protons because it displays the effect of mutarotation, showing two separate signals for the α- and β-anomers. Adjacent peaks having chemical shifts and coupling constants close to those observed for α- and β-D-glucopyranose[2] may be assigned to H-1α and H-1β, as shown in Table I. Published data on the respective anomeric equilibria were used in conjunction with relative peak-heights to differentiate the H-1α and H-1β resonances. However, the H-1 resonances of (1→2)-linked glucobioses were anomalous when compared with those of α- and β-D-glucopyranose and the other reducing glucobioses. The coupling constants were near 7.5 Hz for the trans configuration and near 3 Hz for the cis configuration, in accord with the Karplus relationship, and the J values for the (1→2)-linked glucobioses were also within these ranges.

Anomeric equilibria. — Anomeric equilibria were determined from the intensities of the H-1α and H-1β signals. The observed anomeric compositions of glucobioses and related compounds, for 20–35% solutions in deuterium oxide at equilibrium, are

TABLE II

ANOMERIC COMPOSITION[a] OF REDUCING SUGARS AT EQUILIBRIUM IN DEUTERIUM OXIDE

Compound	*Linkage*	*α-Anomer*	*β-Anomer*
D-Glucopyranose		36[b]	64
Kojibiose	α-(1→2)	52	48
Nigerose	α-(1→3)	37 (25)[c]	63 (75)
Maltose	α-(1→4)	43	57
Isomaltose	α-(1→6)	37	63
Maltotriose α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-D-Glc		43 (42)	57 (58)
Sophorose	β-(1→2)	63 (66)	37 (34)
Laminarabiose	β-(1→3)	43 (40)	57 (60)
Cellobiose	β-(1→4)	36	64
Gentiobiose	β-(1→6)	32	68
Gentiotriose β-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→6)-D-Glc		33 (35)	67 (65)
2-*O*-Methyl-D-glucopyranose		58	42
3-*O*-Methyl-D-glucopyranose		32	68
4-*O*-Methyl-D-glucopyranose		33	67
6-*O*-Methyl-D-glucopyranose		35	65

[a]By integration of the H-1 signal. [b]The values were obtained at room temperature after anomeric equilibrium had been reached. [c]Measured at 90°.

recorded in Table II. In general, the β-anomer preponderated, except for the (1→2)-linked glucobioses. In kojibiose (2-*O*-α-D-glucopyranosyl-D-glucopyranose), the α- and β-anomers were present in approximately equal proportion. In sophorose (2-*O*-β-D-glucopyranosyl-D-glucopyranose), the α-anomer constituted about two thirds of the mixture, contrasting with the preponderance of the β-anomer in D-glucopyranose and most of the glucobioses. The same phenomenon was observed with 2-*O*-methyl-D-glucopyranose; immediately after dissolution, one anomeric-proton signal was present, a doublet at 4.67 p.p.m. ($J_{1,2}$ 8 Hz, β-anomer), and the *O*-methyl signal appeared at 3.60 p.p.m. After 24 h, this anomeric-proton signal diminished, and another *O*-methyl signal appeared, at 3.46 p.p.m. In the equilibrium solution, the ratio of α- and β-anomers was 58:42, whereas the β-anomers preponderated (as shown in Table II) in the case of other mono-*O*-methyl-D-glucopyranoses. P.m.r. studies of these four mono-*O*-methyl-D-glucopyranoses[10] have already been reported with respect to the anomeric proton and the *O*-methyl resonances, and have suggested a dependence on position of the signals for the *O*-methyl group. Horton and co-workers[11] have reported that, in 2-amino-2-deoxy-D-glucose and related compounds, replacement of the C-2 hydroxyl group by an ammonium or acetamido group leads to greater stabilization of the cis relationship of substituents at C-1 and C-2.

The hydroxyl group at the anomeric carbon atom thus appears to favor the axial over the equatorial orientation when the C-2 hydroxyl group is replaced by a methoxyl or glucosyl group. The anomeric effect[1,12] might be outweighed by solvation effects in the 2-hydroxylated derivatives, but no simple explanation for the relationship observed can yet be offered. No detectable proportion of furanose forms were indicated by the spectra.

