Nuclear Magnetic Resonance Studies of 1,6-Linked Disaccharides

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The signals in the ^{13}C n.m.r. spectra of all anomeric forms of the methyl glycosides of D-Glc*p*-(1→6)-D-Glc*p* and D-Glc*p*-(1→6)-D-Gal*p* in water have been assigned. The chemical-shift differences obtained by comparison with relevant monosaccharide derivatives were used to calculate ^{13}C n.m.r. spectra of oligo- and poly-saccharides containing 1,6-linkages. Good agreement between calculated and experimental spectra were obtained for raffinose, stachyose, dextran, and pustulan. The importance of comparing spectra determined at the same temperature is emphasized. Temperature shifts may, in favourable cases, be used for assignment of signals.

N.m.r. spectroscopy has become one of the most versatile methods for structural studies of oligo- and poly-saccharides,[1,2] especially polysaccharides, of bacterial origin, built of repeating units (see, *e.g.*, refs 3—5). N.m.r. methods allow us to obtain information on sugars, non-sugar components, and anomeric configurations without destruction of the material. Both ^{1}H and ^{13}C n.m.r. spectroscopy have been used but, in the former, signals in the region δ_H 3.4—4.2 generally overlap even at high field strengths. Signals of diagnostic value derive from protons upfield and downfield of this region. In spite of the problem with overlapping signals, much information on the structure of a polysaccharide can be obtained by the use of very-high-field spectrometers and two-dimensional n.m.r. techniques. In ^{13}C n.m.r. spectra, determined at 50 MHz or higher, all carbon resonances can often be separated. However, the full potential of ^{13}C n.m.r. spectroscopy is strongly dependent on the establishment of a comprehensive ^{13}C n.m.r. chemical shift data-base for differently substituted sugars. Some useful rules have recently been established by Kochetkov *et al.*[6] and the spectra of a large number of mono- and oligo-saccharides have been recorded and collected.[7,8] Many assignments are, however, tentative. Furthermore, spectra have been recorded with different references and at different temperatures without specification of these, and the value of published data is therefore limited. Glucose oligomers and glucans have been investigated by Usui *et al.*,[9] Colson *et al.*,[10] and Saito *et al.*[11] Their assignments were made, in part, by comparison with methyl glycosides, their mono-*O*-methyl ethers, and relevant oligosaccharides. The value of methyl glycosides and mono-*O*-methyl-substituted glycosides as model substances is, however, limited since the introduction of a methyl group often gives substituent shifts different from those caused by sugar residues. Colson *et. al.* also observed this, and noted that different linkages gave different substituent shifts and that cyclodextrins gave chemical shifts different from those of the corresponding linear structures. This was explained by conformational changes. Usui *et al.*[9] also investigated methyl β-gentiobioside and obtained results essentially the same as those in the present study.

A study on gentiodextrins by Bassieux *et al.*[12] showed that the central unit of gentiotriose constituted a good model for β-1,6-glucans. ^{13}C N.m.r. data on a trisaccharide with 1,6-linkages *N*-glycosidically linked to an amino acid residue have also been published[13] as have data on isomaltotriose,[14] models also serving as guides for the prediction of data for 1,6-glucans.

In order to develop a computer-assisted spectral analysis, studies of a number of oligosaccharide glycosides have been initiated. We now present synthesis and n.m.r. studies of some methyl glycosides of 1,6-linked disaccharides. From chemical shifts of these and relevant monosaccharides, substituent-shift increments are calculated and used to simulate spectra of oligo- and poly-saccharides containing 1,6-linked sugar residues.

Experimental

Materials.—The disaccharides (**1**), (**2**), (**5**), and (**6**) were obtained by the reaction of methyl 2,3,4-tri-*O*-benzoyl-α- and -β-D-glycopyranosides of glucose and galactose with 2,3,4,6-tetra-*O*-α-benzyl-D-glucopyranosyl bromide under conditions of halide-ion catalysis.[15] The disaccharides (**3**), (**4**), (**7**), and (**8**) were obtained by the reaction of the same aglycones with 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide under conditions of silver trifluoromethanesulphonate promotion.[16,17] The coupling products were deprotected by classical methods and purified on a Biogel P-2 column before the n.m.r. spectra were run. The synthesis of compounds (**1**),[18] (**3**),[18,19] and (**4**)[19] have been reported.

