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^{13}C- AND ^{1}H-NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY OF PERMETHYLATED DISACCHARIDES*

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ABSTRACT

The ^{13}C-N.m.r. spectra of permethylated disaccharides composed of D-glucose, or D-galactose, or both residues are analyzed and discussed. Peaks were assigned by correlation of the spectra of the disaccharide derivatives with those of the constituent permethylated monomers. Large shift-increments for skeletal and methoxyl carbons, with respect to the resonance positions of corresponding atoms of the monomers, are explained in terms of steric or proximity effects. The configuration of glycosidic linkages can be deduced from the chemical shifts of the anomeric carbons and from the ^{1}H-n.m.r. data of the attached protons.

INTRODUCTION

The ^{13}C-N.m.r. spectra of several free mono-, oligo-, and poly-saccharides have been published[1–14]. Chemical-shift differences are used to establish type and configuration of the glycosidic linkages. The spectrum of a polysaccharide can give information about the number of monomer units present in the repeating unit. However, ^{13}C-n.m.r. spectral data of completely blocked sugars are scarce; some acetylated compounds[3] and some permethylated monosaccharides[15,16] have been investigated.

In the present study, the ^{13}C-n.m.r. spectra of various permethylated disaccharides were analyzed in order to obtain basic data for the interpretation of spectra of more complex analogs. In the interpretation of the ^{13}C-n.m.r. spectra of permethylated glucopyranose[15] and galactopyranose derivatives[16], the assignments were based on specific labelling and heteronuclear decoupling experiments. The spectra can be subdivided into three groups of resonances, *viz.*: (a) the anomeric carbon atoms between 98–106 p.p.m., (b) the methoxyl carbon atoms between 55–62 p.p.m., and (c) the nonanomeric skeleton carbon atoms between 69–88 p.p.m. Axially substituted anomeric carbon atoms (α-D linkage) resonate at higher field than equatorially substituted ones (β-D linkage). The chemical shifts for C-4 and C-6 in the spectra of

*Dedicated to the memory of Professor W. Z. Hassid.

TABLE I

^{13}C-N.M.R. CHEMICAL SHIFTS OF THE SKELETON CARBONS OF PER-*O*-METHYL DERIVATIVES OF METHYL D-GLUCOPYRANOSIDES, OF METHYL D-GALACTOPYRANOSIDES, OF METHYL GLYCOSIDES OF REDUCING DISACCHARIDES, AND OF NON-REDUCING DISACCHARIDES[a]

Per-O-methyl derivatives of	*C-1*	*C-2*	*C-3*	*C-4*
Methyl α-D-glucopyranoside (1)	98.16	82.58	84.28	80.61
Methyl β-D-glucopyranoside (2)	105.00	84.58	87.21	80.48
Methyl α-D-galactopyranoside (3)	98.80	78.78	80.97	77.25
Methyl β-D-galactopyranoside (4)	105.26	81.50	84.65	76.10
α-D-Glc*p*-(1→1)-α-D-Glc*p* (5)	93.37	82.49	83.88	80.45
	(−4.79)	(−0.09)	(−0.40)	(−0.16)
β-D-Glc*p*-(1→1)-β-D-Glc*p* (6)	98.88	84.43	86.91	80.17
	(−6.12)	(−0.15)	(−0.30)	(−0.31)
Me β-D-Glc*p*-(1→6)-α-D-Glc*p* (7)	98.13	82.50	84.26	80.26
	(−0.03)	(−0.08)	(−0.02)	(−0.35)
Me β-D-Glc*p*-(1→6)-β-D-Glc*p* (8)	104.92	84.55	87.13*	80.15
	(−0.08)	(−0.03)	(−0.08)	(−0.33)
Me α-D-Glc*p*-(1→6)-α-D-Glc*p* (9)	98.20	82.71	84.40	80.30
	(+0.04)	(+0.13)	(+0.12)	(−0.31)
Me α-D-Glc*p*-(1→6)-β-D-Glc*p* (10)	104.94	84.70	87.26	80.33
	(−0.06)	(+0.12)	(+0.05)	(−0.15)
Me α-D-Gal*p*-(1→6)-α-D-Glc*p* (11)	98.16	82.69	84.41	80.34
	(0.00)	(+0.11)	(+0.13)	(−0.27)
Me α-D-Gal*p*-(1→6)-β-D-Glc*p* (12)	104.82	84.61	87.21	80.30
	(−0.18)	(+0.03)	(0.00)	(−0.18)
Me β-D-Glc*p*-(1→4)-α-D-Glc*p* (13)	98.13	81.92*	81.96*	78.20
	(−0.03)	(−0.66)	(−2.32)	(−2.41)
Me β-D-Glc*p*-(1→4)-β-D-Glc*p* (14)	104.87	83.90	85.03	77.87
	(−0.13)	(−0.68)	(−2.18)	(−2.61)
Me α-D-Glc*p*-(1→4)-α-D-Glc*p* (15)	97.73	82.98	84.41	73.32
	(−0.43)	(+0.40)	(+0.13)	(−7.29)
Me α-D-Glc*p*-(1→4)-β-D-Glc*p* (16)	104.82	84.95	87.37	73.18
	(−0.18)	(+0.37)	(+0.16)	(−7.30)
Me β-D-Gal*p*-(1→4)-α-D-Glc*p* (17)	97.99	81.79*	81.95*	78.23
	(−0.17)	(−0.79)	(−2.33)	(−2.38)
Me β-D-Gal*p*-(1→4)-β-D-Glc*p* (18)	104.82	83.82	85.04	77.89
	(−0.18)	(−0.76)	(−2.17)	(−2.59)
Me β-D-Glc*p*-(1→3)-α-D-Glc*p* (19)	97.60	83.47	78.45	78.98
	(−0.56)	(+0.89)	(−5.83)	(−1.63)
Me β-D-Glc*p*-(1→3)-β D Glc*p* (20)	104.86	85.28	81.47	78.90
	(−0.14)	(+0.70)	(−5.74)	(−1.58)
Me β-D-Glc*p*-(1→2)-α-D-Glc*p* (21)	100.40	81.43	83.57	81.03
	(+2.24)	(−1.15)	(−0.71)	(+0.42)
Me β-D-Glc*p*-(1→2)-β-D-Glc*p* (22)	103.33*	79.35	87.68	80.87
	(−1.67)	(−5.23)	(+0.47)	(+0.39)
Me α-D-Glc*p*-(1→2)-α-D-Glc*p* (23)	97.20	75.45	83.42	80.81
	(−0.96)	(−7.13)	(−0.86)	(+0.20)
Me α-D-Glc*p*-(1→2)-β-D-Glc*p* (24)	105.32	76.21	86.11	81.09
	(+0.32)	(−8.37)	(−1.10)	(+0.61)

[a]In acetonitrile-d_3. Shifts on δ scale (p.p.m.). The assignments of the resonances marked with (*) m interchanged. The shift increment, relative to the resonance position of the corresponding carbon i monomer, is given in parentheses.