Assignment of inter-sugar anomeric-proton resonances. — The anomeric-proton resonances for the inter-sugar α-linkage (doublet, $J_{1',2'}$ or $J_{1'',2''}$ ~3 Hz) were concentrated in the region 5.00–5.45 p.p.m., and those of the corresponding β-linkage (doublet, $J_{1',2'}$ or $J_{1'',2''}$ ~7.5 Hz) lay upfield in the region 4.50–4.80 p.p.m. The anomeric-proton resonance of the inter-sugar linkage overlapped in the anomers, except with the (1→2)-linked glucobioses and laminarabiose (3-*O*-β-D-glucopyranosyl-

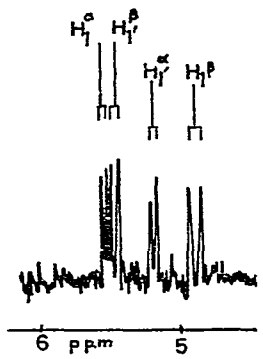

Fig. 1. P.m.r. spectrum of kojibiose at 100 MHz and 90° in deuterium oxide solution.

D-glucopyranose), for which the anomeric proton of the inter-sugar linkage resonated as individual signals (H-1′α and H-1′β in Fig. 1 and Table I) for the α- and β-anomers. To facilitate the assignments, the p.m.r. spectra of methyl glucobiosides were compared with those of the corresponding glucobioses. With sophorose, four peaks (at 4.65, 4.74, 4.77, and 5.46 p.p.m.) were observed in the region for anomeric resonances. The peaks at 4.65 and 5.46 p.p.m. could be assigned to the H-1′α and H-1α pair because of the preponderance of the α-anomer and consequent large peak height. The peaks at 4.74 and 4.77 p.p.m. were thus those for the other anomer, but they could not be specifically differentiated. In kojibiose, the resonance at 4.83 p.p.m. was assigned to H-1β because of the large (trans) coupling (7.3 Hz), and that at 5.39 p.p.m. to H-1′β because of the relative peak-intensity (see Fig .1). The peaks at 5.47 and 5.13 p.p.m. may reasonably be assigned to H-1α and H-1′α, respectively, because the steric environment of the glycosidic proton (H-1′α) should not be much different from that of the analogous proton in the D-glucan that contains α-(1→2) linkages. This dextran will be described later. This interpretation is also supported by ^{13}C n.m.r.[13].

In the (1→1)-linked disaccharides, the anomeric-proton resonances of α,β-trehalose (α-D-glucopyranosyl β-D-glucopyranoside) appeared at 4.67 and 5.24 p.p.m., both as doublets. As expected, the $J_{1,2}$ values were 8 Hz for the trans configuration and 3 Hz for cis. The anomeric-proton signals in α,α-trehalose[4,14] (α-glucopyranosyl α-D-glucopyranoside) and also β,β-trehalose (β-glucopyranosyl β-D-glucopyranoside) were superposed.

Significant variations in chemical shift were observed for the inter-sugar glycosidic proton. Thus the H-1′ signals in the α-glycosidic series showed a difference of ~0.4 p.p.m. between maltose[4] (4-*O*-α-D-glucopyranosyl-D-glucopyranose) and isomaltose (6-*O*-α-D-glucopyranosyl-D-glucopyranose). In the β-glycosidic series there was ~0.2 p.p.m. difference between H-1′ for laminarabiose and for gentiobiose (6-*O*-β-D-glucopyranosyl-D-glucopyranose). To interpret this chemical-shift difference of the glucosidic proton, factors other than equatorial or axial orientation need to be taken into account. Glass[15] has suggested that the electronegativity of the substituent at C-1 is of primary importance, but we consider that steric contributions at the linkage position, as in (1→2)-linked glucobioses, are also a cause of the variation in chemical shifts.