Synthesis of α-Linked Disaccharides (**1**), (**2**), (**5**), *and* (**6**).—For the synthesis of compound (**1**), a solution of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide (2 mmol) in dichloromethane (5 ml) was added to a stirred solution of methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside (1 mmol) and tetraethylammonium bromide (2 mmol) in dichloromethane–dimethylformamide (9:1; 8 ml) containing ground 4Å molecular sieves (2 g). The mixture was stirred at 35 °C for 24 h, when t.l.c. indicated that the reaction was complete. The mixture was diluted with dichloromethane, filtered, and the filtrate was washed successively with water, 5% aqueous sodium hydrogen carbonate, and water. After the filtrate had been dried ($MgSO_4$), filtered, and concentrated to dryness, a syrup was obtained. Chromatography on silica gel with toluene–ethyl acetate (10:1) as eluant gave the target compound. Deprotection was performed by hydrogenation with 10% palladium–carbon in 90% aqueous acetic acid at 400 kPa for 16 h, followed by de-*O*-benzoylation with 0.1M-methanolic sodium methoxide at 25 °C for 2 h. The solution was deionized by passage through Dowex 50 (H^+) and then concentrated. Partition between water and diethyl ether, and gel filtration of the aqueous phase on a Biogel P-2 column eluted with water, gave the pure compound. The oligosaccharide derivatives (**2**), (**5**), and (**6**) were synthesized analogously. Yields and optical rotations are given in Table 1.

Synthesis of α-Linked Disaccharides (**3**), (**4**), (**7**), *and* (**8**).—For the synthesis of compound (**3**), a solution of silver trifluoromethanesulphonate (2.6 mmol) in toluene (2 ml) was added to a stirred solution, at −25 °C, of 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide (2.6 mmol) and methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside (2 mmol) in dichloromethane (5

Table 1. Yield, anomeric purity, and optical rotation of disaccharides (**1**)—(**8**)

Compd.	Methyl glycoside of	Yield[a] (%)	Anomeric purity[b] (%)	$[\alpha]_D^{22}$[c]
(**1**)	α-D-Glc*p*-(1→6)-α-D-Glc*p*	61	94	166
(**2**)	α-D-Glc*p*-(1→6)-β-D-Glc*p*	77	91	51
(**3**)	β-D-Glc*p*-(1→6)-α-D-Glc*p*	88	>99	61
(**4**)	β-D-Glc*p*-(1→6)-β-D-Gal*p*	86	95	−34
(**5**)	α-D-Glc*p*-(1→6)-α-D-Gal*p*	38	92	176
(**6**)	α-D-Glc*p*-(1→6)-β-D-Gal*p*	48	92	87
(**7**)	β-D-Glc*p*-(1→6)-α-D-Glc*p*	53	>99	72
(**8**)	β-D-Glc*p*-(1→6)-β-D-Gal*p*	67	98	−28

[a] Calculated from the amount of aglycone used. [b] Calculated from the intensities of the anomeric protons in the ^{1}H n.m.r. spectra. [c] In water.

ml) containing ground 4Å molecular sieves. The reaction was complete in 15 min and pyridine (2 ml) was added to neutralize the reaction. The reaction mixture was diluted with diethyl ether–ethyl acetate (1:1), and filtered. The filtrate was washed successively with 10% aqueous sodium thiosulphate, water, 2M-sulphuric acid, aqueous ammonia, and water to yield, after concentration to dryness, a syrup containing the expected disaccharide as the major product. The disaccharide was crystallised from ethanol. Debenzoylation was performed as above and the product was purified by chromatography on a Biogel P-2 column.

The oligosaccharides (**4**), (**7**), and (**8**) were synthesized analogously. Yields and optical rotations are given in Table 1. The disaccharides with D-galactopyranosyl residues were also subjected to chromatography on silica gel with toluene–ethyl acetate (12:1) as eluant before deprotection.