C-5	*C-6*	*C-1'*	*C-2'*	*C-3'*	*C-4'*	*C-5'*	*C-6'*
70.98	72.41						
75.38	72.36						
69.88	72.34						
73.86	72.01						
71.54	72.25	93.37	82.49	83.88	80.45	71.54	72.25
(+0.56)	(−0.16)	(−4.79)	(−0.09)	(−0.40)	(−0.16)	(+0.56)	(−0.16)
75.54	72.00	98.88	84.43	86.91	80.17	75.54	72.00
(+0.16)	(−0.36)	(−6.12)	(−0.15)	(−0.30)	(−0.31)	(+0.16)	(−0.36)
70.76	69.25	104.22	84.73	87.19	80.44	75.37	72.33
(−0.22)	(−3.16)	(−0.78)	(+0.15)	(−0.02)	(−0.04)	(−0.01)	(−0.03)
75.00	69.15	104.26	84.50	87.04*	80.30	75.28	72.22
(−0.38)	(−3.21)	(−0.74)	(−0.08)	(−0.17)	(−0.18)	(−0.10)	(−0.14)
70.81	67.04	97.09	82.71	84.16	80.73	71.18	72.49
(−0.17)	(−5.37)	(−1.07)	(+0.13)	(−0.12)	(+0.12)	(+0.20)	(+0.08)
74.94	66.98	96.93	82.60	84.11	80.64	71.09	72.44
(−0.44)	(−5.38)	(−1.23)	(+0.02)	(−0.17)	(+0.03)	(+0.11)	(+0.03)
70.83	67.16	97.79	78.85	80.94	77.30	70.05	72.29
(−0.15)	(−5.25)	(−1.01)	(+0.07)	(−0.03)	(+0.05)	(+0.17)	(−0.05)
74.88	67.07	97.53	78.70	80.82	77.19	69.88	72.15
(−0.50)	(−5.29)	(−1.27)	(−0.08)	(−0.15)	(−0.06)	(0.00)	(−0.19)
70.97	71.67	103.61	85.27	87.60	80.50	75.66	72.42
(−0.01)	(−0.74)	(−1.39)	(+0.69)	(+0.39)	(+0.02)	(+0.28)	(+0.06)
75.42	71.50	103.44	85.12	87.48	80.35	75.51	72.26
(+0.04)	(−0.86)	(−1.56)	(+0.54)	(+0.27)	(−0.13)	(+0.13)	(−0.10)
70.23	72.19	96.84	82.69	83.91	80.38	71.76	72.28
(−0.75)	(−0.22)	(−1.32)	(+0.11)	(−0.37)	(−0.23)	(+0.78)	(−0.13)
74.75	72.14	96.68	82.62	83.89	80.34	71.73	72.23
(−0.63)	(−0.22)	(−1.48)	(+0.04)	(−0.39)	(−0.27)	(+0.75)	(−0.18)
70.90	71.56	104.09	82.16	85.07	76.04	73.78	71.86
(−0.08)	(−0.85)	(−1.17)	(+0.66)	(+0.42)	(−0.06)	(−0.08)	(−0.15)
75.48	71.52	103.93	82.10	85.04	76.00	73.73	71.81
(+0.10)	(−0.84)	(−1.33)	(+0.60)	(+0.39)	(−0.10)	(−0.13)	(−0.20)
70.64	72.54	103.45	85.38	87.04	80.52	75.39	72.42
(−0.34)	(+0.13)	(−1.55)	(+0.80)	(−0.17)	(−0.04)	(+0.01)	(+0.06)
75.08	72.27	103.38	85.28	87.12	80.42	75.40	72.43
(−0.30)	(−0.09)	(−1.62)	(+0.70)	(−0.09)	(−0.06)	(+0.02)	(+0.07)
70.80	72.23	105.20	84.49	87.30	80.33	75.09	72.39
(−0.18)	(−0.18)	(+0.20)	(−0.09)	(+0.09)	(−0.15)	(−0.29)	(+0.03)
75.30	72.23	103.08*	85.01	87.30	80.33	75.30	72.23
(−0.08)	(−0.13)	(−1.92)	(+0.43)	(+0.09)	(−0.15)	(−0.08)	(−0.13)
70.90	72.10	93.56	82.22	83.81	80.36	70.98	72.10
(−0.08)	(−0.31)	(−4.60)	(−0.36)	(−0.47)	(−0.25)	(0.00)	(−0.31)
75.45	72.30	95.22	82.51	83.69	80.36	70.82	72.30
(+0.07)	(−0.06)	(−2.94)	(−0.07)	(−0.59)	(−0.25)	(−0.16)	(−0.11)

the methyl tetra-*O*-methyl-α- (**1**) and β-D-glucopyranosides (**2**), and galactopyranosides (**3** and **4**) are almost independent from the anomeric configuration. The chemical shift for C-6 is practically invariant for the four derivatives. The methoxyl group at C-1 resonates at ~55.3 p.p.m. if it is axially oriented (**1, 3**) and at ~56.9 p.p.m. if its position is equatorial (**2, 4**).