As examples of glucotrioses, we selected maltotriose[15] [*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-glucopyranose], panose [*O*-α-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranose], and isopanose [*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→6)-D-glucopyranose], as these are related to natural oligosaccharides from starch. The glycosidic-proton resonances at the linkage to C-4 in panose (H-1′) and in isopanose (H-1″) appeared at 5.41 and 5.40 p.p.m., respectively. The glycosidic proton at the linkage to C-6 resonated at 5.00 (H-1″ of panose) and 4.99 p.p.m. (H-1′ of isopanose). Thus, the H-1′ and H-1″ signals of these trioses resonated at the same position as observed with the corresponding bioses.

D-*Glucans.* — In extending the study to natural D-glucose polymers[7] the low solubility of glycogen and starch is encountered. However, a β-limit dextrin was found sufficiently soluble to give a spectrum. β-Limit dextrins are degradation products by β-amylase of amylopectin or glycogen. This enzyme hydrolyzes the α-(1→4) linkage of D-glucans from the reducing end of the chain to give maltose, but it can not cleave or by-pass the (1→6) glycosidic linkage. From biochemical-degradation[16] and methylation studies[7], it is known that the differences between β-limit dextrins of various origins arise from the relative ratio of (1→6) to (1→4) linkages. The spectra of β-limit dextrins from animal glycogen (rabbit liver and oyster), plant glycogen (sweet corn; and *Lentinus edodes*, "shiitake"*), and starch (waxy maize and potato) were measured. Maltotriose, panose, and clinical dextran [a dextran containing only α-(1→6) linkages] were selected as the model compounds for aiding peak assignments. In Fig. II, the assignments for the β-limit dextrin of

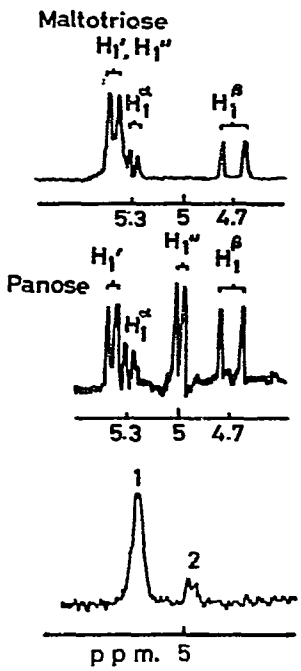

Fig. 2. Comparison of p m.r. spectra, in deuterium oxide, of β-limit dextrin from rabbit-liver glycogen with related compounds.

rabbit liver are shown. All samples were measured at 90°. The relatively intense signals shown as peaks 1 and 2 (5.35 and 4.96 p.p.m.) may be attributed to the glucosidic proton on the C-1 atom linked to O-4 and O-6, respectively, because the

*A kind of mushroom common in Japan and China.

H-1′ and H-1″ resonances of panose (corresponding to the smallest unit of the branched structure) lay in the same positions. Also, peak 1 corresponded to the shift position for the glycosidic protons (H-1′, 1″) of maltotriose, which has only α-D-(1→4) linkage. Peak 2 corresponded to the glycosidic protons of clinical dextran. The intensities of peak 1 and 2 should reflect the ratio of (1→4) to (1→6) linkages. The data obtained are summarized in Table III. The glycosidic proton of the β-limit dextrin resonated about 0.05 p.p.m. to higher field than that for panose and maltotriose. The extent of branching obtained by this direct method agree well with the published values from indirect methods employing degradative procedures[8]. For instance, the ratio of (1→6) to (1→4) linkages in the β-limit dextrin from glycogen was about 1:4, and that for amylopectin was about 1:7. Such differences among β-limit dextrins could be directly detected by this spectroscopic method.

TABLE III

COMPARISON OF THE P.M R. SPECTRA OF β-LIMIT DEXTRINS WITH THOSE OF RELATED COMPOUNDS

Compound	$G_{1\to4}$[a]	$G_{1\to6}$[b]	*Integration*[c]
Panose			
[α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→4)-D-Glc]	5.41	5 00	1:1
Clinical dextran		4.96	
Oyster glycogen[d]	5 34	4.95	5:1
Rabbit-liver glycogen[d]	5.35	4.96	4:1
Lentinus edodes glycogen[d]	5.34	4.95	4:1
Waxy maize amylopectin[d]	5.34	4.95	7.1
Potato starch amylopectin[d]	5.34	4.94	7:1

[a]Chemical shifts in p.p.m. of the glycosidic proton involved in the α-D-(1→4) linkage. [b]Glycosidic proton involved in the α-D-(1→6) linkage. [c]The ratios of α-D-(1→4) to α-D-(1→6) linkages were obtained by integration of the signals due to the glycosidic protons. [d]All β-limit dextrins shown in this Table were measured at 90°.