N.m.r. Spectroscopy.—^{1}H and ^{13}C n.m.r. spectra were recorded for deuterium oxide solutions on a JEOL GX-400 spectrometer using sodium 3-(trimethylsilyl)[$^2H_4$]propionate (TSP) δ_H 0 p.p.m. and dioxane δ_C 67.4 p.p.m. as internal references. ^{1}H N.m.r. spectra were run at 85 °C and ^{13}C n.m.r. spectra at 40 and 70 °C. Proton-decoupled ^{13}C n.m.r. spectra were measured for 0.5M-solutions with an accuracy of ±0.006 p.p.m. and ^{1}H n.m.r. spectra for 0.1M solutions with an accuracy of ±0.0006 p.p.m.

Differential isotope shifts[20] (DIS) were obtained by recording spectra for solutions in deuterium oxide and water containing 5% deuterium oxide. For some of the disaccharides selective decoupling or two-dimensional carbon–proton chemical-shift-correlation spectroscopy was performed for unambiguous assignment of the signals.

Results and Discussion

The disaccharide methyl glycosides used in this study were synthesized by conventional methods as reported in the Experimental section. Their structures were evident from the method of synthesis, ^{1}H n.m.r. data, and optical rotation.

^{13}C N.m.r. spectra for disaccharides (**1**)—(**8**) and the corresponding monomers are given in Table 2. This table also contains the chemical-shift differences obtained by comparing the glycopyranosyl group in the disaccharide with α- or β-D-glucopyranose and the methyl glycoside residue with the corresponding methyl glycoside. The free sugars, instead of the methyl glycosides, are used in the comparison of the chemical shifts of the glucopyranosyl groups because these are unaffected of the methyl group of a methyl glycoside.

Chemical Shifts in the Glucopyranosyl Group.—Inspection of Table 2 shows that a glycoside with a 1,6-linkage gives a ^{13}C n.m.r. spectrum with chemical shifts for the different carbons of the glucopyranosyl group which depend upon its anomeric configuration and the nature of the glycoside residue to which it is linked. Comparison of α-glucosyl groups with β-glucosyl groups [compounds (**1**), (**2**), (**5**), and (**6**) with (**3**), (**4**), (**7**), and (**8**) respectively] shows that the size of the downfield shifts of C-1′ of the former are smaller, reflecting the larger steric interaction in an α-glycoside. Typically shifts around δ_C 99.0 and 103.6 p.p.m., respectively, are obtained. The larger downfield shift of C-1′ for a β-glucosyl group is also accompanied by a larger upfield shift of C-2′, *ca.* 1.1 p.p.m. compared with 0—0.2 p.p.m. for an α-glucosyl group. It can be concluded that the anomeric configuration of the methyl glycoside residue does not significantly affect the chemical shifts of the glucopyranosyl group.

Changing the configuration of the methyl glycoside residue from *gluco* to *galacto* causes minor changes on the chemical shifts of the glucopyranosyl group. Only the difference for C-1′ and C-2′ (0.2 and −0.2 p.p.m. respectively) of the α-glucopyranosyl groups [compounds (**5**) and (**6**) as compared with (**1**) and (**2**)] is of diagnostic value. As is evident from Table 2, chemical shifts of the glucopyranosyl group in the oligosaccharide derivatives and in methyl glucoside differ significantly. Thus to obtain satisfactory comparisons of model substances with larger oligo- and poly-saccharides, the models should be larger than monosaccharides.

Chemical Shifts in the Methyl Glycoside Residue.—As already demonstrated by Dorman and Roberts[21] large chemical-shift changes appear for carbons close to the glycosylated oxygen, *i.e.* in this case for C-6 and C-5, but signals for other carbons are also shifted to a smaller extent.