The analysis of the spectra of the permethylated disaccharides is based on the ^{13}C-n.m.r. data of the monosaccharides **1–4**. In addition, the following assumptions were made: (a) The chemical shifts of the skeleton carbon atoms that are relatively remote from the inter-sugar glycosidic linkage are almost identical with those of the corresponding carbon atoms of the monosaccharide derivatives. It is reasonable to assume that, in these cases, interannular steric perturbations will be relatively unimportant. (b) The chemical shifts of the carbon atoms of the glycoside part (C-1′–C-6′) are expected to be almost independent from the configuration of the methoxyl group at C-1. In the (1→2)-linked glucobioses, significant differences may arise, owing to the small distance between the methoxyl group at C-1 and the glycoside ring.

In this study, the ^{1}H-n.m.r. data of H-1 and H-1′ are also included, in view of their applicability for the determination of the configuration of glycosidic linkages[15–18].

RESULTS

^{13}C-N.m.r. spectra (25.16 MHz) of per-*O*-methyl derivatives of α,α-trehalose (**5**), β,β-trehalose (**6**), and of methyl α-(**7**) and β-gentiobioside (**8**), α- (**9**) and β-isomaltoside (**10**), α- (**11**) and β-melibioside (**12**), α- (**13**) and β-cellobioside (**14**), α- (**15**) and β-maltoside (**16**), α- (**17**) and β-lactoside (**18**), α- (**19**) and β-laminaribioside (**20**), α- (**21**) and β-sophoroside (**22**), and α- (**23**) and β-kojibioside (**24**) were recorded on solutions in acetonitrile-d_3. For derivatives **7–20**, the pure anomeric forms were examined. Generally, all resonances of the skeleton carbon atoms are clearly resolved. Only a few times two resonances coincide, which is recognizable by the enlarged intensity of the peak. The patterns of the methoxy group resonances are complex because of small $\Delta\delta$ values.

Skeleton carbon atoms. — The chemical shift data of the skeleton carbon atoms of the disaccharide derivatives and their increments, relative to the positions of corresponding carbon atoms in the monosaccharide derivatives, are given in Table I.

*Per-O-methyl-α,α- (**5**) and β,β-trehaloses (**6**).* The spectra of **5** and **6** show only six resonances for the skeleton carbon atoms and four for the methoxy groups. Apparently, both D-glucose units are magnetically equivalent. The glycosidically linked carbon atoms resonate at ~4.8 p.p.m. (**5**) and ~6.1 p.p.m. (**6**) upfield from C-1 in **1** and **2**, respectively. The chemical shifts of the nonanomeric carbon atoms differ slightly from those of the corresponding carbon atoms in the D-glucose derivatives.

Methyl per-O-methyl-gentiobiosides (**7** *and* **8**), *-isomaltosides* (**9** *and* **10**), *and -melibiosides* (**11** *and* **12**). The spectra of the permethyl derivatives of the (1→6)-linked disaccharides **7–12** can easily be correlated with those of the constituting monomers **1**, **2**, and **3**. Upfield incremental shifts are observed for δC-1′ and δC-6; this shift for δC-6 in the α-D-(1→6)-linked disaccharides is larger than in the β-D-(1→6)-linked ones. The differences in chemical shift of C-6 and the methoxyl group at C-1 of **7** and **10** are characteristic for these compounds.

Methyl per-O-methyl-cellobiosides (**13** *and* **14**), *-maltosides* (**15** *and* **16**), *and -lactosides* (**17** *and* **18**). The resonances of the glycosidically linked carbon atoms C-1′ and C-4 are shifted upfield; the incremental shifts of δC-4 in the α-D-linked disaccharides (**15**, **16**) is considerably larger than in the β-D-linked analogs (**13**, **14**; **17**, **18**). Cellobiose and lactose differ only in the configuration at C-4′, and there is no evidence for conformational differences around the inter-sugar glycosidic bonds. Therefore, comparison of the spectra of these sugars is helpful in the identification of the resonances of C-2, C-2′, C-3, and C-3′. Significant incremental shifts were observed for δC-3 and, to a lesser extent, for δC-2, δC-2′, and δC-6 in **13** and **14**, and in **17** and **18**, whereas in **15** and **16** appreciable shifts are only found for δC-5 and δC-5′.

Methyl per-O-methyl-laminaribioside (**19** *and* **20**). In addition to the large incremental shifts of δC-1′ and δC-3, significant upfield shifts were also observed for δC-4. A similar phenomenon occurs in the derivatives of the β-D-(1→4)-linked disaccharides for δC-3.

Methyl per-O-methyl-sophorosides (**21** *and* **22**) *and -kojibiosides* (**23** *and* **24**). The α and β anomers of the sophorose (**21**, **22**) and kojibiose (**23**, **24**) derivatives could not be separated, and therefore the anomeric mixtures of these sugars containing about 19% and 28% of the α form, respectively, were investigated. In the assignment of the various signals to specific anomers, relative peak intensities were helpful. However, some peaks of the α and β anomers coincide, which makes the spectrum of the α anomer difficult to be interpreted.

In the spectrum of **21** and **22**, the resonances of the four anomeric carbon atoms are well resolved (Fig. 1). The anomeric carbon atoms of the α anomer (indicated as C-1α and C-1′α) occur about 2 p.p.m. more downfield than expected, but these resonance positions agree well with the spectrum of methyl α-sophoroside (in deuterium oxide: δC-1 = 100.0 p.p.m.; δC-1′ = 105.0 p.p.m.)[10]. This illustrates that the direct environments of C-1α in the methylated and nonmethylated methyl glycosides are identical and that methylation of the compound has only a small influence on the shielding of C-1′α. The chemical shifts for C-1β and C-1′β in the spectrum of **21** and **22** are almost identical (103.33 and 103.08 p.p.m.), so that specific assignment is difficult. A chemical shift of about 103 p.p.m. for C-1β is reasonable, because in free β-sophorose this carbon atom resonates at relatively high field (in deuterium oxide: δC-1β = 95.8 p.p.m.), whereas downfield shifts of about 7 p.p.m. are found for δC-1 upon methyl glycosidation[10]. It has to be noted that the incremental shifts of δC-1α (+2.24 p.p.m.) and δC-1β (−1.67 p.p.m.) have opposite signs. Large differences are