Dextran. — The p.m.r. method should, in principle, be applicable with D-glucans having different kinds of linkage. As an example, the water-soluble dextran[17] from *Leuconostoc mesenteroides* NRRL B-1299 was selected. The structural features of this dextran have been discussed mainly from the results of chemical studies[18]. Methylation analysis[18a] indicated that this dextran contained about 60% of α-D-(1→6) 33% of α-D-(1→2), and 7% of α-(1→3) linkages. The α-D configuration of the linkage was inferred from optical rotatory data[19]. In this high polymer of D-glucose, the chemical shifts of the glycosidic protons would be expected to correspond to those of the α-(1→2)-, α-(1→3)-, and α-(1→6)-linked glucobioses. The glycosidic-proton signals at lower field (in the region of 4.95–5.30 p.p.m.) were the more clearly differentiated. The p.m.r. spectrum of this dextran was measured (*a*) at room temperature and (*b*) at 90°. At room temperature, the signals for the glycosidic protons were broad lines (Fig. III), but the spectrum at 90° displayed sharp lines. This result might indicate variations of a higher-order structure. By making comparisons with the chemical shifts of the corresponding glucobioses, peaks 1, 2+3, and

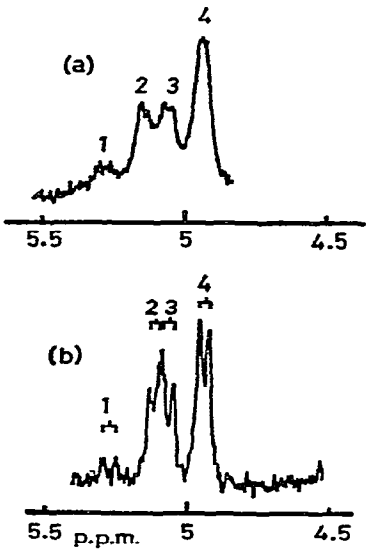

Fig. 3. P.m.r. spectrum of a water-soluble dextran from *Leuconostoc mesenteroides* at 100 MHz in deuterium oxide solution. (*a*) at room temperature; (*b*) at 90°.

4 were attributed tentatively to glycosidic protons involved in the α-(1→3), α-(1→2), and α-(1→6) linkages, respectively. The ratio of intensities of peak 1, 2+3, and 4 (at 90°) is about 8:52:40. If peaks 2 and 3 denote the α-(1→2) linkage, the indicated proportion of such linkages in this dextran is in conflict with the result of methylation[18]. From methylation and hydrolytic data[18], it is concluded that this dextran is branched and has most, if not all, of the branching through an α-(1→2) linkage. Therefore, the signal of the glycosidic proton of the 1,6-di-*O*-substituted residues in the linear portion should differ from that of the 1,2,6-tri-*O*-substituted residues involved in the branching points, because the glycosidic proton in the α-(1→6) linkage intersected by an α-(1→2) linkage would be expected to undergo a steric interaction generated by the cis relationship of substituents at C-1 and C-2. Therefore, either one of signals 2 or 3 may be attributed to the glucosidic proton in a (1→6) linkage. Signals 2 and 3 overlapped each other at 90°, and signal 2 at 90° was shifted slightly upfield of its position at room temperature. This shift, and also the line broadening, may arise from differences of molecular conformation or increased mobility at higher temperature. This temperature effect was found essentially reversible. However, as the branching structure of β-limit dextrin is not altered by such temperature variations, the results suggest that the signals for α-(1→2)-linked unit of branching are affected more easily by temperature variation than the signals for an α-(1→6) linked unit intersected by an α-(1→2) linkage. Signals 2 and 3 should, therefore, be assigned to the α-(1→2)-linked and the α-(1→6)-linked glycosidic proton, respectively. Fig. III suggests that signals 2 and 3 are present in equal proportion. From these results, the ratio of (1→3), (1→2), and (1→6) linkage was