The downfield shift on C-6 is dependent upon the anomeric configuration of the glucopyranosyl group and also upon the configuration at C-4. When the substituent is an α-D-glucopyranosyl group a downfield shift between 5.01 and 5.66 p.p.m. is obtained, but when it is a β-glucopyranosyl group the downfield shift is between 7.70 and 7.86 p.p.m. Typical chemical shifts for C-6 of the different residues should thus be δ_C 66.9 and 69.5 p.p.m. for D-glucosides and δ_C 67.6 and 69.8 p.p.m. for D-galactosides when substituted with an α- or β-D-glucopyranosyl group, respectively. Thus going from *gluco*- to *galacto*-configuration in the methyl glycoside residue induces a downfield shift of 0.7 and 0.3 p.p.m. respectively [compounds (**1**) and (**3**) as compared with (**5**) and (**7**)]. On substitution of C-6 an upfield shift on C-5 is obtained which is somewhat larger when an α-D-glucopyranosyl group is the substituent (1.5 p.p.m. for Glc, 2.0 p.p.m. for Gal), than when it is a β-D-glucopyranosyl group (0.9 p.p.m. for Glc, 1.2 p.p.m. for Gal). Small differences in chemical shift for the *O*-methyl group can also be observed depending upon the anomeric configuration of both sugars. Thus methyl α-glycosides give a signal at δ_C *ca.* 56 p.p.m. and methyl β-glycosides at δ_C *ca.* 58 p.p.m., and if the 6-substituent is α-linked the *O*-methyl resonance appears at higher field than when the 6-substituent is β-linked.

Temperature Dependence.—Spectra of model substances are normally recorded at ambient temperature but those of oligo- and poly-saccharides should preferably be run at elevated temperatures in order to improve resolution. As chemical shifts change with temperature it is necessary to have spectra of model compounds and polysaccharides recorded at the same temperature and to use an internal reference. The chemical-shift differences, δ (70 °C) − δ (40 °C), for compounds (**1**)—(**8**) are given in Table 3, using dioxane, δ_C 67.40 p.p.m., as standard for both temperatures. The general downfield shift, relative to dioxane, upon increase in temperature is far from uniform; in

Table 2. ^{13}C N.m.r. chemical shifts[a] of disaccharides (**1**)—(**8**) and appropriate monosaccharides. Chemical-shift differences are given in parentheses

Methyl glycoside of	C-1′[b]	C-2′	C-3′	C-4′	C-5′	C-6′	C-1	C-2	C-3	C-4	C-5	C-6	OMe
α-D-Glc*p*-(1→6)-α-D-Glc*p*	98.85	72.43	74.11	70.67	72.77	61.65	100.24	72.19	74.31	70.67	71.03	66.92	56.09
(**1**)	(5.86[b])	(−0.04)	(0.33)	(−0.04)	(0.40)	(−0.19)	(0.05)	(−0.04)	(0.21)	(−0.01)	(−1.49)	(5.25)	(0.16)
α-D-Glc*p*-(1→6)-β-D-Glc*p*	98.84	72.41	74.10	70.61	72.74	61.60	104.23	73.99	76.94	70.53	75.18	66.83	57.95
(**2**)	(5.85)	(−0.06)	(0.32)	(−0.10)	(0.37)	(−0.24)	(0.10)	(0)	(0.16)	(−0.16)	(−1.60)	(5.01)	(0.07)
β-D-Glc*p*-(1→6)-α-D-Glc*p*	103.62	74.08	76.78	70.70	76.74	61.86	100.27	72.17	74.02	70.56	71.58	69.48	56.16
(**3**)	(6.78)	(−1.12)	(0.02)	(−0.01)	(−0.02)	(0.02)	(0.08)	(−0.06)	(−0.08)	(−0.12)	(−0.94)	(7.81)	(0.23)
β-D-Glc*p*-(1→6)-β-D-Glc*p*	103.64	74.02	76.72	70.67	76.78	61.83	104.18	73.93	76.72	70.59	75.84	69.52	58.05
(**4**)	(6.80)	(−1.18)	(−0.04)	(−0.04)	(0.06)	(−0.01)	(0.05)	(−0.06)	(−0.06)	(−0.10)	(−0.94)	(7.70)	(0.17)
α-D-Glc*p*-(1→6)-α-D-Gal*p*	99.03	72.24	74.05	70.63	72.82	61.63	100.36	69.15	70.40	70.24	69.49	67.64	56.08
(**5**)	(6.04)	(−0.23)	(0.27)	(−0.08)	(0.45)	(−0.21)	(0.01)	(−0.02)	(−0.06)	(0.05)	(−2.05)	(5.58)	(0.12)
α-D-Glc*p*-(1→6)-β-D-Gal*p*	99.10	72.26	74.03	70.61	72.81	61.61	104.65	71.61	73.75	69.72	73.88	67.50	57.84
(**6**)	(6.11)	(−0.21)	(0.25)	(−0.10)	(0.44)	(−0.23)	(−0.07)	(−0.03)	(−0.04)	(0.10)	(−2.04)	(5.66)	(−0.04)
β-D-Glc*p*-(1→6)-α-D-Gal*p*	103.57	74.09	76.76	70.74	76.81	61.89	100.47	69.12	70.28	70.20	70.30	69.85	56.27
(**7**)	(6.73)	(−1.11)	(0)	(0.03)	(0.05)	(0.05)	(0.21)	(−0.05)	(−0.18)	(0.09)	(−1.24)	(7.79)	(0.31)
β-D-Glc*p*-(1→6)-β-D-Gal*p*	103.55	74.05	76.79	70.74	76.83	61.89	104.68	71.58	73.70	69.70	74.65	69.70	57.95
(**8**)	(6.71)	(−1.15)	(0.03)	(0.03)	(0.07)	(0.05)	(−0.04)	(−0.06)	(−0.09)	(0.08)	(−1.27)	(7.86)	(0.07)
α-D-Glucopyranose	92.99	72.47	73.78	70.71	72.37	61.84							
β-D-Glucopyranose	96.84	75.20	76.76	70.71	76.76	61.84							
Methyl α-D-glucopyranoside							100.19	72.23	74.10	70.68	72.52	61.67	55.93
Methyl β-D-glucopyranoside							104.13	73.99	76.78	70.69	76.78	61.82	57.88
Methyl α-D-galactopyranoside							100.35	69.17	70.46	70.19	71.54	62.06	55.96
Methyl β-D-galactopyranoside							104.72	71.64	73.79	69.62	75.92	61.84	57.88