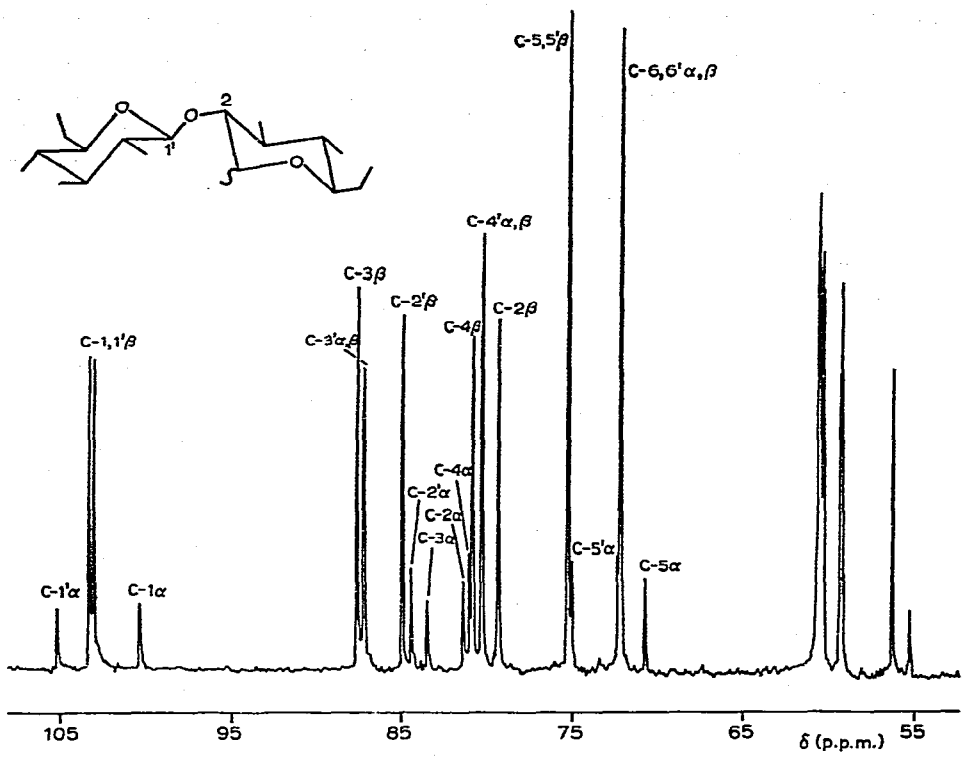

Fig. 1. ^{13}C-N.m.r. spectrum of methyl per-*O*-methyl-α,β-sophoroside (**21** and **22**); 170 mg in 0.5 ml of acetonitrile-d_3; specific width 3076 Hz; acquisition time 1.3 sec; delay 2.0 sec; pulse width 80 μsec; 43200 transients; data 8190; and sensitivity enhancement (t.c.) 0.8 sec.

observed between δC-1′α and δC-1′β. From the complete assignment of the spectrum of the β anomer, it is seen that only the chemical shift of C-1β is rather exceptional. Extending this observation to the spectrum of the α form, the peak at 81.43 p.p.m. must represent C-2α. This means an upfield incremental shift of only 1.15 p.p.m. for this carbon atom, which differs greatly from the shift of δC-2β (−5.23 p.p.m.).

The spectrum of methyl per-*O*-methyl-α,β-kojibioside (**23** and **24**) shows peaks for C-1β and C-1′β at 105.32 and 95.22 p.p.m., respectively (Fig. 2). The chemical shift for C-1β agrees fairly with the corresponding resonance in the spectrum of methyl β-kojibioside (in deuterium oxide: δC-1β = 105.0 p.p.m.)[10]. The resonance of C-1α is found at 97.20 p.p.m. The large incremental shifts, observed for δC-1α and δC-1β in the sophorose derivatives, are not observed for the kojibioside anomers. The shifts for δC-1′α and δC-1′β differ significantly, as was also found for the sophorose derivatives. The assignment of the anomeric carbon atoms in **23** and **24** is in accordance with the relative magnitudes of the residual coupling constants $J_{C-1,H-1}$ and $J_{C-1',H-1'}$ in an off-resonance ^{13}C-{^{1}H} spin-decoupling experiment. The peak at 76.21 p.p.m. is assigned to C-2β, which implies an incremental upfield shift of 8.37 p.p.m. for this carbon atom. The resonance of C-2α coincides either with that of