calculated to be 8:26:66, close to the results of methylation studies[18]. From this interpretation and from methylation studies[18], the number of D-glucose residues in the branch is estimated to be one or two at the most. The foregoing results thus suggest that most of the α-(1→2) linkages of this dextran are at branching points, and they join units that constitute non-reducing residues of the dextran chain. Furthermore, it should be noted that the coupling constants in signals 1, 2, 3, and 4 showed $J_{1,2}$ values near 3.5 Hz, indicating the cis configuration. Hence, each D-glucose residue of this dextran evidently adopts the *C1*(D) chair conformation.

EXPERIMENTAL

Measurement of proton n.m.r. spectra. — Proton n.m.r. spectra were measured at room temperature or at 90° in deuterium oxide at 60 and 100 MHz, by using JEOL C-60 HL 60-MHz and JNM PS-100 100-MHz spectrometers. Chemical shifts were measured in p.p.m. from sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) as an internal standard. The first-order coupling constants (J) are expressed in Herz (Hz). Spectral data were usually obtained at a concentration of 20 to 25%, whereas the spectra of some D-glucans were measured with a saturated solution (5–10%) because of their low solubility. To exchange labile hydroxyl protons with deuterons, the samples were dissolved in deuterium oxide, the solvent was removed under diminished pressure, and the residues were dried in a desiccator on phosphorus pentaoxide. This process was repeated three times.

Materials. — Most materials were prepared by known procedures, as indicated in the following procedures.

Monosaccharides. — The anomeric methyl D-glucopyranosides were synthesized conventionally[20]. The 2-, 4-, and 6-mono-*O*-methyl-D-glucoses were prepared by methylation of the corresponding tetra-*O*-acetyl-D-glucoses[21]. 3-*O*-Methyl-D-glucose[22] was obtained by methylating 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose and then removing the *O*-isopropylidene group by dilute acid.

Oligosaccharides. — α,β-Trehalose[23] and β,β-trehalose[24] were prepared chemically by the method of F. Micheel. α,α-Trehalose was prepared from baker's yeast according to the procedure of T. Sato[25]. Kojibiose was prepared by the method of K. Matsuda[26]. Sophorose[27], gentiobiose[28], and gentiotriose[29] were synthesized by the Koenigs–Knorr condensation. Nigerose[30], laminarabiose[31], isomaltose[32], panose[32], and isopanose[34] were prepared according to known procedures. Maltose and cellobiose were commercial products and were used without further purification.

Methyl glucobiosides. — Methyl β-laminarabioside[35], methyl β-maltoside, methyl β-cellobioside, and methyl β-gentiobioside were prepared by a slight modification of Zemplén's method[20]. Methyl β-kojibioside, methyl α-sophoroside, and methyl β-sophoroside were synthesized as new compounds.

Preparation of methyl β-kojibioside and methyl β-sophoroside. — Five of either octa-*O*-acetyl-β-kojibiose[26] or octa-*O*-acetyl-β-sophorose[36] was dissolved with shaking in cold acetic acid (15 ml) saturated with hydrogen bromide (15 ml) and the

mixture was kept for 12 h at room temperature. The reaction mixture was poured into crushed ice (0.2 kg), and the product extracted with chloroform (3 × 40 ml). The combined extracts were washed with cold water (3 × 40 ml), and then a cold saturated aqueous solution (3 × 40 ml) of sodium hydrogen carbonate. The extract was dried over calcium chloride, evaporated to dryness, and the residual syrup crystallized from ether–petroleum ether. The yields of crude hepta-*O*-acetyl-α-kojibiosyl bromide (**1**) and hepta-*O*-acetyl-α-sophorosyl bromide (**2**) were 4 and 4.2 g, respectively.

The unpurified *O*-acetylglycosyl bromides (compounds **1** and **2**, 4 g) were separately dissolved in methanol (280 ml), and the solutions were stirred for 20 h in the dark with dry silver carbonate (5 g). The reaction mixtures were then refluxed for 30 min.