[a] Chemical shifts are given in p.p.m. relative to internal dioxane (δ_C 67.40 p.p.m.). [b] Primed labels refer to the D-glucopyranosyl group and unprimed to the methyl glycoside residue. [c] Chemical-shift differences are calculated by subtraction of chemical shifts of α-D-glucopyranose and methyl α-D-glucopyranoside from compound (**1**), *etc*, and a positive difference indicates a downfield shift.

Table 3. Chemical-shift differences from variation in temperature[a] ($\Delta\delta_C$ in p.p.m.)

Compd.	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**)	0.13	0.10	0.15	0.24	0.10	0.23	0.05	0.15	0.09	0.29	0.12	0.40	0.04
(**2**)	0.13	0.08	0.15	0.23	0.08	0.21	0.03	0.04	0.10	0.27	0.12	0.42	−0.11
(**3**)	0.05	0.09	0.05	0.16	0.16	0.16	0.04	0.13	0.15	0.30	0.11	0.13	0.03
(**4**)	0.03	0.10	0.16	0.17	0.06	0.18	0.02	0.06	0.16	0.25	0.10	0.09	−0.13
(**5**)	0.13	0.11	0.15	0.19	0.10	0.19	0.08	0.12	0.11	0.10	0.07	0.16	0.06
(**6**)	0.11	0.12	0.14	0.21	0.10	0.20	0.05	0.06	0.13	0.12	0.06	0.16	−0.11
(**7**)	0.04	0.08	0.16	0.16	0.05	0.16	0.07	0.13	0.11	0.08	0.01	−0.11	0.02
(**8**)	0.03	0.10	0.16	0.16	0.06	0.18	0.05	0.08	0.14	0.07	0.02	−0.12	−0.14

[a] $\Delta\delta_C = \delta_C$ (70 °C) − δ_C (40 °C). Dioxane was taken as δ_C 67.40 p.p.m. for both temperatures.

Table 4. Calculated and observed ^{13}C n.m.r. chemical shifts of dextran, →6)-α-D-Glc*p*-(1→, and pustulan, →6)-β-D-Glc*p*-(1→, at 70 °C. Internal standard dioxane was taken as δ_C 67.40 p.p.m.