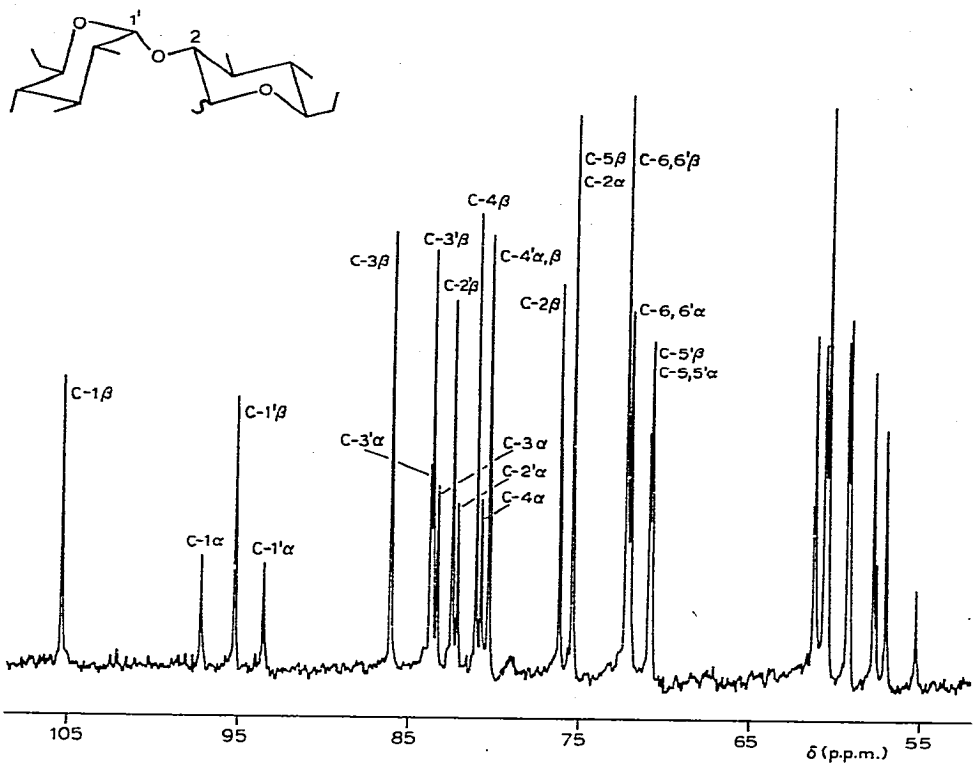

Fig. 2. ^{13}C-N.m.r. spectrum of methyl per-*O*-methyl-α,β-kojibioside (**23** and **24**); 150 mg in 0.5 ml of acetonitrile-d_3; specific width 3076 Hz; acquisition time 1.3 sec; delay 2.0 sec; pulse width 80 μsec; 15453 transients; data 8190; and sensitivity enhancement (t.c.) 0.8 sec.

C-2β or with that of C-5β (75.45 p.p.m.). The latter possibility is the most probable one, in view of the high intensity of this peak.

Methoxyl carbons. — The replacement of a methoxyl group by a glycosidic substituent has only a small effect on the remaining methoxyl resonances in the ^{13}C-n.m.r. spectrum, except when the newly introduced sugar unit gives rise to steric interactions. These interactions will show up clearly in the resonances of the skeleton carbons. Based on this reasoning, the assignment of the methoxyl resonances is made as follows: (a) The incremental substituent effects are minimalized for the methoxyl groups that are not subject to strong steric interactions. (b) When steric interactions are present, as judged from the substituent effects on the skeleton carbon atoms, it is assumed that the substituent effect causes for the corresponding methoxyl resonances shifts, that are equally directed in the α and β anomer of one sugar. The δmethoxyl values and their shift increments with respect to the corresponding δmethoxyl of the monosaccharide derivatives are given in Table II.

Per-O-methyl-α,α- (**5**) *and β,β-trehaloses* (**6**). Only for δMeO-2 in **5** was a significant incremental shift found.

TABLE II

^{13}C-N.M.R. CHEMICAL SHIFTS OF METHOXY GROUPS OF PER-*O*-METHYL DERIVATIVES OF METHYL D-GLUCOPYRANOSIDES, OF METHYL D-GALACTOPYRANOSIDES, OF METHYL GLYCOSIDES OF REDUCING DISACCHARIDES, AND OF NON-REDUCING DISACCHARIDES[a]

Per-O-*methyl derivatives of*	*MeO-1*	*MeO-2*	*MeO-3*	*MeO-4*	*MeO-6*	*MeO-2'*	*MeO-3'*	*MeO 4'*	*MeO 6'*
Methyl α-D-glucopyranoside (**1**)	55.27	58.40	60.71	60.55	59.23				
Methyl β-D-glucopyranoside (**2**)	56.96	60.39	60.74	60.48	59.30				
Methyl α-D-galactopyranoside (**3**)	55.30	58.52	58.11	61.31	59.19				
Methyl β-D-galactopyranoside (**4**)	56.82	60.64	58.27	61.26	59.18				
α-D-Glc*p*-(1→1)-α-D-Glc*p* (**5**)		58.80 (+0.40)	60.78 (+0.07)	60.57 (+0.02)	59.23 (0.00)	58.80 (+0.40)	60.78 (+0.07)	60.57 (+0.02)	59.23 (0.00)
β-D-Glc*p*-(1→1)-β-D-Glc*p* (**6**)		60.42 (+0.03)	60.73 (−0.01)	60.51 (+0.03)	59.29 (−0.01)	60.42 (+0.03)	60.73 (−0.01)	60.51 (+0.03)	59.29 (−0.01)
Me β-D-Glc*p*-(1→6)-α-D-Glc*p* (**7**)	55.33 (+0.06)	58.42 (+0.02)	60.81 (+0.10)	60.68 (+0.13)		60.46 (+0.07)	60.81 (+0.07)	60.46 (−0.02)	59.31 (+0.01)
Me β-D-Glc*p*-(1→6)-β-D-Glc*p* (**8**)	57.05 (+0.09)	60.34 (−0.05)	60.67 (−0.07)	60.67 (+0.19)		60.34 (−0.05)	60.67 (−0.07)	60.42 (−0.06)	59.28 (−0.02)
Me α-D-Glc*p*-(1→6)-α-D-Glc*p* (**9**)	55.34 (+0.07)	58.46* (+0.06)	60.72 (+0.01)	60.48 (−0.07)		58.55* (+0.15)	60.72 (+0.01)	60.54 (−0.01)	59.23 (0.00)
Me α-D-Glc*p*-(1→6)-β-D-Glc*p* (**10**)	56.94 (−0.02)	60.38 (−0.01)	60.67 (−0.07)	60.38 (−0.10)		58.36 (−0.04)	60.67 (−0.04)	60.54 (−0.01)	59.21 (−0.02)
Me α-D-Gal*p*-(1→6)-α-D-Glc*p* (**11**)	55.33 (+0.06)	58.45* (+0.05)	60.69 (−0.02)	60.50 (−0.05)		58.62* (+0.10)	58.12 (+0.01)	61.39 (+0.08)	59.21 (+0.02)
Me α-D-Gal*p*-(1→6)-β-D-Glc*p* (**12**)	56.84 (−0.12)	60.33 (−0.06)	60.60 (−0.14)	60.33 (−0.15)		58.34 (−0.18)	58.09 (−0.02)	61.28 (−0.03)	59.11 (−0.08)
Me β-D-Glc*p*-(1→4)-α-D-Glc*p* (**13**)	55.32 (+0.05)	58.46 (+0.06)	60.07 (−0.64)		59.09* (−0.14)	60.87 (+0.48)	60.74 (0.00)	60.44 (−0.04)	59.48* (+0.18)

TABLE II (*continued*)