After evaporation of the solvent, a red syrup was obtained that crystallized immediately upon the addition of alcohol–ether.

Methyl hepta-*O*-acetyl-β-kojibioside (**3**) was recrystallized from methanol. The product (2 g) had m.p. 142–143° (dec.). The p.m.r. spectrum showed the *O*-methyl resonance at 3.52 p.p.m., and the *O*-acetyl signals at 2.02~2.10 p.p.m. in chloroform-*d*.

Anal. Calc. for $C_{27}H_{38}O_{18}$: C, 49.84; H, 5.98. Found: C, 50.10; H, 5.71.

Methyl hepta-*O*-acetyl-β-sophoroside (**4**) was recrystallized from ethanol. The product (2.2 g) had m.p. 130–131° dec. The p.m.r. spectrum in chloroform-*d* showed the *O*-methyl signal at 3.58 p.p.m., and the *O*-acetyl resonances at 2.00~2.10 p.p.m.

Anal. Calc. for $C_{27}H_{38}O_{18}$: C, 49.84; H, 5.89. Found: C, 49.99; H, 5.95.

The methylated *O*-acetyl sugar (compound **3** or **4**) was dissolved in 0.05M methanolic sodium methoxide (20 ml). The solution was kept for 4 h and then deionized with ion exchangers (Dowex 50-W), and evaporated to low volume.

Methyl β-kojibioside was obtained as amorphous powder, which was dried in a desiccator over phosphorus pentaoxide. The product (0.91 g) had $[\alpha]_D^{20}$ +83° (*c* 0.8, water).

Anal. Calc. for $C_{13}H_{24}O_{11}$: C, 43.82; H, 6.79. Found: C, 43.46; H, 7.10.

Methyl β-sophoroside was obtained from compound **4**. The product was recrystallized from ethanol. The product (0.83 g) had m.p. 240–241° dec., $[\alpha]_D^{20}$ −40° (*c* 0.8, water).

Anal. Calc. for $C_{13}H_{24}O_{11}\cdot 0.5H_2O$: C, 42.73; H, 6.89. Found: C, 42.49; H, 6.47.

Preparation of methyl α-sophoroside. — Methyl 4,6-*O*-benzylidene-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-glucoside (5 g), prepared by condensation of methyl 4,6-*O*-benzylidene α-D-glucopyranoside with tetra-*O*-acetyl-α-D-glucopyranosyl bromide, was suspended in 100 ml of 60% acetic acid. The mixture was heated for 1 h at 80°, and concentrated under diminished pressure. The mixture was repeatedly extracted with pentane, by decantation, to remove almost all of the benzaldehyde, and it was then concentrated under diminished pressure to a syrup, which crystallized from ethanol–ether. The crystals were recrystallized from ethanol to give 2.9 g of product, m.p. 163–164°, $[\alpha]_D^{20}$ +97° (*c* 1, chloroform).

Anal. Calc. for $C_{21}H_{32}O_{15}\cdot 1.5H_2O$: C, 46.07; H, 6.44. Found: C, 46.25; H, 6.15.

The debenzylidated product (1 g) was treated with 0.05M methanolic sodium methoxide (9 ml). Recrystallization from ethanol gave 0.62 g, of the glycoside, m.p. 245–246° dec., $[\alpha]_D^{20}$ +58° (water).

Anal. Calc. for $C_{13}H_{24}O_{11}\cdot H_2O$: C, 41.71; H, 7.00. Found: C, 41.95; H, 6.77.

D-*Glucans.* — β-Limit dextrins were prepared from starch (waxy maize and potato), plant glycogen (sweet corn[37] and *Lentinus edodes* "shiitake"[38]), and animal glycogen (rabbit liver and oyster) by digesting the D-glucan with crystalline soybean β-amylase (a commercial product from Nagase Sangyo Co., Ltd.), according to the method of Whelan[39]. Clinical dextran was a commercial product from Meito Sangyo Co., Ltd., The water-soluble dextran elaborated by *Leuconostoc mesenteroides* NRRL B-1299 was prepared in the same procedure as reported previously[40].

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