	C-1	C-2	C-3	C-4	C-5	C-6
α-D-Glucopyranose	92.99	72.47	73.78	70.71	72.37	61.84
$\Delta\delta_C$ [compare (**1**) C-1′—C-6′]	5.86	−0.04	0.33	−0.04	0.40	−0.19
$\Delta\delta_C$ [compare (**1**) C(1—C(6]	0.05	−0.04	0.21	−0.01	−1.49	5.25
Calc.	98.90	72.39	74.32	70.66	71.28	66.90
Dextran (obs.)	98.69	72.37	74.37	70.71	71.15	66.86
$\Delta\delta_C$ (calc. − obs.)	0.21	0.02	−0.05	−0.05	0.13	0.04
β-D-Glucopyranose	96.84	75.20	76.76	70.71	76.76	61.84
$\Delta\delta_C$ [compare (**4**) C-1′—C-6′]	6.80	−1.18	−0.04	−0.04	0.06	−0.01
$\Delta\delta_C$ [compare (**4**) C-1—C-6]	0.05	−0.06	−0.06	−0.10	−0.94	7.70
Calc.	103.69	73.96	76.66	70.57	75.88	69.53
Pustulan (obs.)	103.76	73.98	76.66	70.66	75.83	69.79
$\Delta\delta_C$ (calc. − obs.)	−0.07	−0.02	0.0	−0.09	0.05	−0.26

some cases even upfield shifts are obtained. The chemical-shift differences range from −0.14 to 0.42 p.p.m. with the majority of values being around 0.1 p.p.m. The largest differences appear for C-4, C-6, C-4′, and C-6′, being especially pronounced for derivatives containing an α-glucopyranosyl group. This may be ascribed to changes in the rotamer distribution of the hydroxymethyl group.

The smallest (the negative) differences appear for the methoxy groups and on C-6 when a β-glucopyranosyl group is linked to galactose.

The temperature-induced shifts may have diagnostic value for assignment of signals, as will be described below.

Calculation of ^{13}C N.m.r. Spectra using the Chemical-shift Increments.—The chemical shifts of a 6-linked glycosyl residue in oligo- or poly-saccharides depend upon, as a first approximation, its two closest neighbours. In order to predict chemical shifts of an α-D-glucopyranosyl residue in dextran, →6)-α-D-Glc*p*-(1→, the influence of O-6 substitution of an α-D-glucosyl group, and the influence of having a 6-linked α-D-glucosyl residue at C-1 should be accounted for. Therefore we start with the chemical shifts of α-D-glucopyranose (Table 2) and add, for each signal, the increments of the methyl glucoside residue of compound (**1**). The shift increments of the glucopyranosyl group of compound (**1**) are then added. As seen from Table 4 the agreement between experimental and calculated values is good, demonstrating that the approach is justified. The largest deviation is observed for C-1, 0.21 p.p.m., and the sum of deviations (absolute values) is 0.50 p.p.m.

A similar calculation of pustulan, →6)-β-D-Glc*p*-(1→, (Table 4) with shift increments of compound (**4**) added to β-D-glucopyranose also gives good agreement, the largest deviation being 0.26 p.p.m. and the sum of deviations 0.49 p.p.m. A calculation of dextran and pustulan using the shift increments from compounds (**2**) and (**3**), *i.e.* disregarding the anomeric nature of the methyl glycoside, gives a sum of deviations of 0.88 p.p.m. and 0.41 p.p.m., respectively. This indicates that a better prediction can be made if both anomeric configurations are available, but that satisfactory results can be obtained with models having the wrong anomeric configuration. However, we still have to investigate whether accurate results can be obtained on calculation of glycosyl residues linked to secondary positions.

In order to test the galactose-containing model substances, raffinose and stachyose were chosen. One of the model substances for the calculation of spectra was sucrose and the spectrum of that substance[22] and of raffinose[23] have earlier been fully assigned. For stachyose,[24,25] however, several but not all signals have been assigned. The remaining signals in the spectrum of stachyose have now been assigned using spin-lattice relaxation times (T_1) and changes in chemical shift with temperature.

It was found that the carbons giving signals at δ_C 99.07, 71.80, 70.51, 70.20, 69.24, and 61.99 p.p.m. relax significantly more slowly than the other carbons and can thus be assigned to the terminal galactopyranosyl group. From the values of raffinose and compounds (**1**), (**2**), (**5**), and (**6**) (Table 3) it is seen that a considerably larger temperature-induced shift of C-6 is obtained for an α-D-glucopyranosyl residue than for an α-D-galactopyranosyl residue. In the spectrum of stachyose the signal at δ_C 67.18 p.p.m. with a large temperature-induced shift could therefore be assigned to C-6 of the α-D-glucopyransoyl residue and that at δ_C 67.54 p.p.m. to C-6 of the α-D-galactopyranosyl residue.