Per-O-methyl derivatives of	*MeO-1*	*MeO-2*	*MeO-3*	*MeO-4*	*MeO-6*	*MeO-2'*	*MeO-3'*	*MeO-4'*	*MeO-6'*
Me β-D-Glc*p*-(1→4)-β-D-Glc*p* (**14**)	56.89 (−0.07)	60.38 (−0.01)	60.07 (−0.67)		59.12* (−0.18)	60.72 (+0.33)	60.72 (−0.02)	60.38 (−0.10)	59.40* (+0.10)
Me α-D-Glc*p*-(1→4)-α-D-Glc*p* (**15**)	55.30 (+0.03)	58.18 (−0.22)	60.28 (−0.43)		59.05* (−0.18)	59.14* (+0.74)	60.66 (−0.05)	60.50 (−0.05)	59.28* (+0.05)
Me α-D-Glc*p*-(1→4)-β-D-Glc*p* (**16**)	56.86 (−0.10)	60.14 (−0.25)	60.14 (−0.60)		59.05* (−0.25)	59.15* (+0.75)	60.64 (−0.07)	60.50 (−0.05)	59.25* (+0.02)
Me β-D-Gal*p*-(1→4)-α-D-Glc*p* (**17**)	55.27 (0.00)	58.41 (+0.01)	59.84 (−0.87)		59.01* (−0.22)	61.07 (+0.43)	58.22 (−0.05)	61.29 (+0.03)	59.21* (+0.03)
Me β-D-Gal*p*-(1→4)-β-D-Glc*p* (**18**)	56.87 (−0.09)	60.31 (−0.08)	59.82 (−0.92)		59.09* (−0.21)	61.00 (+0.36)	58.19 (−0.08)	61.28 (+0.02)	59.18* (0.00)
Me β-D-Glc*p*-(1→3)-α-D-Glc*p* (**19**)	55.28 (+0.01)	58.16 (−0.24)		60.41 (−0.14)	59.23* (0.00)	60.41 (+0.02)	60.70 (−0.04)	60.41 (−0.07)	59.50* (+0.20)
Me β-D-Glc*p*-(1→3)-β-D-Glc*p* (**20**)	56.91 (−0.05)	60.14 (−0.25)		60.65Δ (+0.17)	59.29* (−0.01)	60.32Δ (−0.07)	60.65 (−0.09)	60.32 (−0.16)	59.44* (+0.14)
Me β-D-Glc*p*-(1→2)-α-D-Glc*p* (**21**)	55.31 (+0.04)	←	———	———	59.20* (−0.03)	——— 60.33–60.62 ———		→	~59.3* (0.0)
Me β-D-Glc*p*-(1→2)-β-D-Glc*p* (**22**)	56.32 (−0.64)	←	———	———	59.29* (−0.01)	——— 60.33–60.62 ———		→	59.34* (+0.04)
Me α-D-Glc*p*-(1→2)-α-D-Glc*p* (**23**)	55.28 (+0.01)		61.30 (+0.59)	← ———	59.19* (−0.04)	57.60 (−0.80)	——— 60.47–60.66	→	59.31* (+0.08)
Me α-D-Glc*p*-(1→2)-β-D-Glc*p* (**24**)	57.04 (+0.08)		61.21 (+0.47)	60.47 (−0.01)	59.19* (−0.11)	57.75 (−0.65)	60.66 (−0.05)	60.47 (−0.08)	59.31* (+0.08)

[a]In acetonitrile-d_3. Shifts on δ scale (p.p.m.). The assignments of the resonances marked with * or Δ, respectively, may be interchanged. The shift increment, relative to the resonance position of the corresponding methoxyl carbon in the monomer, is given in parentheses.

Methyl per-O-methyl-gentiobiosides (**7** *and* **8**), *-isomaltosides* (**9** *and* **10**), *and -melibiosides* (**11** *and* **12**). The δMeO values show, in analogy to those of the skeleton carbon atoms, only very small incremental shifts. The interpretation of the methoxyl resonances of the melibiose derivatives (**11** and **12**) is facilitated by the observed differences in the chemical shifts of MeO-3 and MeO-4 in the D-glucose and D-galactose molecules. The identification of the signals of **9** and **10** was made on the basis of the spectra of **11** and **12**. In the spectra of **7** and **8**, all methoxyl resonances, except those of MeO-1, MeO-2, and MeO-6′ in **7**, and MeO-1 and MeO-6′ in **8** are concentrated at 60.3–60.8 p.p.m. These small $\Delta\delta$ values complicate the analysis of these peaks.

Methyl per-O-methyl-cellobiosides (**13** *and* **14**), *-maltosides* (**15** *and* **16**), *and -lactosides* (**17** *and* **18**). In the spectra of **13** and **14**, **15** and **16**, and **17** and **18**, the chemical shifts of MeO-2′ and MeO-3 show significant increments. Downfield shifts were observed for δMeO-2′ and upfield shifts for δMeO-3.

Methyl per-O-methyl-laminaribiosides (**19** *and* **20**). Large incremental shifts of δMeO-2 and δMeO-2′, which might be expected in analogy to those observed for δMeO-3 and δMeO-2′ in the β-D-(1→4)-linked disaccharides, do not occur in **19** and **20**.

Methyl per-O-methyl-sophorosides (**21** *and* **22**) *and -kojibiosides* (**23** *and* **24**). Relatively large upfield incremental shifts for δMeO-2′ and downfield shifts for δMeO-3 were found in both **23** and **24**. The directions of these shifts are opposite to those observed for the substituents in the same positions of the α-D-(1→4)-linked sugars. Surprisingly, δMeO-1 is quite normal for both anomers. The resonances of MeO-3′, -4, -4′, -6, and -6′ of the α-D anomer coincide with the peaks of the β-D anomer. Therefore, they could not be analyzed.

The chemical shift for MeO-1α in the spectrum of **21** and **22** is in agreement with the resonance positions of glycosidic methyl groups in other α-D anomers, whereas δMeO-1β has shifted upfield.

^{1}H-N.m.r. data of the anomeric protons. — ^{1}H-N.m.r. chemical shifts and coupling constants of the anomeric protons of compounds **1**–**24** are given in Table III. The signals appear as doublets in a region downfield from the other protons. The coupling constants are almost identical with those of the corresponding monomers **1** and **2**, and **3** and **4**, except for H-1α and H-1β in the sophorose and kojibiose derivatives (**21**–**24**), where smaller values are observed.

The δH-1 values of the disaccharide derivatives and the corresponding monomers are consistent, but the H-1′ resonances are shifted downfield. In both anomers of each permethylated disaccharide, δH-1′ is almost the same, except for the (1→2)-linked sugars.

Previously, Minnikin[18] reported δH-1 and δH-1′ values for some of the discussed compounds in chloroform-*d* and benzene-d_6 solution. He distinguished H-1 and H-1′ by the different aromatic-solvent-induced shifts; when chloroform-*d* was replaced by benzene-d_6, the H-1 signal was shifted upfield and that of H-1′ downfield. However, we found (see Table IV), that similar shifts did not take place for the

H-1α and H-1β signals of the per-*O*-methyl sophorose derivatives (**21** and **22**) and for the H-1'α signal of the per-*O*-methyl kojibiose derivative **23**. Generally, the data obtained from acetonitrile-d_3 solutions differ slightly from those of the chloroform-*d* solutions.

TABLE III

^{1}H-N.M.R. DATA OF THE ANOMERIC PROTONS OF PER-*O*-METHYL DERIVATIVES OF METHYL D-GLUCOPYRANOSIDES, OF METHYL D-GALACTOPYRANOSIDES, OF METHYL GLYCOSIDES OF REDUCING DISACCHARIDES, AND OF NON-REDUCING DISACCHARIDES[a]

Per-O-methyl derivatives of	*H-1'* (α-D *linkage*)		*H-1α*		*H-1'* (β-D *linkage*)	*H-1β*
Methyl α-D-glucopyranoside (**1**)			4.75	(3.5)		
Methyl β-D-glucopyranoside (**2**)						4.11 (7.8)
Methyl α-D-galactopyranoside (**3**)			4.77	(~2.2)		
Methyl β-D-galactopyranoside (**4**)						4.09 (7.5)
α,α-Trehalose (**5**)	5.10	(3.4)				
β,β-Trehalose (**6**)					4.58 (7.5)	
Methyl α-gentiobioside (**7**)			4.77	(3.4)	4.25 (7.5)	
Methyl β-gentiobioside (**8**)					4.26 (7.5)	4.12 (7.5)
Methyl α-isomaltoside (**9**)	4.93	(3.5)	4.76	(3.5)		
Methyl β-isomaltoside (**10**)	4.95	(3.5)				4.13 (7.5)
Methyl α-melibioside (**11**)	4.96	(~2.2)	4.77	(3.5)		
Methyl β-melibioside (**12**)	4.97	(~2.2)				4.14 (7.5)
Methyl α-cellobioside (**13**)			4.77	(3.5)	4.28 (7.7)	
Methyl β-cellobioside (**14**)					4.29 (7.7)	4.14 (7.6)
Methyl α-maltoside (**15**)	5.45	(3.6)	4.77	(3.4)		
Methyl β-maltoside (**16**)	5.45	(3.6)				4.16 (7.4)
Methyl α-lactoside (**17**)			4.75	(3.4)	4.24 (7.4)	
Methyl β-lactoside (**18**)					4.26 (7.2)	4.13 (7.6)
Methyl α-laminaribioside (**19**)			4.77	(3.5)	4.68 (7.5)	
Methyl β-laminaribioside (**20**)					4.69 (7.6)	4.13 (7.8)
Methyl α-sophoroside (**21**)			4.74	(2.8)	4.34 (7.5)	
Methyl β-sophoroside (**22**)					4.60 (7.8)	4.21 (6.8)
Methyl α-kojibioside (**23**)	5.05	(3.5)	4.81	(3.1)		
Methyl β-kojibioside (**24**)	5.39	(3.3)				4.22 (7.1)

[a]In acetonitrile-d_3; δ p.p.m., (*J*) Hz.

TABLE IV

^{1}H-N.M.R. DATA OF METHYL PER-*O*-METHYL-α,β-SOPHOROSIDE (**21** AND **22**) AND -α,β-KOJIBIOSIDE (**23** AND **24**) IN VARIOUS SOLVENTS[a]

Compounds	*Solvent*	*H-1α*	*H-1'α*	*H-1β*	*H-1'β*
21 and **22**	$CDCl_3$	4.87	4.34	4.28	4.57
21 and **22**	C_6D_6	4.99	4.47	4.29	4.79
23 and **24**	$CDCl_3$	4.83	5.02	4.31	5.47
23 and **24**	C_6D_6	4.77	4.96	4.16	5.64

[a]Chemical shifts on δ scale (p.p.m.).

DISCUSSION

The complete ^{13}C-n.m.r. spectra of all investigated disaccharide derivatives can be subdivided into three parts, *viz.*: (i) the anomeric carbon atoms from 93.0 to 105.5 p.p.m., (ii) the nonanomeric skeleton carbon atoms from 66.5 to 88.0 p.p.m., and (iii) the methoxyl carbon atoms from 55.0 to 61.5 p.p.m. Separate ranges can be observed for the anomeric carbon atoms in α-D glycosidic linkages: C-1α (97.0–100.5 p.p.m.), C-1′ (93.0–98.0 p.p.m.); and in β-D glycosidic linkages: C-1β and C-1′ (103.0–105.5 p.p.m., except in **6**).

In the ^{1}H-n.m.r. spectra, four separate regions can be distinguished for the different types of anomeric protons, *viz.*: H-1α (4.73–4.81 p.p.m.), H-1β (4.09–4.22 p.p.m.), H-1′ in α-D linkages (4.90–5.45 p.p.m.), and H-1′ in β-D linkages (4.24–4.70 p.p.m.).

Hence, the anomeric configuration(s) of glucopyranosyl and galactopyranosyl units in permethylated carbohydrates can be deduced from the chemical shifts of the anomeric carbon atoms, as well as of the anomeric protons. For this purpose, the coupling constants with the anomeric proton, *i.e.* $J_{H-1,\,H-2}$ ($J_{H\text{-}1',\,H\text{-}2'}$) and $J_{C-1,\,H\text{-}1}$ ($J_{C-1',\,H-1'}$)[19,20] and the carbon chemical shift of MeO-1 are also useful. Table II shows that axially oriented (α) MeO-1 groups resonate at ~55.3 p.p.m., whereas equatorially oriented (β) MeO-1 groups resonate at 56.8–57.1 p.p.m. (except for **22**).