The temperature-induced shifts in sucrose, raffinose, and stachyose (Table 5) show very little variation on elongation of the oligosaccharide chain. Such temperature shifts may therefore be a useful tool in the assignment of oligosaccharide spectra.

Table 5 shows the ^{13}C n.m.r. chemical shifts of raffinose, stachyose, and pertinent references, together with the calculated chemical shifts of raffinose and stachyose.

The calculation of the chemical shifts for raffinose was made

Table 5. ^{13}C N.m.r. spectra of raffinose and stachyose and pertinent model substances together with changes in chemical shifts induced on heating[a]

			α-D-Gal*p*-(1→				→6)-α-D-Gal*p*-(1→					
α-D-Gal	93.18	69.35	70.13	70.25	71.30	62.05						
Sucrose												
Raffinose							99.46	69.39	70.45	70.19	71.82	62.00
Raffinose (calc.)							99.03	69.31	70.46	70.21	70.70	61.86
$\Delta\delta_C$							(−0.43)	(−0.08)	(0.01)	(0.02)	(−0.12)	(−0.14)
Stachyose	99.07	69.24	70.51	70.20	71.80	61.99	99.34	69.35	70.38	70.28	69.73	67.54
Stachyose (calc.)	99.22	69.12	70.40	70.17	71.75	61.84	99.05	69.29	70.40	70.26	69.85	67.44
$\Delta\delta_C$	(0.15)	(−0.12)	(−0.11)	(−0.03)	(−0.05)	(−0.15)	(−0.29)	(−0.06)	(0.02)	(−0.02)	(0.08)	(−0.10)
Sucrose[b]												
Raffinose[b]							0.10	0.05	0.11	0.10	0.02	0.03
Stachyose[b]	0.14	0.08	0.10	0.07	−0.01	0.01	0.10	0.06	0.11	0.08	0.07	0.14
			→6)-α-D-Glc*p*-(1→						→2)-β-D-Fru*f*			
α-D-Gal												
Sucrose	93.02	72.02	73.63	70.38	73.34	61.35	62.74	104.63	77.91	75.24	82.28	63.21
Raffinose	92.97	71.93	73.69	70.58	72.31	67.22	62.81	104.71	77.84	75.24	82.25	63.30
Raffinose (calc.)	93.07	71.98	73.84	70.37	71.85	66.60	62.74	104.63	77.91	75.24	82.28	63.21
$\Delta\delta_C$	(0.10)	(0.05)	(0.15)	(−0.21)	(−0.46)	(−0.62)	(−0.07)	(−0.08)	(0.07)	(0)	(0.03)	(−0.09)
Stachyose	92.98	71.93	73.73	70.65	72.22	67.18	62.82	104.74	77.86	75.25	82.26	63.31
Stachyose (calc.)	93.07	71.98	73.84	70.37	71.85	66.60	62.74	104.63	77.91	75.24	82.28	63.21
$\Delta\delta_C$	(0.09)	(0.05)	(0.11)	(−0.28)	(−0.37)	(−0.58)	(−0.08)	(−0.11)	(0.05)	(−0.01)	(0.02)	(−0.10)
Sucrose[b]	0.04	0.10	0.17	0.23	0.08	0.26	0.36	0.10	0.43	0.29	0.08	0.03
Raffinose[b]	0.04	0.08	0.13	0.22	0.06	0.30	0.35	0.08	0.41	0.27	0.07	0.03
Stachyose[b]	0.04	0.11	0.11	0.23	0.07	0.30	0.33	0.09	0.41	0.27	0.07	0.01

[a] For the calculation of the spectra of raffinose and stachyose see text. [b] Chemical-shift differences = δ_C (70 °C) − δ_C (40 °C).

from the chemical shifts of sucrose and α-D-galactopyranose. As the methyl glycoside of α-D-Gal*p*-(1→6)-α-D-Glc*p* was not available, the chemical-shift increments of compound (**1**) were used for the α-D-galactopyranosyl group and the α-D-glucopyranosyl residue with the assumption that the shift increments for the former should be closely similar to those of compound (**1**). The spectrum of stachyose was calculated from that of α-D-galactose and the calculated spectrum of raffinose. Shift increments were taken from compound (**5**), the model substance being closest to α-D-Gal*p*-(1→6)-α-D-Gal*p*. These increments were added to the α-D-galactopyranosyl units.