As shown in Table I, substantial upfield incremental shifts are observed for the carbon atoms involved in the inter-sugar glycosidic linkage (again with the exception of **22**), whereas the shifts for the nonanomeric carbon atoms of α-D-linked disaccharides are larger than those of β-D-linked. These incremental shifts cannot be due to differences in electronegativity or anisotropy of the substituents, since these effects would introduce smaller shifts. The observed difference between the shifts depends on steric effects. A sterically hindered carbon atom is more shielded than a similar, not hindered one, the so-called "steric compression effect"[21]. In ^{1}H-n.m.r. this effect is present too, but it is less evident. In contrast to carbon atoms, protons are deshielded by steric hindrance[22]. These trends in the chemical shifts are also observed for δC-1′ and δH-1′ when compared to δC-1 and δH-1 in corresponding anomeric configurations. Thus, the replacement of a methoxyl group by a glycosyl residue (with retention of configuration) gives rise to steric interactions, that reflect the larger steric requirement of a glycosidic substituent. Interannular steric hindrance for the nonanomeric carbon atoms involved in an α-D glycosidic linkage, is stronger than for those involved in a β-D linkage, as is shown by the relatively large incremental shifts of these carbon atoms in **9–12**, **15**, **16**, **23**, and **24**.

The small incremental shifts observed for the carbon atoms not involved in the glycosidic bond of the (1→1)- and (1→6)-linked disaccharide, and (to a lesser extent) also of the α-D-(1→4)-linked glucobiose derivatives, suggest that interannular steric interactions are almost absent for these carbon atoms. This implies, particularly for the α-D-linked sugars, that the (folded) conformations at the glycosidic bond, in which

the angle between the planes of the two pyranose rings is close to the minimum value, do not contribute appreciably to the total conformer population. This conclusion is supported by the observation that δC-3′ and δC-5′, in the α-D-linked sugars, show only small incremental shifts when the methoxyl substituent at C-1′ is replaced by a glycosyl residue. Therefore 1,3-diaxial interaction between this substituent and C-3′–H and C-5′–H is restricted mainly to the interaction of the axial protons and the glycosidic oxygen atom.

Significant upfield incremental shifts were observed for C-3 in the β-D-(1→4)-linked and for C-4 in the β-D-(1→3)-linked disaccharide derivatives. The magnitude of these shifts suggests a contribution of the steric compression effect to the shielding of these carbon atoms. It is of interest that a similar shift for C-3 in the α-D-(1→4)-linked sugars was not observed, although substantial differences in steric hindrance of C-3 between α- and β-D-(1→4)-linked disaccharides cannot clearly be deduced from molecular models. The presence of steric perturbations in the inter-sugar glycosidic region of the β-D-(1→4)-linked disaccharides is reflected too in the incremental shifts of δMeO-3 and δMeO-2′ (Table II). The α-D-(1→4)-linked disaccharides show a similar effect.

In the permethylated (1→2)-linked disaccharides **21**–**24**, the pattern of the incremental shifts is less evident than for the other disaccharides. As a consequence of the close proximity of the methoxyl aglycone to the inter-sugar glycosidic bond, change of anomeric configuration at C-1 may give rise to large differences in the incremental shifts of the carbons (C-1, C-1′, C-2, and MeO-1) and of the protons (H-1 and H-1′) involved in the glycosidic linkages. The shielding states of C-1 and C-1′ are differently associated with the shielding states of the attached protons. Surprisingly, no strong incremental shifts were observed for δC-1, δH-1, and δMeO-1 in **23** and **24**, which reflects a rather small over-all effect of the α-D-(1→2)-glycosidic linkage on the methyl glycosidic group. However, the relative small coupling constants $J_{H-1,H-2}$ show that still some deformations in the MeO–C-1–C-2–OGlc*p* moiety of **21**–**24** are present (Table III). The deviation of the dihedral angle $\Phi_{1,2}$ between H-1 and H-2, with regard to $\Phi_{1,2}$ in **1** (47°) and in **2** (147°), respectively, suggests a flattening of this ring fragment in the β-D anomers[15,23]; in the α-D anomers this flattening is less pronounced. Evidently, significant differences exist between the preferred conformations at the glycosidic linkages of **21**–**24**.

In conclusion, the shift increments of skeletal and methoxyl carbon atoms at various locations of the disaccharide molecules show significant differences in steric interaction of the monomeric units. These differences depend on the type and configuration of the glycosidic bonds. The incremental shifts of the carbon atoms in the vicinity of the glycosidic linkages can give valuable information concerning the conformations of these bonds. For a more detailed conformation analysis, high-resolution, single-resonance spectra are required.

EXPERIMENTAL

Laminaribiose was prepared from laminaran of *Laminaria hyperborea* (Koch Light Lab. Ltd.)[24]. β,β-Trehalose was kindly supplied by Dr. G. G. Birch, the other disaccharides were commercial products.

Sugars were methylated according to the method of Kuhn *et al.*[25]. The separation of permethylated α and β anomers was obtained by t.l.c. on 0.5-mm precoated plates of Silica Gel (Merck) with 3:2 (v/v) hexane–acetone, and detection under u.v. light after spraying with 1% Morin in methanol. The components were extracted from the silica with chloroform.

^{13}C-N.m.r. spectra, decoupled for proton noise, were recorded at 25.16 MHz on a Varian XL-100-15 FT spectrometer, operating in the deuterio-lock mode at a probe temperature of 30°, for solutions (5–20%) of the disaccharide derivatives in acetonitrile-d_3. ^{1}H-N.m.r. spectra of the same samples of disaccharide derivatives in acetonitrile-d_3 were recorded on a Varian HA-100 spectrometer (Organic Chemical Institute T.N.O., Utrecht) at 25°. Chemical shifts are given on the δ scale, relative to that of internal tetramethylsilane, with an accuracy of 0.04 p.p.m. for ^{13}C-n.m.r. spectra and 0.01 p.p.m. for ^{1}H-n.m.r. spectra. The accuracy of the coupling constants is about 0.2 Hz.

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