The agreements, both for raffinose and stachyose, between the observed and calculated spectra are good, demonstrating that the assumptions, upon which the calculations were based, were reasonable. The largest deviations in stachyose, more than 0.2 p.p.m., were observed for C-4, C-5, and C-6 (−0.28, −0.37, and −0.58 p.p.m.) of the α-D-glucopyranosyl residue and for C-1 (−0.29 p.p.m.) of the internal α-D-galactopyranosyl moiety, all carbons being close to each other. The same effect was present for raffinose. The reason for this difference may be that not the best models were used. The differences may, however, also be due to a conformational change when going from sucrose to raffinose. According to Bock and Lemieux[22] the rotation of the C-5 to C-6 bond in the α-D-glucopyranosyl group of sucrose is restricted because of interaction between the C-6 hydroxymethyl groups of the glucosyl and fructosyl units. This interaction should be more severe when O-6 in the glucose moiety is glycosylated, and the anomalous shifts could thus be due to a change in the rotamer distribution.

Proton Shifts.—The chemical shifts of the anomeric protons of compounds (**1**)—(**8**) dextran, pustulan, and stachyose are given in Table 6. The chemical shifts for 1′-H of the α- and β-D-glucopyranosyl groups vary by less than 0.03 p.p.m. One should therefore expect chemical shifts of δ_H *ca.* 4.96 and *ca.* 4.51 to be typical for terminal α- and β-D-glucopyranosyl groups in oligo- and poly-saccharides, when linked to O-6 of D-glucopyranosyl

Table 6. ^1H N.m.r. chemical shifts[a] and coupling constants of anomeric protons at 85 °C

Compound	1′-H	$J_{1,2}$	1-H	$J_{1,2}$
(**1**)	4.966	3.8	4.815	3.8
(**2**)	4.964	3.7	4.384	7.3
(**3**)	4.495	8.0	4.805	3.8
(**4**)	4.516	7.9	4.373	8.0
(**5**)	4.950	3.7	4.838	2.7
(**6**)	4.958	3.8	4.326	7.9
(**7**)	4.503	7.9	4.845	3.2
(**8**)	4.524	8.1	4.327	7.8
Dextran	4.971	3.6		
Pustulan	4.526	7.9		
Stachyose	~4.99[b]			
(D-galactosyl residues)				

[a] Chemical shifts are given in p.p.m. relative to internal TSP (δ_H 0.00). [b] Overlapping signals with couplings of higher order.

or D-galactopyranosyl residues. The similarity of those chemical shifts with those of the D-glucopyranosyl residues in dextran and pustulan and of the D-galactopyranosyl residues in stachyose shows that substitution of a glycopyranosyl residue to O-6 does not significantly alter the chemical shift of 1-H. The higher value (0.02 p.p.m.) for the D-galactopyranosyl residues in stachyose is also seen for the methyl D-galactopyranoside residues of compounds (**5**) and (**6**) compared with those of the corresponding *gluco*-derivatives.

Conclusions

The successful calculation of ^{13}C n.m.r. chemical shifts of 1,6-linked D-gluco- and D-galacto-pyranosyl residues in oligo- and poly-saccharides from the spectra of 1,6-linked disaccharide glycosides indicates that such calculations may be of general value. When the spectra of suitable reference substances are available it should thus be possible to determine the structures of oligosaccharides and polysaccharides composed of oligo-

saccharide repeating units, provided that the sugar components and positions in which they are linked are known. Computer-assisted creation of spectra and comparison of calculated and observed spectra should facilitate such studies. Complications may, however, be expected for sterically crowded structures.

It is essential that all spectra are determined at the same temperature, *e.g.* 70 °C, and with an internal reference, *e.g.* dioxane, in order to obtain reliable data.

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